Characterisation of soft tissue and skeletal bullet wound trauma and three-dimensional anatomical modelling

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Contents

Thesis Abstract

Effects of firearm projectiles causing injuries to human vital organs were investigated. In this study, the effects of high and medium velocity projectiles on thoracic organs (hearts and lungs) and abdominal organs (livers and kidneys) were investigated using fresh porcine organs and tissue simulants. Furthermore, characteristics of wounds caused by bullets to the cranium, ribs, sternum, vertebrae, scapula and pelvis were analysed. The direction of bullet entry, the manner of death and the mechanisms that caused bone injuries were also determined.

A mounted, remotely operated firearm was used to fire a spherical projectile at 900m/s and 500m/s. Doppler radar, infrared sighting screens and high-speed video cameras were used to determine the velocities of the projectiles during their passage through 50mm cubes of fresh porcine lungs, livers, kidneys and hearts, and ballistic tissue simulants. The organs were tested at room (16°C) and core body (37°C) temperature. Time and temperature associated changes in porcine organs were histologically analysed. Two skeletal collections with documented cases of firearm trauma were used for skeletal analyses.

Energy loss from projectiles penetrating porcine organs tested at 16°C and 37°C were not significantly different. Histological features of porcine organs did not change during the time of heating from refrigeration to core body temperatures. The energy loss from projectiles penetrating organ simulants at the two tested velocities were different from those measured in porcine organs. This may be the result of differences in densities between the simulants and porcine organs.

In skull bones, shape and extent of wounds varied according to the projectile entry energy. Small nicks, circular wounds, or butterfly-like mid-shaft and comminuted fractures were seen in ribs. Injuries in the sternum and ilium were circular in appearance. In the vertebrae, shattering of the vertebral bodies, small fractures and missing segments of the pedicles

occurred. Wound characteristics of scapulae varied according to the bone thickness across the scapula. Circular wounds with/without internal bevelling and larger irregularly shaped injuries with/without external bevelling were identified as entry and exit wounds, respectively. High velocity projectiles caused radiating and concentric fractures in brain cases.

Skeletal wound characteristics, location and number of wounds, and projectile path allowed the determination of the manner of death. Fracture patterns varied according to the physical properties of bone and projectile entry energy. Re-heating of organs to core body temperature in ballistic research was not necessary. Furthermore, 50mm cubes of organ tissue were adequate to significantly reduce the projectile velocity from entry to exit. New organ simulants with densities similar to soft tissues of human organs or simulants where the density could be altered to match that of the test organ should be used in future ballistic research. Simulants used to represent bone should behave like fresh bone. Accurate information generated by such simulants is of value for the construction of digital programmes or 3D-models to predict how different types of ammunition fired from a variety of firearms cause injury to the human body. They will also be valuable in the development of new ammunitions, firearms, protective body armour and medical treatment of such injuries.

Originality statement

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Thesis Style and Layout

This thesis is submitted in the style of a 'Thesis by Publication'; and as such, each chapter is a standalone paper. Part I of this thesis introduces the topic of ballistics trauma and presents the research aims and objectives and is followed by two chapters. Chapter 1 provides an in-depth analysis of the topic of ballistics, anatomical modelling, soft and hard tissue trauma of the body and provides an insight into the research aims of my doctoral studies. Chapter 2 provides an in depth look at the physical principles and biomechanics associated with ballistic skeletal injuries.

As each study uses different methods, specific methods and materials relating to each research task are described in detail in the methods subsection of the respective publication. The results chapter is replaced by seven research articles (Chapters 3 to 9), split into two parts. Part II of this thesis describes ballistic experiments utilising porcine models and ballistics simulants (Chapters 3, 5 and 6) and a histological experiment on porcine organs (Chapter 4). Part III of this thesis (Chapters 7 to 9) describes skeletal characteristics of ballistics trauma and what can be determined from the skeleton using forensic, anthropological and osteological methods.

Author contributions for each publication are outlined in the preceding 'Statements of Authorship', and the context of the article with respect to the thesis is described in the Context subheading. Each article format remains as is in the submitted or published version, complying with the specific journal requirements. As such, formatting may be slightly different including referencing and style of English (American/Australian English).

Finally, the research is drawn together in Part IV where a Summary of Results and Discussion is presented followed by the Conclusions and Future Directions of the research. Published manuscripts as seen in the respective Journals are found in the Appendices.

Publications

Published Articles

Humphrey, C and Kumaratilake, J (2016) Ballistics and anatomical modelling – a review. *Legal Medicine*. 23:21-29. doi:10.1016/j.legalmed.2016.09.002

Humphrey, C, Henneberg, M, Wachsberger, C, Maiden, N and Kumaratilake, J (2017) Effects of re - heating tissue samples to core body temperature on high - velocity ballistic projectile–tissue interactions. *Journal of Forensic Sciences*. doi: 10.1111/1556-4029.13473 [epub ahead of print]

Humphrey, C, and Henneberg, M (2017) Anthropological analysis of projectile trauma to the bony regions of the trunk. *Anthropological Review.* 80(2);207-218. doi: 10.1515/anre-2017-0015

Humphrey, C, and Kumartilake, K (2017) A histological analysis of visceral organs to evaluate the effect of duration of heating from refrigeration to core body temperature for ballistics investigations. *American Journal of Forensic Medicine and Pathology.*

doi: 10.1097/PAF.0000000000000345 [epub ahead of print]

Humphrey, C, Henneberg, M, and Kumaratilake, J (Accepted 19th October 2017) Variability of characteristics of cranial trauma in skeletal material. *Anthropologischer Anzeiger* [epub ahead of print]

Humphrey, C, Henneberg, M, Wachsberger, C, and Kumaratilake, J (Accepted 15th November 2017) The deceleration of a spherical projectile passing through porcine organs at laboratory temperature (16°C) and core body temperature (37°C). *Journal of Forensic and Legal Medicine.*

Under Review

Humphrey, C, and Henneberg, M. When physics meets ballistics bone injury. *Journal of Trauma and Acute Care Surgery*

Humphrey, C, Henneberg, M, and Kumaratilake, J. Reconstructing the life and manner of death from skeletal remains in a case of a Hispanic man who died of gunshot wound in 1962, St Louis, Missouri. *Journal of Anthropology*

Humphrey, C, Henneberg, M, Wachsberger, C, and Kumaratilake, J. Comparison of porcine organs and commonly used ballistics simulants when subjected to impact from steel spheres fired at supersonic velocities. *Forensic Science International*

Published Conference Abstracts

Humphrey, C, Henneberg, M, and Kumaratilake, J (2016) Osteological analysis can still shed light on a recent forensic case. 85th Annual Meeting of the *American Association of Physical Anthropologists*.

Humphrey, C, and Henneberg, M (2017) Reconstructing the manner of death from cranial trauma. 44th Annual North American Meeting of the *Paleopathology Association*.

Conference Attendances

2015

84th Annual Meeting of the American Association of Physical Anthropologists – St Louis, Missouri, United States of America

2016

85th Annual Meeting of the American Association of Physical Anthropologists – Atlanta, Georgia, United States of America

2017

86th Annual Meeting of the American Association of Physical Anthropologists – New Orleans, Louisiana, United States of America

42nd Human Biology Association Meeting - New Orleans, Louisiana, United States of America

44th Annual North American Meeting of the Paleopathology Association - New Orleans, Louisiana, United States of America

International Research Attendances

2015

Smithsonian Institution, Washington DC, USA

2016

Smithsonian Institution, Washington DC, USA

Cleveland Museum of Natural History, Cleveland, Ohio, USA

Scholarships and Awards

2015

Australian Government Post Graduate Scholarship

2016

Australian Government Post Graduate Scholarship

2017

Australian Government Post Graduate Scholarship

Adelaide Medical School Research Travel Award

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Part ONE

Part I provides the background to ballistics and anatomical modelling. The Introduction provides introductory remarks, discusses the prevalence of firearm use and the toll this has taken in the world. It also provides a brief overview of current research into ballistics trauma and the research aims and objectives of this PhD. Chapter 1 is a published review providing an in depth look at ballistics, the current research in this field, mechanisms of wounding in soft tissues and anatomical modelling of soft tissues in the thorax and abdomen. It shows where research is required in relation to computer modelling of the effects of penetrating projectiles in causing injuries to organs. In addition, the advantages and disadvantages of the use of currently available ballistics simulants to investigate the effects of penetrating projectiles in causing injuries to body tissues have been discussed. Chapter 2 is a manuscript submitted for publication and discusses the physical principles and biomechanics of how projectiles cause injuries to both cranial and infra-cranial skeletal tissues. This manuscript highlights the need for collaborations between physicists and medical professionals to understand the complex nature of ballistic injuries to the human body. Furthermore, the current bone simulants and their value for anatomical modelling of bone injuries are discussed.

Introduction

Firearms have been used in the world for centuries, in the armed forces, law enforcement agencies, by criminals and various other groups. Casualties resulting from firearms in humans, including armed and unarmed civilians, are enormous. The injuries caused by firearms could vary from minor to severe and could cause short to long term (i.e. lifelong) disabilities. Therefore, the cost of treatment of such injuries has become a huge financial burden to the victim, family and ultimately to the country and the society (i.e. taxpayers) (Cook et al., 1999). In the United States, the cost has been estimated at US $100 – 174 billion annually (i.e. AUD $134 – 234 billion) (Rhee et al., 2016). In addition to the financial cost, long-term disabilities caused by firearm injuries affect the mental health of the victim as well as that of the immediate family. Furthermore, caring for individuals with such disabilities becomes a burden for the family and close friends.

In 1996, the Port Arthur massacre in Tasmania, Australia, led to the introduction of strict firearm controls in Australia (National Firearms Agreement, NFA, 1996), including prohibition of certain types of firearms (e.g. fully automatic rifles) and a buy-back scheme, which saw over 600,000 firearms destroyed by the police (Baker & McPherdran, 2007). This, in turn, reduced the number of casualties resulting from firearms in Australia, particularly mass shootings.

Research has been carried out to investigate the effects of firearms and their ammunition in causing injuries to the human body including the vital organs, which could lead to immediate incapacitation. Available firearms in the world have been divided into three general categories; handguns, rifles and shotguns (Stefanopoulos et el., 2014). The ammunition that could be used with these weapons varies markedly. Therefore, each type of ammunition and the firearm should be investigated to learn and understand the mechanisms that are involved in causing injuries to the human body and its organs (i.e. hard and soft organs).

Characteristics and the extent of injury caused by a bullet penetrating the human body are determined by the bio-physical properties of the organ/tissue (i.e. elasticity, density and size) and the bullet (i.e. shape, construction, velocity, mass and energy) (Fackler, 1986; Hollerman et al., 1990). The mechanisms that cause the injury include; a pressure wave, temporary cavity and permanent cavity caused by the bullet, and yawing, tumbling and fragmenting of the bullet on impact with the body/organ (Stefanopoulos et al., 2014). Accurate understanding of the mechanisms involved in causing injuries to the human body by different projectiles is necessary for:

- The designing and production of protective body armour
- The development of new ammunitions with different wounding capabilities
- The planning of fighting strategies to minimise injuries to the armed forces and maximise the injuries to the enemy
- The planning of methods of treatment to minimise the adverse effects of the injury
- The training of surgeons in the treatment of ballistics injuries.

Furthermore, accurate information is important in the development of new ammunition and weapons to cause death instantaneously, cause death of a few people from a single projectile or cause suffering for a long period of time. On the other hand, use of sophisticated instruments (imaging devices, computer programmes, etc.), improved organ simulants (i.e. materials to represent soft organs and bones) or animal models are necessary to generate accurate results. Generation of accurate results may also assist in the production of new computer programmes or computer based anatomical models that will predict accurately the effects of different ammunitions and weapons in causing injuries to the human body.

Previous research in the field of ballistics trauma has utilised cadaveric tissues, animals, ballistics ordnance gelatine, synthetic tissue simulants, physical anatomical models and

computational anatomical models (Bir, 2000; Shantz, 1978; Sellier, 1994; Mabbott et al., 2016). The human body is heterogeneous in its organ and tissue composition thus, their bio-physical properties vary across various locations on the body. The above-mentioned resources used in ballistics research to represent the human body structures have their own limitations, because, a single simulant could not represent the heterogeneous bio-physical properties of different organs/tissues across different diameters of the body. Torso and some cranial and maxillofacial models to investigate non-penetrating and penetrating projectile trauma have been developed (Roberts et al., 2007; Biermann et al., 2006; Thali et al., 2002). However, the development of digital programmes/models for the accurate prediction of the effects of penetrating projectiles on the human body need to consider the following aspects:

- The bio-physical properties of each organ/tissue in the body (i.e. elasticity, density, size), including the body wall and in-situ arrangement of the organs/tissues within the body cavities
- The physical properties of the projectile (i.e. weight, size, shape, construction)
- The dynamic properties of the projectile in flight (i.e. the behaviour of the projectile after leaving the muzzle of the firearm and on impact)
- The in-situ arrangement of organs/tissues (including the body wall) within each part of the body. The exit velocity of a projectile from one organ/tissue is the entry velocity of the projectile into the adjacent organ/tissue. The bio-physical properties of one organ/tissue are different from the other, thus the entry and exit velocities of the projectile for each organ/tissue need to be determined. Furthermore, the direction (anterior-posterior, posterior-anterior, right-left, left-right, superior-inferior and inferior-superior) and the angle of entry of the projectile into the body have to be taken into consideration in the determination of entry and exit velocities for each organ/tissue for their in-situ location in the body.

Therefore, for the development of 3D anatomical models or computer programmes for ballistic research, there needs to be a lot more research to generate the essential basic information.

A comprehensive review of the literature in relation to the effects of projectiles in causing injuries to the human body and its organs has been presented in Chapter 1.

Research Aims and Objectives

Aims

To determine:

1. Whether the vital organs of the thorax and abdomen respond differently to medium and high velocity projectiles penetrating them
2. Whether simulants used in ballistics research could accurately mimic the injury patterns seen in the organs of the body that result from medium and high velocity projectiles
3. Whether temperatures at which the body organs are experimented on in ballistics research could alter the effects of projectile-tissue interactions
4. The histological changes (i.e. at light microscopic level) occurring in the body organs (heart, lung, liver and kidney) during the period of re-heating from refrigeration temperature to the core body temperature
5. The variation in the characteristics of skeletal wounds caused by projectiles and whether these characteristics could be used to identify the site of entry of the projectile and to predict the manner in which the individual died.

Objectives

1. Establish a method to fire spherical steel projectiles of same size and weight repeatedly at chosen test velocities that represent a currently used military weapon (i.e. to eliminate variations resulting from projectile construction) and determine:
    a. Whether the length of time taken to re-heat porcine organs to core body temperature could affect the energy loss from projectiles penetrating the porcine organs at entry velocities of 500 m/s and 900 m/s (Chapter 3)

b. The length of time taken to re-heat porcine organs from refrigeration temperature to core body temperature (Chapter 4)

c. Whether the length of time taken to re-heat porcine organs to core body temperature from refrigeration temperature alters their morphology as determined by histological techniques (Chapter 4)

d. Whether the deceleration of projectiles travelling through porcine organs/tissues at laboratory temperature (16°C) is different from the deceleration of projectiles passing through organs at core body temperature (37°C) (Chapter 5)

e. The energy loss of the projectiles when penetrating porcine organs (Chapter 5 and Chapter 6).

2. Identify the organ simulants that could represent the bio-physical properties of porcine organs by:

    a. Determining whether the energy loss of both high and medium velocity projectiles penetrating through ballistics simulants (10% Ballistics Gelatine, 20% Ballistics Gelatine and Clear Gel) is similar to the energy loss from projectiles penetrating through the porcine organs (Chapter 6)

    b. Determining if previously published low velocity results could be compared with those of the medium and high velocity projectiles (Chapter 6).

3. Evaluate skeletal ballistics injuries to the cranium and post-cranial skeleton by:

    a. Analysing the bullet wound injuries of two museum skeletal collections with known ballistics trauma history (Chapters 7 to 9).

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Chapter 1

1 Ballistics and anatomical modelling - a review

Caitlin Humphrey and Jaliya Kumaratilake

1.1 Statement of Authorship

Manuscript Details

Title of Paper	Ballistics and anatomical modelling – a review
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Humphrey, C and Kumaratilake, J (2016) Ballistics and anatomical modelling - a review. Legal Medicine. 23:21-29

Principal Author

Name of Principal Author (Candidate)	Caitlin Humphrey
Contribution to the Paper	Literature review, interpretation, writing and editing manuscript
Overall percentage (%)	80%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 13/9/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	**Jaliya Kumaratilake**
Contribution to the Paper	Manuscript writing and editing
Signature	Date 13.09.2017

1.2 Context

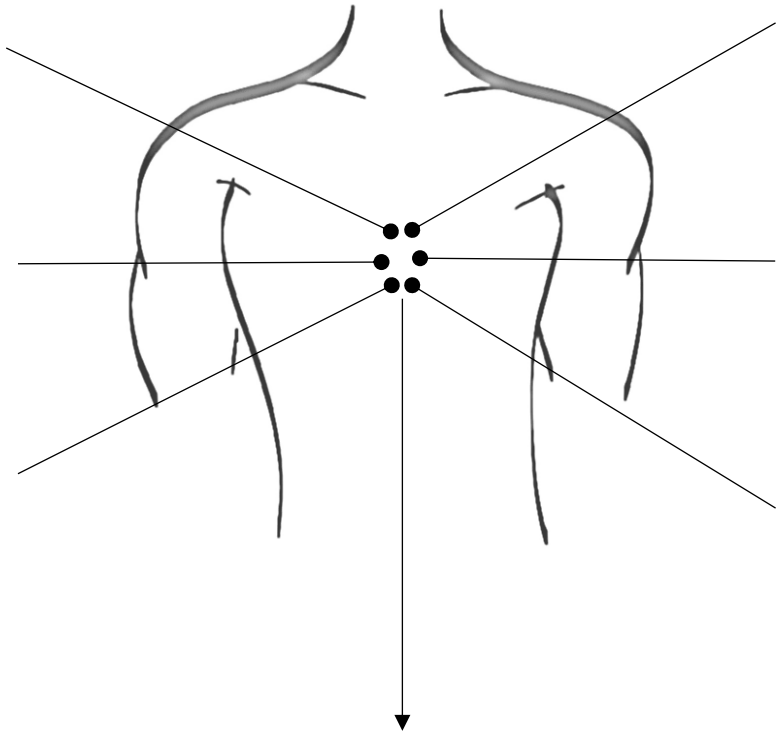
An in-depth review of the literature is presented in Chapter 1. This review describes the basics of ballistics and ammunitions, which are necessary to understand the mechanisms of wounding in soft tissues of the human body. Furthermore, presented here is an analysis of currently used anatomical models in ballistics research and an account of what is required to generate an accurate anatomical model/s that could represent the heterogeneity in the bio-physical properties of the organs in the human body.

1.3 Abstract

Ballistics is the study of a projectiles motion and can be broken down into four stages: internal, intermediate, external and terminal ballistics. The study of the effects a projectile has on a living tissue is referred to as wound ballistics and falls within terminal ballistics. To understand the effects a projectile has on living tissues the mechanisms of wounding need to be understood. These include the permanent and temporary cavities, energy, yawing, tumbling and fragmenting. Much ballistics research has been conducted including using cadavers, animal models and simulants such as ballistics ordnance gelatine. Further research is being conducted into developing anatomical, 3D, experimental and computational models. However, these models need to accurately represent the human body and its heterogeneous nature which involves understanding the biomechanical properties of the different tissues and organs. Further research is needed to accurately represent the human tissues with simulant sand is slowly being conducted.

1.3.1 Graphical Abstract

In anatomical modelling for the purpose of penetrating ballistics trauma the following aspects need to be included:



1.4 Introduction

Ballistics is the study of projectiles in motion. This includes the investigation of the projectile and the changes occurring during its motion from the barrel to its target. This motion can be divided into following four stages.

i) Internal ballistics - the study of the projectile during the acceleration phase within the barrel of the firearm

ii) Intermediate ballistics – the study of the projectile within the first few centimetres after the projectile leaves the barrel

iii) External ballistics - the study of the projectile's flight from the first few centimetres after leaving the barrel to the target

iv) Terminal ballistics - the study of the projectile during the penetration of the target (i.e. solid material) [1-4]. If the target is a living animal (i.e. including humans), the study is referred to as wound ballistics and investigates the effects of interaction of the projectile and the tissue [3, 5-8].

Ballistics related research has been conducted to investigate, the flight patterns of different bullets, the lethality of ammunition types and their effects on targets and to develop body armour and protection methods against different types of ammunition.

1.5 Overview of Firearms and Ammunition

Thousands of different types of firearms have been manufactured, but they are broadly grouped into three classifications; rifles, handguns and shotguns.

1.5.1 Rifles

Rifles have a distinctively longer barrel and imparts high energy and velocity to a bullet, thus has a greater wounding potential at long distances than the other two categories [1]. Rifling,

which involves cutting of spiral parallel grooves into the inner surface of the barrel (i.e. the bore), is an important feature. This feature, causes a rotational motion of the bullet along its longitudinal axis, creating a gyroscopically stabilised spin on the bullet during its flight. Thus, increases the range and accuracy and also reduces the tendency of the bullet to tumble in flight [3, 5, 9-12].

1.5.2 Handguns

Handguns have short barrel lengths, are concealable and are commonly used at close ranges [13]. The amount of energy generated is low and causes minor temporary cavitation, thus the wounding is limited to the permanent wound cavity caused by the bullet. However, there is a wide variety of expanding and deforming bullet types, which maximises the wounding potential within the permanent cavity [13].

1.5.3 Shotguns

Shotguns generally do not have riffled barrels and are called smooth bore firearms. They are designed to fire multiple shot-shell pellets instead of a single bullet, thus are commonly used for bird hunting or firing at fast moving targets. When shot-shell pellets are fired, they spread out as the distance from the barrel increases. This spread of pellets could be reduced by installing a choke mechanism at the end of the barrel to reduce the diameter of the nozzle. The wounding characteristics of shot-pellets are unique due to the type of ammunition used. At close range, the pellets stay grouped and act as one single mass and create a significant and distinct wound. Most often, the pellets will not exit the body, as they lose their kinetic energy and momentum rapidly. At a greater distance, the pellets spread and cause multiple injuries as they enter the body. However, each pellet will lose kinetic energy due to air drag and thus may not penetrate tissue at extreme ranges [13].

1.6 Mechanisms of Bullet Wounding

The mechanisms by which a bullet causes trauma is determined by the shape, construction, mass and velocity of the bullet, and the bio-mechanical properties of tissues, such as elasticity and density [11, 14] (Figure 1-1). Mass and velocity of a bullet determine the potential to destroy tissues, while the shape and construction account for the amount of tissue damage [15, 16]. Mechanisms for tissue injury resulting from bullets have been investigated extensively by European and American medical researchers [13, 17-23].

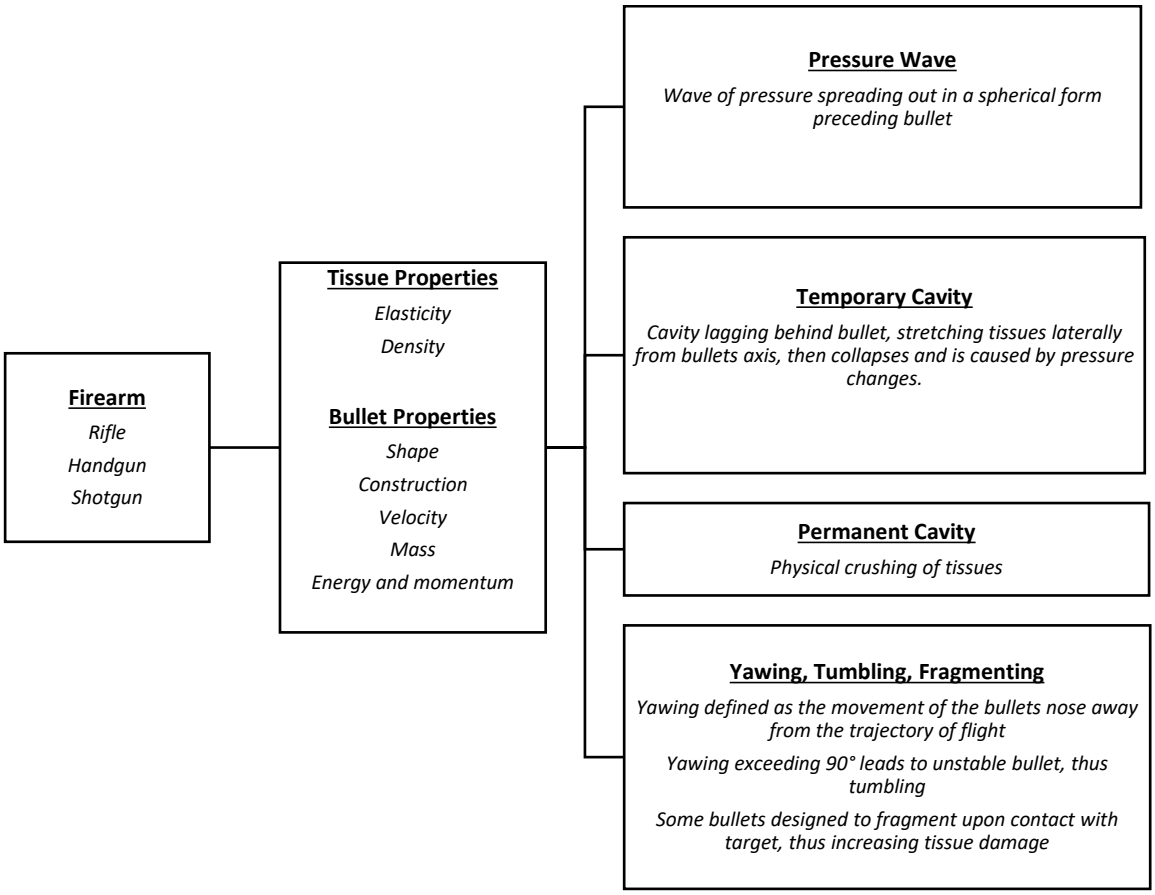


Figure 1-1 Flow chart of the factors determining the wounding in tissues

The factors that determine the extent of wound damage are as follows:

1.6.1 Density, Viscosity and Elasticity of Tissues

The density, viscosity and elasticity are the properties of a tissue that will determine the amount of injury that could be caused by a projectile [5, 12, 24]. The human body is heterogeneous. Stress-strain response of different organs/tissues (i.e. including internal organs) [25]; elastic properties of ribs, sternum, bone replacement materials [26] and vertebral column [27, 28] have been determined and they vary among the organs/tissues. Therefore, the amount and the type of injury caused by a projectile of same shape, size, mass and velocity to an organ/tissue in the body may vary according to the variation in the physical properties of them. The wounding potential of a projectile is also proportional to the amount of energy the projectile delivers to the tissues. However, the net effect of an injury caused by a projectile on an individual may depend on the specific functions lost to the body by the wound caused to the organ/tissue [5, 29].

1.6.2 Energy and Momentum of the Projectile

A widely accepted determinant of the wounding power of a bullet is the mass and velocity of the bullet, thus its momentum. The latter, is directly proportional to the amount of energy transferred into the tissues by the bullet, during its passage through the tissue [30]. Some research believes it to be the most important factor to cause tissue damage [10, 31], however other mechanisms can influence the wounding potential. Momentum is an important factor that could be used to estimate the wounding potential of a bullet, but has been found to underestimate the wounding potential [32]. The amount of energy carried in a bullet is dependent on the amount of gun powder ignited to fire the bullet, and the distance from the muzzle of the firearm to the tissues. A high powered firearm fired closely to the tissues will transfer more energy into the tissue than a bullet that has spent a significant amount of time

in flight before interacting with the tissues, i.e. the bullet has lost energy during the flight [5, 33]. A bullet could dissipate a small amount of its energy, if it passes directly through the organs/tissues and exits from the other side of the body. On the other hand, a bullet that stops within the body will transfer all its energy into the organs/tissues of the body. A bullet that is designed to fragment on impact will be an example of a projectile that will transfers its energy into the tissues and will most likely stop within the body. This is in comparison to a bullet that is designed to stay intact during its passage through tissues, thus exits the body retaining a large proportion of its energy and continues in flight [5].

Projectiles have been classified into low and high velocity categories [10, 34], however, the definitions of the two categories vary among publications. Bellamy and Zajtchuk [35] have defined high velocity as speeds exceeding 600 m/s, while Rozen and Dudkiewicz [10], have described it as speeds greater than that of sound in air (1100 feet/s or 335m/s). The velocity of projectiles fired from military rifles ranges from 700 – 950 m/s and that from handguns ranges from 250 – 370 m/s [5, 12]. Using the velocity of bullets, shotguns are generally classified as low velocity weapons [10, 36, 37]. However, the latter firearms cause major damage to bones, joints and soft tissues, including nerves and vascular structures due to the spread of the shot pellets [10, 36, 37]. These findings may indicate that both high and low velocity weapons cause severe damage to organs/tissues of the body. The use of impact velocity of a projectile, as the sole indicator of the extent and severity of an injury may be misleading [5, 31, 38-42]. For example, a large calibre bullet that has a large mass but travelling at a slower velocity will still cause massive tissue damage through the formation of a permanent cavity i.e. the bullet crushes the tissues.

1.6.3 Permanent Cavity

A permanent cavity or wound track is formed as the bullets frontal surface physically crushes the tissues it penetrates. The damage to the tissues is permanent and varies with different calibres of bullets. The path formed is not necessarily a straight line due to differences in tissue densities and contact with bones that causes the bullet to deflect [1, 12, 14]. The size and shape of the permanent cavity could be affected by three mechanisms; i) projectile yaw, where at the point of strike, the bullet yaws by 90° and travels with its long axis striking the tissues, leading to a cavity that is 10-14 times the size of the diameter of the projectile [3, 6, 10, 14]; ii) bullet deformation, where mushrooming or flattening of the tip increases the bullets diameter and iii) bullet fragmentation, which may occur with high velocity bullets or on contact with bones [14, 20, 43, 44] (described in detail later). A permanent cavity is usually surrounded by an area of cellular and endothelial damage (i.e. in the skeletal muscles), bruising, necrotic tissues or an area of haemorrhage [5, 35].

1.6.4 Pressure Wave and Temporary Cavitation

A pressure wave is formed at the point of impact of the bullet with the organ/tissue and spreads out rapidly in a spherical form preceding the bullet. It has been described to be travelling at approximately the speed of sound in water (~1500m/s) [1], which is different from the speed of sound in air (335m/s) [12]. These waves progress through tissues and dissipate the energy they are carrying s into the surrounding structures [45, 46]. The ability of this wave to cause tissue damage is disputed by some authors, as their duration is short (~2µsec), thus often deemed to play no part in wounding [14, 18, 38, 47-49]. However, injuries to the capillary endothelium in animal tissues [50]; traumatic brain injury [51], central and peripheral nervous system [50] have been linked to the pressure wave. The magnitude of the pressure wave has been measured to be as high as 60 atm [12]. Evidences are increasing to support the theory of

remote injuries by this pressure wave [52, 53]. This pressure wave could be closely linked to the temporary cavity, which is generally seen only in high velocity bullets. One study [50], found that the pressure waves come in small bursts and oscillates, moving through the body with a velocity that is similar to that of sound in water. They also found that the function of endothelial cells in smaller vessels were influenced by these waves, however it is unknown if it is a temporary or permanent effect.

Approximately 1-4 m/s after the bullet hits the body, a temporary cavity, lagging behind the bullet, is formed in body tissues. This is due to the movement and interaction of the bullet with the tissue causing changes in pressure, which leads to dislocation of tissues laterally from the bullets axis by stretching [1, 5, 10, 17, 29, 33, 54-56]. It could reach a size of up to 11-12.5 times the diameter of the bullet rapidly and has been described as a significant aspect of the wounding process [22, 57, 58]. This cavity collapses just as quickly as it is formed and other outward motions occur in elastic tissues and continues even with the bullet moving a distance away [20, 56]. Similar to pressure waves, the tissue damage caused by the temporary cavity varies greatly according to the size, the anatomical location of the cavity and the direction of least resistance taken by the pressure wave in the tissue [19]. The temporary cavity may cause significant injury by exceeding the elastic limit of a tissue, i.e. in tissues with minimal amount of elastic elements e.g. brain, liver, kidneys, spleen, pancreas, capillaries. Tissues that are more elastic (e.g. muscles, lungs) are fairly resistant to damage from this cavitation process [14, 18, 20, 21, 55, 59, 60]. Temporary cavitation could fracture bones that are located some distance away from the path of the projectiles, particularly with high velocity bullets [6, 11, 12, 14, 18, 57, 58, 61]. Yawing or tumbling in a projectile increases the size of the temporary cavity and tissue disruption. Vascular damage during temporary cavitation is extensive, small blood vessels such as capillaries are particularly affected [5, 33]. Clothing has been found to increase

the risk of an indirect fracture and a larger temporary cavity, potentially due to the clothing causing rapid yaw and possibly fragmentation of the projectile [54].

1.6.5 Yawing, Tumbling and Fragmenting

A stable bullet in flight is described as travelling nose-on with its central axis close to the trajectory. If the axis deviates in a tangent from the trajectory, it is referred to as yawing [5, 14, 57]. Rifles and handguns significantly reduce this yawing during flight by having rifling within the barrel of the firearm, thus promoting spin on the bullet to stabilise it during the flight. The stabilising action is overcome when it enters a higher density target, such as the human body [14, 29, 35, 62]. If the yaw is exceeded approximately by 15°, it causes the bullet to become unstable [22]. Once unstable, yawing will continue and eventually exceed 90°, at which point the bullet will begin to tumble [5, 35]. Once the gyroscopic stability is lost, drag increases leading to increases in permanent and temporary cavitation's, thus increasing the tissue damage [63-65]. In this situation, in some bullets, the entire length of the projectile acts against the tissues, increasing the amount of injury [1, 5, 11, 13, 22, 35, 38, 42, 53, 66-69]. It is common for some bullets such as 7.62x39mm Soviet to yaw to 180° and travel base forward until it exits the tissues or its energy has dissipated [11, 14, 18].

Some bullets are designed to fragment or deform upon contact with the target, thus increasing potential tissue damage. If a bullet makes contact with bone, fragments of the bone can become secondary projectiles and increase the tissue damage [63, 70]. When a tissue is perforated, it loses its elasticity and tears or becomes detached as it can no longer absorb the stretching caused by the temporary cavity formation [18, 44, 58]. Thus, an assumption can be made that a fragmenting or deforming bullet will create more tissue damage than one that is designed not to fragment [2, 16].

1.7 Ballistics Research

Wound ballistics research has important applications in commercial, military, civilian and medical areas relating to the human body. In this research, many aspects in relation to flight patterns of bullets, wounding potential of different ammunitions, and development of new ammunitions and protective body armour have been investigated. A summary of the current materials and the advantages and disadvantages associated with each material can be seen in Table 1-1.

Table 1-1 Overview of the advantages and disadvantages associated with currently used materials in ballistics experiments

Current Material	*Advantages*	*Disadvantages*
Human Cadavers	• Actual human organs/bodies	• Alterations in tensile strength, elastic modulus, biomechanical properties [75-82] • Dehydration and redistribution of water during freezing [80-82] • Loss of cellular fluid, cell shrinkage, extracellular fractures, haemolysis [74, 83-85] • Elderly and tensile strength and elasticity increase with age [71, 74, 97] • Ethical issues arise with use of human tissues
Animals	• Wide variety of animals [30, 89-91] • Well known pigs closely resemble human tissues	• Skin and subcutaneous tissue of pigs are thicker than humans [89] • Ethical and moral issues may arise
Ballistics Ordnance Gelatine (10%/20%)	• Derived from animal products [89, 92-94] • Removes ethical issues • Accepted as a human soft tissue simulant mainly representative of a porcine thigh [59] • Transparent nature allows visualisation and photographic representation [88, 96, 97] • Elasticity similar to some human tissues [2]. • Calibration is needed to match physical properties of live porcine thigh tissues [88, 100, 101]	• Lacks bio-mechanical properties of all tissues and organs • Radial cracks occur when penetrated by a bullet [88] • Only represent porcine thigh • Affected by bacterial contamination, decomposition and short storage life [113]
Synthetic simulants	• No ethical issues • Risk of infection reduced • Allows photography and observation due to transparent nature [38, 88, 89] • Great variety in types [65, 111, 112, 113] • Some can be reconstituted [65] • Unaffected by bacterial contamination [113]	• Do not replicate the heterogeneous nature of tissues/organs of human body • Some are non-elastic, thus not showing all wounding mechanisms [18, 38, 89, 90, 96, 106-110]

1.7.1 Materials Used for Research

1.7.1.1 Cadavers

The aim of many ballistic research is to investigate ballistic wounds or wounding potential of bullets on human body, organs or tissues. In such research, the organs/tissues used should retain the normal/natural biomechanical properties, particularly the tensile strength. Thus, organs and tissues from fresh human cadavers have been used [65, 71-74]. Similarly, organs/tissues obtained from frozen and thawed, un-embalmed human cadavers have also been used in ballistic wound research. In such research, controversial findings have been reported in relation to bio-mechanical properties (i.e. particularly tensile strength) of organs/tissues. These findings ranged from no effect on biomechanical properties [75, 76], to decrease in tensile strength [77-79], and increase in tensile strength and elastic modulus [80-82]. The latter changes have been linked to dehydration and redistribution of water during freezing [80-82]. Histological changes seen in frozen-thawed tissues are loss of cellular fluid, cell shrinkage, extracellular fractures and haemolysis [74, 83-85]. Furthermore, in collagen rich tissues, the collagen fibres became more prominent with freezing and such tissues resisted deterioration better than other soft tissues with low collagen content [85]. In addition to organs /tissues from unfixed cadavers, those from embalmed cadavers have been used in ballistic wound research. Embalming alters the biomechanical properties of both bones and soft tissues [86], thus not suitable for ballistic research. Cadavers used for research are generally from body-donation programmes. Thus, they are usually from elderly people. The elasticity and tensile strength of organs/tissues alter with increasing age [71, 74, 87], thus such organs/tissues will not be suitable for ballistic wound research. Furthermore, use of human cadavers for ballistic research could generate ethical issues. Taking these into account, the use of human cadavers for ballistic research may be discouraged [88-90].

1.7.1.2 Animal Models

In ballistics research, live and deceased animals including horses, cattle, goats, sheep, dogs and pigs have been used extensively were extensively used to conduct trauma studies [30, 89-91], however are being less used today. The use of pigs as a substitute for human tissue is a common scientific practice as they are anatomically similar to humans. However, the skin and subcutaneous tissue of pigs are thicker than humans [89], thus pigs may not be an ideal substitute for humans in the investigations involving skin and subcutaneous tissues. Furthermore, the use of live large animals in ballistics testing or even other scientific research may cause ethical and moral issues. Therefore, their use must be carefully scrutinised.

1.7.1.3 Ballistic Ordnance Gelatine (10% and 20%)

Ordnance gelatine is a product derived from collagen proteins in animal products through a process of hot water acidic extraction and available in consistencies between 50 and 300 Bloom (strength and stiffness measure of gelatine) [89, 92-94]. The strength and stiffness is not solely determined by the Bloom Number, but also the concentration and temperature during preparation [89].

Ballistic ordnance gelatine is currently an accepted human soft tissue simulant used in ballistic testings, if it is calibrated to a set standard [59]. Calibration only began in the mid-1980s when it was recognised that uncalibrated gelatine had deficiencies [88, 100, 101]. Calibration of the gelatine was carried out using a small number of samples, thus a disagreement exists in relation to the results [23, 53]. Some tests are being conducted into the resemblance of these simulants to that of porcine tissues [103, 104]. It has been found to replicate the mechanical properties of skin, fat, fascia and muscle tissue of a porcine thigh [59]. As porcine tissues are similar to that of human tissues, it has led researchers to accept that the tissue penetration characteristics by projectiles is the same as what would occur in the soft tissue of humans [59, 65, 88-90, 95, 101,

102]. It is advantageous as it allows the visualisation and photographic representation of the projectiles wound profiles [88, 96, 97] as well as reproducing elasticity similar to some human tissues [2], it lacks the bio-mechanical properties of other tissues and organs, thus may not accurately representing all soft tissues of the human body [98]. Furthermore, radial cracks occur, when the gelatin is penetrated by a bullet, which is different to the behaviour of the human tissues in response to penetration by a bullet [88]. This could create difficulties in translating the findings generated in ballistics ordnance gelatine to real wounding occurring in the human body [90, 99].

The North Atlantic Treaty Organisation (NATO) currently uses ordnance gelatine 20% at 10°C ± 2°C with a Bloom Number between 250 and 300, although there is no calibration standard for this concentration [65]. The FBI standard formulation of 10% at 4°C is calibrated in accordance with the method developed by Fackler and is considered to be a better soft tissue simulant than the NATO formulation [23, 90, 99, 105]. However, both simulants vary and are being used in ballistics research.

Even though ballistic ordnance gelatine allows for the investigation of general tissue trauma resulting from projectiles without the ethical issues arising from the use of animals or cadavers, it only resembles porcine tissues, and in particular porcine thigh muscle. Thus, it does not accurately replicate the biomechanical properties and responses of the heterogeneous tissues and organs within the human body.

1.7.1.4 Synthetic Tissue Simulants – Old and New

The use of synthetic tissue simulants in wound ballistics research is common. It is preferred over biological tissues as there are no ethical issues, the risk of infection is reduced, they allow photography and observation of how the projectiles cause injuries, particularly when the tissue simulants are transparent [38, 88, 89]. Soap, wet packs, and clay have commonly been used for

research. The non-elastic nature of simulants such as clay and soap allow the maximum size of the temporary cavity to be viewed frozen in place, however the permanent cavity cannot be seen. Therefore, if these materials are solely used for wound ballistics research, invalid conclusions could be made [18, 38, 89, 90, 96, 106-110].

New homogenous synthetic simulants are being developed which address the issues occurring with ballistic ordnance gelatine [65]. Perma-Gel is a clear synthetic medium which is stable at room temperature and has similar properties to 10% ordnance gelatine at 4°C. It can be reconstituted and is gaining acceptance, particularly among researchers in the USA [65]. Physically Associating Gelatine's (PAG) are also being developed in the USA and are derived from triblock copolymers, which exhibit deformation behaviours similar to ballistics ordnance gelatine and therefore, it is an alternative tissue simulant [111, 112]. Another suitable soft tissue simulant is transparent gel candle, created from kraton and white paraffin oil and meets FBI calibration standards. This has an additional advantage of allowing improved photography of the permanent and temporary cavities due to its transparency. Additionally, this product is unaffected by bacterial contamination unlike ordnance gelatine [113]. However, these new synthetic simulants are still homogenous and the findings in these simulants cannot be describe all the wounding occurring in the human body and its organs.

1.7.2 Anatomical Models

Simulating all the different organs and tissues within the human body is a difficult task. A lot of research has and is being conducted towards developing anatomical models for various reasons including motor vehicle collision investigations, projectile testings and the development of body armour. Simulating the human body involves producing synthetic bone, blood, organs, skin and muscles and can eventually lead to computational Finite Element Analysis models. A summary can be seen in Table 1-2.

1.7.2.1 3D Modelling/Experimental Models

Anthropometric dummies of the human torso have been created for various research fields including blast tests, ballistics and car crash investigations. In earlier years, the dummies consisted of wood, water and plastic with foam for the lungs, water filled torso and included pressure transducers in the airways [114, 115]. These models have developed over time with the Defence Science and Technology Organisation of Australia (DSTO) creating a torso model referred to as AUSMAN. This was made from polyurethane and a stainless steel rib cage, and used for conducting blast investigations. The Naval Research Laboratory (NRL) created a torso using ordnance gelatine with a rib cage enclosed. This torso used a different material (silicone gel) for the lungs. Crash test dummies have been created and developed for the use in non-penetrating ballistic impact testing [116]. Roberts, Merkle [117] created a physical human surrogate torso model (STM), which incorporated anthropometric values for the design of organs and bones. The bones were created to have tensile properties similar to that of cancellous bone and organs were created from silicone gel. These models have created a basis for anatomical modelling, but do not include all the aspects of the human torso and the bio-mechanical properties of the different organs.

1.7.2.2 Computational Models

A mathematical computational modelling method, namely Finite Element Analysis (FEA), breaks down a problem into a finite number of simple problems using linear and non-linear equations in 2D and 3D computer programs. This technique has advanced medical research and allowed physical surrogates of the human body to be created for us in, but not limited to, non-penetrating ballistic impact and blast research [118-120], along with modelling traumatic brain injuries [121]. In 1998 a 3D model of a sheep thorax that responded to blast impacts was created and included the lung, small intestine, large intestine, all of which assumed linear

viscoelastic properties [122]. Further research provided finite element models for frontal impact simulation including a realistic skeletal structure, but did not include the internal organs [123]. Eventually, models included both the musculoskeletal structures and internal organs [27, 124]. A Human Surrogate Torso Model (HTSM) has been created to investigate trauma resulting from the impact on ballistic resistant vests [119, 120] and represents a 5th male percentile in size, where the internal organs were fabricated to have the same density as porcine organs. These have been designed to replicate as accurately as possible, bone, cartilage, soft tissues and connective tissues of the body. However, some of these models lack correct anthropometric measurements with appropriate soft and hard tissue properties, and are designed to study only non-penetrating injuries [119]. There is currently no computational model that is usable for penetrating ballistics trauma. Multiple frangible surrogate leg models (FSL) have been created including one by The Australian Defence Science and Technology Group (DTSG) which are anatomically accurate and constructed from materials which simulate bone, cartilage, soft tissues and connective tissue. These models are being used to study the loading response in landmine lower leg injuries [72, 125-129]. Human surrogate models, including the thorax and abdomen, are already being created for non-penetrating ballistic and blast injury evaluations. The Visible Human Project and associated projects using this data has incorporated very detailed aspects of the human body for anatomical study purposes [130]. However, the development of a surrogate model incorporating the bio-mechanical properties of the heterogeneous nature of the internal human organs and tissues has not been created but would be of great benefit to investigate penetrating ballistic trauma.

Table 1-2 Summary of some anatomical models being used for various purposes

Model	*Reference*	*Details*	*Use*	*Advantages*	*Disadvantages*
Dummy torso	114, 115	Wood, water, plastic, foam lungs, water filled torso, pressure transduce in airways	Assessing hazards, biodynamics of impact associated with pressure loads	Measures pressure through airways	Not accurate representation of human body and organs
AUSMAN torso	DSTO	Made from polyurethane with a stainless steel rib cage	Blast investigations	Includes rib cage	Not accurate representation of human body and organs
Torso	Naval Research Laboratory (NRL)	Ordnance gelatine with rib cage enclosed Silicone gel for lungs	Non-penetrating ballistics impacts	Includes rib cage	Ordnance gelatine cannot represent all organs of torso
Test dummy	116	Ribs, spine, transducers	Non-penetrating ballistic impacts	Transducers	Not usable for penetrating ballistic trauma Excludes soft tissues and elastic and physical properties of organs
Physical surrogate torso model (STM)	117	Organs created from silicone gel	Non-penetrating ballistics impacts	Anthropometric values included Bones have tensile properties of cancellous bones	Not accurate representation of human body and heterogeneous nature of tissues/organs
Human torso Finite Element Model (HTFEM)	118-120	Finite Element Analysis (FEA) including heart, lungs, liver, stomach, ribs and sternum	Non-penetrating and blast research	Computer model including some properties of tissues and bone Pressure sensors in organs	Does not include all aspects of organs and anthropometric values
Sheep thorax	122	Includes lung, small intestine, large intestine	Blast impacts	Viscoelastic properties of the organs modelled	Does not include many critical organs Non-human
FEA model	123	Computer model	Frontal impact simulation	Realistic skeletal structure	No internal organs
FEA model	27	FEM computer model of thorax, abdomen, shoulder, head-neck of average adult male	Stress analysis under loading	Musculoskeletal structures and internal organs included Validated against post mortem human subjects	Not accurate for use in penetrating ballistics trauma
FEA model	124	FEA model of human thorax	Impact loading onto protective body armour from a projectile	Elastic properties for skeleton Viscoelastic properties for muscle Validated against cadaver tests	Non-penetrating ballistics trauma
Human Surrogate Torso Model (HTSM)	119, 120	Torso model representing 5th male percentile in size	Trauma resulting from impact on ballistic resistant vests	Internal organs matched to density of porcine organs Replicate bone, cartilage, soft tissues and connective tissues	Does not included accurate anthropometric measurements or torso and internal size of organs Soft and hard tissue properties not all included Non-penetrating trauma only
The Visible Human Project	130	Thorax and abdomen computer model	Non-penetrating ballistic and blast injury evaluations	Detailed aspects of human body for anatomical study purposes	Non-penetrating trauma only
Frangible Surrogate leg model (FSL)	DSTO [72, 125-129]	Constructed from materials that simulate bone, cartilage, soft tissues and connective tissue	Land mind wounds of lower leg	Anatomically accurate leg	Not torso and thorax

1.8 Biomechanical Properties of Tissues and Organs

To create accurate anatomical models of the human body, the biomechanical properties of the tissues and organs need to be known. This is a widely researched area and it is not possible to include all the relevant literature here. However, the important biomechanical properties include tensile strength, strain, breaking strength, viscosity, viscoelasticity, elasticity and density [71-73, 87]. Over the year's research has been conducted regarding the elastic properties of the ribs, sternum and the vertebral column [26-28]. Other research has included tests on silicon gel simulants under strain to determine the internal organ properties [25], along with testing various organs under compression loads [131]. Liver, kidney and spleen tissues have been tested under static and dynamic elongation tests [132-138] and shear tests [139-141].

1.9 Conclusions

Wound ballistics research has a variety of applications, but is limited by currently available simulants. Ballistics ordnance gelatine is the most popular simulant, but has its limitations as outlined. All of these simulants are homogeneous and do not take into account the heterogeneous nature of the human body, where skin, bone and tissues all have different compositions. Three dimensional modelling will need to incorporate modelling the internal organs and their heterogeneous nature, size and location. Although progress has occurred over the years in anatomical modelling, further work needs to occur to develop an accurate model for penetrating ballistics trauma. The use of computed tomography may be applicable in gaining accurate sizing of organs. The addition of bone and skin simulants to form experimental models of the human body is a first step, but more advanced anatomical models are required for research in the field of ballistics. This will in turn lead to the development of computer models and simulations.

1.10 References

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Chapter 2

2 Ballistic bone injury is in need of more interpretation based on physical principles

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
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2.2 Context

Soft tissues and skeletal tissues react differently to ballistic projectiles due to the differences in their composition that leads to the differences in their bio-physical properties. Therefore, the mechanisms involved in causing wounds in the two tissue types are different. Wounding mechanisms of soft tissue have been discussed in Chapter 1. The biomechanics involved in the interaction of projectiles with bones and the resultant injuries, particularly in relation to the composition and the biological properties of the bone are presented in this Chapter. The bones of the skeleton vary in size, shape and structural composition, thus, the fracturing mechanisms among bones could vary. Therefore, in the preparation of bone simulants to be used in ballistic research, the bio-physical properties of different bones have to be taken into account. These bone simulants should be able to represent fresh live bone. This chapter also highlights the need for further research into bone injuries from projectiles with the interpretation based on physical principles.

2.3 Abstract

Bone is one of the strongest biological materials, is light weight, provides support, protection and aids in locomotion. It is comprised of collagen providing the strength, elasticity and flexibility and hydroxyapatite, providing the rigidity, hardness and strength. Bone responds to different forces in different ways depending on a number of aspects, including location, composition, shape of a bone element, age, and strength, velocity and direction of force. With a projectile penetrating bone, the energy the projectile carries overcomes the ability of the bone to resist deformation. Once the yield point is reached, the bone will fracture. The way in which bone fractures can be used to determine much about the projectile. In ballistics research, much is known about the characteristics of soft and hard tissues, however less is known about the physical principles associated with bone fracturing. Anatomical modelling, both physical and computational can greatly improve this research field and have the ability to predict wounding in the human body to both soft and hard tissues, however, further research is required to create anatomically accurate models.

2.4 Introduction

The main supporting tissue of the body is bone. It is one of the strongest biological materials and is very lightweight, constituting approximately 18% of the weight of the entire body (1). It provides support, protection and aids in locomotion and movement of body parts, facilitating proper muscle function. In addition, bone stores minerals, assists in endocrine control, provides a source of calcium ions, houses erythropoietically active red bone marrow in spaces enclosed within the trabecular bone producing blood cells and storing triglycerides in yellow bone marrow (1-4). Biomechanically, bone functions as a shield for vital and delicate organs and structures and provides levers for muscles to execute movements (1, 3).

2.5 Gross Anatomy of Bone

2.5.1 Types of Bone

In the human body, bones have been categorised according to the shape: flat (e.g. skull bones), long (e.g. humerus, femur), short bones (e.g. phalanges) and irregular bones (e.g. vertebrae) (2, 7, 8). Flat bones are those of the cranial vault, shoulder blades, pelvis and rib cage, long bones are tubular in shape with expanded ends, short bones are similar to the long bones, however shorter. Irregular bones, including vertebrae, ankle and wrist, are blocky and of varied shapes (2, 7). The bones are further subdivided into bones formed by endochondral and intramembranous ossification. In the former, the skeletal element is first formed as cartilaginous structure and then undergoes ossification, while in the latter bone directly develops from mesenchymal tissue. The initial bones formed by both mechanisms are remodelled and replaced with new bone during life. Bones formed by both processes are identical in their microstructure (2, 7, 8).

2.5.2 Structures

The outer surface of bone is covered by periosteum, except where they articulate with another bone where the surface is covered with hyaline cartilage. Periosteum consists of outer fibrous and inner cellular layers. In actively growing bones the inner layer is more cellular and contains osteoprogenitor cells, while in mature bones, this layer is less defined and has relatively few cells, the periosteal cells that could become osteoblasts under appropriate stimulations (2, 7, 8).

Beneath the periosteum, the bone is dense and forms the outer compact bone, while the inner bone forms a spongelike meshwork, trabecular/spongy bone. The space within the meshwork is continuous and occupied by red marrow and blood vessels. The bone (compact and spongy) facing the cavity is lined by endosteum, which is one cell layer thick and consists of osteoprogenitor cells (2, 7, 8).

Mature compact bone consists of cylindrical units referred to as osteons/Haversian systems. They are made of concentric lamellae of bone matrix arranged around a central canal, the osteonal canal/Haversian canal. The latter contains blood vessels and nerves supplying the osteon. The mature spongy bones are lamellated similar to compact bones, but arranged as trabeculae/spicules. If these trabeculae are thick, they may contain osteons (2, 7-10).

2.5.3 Molecular Structure of Bone

No matter the type of bone, the basic molecular structure is similar and is a composite of two materials: collagen and a hydroxy calcium phosphate (hydroxyapatite). The collagen component intertwines to form the flexibility of the bone while the inorganic hydroxyapatite weaves through the collagen matrix to create strength and rigidity (2, 4, 8).

2.5.4 Histology of Bone

Two histological types of bone occur: immature and mature bone. Immature bone forms first in prenatal life, and is usually temporary. Immature bone is coarsely bundled and woven bone (bundles of collagen fibres are orientated in a random pattern) and with a greater proportion of osteocytes than mature bone (2, 4).

Mature bone consists of lamellae which include the collagen fibres (i.e. organic matrix, which is approximately 30% of the bone) and are arranged in an orderly pattern parallel to one another, while the collagen fibres of the adjacent lamellae are arranged in different directions (1). These arrangements of collagen fibres give the osteons great strength in tension, elasticity and flexibility. The inorganic matrix, calcium phosphate, a crystallized mineral (i.e. ~60% of the bone) provides the lamellae with rigidity, hardness and strength in compression. The remaining 10% of bone is comprised of water (5, 7, 11-15). Bones are dynamic structures undergoing remodelling, i.e. the arrangement of osteons and trabecules changes according to the variations in the action of tension and compressive forces on the bone. This occurs by reabsorption of the existing osteons and deposition of new osteons according to the changed tension and compressive forces acting on the bone (7).

The unique way in which bones respond to different forces, the specific bone, location, composition of bone, individuals' age and the presence/absence of pathological conditions can provide information that can lead to interpreting the mechanisms of the injury (5, 11, 16-18).

2.6 Ballistics Trauma to Bone

Trauma to bone follows the basic principles of mechanics where the ability for bone to resist deformation is overcome and therefore bone breaks. The loading mode of the force applied to bone and the ultimate strength of bone are quantities that determine the ability of bone to resist deformation. However, due to bones being biological entities of complicated shapes and

complex microstructures, detailed application of physical principles is in need of extension to understand how the kinetic energy of a projectile affects various bones in the body. The biomechanical behaviour of bone also depends on the direction in which the force is applied as well as the speed of application. Bone is an anisotropic material whose mechanical properties differ under different loading directions, rather than the ideal isotropic material (10).

Young's modulus is a measure of the stiffness or rigidity of a solid-state material (4, 19). This allows for interpretations related to stress and strain, where elastic deformation occurs as a reaction to the force acting on the bone up to a certain point (yield point) at which the trauma becomes irreversible i.e. the bone will not return to its original shape once the force is removed. However, depending on the shape of the bone, the location at which the force is applied and underlying architecture of compact and trabecular bone, the same force may have different effects. The Young's modulus applies reasonably well to bone tissue of homogeneous structure at locations of geometrically simple architecture, e.g. flat cranial vault or tubular shaft of a long bone. Fractures occur when the force acting on the bone is greater than what the bone strength can withstand, and the minimum fracturing force measures the ultimate strength of the bone (3, 19). The force acting on the bone can be measured by the stress, which is equal in magnitude but opposite in direction to the force that is applied and is often measured in Pascals (force per unit area), equivalent to one Newton of force distributed over one square metre (19). Due to its collagen content, bone is partly elastic. The strain of bone has been documented to not exceed 3% (20). The elastic modulus of bone i.e. its stiffness has been found to vary between 15 and 25 GPa, while bone strength is a few hundred MPa and its ability to resist fracture is approximately 3-10 MPa/m (21). The strength of bone will be dependent on the direction in which the force is applied and thus the direction in which the fracture forms. A fracturing force running along the long axis of the bone will cause a crack quicker than one that is perpendicular to the long axis (10).

2.7 Force Acting on Target/Impact Force

The force of a projectile is known as the change in the state of movement of that bullet. Using Newton's laws of motion the velocity of a object remains constant unless a force acts upon it; the force is equal to the change in momentum over time, and; for every force, there is an opposing force of the same magnitude (4, 22).

The laws of conservation also apply in ballistics.

1. The law of conservation of mass: the mass of an object remains constant i.e. mass is neither created nor destroyed.
2. The law of conservation of momentum: the total momentum remains constant when no external forces act against the object
3. The law of conservation of angular momentum: the total momentum remains constant where no external moment is acting
4. The law of conservation of energy: the sum of all forms of energy (potential, kinetic and rotation energy) remains constant in a frictionless system where no energy enters or leaves the system.

2.8 Energy

The ability for a projectile to cause wounding is due to the energy which it carries during flight (kinetic energy) that is converted into work. Energy and work can never be created or destroyed, they can only be converted to each other. The work occurs when the bullet penetrates tissue, deforms and destroys the tissue because kinetic energy that it carries is dissipated into tissues. The amount of damage caused to the tissues will depend not on the total energy the bullet contains, but the energy which is transferred from the bullet into the bone (23), as well as the surrounding tissues. If this energy exceeds what the bone can absorb, it will fracture (3).

2.9 Kinetic Energy Transfer

2.9.1 Slow Force

Slow force fractures could result from sharp objects, blunt objects, beatings, falls from heights and motor vehicle accidents. The forces causing these injuries are often results of a mass of an object moving at speeds measured in kilometres per hour. A slow loading force allows the bone to respond and compensate for the stress e.g. deform and once the stress is removed to return to its original shape. If the force is too great the bone will fail (i.e. cause fracture) (5, 24).

2.9.2 Sudden Force

Sudden force fractures result from forces produced by a mass moving at speeds measured in meters per second. These are ballistic injuries resulting from high velocity projectiles (e.g. bullets) and the fracture patterns vary. When the force acts rapidly, the bone absorbs the energy and shatters, with no time to compensate for the increased stress (5, 11, 18, 24, 25).

Sudden force fractures could further be subdivided into fractures caused by direct or indirect impact (24, 26). A direct fracture would be the result of the projectile directly contacting the bone. Indirect fractures occur when a bullet passes at close proximity to the bone (26). When a bullet enters the body, it produces an initial shock wave and forms a temporary cavity. The temporary cavity is produced by the action of shockwaves that push tissues outward from the bullet trajectory (27, 28). There is yet another possibility that a projectile impact on the bone may cause an indirect, distant fracture. This is suggested by an analogy to situations in which a fracturing force acting approximately along the main axis of a long bone is transmitted by the shaft to the joint and from there it produces a fracture at the other bone linked to this joint, as happens when a person falls on outstretched hand suffering fracture to the supracondylar region of the humerus or to the clavicle. This requires that the direction of projectile impact is approximately along the main axis of a bone (26). The speed at which the force is loaded into

the tissues is an important factor in determining whether fractures occur as tissues are strain-rate dependent. Thus, a high velocity bullet passing through tissues may not have sufficient time to transfer its energy to cause a large break and produces a localised fracture only, while a lower velocity bullet acting on the same tissue with the same amount of energy may cause a larger break to the bone. This highlights how complex ballistics bone fractures can be based on ballistics injury mechanisms.

The basics of physics are needed to be understood to further understand how a projectile injures bone. A projectile in flight will include components of velocity, acceleration or deceleration and rotational movement. Not all projectiles have rotational movement, however with reference to a spin-stabilised bullet, the bullet rotates around its longitudinal axis to cause stabilised flight. With this type of bullet, the circumferential velocities are large even when the forward velocity is moderate and the calibre of the bullet is small.

2.10 Projectile and Firearm Type

Firearm type i.e. shotgun, handgun or rifle will also affect the wounding of the body. Shotguns more often than not fire multiple pellets (single shots can occur), while rifles and handguns fire single projectiles. A shot gun fired from a distance will cause less trauma as the pellets spread and lose velocity relatively quickly, compared to when fired at close range. Then the pellets could enter as a single mass and cause massive injury due to large mass. Variation in shotgun wounding patterns depends on the gauge, choke and proximity of the weapon to the body (5, 29-31). Rifles are high velocity weapons and impart high kinetic energy to the projectile. If fired at close range, the bullet causes significant injuries to the body by transferring high kinetic energy. Variations in a rifle's ammunition (e.g. metal jacketed, hollow point) will determine the extent of damage caused to the body. Handguns generally impart less energy to the projectile and therefore are often less destructive than rifles (5, 29-31). However, some handguns, can

produce muzzle energies similar to some rifles (e.g. .44” Magnum, .460” S&W Magnum and .500” S&W Magnum).

2.10.1 Ammunition

A bullet is designed for a specific purpose i.e. to transfer its energy to the target to achieve a given effect by converting that energy into work once the target is reached. Bullets can be divided into three categories based on their penetration behaviour. Shape stable bullets maintain their shape within the target and only undergo minor structural changes upon impact. Deforming bullets, deform upon impact on the target and lose a small percentage of their total mass. Fragmenting bullets are designed to fragment upon impact with the target or within the target. However, not every bullet can be categorised neatly into the above categories as their penetration behaviour also depends upon their impact velocity and target medium. For example, a bullet may fragment at high velocity, deform at medium velocity and be stable within the target at a low velocity (32).

2.11 Fractures to Bones

Terminologies used to describe fractures are based on the shapes or patterns of the fractured surface (26), i.e. transverse, oblique, spiral, comminuted, compression, compacted, compounded and greenstick fractures (Table 2-1) (5, 24, 26). The types of forces (e.g. tension, compression) acting on the bone to cause the different fracture types are often working in combination to create a fracture in real life situations, while in an experimental situation a pure force will cause the type of fracture pattern.

Table 2-1 - Descriptions of the types of bone fractures [5, 22, 24].

Fracture	*Description*
Transverse	Bone fractures perpendicular to its long axis under tension
Oblique	Bone fractures at 45-degree angle to its long axis under bending and compression
Spiral	Fracture encircles around axis causing oblique-like fracture under torsion
Comminuted	Bone fractures into two or more pieces under combination of forces
Compression	Associated with compression of small bones, often vertebrae when vertebral body weakens and compacts
Compounded	Bone fractures and pierces the skin
Compacted	Bone fractures and is forced into other bone sections
Greenstick	Incomplete fracture that does not break through entire bone
Butterfly	Bone fractures under tension, compression and bending causing a triangular fragment and two segmented pieces

2.11.1 Force Dissipation

To understand how the body is injured by a projectile, fluid dynamics plays a role, as the bullet will act in the body similar to actions in a viscous liquid. The behaviour of the bullet in the tissues depends on the impact conditions which are linked to the bullet's movement in air. Studying the movement of a bullet in a medium (e.g. the body) there is no difference if that medium is stationary or in motion or vice versa for the bullet. With the laws of physics, energy is neither created nor destroyed, and often when referring to ballistics, part of the energy is converted into heat (23).

When a bullet travels through air for any period, a small amount of its energy is dissipated into the surrounding air because the air produces resistance, or an air drag effect on the bullet. Most bullets are designed to travel through air in the most effective way without losing much of their energy until penetration of the target.

As a bullet penetrates the body it first comes in contact with external materials (i.e. clothing). Clothing can consist of a variety of materials including wool, leather and cotton. In stabbing events, this provides resistance to the penetrating force of a knife (33). The same occurs in ballistics events, however, to a lesser effect due to the greater energy of the penetrating bullet.

In many war zones and in some civilian settings, bullet proof vests or body armour are worn to reduce the chance of life threatening injuries. They dissipate the force and reduce the penetration ability of a projectile, as they cover the major organs of the thorax (i.e. heart). This is the main way to prevent injury to the body in settings where ballistic impacts may occur. Body armour is often produced from very tough woven or laminated fibres in numerous layers. Torso body armour has been found to stop 9mm bullets and with an insertion of front and back ceramic plates it can stop 7.62mm bullets (34), however there is an increase in injuries to the side of the torso in spite of wearing the armour. These protective vests often deform or catch the bullet and distribute the force over a larger section of the vest, thus preventing penetration (34). However, the energy is still transferred into the wearer and instead of penetrating behaviour, it becomes similar to blunt force trauma. Early investigations into behind body armour trauma from projectiles (i.e. nonpenetrating ballistic injury) found that the liver, heart, spleen and spinal cord are still vulnerable to injury (35). This was shown through other studies to be due to the propagation of shock, stress and shear waves occurring through the body (36). Research has been conducted into the effects behind these protective vests and the distribution of the energy into the body and the associated wounds. Serious injury including pulmonary contusions and lacerations was found (37, 38). Research has further progressed into the use of finite element analysis of the human torso to assess the effectiveness of body armour and the behind armour injuries which occur (39). The use of body armour decreases mortality but is deficient in many aspects, particularly from the side and therefore further work needs to occur in this field to develop body armour which has the ability to prevent severe life-threatening injuries without sacrificing the ability of the individual to move freely (34). Body armour and prevention of injury is also limited to the weight and heat load it causes to the wearer, i.e. in very hot climates body armour would be limited, although some modern designs incorporate cooling mechanisms.

Before a bullet contacts bone, it must penetrate skin, fat, fascia and possibly muscles and organ tissues which all resist penetration to some degree, depending on their elasticity and density. The transfer of energy in soft tissues is in the form of temporary and permanent cavity. A temporary cavity stretches the tissues away from the wound track and, depending on the properties of the tissues, either collapses back to its original shape and position, collapses to a different location or does not collapse back at all (i.e. permanent damage). The damage caused by a temporary cavity depends on the properties of the soft tissue (i.e. density, elasticity, viscosity). For example, lung tissue is more elastic than the brain tissue and thus will most likely return to normal shape and function when the temporary cavity collapses, whereas the brain will have permanent damage (32, 40-47).

2.11.2 How Does Bone React to Impact?

2.11.2.1 Force Categories

If a bullet penetrates into the body dissipating some but not all of its energy into the surrounding tissues, the forces that could act on bones can be categorised into compression, tension, shearing and torsion forces, depending on the angle at which the force acts against a bone. Bones could withstand compression forces (perpendicular to the surface) well, while they react weaker when subjected to tension (tensile: perpendicular) and shearing forces (parallel to the surface) (23). The factors that hold the body together and act against the body are referred to as intrinsic and extrinsic respectively, and their interplay will determine whether or not a bone will fracture (5). Extrinsic factors will include the direction, magnitude and rate at which force is applied (4). When the bone's strength and viscoelasticity are overcome, the bone will fracture (i.e. its ability to resist the force fails) (5, 24, 48). The "toughness" of bone refers to the amount of energy required to fracture the bone, where some energy is absorbed prior to the generation of a crack (i.e. diffused), some energy is required to start the crack and the

remaining energy is required to drive the crack, fracturing the bone (49). This can also be described as the strength of bone which is a measure of the load at which a specimen breaks (50). With ballistics injuries, the bone is subjected to a high energy trauma, which forms multiple cracks in the bone due to the concentration of energy that needs to be released in a short period (3).

Different types of bones will respond differently in different loading situations. Cortical and trabecular bones withstand compression forces better than tension and shearing forces. However, trabecular bones could withstand forces longer before failure (51, 52). In life, bones could withstand the forces up to the "injury-threshold" and the level of the "injury-threshold" could vary for each of the force categories and each bone of the body. When the magnitude of a force exceeds the bone's threshold, traumatic injury to a bone may occur. The composition of both compact and cancellous bone will also vary depending on age, anatomical site and physiological function, which in turn create differences in how bone will react under loading forces (10).

Forces can be further categorised according to the direction they act on a bone. The type and level of injury will vary depending on the direction and the magnitude of the force. In real-life situations, it is common to find all types of directional forces occurring within an injury, i.e. tension, compression, shearing and torsion (5, 18).

2.12 Current Knowledge About Ballistics Skeletal Trauma Characteristics

2.12.1 Cranial Trauma

Ballistics cranial trauma displays two types of fractures; radiating and concentric (Figure 2-1) (5, 24), due to the increased intracranial pressure needing release. Radiating fractures appear first, and span away from the wound, while concentric fractures are arched/circular fractures

forming between the radiating fractures (5, 24). Wounds on the cranium often display internal and external bevelling, being a distinguishing feature for entry and exit wounds, respectively (5, 53, 54). The presence of this pattern of bevelling can lead to the identification of entrance and exit wounds, direction and angle of the wound and perimortem body position (29, 31, 53, 55, 56). If the firearm is discharged in direct contact with the cranium and it is produces high velocity projectile, the extent of fracturing will be great and there would be outward displacement of portions of the skull. In comparison, a low velocity projectile discharged in contact with the cranium will have less fracturing and minimal to no blowout of the brain tissues (10). The shape of the resultant bone wound will depend on the angle of impact. Entrance wounds from projectiles contacting and penetrating perpendicular to the bone are often round or ovoid with sharply defined outer margins and at times internal bevelling. When the bullet penetrates at an angle, the defect will contain a punched out ovoid shape with a trapezoid shape in front, described as a keyhole defect (10). Exit wounds are more often than not irregular in shape and larger than the entry wounds with external bevelling (55, 57).

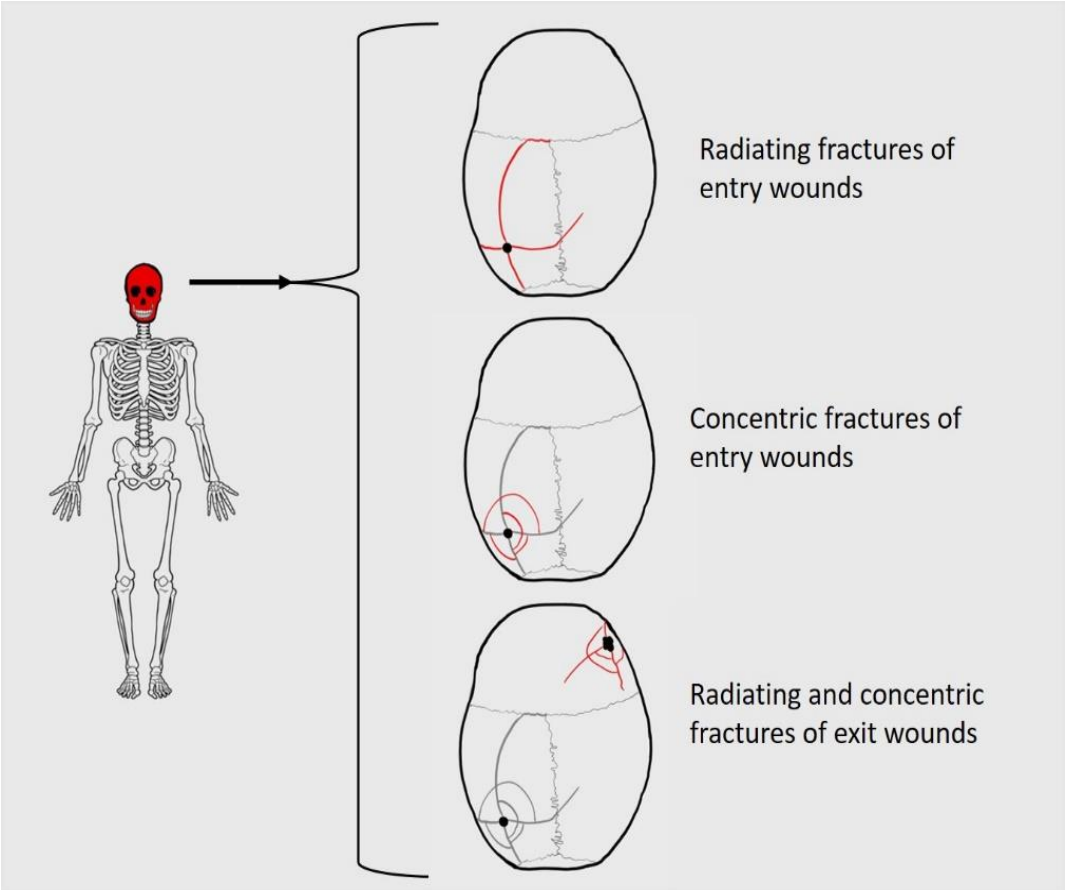

Figure 2-1 Fractures often associated with cranial gunshot trauma, but can be seen in any brittle material (e.g. glass). Entry wounds often appear circular and may show radiating fractures caused by the impact, which travel at velocities at times greater than the projectile itself and arrest at suture lines or previous fractures. Concentric fractures form between radiating fractures and appear as circular fractures, caused by the waves of pressure coming from the centre of the wound. Exit wounds may also show these fracture patterns. Radiating fractures occur first due to the intracranial pressure being greater than what the cranial bone can withstand, therefore force the bone to fracture outwards. Concentric fractures occur second, when intracranial pressure was not relieved through the radiating fractures. The greater the number of fractures (radiating and concentric) the greater the kinetic energy that was imparted to the bone upon impact (5). Puppe's law of sequence can be used to describe fracture order when multiple fractures and wounds are present. This law states that the first fractures will form normally, while those caused by subsequent injuries will terminate at a previous fracture (5, 6) [Image by CH].

2.12.2 Infra-cranial Bone Wounds

Gunshot entrance wounds in long bones differ depending on the composition of the bone (cancellous or compact). In the proximal and distal ends of long bones, vertebrae, hands and feet, the entrance wounds may appear as smooth and round due to the thin cortical bone. Entrance wounds at the ends of the shaft (metaphyses) are sharp and oval-shaped with few radiating fractures. They have been described as being similar to a drill hole (58). Entrance wounds in the mid-shaft of long bones produce comminuted fractures and extreme fragmentation (5, 58-61). Exit wounds in post cranium are often irregular, fractured. The differences in fracture patterns to the metaphyses and the proximal and distal ends occur due to the composition of the penetrated bone. The proximal/distal ends are composed of cancellous tissues that can act to decrease the amount of kinetic energy lost upon impact. The spongy bone dissipates the pressure, which results in less trauma (58). The bone shaft is a hard-cortical section and the strength of this section of the bone provides greater resistance to penetration and can withstand a large amount of pressure upon impact. However, the high energy of a projectile which forms a temporary cavity, causes the structure to fail and therefore fracture (58). These types of fractures can be defined by the fracture classifications defined earlier or seen in Figure 2-2. Cylindrical bone (i.e. long bones) under ballistic load will more often than not act like a brittle material, where the application of force at low velocity to the anterior mid-diaphysis will follow a reproducible fracture pattern which includes an indentation of the projectile, cone cracks followed by radial cracks which form a butterfly-like fracture (62). A projectile travelling at a higher velocity will have the added effect of shockwaves and cavitation which would contribute to the fracturing producing increased indentation in the bone (62). Wounding to the ribs may form cone shaped spalling and clear entry and exit wounds, comminuted fracturing and small nicks on the edges of the bone (63, 64).

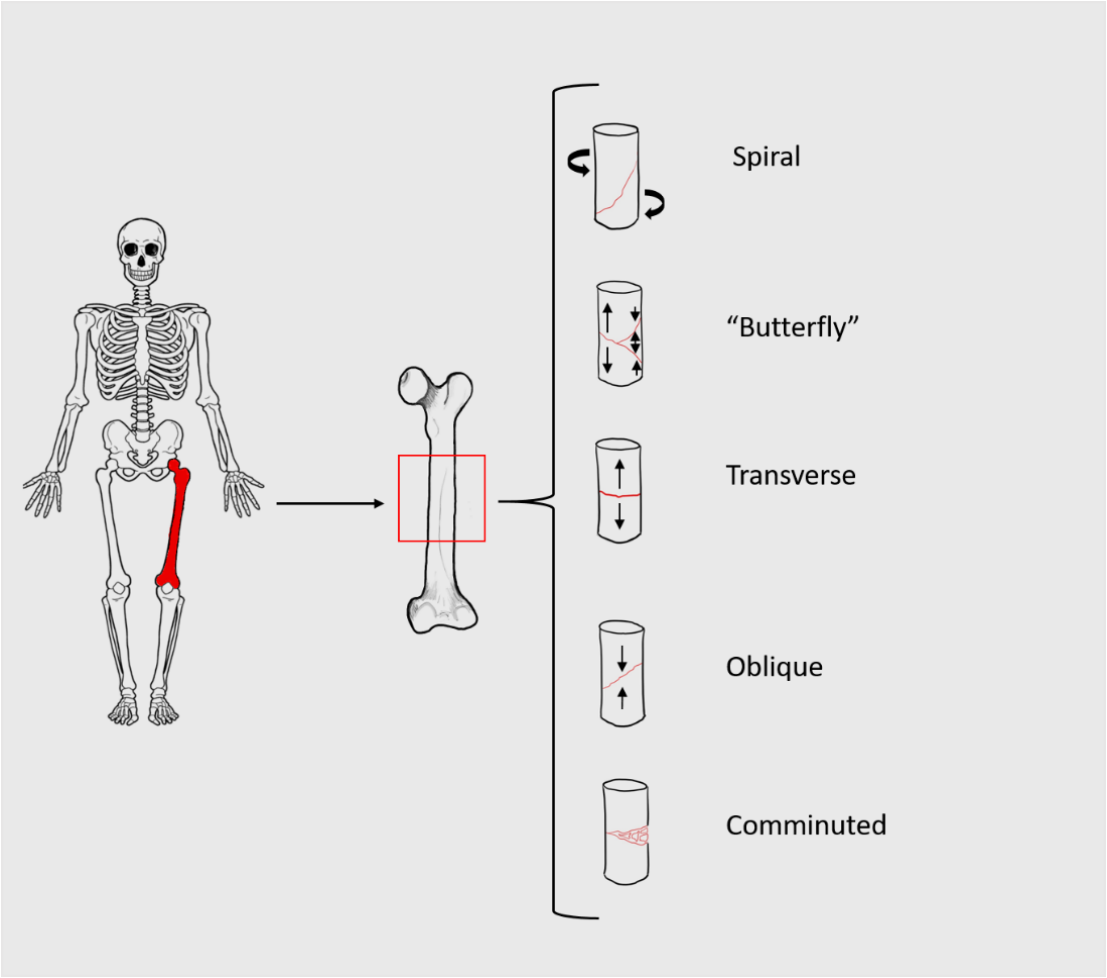

Figure 2-2 Common fracture types to long bones such as the femur. Spiral fractures are due to torsion forces acting on the bone, where the fracture runs obliquely around the bone’s axis. “Butterfly” fractures are a result of a combination of forces (tension, compression and bending), breaking in two pieces with a triangular fragment. Transverse fractures occur due to tension, where the bone fractures perpendicular to its axis. Oblique fractures are formed due to bending and compression forces and occur at approximately 45 degrees to the bones axis. Comminuted fractures occur due to high velocity loading and result in two or more pieces of bone fragments. The latter fracture types is often associated with ballistics injuries due to the transfer of high energy and high speed (5) [image by CH].

2.13 Currently Known Modelling of Trauma

Trauma modelling has been developing into a technique that is vastly beneficial to many areas of the scientific community, including car crash testing, non-penetrating trauma and blast trauma (32, 39, 65-69). It is slowly becoming more prominent in ballistics studies, particularly in the development of body armour and further understandings of what occurs in the human body when penetrated by a projectile (32). The current models being used to investigate ballistics trauma either lack vital organs, have inaccurate representation of the organs and their biomechanical properties or lack hard tissues or they are not accurately represented. This occurs particularly with thoracic and abdominal modelling, potentially due to the complexity of this anatomical region. Researchers are slowly studying the accuracy of ballistics gelatines (70-73), and the idea of anatomical modelling for ballistics purposes is beginning to take form. Developing models that can expand the knowledge and understanding of how the body reacts to projectiles may also lead to better emergency treatments and training of surgeons in this field.

Trauma to osseous tissues, particularly fracture formation from projectile injury, is of interest to forensic scientists and ballisticians and has scarce information and research surrounding the development of models for experimental investigations. The combination of intrinsic and extrinsic factors and heterogeneous nature of bones make it difficult to develop models to represent wounds resulting from projectiles. Computer technology, rather than physical models has been used to model the loading on bones (i.e. Finite element analysis: FEA). Finite element analysis is a computer method, which takes complex geometries and reduces them into a finite number of elements which have simpler geometries. Combining digitised images of bone samples with properties of the bone and applying loads, an output of the strain distribution can be digitally obtained. Although, FEA was introduced to the forensic anthropology field in 2003 by Berryman et al., (74), this technology has not been introduced to

study ballistic trauma until recently. Finite element analysis models have been used to simulate cranial impacts, particularly in the parietal bone where injuries produced comminuted fragments, which are similar to what has been observed in autopsies (75). Mandible models have also been created (76-78). However, FEA models have not been expanded into investigations of ballistic trauma in the bones of the thorax and abdomen (ribs, sternum, clavicle, scapula, vertebrae), possibly due to the complexity of this region of the body. Development of both FEA and 3D models would lead to better understanding of the biomechanics of ballistic trauma to bone, as well as to soft tissues.

Accurate information in relation to the structure, texture (i.e. biomechanical properties) and the three-dimensional arrangement of hard and soft tissues in the body is necessary to create anatomical models. This would include creating surrogate bone material as well as soft tissue simulants and understanding the characteristics of bone injuries from bullets. Research is being conducted into soft tissue simulants and their accuracy at representing the organs of the body (69). Research into surrogate bone material and its use in ballistics trauma is still in its infancy. A "skin-skull-brain model" has been created, which consists of an artificial silicon cap to simulate the skin: layered polyurethane to simulate the external table, internal table and the porous diploe of the skull, and 10% ballistics ordnance gelatine to represent the brain. This model has been used in numerous investigations and has generated reproducible results demonstrating its suitability as an experimental model. The skin-skull-brain model has produced "bone" injuries that were comparable to those of real gunshot wounds (79-81). The properties that are required to be replicated in a surrogate skull, include the fracture toughness, tensile strength and bending strength (67). Roberts et al., (67) investigated another surrogate material for the skull, where experiments were conducted on bending fracture toughness, tensile strength and drop tower tests. The material consisted of two different epoxy resin systems with milled glass fibres, which represented the strength of the cranial tables, and

three layers of low density foam to represent the biomechanical properties of the diploe (67). This material produced fracture patterns similar to intact human skull on impact tests.

Ragsdale and Josselson (82) investigated ballistic trauma patterns of long bones using a model, where fresh long bones were embedded in a 20% gelatine block. They found that fracture variations occurred due to differences in weapon calibre and design, and that the destructive force measured through comminution of bone segments can differ greatly even with the same temporary cavity size. Kieser et al., (72) has also used fresh long bones (deer femora) embedded in 20% ballistic gelatine blocks, discovering that the expanding temporary cavity affects the weakest part of the bone causing a wedge-shaped fracture. Zhang et al., (83) also used the same technique of embedding bone in gelatine, however used porcine marrow bones in 10% ballistics gelatine. They found that with increasing velocities of the bullets, the stress the bone is under increased and if the bullet penetrated the bone from a distance, no fractures occurred.

Kneubuehl and Thali (84) designed synthetic bone (polyurethane) with ordnance gelatine injected into the bone's hollow to mimic bone marrow. Periosteum was simulated with a latex layer. This simulated bone was then embedded into gelatine, fired into and compared with experimental shots fired at swine bones. The simulated bone resembled the biological swine bone in energy and velocity loss, bone fragmentation, bullet deformation and wound channel.

A skin simulant has been created for ballistics experiments based upon the mechanical and ballistic properties of the human skin obtained from cadavers (85). This research found that semi-finished chrome tanned upholstery "crust" cowhide in a thickness of 0.9-1.1 mm created similar threshold velocities, tensile strengths and elongation at break to human skin (85).

Bone simulants that could generate consistently the pathophysiological features of fractures are being investigated. Two currently available bone simulants (Sawbones and Synbone), commonly used in orthopaedic training and testings have been compared with human bones

in ballistics experiments (86). In this study, Bir et al., (86) conducted experiments on direct and indirect loading of post mortem human femora and two bone simulants. Findings indicated that the bone simulants had the velocity tolerance of, but not the biomechanical properties of ballistic trauma of human femora. They concluded that a bone surrogate for ballistics purposes has not yet been developed and required further research.

2.14 Conclusions

Although much research has been conducted into the wounding of both soft and hard tissues of the human body, further documenting cases of skeletal trauma both cranial and post cranial leads to identifying the characteristics of projectile trauma and any abnormalities that stray from the norm. The use of anatomical models would greatly expand this research field and has the ability to improve trauma modelling, body armour and protection and ammunition potential. A simulant bone which reflects the complex mechanical properties of live bone will be of great benefit to the scientific community. To create a synthetic bone, the physical characteristics of ballistics injuries and bone microanatomy need to be determined. This will all lead to the ability to predict what will happen to the human body when penetrated by different projectiles and the advantages of knowing this. Physical models will also aid hands on trauma treatment for medical professionals. Physical anatomical models may also be replaced by finite element computer models, which have the ability to build complex geometries and conduct multiple virtual experiments, however these require knowledge of computer coding which is another field entirely.

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Part TWO

Part II of this thesis describes the ballistics experiments conducted. These ballistics experiments included the methodology formulation and aimed to determine which currently available simulants can represent which organs of the human body. Also presented in this part of the thesis is a histological analysis which clarified the findings of one ballistics experiment.

Chapter 3

3 Effects of re-heating tissue samples to core body temperature on high velocity ballistic projectile-tissue interactions

Caitlin Humphrey, Maciej Henneberg, Christian Wachsberger, Nicholas Maiden and Jaliya Kumaratilake

3.1 Statement of Authorship

Title of Paper	Effects of Re-heating tissue samples to core body temperature on high-velocity ballistic projectile-tissue interactions
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Caitlin Humphrey
Contribution to the Paper	Data collection, data analysis and interpretation, writing and editing manuscript
Overall percentage (%)	60%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 7.11.17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Nicholas Maiden
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Contribution to the Paper	Data interpretation; manuscript writing.
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Name of Co-Author	Maciej Henneberg
Contribution to the Paper	Data statistical analysis and interpretation; manuscript writing
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Please cut and paste additional co-author panels here as required.

3.2 Context

Porcine organs/tissues have been used frequently in ballistic research, due to the similarities between human and porcine tissues. The ethical concerns in relation to the use of human cadavers or cadaveric materials in this type of research are also of importance. Injuries caused to body tissues/organs by projectiles penetrating through them, largely depend on the bio-physical properties of the organs/tissues. Therefore, the commonly accepted norm was that the organs/tissues used in ballistic research should closely mimic the living conditions. Furthermore, after the removal of organs/tissues from an animal, to minimise autolytic degradation, they are immediately refrigerated. The current practice is to re-heat the refrigerated organs/tissues to core body temperature of 37°C before subjecting them to ballistic testing (Maiden, 2014). The effects of the time taken to re-heat organs/tissues to the core body temperature on the physical properties of the organ/tissue could affect the loss of energy from high velocity projectiles penetrating through such organs. An experiment was conducted to investigate these aspects and the details of the experiment and the findings are presented in this chapter.

3.2.1 References

Maiden, NR (2014) The assessment of bullet wound trauma dynamics and the potential role of anatomical models. Doctor of Philosophy Dissertation, University of Adelaide, School of Medical Sciences.

3.3 Abstract

Damage produced by high-speed projectiles on organic tissue will depend on the physical properties of the tissues. Conditioning organic tissue samples to human core body temperature (37°C) prior to conducting ballistics experiments enables their behavior to closely mimic that of living tissues. To minimize autolytic changes after death, the tissues are refrigerated soon after their removal from the body and re-heated to 37°C prior to testing. This research investigates whether heating 50-mm-cube samples of porcine liver, kidney, and heart to 37°C for varying durations (maximum 7 h) can affect the penetration response of a high-speed, steel sphere projectile. Longer conditioning times for heart and liver resulted in a slight loss of velocity/energy of the projectile, but the reverse effect occurred for the kidney. Possible reasons for these trends include autolytic changes causing softening (heart and liver) and dehydration causing an increase in density (kidney).

3.4 Introduction

The injury effects of high-velocity firearm projectiles, such as rifles (approximately 900 m/s), have been investigated using human cadavers (1,2), synthetic materials, ballistics gelatines (3,4), and animals/animal tissues (5,6). Areas of the body commonly affected by high-velocity projectiles and those fired from low-velocity firearms, such as handguns or low-powered rifles (approximately 300 m/s), have been investigated previously, and a number of different injury scoring systems have been developed (7,8). Porcine organs have been commonly used as substitutes for human organs in ballistic tests.

As the human body is maintained at a constant core temperature of 37°C (9), it is believed that investigations on cadaveric material, including porcine organs, be conducted at living tissue temperature to recreate the physical properties of the living tissue and provide a suitable basis for interpreting the results. This becomes important as the physical properties of an organ, particularly its elasticity and texture, may vary with changes in temperature. Temperature dependence also applies to organ/tissue simulants such as ballistics gelatine, which need to be tested at a particular temperature in order to accurately mimic the behavior of those tissues (10). Furthermore, when human or animal tissues are removed from a recently deceased body, they need to be cooled rapidly to a refrigeration temperature of not more than 4°C, in order to minimize autolytic changes and to preserve the physical properties of the organs (11).

This introduces a dilemma for the wound ballistics investigator who requires the test organs to behave in a manner representative of living tissue, and this requires the organs be conditioned at 37°C for the duration of the testing. It is unknown whether during this time, the samples may become subject to change resulting from autolysis, dehydration, and partial denaturation. This may also affect tissue simulants such as ballistics gelatine that is moisture dependent and subject to drying and polymerization (10,12).

This study describes an experiment that was carried out to investigate the effect of conditioning time of porcine liver, kidney, and heart (without pericardium) tissue samples stored at 37°C on the penetration behavior of 6.35-mm-diameter steel sphere projectiles fired at high velocity (~900 m/s). This experiment is being conducted to determine whether the length of time the tissues are re-conditioned to core body temperature affects their bio-mechanical properties for ballistics studies purposes. The results of this study will be used for further analyses on tissues and their retardation effect on projectiles traveling at high velocity, which will ultimately be used to develop a complex physical human anatomical torso model simulant for use in wound ballistics studies. In a forensic setting, these results may not directly be useful; however, an accurate physical anatomical model will aid in a variety of trauma studies (e.g., sharp, blunt and ballistic trauma) as well as development of body protection systems.

3.5 Materials and Methods

3.5.1 Equipment

Live firing experiments were undertaken at an indoor ballistic test facility located at the Defence Science and Technology Group (DSTG), Edinburgh, South Australia. A purpose built remotely operated 7.62 9 51 mm firearm, fitted to a fixed mount, enabled the firing of specially prepared ammunition. The test projectiles comprised 6.35-mm-diameter steel spheres (mass = 1.043 g) encased within frangible plastic sabots. The cartridge was filled with a single base (nitro-cellulose) gun propellant to provide a projectile launch velocity of approximately 900 m/s. This velocity was chosen as it would provide impact energies analogous to a military 5.56 mm caliber cartridge. Use of steel sphere projectiles eliminated uncontrolled effects associated with bullet tumbling, breakup, or distortion. The gun was set up at a distance of 10 m from the test specimen. This distance would enable the plastic sabot to separate cleanly from the sphere and not impact the test specimen, while providing the highest possible accuracy and impact

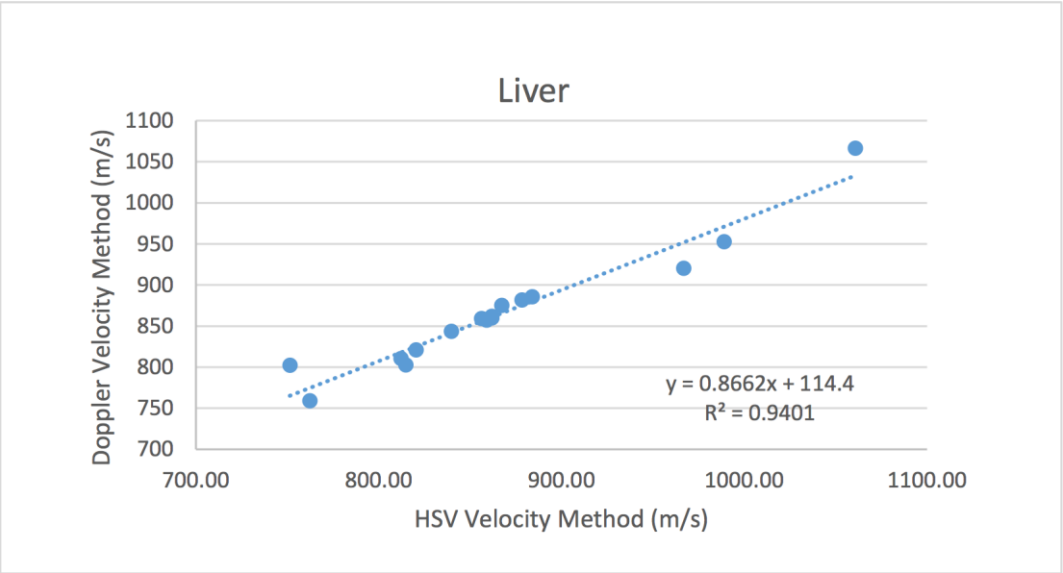
velocity. A Doppler radar was used to track the spherical projectiles velocity from the firearm muzzle to the test specimen. A pair of optical infrared sighting screens recorded the spherical projectiles residual velocity after having passed through the test specimen. Two high-speed video cameras provided top and side views of the test specimen. High-intensity LED lighting enabled the cameras to be operated at 10,000 frames per second with a shutter speed of 1/150,000th of a second which provided sufficient image resolution and number of frames to undertake a post firing analysis of the shot line, target thickness and projectile entry and exit velocity.

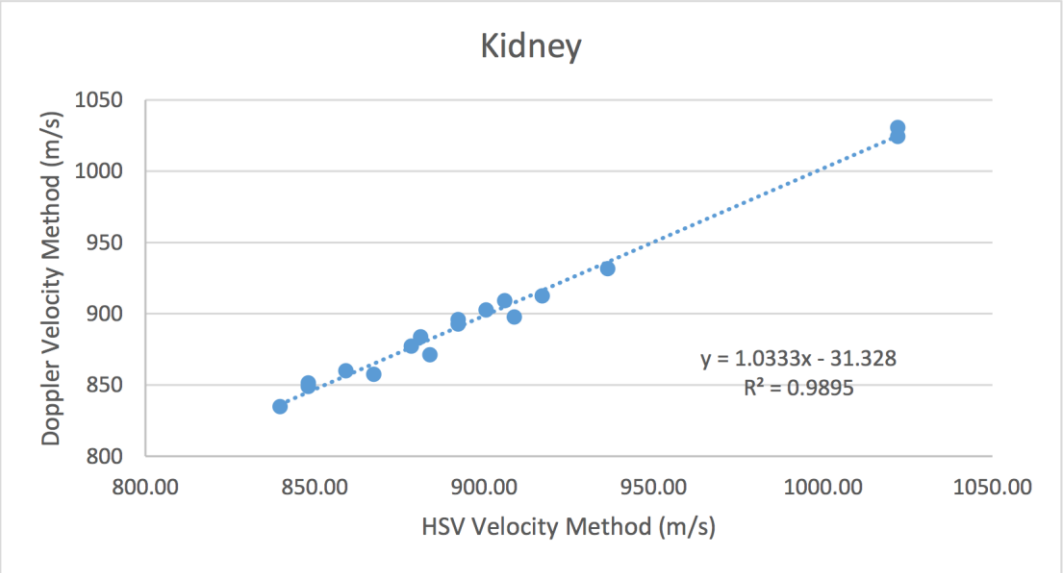
3.5.2 Materials

Porcine organs including the heart (without pericardium), liver, and kidneys were obtained from freshly slaughtered pigs (approximately 80 kg) at a local abattoir. On the day of slaughter, the organs were cut into approximately 50-mm-sided cubes and individually placed in zip-lock plastic bags (to facilitate mounting of the specimen on to the sample holder and to minimize dehydration of the tissue) and refrigerated at 4°C until they were needed for ballistic experiments. The tissue samples were kept in their zip-lock bags and placed in a temperature conditioning chamber set at 37°C for 1–7 h until such time as they were required for testing. A specimen holder had been specifically constructed to support the tissue sample in its zip-lock bag in an upright position and minimize distortion of its cubic shape. We have fired the projectile through the walls of an empty ziplock bag situated 10 m from the muzzle and found that the exit velocity of the projectile after passing through the walls of the bag (with no specimen, expanded to 50 mm) was the same as the velocity of the unopposed projectile at the same distance from the muzzle of the firearm. Therefore, there was no noticeable velocity loss of the projectile caused by the plastic walls of the bag. The steel spherical projectiles were discharged from a distance of 10 m. The entry and exit velocities were recorded by the Doppler/IR sight screens (Doppler velocity method), shot line thickness was measured from

video recording and entry and exit velocities were also calculated from the high-speed video recording (HSV velocity method).

The projectiles entry velocities were obtained by the two methods—Doppler velocity method and high-speed video calculations—were compared to determine reliability of the test methods. Figure 3-1 shows correlations of results obtained by the two methods. Coefficients of determination (r2) for all three organs indicate that results provided by the two methods were practically identical and assured their accuracy.





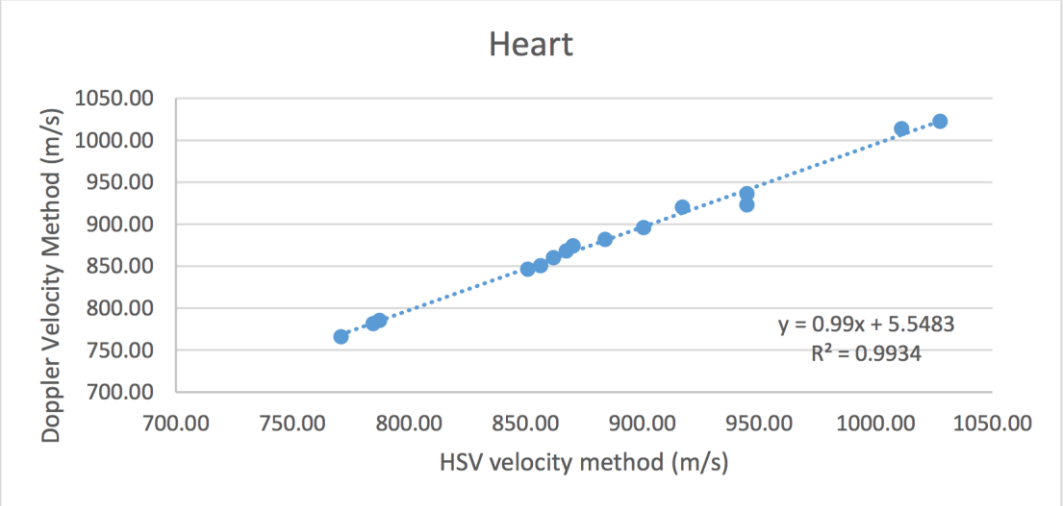


Figure 3-1 Correlation of projectiles entry velocity results obtained using the Doppler method and the High Speed Video (HSV) method for the three organs (kidney, liver and heart)

3.5.3 Statistical Analysis

Independent sample t-tests, assuming differences in standard deviations, were used to compare mean entry and exit velocities and analysis of variance (ANOVA), comparing several means in separate samples at the same time was used to compare velocities in different organs. Linear regressions of time in the conditioning chamber at 37°C and velocity loss (m/s) were applied to determine the relationship between conditioning time and ballistic test results.

3.6 Results

Entry and exit velocity means for each organ (Table 3-1) from both velocity measuring methods are presented, along with mean energy loss (J/m) per tissue thickness. No significant differences were noted between the two methods for the liver, kidney, heart for entry velocities ($p = 0.9461; 0.9418; 0.9061$), or the exit velocities ($p = 0.7987; 0.8719; 0.6692$). The average entry velocities of the spherical projectiles did not differ significantly between the various organs, permitting a direct comparison of the results (Table 3-2).

Table 3-1 - Mean and standard deviation (SD) of entry and exit velocities from both velocity measuring methods and mean energy loss (J/m)

	HSV					*Doppler/Chronograph*				
	Entry		Exit			Entry		Exit		
	Mean Velocity (m/s)	SD	Mean Velocity (m/s)	SD	Mean Energy loss (J/m)	Mean Velocity (m/s)	SD	Mean Velocity (m/s)	SD	Mean Energy Loss (J/m)
Liver	867.6	77.5	639.9	60.9	3452.3	865.8	69.2	634.7	57.2	3481.3
Kidney	900.2	52.7	716.0	53.0	3662.3	898.9	54.8	713.2	47.2	3703.1
Heart	885.3	75.8	645.9	58.4	3278.3	882.0	75.3	636.6	58.4	3333.9

Table 3-2 – p-values for comparison of entry velocities using HSV velocity method above diagonal and Doppler method below diagonal. No significant differences seen in entry velocities.

	Liver	*Kidney*	*Heart*
Liver		0.5281	>0.9999
Kidney	0.4650		>0.9999
Heart	>0.9999	>0.9999	

3.6.1 Heart

Slight surface changes occurred in the heart as the time spent in the temperature chamber increased. The color changed from the normal red to a brownish red color. The median time the heart samples (n = 15) spent in the temperature conditioning chamber was two hours (1.0–4.98 h). Mean tissue thickness was 58.97 mm (SD 5.6). Significant differences were seen between the entry and exit velocities for both methods, HSV p =<0.0001, Doppler p < 0.0001 (Figure 3-2). The average velocity loss was HSV 239.4 m/s (SD 31.4), Doppler 245.3 m/s (SD 27.4). As the time spent in the conditioning chamber at 37°C increased, the loss in velocity decreased for the heart. Both methods of measuring velocity show this trend (Figure 3-3). A partial correlation coefficient between the velocity loss and time with thickness of the tissue samples remaining statistically constant for each test was calculated as -0.618. This is a significant correlation and as it is negative, as time increases the velocity loss decreases.

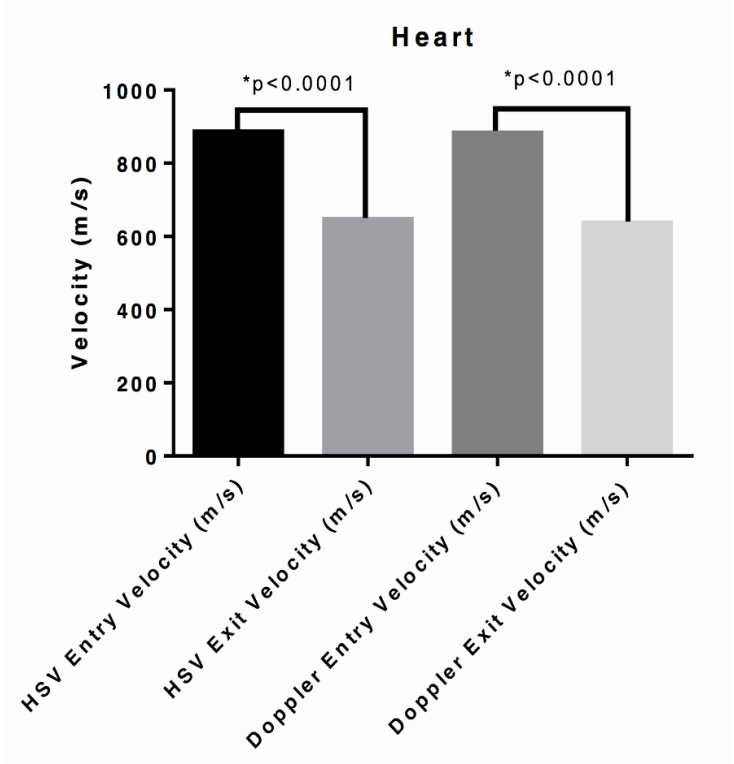


Figure 3-2 Comparison of mean entry and exit velocities for the two methods, showing significant differences between the entry and exit velocity

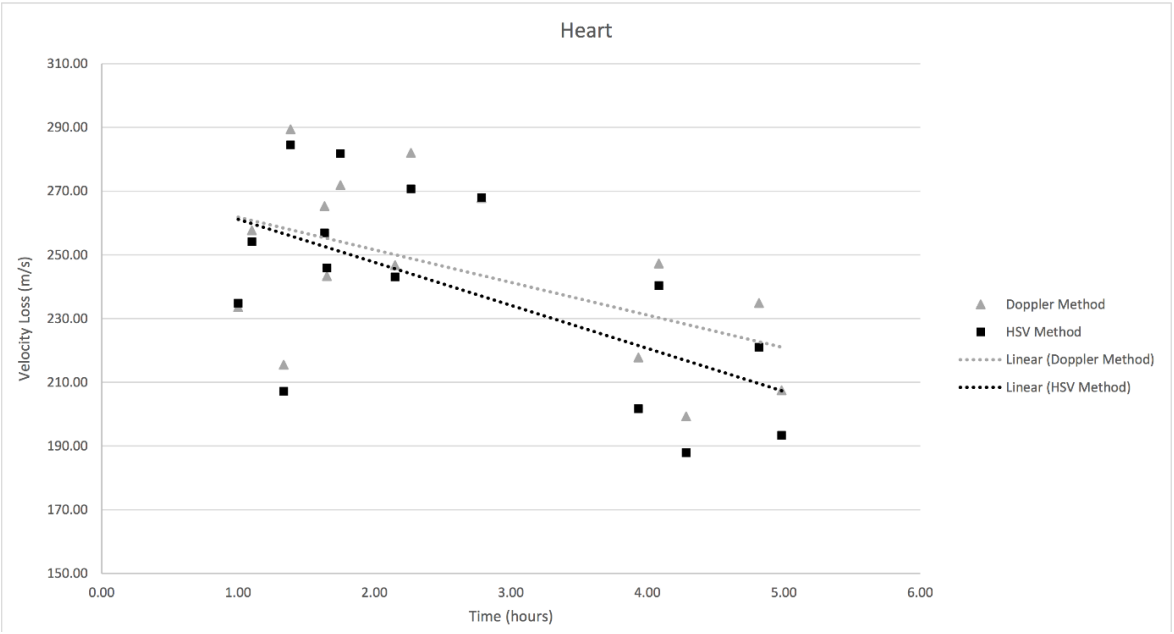


Figure 3-3 Velocity loss of projectile through the heart as time spent in conditioning chamber at 37°C increases, using both methods of velocity measurements

3.6.2 Liver

The appearance of the surface of the liver tissue altered as the time spent at 37°C increased. As the time increased, the surface color became a darker brown, resembling the appearance of slightly cooked liver. The median time liver samples (n = 17) spent in the temperature conditioning chamber was two hours (0.8–6.78 h). Mean tissue thickness was 52.81 mm (SD 8.7). Significant differences were seen between the entry and exit velocities for both methods, HSV p =<0.0001, Doppler p < 0.0001 (Figure 3-4). The average velocity loss was HSV 227.7 m/s (SD 42.8), Doppler 231.1 m/s (SD 35.0). As the time spent in the conditioning chamber at 37°C increased, the loss in velocity decreased for the liver. Both methods of measuring velocity show this trend (Figure 3-5). A partial correlation coefficient between the velocity loss and time, with thickness of the tissue samples remaining statistically constant for each test, was calculated as -0.502. This is a significant correlation and as it is negative, as time increases the velocity loss decreases.

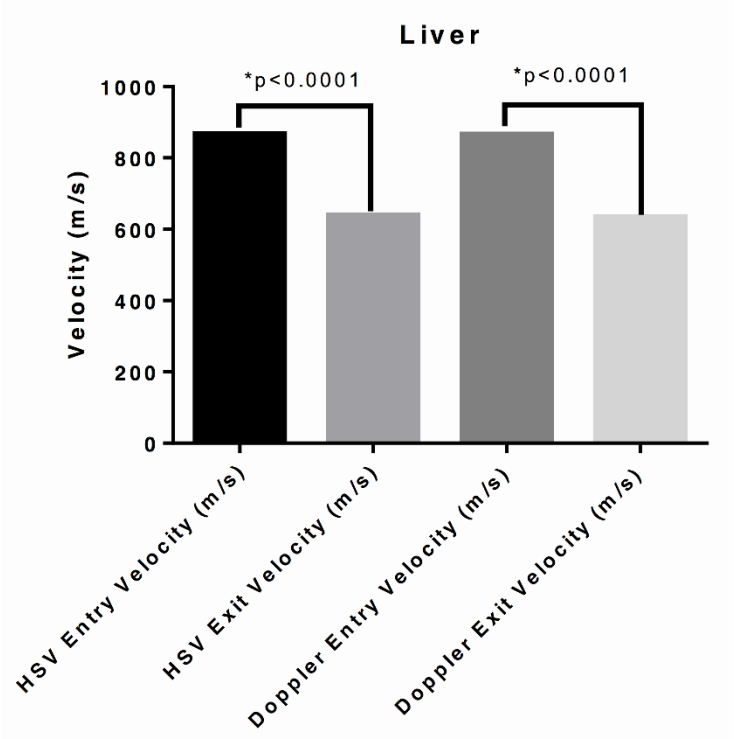


Figure 3-4 Comparison of the mean entry and exit velocities for both methods, showing significant differences between the entry and exit velocity

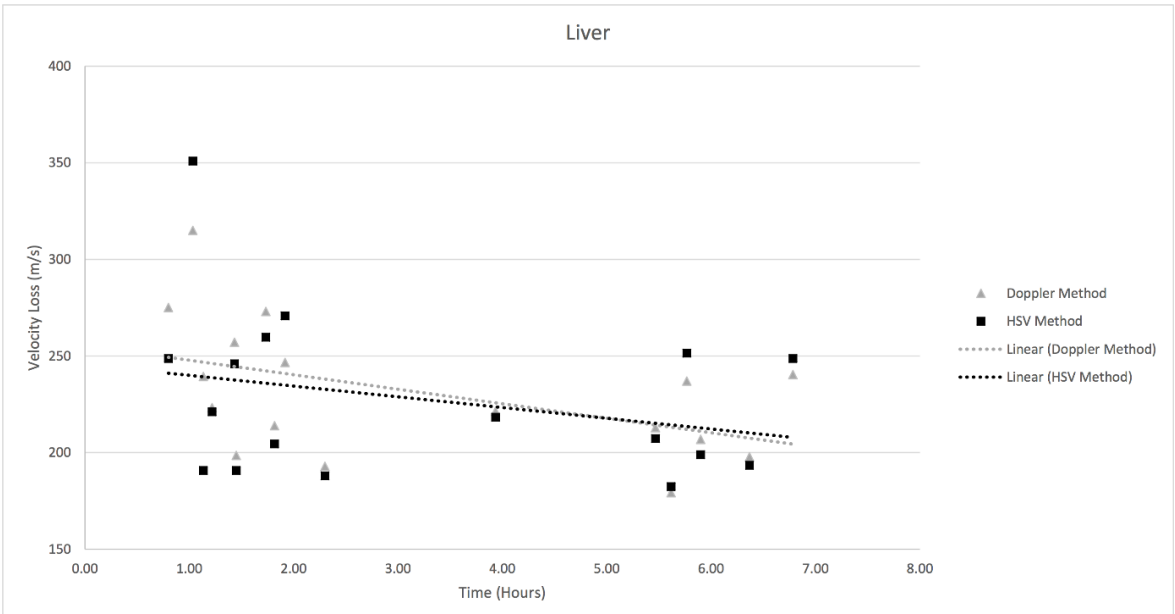


Figure 3-5 Velocity loss of projectile through the liver as time spent in conditioning chamber at 37°C increases, using both methods of velocity measurements

3.6.3 Kidney

The external surface of the kidney had slight visual changes in the color, resembling that of slightly cooked meat. The median time kidney samples (n = 17) spent in the temperature conditioning chamber was two hours (0.97–4.63 h). Mean tissue thickness was 42.64 mm (SD 5.6). Significant differences were seen between the entry and exit velocities for both methods, HSV p =<0.0001, Doppler p < 0.0001 (Figure 3-6). The average velocity loss was HSV 184.3 m/s (SD 24.7), Doppler 185.7 m/s (SD 27.1). As the time spent in the conditioning chamber at 37°C increased, the loss in velocity increased for the kidney. This, however, is not significant increase. Both methods of measuring velocity show this trend (Figure 3-7). A partial correlation coefficient between the velocity loss and time, thickness of the tissue samples remaining statistically constant for each test, was calculated as +0.290. This is not a significant correlation, and as it is positive, as time increases the velocity loss also increases.

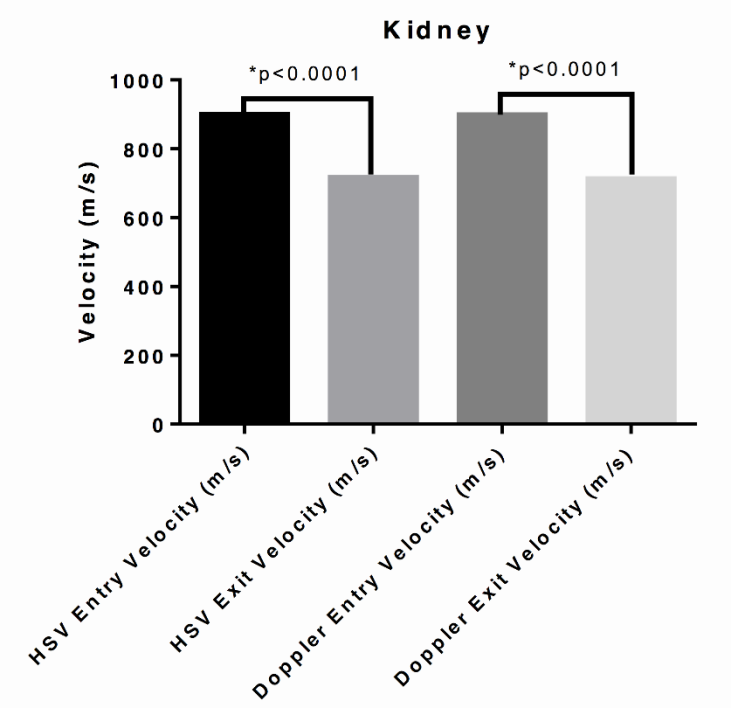


Figure 3-6 Comparison of the mean entry and exit velocities of both methods, showing significant differences between entry and exit velocities

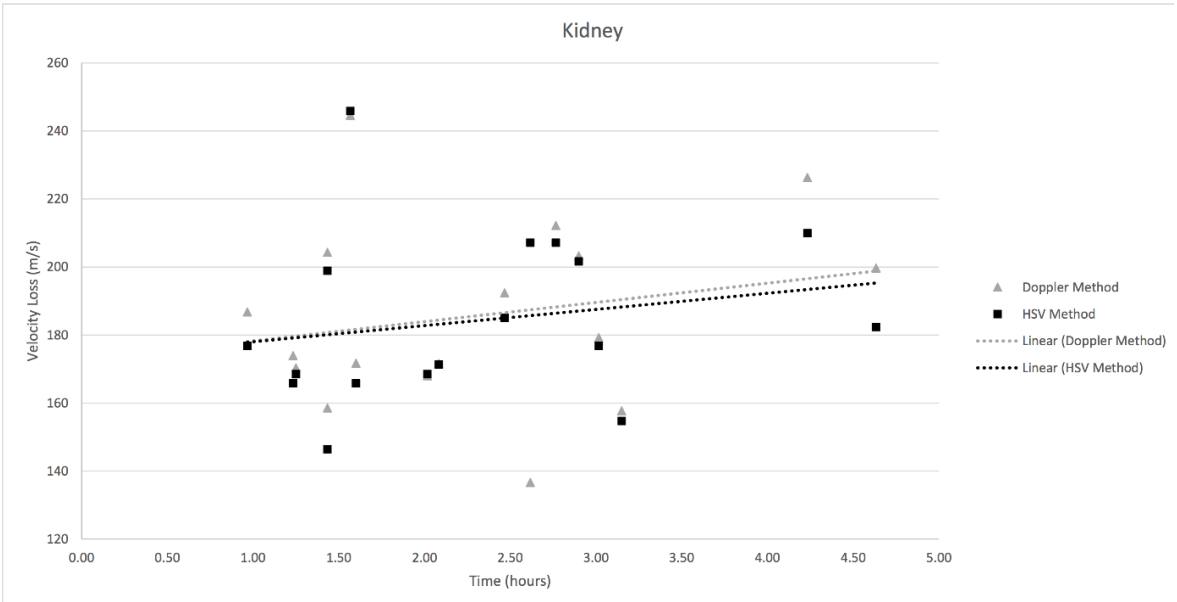


Figure 3-7 Velocity loss of projectile through kidney as time spent in conditioning chamber at 37°C increases, using both methods of velocity measurements

3.7 Discussion

The entry velocities of the projectiles into all three tissues did not vary significantly for either of the velocity measuring methods, indicating that the specially built ammunition maintained the velocity within a range that allows comparisons between tissues. Viewing of the high-speed video recordings showed no sample distortion or movement during impact, thus not affecting the projectiles. A strong correlation is seen between the two methods for measuring velocity; thus, the results of both methods are similar. It is therefore acceptable to use either method for future ballistics testings.

The mean velocity of the projectiles reduced significantly from the entry to exit in the heart, liver, and kidney specimens. This indicates that the projectiles lost velocity and thus energy during their passage through each tissue. Furthermore, the thickness of the tissue specimens proved sufficient to reduce the velocity of ballistic projectiles in adequate magnitude to allow comparisons between tissues.

As a high-velocity projectile passes through a tissue, it loses velocity and thus energy (based on the equation for energy, $K = \frac{1}{2}mv^2$). The resultant damage to the tissue occurs ultimately from two sources. The change in pressure occurring on initial penetration and a pressure wave causes the temporary cavity, which stretches the tissues away from the projectile, before collapsing. In the literature, it is suggested that the damage to the tissue is greatest in the least elastic tissues. A permanent cavity occurs as the projectile crushes the tissues leaving a permanent passage through the tissue, while a temporary cavity occurs by stretching the tissues away and then collapsing (13–15).

Organs which are most susceptible to this stretch injury would have low elasticity that is close to water density, such as the liver, while the heart would be less susceptible (16). It would be expected that in tissues which are least elastic, the loss of velocity would be greatest, as these

tissues lack the ability to stretch and collapse without damage. However, in this study, the injury to the heart was the greatest and that to the kidney was the least, which disagrees with the elasticity concept. The explanation may lie in the fact that the friction coefficients of these tissues and their densities differ in opposite directions to elasticity.

The rate of decrease in the velocity of a projectile when it is traveling through a tissue depends on the size and mass of the projectile (i.e., cross-sectional area and length), composition and thickness of the tissue (15). In this experiment, the size of the projectile and the thickness of the tissue is relatively constant, and thus, the observed differences in the reduction in the velocities among the tissues resulted from the differences in the composition of the tissues. Heart has densely arranged cardiac muscle fibers surrounded by fibrous connective tissues. In addition, the heart consists of an epicardium that consists of connective tissue containing blood vessels and pericardium. The latter has a dense parietal fibrous pericardium (9). The heart samples used here consisted of myocardium without the pericardium. Liver consists largely of parenchyma and portal triads rich in fibrous tissue. Liver parenchyma consists of cords of hepatocytes surrounded by sinusoids and veins (9). Thus, liver is less dense than the heart, particularly the myocardium. Kidney has a capsule and renal parenchyma and has very much less fibrous connective tissue than the liver (9). Thus, liver could be considered as denser than the kidney, which has been found in density values (17).

The liver and heart showed decreases in velocity loss over the period of time at 37°C; however, the kidney showed a slight increase. With a linear trend for time spent at 37°C and velocity loss, whether negative or positive, the composition of the tissues did not vary significantly over the maximum time in this experiment to alter the velocity loss of the projectile. The time spent in the hot conditioning chamber was not of a length that would show a clear change in the velocity loss of a projectile penetrating it. However, as these samples have been removed from the body

and removed of the blood flow, the histology of the samples may vary, but is not visible to the naked eye and future research will investigate this aspect.

Due to no significant differences, heating the tissues to 37°C and retaining them at that temperature in the temperature conditioning chamber until tested did not have an effect on the size of the reduction in the velocity from the point of entry to exit from the tissues. However, organs/tissues of the body undergo autolytic changes and microbial breakdown from the time of an animal's/human's death. Therefore, in this experiment, the tissues were refrigerated immediately after the slaughter of the pigs to minimize and reduce these changes. The pigs were slaughtered under the sterile conditions of an abattoir; thus, the microbial breakdown of the tissues would have been minimal. However, heating of the specimens to 37°C and maintaining them at that temperature in the temperature conditioning chamber accelerates autolytic and microbial breakdown of the organs/tissues. Thus, the length of time the tissues kept in the temperature conditioning chamber determines the degree of autolytic and microbial breakdown. The amount of enzymes present within the cells of a tissue may also contribute to the rate of autolytic degradation. Possible reasons for the decrease in velocity loss over time in the heart and liver are the tissue became softer possibly due to autolytic changes, while the increase in the velocity loss seen in the kidney may be caused by the kidney tissues becoming denser possibly due to dehydration.

In all tissues placed in the temperature conditioning chamber, some discoloration that was visible to the naked eye occurred. This change was limited to the surface of the tissue and may have resulted from the loss of moisture from the surface of the tissues. The temperature of the chamber was maintained at 37°C; thus, the tissues would not have become cooked or burnt, as a much higher temperature would be needed for this to occur. Furthermore, this temperature does not appear to affect the physical properties of tissue components. Therefore, these organs are unlikely to change their resistive characteristics when penetrated

by high-velocity projectiles. It is possible, however, that prolonged exposure to a 37°C environment could result in an increase in the activity of the intracellular enzymes leading to autolytic degradation of the tissues. Further studies would be required to determine this.

3.8 Conclusion

While the findings of this study reveal no statistical differences in the velocity loss of a high-speed projectile and the duration the organ samples were maintained and tested at 37°C, the slight changes in physical appearance suggest that autolysis and dehydration is possibly occurring. Future investigations will determine the extent of autolytic and dehydration effects; these organs are undergoing during the heating phase. Without further studies determining the extent of enzymatic activity and dehydration, it would be best practice to minimize the length of time organ tissues remain at 37°C after removal from the body; however, a specific time frame cannot be concluded from this study alone.

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Chapter 4

4 A histological analysis of visceral organs to evaluate the effect of duration of heating from refrigeration to core body temperature for ballistics investigations

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4.1 Statement of Authorship

Manuscript Details

Title of Paper	A histological analysis of visceral organs to evaluate the effect of duration of heating from refrigeration to core body temperature for ballistics investigations
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Principal Author

Name of Principal Author (Candidate)	Caitlin Humphrey
Contribution to the Paper	Concept, methods, data collection, data analysis and interpretation, writing and editing manuscript
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 13/9/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	**Dr Jaliya Kumaratilake**
Contribution to the Paper	Data collection; data interpretation; manuscript writing and editing
Signature	Date 13.09.2017

4.2 Context

In the experiment described in Chapter 3, porcine organs were re-heated from refrigeration to room temperature (16°C) and core body temperature (37°C) and maintained at these temperatures until tested. This time duration varied approximately from 2 to 7 hours, thus, it was expected that the autolytic degradation changed the physical properties of these organs. However, the energy loss from the projectiles were not significantly different between the two tested temperatures. These findings indicated that the duration taken to re-heat and test the organs had not affected the bio-physical properties of the tissues. A further experiment was conducted to investigate the microscopic (i.e. histological) changes that could occur in porcine organs (heart, lung, liver and kidney) in the time interval taken to re-heat them from refrigeration temperature to core body temperature of 37°C. This chapter describes the experiment and the findings.

4.3 Abstract

Animal organs have been used in ballistics research to investigate the effects on human organs. Such organs are refrigerated until the investigation to minimise autolytic degradation and at times have been re-heated to the human core body temperature to simulate the in-situ environment. The aim of this investigation was to study the micro structural changes that may occur in fresh chilled visceral organs of the thorax and abdomen (ie, heart, lung, liver, and kidney) during the period of reheating to 37°C. Fifty-millimetre cubes of porcine heart, lung, liver, and kidney were taken rapidly after slaughter, chilled overnight and the next morning were reheated to core body temperature (37°C). Histological changes occurring in the tissues during the re-heating phase were investigated. The findings indicated that no cytoplasmic or nuclear changes occurred in any of the tissues during the period of re-heating. Therefore, reheating of animal organs to the human core body temperature is not necessary, if the organs are refrigerated.

4.4 Introduction

The manner in which injuries are caused by projectiles passing through human organs/tissues have been investigated using animal tissues[1-4] and/or tissue simulants (e.g. ballistic simulants).[5-12] Porcine tissues/organs are the commonly used animal resource in ballistics and other scientific research, as the gross and micro structures are similar to those of the human organs/tissues.[1,13,14] Tissues removed from live animals after anaesthesia (ie, including biopsies) or after slaughter undergo degenerative changes, which results from the cutting off of circulation, and the rates of degradation vary among different organs and tissue components within an organ. The changes tissues undergo are aerobic to anaerobic respiration, molecular, ultrastructural, micro-structural and gross anatomical in progressive stages.[15,16] Minimizing these changes are very critical in ultrastructural and light microscopic research investigations, pathological diagnosis, and tissue/organ transplants.[17] The effects of tissues on projectiles passing through, particularly on the retardation of velocity and the loss of energy, depend on hardness and fibroelastic properties of the tissues.[4] The hardness and the fibroelastic properties of tissues depend on the amount of ossification and/or calcification, and the distribution of collagen and elastic tissues, respectively. These tissue components are more resistant to degradation than the cellular components of tissues. Furthermore, to simulate the fibroelastic properties of organs/tissues to those of the living human, the ballistic investigations were traditionally carried out at the core human body temperature, 37°C. Therefore, organs removed from slaughtered animals are immediately chilled to 4°C (ie, to minimize degenerative changes) and reheated to 37°C before ballistic testing. In a recent ballistic investigation, porcine organs cut to cut into 50 mm^3 blocks were maintained at 37°C for 1 to 7 hours before testing. Interestingly, the velocity loss from the projectiles passing through them was not significantly different between the times they spent at 37°C.[4] These findings indicated that the degradation of the tissue components determining the hardness and fibroelastic properties of the tested

organs during the period of reheating was not adequate to cause a significant change in reductions of the velocity or energy loss from the projectiles.

The aim of this investigation was to study the microstructural changes that may occur in fresh chilled visceral organs of the thorax and abdomen (ie, heart, lung, liver, and kidney) during the period of reheating to 37°C.

4.5 Methods

Hearts, lungs, livers, and kidneys were obtained from a local abattoir from 4 freshly slaughtered pigs (each 80-kg body weight). Approximately 50-mm cubes were cut from each organ, placed in individual ziplock plastic bags and refrigerated overnight at 4°C. The next morning, each sample was photographed and weighed, and the temperature at the surface and at the center of the block was measured. Furthermore, an approximately 5 X 5 X 10-mm piece of tissue was dissected off and fixed in 10% buffered formalin for histological investigation. Thereafter, the tissues were heated in a temperature-conditioning chamber set at 37°C. Every 10 minutes, the temperature on the surface and at the center of the tissue block was measured until the latter temperature reached 37°C. Then, the tissue was weighed and photographed, and another sample (approximately 5 X 5 X 10-mm piece) was taken and placed in a 10% buffered formalin for histological investigation. Surface temperature was measured using a HP-880EK digital infrared noncontact thermometer (accuracy ±0.5°C) (Zhuhai Jida Huapu Instrument Co., Ltd. China), and the temperature at the center of the tissue block was measured using a LCD Probe Thermometer (accuracy ±1.0°C)(Tech Brands, Australia). The core temperature was measured by inserting the probe towards the center of each block of tissue.

Heart, lung, liver, and kidney samples fixed in 10% buffered formalin were dehydrated through a graded series of ethanol and embedded in paraffin wax as described by Pearse.[18] Five-micron-thick sections were cut, mounted onto glass slides, and stained with hematoxylin-eosin as

described by Pearse[18] and examined using an Olympus BX 50 microscope (Olympus Corporation, Tokyo, Japan) coupled to a digital imaging system (consisting of Olympus SC 100 digital camera [Olympus Corporation, Tokyo, Japan] and Stream Essentials imaging software [Olympus Corporation, Tokyo, Japan]).

4.6 Results

The mean weights of the organs in grams (±SEM) at the commencement of the experiment (ie, refrigerated samples) were 106.2 ± 11.0 g (lung), 112.5 ± 7.2 g (kidney), 119.3 ± 4.5 g (liver), and 141.1 ± 2.8 g (heart). Once the tissues reached the core temperature of 37°C, the mean weights of the organs (±SEM) were 104.8 ± 11.0 g (lung), 109.6 ± 4.2 g (liver with no fluid), 112.3 ± 7.2 g (kidney), 116.1 ± 5.3 g (liver with fluid), and 141.0 ± 3.3 g (heart). The weights of the organs at the commencement of the experiment were not significantly different from those when the core temperature reached 37°C ($P > 0.05$).

The time taken to reach the core temperature of each organ to 37°C varied among samples and among organs. This time ranged from 130 to 140 minutes for the kidneys, 140 to 150 minutes for the hearts, and 160 to 200 minutes for the lungs. In each liver sample, the time taken to raise the core temperature to 37°C was 180 minutes (Figure 4-1 to Figure 4-4). Surface temperatures of heart, liver and lung samples increased quicker than the core temperatures during the early stages of heating, whereas in the later stages of heating, surface temperatures of these 3 organs dropped quickly below the core temperature during the periods of temperature measurement. The surface and core temperatures of the kidney samples remained relatively the same throughout the experiment (Figure 4-1 to Figure 4-4). In each organ, the temperature increased at an approximate rate of 1°C per 10 minutes, and as the temperature approached 37°C, the rate of increase slowed down (Figure 4-1 to Figure 4-4).

The color of the surface in each organ altered at a different time of heating, and it is indicated on Figure 4-1 to Figure 4-4 by arrows. The surface color changed first in the kidney samples, followed by the liver, heart, and lung samples. Figure 4-5 shows the changes in surface color in the organ samples.

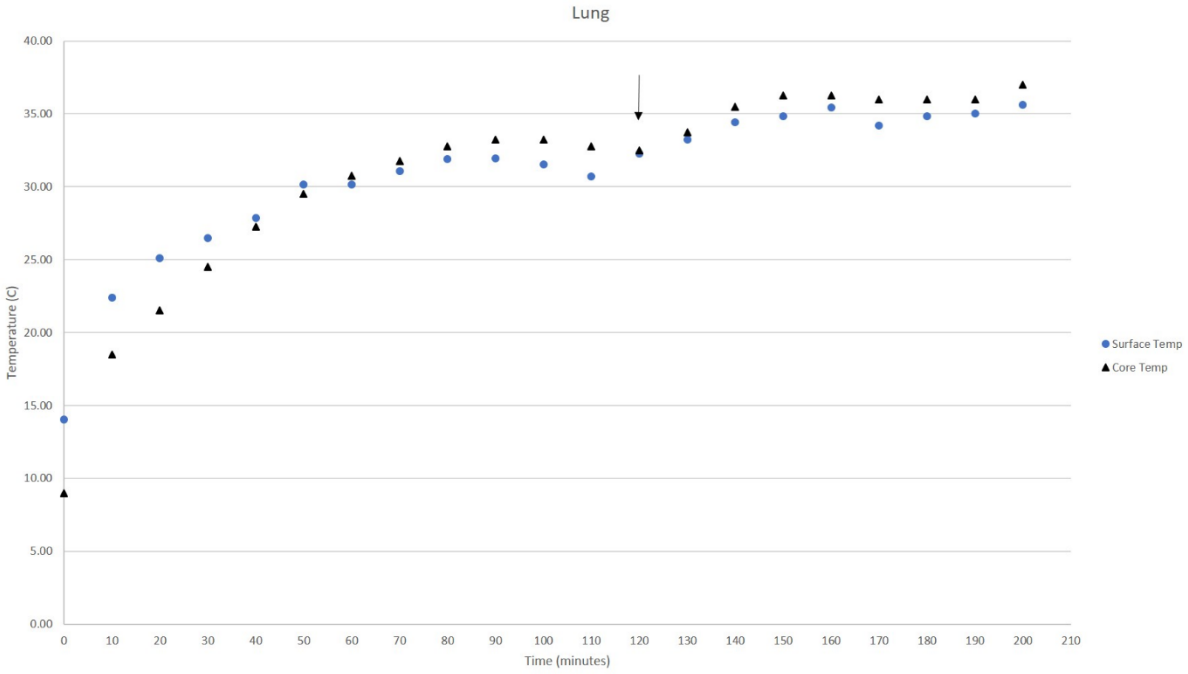

Figure 4-1 The core and surface temperatures of the lung samples measured at 10 minute intervals during the time taken to reach the core temperature to 37°C in the temperature conditioning chamber. The arrow indicates the approximate time at which a surface color change occurred.

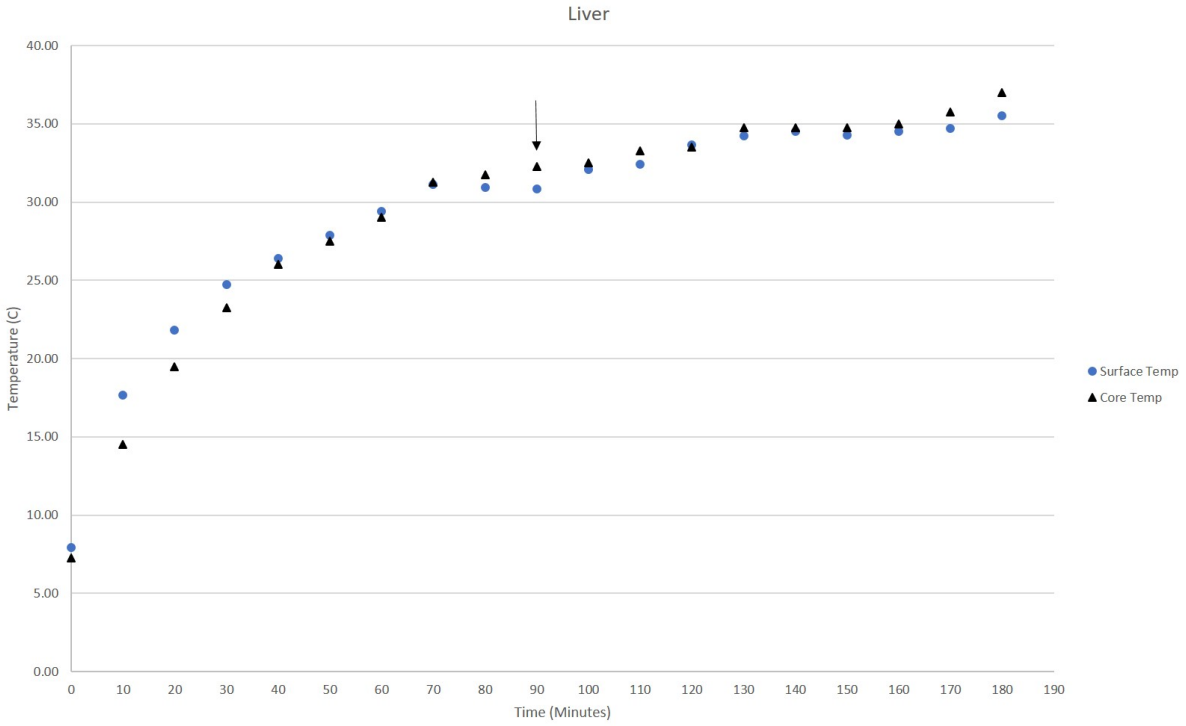

Figure 4-2 The core and surface temperatures of the liver samples measured at 10 minute intervals during the time taken to reach the core temperature to 37°C in the temperature conditioning chamber. The arrow indicates the approximate time at which a surface colour change occurred

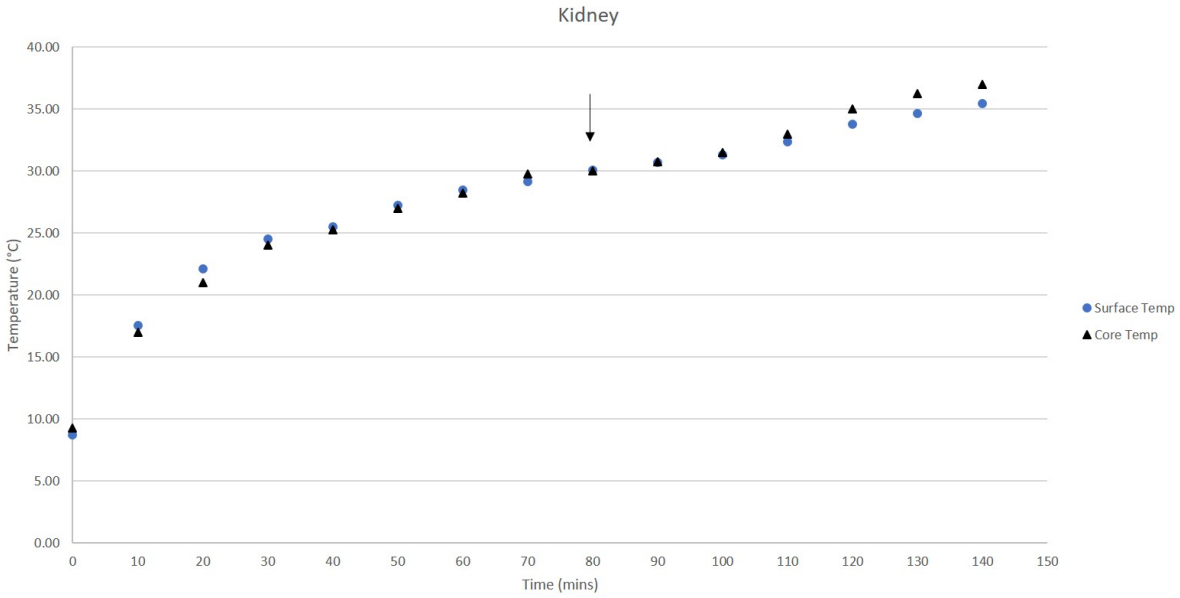

Figure 4-3 The core and surface temperatures of the kidney samples measured at 10 minute intervals during the time taken to reach the core temperature to 37°C in the temperature conditioning chamber. The arrow indicates the approximate time at which a surface color change occurred

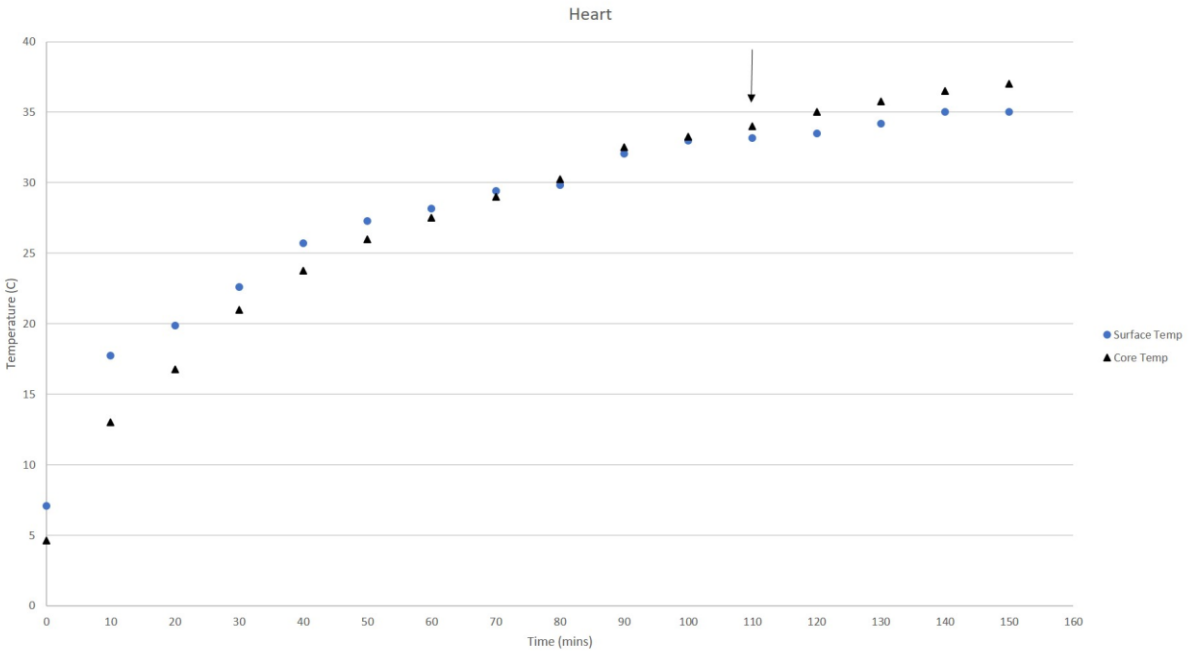

Figure 4-4 The core and surface temperatures of the heart samples measured at 10 minute intervals during the time taken to reach the core temperature to 37°C in the temperature conditioning chamber. The arrow indicates the approximate time at which a surface color change occurred.

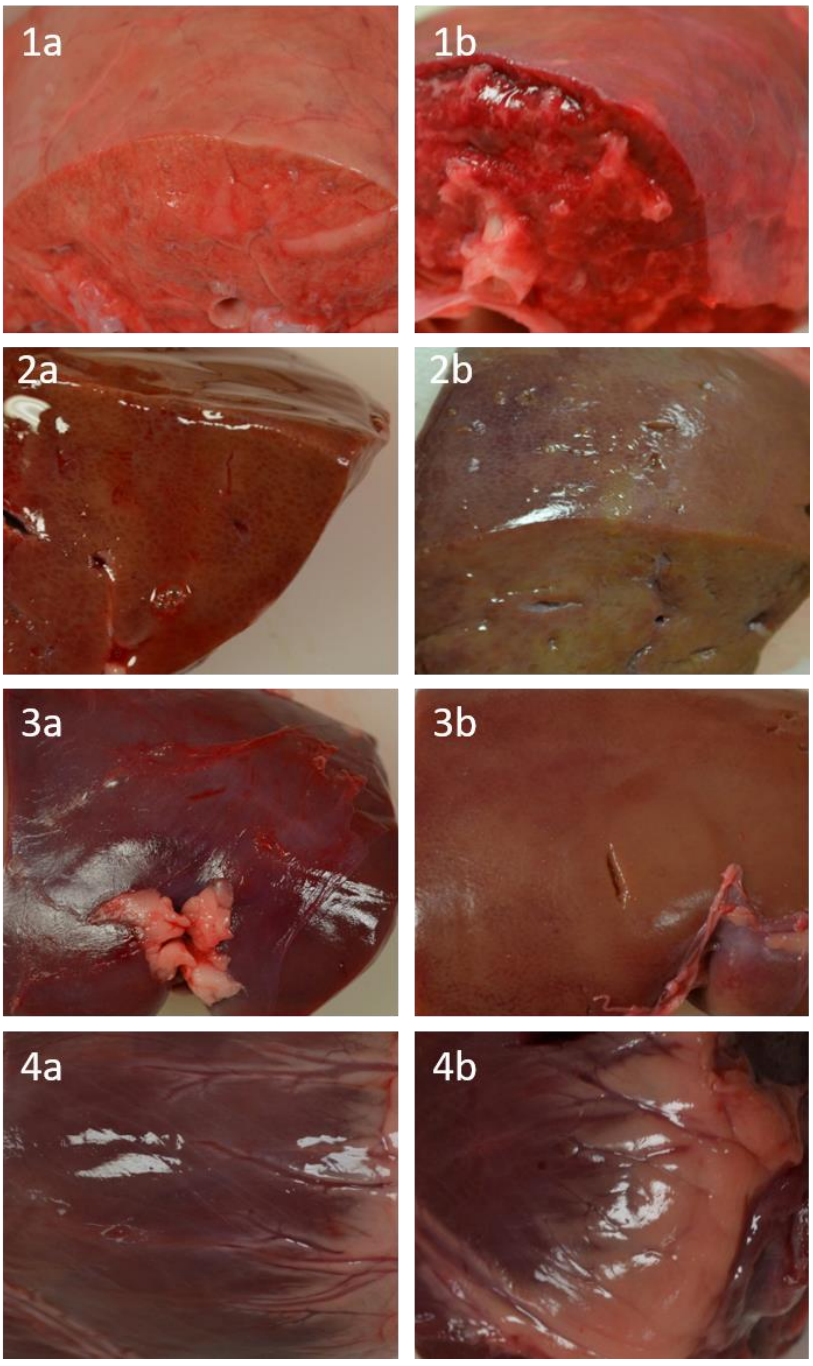

Figure 4-5 Surface colour of the lung (1), liver (2), kidney (3) and heart (4) at removal from 4°C (a) and during the heating phase in the conditioning chamber (b)

4.6.1 Microstructural (Histological) Changes:

Heart. In samples taken either at 4°C or at core 37°C, abnormalities (ie, changes in staining color or the structure) were not seen in the cytoplasm and nuclei of cardiac muscle cells, cells of the connective tissue matrix (ie, tissues among cardiac muscle cells), walls of blood vessels and red blood cells in the capillaries (Figure 4-6 A and B). However, in the cardiac muscle cells that were at and near the cut surface of the tissue block, the cytoplasm was less dense (note the staining color was similar to the rest of the cardiac muscle cells), cross striations were very prominent, and the nuclei showed varying degrees of chromatin condensation (Figure 4-6 C). This change was seen in samples taken at both 4°C and 37°C, but the chromatin condensation of the nuclei was seen in more cardiac muscle cells of the samples taken at 37°C.

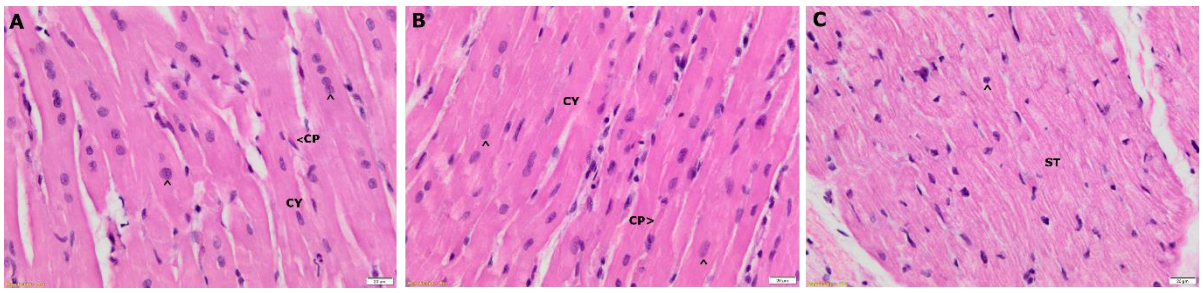

Figure 4-6 Heart tissue viewed at 20x magnification. A: refrigeration temperature; B: 37°C; C: 37°C. ^ nuclei, CP capillary, CY cytoplasm, ST striations

Lung. In samples taken either at 4°C or at core 37°C, abnormalities (ie, changes in color or structure) were not detected in the cytoplasm and nuclei of epithelial cells of alveoli, bronchi and bronchioles, and walls of blood vessels, bronchi and bronchioles (Figure 4-7 A and B). In the lung samples taken at 4°C, more alveoli were open and distended compared to the lung samples taken at 37°C. In lung tissues taken at both temperatures, areas of compact cell densities were seen; such regions were more common in the samples taken at 37°C (Figure 4-7 C).

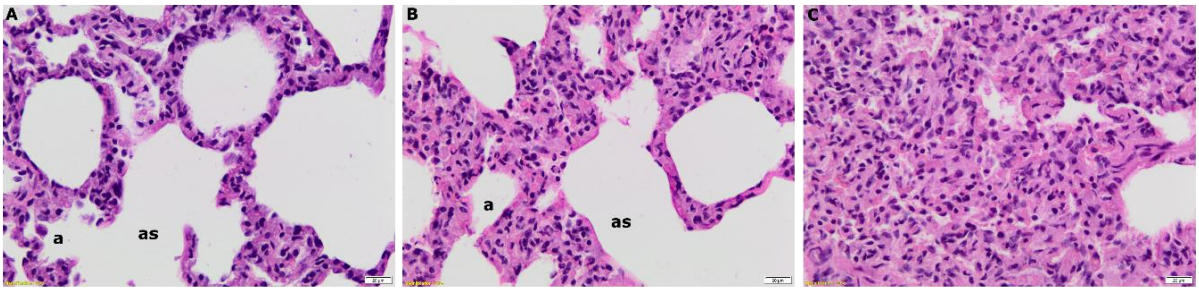

Figure 4-7 Lung tissue viewed at 20x magnification. A: refrigeration temperature; B: 37°C; C: 37°C. a: alveoli, as: alveoli sac

Liver. Liver samples taken either at 4°C or at core 37°C did not show abnormalities (ie, changes in color or structure) in the cytoplasm and nuclei of hepatocytes, Kupffer cells, walls of sinusoids and central veins, and bile ducts and vascular elements of portal tracts (Figure 4-8 A and B).

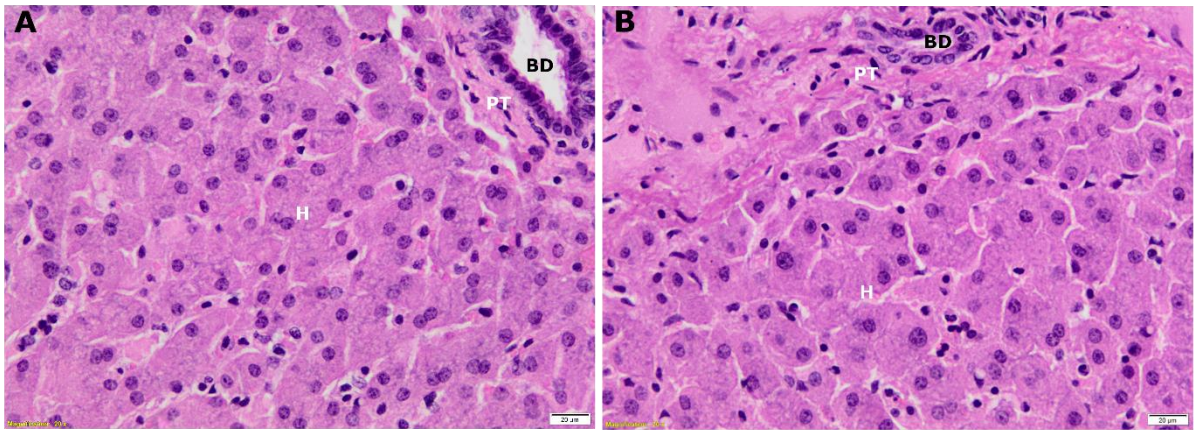

Figure 4-8 Liver tissue viewed at 20x magnification. A: refrigeration temperature; B: 37°C. H hepatocyte, PT portal tubule, BD bile duct

Kidney. Kidney samples taken either at 4°C or at core 37°C did not show abnormalities (ie, changes in color or structure) in cytoplasm and nuclei of cells of glomeruli (including Bowman capsules), tubules, and walls of blood vessels (Figure 4-9, A and B).

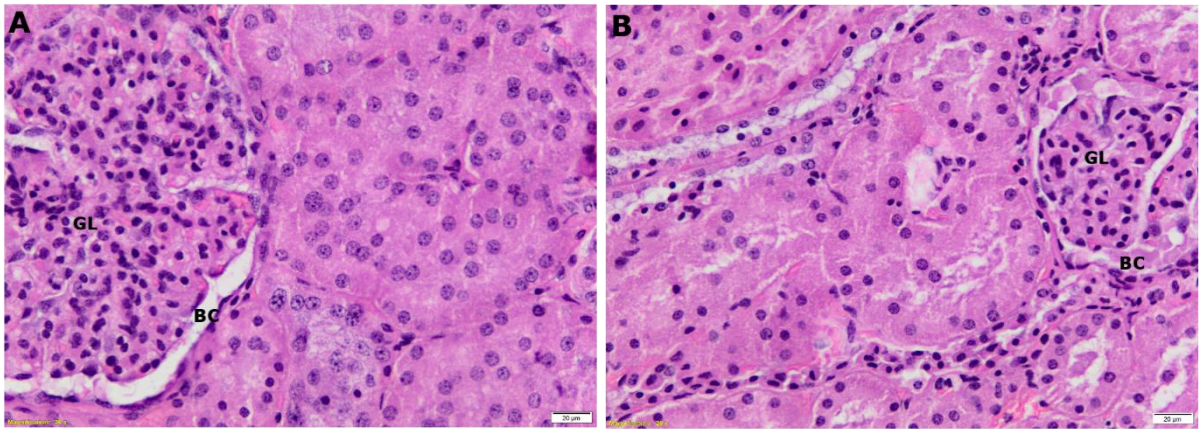

Figure 4-9 Kidney tissue viewed at 20x magnification. A: refrigeration temperature; B: 37C. GL glomerulus, BC Bowmen's capsule

4.7 Discussion

Cells of organs removed from slaughtered animals or for human organ transplantation are commonly expected to undergo degeneration and necrosis followed by autolysis after the removal. These changes could commence from the time of slaughter of an animal or removal from a live animal or human body.[19,20] This is the reason why tissues are fixed in chilled fixatives immediately after removal from the body for ultrastructural investigations. However, cytoplasmic and nuclear changes that indicate cell degeneration, necrosis, or autolysis at light microscopic level was not seen in the heart, lung, liver, and kidney samples removed from the slaughtered pigs, even after warming for 130 to 200 minutes in a temperature-conditioning chamber (Figure 4-6 to Figure 4-9). These are novel and interesting findings. Complete obstruction to a coronary artery or a segmental artery of the kidney or liver leads to infarction of the respective end-artery areas of the blocked artery in heart,[21] kidney,[22,23] and liver[24] within a short time. In lungs, such infarctions are less frequent because of the double circulation and possibly diffusion of oxygen into the lining cells of the alveoli. Heart, kidney, and liver are highly functionally active organs; thus, their oxygen demand is very high. Therefore, cutoff of oxygen supply for a short period because of the obstruction of an artery causes infarction in the end-artery area of the obstructed artery. Slaughtering of an animal causes cessation of functioning of the vital organs such as heart, lung, kidney, and liver rapidly; thus, their oxygen demand may drop markedly. Therefore, the cells in these organs could survive for a longer period without an oxygen supply or immersion in a specific buffer.[20] This may be the reason for the cells in the organs of the current investigation to remain unchanged during the period of investigation (Figure 4-6 to Figure 4-9). Refrigeration (at 4°C) of the 50-mm^3 blocks of the organs could have further reduced the metabolic rate of the cells and protected them until commencement of the experiment and gradual warming up to the core temperature of 37°C. The changes seen in

the cardiac muscle cells, at the cut end of the block (Figure 4-6), could be the effects of direct physical injury of cutting.

Porcine organs have been used in ballistic research to investigate injury patterns of projectiles passing through human organs.[5] In such experiments, the organs have been warmed to 37°C to simulate the human in-situ condition.[4] Projectiles penetrating body organs lose their energies and reduce velocities. The amount of energy loss and the reduction in velocity depend on the fibroelastic properties of each organ. Fibroelastic properties of organs are determined primarily by the connective tissue matrix of the organ.[5] Connective tissues, particularly the fibrous elements, are more resistant to autolytic degradation than the cellular elements of the organ. Current findings clearly indicate that the cellular elements do not undergo autolytic degradation during the period of warming from refrigeration temperature to core temperature of 37°C (Figure 4-6 to Figure 4-9). Therefore, the loss in energy and the drop-in velocity of projectiles penetrating fresh refrigerated porcine tissues should not be different from those of projectiles passing through porcine organs warmed to core temperature of 37°C. Therefore, in ballistic research, warming of body organs under investigation to the human core body temperature of 37°C is not necessary.

The areas of cell densities seen in the lung samples (Figure 4-7) are regions of alveolar collapse resulting from the escape of air trapped in them. During warming of the lungs, air from numerous alveoli has escaped, causing the increase in the number of areas of cell densities seen in the lung samples warmed to core temperature of 37°C. Heat would have also lost with the air escaping from the alveoli leading to the observed drop in temperature of the lung samples, before the final rise in temperature (Figure 4-1).

The cells of the liver samples warmed to 37°C were like those of the liver samples before warming and did not show any evidence of shrinking (Figure 4-8). Therefore, the 6.5 g of fluid

that drained out from the liver samples during warming of the tissue, may not have come from the liver cells. Liver is the organ that generates approximately 25% to 50% of the lymph drained via the thoracic duct.[25,26] Therefore, the fluid that drained out could be the lymph that was in lymphatic vessels. Furthermore, liver contains large volume of blood in branches of the portal vein, sinusoids, central veins and the remainder of the venous tree. Therefore, some of the fluid could also be the serum that escapes out (ie, from cut surfaces) after clotting of the blood.

4.8 Conclusion

The degenerative changes in the cells of the organs removed rapidly from the body and chilled quickly are minimal. During reheating to core body temperature (37°C) after refrigeration, the cells remain unchanged during the period of reheating. The time taken for reheating of the organ to human core body temperature, 37°C, depends on the specific organ. In ballistic research, heating of the chilled organ to human core body temperature is not necessary.

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Chapter 5

5 The deceleration of a spherical projectile passing through porcine organs at laboratory temperature (16°C) and core body temperature (37°C)

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5.1 Statement of Authorship

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
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5.2 Context

The two previous chapters (Chapter 3 and Chapter 4) described experiments that investigated the effects of the time taken to re-heat porcine organs to core body temperature on the energy loss from high velocity projectiles penetrating through the porcine organs and on the micro-structure of the porcine organs.

An experiment has been conducted to investigate the effects of low velocity projectiles (~200 m/s) penetrating through porcine organs at room temperature (16°C) and re-heated to core body temperature (37°C) on the loss of energy from the projectile. This indicated that there is a difference between the two temperatures (Maiden, 2014). The current experiment was conducted to investigate the effects of medium (500 m/s) and high (900 m/s) velocity projectiles penetrating through porcine organs (hearts, lungs, livers and kidneys) at room temperature (16°C) and core body temperature (37°C) on the loss of energy from the projectiles. This chapter describes the experiment, findings and the comparison of energy loss from the projectiles between the two temperatures.

5.2.1 References

Maiden, NR (2014) The assessment of bullet wound trauma dynamics and the potential role of anatomical models. Doctor of Philosophy Dissertation, University of Adelaide, School of Medical Sciences.

5.3 Abstract

Continued conflicts in the world lead to an interest in the interaction of projectiles with the human body and the efforts to protect the vital organs from these projectiles. Tissues excised from various body organs of experimental animals have been used in previous ballistics research to investigate the traumatic effects of a projectile. After removal from the body, organs were refrigerated to prevent decomposition. Before testing, organs were warmed to the temperature of the laboratory (16-18°C), or kept chilled and firing tests conducted. The results may not be representative because physical properties of tissues may differ at the body temperature (37°C). In the present study, porcine tissues (lung, liver, kidney, heart) were tested for a difference in the deceleration of a spherical projectile with high (~900m/s) and medium (~500m/s) entry velocity at 16°C and 37°C. The mean deceleration of medium velocity projectiles travelling through tissues conditioned to 16°C in the descending order was lungs ($6.8 \times 10^{5} \pm 3.2 \times 10^{4}$ m/s^2), hearts ($8.0 \times 10^{5} \pm 3.9 \times 10^{5}$ m/s^2), livers ($8.9 \times 10^{5} \pm 1.2 \times 10^{4}$ m/s^2) and kidneys ($9.5 \times 10^{5} \pm 6.8 \times 10^{4}$ m/s^2) and at 37°C, lungs ($6.5 \times 10^{5} \pm 2.2 \times 10^{4}$ m/s^2), livers ($7.8 \times 10^{5} \pm 3.7 \times 10^{4}$ m/s^2), kidneys ($8.6 \times 10^{5} \pm 6.1 \times 10^{4}$ m/s^2) and hearts ($8.9 \times 10^{5} \pm 1.8 \times 10^{4}$ m/s^2). These do not differ significantly for any organ. Similary, the results did not differ significantly at a high velocity for any organ. This is potentially due to the high entry velocities (500-900m/s) producing such high energy that can not be seriously reduced by the resistance of a tissue in an average size organ. Thus, heating of the tissues to 37°C is not necessary for ballistics experiments conducted at entry velocities that range between 500 and 900m/s. This may serve as a guideline to produce artificial testing materials.

5.4 Introduction

In ballistics research, the use of live humans to investigate the effects of projectiles passing through different body tissues is unethical and illegal. Ethically approved research has been conducted on human cadavers, however, the bio-mechanical properties, tensile strength, water distribution, histology, cellular fluid and cell size of the organs and tissues of embalmed or frozen cadavers are just a few properties that are altered [1-9]. An alternative is to use animal tissues and tissue simulants including the Federal Bureau of Investigation (FBI) and The North Atlantic Treaty Organization (NATO) standard ballistic gelatine [10]. Porcine tissues have been widely used in ballistics research due to their similarity to human tissues, however other animal tissues have also been used including those of dogs, goats, sheep and horses [11-14].

The simplest way to investigate the effects of projectiles passing through the body tissues is to conduct the experiment at room (laboratory) temperature of 16-18°C. However, whether these findings could represent the effects of similar projectiles passing through the same tissues at 37°C (i.e. the core body temperature of humans) is not certain. Previous research has been conducted using steel spheres as projectiles fired at a low velocity (<200m/s) into porcine tissues [15]. Although this study did not directly compare the ballistic behaviour of porcine tissue at these two temperatures, it was found that heart and lung were comparable to each other in terms of energy loss both at room temperature and body temperature. The energy loss of heart, lung and hindquarter muscle decreased with increasing temperature, while in liver, kidney and adipose tissue, the energy loss increased with increasing temperature [15].

The focus of the current research is to determine whether the deceleration of projectiles travelling through porcine organ tissue at laboratory temperature of 16°C is different from the deceleration of projectiles passing through same tissues maintained at 37°C at both a high (900m/s) and medium (500m/s) entry velocity. Thus, the aim is to determine whether an organ

tissue at laboratory temperature could represent an organ at core body temperature. Furthermore, the data published by Maiden, Musgrave[15] for low velocity projectiles were reanalysed to allow a direct comparison between the three velocities.

5.5 Materials and Methods

5.5.1 Equipment

A series of experiments were conducted to investigate the effects of projectiles travelling through porcine and human tissue simulants in an indoor ballistic test facility located at the Defence Science and Technology Group (DSTG), Edinburgh, South Australia. The methods follow methods previously used by Humphrey, Henneberg[16](p2). Specially prepared ammunition was fired from a purpose built 7.62 x 51 mm gun, mounted on a firm support. The gun was remotely operated. The gun fired steel spheres of 6.35 mm diameter and 1.043g mass. These spheres were encased within frangible plastic sabots[16]. Steel spheres were used instead of actual rifle bullets, as they do not tumble, nor distort upon impact with soft tissues. This removed the effects of tumbling, break up, distortion and fragmenting and allowed consistent repeatable interactions with the tissues. Therefore, the findings could be compared between tissues.

A specific projectile launch velocity was regulated by altering the quantity of the ammunition's single base (i.e. nitro-cellulose based) gun propellant. The steel sphere projectiles were fired at two different velocities, 900 m/s and 500 m/s. The former velocity provides an impact energy analogous to a military 5.56 calibre cartridge at its typical engagement range of ~250m, while the 500 m/s velocity represents the velocity of the same cartridge at the operationally effective range limit (~600m).

In the previous paper,[16](p2), it was described as follows, "*the gun was set up at a distance of 10 m from the test specimen. This distance would enable the plastic sabot to separate cleanly from the ball and not impact the test specimen, whilst providing the highest possible accuracy and impact velocity*. *A Doppler radar was used to track the spherical projectiles velocity from the firearm muzzle to the test specimen. A pair of optical infra-red sighting screens recorded the projectiles' residual velocity after having passed through the test specimen. Two high speed video cameras provided top and side views of the test specimen. High intensity LED lighting enabled the cameras to be operated at 10,000 frames per second with a shutter speed of 1/150,000th of a second. This provided sufficient image resolution and number of frames to undertake a post firing analysis of the shot line, target thickness and projectile entry and exit velocities*[16] (Figure 5-1).

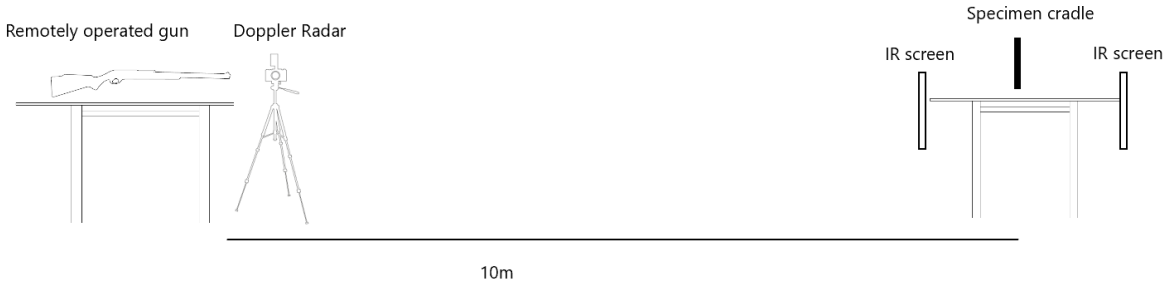

Figure 5-1 Schematic diagram of the test equipment set up

5.5.2 Materials

Porcine organs, lung, liver, kidney and heart (without pericardium), were obtained from freshly slaughtered pigs (approximately 80kg) at a local abattoir. *On the day of slaughter, the organs were cut into approximately 50 mm sided cubes and individually placed in zip lock plastic bags (to facilitate mounting of the specimen on to the sample holder and to minimise dehydration of the tissue) and refrigerated at 4°C until they were used for ballistics experiments* [16](p2) (approximately minimum 24 hours, maximum 3 days). Due to the nature of the lung tissue, the equipment cut the samples to approximate 50mm cubes, however, once cut, the tissue deflated creating slightly smaller cubes. Before the experiment, organs were transferred to a temperature conditioning cabinet at 16°C or 37°C. From a previous experiment it was found that a slight loss of energy of a steel spherical projectile occurred with heart and liver tissues conditioned for a long period of time (max 7 hours) compared to a short time frame (2 hours), and a reverse effect was found for the kidney tissues [16]. Therefore, for this experiment the tissues were conditioned for a minimum period of 2 hours and no longer than seven hours, to ensure that the samples were evenly heated to the specific temperature. The lower temperature of 16°C represented the laboratory test temperature used by Maiden [15], whilst the temperature of 37°C is the core temperature of the human body. To support tissue samples, a specific specimen cradle was built. Prior to each firing, the sample was collected from the temperature conditioning cabinet, brought to the test area and mounted onto the holder in the upright position. The time each specimen was stored in the temperature conditioning cabinet and the time taken to remove them and conduct the firing was recorded.

5.5.3 Testing

The spherical projectiles were fired into the fresh test specimens at either high velocity or medium velocity, from a distance of ten metres. Doppler/IR sight screens were used to record

the entry and exit velocities of the projectiles, as well as high speed video recording. [16]. Previous research found that the coefficients of determination between the results provided by the Doppler velocity method and the HSV methods were practically identical assuring the accuracy of these measuring devices [16]. For the purpose of these results, only the Doppler/IR sight screens method results are reported. The shot line thickness was measured from video recordings. The energy loss was calculated per metre of tissue thickness penetrated (J/m), using the equation $K = \frac{1}{2}mv^2$. Regular temperature measurements were taken of the test environment so that the impact of a long-time interval between sample removal from the temperature conditioning cabinet and firing of the projectile could be evaluated. Temperatures in the test environment ranged from 14.9°C to 24.0°C (mean 18.6°C, SD 3.2).

5.5.4 Analysis

Data were standardised for tissue thickness by determining regressions of the energy loss on the actually measured thickness of the tissue. Various regression lines were fitted to the bivariate data in order to determine which gives the best fit (greatest determination coefficient). In linear relationships, the energy loss was directly divided by the tissue thickness. The percentage energy loss was corrected for tissue thickness and the deceleration (negative acceleration) of the projectile was calculated using the equation for acceleration, $a = \frac{v^2-u^2}{2s}$, where v = exit velocity, u = entry velocity and s = tissue thickness. In curvilinear relationships, a regression equation was used.

5.5.5 Low Velocity Data

Raw data from Maiden, Musgrave[15] research were obtained, with entry velocities approximately 200m/s. The energy loss (J) of these data was corrected for the tissue thickness, in order to compare with the current findings. Correlation between the energy loss and the thickness of tissues was determined. The correlation in some cases was linear, while in others

it was curvilinear. In linear relationships, the energy loss was directly divided by the tissue thickness, while in curvilinear relationships the energy loss was adjusted using a regression equation. New values of the percentage energy loss were corrected for tissue thickness and the decelerations of the projectiles were calculated using the equation for acceleration (see above).

5.5.6 Statistical Analysis

One-way ANOVA with Bonneferoni's post hoc test was used to determine statistical differences between the deceleration of the projectile through the organs at laboratory temperature (16°C) and body temperature (37°C) at different velocities.

5.6 Results

Results of the mean tissue thicknesses, entry and exit velocities and energy and the percentage of energy losses adjusted for the thickness of tissues are presented in Table 5-1. The minimum percentage energy loss that was adjusted for the thickness of the tissues was seen in lungs with the low velocity projectiles at 37°C (29.4%, SD 3.1), while the maximum was found in the livers with the low velocity projectiles at 16°C (53.9%, SD 8.5).

The mean (±SEM) deceleration of high velocity projectiles travelling through tissues conditioned to 16°C in the descending order was lungs ($2.777x10^6 \pm 1.3x10^5$ m/s^2), hearts ($3.436x10^6 \pm 1.2x10^5$ m/s^2), livers ($3.639x10^6 \pm 1.6x10^5$ m/s^2) and kidneys ($3.960x10^6 \pm 2.7x10^5$ m/s^2). The mean deceleration at 37°C in descending order was lungs ($2.760x10^6 \pm 1.9x10^5$ m/s^2), hearts ($3.196x10^6 \pm 1.6x10^5$ m/s^2), livers ($3.397x10^6 \pm 1.7x10^5$ m/s^2) and kidneys ($3.542x10^6 \pm 9.9x10^4$ m/s^2). No significant differences were seen between the temperatures for any organ (Figure 5-2).

The mean deceleration of medium velocity projectiles travelling through tissues conditioned to 16°C in the descending order was lungs ($6.8x10^5 \pm 3.2x10^4$ m/s^2), hearts ($8.0x10^5 \pm 3.9x10^5$ m/s^2), livers ($8.9x10^5 \pm 1.2x10^4$ m/s^2) and kidneys ($9.5x10^5 \pm 6.8 x10^4$ m/s^2) and at 37°C, lungs ($6.5x10^5 \pm 2.2 x10^4$ m/s^2), livers ($7.8x10^5 \pm 3.7x10^4$ m/s^2), kidneys ($8.6x10^5 \pm 6.1x10^4$ m/s^2) and hearts ($8.9x10^5 \pm 1.8x10^4$ m/s^2) (Figure 5-2).

The mean deceleration of low velocity projectiles (entry velocities approximately 200m/s) travelling through tissues conditioned to 16°C in the descending order was livers ($2.3x10^5 \pm 7.1x10^3$ m/s^2), hearts ($3.0x10^5 \pm 7.2x10^3$ m/s^2), lungs ($3.1x10^5 \pm 1.3x10^4$ m/s^2) and kidneys ($3.3x10^5 \pm 1.8x10^4$ m/s^2) and at 37°C, hearts ($2.6x10^5 \pm 5.1x10^3$ m/s^2), lungs ($2.8x10^5 \pm 1.3x10^4$ m/s^2), livers ($3.3x10^5 \pm 1.8x10^4$ m/s^2) and kidneys ($3.6x10^5 \pm 1.1x10^4$ m/s^2).

No significant differences were observed in the deceleration of projectiles travelling through any tissues at high or medium velocities at either temperature. At low velocity, a significant difference occurred for the liver, however this can be classed as insignificant when Bonferroni's correction to the test probability values for multiple comparisons is made.

The mean time taken for each specimen to be removed from the hot conditioning chamber until fired into at high entry velocities ranged from 3.2 minutes (SD 0.5) for hearts to 4.0 minutes (SD 0.5) for lungs. At a medium entry velocity, the time ranged from 2.7 minutes (SD 0.2) for livers to 4.1 minutes (SD 2.2) for hearts. The surface temperature taken immediately before being fired into for the organs ranged between a minimum of 36.5°C for hearts to a maximum of 37.1°C for lungs. The surface temperature would decrease in an ambient room environment before the core temperature decreased. With non-significant results observed in deceleration of the projectile, the time taken from removal from the hot chamber to being fired into did not affect the results.

Significant differences occur between the entry and exit velocities and energies for all organs at whatever temperature at the three velocities ($p<0.0001$). Within the same organ no significant differences occur between the two temperatures.

The mean deceleration of the three velocity projectiles through each of the tissues can be seen in Figure 5-2, while the mean energy loss per thickness of tissue can be seen in Figure 5-3.

Table 5-1 Means and standard deviations (SD) for organs at 16C and 37C at high, medium and low velocities

	Organ	*Temp. (°C)*	*N*	*Tissue Thickness (mm)*	*SD*	*Entry Velocity (m/s)*	*SD*	*Exit Velocity (m/s)*	*SD*	*Entry Energy (J)*	*SD*	*Exit Energy (J)*	*SD*	*% Energy Loss for total thickness of tissue sample*	*SD*	*Energy loss (J/mm) adjusted for tissue thickness*	*SD*
	Lung	16	10	49.6	7.1	877.1	41.0	705.2	35.2	401.9	37.5	259.9	28.8	35.6	3.2	2.895	0.4
		37	10	48.5	8.1	876.3	38.8	712.6	39.1	401.2	35.6	265.6	28.4	34.7	6.5	2.879	0.6
	Liver	16	10	51.3	7.5	894.8	65.3	654.9	41.4	419.5	61.2	224.5	28.4	46.5	3.2	3.796	0.5
High		37	15	50.5	6.3	866.5	70.5	641.4	57.1	393.9	67.4	216.2	40.0	45.4	2.7	3.543	0.7
Velocity	Kidney	16	10	39.7	5.6	881.8	70.6	677.4	76.8	407.9	67.6	242.1	52.0	40.5	7.9	4.131	0.9
		37	15	42.4	5.8	894.8	44.7	708.5	44.7	418.5	43.6	262.8	34.3	37.5	2.6	3.695	0.4
	Heart	16	10	52.4	4.2	884.9	42.3	651.4	26.2	409.2	39.9	221.6	17.9	45.9	2.8	3.585	0.4
		37	15	59.0	5.6	882.0	75.3	636.6	58.4	408.4	70.2	213.0	39.1	48.1	3.3	3.334	0.6
	Lung	16	5	65.4	9.7	450.6	10.6	338.1	18.5	105.9	5.0	59.8	6.3	43.8	2.9	0.71	0.07
		37	5	65.9	5.1	447.6	7.2	338.5	8.1	104.5	3.4	59.8	2.8	42.7	2.3	0.68	0.05
	Liver	16	5	56.9	6.7	458.3	12.7	329.4	9.9	109.6	6.1	56.6	3.4	48.5	3.1	0.93	0.03
Medium		37	5	51.7	9.1	442.1	8.9	336.6	21.1	102.0	4.1	59.3	7.5	41.6	3.3	0.82	0.09
Velocity	Kidney	16	5	45.4	4.3	449.6	13.0	342.0	13.3	105.5	6.1	61.1	4.7	42.3	4.8	0.99	0.16
		37	5	46.9	6.9	452.6	12.4	353.8	12.3	106.9	5.9	65.3	4.6	39.3	4.3	0.9	0.14
	Heart	16	5	55.5	5.1	442.4	18.3	327.7	13.2	102.2	8.3	56.1	4.5	45.3	3.4	0.83	0.09
		37	5	54.5	2.7	457.9	5.5	336.2	2.6	109.4	2.6	58.9	0.9	46.1	1.5	0.93	0.04
	Lung	16	18	19.5	4.7	183.5	1.5	146.7	9.3	5.9	0.1	3.8	0.5	36.1	4.9	0.11	0.02
		37	20	18.4	6.1	182.3	1.6	153.2	7.2	5.8	0.1	4.1	0.4	29.4	3.1	0.09	0.01
	Liver	16	24	40.2	12.9	183.9	1.8	126.3	16.9	5.9	0.1	2.8	0.7	53.9	8.5	0.08	0.01
Low		37	20	20.5	9.1	182.1	2.7	143.2	13.3	5.8	0.2	3.6	0.7	37.8	4.2	0.11	0.01
Velocity	Kidney	16	19	20.3	6.8	185.8	1.6	147.9	9.2	6.0	0.1	3.8	0.5	36.4	1.6	0.11	0.004
		37	20	15.9	2.9	182.1	2.3	147.2	7.0	5.8	0.1	3.8	0.4	35.0	5.1	0.13	0.02
	Heart	16	16	23.0	3.5	185.5	1.1	143.4	3.7	6.0	0.1	3.6	0.2	40.6	3.9	0.11	0.01
		37	22	29.8	5.9	183.2	1.8	135.3	7.8	5.8	0.1	3.2	0.4	46.0	4.1	0.09	0.01

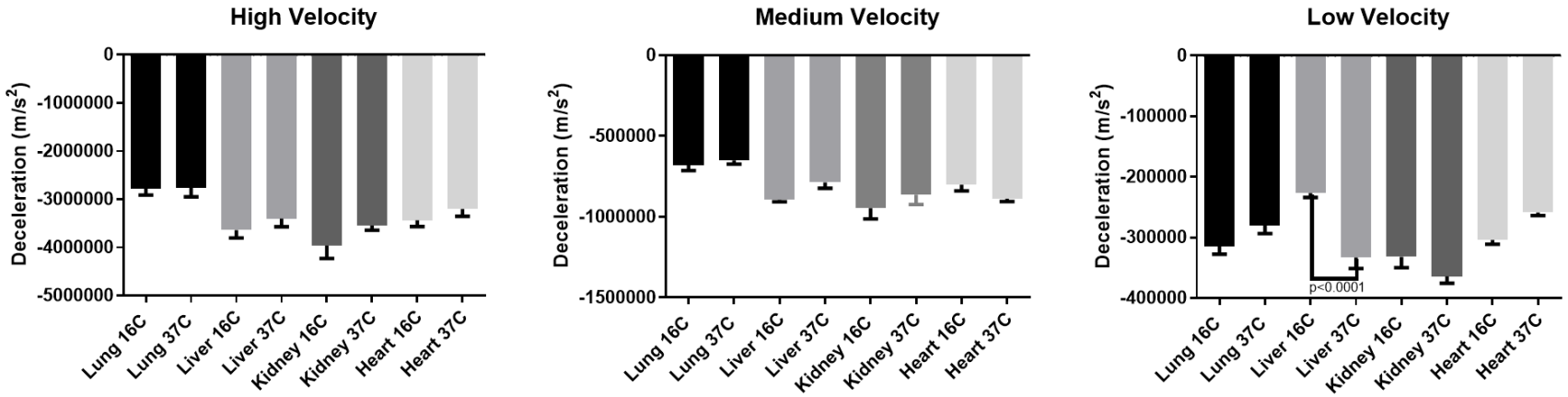

Figure 5-2 Mean deceleration and SEM of projectiles travelling at three different velocities through the lung, liver, kidney and heart tissues at 16°C and 37°C

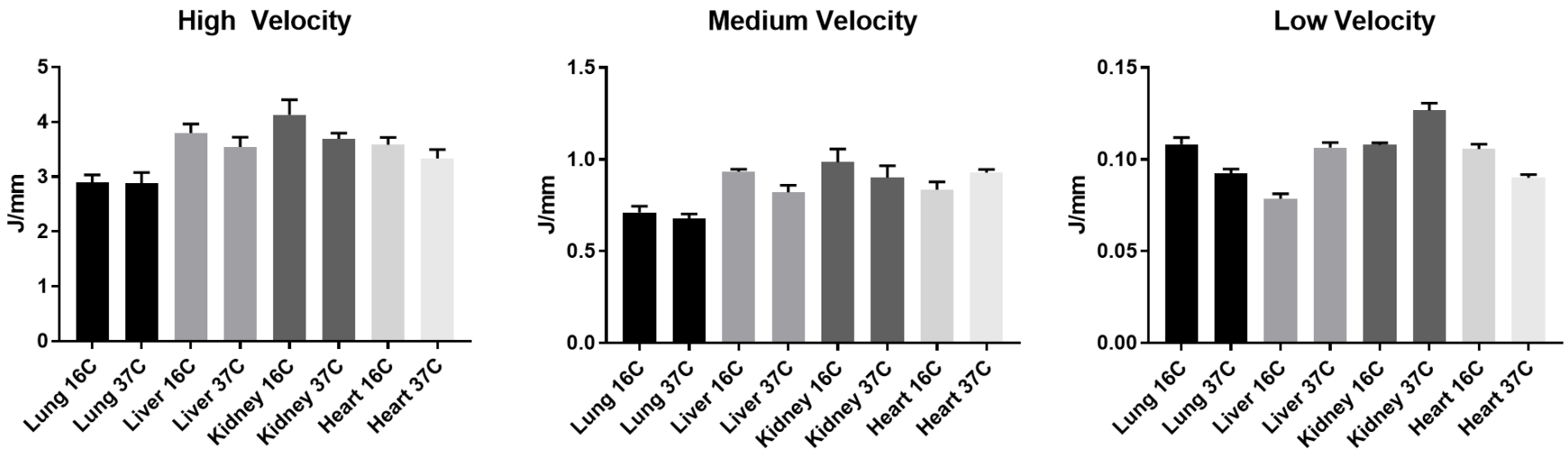

Figure 5-3 Mean energy loss per millimetre of tissue (J/mm) and SEM of projectiles travelling at three velocities through the lung, liver, kidney and heart tissues at 16°C and 37°C

5.7 Discussion

In all body tissues investigated at 16°C and 37°C, the entry velocities, irrespective whether high, medium or low, decelerated during the passage of the projectile through the tissues to a level that the exit velocities were significantly lower than the entry velocities ($p<0.0001$). An identical pattern was seen in relation to the entry and exit energies ($p<0.0001$). These indicate that the thickness of each tissue used for the investigation was adequate to cause an energy loss during the passage of the projectile through the tissue to reduce the exit velocity to a level significantly lower than the entry velocity (Table 5-1). However, when the loss of energy of the projectiles in the tissues was adjusted to the tissue thickness, there were no differences between the two temperatures at any of the projectile entry velocities tested (Table 5-1 and Figure 5-1). This clearly indicates that the deceleration resulted from the loss of energy of the projectile as a function of the thickness of the tissue, rather than the temperature. Therefore, effects of a projectile travelling through an organ/ tissue at room temperature may not be different from the effects resulting at 37°C. This may indicate that ballistic testing, where animal organs/tissues are used, could be carried out at room temperature. However, it could be hypothesised that the results of this study are related to the density of the tissue, which is unlikely to change within the temperature limits of this study. Only severe dehydration and hardening would result in changes and this would most likely occur over a long period of time.

The deceleration or loss of energy of a projectile travelling through a body organ (Table 5-1 and Figure 5-1) results from the resistance offered by the tissue. A tissue consists of cells and connective tissue matrix (i.e. including the capsule). Major part of the resistance comes from the connective tissue matrix. In the current experiment, tissues were heated in the temperature conditioning chamber from 4°C to 16°C or 37°C. After the removal of a tissue/organ from the body, decomposition by autolytic changes sets in (i.e. by release of the lysosomal enzymes stored within cells)[16]. Refrigeration, slows down the autolytic changes by

reducing the activity of autolytic enzymes and therefore prolongs the duration at which the organ is viable without oxygen supply[17]. The opposite occurs when heating of the tissues occurs, accelerating the autolytic changes. These changes of the connective tissue matrix will be slower than those of the cells. The autolytic changes of the tissues heated to 37°C should be greater than those heated to 16°C. Therefore, the thickness adjusted energy loss from the projectile travelling through the tissue should be lower in tissues heated to 37°C than those heated to 16°C. A similar trend was seen in lungs, livers and kidneys between the two temperatures at all three velocities tested (Table 5-1), but these differences were not statistically significant. However, in the hearts tested at all 3 velocities, the thickness adjusted loss of energy tended to be greater at 37°C than at those at 16°C (Table 5-1). This could be due to some factor other than autolytic changes, possibly setting in of rigor mortis, where timing is affected by temperature [18].

5.8 Conclusion

The findings of the current research suggest that when conducting energy loss tests through organs, for the purpose of ballistics testing, the effect of the temperature of the organ (i.e. in the range from 16°C to 37°C) is negligible, therefore the testing could be conducted at room temperature to evaluate the effects at human body temperature. Other aspects relating to ballistics studies such as the elastic properties, stretching and tearing of tissues, were not investigated, however there is a possibility that these would be directly attributed to the cellular structure of the tissues. The effect of temperature change on the elastic properties of tissues was not studied, and thus the conclusion that the temperature is negligible, is only applicable to this type of ballistic studies. Furthermore, any simulants developed to represent the human organs/tissues at core body temperature could be tested at the laboratory temperature.

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Chapter 6

6 Comparison of porcine organs and commonly used ballistic simulants when subjected to impact from steel spheres fired at supersonic velocities

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6.1 Statement of Authorship

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
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6.2 Context

Tissue simulants have been used more often than animal tissues (e.g. porcine) in recent ballistics research, as they are easy to prepare, store and use. They also have the advantage of transparency, particularly in studying the temporary cavitation effect. Different simulants are available on the market, each has its advantages and disadvantages. The major disadvantage is that these simulants are homogeneous and could only represent a single organ, thus not able to represent the heterogeneity of the multiple organs within the human body.

A study conducted by N, Maiden (2014), investigated the energy loss of low velocity projectiles penetrating simulants and organ tissues and determined that the lungs behaved similar to the heart as well as 10% ballistics gelatine. The biological and physical properties of the lung and heart are completely different; thus, it is difficult to interpret these results. The lung primarily comprises of connective tissues and blood vessels that are organised around air-filled alveolar spaces, while the heart predominantly consists of compact muscle tissue. It would be correct to assume these anatomical differences could affect the loss of energy from projectiles penetrating through the tissues at different rates. If the lung behaves similar to the heart at low velocity, it is worth investigating whether this similarity persists when these tissues are penetrated by medium and high velocity projectiles. If effects of all three entry velocities on the body organs are similar, one anatomical model could be created to investigate all projectile velocities. However, if differences occur, then a different anatomical model would be required for each of the different velocities.

This chapter describes an experiment that compared the effects of medium and high entry velocities of projectiles penetrating porcine organs. In addition, three ballistics simulants were tested to determine which simulants could represent the bio-physical properties of each body organ. This experiment also determined whether the same comparisons could be made across

medium and high velocity projectiles. A brief comparison has been made with the previously investigated low velocity projectiles, although it is noted that a different experimental method was used (Maiden, 2014). Furthermore, this chapter discusses whether new types of simulants are needed to accurately represent the bio-physical properties of each body organ and could they be used to create an anatomically accurate physical model to use in future ballistics research.

6.2.1 References

Maiden, NR (2014) The assessment of bullet wound trauma dynamics and the potential role of anatomical models. Doctor of Philosophy Dissertation, University of Adelaide, School of Medical Sciences.

6.3 Abstract

Firearm injuries are common in the world today, in both military and civilian settings. Research into the effects different ammunition has on the human body has been conducted using tissue simulants such as Ballistics Ordnance Gelatine. Previous research has found that with low velocity projectiles, the tissue simulants only represent a selected few organs, as the human body and its organs/tissues are not homogeneous. The aim of this research was to determine which ballistic simulants best represent the abdomen and thorax, for the purposes of anatomical modelling. A mounted firearm was used to fire specially made ammunition containing a sabot and steel spherical projectile at medium (~500 m/s) and high (~900 m/s) velocities. Test specimens of 50mm cube porcine tissues (lung, liver, kidney and heart) and ballistics simulants (20% and 10% Ballistics Gelatine and Clear Gel) were shot at and the energy loss determined using Doppler radar, optical infra-red sighting screens and high speed video. This study determined that the conclusions drawn from studies in these tissue simulants may not be an accurate representation of what occurs in human tissues and that different impact velocities produce differing results. New simulants are required to better represent the energy loss of a projectile through the organs of the abdomen and thorax and the results of this study may guide this development. Further experiments will be required to determine different concentrations of gelatine and their accuracy at representing the heterogeneous nature organs of the human body.

6.4 Introduction

Injuries resulting from firearms are common in wars and to a much lesser extent in civilian populations. In the recent past, global conflicts leading to wars have increased and the casualties resulting from firearms have also markedly increased. Furthermore, advances in technology have led to the development of different types of firearms and ammunition, thus the effects of the projectiles fired from these weapons will vary. Therefore, there is a need to understand the mechanisms by which penetrating projectiles cause injury to body tissues and the associated wounds. Understanding the mechanisms of wounding could assist in the development of future weapons, particularly ammunition, in the training of medical personnel for the planning and treating wounds in a rational and timely manner, and in the development of body armour [1].

Currently, tissue simulants such as Ballistics Ordnance Gelatine in concentrations of 10% and 20% at temperatures of 4°C and 10°C, respectively, are being used to evaluate the effects of different projectiles on human body tissues. These simulants, after calibration, claim to simulate the soft tissues and organs of the human body, however, they have only been found to replicate the mechanical properties of skin, fat, fascia and muscle tissue of the porcine thigh [2]. Porcine tissues have been accepted as similar to those of human tissues, thus it has lead researchers to accept that the projectile-tissue behaviour of porcine tissue is the same as the human tissues [3]. The use of tissue simulants does not lead to ethical issues and can generate photographic evidence for the visualisation of wounding paths, however, they lack the bio-mechanical properties of different human tissues and organs that are commonly affected. Thus, these simulants are unlikely to represent the critical organs that are important to ballistic research.

The human body (i.e. the wall and the internal organs) is not homogenous, thus the effects of a projectile on causing tissue damage will depend on the viscoelastic properties of the tissues. They may react in different ways to different projectiles, thus the effects may vary according to the physical properties of the projectile, i.e. shape, construction, velocity, mass, energy and momentum [1, 4]. In addition, the effects of the projectile on body tissues will also depend on the type of the firearm and the distance from the body at which the weapon was discharged from. Firearms are classified into three categories: handguns, rifles and shotguns. Each type of firearm and its ammunition is distinct and would produce varying trauma to the human body [5]. This clearly indicates that the damage to a tissue by a projectile penetrating through the tissue does not totally depend on the amount of energy delivered to the tissue by the projectile, rather a combination of multiple factors including composition of tissues. Furthermore, the net effect of a projectile penetrating through the body of an individual will depend on the vital functions of the affected tissues and the amount of haemorrhage caused by the injury [6, 7].

To accurately model the human body and the internal organs and tissues for the prediction of the injury patterns (i.e. effects) that could result from projectiles penetrating through body tissues the mechanisms of wounding need to be understood. The mechanisms of wounding include the following:

The physical properties of the projectile i.e. shape, construction, mass and velocity. The mass and velocity of the bullet (by Newton's Laws) determine the potential energy and momentum and thus the ability to destroy tissue. The shape and construction of the bullet determine how much tissue is damaged. For example, a bullet designed to fragment upon impact on its target will have extensive damage due to the bullet fragmenting and increasing the number of projectiles travelling through the tissue. In comparison, a bullet designed to not fragment, however tumbles during flight (air or target) will produce a different wounding pattern, and the

same occurs with a bullet designed to stay intact, thus having the ability to penetrate a body and exit with substantial amounts of energy still available [1, 4, 8].

The firearm and its ammunition. Three different categories of firearms exist: handguns, shotguns and rifles. Each being distinct from the other and containing different ammunition. Handguns have short barrels and generate low amounts of energy. Shotguns are smooth bore firearms (i.e. no rifling in the barrel) and fire multiple shot-shell pellets compared to a single bullet. The type and size of the pellets can vary and the wounding pattern is distinct, however kinetic energy is lost quickly due to air drag and the pellets may not penetrate at great ranges. Rifles have a longer barrel that is rifled to stabilise the bullet during flight and have the ability to produce high energy and velocity to a bullet [3, 5, 9, 10].

The biomechanical properties of the tissues/organs forming the part of the body through which the projectile is passing through. The composition of the body in relation to tissue type and organs vary, particularly along the transverse, sagittal and vertical diameters in the head, thorax, abdomen and pelvis. Information in relation to reduction of velocity/energy across each organ and tissue along a diameter from the point of entry of the projectile is essential. Also, there is a need to consider the effects of a projectile entering the body at an angle to the three basic directions.

The three-dimensional arrangement and volume composition of each organ and tissue in the body/body cavities. The analysis and evaluation of this aspect is also essential to create an anatomically accurate representation of the human body for penetrating ballistics trauma investigations.

During the use of firearms in war and civilian altercations, the commonly affected organs are those that are in the centre of mass as this is the largest target area. Thus, the abdomen and thorax are commonly affected. In the thorax and abdomen are heart, lungs, intestines, liver and

kidneys. The reduction in velocity of a projectile passing through an organ results from its tensile strength, strain, elasticity and density [11, 12] and these bio-mechanical properties vary among organs and tissues. Thus, the reduction in the velocity or the energy in a projectile passing through an organ/tissue is different among different organs [13].

Anatomical models of the thorax and abdomen for non-penetrating wounds have been generated [14, 15] and other various models of the human body including head models [3]. A method has been developed for the generation of baseline data to establish an anatomical model to investigate the effects of projectiles passing through human tissues using human tissue simulants and porcine tissues [16]. In this investigation, steel spheres were fired at subsonic velocities through ballistic ordnance gelatine, and synthetic gelatine. The reduction in energy levels of the spheres, when passing through the organ simulants were compared with those of porcine organs. The findings of the study indicated that the tissue simulants represented only a few selected organs [16].

This study investigated the effects of the passage of spherical projectiles fired at supersonic velocities through commonly affected organs of human thorax and abdomen. Porcine heart, lungs, liver, kidneys and ballistic simulants were used to determine the reduction in the velocity and the energy loss from the projectiles passing through the tissues and simulants. Effects of two velocities were investigated. The final aim of the investigation is to develop a physical anatomical model for ballistic impact research.

6.5 Methods

6.5.1 Materials

Porcine organs, lung, liver, kidney and heart (without pericardium), were obtained from freshly slaughtered pigs (approximately 80kg) at a local abattoir. On the day of slaughter, the organs were cut into approximately 50 mm sided cubes and individually placed in zip lock plastic bags (to facilitate mounting of the specimen on to the sample holder and to minimise dehydration of the tissue) and refrigerated at 4°C until they were needed for ballistics experiments. Organs were heated in a conditioning cabinet to 37°C, the core body temperature of the human body. Prior to each test, the sample at 37^{0} C was collected from the conditioning cabinet, brought to the test area and placed in its holder in an upright position in preparation for firing. Times each specimen was stored in the conditioning cabinet and times taken to remove them and conduct the firing were also recorded.

Three ballistic simulants were examined: 20% ballistic ordnance gelatine conditioned at 10°C, 10% ballistic ordnance gelatine conditioned at 4°C and a synthetic product manufactured by US company Clear Ballistics, herein referred to as Clear Gel, was conditioned at 16°C prior to testing. The 20% ballistic ordnance gelatine was made as per military Standard D14 and the 10% ballistic gelatine was made to FBI standards. As the moulds in which these gelatines were formed were much larger than required, a wire cutter or sharp knife was used to cut the gelatines into 50 mm cubes. Prior to conducting tests firing into gelatine samples, an accepted calibration procedure for 10% ballistic gelatine [2] was performed, as no calibration method has been outlined for the other formulations. This involved firing steel ball bearings (BB) into the gelatine blocks three times and the depth of penetration was measured to compare with published literature [2]. This allowed only calibrated blocks of gelatines to be used in the experiment.

6.5.2 Testing

6.5.2.1 Equipment

The firing experiment was conducted at the indoor ballistic testing facility at the Defence Science and Technology Group (DSTG), Edinburgh, South Australia. A remotely operated 7.62 x 51 mm gun fitted to a fixed mount was used to fire a cartridge containing specially prepared ammunition (6.35 mm diameter steel spheres, mass 1.043g, encased in a frangible plastic sabot). In this study nitro-cellulose based gun propellant was used. Steel spheres were fired at two velocities, high and medium, by altering the amount of the propellant. The high and medium velocities ~900 m/s and ~500 m/s respectively of the steel sphere were analogous to impact energies of a military 5.56 mm cartridge and the same cartridge firing the same size (i.e. weight and size) steel sphere at the operationally effective range. The velocities generated by the established amounts of propellant were repeatable.

A specimen cradle device was specifically constructed to mount the tissue in its zip-lock bag in an upright position 10 m from the nozzle of the gun. This distance would enable the plastic sabot to separate cleanly from the steel sphere and not impact the test specimen, whilst providing the highest possible accuracy and impact velocity.

A Doppler radar was used to track the spherical projectiles velocity from the firearm muzzle to the test specimen. A pair of optical infra-red sighting screens recorded the projectiles residual velocity after having passed through the test specimen. Two high speed video cameras provided top and side views of the test specimen. High intensity LED lighting enabled the cameras to be operated at 10,000 frames per second with a shutter speed of 1/150,000th of a second which provided sufficient image resolution and number of frames to undertake a post firing analysis of the shot line, target thickness and projectile entry and exit velocities.

The spherical projectiles were fired into fresh test specimens in their zip-lock bags at either high velocity (~900 m/s) or medium velocity (~500 m/s), from a distance of ten metres. The entry and exit velocities were recorded by the Doppler/IR sight screens (Doppler velocity method), shot line thickness was measured from video recording and entry and exit velocities were also calculated from the high speed video recording (HSV velocity method). The energy loss was calculated per metre of tissue thickness penetrated (J/m), based on the equation of $K = \frac{1}{2}mv^2$. Regular temperature measurements were taken of the test environment so that the impact of a long dwell time between samples being removed from their conditioning cabinet and the time the firing occurred could be examined. For the purposes of these results only the results of the Doppler/IR sight screens method is reported.

6.5.2.2 Experimental Limitations

The ball bearing accuracy to strike completely within the 50x50 mm target area was not always consistent owing to shot-to-shot variations in the break up characteristics of the sabot material. The sabot had been experimentally manufactured from Acrylonitrile Butadiene Styrene (ABS), an inexpensive thermoplastic material, using a computer controlled rapid prototyping machine. This experimental approach was selected as it represented the best compromise between a fast shooting turnaround time and target accuracy, but nonetheless led to variations in the sizes of the various sample lots that could be examined. Furthermore, the available time to use the indoor range was severely limited owing to competing with high priority operational requirements; therefore, very few repeats were possible. The sample size of each test group can be seen in Table 6-1.

When comparing the deceleration of a projectile in tissues at 16°C and 37°C, no significant differences are found at a high or medium impact velocity ($P<0.05$). As a result of these findings the data presented here for the organs were independent of temperature.

Table 6-1 - Sample size of each sample at high velocity and medium velocity

	Sample size (n)	
	High Velocity	Medium Velocity
Lung	20	10
Liver	25	10
Kidney	25	10
Heart	25	10
20% ballistics gel @ 10°C	10	5
10% ballistics gel @ 4°C	10	5
Clear gel @ 16°C	10	5

6.5.2.3 Analysis

Data were standardised for tissue thickness by determining the correlation between the energy loss and the depth of tissue. For linear relationships the energy loss was directly divided by the tissue depth. The percentage energy loss corrected for tissue thickness was obtained and the deceleration (negative acceleration) of the projectile calculated using the equation for acceleration, $a = \frac{v^2-u^2}{2s}$, where v = exit velocity, u = entry velocity and s = tissue thickness.

6.5.2.4 Statistical Analysis

GraphPad Prism was used to conduct one-way ANOVA with Bonferroni's multiple comparisons post hoc statistical tests on the data to compare the various organs at both high velocity and medium velocity firings. These results were also compared with the three simulants.

6.6 Results

The mean entry and exit velocities, and the mean entry and exit energies of the projectiles passing through porcine lung, liver, kidney and heart and the three human tissue simulants are presented in Table 6-2. In addition, means for calculated energy differences, reductions in energy per metre of tissue, reductions in energy adjusted for average tissue thickness,

percentage energy reductions adjusted for tissue thickness and deceleration of the projectiles when passing through the porcine tissues and the three human tissue simulants are also presented in Table 6-2. The values in Table 6-2 are for both high and low velocity projectiles. No significant differences occur between the entry velocities of each of the organs/simulants at high or medium velocities (Table 6-3). Significant differences occur between the entry and exit velocity for all organs and simulants at medium and high velocity ($p<0.0001$).

Direct comparisons of the percentages of energy loss from high and medium velocity projectiles entering and travelling through porcine lungs, liver, kidney and heart and three human tissue simulants are presented in Table 6-3 and Table 6-4, respectively.

At high entry velocity, the mean percentage of energy lost adjusted for the thickness of tissue penetrated in ascending order (presented as mean ± SEM) was lung (35.1 ± 1.1%), kidney (38.7 ± 1.1%), clear gel at 16°C (40.5 ± 0.5%), 10% ballistics gel at 4°C (45.8 ± 0.5%), liver (45.8 ± 0.6%), heart (47.2 ± 0.6%), and 20% ballistics gel at 10°C (48.0 ± 1.0%).

At medium entry velocity, the mean percentage of energy loss adjusted for the thickness of tissue penetrated in ascending order (presented as mean ± SEM) was kidney (40.8 ± 1.5%), clear gel at 16°C (41.6 ± 0.8%), lung (43.3 ± 0.8%), 10% ballistics gel @4°C (43.6 ± 0.5%), liver (45.1 ± 1.5%), heart (45.7 ± 0.8%), and 20% ballistics gel at 10°C (49.2 ± 1.1%).

Figure 6-1 to Figure 6-4 show visually the effect of a projectile penetrating each tissue sample and simulant at high and medium velocity. Each tissue sample is photographed at 16°C and 37°C, although it has previously been found that no significant differences occur between the temperatures. Back splatter occurs in all organs and simulants, however the simulants produce less back splatter, which is more contained due to the nature of gelatine.

Table 6-2 Summary for the energy lost from the high and medium velocity projectiles entering and travelling through porcine lung, liver, kidney and heart, and three tissue simulants 10% Ballistics gelatine @ 4°C, 20% Ballistics gelatine @10°C and Clear Ballistics gel @16°C

	Organ	*N*	*Tissue Thickness (mm)*	*SD*	*Velocity In (m/s)*	*SD*	*Velocity Out (m/s)*	*SD*	*Energy In (J)*	*SD*	*Energy Out (J)*	*SD*	*Energy Difference (J)*	*SD*	*Energy lost per m of tissue (J/m)*	*SD*	*Energy loss adjusted for Av tissue thickness (J)*	*SD*	*% Energy Loss adjusted for tissue thickness*	*SD*	*Deceleration (ms²)*	*SD*
High Velocity	Lung	20	49.0	7.5	876.7	38.8	708.9	36.4	401.6	35.6	262.7	26.5	138.8	18.8	2887.1	527.2	141.5	25.8	35.1	5.0	2.8e6	5.1e5
	Liver	25	50.9	6.6	877.8	68.6	646.8	50.8	404.2	65.0	219.5	35.4	184.7	36.9	3643.9	634.7	185.3	32.5	45.8	2.9	3.5e6	6.1e5
	Kidney	25	41.3	5.8	889.6	55.5	696.1	60.1	414.3	53.4	254.5	42.5	159.8	35.2	3869.0	645.6	159.6	25.2	38.7	5.4	3.7e6	6.3e5
	Heart	25	56.4	5.9	883.1	63.1	642.6	48.0	408.7	59.0	216.5	32.1	192.3	31.0	3434.0	558.7	193.1	31.7	47.2	3.2	3.3e6	5.4e5
	10% Ballistics Gel @4C	10	54.3	2.9	925.5	81.5	673.1	54.9	449.8	77.5	237.7	38.2	212.1	39.8	3898.9	668.5	205.8	35.3	45.8	1.7	3.7e6	6.4e5
	20% Ballistics Gel @10C	10	54.0	3.8	860.3	44.9	621.6	33.0	386.9	40.0	202.0	21.3	184.9	19.1	3437.9	437.8	185.8	23.7	48.0	3.1	3.3e6	4.2e5
	Clear Gel @16C	10	51.3	2.9	896.2	60.6	691.1	44.1	420.6	56.0	250.0	31.4	170.6	26.4	3328.2	506.6	170.8	26.0	40.5	1.5	3.2e6	4.9e5
Medium Velocity	Lung	10	65.6	7.3	449.1	8.7	338.3	13.5	105.2	4.1	59.8	4.6	45.4	5.2	694.5	62.6	45.6	4.1	43.3	2.5	6.7e5	6.0e4
	Liver	10	54.3	8.0	450.2	13.4	333.0	16.0	105.8	6.4	57.9	5.7	47.8	9.3	876.6	84.6	47.8	6.4	45.1	4.7	8.4e5	8.1e4
	Kidney	10	46.1	5.4	451.1	12.1	347.9	13.5	106.2	5.7	63.2	4.9	43.0	4.5	943.1	148.3	43.5	6.6	40.8	4.6	9.0e5	1.4e5
	Heart	10	55.0	3.9	450.2	15.1	331.9	10.1	105.8	7.0	57.5	3.4	48.3	4.4	880.6	82.5	48.4	4.3	45.7	2.5	8.4e5	7.9e4
	10% Ballistics Gel @4C	5	54.7	1.0	464.2	13.6	345.4	8.6	112.4	6.5	62.2	3.1	50.2	3.8	917.4	60.8	49.0	3.2	43.6	1.2	8.8e5	5.8e4
	20% Ballistics Gel @10C	5	50.8	0.6	428.9	35.7	306.1	27.6	96.4	15.6	49.2	8.6	47.3	7.1	930.7	147.5	47.5	7.5	49.2	1.1	8.9e5	1.4e5
	Clear Gel @16C	5	51.8	3.2	449.8	17.2	343.9	11.9	105.6	8.0	61.7	4.2	43.9	4.6	848.3	89.5	44.0	4.6	41.6	1.7	8.1e5	8.6e5

Table 6-3 Results of tests of statistical significance between the entry velocities of the organs and simulants, high velocity above the diagonal and medium velocity below.

	Lung	*Liver*	*Kidney*	*Heart*	*10% Ballistics gel @4°C*	*20% Ballistics gel @10°C*	*Clear gel @16°C*
Lung		>0.10	>0.10	>0.10	0.78	>0.10	>0.10
Liver	>0.10		>0.10	>0.10	0.73	>0.10	>0.10
Kidney	>0.10	>0.10		>0.10	>0.10	>0.10	>0.10
Heart	>0.10	>0.10	>0.10		>0.10	>0.10	>0.10
10% Ballistics gel @4°C	>0.10	>0.99	>0.10	>0.10		0.34	>0.10
20% Ballistics gel @10°C	0.58	0.43	0.34	0.43	*0.03		>0.10
Clear gel @16°C	>0.10	>0.10	>0.10	>0.10	>0.10	0.99	

**significant given as p<0.05 however applying Bonferroni correction removes significance*

not significant p ≥ 0.05

Table 6-4 Results of tests of statistical significance between the percentages of energy lost from high velocity projectiles entering and travelling through porcine lungs, kidney, liver and heart and the three ballistics simulants.

	Lung	*Liver*	*Kidney*	*Heart*	*10% Ballistics gel @4°C*	*20% Ballistics gel @10°C*	*Clear gel @16°C*
Lung		*<0.00	*0.04	*<0.00	*<0.00	*<0.00	*0.01
Liver			*<0.00	>1.0	>1.0	>1.0	*0.00
Kidney				*<0.00	*<0.00	*<0.00	>1.0
Heart					>1.0	>1.0	*0.00
10% Ballistics gel @4°C						>1.0	0.06
20% Ballistics gel @10°C							*0.00
Clear gel @16°C							

**significant given as p<0.05*

not significant p ≥ 0.05

Table 6-5 Results of tests of statistical significance between the percentages of energy lost from medium velocity projectiles entering and travelling through porcine lungs, kidney, liver and heart and the three ballistics simulants.

	Lung	*Liver*	*Kidney*	*Heart*	*10% Ballistics gel @4°C*	*20% Ballistics gel @10°C*	*Clear gel @16°C*
Lung		>1.0	>1.0	>1.0	>1.0	*0.04	>1.0
Liver			0.13	>1.0	>1.0	0.54	>1.0
Kidney				*0.04	>1.0	*0.00	>1.0
Heart					>1.0	>1.0	0.56
10% Ballistics gel @4°C						0.20	>1.0
20% Ballistics gel @10°C							*0.01
Clear gel @16°C							

**significant given as p<0.05*

not significant p ≥ 0.05

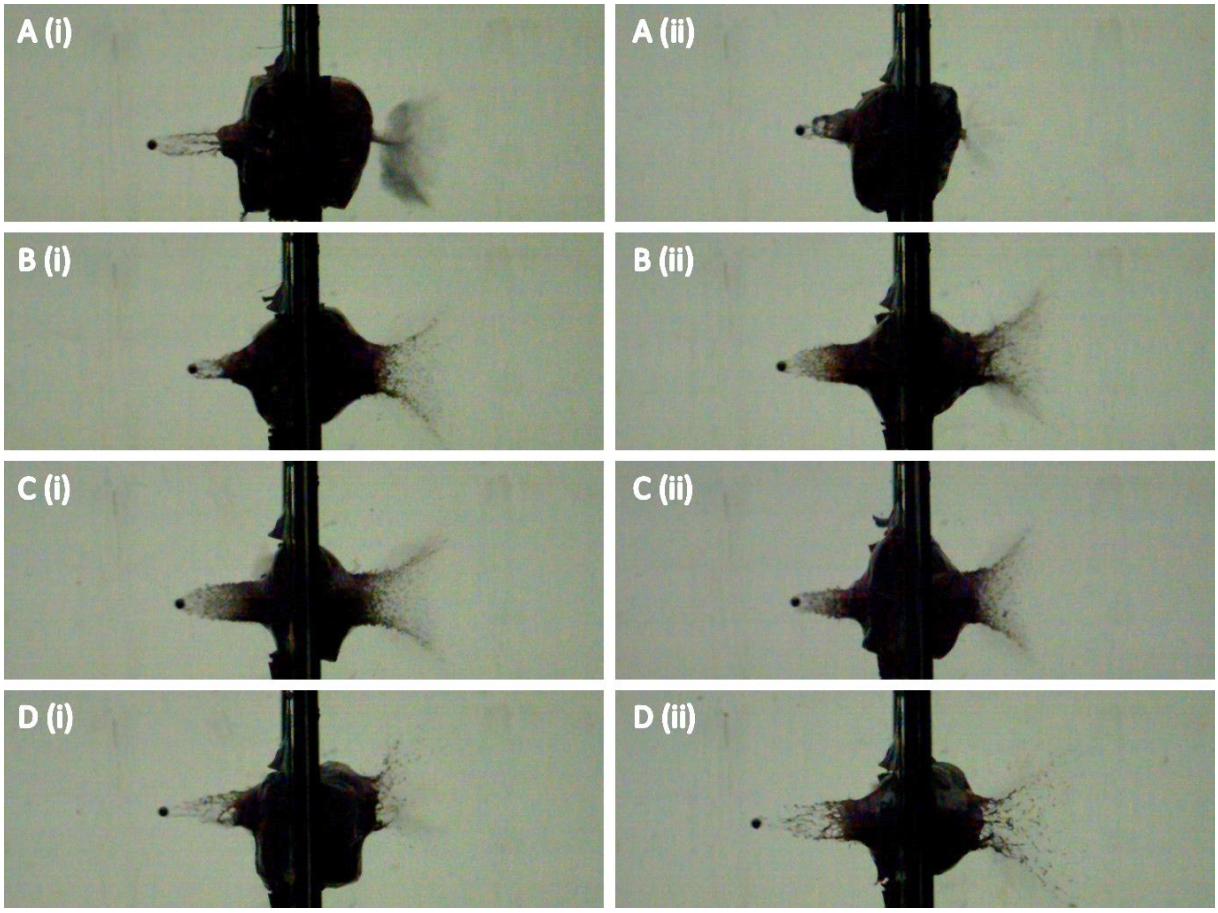

Figure 6-1 High speed still images of medium velocity firings of A (lung), B (liver), C (kidney) and D (heart at 16°C (i) and 37°C (ii)

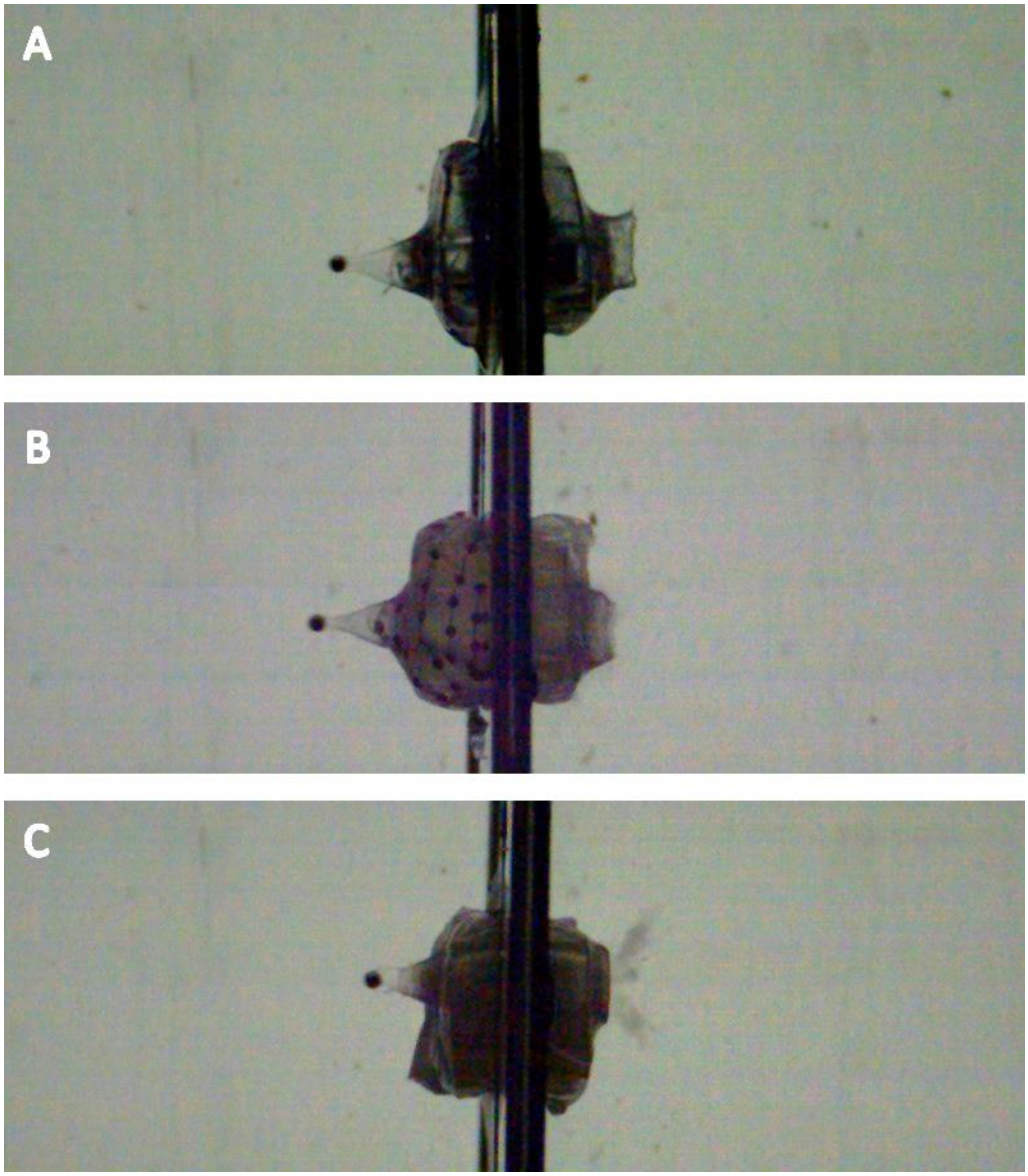

Figure 6-2 High speed still images of medium velocity firings of A (clear gel at 16°C), B (10% ballistics gel), and C (20% ballistics gel)

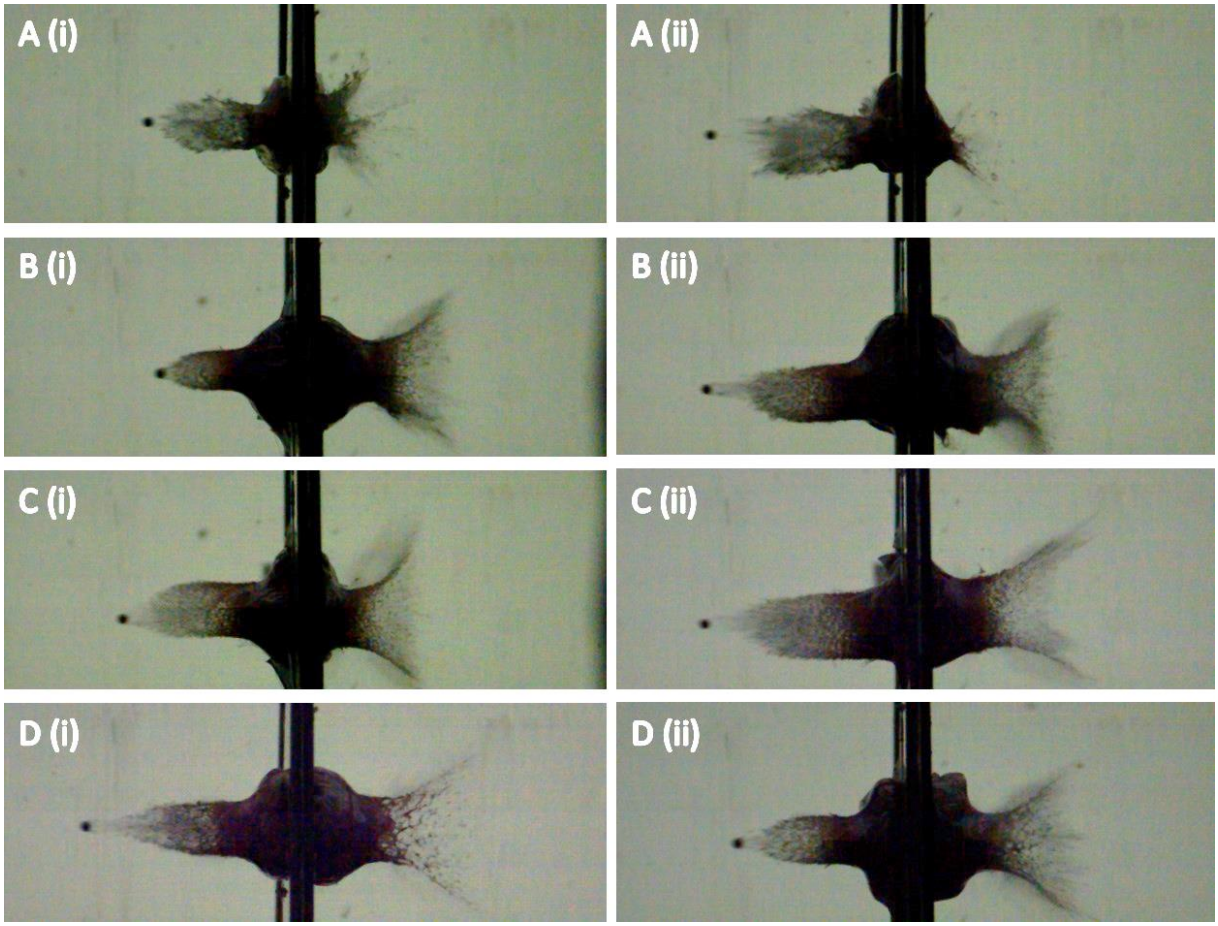

Figure 6-3 High speed still images of high velocity firings of A (lung), B (liver), C (kidney), and D (heart) at 16°C (i) and 37°C (ii)

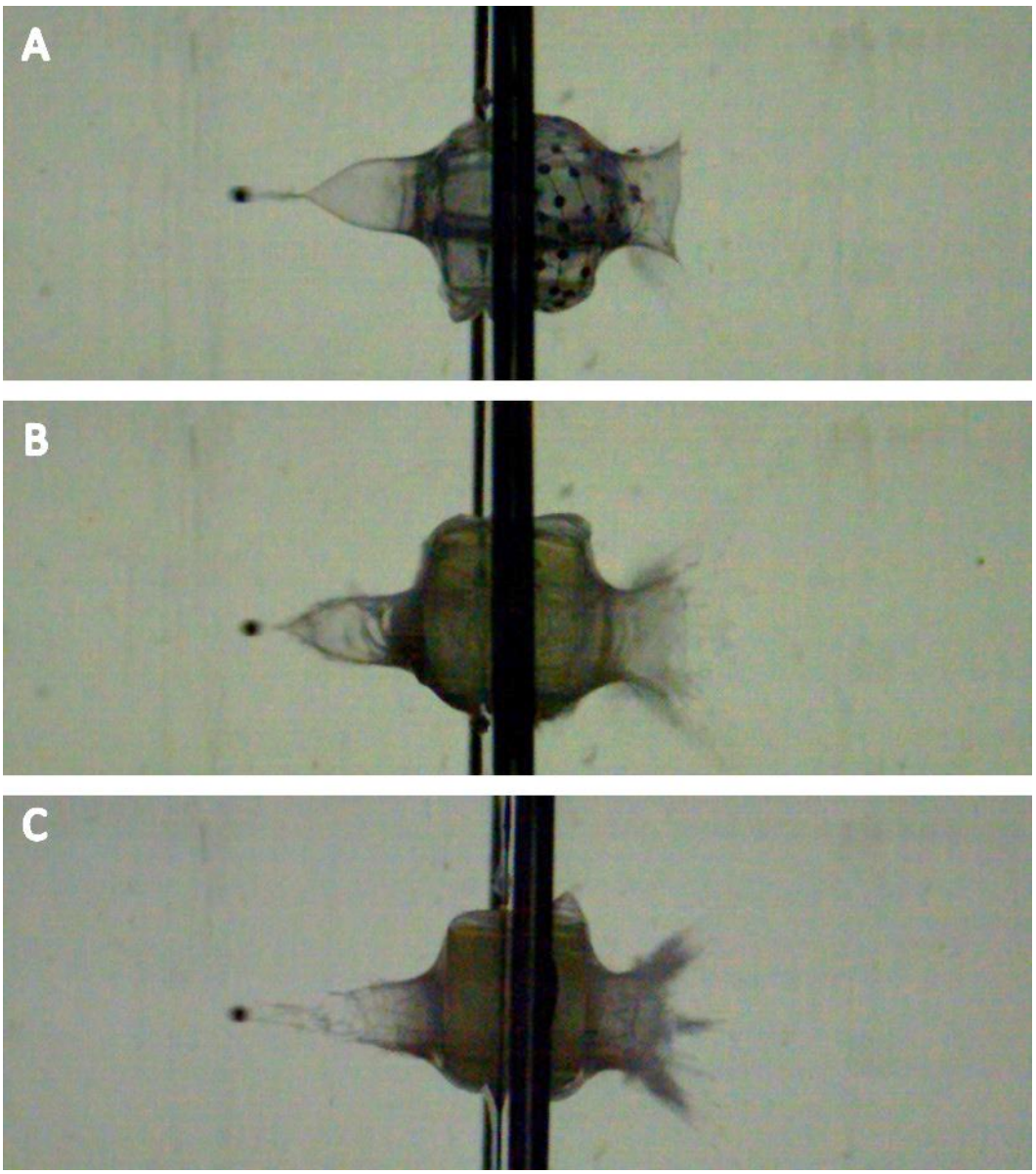

Figure 6-4 High speed still images of high velocity firings of A (clear gel at 16°C), B (10% ballistics gel), and C (20% ballistics gel)

6.7 Discussion

The use of steel spheres (6.35 mm, 1.034 g) as the projectile passing through the tissues, eliminated the effects of tumbling, break up, distortion and fragmenting, allowing consistent and repeatable interactions with the tissues. Furthermore, the use of frangible plastic sabots to encase the steel sphere and ammunition and placing of the tissue target at 10.0 meters from the muzzle of the gun prevented interference of the plastic sabots with the steel projectile or the impaction of the former with the tissue target. Mounting of the gun to a fixed structure and using the same gun to fire the projectiles minimised the variations that could result from the gun between firings.

The variations seen in the entry velocities of the steel spheres (i.e. in both high and medium velocity firings) between firings through each organ/simulant were not significantly different (Table 6-2), indicating that the projectile velocities generated by the ammunition used with each velocity group were consistent between firings. Therefore, the ammunition produced for each group generated reliable and reproducible velocities of the projectile. These findings clearly indicate that in this study, the variables that could have resulted from the gun, ammunition and the physical properties of the projectile were minimised. Therefore, the significant reduction ($p<0.0001$) in the velocity (i.e. deceleration) of the projectiles from the entry to the exit through the tissues/organs or the tissue simulants is likely to be related to the physical properties of the tissues/organs or the tissue simulants investigated. Similarly, the energy lost from the projectile from the entry to the exit through the tissue/organs or tissue simulant is most likely to be related to their physical properties.

The tissues/organs (lung, heart, liver and kidney) investigated in this study consist of tissue parenchyma (i.e. specific functional tissue cells) supported by connective tissue matrix elements (i.e. including connective tissue cells). The entry velocity of the projectiles into the

tissue samples or the tissue simulants was not significantly different among samples. The velocity of the projectiles reduced significantly during their passage from the entry to exit through the tissues or the tissue simulants ($p<0.0001$). As energy is related to the velocity, the energy would also reduce significantly. These findings indicate that the approximate 50 mm blocks of tissues/tissue simulants used in the study were of adequate thickness to reduce the velocity and the energy from projectiles in significant amounts during their passage through the tissues/tissue simulants. The reduction in the velocity or the loss of energy from the projectiles could have resulted primarily from the resistance offered by the connective tissue matrices of the tissues.

The percentage energy loss from high velocity projectiles entering the porcine lungs was significantly lower than the porcine livers, kidneys, heart and the 3 tissue simulants tested (Table 6-2; Table 6-4; Table 6-5). Therefore, a new tissue simulant needs to be developed to simulate the loss in energy of lungs. The 10% ballistic gelatine at 4°C and 20% ballistics gelatine at 10°C have been found to be suitable soft tissue simulants, when calibrated. Penetration tests conducted on porcine tissue have revealed that the above tissue simulants replicate the mechanical properties of skin, fat, fascia and muscles of a porcine thigh [2]. Physical properties of porcine tissues have been claimed to be similar to human tissues, thus the above ballistic simulants may simulate human tissues/organs [17-23].

The percentage energy loss from the high velocity projectiles entering both porcine liver and heart were not different from those entering 10% ballistic gel at 4°C and 20% ballistic gel at 10°C, while those entering kidney was similar to those entering clear gel at 16°C. Furthermore, the percentage energy lost from the high velocity projectiles entering porcine liver was similar to those entering heart (Table 6-4).

The percentage energy loss from medium velocity projectiles entering the porcine lungs was similar to those entering liver, kidney, heart, 10% ballistic gel at 4°C and clear gel at 16°C (Table 6-5). In a study conducted previously using low velocity projectiles, lungs behaved similar to the heart and the 10% ballistics gel formulation [16]. Similarly, in the current study, the liver was not different from kidney, heart, 10% ballistic gel at 4°C, 20% ballistic gel at 10°C and clear gel at 16°C in relation to the percentage energy loss from medium velocity projectiles entering the respective tissues and tissue simulants. In the same context kidney was different from heart and 20% ballistic gel at 10°C; while kidney and heart were similar to 10% ballistic gel at 4°C and clear gel at 16°C; and 10% ballistic gel at 4°C, 20% ballistic gel at 10°C and clear gel at 16°C respectively (Table 6-5).

The reasons for the differences in the behaviour seen in relation to the percentage of energy loss and the reduction in velocity from high and medium velocity projectiles entering the test tissues and tissue simulants are not clear. The elements that could reduce the velocity of a projectile penetrating through a tissue are fibrous connective tissue elements and gelatinous intracellular matrix components. The calculated percentage energy losses have been adjusted to the tissue thicknesses, thus the effects of fibrous connective tissue elements and gelatinous intracellular matrix components in tissues tested in the two velocity groups should be similar. The only difference is that the projectile tissue interaction is longer at a medium velocity.

The result that the 10% ballistics gel at 4°C and the 20% ballistics gel at 10°C did not differ significantly from each other at both high and medium entry velocities, differs from what has been previously found [16]. A lengthened curing time may have altered the density of the 10% ballistics gelatine to be closer to that of the 20% ballistics gel, thus leading to no significant differences, however, 20% gelatine and 10°C and 10% gelatine at 4°C are expected to be similar when prepared and stored correctly. Different curing times have been found to affect penetration ability of projectiles [24].

Visually, a difference could be seen between the organs and simulants at both medium and high velocity Figures 6; 1-4). The simulants absorb the energy from the projectile, expand and then contract back to their original shape. The organs contain some viscoelastic properties (particularly the lungs), however the expanding pressure wave when the projectile penetrates the tissue exceeds the natural elastic properties of the organs, thus they overstretch and fail. Splatter from the organs projects forwards and backwards and the degree of splatter depends on the organ and velocity. A greater degree of splatter was seen in higher velocity testings and for the liver, kidney and heart. As the lung is the most elastic out of these four organs, it produced the least splatter. These splatter patterns can vary with organ and projectile impact velocity, thus visually it would be difficult to distinguish organs based on splatters.

6.8 Conclusion

This research has established a basis for future research into whether or not the currently used ballistics ordnance gelatine and synthetic simulants can accurately represent the critical organs of the body at two different entry velocities. This is important as the conclusions drawn from studies using tissue simulants may differ significantly from what occurs in different human tissues, and at different velocities of projectiles.

As the results suggest, these types of simulants may be able to represent the different organs. Future testing could be conducted that uses a different percentage of gelatine mixture i.e. making it denser or less dense, the energy absorption behaviour of projectiles may be able to more accurately match one of the organs. This may lead to the use of these gelatine formulations in anatomical modelling for the purposes of penetrating ballistics testing. The new gelatine formulations would need to reflect the differences in energy loss through the organs at the different velocities to be accurate.

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Part THREE

Part III investigates skeletal trauma from firearms. The three chapters presented in this part describe osteological and anthropological examinations of the trauma produced from ballistic wounds. Two skeletal collections located at museums in the United States of America with documented gunshot trauma were analysed.

Chapter 7

7 Reconstructing the life and manner of death from skeletal remains of a case of a Hispanic man who died of gunshot wound in 1962, St Louis, Missouri

Caitlin Humphrey, Jaliya Kumaratilake and Maciej Henneberg

7.1 Statement of Authorship

Manuscript Details

Title of Paper	Reconstructing the life and manner of death from skeletal remains in a case of a Hispanic man who died of gunshot wound in 1962, St Louis, Missouri
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Journal of Anthropology

Principal Author

Name of Principal Author (Candidate)	Caitlin Humphrey
Contribution to the Paper	Travel, data collection, anatomical analysis and interpretation, photography, writing and editing manuscript, manuscript revisions
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 13/9/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	**Maciej Henneberg**
Contribution to the Paper	Anatomical analysis and interpretation; manuscript writing and editing, manuscript revisions
Signature	Date: 13.09.17
Name of Co-Author	**Jaliya Kumáratilake**
Contribution to the Paper	Anatomical analysis, manuscript writing and editing
Signature	Date: 13.09.2017

7.2 Context

One major challenge forensic anthropologists face is the need to reconstruct the manner of death of an individual after the analysis of the injuries of the body. In firearm trauma, the cause of death could be determined according to the organ or the region of the body affected by the bullet(s). Determination of the manner of death (i.e. suicide, homicide, or accident) often requires detailed meticulous analysis of the skeletal materials available. Such details are critical when designing bone simulant material which is required to act like fresh live bone. This chapter documents a case from the Smithsonian Institution in Washington, D.C., United States of America.

7.3 Abstract

Bio-archaeological methods can be used to study skeletons of persons who died from gunshot wounds. We present a 55-year-old case where only a skeleton is available for examination, in order to illustrate the amount of information that can be obtained from a skeletal analysis, which may be helpful in archaeological cases of gunshot wounds. The entire skeleton of a Hispanic male aged 30 years, died in 1962, from the Terry Skeletal Collection, was analysed due to the presence of a gunshot wound to the skull. A small circular entry wound was located in the right pterion and a small circular exit wound on the left pterion. There was damage to the skull base and radiating fractures in the skull vault. This indicates the possibility that a small calibre, high energy projectile, fired from a relatively short distance between the muzzle of the weapon to the skull, entered the skull at an angle of 10 degrees upwards from the horizontal plane. Long bones were robust indicating an athletic body build. Gold dental fillings and very tall stature (~2.5SD above mean) suggest this individual came from a wealthy environment. The most likely manner of death was suicide.

7.4 Introduction

Forensic anthropology has developed a large number of methods to reconstruct the life of an individual from their skeleton including lifestyle, pathologies and manner of death. In forensic investigations of crime where a skeleton is present, a forensic anthropologist is expected to reconstruct the biological profile of the deceased (i.e. sex, age at death, body build, stature and any identifying characteristics of the individual), while establishing the cause of death is the role a forensic pathologist. However, a forensic anthropologist can assist in interpreting the skeletal signs which may lead to identifying a possible cause of death. When, however, a skeleton of a person who died many years ago is found and is not related to any active criminal investigations, it is difficult to expect the involvement of a forensic pathologist. The case may be of historical, rather than judicial, interest and in this situation forensic anthropologists may attempt to determine the cause of death from the signs on the skeleton. Biological anthropologists have good knowledge of paleopathology including signs of various infectious and degenerative diseases on the skeleton. Working often with archaeological or historical samples, they however, have less opportunity to study bullet wound skeletal trauma. Ballistics is a well-developed discipline often used in forensic situations. Most often ballistic analysis applies to victims of recently inflicted wounds allowing analysis of all tissues of the body. Less often skeletal remains that bear signs of destruction by a firearm projectile become an object of forensic interest.

Wound ballistics is an aspect of terminal ballistics, which studies the effects of projectiles on living tissues [1-4]. The mechanisms by which a bullet creates a wound are determined by both the properties of the bullet (e.g. shape and construction), and the characteristics of the tissues (e.g. elasticity and density). Although in a case of skeletal remains only destruction of bone tissue can be directly observed, with the knowledge of all aspects of terminal ballistics, it is

possible to deduct effects of a projectile on soft tissues of the victim. This creates a possibility of interpreting whether the observed trauma was a direct cause of death or not. Estimations of the size of the projectile, of its velocity at entry and exit and the direction of its path allow suggestions to be made concerning the type of weapon used and distance and direction from which a projectile was fired [5]. Such studies of skeletal remains are useful in older cases, archaeological remains and old war crimes, historical personalities and mass graves [6-8]. During a survey of the Terry Collection at the National Museum of Natural History of the Smithsonian Institution in Washington D.C. a skeleton whose skull had a gunshot wound was encountered. The death occurred 55 years ago (1962) and only routine information about sex, age, ancestry and the fact that it had traces of a gunshot wound were recorded. No information on the specific cause and manner of death nor of his origins and lifestyle was available. Thus, we have conducted an anthropological forensic analysis of the skeleton to obtain as much information about this individual as methods of this analysis will allow. This produces an example of information that can be obtained from a skeleton. This paper presents a case of an osteological analysis of the skeleton with a gunshot wound to the skull, 55 years after death.

7.5 Methods

Records from the Smithsonian indicate that individual 1569 was a Hispanic male, aged 30 years, who died in 1962. The cause of his death was recorded as a gunshot wound to the head without further explanation. The entire skeleton of individual 1569 was studied by macroscopic observation and osteometric measurements. Since the skull cap was removed at autopsy, there was a horizontal cut around the braincase. The exit wound caused by the projectile was trans-sected by the cut that resulted in small fragments of the bone surrounding the wound to be missing. These fragments were reconstructed using plasticine prior to the analysis. The gunshot wounds were assessed using knowledge of osteology, ballistics and anatomy. Basic

osteometric dimensions of the postcranial skeleton were measured. Since the sex and age at death were known, there was no need to apply anthropometric methods of their estimation beyond cursory observations confirming correctness of the records.

7.6 Results

7.6.1 The Skull

7.6.1.1 Right Side – Point of Entry

The projectile entered the greater wing of the right sphenoid bone at pterion, producing a circular wound with a diameter of 6 mm (Figure 7-1). Only radiating fractures appeared leading away from the entrance wound, no concentric fractures present.

Fractures. Fracture one (red, Figures 7; 1-3) runs supero-posteriorly, obliquely away from the wound, passing approximately 10mm above the squamous suture. It continues into the parietal bone towards the midline. At the sagittal suture the fracture turns posteriorly and travels vertically close to the midline towards the lambdoid suture, deviating obliquely to the left and fading.

Fracture two (blue) runs supero-inferiorly on the greater wing of the sphenoid almost parallel to the posterior aspect of the zygomatic bone. It changes direction to travel horizontally posteriorly to the squamous part of the temporal bone and continues just above the mastoid into the occipital bone, where it terminates (Figure 7-1).

Fracture three (green) travels anteriorly, horizontally towards the lateral most part of supraorbital torus then turns vertically running parallel to the supraorbital torus of the frontal bone, then ascends the frontal squama and eventually sharply deviates posteriorly to run

obliquely across the parietal bone to join fracture one (red) in the middle of the parietal bone (Figure 7-1 and Figure 7-2).

Fracture four (yellow) occurs in the middle of the zygomatic arch and runs vertically (Figure 7-1).

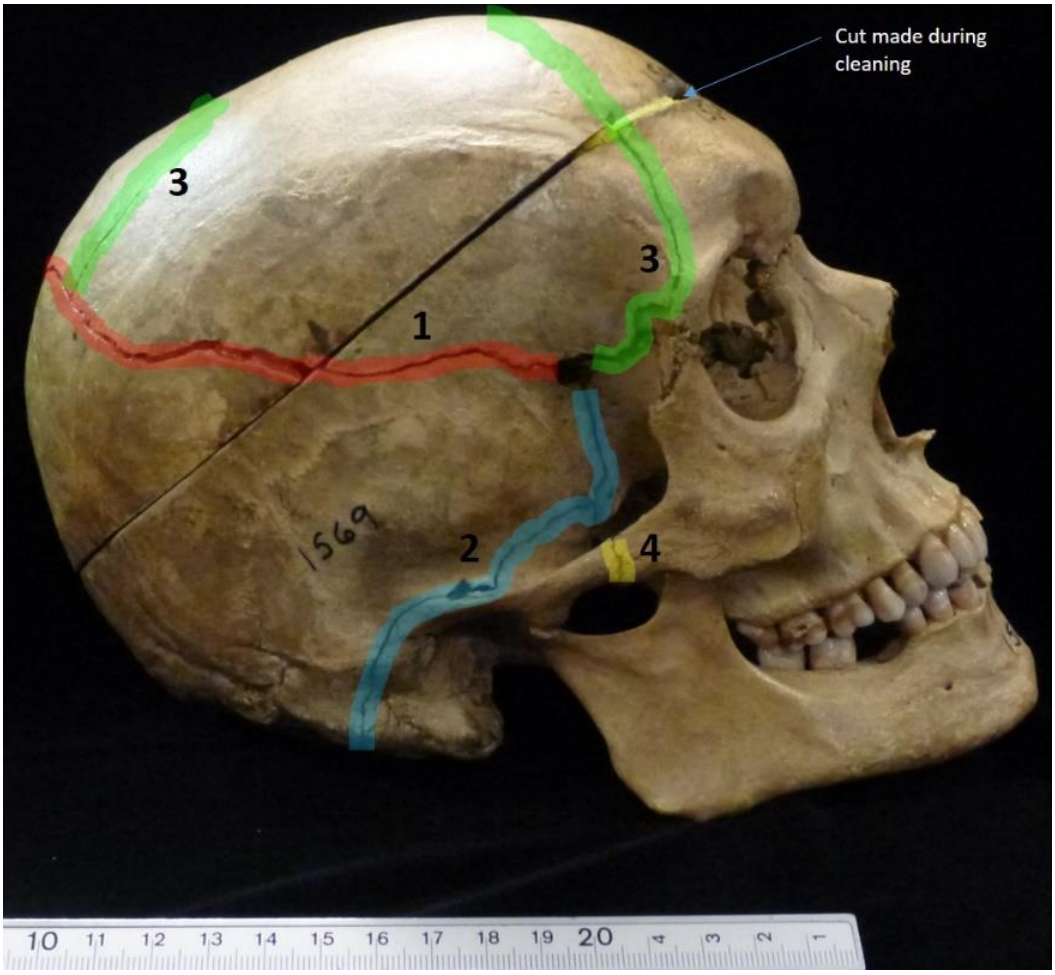

Figure 7-1 Entry wound located in right pterion. Four fractures labelled

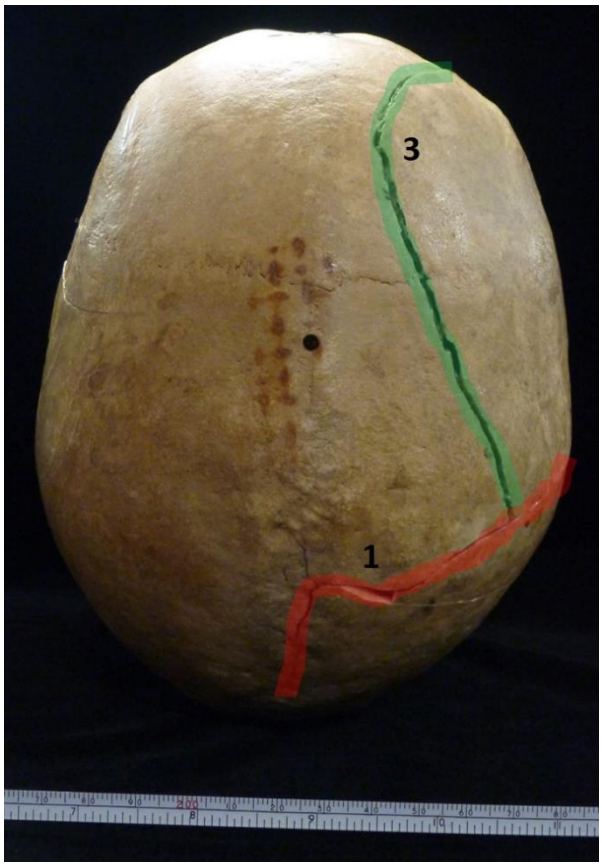

Figure 7-2 Fractures one and three as seen from above

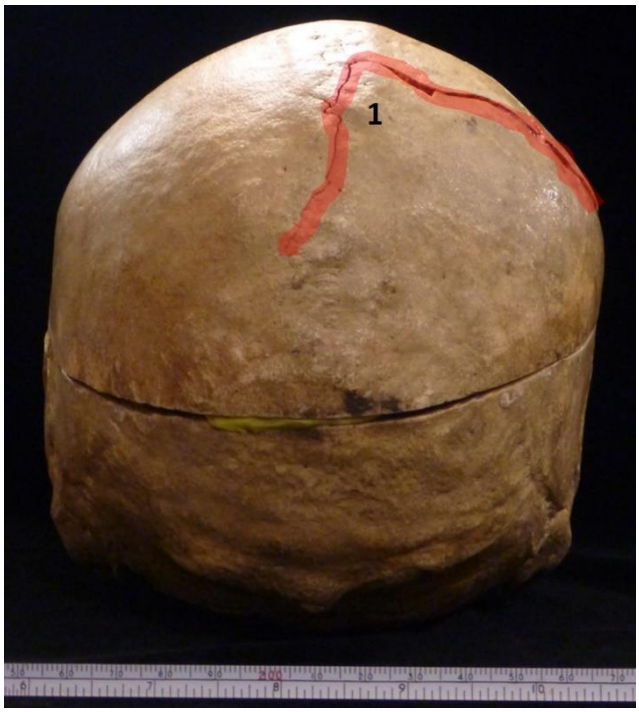

Figure 7-3 Fracture one as seen from rear

7.6.1.2 Left Side of Specimen – Point of Exit

A large oval exit wound (41.1 x 22.2 mm) appears on the left pterion. The damage caused by the projectile is not a clear small circular wound, as with the entry wound. There are missing parts of bone from the superior part of the greater wing of the sphenoid bone and the squamous part of the temporal bone, anterior to the exit wound (Figure 7-4). Pronounced external bevelling is also seen (Figure 7-5).

Reconstruction of the exit wound area, allowed for the measurement of 9 mm of a small circular exit wound. There is also the possibility that the temporal squamous is chipped, and bone pieces are missing (Figure 7-4). Post-mortem autopsy cut intersects the exit wound area and is a possible cause of missing fragments of bone. It is unknown if the autopsy cut affected the appearance of the exit wound.

Fractures. Fracture five (dark blue) (Figure 7-4) extends upwards from the superior margin of the exit wound on the squamous part of the temporal bone, crosses the squamous suture and extends to mid parietal bone where it terminates.

Fracture six (pink) (Figure 7-4) travels in a curved path around the superior and posterior margin of the exit wound. It runs parallel to the superior posterior margin of the wound and crosses fracture five running anteriorly into the orbit through the zygomatic process of the frontal bone, across the supra-orbital margin and into the orbit (medial to the frontal process of the zygomatic bone).

Fracture seven (purple) (Figure 7-4) commences from fracture six at the margin of the zygomatic process of the frontal bone. It runs posteriorly obliquely downwards to the root of the zygomatic process where it continues along the temporal line. It ceases at the squamous suture. Fractures six and seven surround the exit wound.

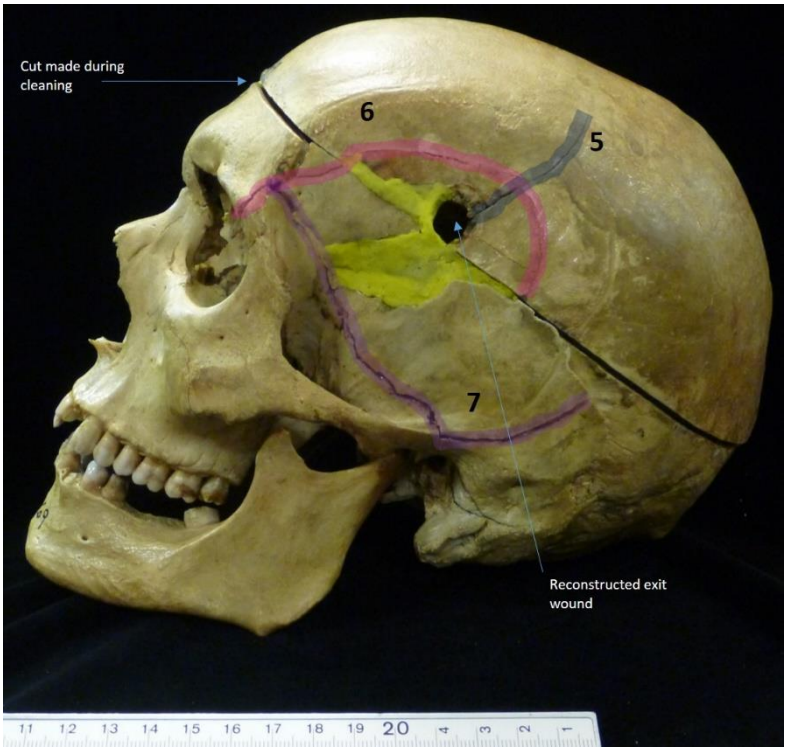

Figure 7-4 Left view of skull with exit wound and labelled fractures. Missing bone pieces reconstructed and plasticine (yellow), exit wound visible.

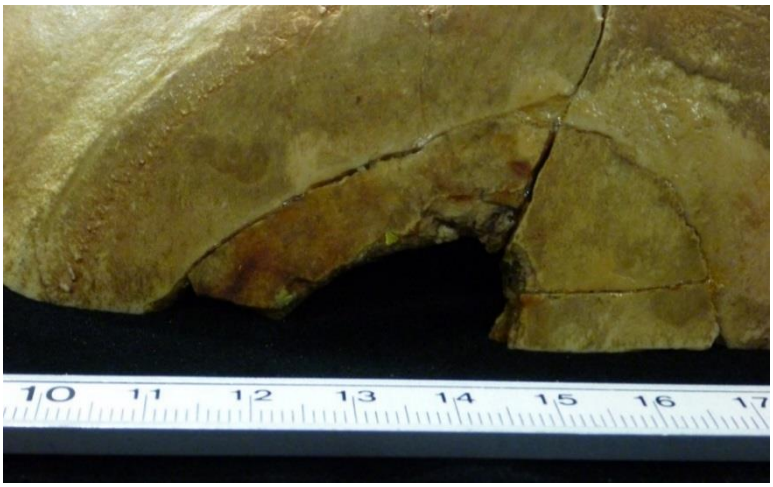

Figure 7-5 Close view of exit wound, not reconstructed, showing external bevelling

7.6.1.3 Frontal View

From the frontal view of the specimen, the fronto-nasal and maxillo-frontal sutures appear to be traumatically separated (fracture eight; orange). The fracture runs from the left to the right orbit. A small fragment of the frontal bone is missing from the specimen's left orbit and a small fracture is extending superiorly from the orbit approximately to the midline where it bifurcates and ceases (fracture nine; white) (Figure 7-6).

7.6.1.4 Projectile Path

The path of the projectile runs obliquely, slightly upwards, through the inferior part of the right anterior cranial fossa towards the left of the anterior cranial fossa (Figure 7-6). This wound can be described as a through and through skull bullet wound [9, 10].

Figure 7-6 Frontal view showing projectile path, fracture one, eight and separation of sutures

7.6.1.5 Internal View of Skull

The appearance of the interior of the skull shows that the medial part of the anterior cranial fossa floor is missing from the mid lesser wing of the sphenoid bone to the crista galli. Most of the left anterior cranial fossa floor is also missing. The medial part of the anterior cranial fossa up to the lesser wing of the sphenoid bone is remaining. The horizontal (cribriform) plate of the ethmoid bone has been lost. The fractures described above are visible in the interior of the skull (Figure 7-7 and Figure 7-8).

Figure 7-7 Internal view of skull

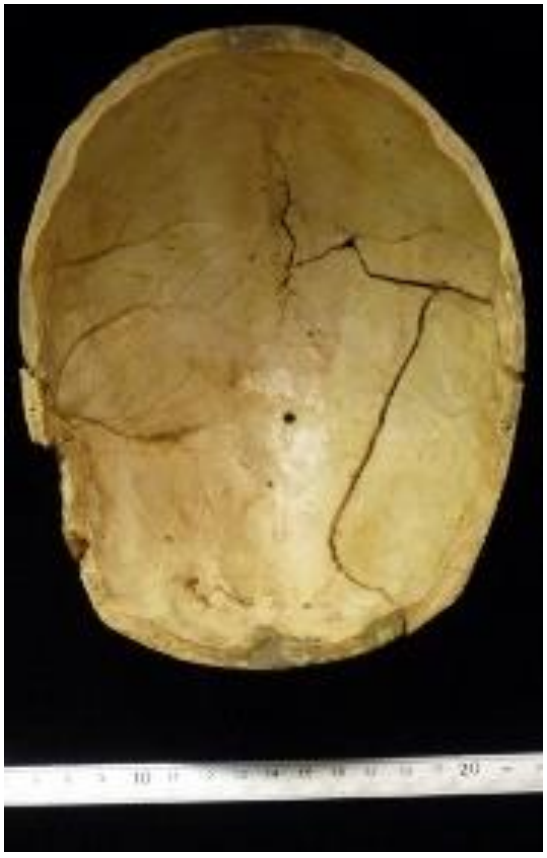

Figure 7-8 Inside of skull cap

7.6.2 Teeth

All maxillary teeth are present bar the left lateral incisor that was lost post mortem (Figure 7-9). The central left incisor appears broken possibly post mortem. Morphology of the anterior teeth up to the second premolar is normal. First right molar has a class one amalgam restoration on its occlusal surface. The second molar has a class II gold restoration on the occlusal and distal surfaces. Third right molar has a class II porcelain restoration on the distal surface. First left molar has a class I gold restoration on the occlusal surface. Second molar has normal morphology. Third molar has a small class I amalgam restoration.

The right and left mandibular central incisors are broken most likely post mortem (Figure 7-10). The right lateral incisor is missing post mortem, the right canine is broken, possibly post mortem. Both first pre-molars have normal morphology. Both second pre-molars have been subject to restorations. The left with amalgam and the right has the filling material missing. Both first molars are missing ante mortem, probably extracted since there is no evidence of inflammation. The second left molar was also lost, probably extracted. While the right second molar was prepared for class II restoration most of the filling substance is missing. Both third molars have class I restorations, right with gold, left with amalgam [11].

Figure 7-9 Maxillary teeth

Figure 7-10 Mandibular teeth

7.6.3 Long Bones

The humeri of the individual varied in length and circumferences. The right humerus was shorter (359mm) and had a larger circumference (84mm) than the left (374mm; 70mm). Otherwise, morphology was normal (Figure 7-11).

Figure 7-11 Humeri of individual showing differences in total length and thickness

7.6.4 Stature

Table 7-1 Reconstruction of stature using Trotter and Gleser regression equations [36-38], methods for both white and Mexican male, using right and left limb measurements for each long bone

	Humerus		*Radius*		*Ulna*		*Femur*		*Tibia*		*Fibula*	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Max Length (cm)	36.0	37.4	28.3	28.1	30.0	29.7	52.8	53.1	44.7	44.4	42.8	43.0
Stature by White Male equation	182.1	186.2	186.7	185.9	188.4	187.2	188.0	188.7	190.1	189.4	186.7	187.3
Stature by Mexican Male Equation	179.0	183.1	181.2	180.5	181.4	180.3	187.5	188.2	186.1	185.4	182.4	182.9

7.6.5 Other Findings

No arthritic evidence occurs in the pelvis. The sacrum appears normal. Calcaneus is large in both feet. No gross differences in size of hands. Spinous processes of lumbar vertebrae have mostly normal morphology. Slight Schmorl's nodes on the upper surfaces of 4th lumbar and 11 thoracic vertebral bodies. Serious pitting on the inferior body of L5 and corresponding superior S1 (sacrum) (Figure 7-12).

Figure 7-12 Lumbar 5 vertebrae showing pitting

7.7 Discussion

The skull injury described can be defined as a perforating wound as it enters and exits the skull. This is in contrast to a penetrating wound where the bullet enters, but does not exit [9, 10]. Penetrating wounds are often caused by low impact velocity projectiles whereas perforating wounds are typically produced by a higher velocity projectile [10] Since the bullet exited the skull in this case, it is assumed that it initially had a high velocity due to the small size and circular punched-out appearance of the entry wound. The anatomical region in which the bullet entered the skull, the pterion area and temporal region, has been described as the most common site for suicidal gunshot entrance wounds. This can also occur in homicide shootings; however, it is less likely [12-18]. The anatomical location in combination with the right to left, upwards directionality of the projectile indicates that suicide is the most likely manner of death.

7.7.1 Bevelling

The appearance of a clean, circular, punched out appearance of the wound on the right pterion region suggests this to be the entry wound of the bullet as is seen with most entry wounds [19, 20]. Exit wounds tend to be larger and of an irregular shape. Although it is not conclusive that the exit of a bullet leaves a larger wound, the appearance of the suspected exit includes external bevelling. Although bevelling is not always present in exit wounds, if it is there it indicates the wound is the exit [2, 19, 20]. It is more obvious in thicker bone, such the parietal bone, where a funnel-shaped wound tract is possible [21]. There is also the possibility that the increased pressure inside the brain case forces a backward pressure through the entry wound. This will also occur due to the temporary cavity and may contribute to external bevelling, along with the angle of the shot, the twisting force of the rotating bullet, blow-back effect, the velocity, shape and size of the bullet and the resistance of the skull [20, 22].

7.7.2 Trajectory

The intracranial trajectory of the bullet from right-to-left, front-to-back and upward in the horizontal plane has been found to be typical of right handed individuals [16], along with being an indicator of suicide [13, 23]. In this case, the anatomical site of bullet entrance in combination with the projectile path indicates that this is more likely to be a case of suicide, than a homicide. Homicide cases occur in a dynamic situation where the motion of those involved, the victims' resistance and distances create a projectile path that varies from the typical suicidal projectile path, even if the entrance wound is in the same anatomical location [14]. Observations of the skull interior and knowledge of cranial anatomy indicate that the bullet produced limited tissue damage to areas that cause immediate incapacitation, since it passed through frontal lobes and thus it was possible for the individual to survive for a short period after being shot. This has been found to occur in other cases [24, 25]. Without radiological testings of the soft tissues, it is difficult, if not virtually impossible, to conclude the exact effects the bullet had on the soft tissues.

The brain has lesser hardness, density and strength than bone; therefore, it is easier penetrated by a bullet. The bullet would keep a substantial amount of the energy while passing through the brain, in comparison to losing energy when penetrating bone [9, 10]. The bullet penetrated a thin layer of bone at the pterion, lost some energy and then passed easily through soft brain tissue exiting on the other side of the skull. As a bullet penetrates the cranial cavity filled with inelastic cerebro-spinal fluid and brain tissues, a sudden increase in intracranial pressure occurs, causing compressive forces against the brain case [9, 26]. This can contribute to secondary fractures [26, 27], especially those of the fronto-nasal and fronto-maxillary sutures independent from whether they are in contact with the point of entry and/or exit (i.e. sutures separate due to increased cranial pressure). The first fractures to occur are those referred to

as radiating fractures, spreading away from the wound. Secondly, concentric fractures occur, which span between the radiating fractures, as appears in the exit wound of this case. Only radiating fractures appear on the entrance wound, while concentric and radiating fractures occur on the exit wound. If the intra-cranial pressure exceeds the ability of the skull to withstand it, indirect fractures of the vault at sites away from the projectile path may occur [9]. In this skull, linear fractures of the thin orbital plates occurred due to this mechanism of increased cranial pressure [10]. Fractures cross the regions of less resistance and often follow suture lines rather than crossing them. If the force is great, the fractures travel longer distances. Fractures often terminate at previously made fractures and this can aid in sequencing the fractures [28].

7.7.3 Type of Weapon

An entry wound with a minimum diameter of 6mm would suggest that the calibre of the projectile used can be no larger than 6mm or 0.243 inches. The range of calibres that are equal to or less than 6mm is inclusive of rifles and hand guns. Shotguns can be ruled out due to the distinctive trauma associated with the projectiles, along with deforming and fragmenting bullets due to the extent of damage associated with those types of bullets. De la Grandmaison, Fermanian [29], have determined that with suicide from long barrelled weapons the site of entry and its associated wounding is limited due to the ability to reach the trigger. The most common sites are therefore the mouth, chest and front of the head. Based on this, a rifle was most probably not used to inflict the presented trauma. One previous researcher has determined a relationship between the entrance size and the calibre of the weapon [30]. In comparisons between calibre and cranial entrance defects from 73 autopsy specimens, it was found that the strongest relationship was between calibre and minimum diameter of the entrance defect. In this study the minimum diameter for the entrance wound was 6mm, which

would indicate a projectile within the range of small calibre, similar to a .22. however, difficult to distinguish wounds caused by .22-calibre and .25-calibre [30]. Variations in the wound size may result from the "*bullet shape, surface treatment, strength characteristics, loss of gyroscopic stability, intermediate targets and tangential impacts*" [31] p1). A large variety of calibres and variations in their manufacturing make it difficult to conclude on an exact calibre.

Due to the extensive radiating fractures, it could be suggested that the initial energy of the bullet was high. High kinetic energy with a small clean circular entry wound may occur if the firearm is discharged in direct contact with the individual's head.

7.7.4 Handedness

It has been found that a statistically significant correlation exists between the site of the entrance wound and the handedness of the individual [16]. A gunshot entrance wound in the right temporal region is typical of a right-handed individual and vice versa for a left-handed individual. Although not common, it has been observed that a right handed individual shot themselves in the left temple and vice versa [16]. By this classification, the individual studied here was right handed with a gunshot wound to the right temple region.

The humeri of this individual are of different lengths and circumferences. The right humerus is shorter, however has a greater circumference. A greater circumference suggests a greater use of that arm. This asymmetry of the humeri is unusual because in right handed people the right humerus is both longer and thicker [32, 33]. It is possible that this individual was left handed as a child, which affected the length of the bone during the growth period. As an adult he however, was forced by some circumstances to use the right arm more often. This may have occurred as his trade/job likely forced him to operate a tool or control with his right hand, without being given a choice of which hand to choose, for example a steam locomotive operator, a soldier or driving of a manual transmission automobile.

7.7.5 Stature

We used equations constructed for American Males born in the mid-20th Century by Trotter and Gleeser, since these males are the closest available match for the date of birth and living circumstances available to the man in question. Using regression equations, a reconstructed living stature minimum of 179.03±4.20 cm was found using the right humerus in a Hispanic equation and a maximum of 190.10±4.00 cm using the right tibia and the White regression equation (Table 7-1), while the Mexican equation gave the maximum of 188.2cm for the femur. It can be assumed for purposes of further discussion that the man in question was approximately 185 cm tall.

Mexican Americans aged 20-39 years have been measured to have a height of 169.7 cm (SEM 0.4). Non-Hispanic white males aged 20-39 years have also been measured to have a mean height of 178.2 (SEM 0.2) [34]. Others have reported mean stature of Mexican-American men to be 166.9, SD=6.3 cm, with a maximum height of 188.3cm [35]. The man in question is most likely to have stature some 2.4 to 2.9 standard deviations above the Hispanic mean. He was therefore unusually tall – fitting around 95 % of the stature distribution. This suggests that, this individual grew up in a good environment with a good diet and good health care.

7.7.6 Teeth

Dental care in the United States of America in 1950s was expensive and thus, the individual in question should either have significant financial resources or access through an employer.

From the appearance of this individual's teeth, he attended dentists regularly though at various times during his life because his dentition has many signs of extractions or restorations with the use of different materials including gold. There are no carious lesions left open, and even the smallest of cavities have been restored. This suggests that this individual was of a

meticulous character and substantial wealth or belonged to an organisation in which dental health care was provided.

7.8 Conclusion

This Hispanic individual had access to substantial resources, he was meticulously aware of his dental health. His body was large and robust. His activities put significant physical strain on his upper limbs as evidenced by asymmetry of the humeri. The location of the trauma and the path of the projectile is classic for a suicide by a right-handed individual. Within the brain, a temporary cavity and pressure wave would occur and cause damage to the cellular structure. The temporary cavity could also contribute to the extensive fractures related to both the entry and exit wounds. With only a skeletonised skull, the assessment of the brain injury is difficult and only a rough estimate of the anatomical regions that were affected can occur. This suggests that with the damage limited to frontal lobes the individual may not have lost consciousness immediately, but the injury could have been sufficiently fatal due to haemorrhaging. This case study has demonstrated how biological anthropology can help to add information about an individual's identity, reconstruct the events surrounding the death and suggest a conclusion on the manner of death. Although atypical findings do occur in gunshot cases, the probability of the manner of death being suicide is higher than a homicide based on the characteristics of the skeletal wound.

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Chapter 8

8 Variability of characteristics of cranial trauma in skeletal material

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8.1 Statement of Authorship

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 18/9/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Maciej Henneberg
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Contribution to the Paper	Anatomical analysis, manuscript writing and editing
Signature	Date 18.09.2017

8.2 Context

This chapter describes thirteen cases of documented cranial trauma from the Cleveland Museum of Natural History, Ohio, United States of America, in particular the Hammann-Todd Osteological Skeletal Collection. Some of the documentations of the cases indicated the manner of death as homicide or suicide, but for the remaining the manner of death was not known. Analysis of the skulls with projectile trauma from a forensic skeletal collection led to the understanding of the types and characteristics of wounds that could result from projectiles.

8.3 Abstract

In cranial firearm trauma, where soft tissues have decomposed, determining the manner of death can pose difficultly for a forensic anthropologist. Detailed analysis of skeletal wounds may lead to reconstruction of the events surrounding death, however considerable research has been conducted on soft tissue details and less has focused on skeletal wounds alone. Macroscopic descriptive observations were carried out on thirteen skulls from the Hamman-Todd Human Osteological Collection at the Cleveland Museum of Natural History, Ohio which had documented cranial projectile trauma to analyse wound location, shape, size, beveling and projectile paths. This sample of skeletons provides a non-arbitrary selection of individuals which were unclaimed bodies from a disturbed social environment. Nine entry wounds were located on the right side of the skull, 3 located on left side of skull and one had entry under the right side of the skull. Entry wounds were often oval or round in shape. Exit wounds were not present in all cases, however, when present they were of irregular shape. Radiating fractures occurred in 8 entry wounds and 8 exit wounds. Concentric fractures accompanied 3 exit wounds. Three entry wounds had clear internal beveling. Three cases had exit wounds with external beveling. One showed signs of combination beveling on the entry wound. Beveling, if present, can lead to identifying the entry/exit wound. Radiating fractures form when the intracranial pressure is too great and if the pressure is not relieved, concentric fractures form secondarily. Based on the characteristics of the cranial projectile trauma one can suggest manner of death, however, it is not possible to reach a firm decision.

8.4 Introduction

Identification and interpretation of gunshot trauma to the skeleton is required of forensic anthropologists and in historical bioarchaeology. Firearms have ben commonly used in human conflicts for the last 500 years, and it seems to be of some use for anthropologists to understand how high-powered projectiles affect bones.

When a projectile enters the body, it often contacts osseous tissues and with head trauma, osseous tissue involvement nearly always occurs. When death is recent, soft tissue damage can help to determine the manner of death. There are also other indications of a manner of recent death such as presence of suicide notes, fingerprints, DNA analysis, position of body and the weapon. Abrasion rings on the skin can determine the distance between the muzzle of the firearm and the body, which can aid in determining the manner of death (Chapman 2007; DiMaio 2015). However, with less recent death, when skeletal material is the only material left to analyse, the characteristics of defects in the bone can provide information concerning the causative weapon, projectile characteristics, sequence of wounds and shooter(s) individual traits (e.g. handedness, height), which can be useful to law enforcement officials (Chapman 2007).

In less recent cranial firearm trauma (defined here as a situation when soft tissues have decomposed leaving only hard tissues), determining the manner of death can be difficult. Detailed analysis of the skeletal trauma may lead to a reconstruction of the events surrounding the death. A considerable amount of previous research has focused on autopsy and soft tissue details establishing the basis of gunshot wound characteristics including indicators of homicides and suicides (Stone 1992; Druid 1997; Karger et al. 2002; Desinan & Mazzolo 2005; Balci et al. 2007; de la Grandmaison et al. 2008). Autopsy findings are often analysed over a period of time, for example Solarino et al. (2007), who analysed fatal gunshot wounds in Bari, Italy between

1988 and 2003. Other studies have analysed historical gunshot wounds from the 1900s (Bailey & Mitchell 2007). More recently, descriptions of the range of ballistics trauma to the post cranial skeleton have been published (Humphrey & Henneberg 2017), including the ribs, vertebrae, scapula, sternum and the pelvis.

Physics, ballistics and anatomy are well known fields, so theoretically one could predict what happens when a bullet hits a bone. However, many variations occur in the wounds with different bullet types, calibres, velocities and angles of penetration, leading to a wide range of wounds. The aim of this study is to analyse and present a range of cranial trauma caused by projectiles evident in a collection of skeletons of unclaimed deceased persons located at the Cleveland Museum of Natural History. A collection of unclaimed bodies provides a non-arbitrary selection of individuals who are often from a disturbed social environment with a lack of family members to claim their bodies after death. This may indicate a specific set of circumstances under which the people have encountered traumatic deaths.

8.5 Materials and Methods

The Cleveland Museum of Natural History has collected skeletons of unclaimed deceased persons of the Cleveland, Ohio region between the years 1912 and 1938 (The Hamann-Todd Human Osteological Collection). In this collection, there are thirteen skeletons with evident cranial projectile trauma. Macroscopic descriptive observations were carried out on each case to analyse wound location, shape, size, beveling, fractures and projectile paths.

8.6 Results

8.6.1 The Sample

Thirteen cases were analysed, 12 were male and 1 female. The mean age was 38.5 years (SD 13.5), with a minimum of 25 and a maximum of 78 years. Eight cases had ancestry documented as white, while 5 had it documented as black. The cause of death for all cases was documented as gunshot wound, however, the manner of death was recorded in eight cases as unknown, two cases as murder/homicide and three cases as suicide (Table 8-1).

Table 8-1 Summary table of the gunshot wound trauma to the cranium in cases from the Cleveland Museum of Natural History

					Wound Location		*Beveling*		*Fracture Patterns*						
									Entry		Exit				
Manner of death	Specimen	Sex	Age	Race	Entry	Exit	Entry	Exit	Radiating	Concentric	Radiating	Concentric	Projectile Path	Comments	Manner of death based on characteristics
Homicide	2005	M	53	B	Right asterion region	Right pterion region	no/minimal	external	yes	-	yes	yes	Back to front, upwards, right	exit fractures comminuted	homicide
Homicide	1313	M	35	B	Left temporal, inferior temporal line	-	internal	-	yes	-	-	-	Left to right	external flaking/chipping on entry wound	homicide
Suicide	875	M	35	W	right greater wing of sphenoid	left temporal, near sqaumosal suture and inferior temporal line	-	external	yes	-	yes	-	right to left, slightly front to back and upwards	external flaking/chipping on entry wound	suicide
Suicide	889	M	36	W	frontal bone, right side of midline	lambdoid suture, left of midline	internal	external on posterior-inferior aspect	yes	yes	yes	yes	right-left, front-back, downwards	extensive fractures	suicide
Suicide	1080	M	38	W	right sphenoid-temporal suture region	-	internal	-	-	-	-	-	right-left		suicide
Unknown	1214	F	32	B	Left occipital, near asterion	Right temporal, near squamosal suture	internal	external	yes	no	no	no	left to right, back to front, upwards		***requires more details: possible homicide***
Unknown	1468	M	37	B	1. right maxillae, through zygoma 2. left mandible	left zygoma, maxillae	internal on zygomatic	external on left zygomatic arch, maxillae. Left mandible internal and external	comminuted	comminuted	comminuted	comminuted	right to left	comminuted maxillae, mandible and zygoma. Multiple bullets through cranium, one in scapula	***homicide***
Unknown	1903	M	32	B	right parietal, posterior to pterion	rebounded on left parietal-frontal suture. Exit on left mandible	internal		yes	-	yes	yes	right to left, upwards	projectile rebounded off left side, creating fractures, exited right mandible. Energy was not great enough to penetrate left side. Bullet wound to femur	***homicide***
Unknown	193	M	33	W	right pterion region	left pterion region	-	external	-	-	yes	-	right to left		***requires more details***
Unknown	575	M	25	W	right sphenoid-frontal suture	right frontal bone, above right orbit ridge	internal on anterior aspect	external	yes	-	-	-	back-front, right-left, upward	comminuted orbits	***requires more details***
Unknown	1926	M	30	W	right base of skull	left base of skull		external	yes	yes	yes	yes	right-left, upwards	severe comminuted skull including fractured parietal, frontal and temporal bones, orbits, ethmoid, mastoid process, maxillae. Mandible and cervical vertebrae intact.	***requires more details: possible homicide***
Unknown	1576	M	78	W	right temporal, above ear canal	left pterion region	external flaking and chipping	external on anterior-superior aspect	-	-	yes	-	right-left, back-front, upwards		***requires more details***
Unknown	749	M	36	W	right frontal, above pterion	left coronal suture	internal	external	-	-	yes	-	right-left, front-back, upwards		***requires more details***

8.6.2 Number of Wounds

Most skulls in this sample had more than one wound: 8 had clear entry and exit wounds (Figure 8-1 to Figure 8-8). Two skulls (1080, 1313; Figure 8-9 and Figure 8-10) had only entry wounds, no exit wound visible. The skull 1926 had radiating fractures indicating entry of the bullet into the head just below the right part of the skull base and exit on the left but projectile path must have passed only through soft tissues while skull trauma was caused by the transfer of energy (Figure 8-11). One male, had an entry wound, a wound caused by the projectile rebounding inside the skull, partially breaking the vault from inside and a further wound to the mandible. It is unclear whether the mandible wound has been caused by the projectile (1903; Figure 8-12 and Figure 8-13). Another case (1468; Figure 8-14) had three wounds in its jaws – entry in the right maxilla and exit in the left maxilla and a wound to the left side of the mandible. These latter two cases (1903 and 1468) also had wounds in the scapula and the femur, respectively.

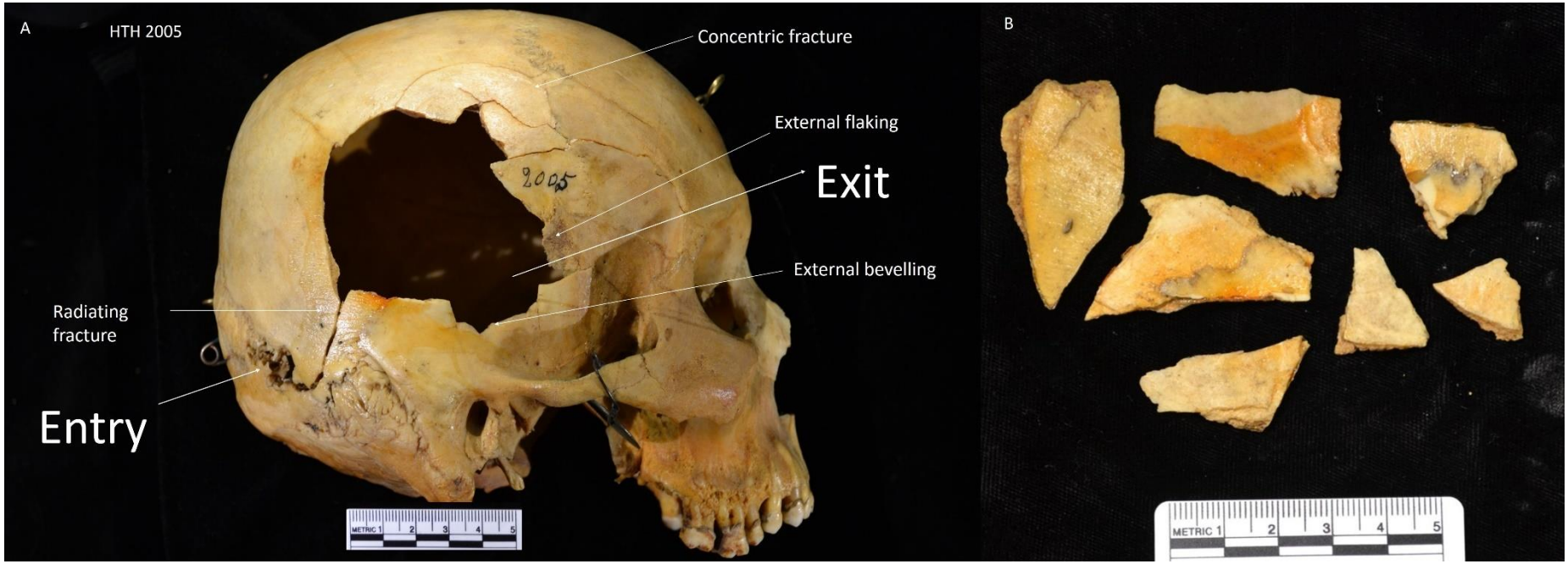

Figure 8-1 Individual HTH 2005. Male, 53 years of age, black. All trauma has been labelled, use as reference for all figures.

Figure 8-3 Individual HTH 875. Male, 35 years of age, white.

Figure 8-2 Individual HTH 889. Male, 36 years of age, white.

Figure 8-4 Individual 1214. Female, 32 years of age, black.

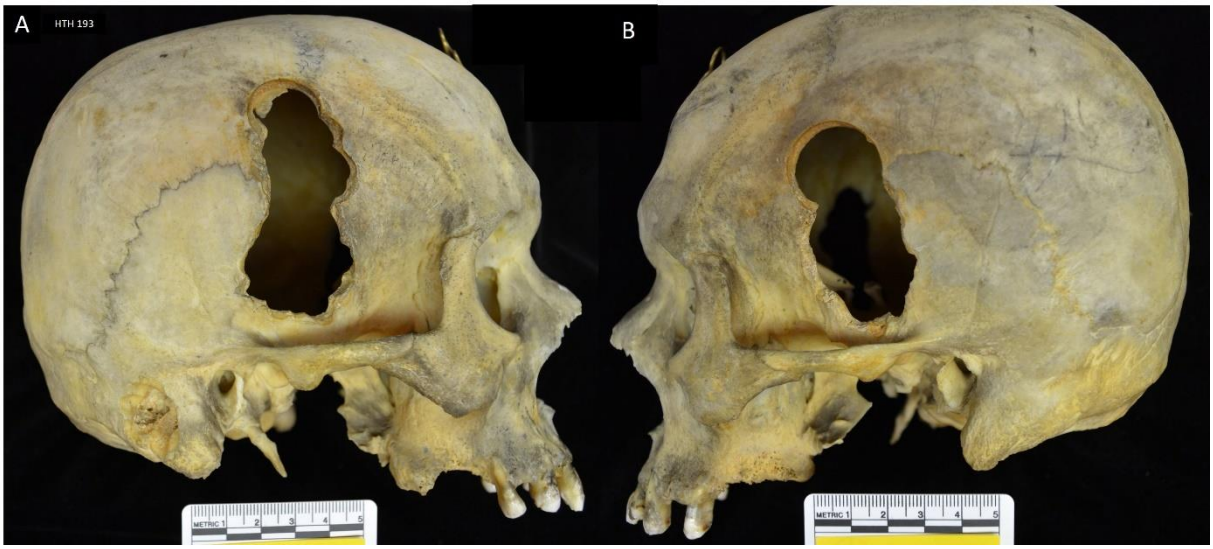

Figure 8-5 Individual 193. Male, 33 years of age, white.

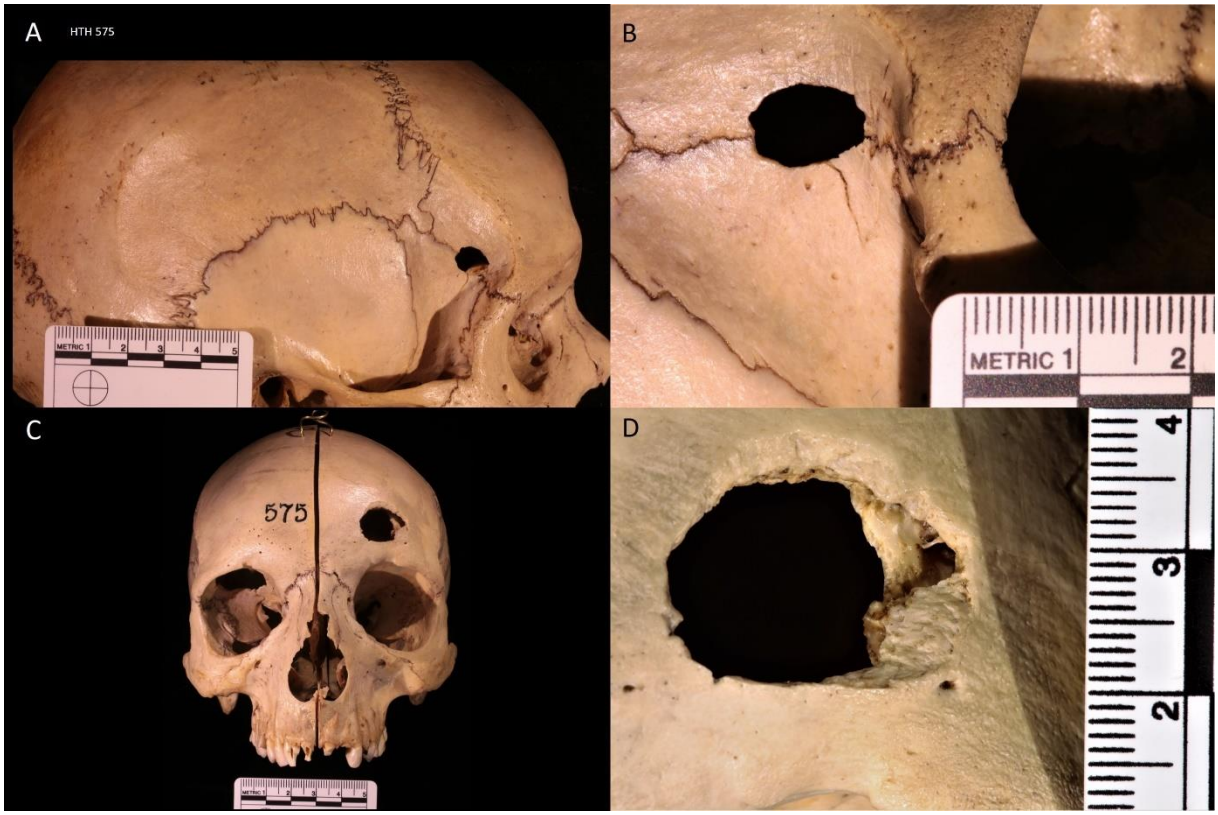

Figure 8-6 Individual 575. Male, 25 years of age, white.

Figure 8-7 Individual 1576. Male, 78 years of age, white.

Figure 8-9 Individual 749. Male, 36 years of age, white.

Figure 8-8 Individual HTH 1080. Male, 38 years of age, white.

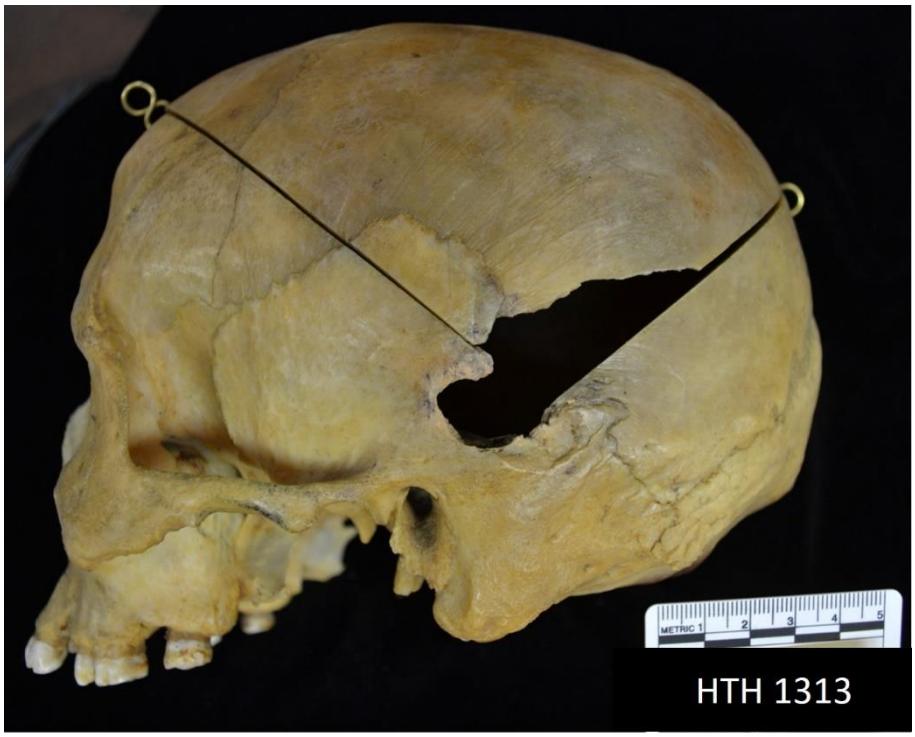

Figure 8-10 Individual HTH 1313. Male, 35 years of age, black.

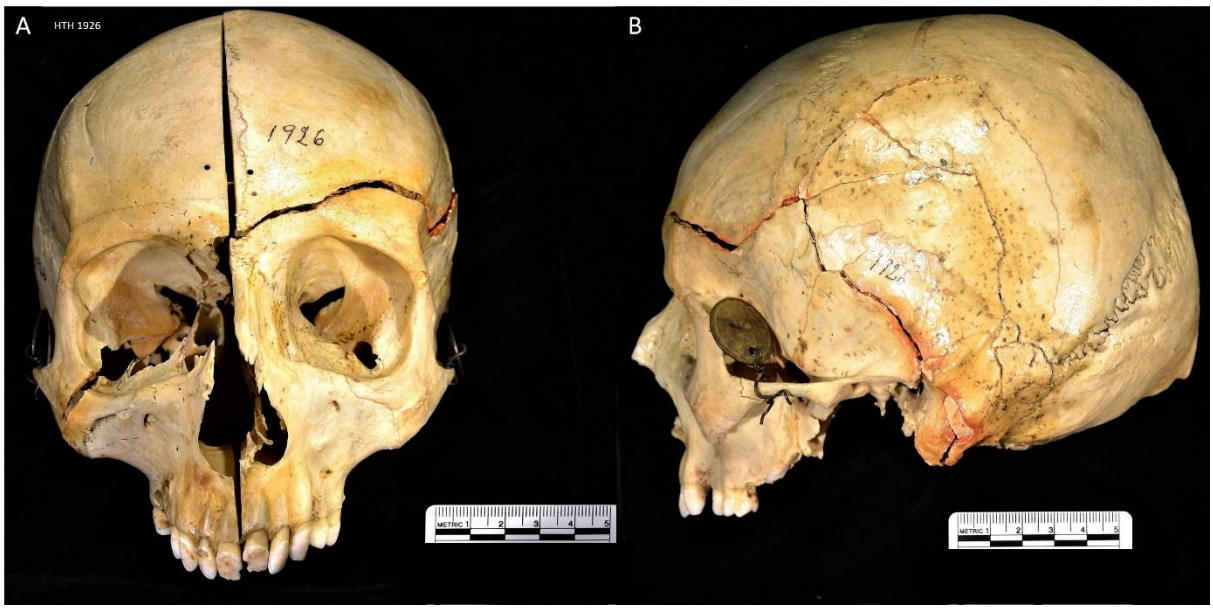

Figure 8-11 Individual 1926. Male, 30 years of age, white.

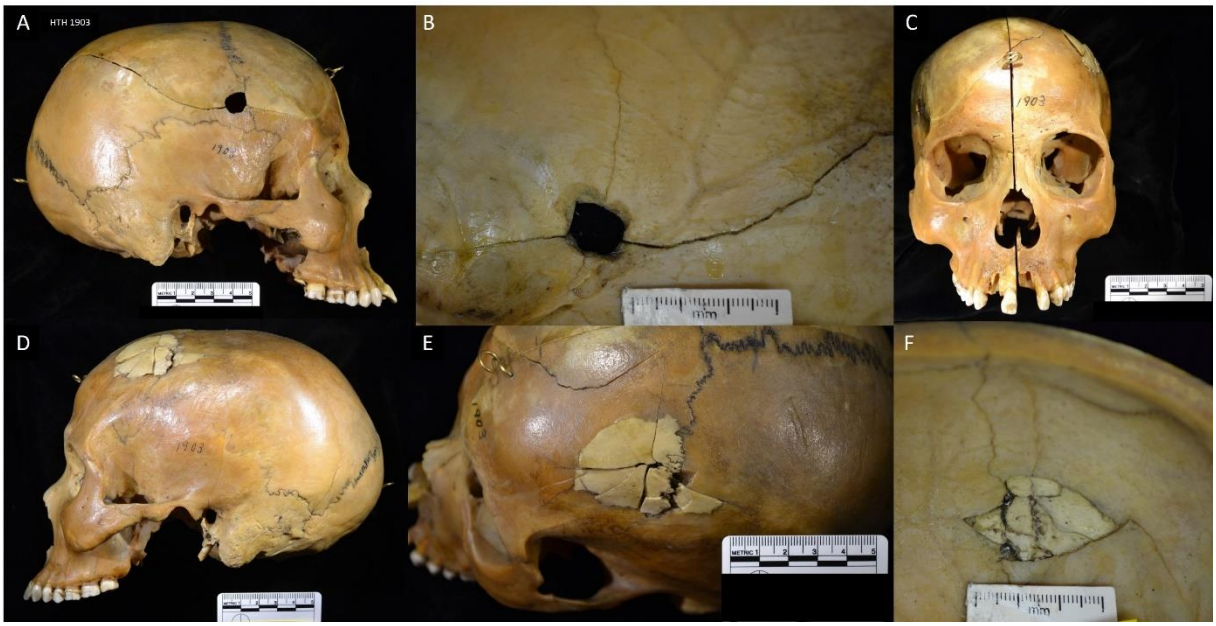

Figure 8-12 Individual 1903. Cranium. Male, 32 years of age, black.

Figure 8-13 Individual 1903. Mandible. Male, 32 years of age, black

Figure 8-14 Individual 1468. Male, 37 years of age, black.

8.6.3 Entry Wounds

Nine entry wounds were located in the right side of the skull; 3 were located on the left of the skull and in one case (1926; Figure 8-11) the projectile entered under the right side of skull. Internal beveling was present on three entry wounds in the brain case (889, 749, 1903; Figure 8-3, Figure 8-8 and Figure 8-12). One case had a combination of internal and external beveling (875;Figure 8-2), one reverse (1576;Figure 8-7) and one case had no beveling (575; Figure 8-6). In the case 1468 the maxilla had internal beveling (Figure 8-14). It is difficult to determine if the wound to the left side of the mandible of this individual is an entry or exit as it mostly broke off teeth, the mandible has just a linear vertical fracture at the position of the second left premolar. Entry wounds were most often oval or round in shape. The distribution of entry wounds can be seen in Table 8-2.

Table 8-2 Distribution of entry wounds in sample analysed

	Homicide	*Suicide*	*Unknown*
Head			
Right face			maxilla *1
Left face			
Right temple		2	5
Left temple	1		
Back of head	1 (asterion)		1
Middle forehead			
Forehead others		1	
Mandible/mouth			*1
Nape/neck/skull base			1

**same individual, multiple wounds*

8.6.4 Exit Wounds

Five exit wounds were located on the left of the skull, while three were located on the right. Three skulls presented with no exit wounds. One case (1903; Figure 8-12 and Figure 8-13) was different from the standard entry and exit wounds indicating a straight path of the projectile entering on the right hitting the left parietal bone that fractured, but the projectile did not have enough energy to penetrate the bone to exit. This individual also had a comminuted fracture of the right mandibular ramus, however it is difficult to judge how this injury occurred. Case 1468 only had a clear exit wound in the left maxilla (Figure 8-14). The wound to the mandible is difficult to interpret. Exit wounds appeared larger than their corresponding entry wounds and had more irregular shapes that included oval, round and comminuted.

8.6.5 Beveling

Three entry wounds had clear internal beveling. Three other cases had exit wounds with external beveling. One case (875; Figure 8-3) presented both internal and external beveling of the entry wound. Three cases had no beveling of the entry wound and one case had no beveling of the exit wound. Those without clear entry or exit wounds (i.e. 1926; Figure 8-11) obviously had no beveling.

8.6.6 Fractures

Eight brain cases presented with radiating fractures on the entry wound. Four of those cases also had radiating fractures of the exit wound. Four other cases had radiating fractures only of the exit wound. Concentric fractures appeared on no entry wounds. However, three cases had concentric fractures on the exit wound. One case had no fractures of the entry wound. One case had commingled exit wound (2005; Figure 8-1) and another case had a commingled wound to the vault resulting from the projectile bouncing from its inner side (1903; Figure

8-12). Two cases (875, 889; Figure 8-3 and Figure 8-2) had Le Fort II fractures. Two mandibles were fractured (1903, 1468; Figure 8-12 and Figure 8-14).

8.6.7 Projectile Path

With nine entry wounds located on the right side of the skull, and one skull having indication of the entry under its right side, the most common projectile path was right to left. This is true of two recorded suicides whose right to left path deviated slightly towards the back. The third suicide had front to back path. One homicide had a path back to front and the other left to right. One skull with unknown cause of death had a left to right path. The remaining seven skulls with right to left projectile path had cause of death documented as unknown. Altogether there were eleven pathways running roughly parallel to the transverse plane and only two running roughly parallel to the sagittal plane.

8.7 Discussion

This collection of skeletons is of unclaimed individuals, obtained by Thomas Wingate Todd and Carl Hammann who founded this collection in the early 20th century. It was documented by Todd that these individuals were "…gathered by accident, misfortune or design into a common net by lack of funds for burial … relatively low stratum of society subjected to hazards in many ways comparable with the risks of medieval and primitive populations" (Mangels 2017 <https://www.cmnh.org/science-news/blog/march-2017/what-the-bones-say>). If these individuals died in a socially stable environment, there would have been a family member who would have claimed the body. The tragic ending that these individuals have come to reflects hard lives that Clevelanders lived during the early 20th century. Most of the skeletons in this collection were of immigrants with European descent, working in the factories and African-Americans who migrated after the first World War from the South in search of jobs (Mangels

2017). In the sample studied here, eight were white and five were black, reflecting the overall state of the collection.

The age range of the majority of these individuals: 31 - 40 years (69.2%) was similar to the age distribution described in other investigations of firearm injuries (de la Grandmaison et al. 2008).

A bullet impacting on a bone, particularly the flat skull vault, causes a plug of bone to shear off in front of it creating beveling, which can be either internal, external or reverse (Symes et al. 2012). Arrangement of beveling in relation to the wound, assists in determining the direction of the projectile-bone contact. Internal and external beveling are characteristics of entry and exit wounds respectively. Beveling surrounding uniformly the entire entry and exit wounds indicates that the path of the bullet is perpendicular to the surface of the bone. Presence of beveling only on a part of the entry wound indicates that the bullet travelled at an angle to the surface of the bone. Characteristics of the beveling also help to identify the perimortem position of the victim (Berryman & Symes 1998; Berryman & Gunther 2000; DiMaio 2015). Eight skulls studied here had entry wounds identified by the presence of internal beveling. In two skulls (1214 and 749; Figure 8-4 and Figure 8-8), the internal beveling uniformly surrounded the entry wound, indicating the paths of the projectiles were perpendicular to the surface of the skull. Internal beveling on the entry of skull 575 (Figure 8-6) was seen on the anterior aspect only, indicating that the projectile penetrated the skull at an angle to the surface.

Eight exit wounds had evidence of external beveling. The external beveling of the exit wound on skull 889 was on the postero-inferior aspect, suggesting that the projectile exited the skull at an angle to the surface of the bone. In individuals 2005, 875, 193, 1576 and 1903 (Figure 8-1, Figure 8-3, Figure 8-5, Figure 8-7, and Figure 8-12, respectively), the skull wounds in the right asterion region, right greater wing of the sphenoid, left mandible, right pterion region and right temporal above the ear canal had neither external nor internal beveling. However, in skulls of

individuals 2005, 875, 193 and 1576 the other wounds were identified as the “exit wound” from the presence of external beveling. Therefore, in these skulls, the wounds without beveling are the “entry wounds” through which the projectile entered the skulls. Non-bevelled wounds are most likely due to low energy of the projectile, as indicated by the appearance of the exit wounds they were coupled with, and the lack of extensive fractures.

Cranial gunshot wounds may result from homicides or suicides. It is often found that entry wounds in homicides are variable in their locations, while suicide entry wounds are more often found in the right temporal region, frontal bone or in the mouth (Stone 1992; Cina et al. 1999; Karger et al. 2002). In the present study, the cases documented as homicides had entry wounds varying: back right side and left side, while most suicides had entry wounds on the right side, particularly in the temporal region. The unknown cases had varying entry locations, most being located on the right temporal region as is common in suicides, although the right temporal region has been found in a few homicide cases as well (Druid 1997).

Exit wounds are not always present in cranial trauma, such as with cases 1080, 1313 and 1903 of the present study (Figure 8-9, Figure 8-10, and Figure 8-12, respectively). This may indicate that the projectiles were of low entry velocity and did not have adequate energy to penetrate the skull bone for the exit from the skulls. There is also the possibility that upon entry to the skull the projectiles altered their course (i.e. tumbling), which reduced the momentum of the projectile by contact with meninges and brain tissues, thus causing a lack of energy to exit the skull.

The appearance of the exit and entry wounds studied here and the pattern of the overall trauma of all of these individuals indicate that the wounds were caused by fairly low energy projectiles i.e. low powered weapons, in comparison to high powered rifles which would create a large temporary cavity due to the greater velocity which increases the energy (Holt and

Kostohryz 1983; Humphrey & Kumaratilake 2016). It is most likely that the weapons used were of small calibre. Injuries described here would also be classic of an urban society compared to a military setting because military weapons are often high powered.

Reverse beveling - in the opposite direction to the projectile direction of entry - is an atypical pattern of injury (Bhoopat 1995). This type of injury results when a weapon is discharged in direct contact with the skull, where the intra-cranial pressure becomes so great that it gets expelled backwards through the entrance wounds towards the weapon (Chapman 2007). In skull 875 (Figure 8-3), the entry wound has minimal internal beveling and external chipping/flaking/beveling. Keyhole entry wounds are another example, where entry and exit wounds are difficult to differentiate from one another, due to the presence of characteristics of both entry and exit wounds (Berryman & Gunther 2000). Keyhole wounds occur when the bullet penetrates the bone tangentially and causes circular/oval defect and radiating fractures. As the bullet travels through the bone the pressure causes concentric fracture between the radiating fractures which then lifts outwards creating a wound that resembles a keyhole (Berryman & Symes 1998; Quatrehomme & İşcan 1999; Harada et al. 2012; Symes et al. 2012; DiMaio 2015). This type of wound was not present in any of the skulls investigated here.

After a bullet impacts the skull, fractures appear as a result of the increased intracranial pressure, overcoming the strength of the bone, with radiating fractures occurring first and concentric second (Chapman 2007; Symes et al. 2012). Each fracture can be distinguished from each other and the sequence in which they were formed could be determined, as a new fracture line will arrest at a previously formed fracture. Such radiating and/or concentric fractures were seen in association with the projectile injuries of eight skulls (2005, 875, 889, 1214, 749, 1313, 1926 and 1903, Figure 8-1 to Figure 8-4, Figure 8-8, Figure 8-10, Figure 8-11, and Figure 8-12, respectively). High velocity projectiles will often cause greater fracturing due

to the high-energy dissipating into the cranium, however, structure and the composition of the bullet and the distance at which it was fired from will also contribute to the occurrence of the fractures and their severity. Therefore, the above fractures would most likely have resulted from high energy projectiles. Low velocity firearms generate less initial energy upon penetration of the cranium, thus will cause less severe fractures or no fractures, because the cranium's threshold for intracranial pressure has not been overcome. Therefore, non-fracture associated injuries of the skulls 1080 (Figure 8-9) and 1576 (Figure 8-7), may have resulted from low velocity firearms.

Determination of the path taken by a projectile is important in forensic investigations. Identification of the entry and exit wounds, and the location of beveling in relation to the entry and exit wounds are vital in determining the direction the projectile travelled. As evidenced in Table 8-2, suicides usually do not produce back to front paths (Examples – skulls 875 and 889; Figure 8-2 and Figure 8-3). Karger et al. (2002), found that left to right trajectories were not typical for suicides, but existed (Example – skull 1080), de la Grandmaison et al. (2008), support this, saying that left to right trajectories were more common in homicide cases. However, Balci et al. (2007) and Karger et al. (2002) have described that the most common trajectory taken by a suicide bullet is front to back and upwards. It seems that the characteristics of suicide wounds depend on the weapon used – handgun versus a long-barreled weapon – and the handedness of individuals, which is most often, though not exclusively, right. Back to front trajectories in combination with an entry wound in the right temple are indicative of homicide (Druid 1997) (Example – skull 2005). Often a homicide can be planned to mimic the most common characteristics of suicides including anatomical location and direction of the projectile. The homicide cases described here also contradicted what has been previously found as typical horizontal and downwards trajectories (Suwanjutha 1988). The small number of cases presented here is not adequate to determine whether these variations occur more often.

Concerning the sagittal plane direction, variations occurred in both suicide and homicide. Based on the projectile path alone, one cannot say for certain that the manner of death was homicide or suicide as variations in the positioning of a weapon can create wounds which mimic homicides, and suicides and vice versa. A case described by Durak et al. (2006), shows a man with a firearm set up to be remotely fired into the individual's back i.e. suicide, but anatomical positioning of wound is more like that of a homicide.

Two individuals (1468 and 1926), where the manner of death was unknown, had multiple wounds. Skeleton 1926 had two wounds to the skull and one to the scapula, while the other had a wound to the cranium and to the femur (Figure 8-11). The presence of more than one wound can be a result of either suicide or homicide, however, multiple firearm wounds strongly suggest that they were a result of homicide, rather than suicide or accident (Druid 1997; Desinan & Mazzolo 2005; de la Grandmaison et al. 2008; Jacob et al. 1989; Karger 1995, Karger & Brinkmann 1997; Sekula-Perlman et al. 1998; Karger et al. 2002). If a bullet penetrates the cranium, incapacitation occurs relatively quickly if the vital regions are damaged and the energy transfer to the tissues is substantial. Although it is uncommon to have wounds to the post cranial skeleton and to the cranium in suicide cases, such situations have been documented (Eisele et al. 1981; Hudson 1981; Introna & Smialek 1989; Betz et al. 1994; Karger & Brinkmann 1997). Basing a decision about the manner of death on the number of shots may lead to inaccurate outcomes as multiple wounds can appear in either manner of death. It is possible to distinguish the sequence of bullets and whether they penetrated the critical central nervous system centres and resulted in immediate incapacitation. Were immediate incapacitation not the result of the first shot, further wounds inflicted by the suiciding person are possible.

Each of the characteristics of gunshot wounding described above can be used to aid in determining the manner of death. Identifying the projectile path using all the available

characteristics including fractures and beveling can aid the determination of the manner of death (homicide, suicide or accident). Inaccurate interpretations may also occur if these characteristics are relied solely upon, as atypical suicides and homicide cases do occur, along with cases which mimic other manners of death. Interpreting osseous tissue trauma to determine the manner of death is difficult. The use of all available tissues (including soft tissues), along with features of the scene would be advantageous. Traumatic lesions associated with recoil of a weapon, although rare, very significantly suggest that the manner of death was suicide (de la Grandmaison et al. 2008). If no other factors are present, then care is needed to form an objective point of view on the manner of death. Though individual decisions may be difficult, the statistical overview of the pattern of cranial injuries in the sample studied indicates predominance of suicides with low-powered weapons. Taking into account the socio-economic background of the collection, such finding is not surprising.

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Chapter 9

9 Anthropological analysis of projectile trauma to the bony regions of the trunk

Caitlin Humphrey and Maciej Henneberg

9.1 Statement of Authorship

Manuscript Details

Title of Paper	Anthropological analysis of projectile trauma to the bony regions of the trunk
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Caitlin Humphrey
Contribution to the Paper	Travel, data collection, anatomical analysis and interpretation, photography, writing and editing manuscript, manuscript revisions
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 18/9/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Maciej Henneberg
Contribution to the Paper	Anatomical analysis and interpretation; manuscript writing and editing, manuscript revisions
Signature	Date 18.09.17

9.2 Context

Similar to cranial trauma (Chapter 8), variations often occur among wounds in the infra-cranial skeleton. In anatomical modelling in relation to injuries caused by projectiles, priority was often given to the thorax and abdomen, as they contain the vital organs. Furthermore, the thorax and abdomen have a larger body mass compared to the rest of the body, thus are easy to aim at in firearm incidents (i.e. centre of mass). The skeletal structures within this region included the ribs, sternum, clavicle, vertebrae and scapulae. When this area is penetrated by a projectile, it is likely to cause injuries to the skeletal elements. Ribs support a large area of the thorax; thus, only small calibre bullets could travel through the intercostal spaces without encountering the osseous tissues. Most of the trauma descriptions presented in the literature are derived from autopsy findings. Injuries present on skeletal materials of the trunk are less often used to describe the cause of death or the manner in which the death occurred. This chapter presents a detailed analysis of injuries caused by projectiles on the infra-cranial skeleton, with the aim of broadening the understanding of many aspects of ballistic injuries.

9.3 Abstract

Ballistics literature often focuses on soft tissue injuries and projectile trauma to the cranium. Minimal details on the bony characteristics of projectile trauma to the thorax/abdomen regions have been published. This study aims to analyse projectile trauma to the bony trunk region including the ribs, vertebrae, scapula, sternum and the hip bone to form a better understanding of the characteristics and biomechanics of skeletal trauma caused by a projectile and contribute to the existing database on skeletal trauma caused by projectiles. Fourteen cases of documented projectile trauma to the bony regions of the trunk from the Hamman-Todd Human Osteological Collection at the Cleveland Natural History Museum, Ohio were analysed. Of the 14 individuals with gunshot wounds examined, 40 wounds occurred to the bones. Twenty-four injuries to the ribs, 1 ilium, 11 vertebrae, 3 scapulae, and 1 sternum. Fracture patterns, heaving and bevelling can be used to determine the direction of travel of the projectile which can be evident on the ribs, sternum, scapula and ilium. It is critical to understand the wounding patterns associated with projectile trauma to the torso region as this is often targeted, due to being the centre of mass.

9.4 Introduction

Much research in ballistics has focused on soft tissue injuries including autopsy features and experimental aspects utilising animal models, ballistics simulants and dummy models (Bir et al. 2016; Humphrey et al. 2017; Humphrey and Kumaratilake 2016; Jönsson et al. 1988; Mabbott et al. 2016; Schantz 1978). When it comes to bony injuries from projectiles, a lot of research has been conducted into maxillofacial ballistics trauma (Berryman et al. 1995; Lahren et al. 1987; Stefanopoulos et al. 2015; Viel et al. 2009). The literature has been able to document the characteristics of projectile skeletal trauma to the skull as well as soft tissue characteristics, however minimal details on the thorax/ abdomen bony regions in particular the scapula, ribs, sternum and vertebrae have been published (Langley 2007).

In 1995, Ubelaker (1995), published an anthropological analysis of an individual who was assassinated sustaining gunshot trauma. The analysis produced evidence for projectile path and thus direction of fire based on the displacement of bone fragments, fractures, bevelling and appearance of the fractures. More recently, Langley (2007), produced an anthropological analysis of gunshot wounds to the chest region with the focus on assessing the usefulness of the characteristics of the wounds in determining the direction of fire. Using 54 documented cases of gunshot wounds to the thorax, Langley (2007), found that due to the ribs occupying a significant portion of the thorax, they were commonly hit by a projectile and that the bullets leave distinctive marks on ribs which are able to determine the direction of fire. Langley concluded that further analyses of the bony structures of the thorax are needed to get a better understanding of the biomechanics associated with bony projectile trauma to the thorax. Most forensic anthropology text books also describe bone trauma from projectiles (Byers 2015; DiMaio 2015; Dirkmaat 2014). Many other researchers have investigated the biomechanics of gunshot wounding, characteristics of wounds caused by different projectiles, how low velocity

projectiles cause injury, prediction of the calibre of the weapon and manner in which death occurred (Berryman et al. 2012; Berryman and Symes 1998; de la Grandmaison et al. 2001; DiMaio 2015; Lahren et al. 1987; Langley 2007; Smith and Wheatley 1984; Spitz and Spitz 2006). However, misinterpretations have been noted, thus there is a need to understand the relationships between soft and hard tissue trauma injuries (Fackler 1987; Fackler 1988). Symes et al. (2012), have suggested that the interpretation and assessment of high velocity impact to osseous tissue is only in its early stages and therefore more research is necessary to properly understand and accurately interpret the bone injuries resulting from projectiles.

This study aims to analyse projectile trauma to the bony trunk region including the ribs, vertebrae, scapula, sternum and the hip bone to form a better understanding of the characteristics and biomechanics of skeletal trauma caused by a projectile. This study will contribute to the existing literature on skeletal trauma caused by projectiles.

9.5 Materials and Methods

The Hamman-Todd Human Osteological Collection at the Cleveland Natural History Museum, Ohio, contains 44 documented cases of gunshot trauma. Two cases had been returned to their respective families; twelve had unknown location of wound or not visible wound (i.e. soft tissue trauma only); 3 had wounds to the limbs; thirteen had wounds to the cranium and fourteen had wounds to the torso region, that were sustained in real life situations. The fourteen torso region cases are discussed here. Each case was documented as gunshot wound being the cause of death, and where known, the manner of death was also documented (4 homicide, 10 unknown). The age and sex were noted from the Museums records (mean age 29.86 (SD 7.57); sex: female 14.2%, male 85.7%). Causative weapon was not known in the cases; however, no shotgun injuries were present due to the unique wounding properties of these types of

weapons. Shots expelled from such a firearm form a large cloud of pellets that expands in diameter as distance increases. Due to the small size of each pellet, its kinetic energy is low and therefore one could expect multiple low impact injuries.

A visual examination of the entire skeleton (where present) occurred to determine the location of the wounds. A Canon 5D Mark III with macro lenses camera was used to photograph the wounds. No autopsy records or pathologists evaluations were available to assess soft tissue details, thus only anthropological analysis of the wounds occurred.

9.6 Results

Of the 14 individuals with gunshot wounds examined in this study, 40 wounds occurred to the bones. Twenty-four injuries to the ribs, 1 ilium, 11 vertebrae, 3 scapulae, and 1 sternum.

9.6.1 Vertebrae

In the vertebrae, the injuries were often a shattering of the vertebral body, small clear fractures, and missing segments of bone including the pedicle. This occurs in the cervical, thoracic and lumbar vertebrae (Figure 9-1 to Figure 9-4).

Figure 9-1 Vertebrae of Individual HTH 524, displaying 7th, 8th and 9th vertebral body shattering

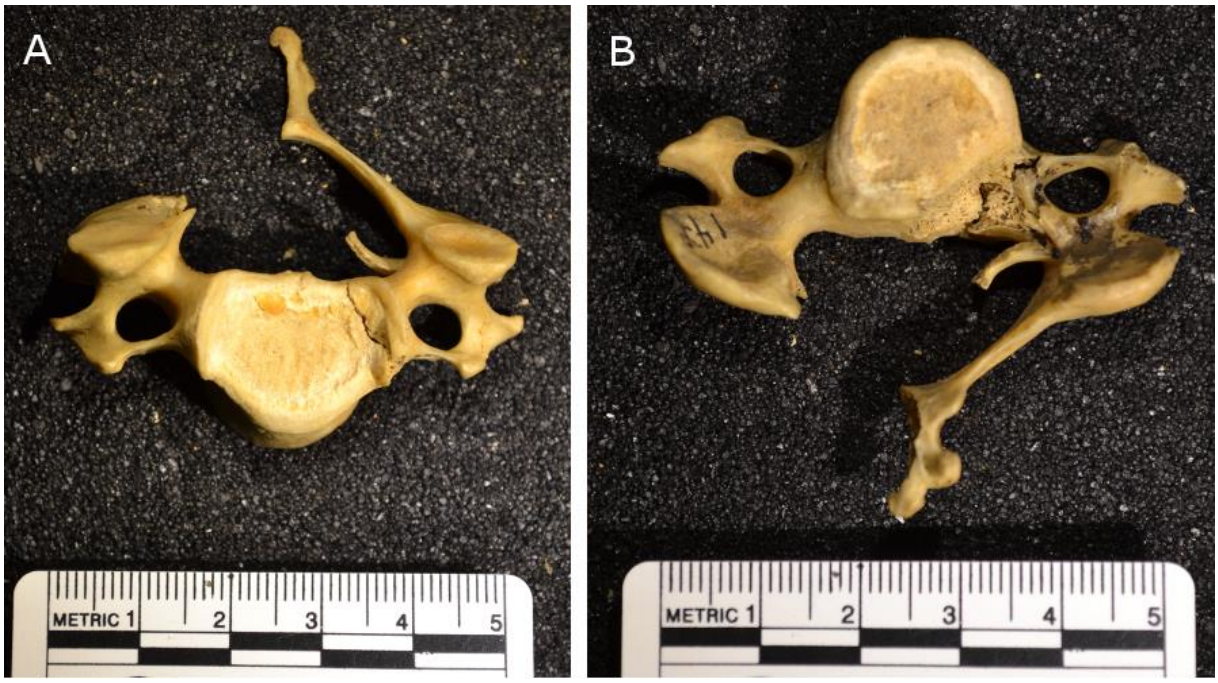

Figure 9-2 Vertebra of Individual HTH 1430, displaying clear fracture and missing segment of cervical vertebra (A, B)

Figure 9-3 Vertebrae of Individual HTH 1812, displaying fracture and missing segments of Lumbar 4

Figure 9-4 Vertebrae of Individual 659, displaying vertebral shattering of thoracic 3, 4, 5 and 6 (A, B)

9.6.2 Sternum

The sternum, particularly the corpus sterni (Figure 9-5), showed both an entry and exit wound which were distinguishable from each other, thus the ability to determine the projectile path. The entry wound was smaller with radiating fractures. The exit wound, larger and more irregular in shape, also produced radiating fractures, however these heaved outwards.

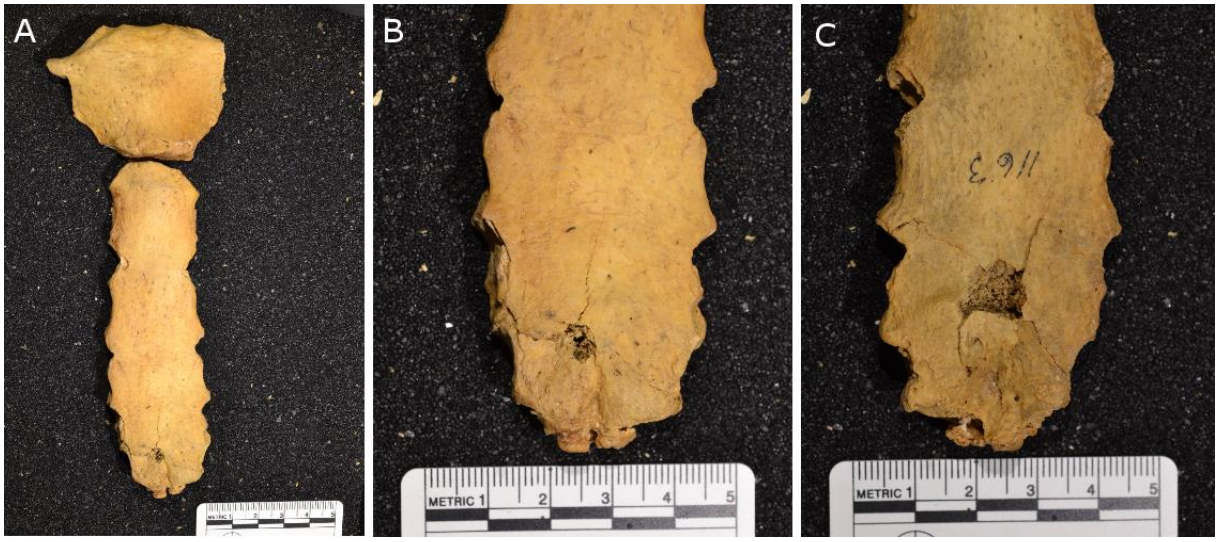

Figure 9-5 Sternum of Individual HTH 1163. Full sternum displaying entry wound (A), entry wound (B), and exit wound (C), with small radiating fractures

9.6.3 Ribs

The ribs showed varying types of wounds. The first being small nicks in the bone as the projectile passed and minimally made contact with the bone. These present as semi-circular/oval defects on either the caudal or cranial edge of the ribs (Figure 9-6 to Figure 9-11). The diameter of the wound may reflect the calibre of the bullet, being large or small, however, such evidence is not definitive. The second type are fractures on either the sternal or vertebral end of the rib. These fractures run along the length of the rib (Figure 9-12 and Figure 9-13). Other fractures can occur mid-shaft, often fracturing a rib completely into separate pieces, two or more which can be reconstructed (Figure 9-15). The third is a combination of a small circular nick in the bone, with fractures that run along the edge of the rib, some appearing similar to spiral fracture patterns (Figure 9-8 and Figure 9-9). What has been referred to as butterfly fractures in long bones, may also occur in these types of rib fractures (Figure 9-15). When the circular defect is present, the entry and exit side may be distinguished with the use of bevelling on the exit wound, entry wound is circular and smaller than the exit, depressed or heaving fractures (Figure 9-8, Figure 9-9 and Figure 9-11). Multiple fractures may appear in the same individual, which can be difficult to distinguish as peri- or post-mortem (Figure 9-16 and Figure 9-17). These fractures can be simple transverse fractures across the width of the rib, or run along the rib, as in oblique fractures. If the bullet arrests in the rib, it may form a wound similar to Individual HTH 985 (Figure 9-18), where the bone remodels around the bullet (as would occur if the bullet was not removed and the patient survived).

Figure 9-6 Superior view of Individual HTH 65 right 4th rib (A) with arrow indicating wound on cranial edge of the sternal end. Close view of wound (B), indicated by arrow

Figure 9-7 Inferior view of Individual HTH 65, left 4th and 5th rib (A), with arrow indicating wound on caudal edge of sternal end of 4th rib. Close view of wound (B).

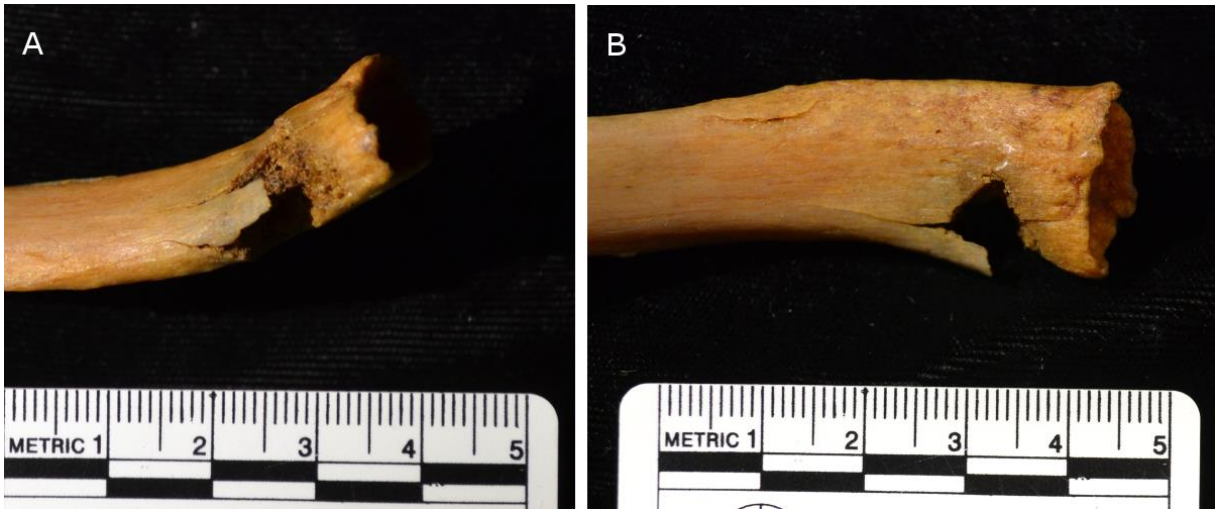

Figure 9-8 Individual HTH 1662, displaying circular defect in sternal end, caudal edge with flaking inwards (A, B)

Figure 9-9 Right 8th rib of individual HTH 596 (A). Wound located on cranial edge on articulating end of rib. Fractures running along length of rib in spiral pattern (B), and the clear circular entry wound (C).

Figure 9-10 Individual HTH 1238 left 9th rib displaying bone nick on cranial edge with depressed bone (A, B), and small radiating fractures lifting outwards (B)

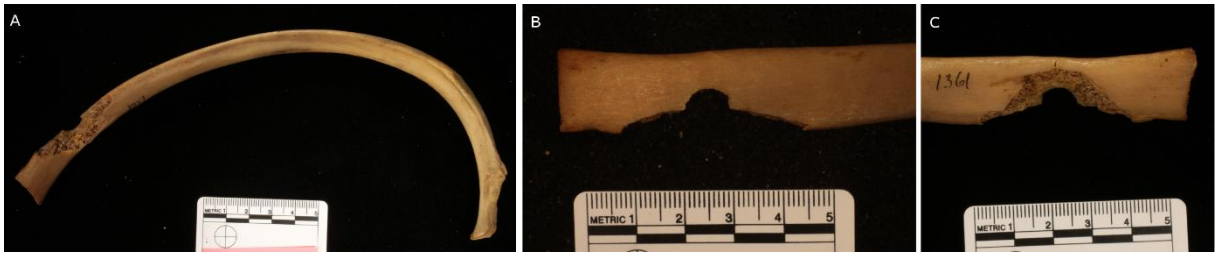

Figure 9-11 Individual HTH 1361. Left 3rd rib displaying circular defect on sternal end, caudal edge (A). Clear circular entry with missing segments (A), external bevelling and flaking of exit wound (B).

Figure 9-12 Individual 524 displaying fracture running along length of rib on caudal edge of articulating end

Figure 9-13 Individual HTH 2104. Right 5th and 6th ribs with fractures running along cranial surface edge of sternal end (A). Left 1st rib displaying small notch, and other rib with damage to sternal end

Figure 9-14 Individual HTH 1361. Right 6th rib displaying fracture through shaft bending laterally

Figure 9-15 Individual 1238 right 3rd rib. Caudal edge on articulating end displaying oval defect with radiating fractures similar to a butterfly pattern seen in long bone fractures

Figure 9-16 Individual HTH 1163 displaying commingled ribs with various fractures with no clear entry of exit wounds

Figure 9-17 Individual HTH 659. Fracture of left 3rd rib through shaft (A, B). Right 2nd, 3rd, 4th ribs with no distinct entry or exit wounds but fractures through shaft (C). Left 2nd rib with fracture through and along shaft (D)

Figure 9-18 Individual HTH 985. Wound on right 7th rib. Potentially due to bullet arresting in rib and bone displaying signs of remodelling around bullet

9.6.4 Scapulae

Due to the irregularity and non-uniform thickness of the scapula, the wounds vary. The three injuries to the scapulae in this study showed that when a bullet penetrates the subscapular fossa (Figure 9-19) a clear circular wound is visible but fracturing into segments occurs. In individual HTH 1709 (Figure 9-20), both the left and right scapulae were injured. The fracturing pattern on the left scapula shows in the infraspinous fossa fractured fragments heaving in the dorsal direction. The right scapula shows similar fracturing of the fragile and thin infraspinous fossa as well as the vertebral border of the scapular spine. In this individual, no clear entry or exit wound was visible. Most likely the projectile entered the thorax anteriorly and caused cavitation that produced injuries to both scapulae.

Figure 9-19 Individual HTH 659 with circular wound in subscapular fossa with fracturing

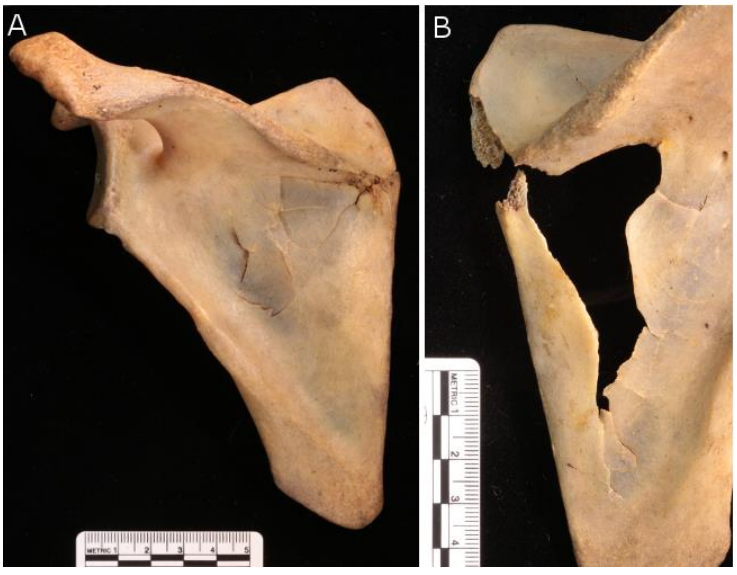

Figure 9-20 Individual HTH 1709. Left scapula with damage to infraspinous fossa (A), while right scapula shows damage to infraspinous fossa and vertebral border of scapula spine and missing segments (B)

9.6.5 Ilium

The ilium also showed distinct entry and exit wounds. The entry was oval in shape with depressed bone fragments and small radiating fractures. The exit wound, had significant external bevelling.

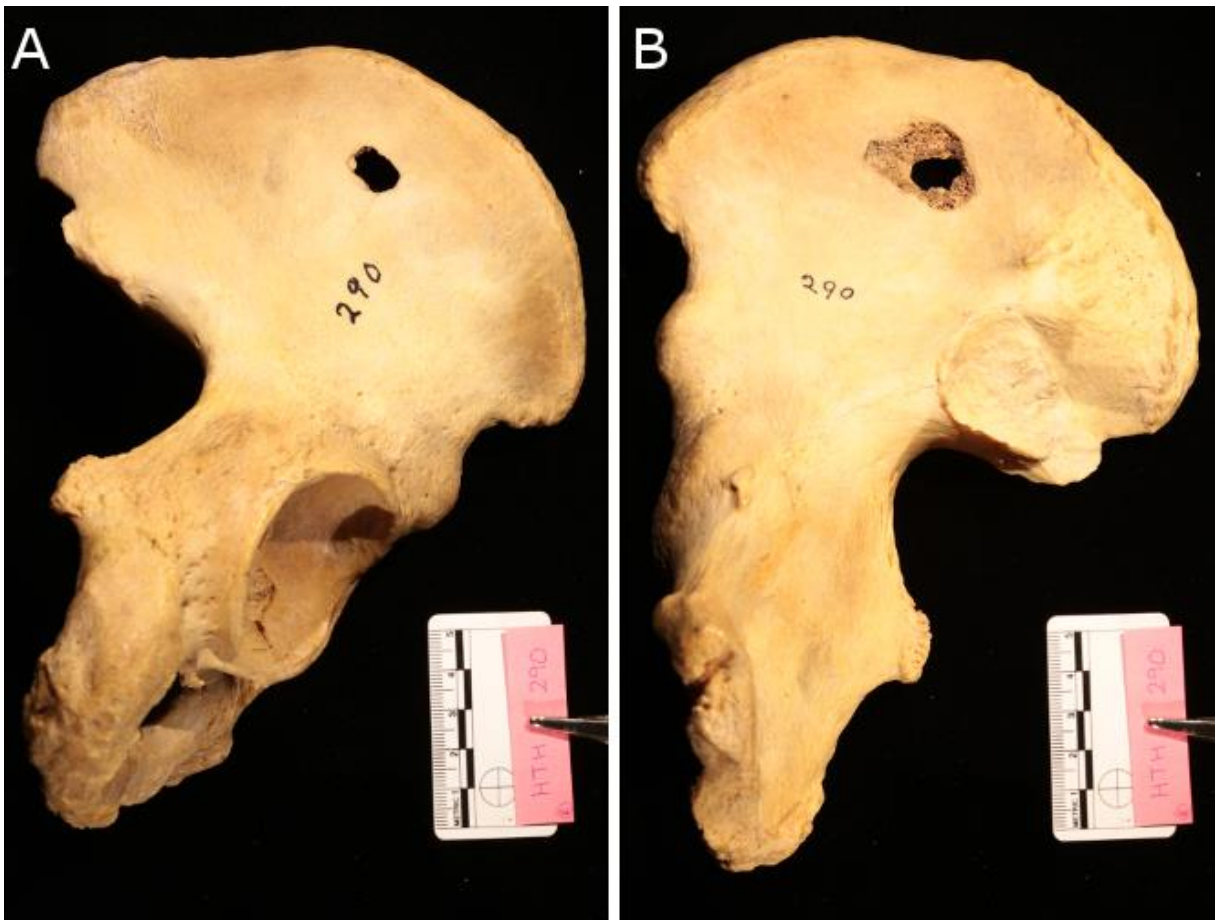

Figure 9-21 Individual HTH 290 with wound on left ilium displaying circular wound (A), and clear exit wound with external bevelling (B)

9.7 Discussion

When firing a weapon at an individual, the majority of people will aim for the centre of mass, this being the thorax and abdomen region or torso. In classical training of military and police, the trainees are instructed to aim at the centre of mass of the body, which is located in the torso region. Within the torso are major organs and thus death is highly likely. However, along with the soft tissues, there are also bony structures including the sternum, vertebrae, ribs, scapula, clavicle and lower down the pelvis. The projectile is therefore often to produce skeletal trauma to these regions in the victim. However, it has been found that a bullet can pass through the intercostal spaces without leaving evidence on the bone (Langley, 2007). The analysis of soft tissue, when available, can determine many details about an individual's death, however, the analysis of the skeletal elements by a forensic anthropologist can provide additional support and is critical when only skeletal material is available.

Unlike soft tissues, bone offers more resistance against penetration by a bullet due to its composition, hardness, density and strength (Bartlett 2003; Janzon et al. 1997; Stefanopoulos et al. 2015). Due to its nature, bone tissue cannot absorb the energy transferred from a bullet to the extent in which soft tissues do. Instead, bones act like a brittle material (Kieser et al. 2014; Stefanopoulos et al. 2015), when the stress/strain is beyond that they can rebound from, fractures occur. Ballistics fractures fall within sudden force and high speed category (Chapman 2007; Hamblen et al. 2007), and such fractures could result from direct impact of projectiles with excessive speed or a projectile at a vulnerable point in the bone (Byers 2015; Chapman 2007). The force exerted must be greater than what the bone can withstand and often occurs with projectiles moving at velocities ranging between 61 and 171 metres per second. When this occurs, the bone will fracture (DiMaio 2015; Harvey et al. 1962; Sellier and Kneubuehl

1995). Huelke et al. (1968) found that for visible damage of the bone, a velocity of 213.36 m/s or more was required.

The skeletal wounding potential of a projectile will depend on numerous factors associated with the projectile itself as well as the bone which it makes contact with. These will affect the characteristics of the injury. The type of bone which the bullet contacts will affect the characteristics of that injury. Bone consists of, on a molecular level, collagen and a calcium phosphate (hydroxyapatite) which give it its flexibility, strength and rigidity. Bone can be categorised according to the shape (i.e. long, short, flat, irregular). Projectile injuries to the flat bones, such as the cranial vault, have the distinguishing feature of bevelling which can determine the entry and exit wound (Chapman 2007; DiMaio 2015; Quatrehomme and İşcan 1997, 1998a,b, 1999). The ribs have an oval elongated cross-section, and contain some tubular properties, although the entire inside of the rib is comprised of trabecular spongy bone. Wounds in the ribs may also show bevelling or flaking in the direction of bullet travel, as seen in (Figure 9-9, Figure 9-11 and Figure 9-15). This bevelling appearance of wounds is critical in determining the direction of travel of a bullet, and as seen in this study, can be seen in the ribs (Figure 9-9, Figure 9-11 and Figure 9-15), sternum (Figure 9-5), ilium (Figure 9-21). It is less seen in the scapula (Figure 9-19 and Figure 9-20), potentially due to the thin nature of this bone compared to the thicker sternum which has a greater trabecular bone content (Tersigni-Tarrant and Shirley 2012).

The direction in which the force of the bullet penetrates the bone will also determine the characteristics of the wounds. If a bullet penetrates the thorax cavity, travelling through the intercostal spaces, no bone defects may appear. However, if the bullet contains a significant amount of energy which is deposited into the surrounding tissues by Newtons Laws, a temporary cavity will occur which may cause fractures in the midshaft of the ribs (Figure 9-15,

Figure 9-17 and Figure 9-16). Unlike in soft tissues, the temporary cavity in bone tissue is not followed by the collapse of the cavity, rather the lack of elasticity causing a pulverisation effect to the bone (i.e. fracture) (Huelke et al. 1968; Janzon et al. 1997; Stefanopoulos et al. 2015). The transfer of energy to bone in ballistic injuries is less understood (Kieser et al. 2014; Molde and Gray 1995; Stefanopoulos et al. 2015), in comparison to that in soft tissues. The soft tissues, due to their elastic properties, are able to absorb the energy transferred and revert back to their normal state, unless their elasticity is overcome. In bones, this elasticity value is less than that in soft tissues i.e. less energy is required to fracture the bone and it acts in a brittle manner. The amount of energy transferred to bone is influenced by the amount of contact time between the bullet and the bone, and this is inversely proportional to the velocity. Thus, a bullet travelling with low velocity will have more contact with the bone compared to a high velocity bullet and therefore it is possible for these slow bullets to cause more damage (Rothschild 2011; Stefanopoulos et al. 2015). However, high velocity bullets can have an explosive effect where when penetrating soft tissues indirect fractures are caused to nearby bones (e.g. ribs or long bones) due to the expansion of the temporary cavity and the transfer of high energy (Clasper 2001; Hollerman et al. 1990; Humphrey and Kumaratilake 2016; Janzon 1983; Mellor 1994; Stefanopoulos et al. 2015).

A bullet may also contact the bone at an angle, such as with the scapula (Figure 9-20), and the bone will fracture in a comminuted way. When this occurs, the way the bone fractures (e.g. heaves outwards) can be used to determine the direction in which the bullet travelled. When the bullet penetrates any bone perpendicular to the surface, it is highly likely that the bullet will completely penetrate the bone, and a clear entry/exit wound will appear. This could occur here in the sternum (Figure 9-5), scapula (Figure 9-19), ilium (Figure 9-21). When the bullet penetrates the ribs, perpendicular, it may not make contact with the whole rib and therefore produce a nick in the rib. This nick will be oval/circular in shape, mimicking the shape of the

bullet (Figure 9-6 to Figure 9-11). If high energy, the rib may also fracture along the length of the rib (Figure 9-8, Figure 9-12, Figure 9-13 and Figure 9-14).

Fractures in the ribs, and also the vertebrae, may also be due to the passing of a bullet in close proximity to the bones, however not directly penetrating them. This would possibly only occur if the energy of the bullet is high enough to transfer the energy to the surrounding tissues and overcome the strength of the bone. With the vertebrae, as also found by Langley (2007), there is no clear entry or exit wounds, and the vertebral body often is comminuted (Figure 9-1 to Figure 9-4). Secondary missiles occur when minute fragments from the impacted bone cause their own permanent cavity. This can also occur with fragmenting bullets. This causes further trauma to other portions of the body that can magnify the damage beyond that of the simple drilling effect of the bullet itself (Harger and Huelke 1970). These are most often seen through radiographic studies (Amato et al. 1989). It has been found through experimental studies that the secondary fragments (e.g. bullet fragments, jacketing and bone shards) have the same possibility of lethality as the original bullet. The amount of damage increases if the impact velocities are great (i.e. 243 m/s) (Harger and Huelke 1970). It could be presented as jagged edges, blown out fracture edges, large quantities of bone loss (Robens and Küsswetter 1982).

The fractures to the ribs may also be able to be described by the general types of fracture terminology based on the pattern of the fracture which reflects the type of force acting on the bone. For example, transverse fractures where the bone fractures perpendicular to its long axis under tension occur often (Figure 9-17 and Figure 9-16), oblique fractures where a 45 degree angle to its long axis under bending and compression occurs (Figure 9-16), spiral fractures, often occurring in long bones, have been noted in the rib cases (Figure 9-8). As ribs and shafts of long bones have a shape of oval tube, the force acting on these bones in torsion will create an oblique-like fracture which encircles the axis of the ribs. An interesting find was in one case

(Figure 9-14) where a fracture similar to a butterfly fracture occurred. The bone is penetrated by a bullet, where the force is a combination of tension, compression and bending creating a nick in the bone directly from the bullet and a triangular fragment and two segmented pieces (Figure 9-14). This often occurs in long bones (Symes et al. 2012) and in blunt force trauma. The force acting on in this type of wound produces angulation fractures (Ubelaker 1995).

As with gunshot wounds to the cranium, bevelling is a distinguishing feature of entrance and exit wounds. This characteristic has been seen here on some of the ribs (Figure 9-11) as well as on the ilium (Figure 9-21). The heaving of a fracture in a particular direction or displaced fragments of bone can also determine the direction of travel (e.g. Figure 9-15).

9.8 Conclusion

It is critical to understand the wounding patterns associated with projectile trauma to the torso region as this is the centre of the mass and is often targeted. The fracture patterns on the ribs can be used to determine the direction of travel of the projectile. Further analysis of more specimens will provide a greater understanding of these wounding patterns and controlled experimental studies may lead to the development of a bone simulant which is able to be used in these experiments.

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Part FOUR

Part IV provides a summary of the results and discussion of the PhD research. This part of the thesis also provides the conclusions and future directions of the research.

Summary of Results and Discussion

The absence of significant variations in entry velocities of the projectile, between firings in either the high or medium velocity experiments (Chapter 3, 5, and 6) confirmed the reproducibility of the entry velocities. The use of a remotely operated firearm, fitted to a fixed mount with specially built ammunition, which contained a cartridge that allowed adjustments to the amount of gun propellant assisted with firing the projectile without significant variations in velocity between firings. Using a fixed distance (10 metres) between the muzzle of the firearm and the test porcine organ/simulant allowed the frangible 3D printed sabot to separate from the steel spherical projectile before penetration of the test porcine organ/simulant. In addition, the use of a non-deforming steel spherical projectile encased in the frangible 3D printed sabot eliminated the uncontrolled effects associated with bullet tumbling, breakup and/or distortion due to their constructions.

Due to these methods the direct effect of the entry velocity of the projectile in causing injuries to the test porcine organs (heart, lung, liver and kidney) and organ simulants was determined. The reproducibility of the results made it possible to compare between experiments. The determination of the amount of the single base gun propellant (nitro-cellulose) required to generate entry velocities of 900 m/s and 500 m/s of projectiles and construction of the special frangible 3D printed sabot were carried out in a previous pilot study.

The significant reduction in entry velocities or the calculated energy of the projectiles during their passage through the tested porcine organs (i.e. from entry to exit) (Chapters 3 and 5) indicated that the size of the organs used (50mm cubes) was adequate to investigate the effects of the projectile and its entry velocity in causing the injuries. Furthermore, the use of zip-lock plastic bags to enclose the organs did not significantly affect the penetration ability of the projectile or the velocity loss (Chapters 3, 5, and 6) as the bags were used with each tested

organ and simulant. The bags helped to attach the organs to the firing setup, assisted in keeping the organs in the upright position, minimised distortion of their cubic shape and may have minimized dehydration of the organs. The amount of injury and the reduction in entry velocity or the energy of the projectile penetrating through the tested organs depends on the bio-physical properties (i.e. elasticity, density, viscosity) and the penetration depth of the organs, as well as the size, energy, momentum and construction of the projectile (Stefanopoulos et al., 2014; Chow, 2001; La Garde, 1916). The use of the same size steel spheres (6.35-mm-diameter and mass 1.043 g), same size organ cubes, pre-determined amounts of gun propellants and the same firearm allowed the determination of the direct effects of the tissue composition of each organ in reducing the entry velocity or energy of the projectile penetrating through the organ. This also allowed the comparison of results between the two tested velocities.

The core body temperature of the human body is 37°C. Early understanding was that the bio-physical properties of body organs/tissues changed with variation in temperature. In scientific research the common practice is to harvest body organs immediately after slaughtering the animal and refrigerate them until experimentation or use directly after slaughter. In some ballistic experiments the organs have been re-heated to 37°C and used for testing to simulate the bio-physical properties of live organs/tissues. In the experiment that was conducted to investigate the effect of the temperature of porcine organs in reducing the entry velocity or energy from the projectiles passing through these porcine body organs (lungs, livers kidneys and hearts), the findings indicated that the reduction in velocity of the projectiles passing through the organs at room temperature (16°C) was not significantly different from those of projectiles passing through organs at core body temperature (37°C) (Chapter 5). These findings indicated that the bio-physical properties of the organs did not change during the period of re-heating the organs from 16°C to 37°C.

A second experiment was conducted to investigate the morphological changes that could occur during the period of re-heating the body organs from refrigeration temperature to 37°C. Findings of this experiment indicated that no morphological changes occurred (i.e. at light microscope level) in any of the organs investigated (Chapter 4), thus confirming the findings of the first experiment (Chapter 3). In the morphological investigation, porcine organs were harvested immediately after slaughter and refrigerated. The process of cooling organs rapidly after harvesting has been used in organ transplant procedures (Belzer & Southard, 1988). Therefore, the cells and tissues of the porcine organs would have remained unchanged in the experiment described in Chapter 3 and the bio-physical properties of organs behaved similarly at 16°C and 37°C.

In high velocity experiments, the percentage energy loss from the projectile penetrating the porcine organs determined the following:

- The results in the lungs were significantly lower than that in the livers, kidneys, hearts and the three tissue simulants (Chapter 6). This suggests that the tissue composition of the lung is less dense than that in the liver, kidney, heart and the tissue simulants. Therefore, these three tissue simulants did not represent the bio-physical properties of lungs. A new simulant that could represent the lungs is required to be tested or developed.
- The results in the livers were similar to that from projectiles penetrating through the hearts. These in turn were not different from the percentage energy loss of projectiles entering the 10% ballistic gel at 4°C and 20% ballistic gel at 10°C (Chapter 6). These two gelatine simulants represented the bio-physical properties of both porcine liver and heart.

- The results in the kidneys were similar to that from projectiles penetrating through the clear gel at 16°C (Chapter 6), thus this simulant represented the bio-physical properties of the porcine kidney.

The reductions in entry velocity and energy of projectiles that entered porcine organs at medium velocity were significantly lower than those of projectiles entering porcine organs at high velocity (Chapter 5). Projectiles travelling at a medium velocity penetrate a smaller distance of tissue compared high velocity projectiles in the same time frame. Therefore, the high energy and the greater distance of penetrated tissue in high velocity projectiles transfers more energy to the tissues in the same given time.

In medium velocity experiments, the percentage energy loss from the projectile penetrating the porcine organs determined the following:

- The results in the lungs were similar to those that penetrated the liver, kidney, heart, 10% ballistic gel at 4°C and clear gel at 16°C
- The results obtained for the liver were not different from the kidney, heart, 10% ballistic gel at 4°C, 20% ballistic gel at 10°C and clear gel at 16°C. However, the percentage loss of energy from the projectiles that penetrated the kidneys was different from those that penetrated the hearts and 20% ballistic gel at 10°C; while findings for the kidneys were similar to those of the 10% ballistic gel at 4°C and clear gel at 16°C; and findings for the hearts were similar to those of the 10% ballistic gel at 4°C, 20% ballistic gel at 10°C and clear gel at 16°C (Chapter 6).

The findings of a low velocity projectile interaction with porcine organs and simulants experiment were different from those reported here for medium and high velocity projectiles (Maiden, 2014). The methods used in the low velocity study were different from those used in the current studies. The firearm, ammunition, size and weight of the projectile, size of test

organs and organ simulants and the distance between the muzzle of the firearm and the test organ were different from those used in the current experiments (Maiden, 2014). Thus, the findings of the medium and high velocity experiments could not be directly compared with those of the low velocity experiment without taking into consideration the differences in methodologies used.

The analysis of the cranial and infra-cranial projectile injuries of the two skeletal collections in the United States of America revealed that projectiles caused wounds to the bones with distinctive characteristics (Chapters 7-9). In gunshots to the cranium, the impact of the bullet with the bone caused shearing-off of a piece of bone in front of the projectile. Depending on the energy of the projectile, it may or may not exit the skull from the opposite side. Low velocity projectiles might not have adequate energy remaining to penetrate through the bone again after initial penetration of the bone and travel through the brain tissues.

As the projectile penetrates bone, a fragment is sheared-off in front of the projectile. As this bone fragment is sheared-off it may leave a bevelled mark on the surface of the bone, in the direction of projectile travel. Hence, at the entry site of the projectile into the skull, the bevelling will be internal, while at the site of exit of the projectile from the skull, the bevelling will be external (Chapters 7 and 8). When present, bevelling will assist in identifying the entry and exit wounds of the projectile and as such the projectile path. Uniform or non-uniform distribution of the bevelling surrounding the entry and/or exit wounds may indicate that the bullet entered/exited the skull perpendicular or at an angle to the surface of the bone.

The anatomical location of the wound in the cranium and the number of wounds may assist in the differentiation between suicide and homicide. Bullet entry wounds located at the right temple are often associated with suicide. Multiple wounds are more than likely associated with homicide.

Extensive fracturing of the cranium including radiating and concentric fractures results from high energy projectiles. Fractures will follow the path of least resistance and terminate at a previously formed fracture or suture, allowing sequencing of fractures to occur (Chapter 8).

Gunshot injuries of the thorax and abdomen could involve the vertebral column, ribs, sternum, scapula and pelvic bones. Injuries commonly caused by projectiles in the infra-cranial skeleton are as follows:

- Vertebrae - shattering of the vertebral body and fracture of the pedicle (Chapter 9)
- Sternum - circular holes with radiating fractures that spread away from the wound. Flaking may occur in the direction the projectile travelled (Chapter 9)
- Ribs - small nicks in the bone, fractures of the mid-shaft, and butterfly-like and comminuted fractures (Chapter 9)
- Scapula - shattering of subscapular fossa and wounds of variable appearance due to the irregularity and non-uniform thickness of the scapula (Chapter 9)
- Ilium - open holes with internal or external bevelling as in cranial wounds (Chapter 9).

In all the above thoracic and abdominal bones, except vertebrae, the entry and exit wounds could be identified, thus the direction of travel of the projectile and the path could be determined.

Due to the nature of the calcified matrix of bone (i.e. the density and hardness) the projectile will meet with greater resistance as opposed to soft tissue. The inherent brittleness of bone causes it to fracture with the transferred projectile energy. Simultaneously, the transferred energy radiates along the bone away from the site of impact. Fractures follow the path of least resistance in the bone matrix. Such fractures could extend up to previously formed fractures or syndesmoses (i.e. sutures) and terminate, allowing the sequencing of fractures (Chapter 2).

Impact of a projectile at an angle to the bone surface will cause more energy to radiate in one direction, thus the radiating fractures could be skewed to that direction.

The analysis of the above discussed factors may generate adequate information to reasonably assist in determining the manner in which the death occurred (i.e. suicide, homicide or accident).

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Conclusions

1. In ballistic research, where the aims are to investigate the effects of projectiles in causing injuries to the human body, the following methodology could be used for the standard methods in ballistics research to generate accurate and reproducible results:
    - Specimens (organs or simulants) cut into 50mm cubes and enclosed in zip-lock plastic bags mounted in an appropriate device to ensure perpendicular penetration by the projectile
    - A remotely operated firearm, fitted to a fixed-mount with a distance of 10m between the muzzle and specimen
    - Specially prepared ammunition to allow for varying projectile velocities (i.e. adjustable amounts of gun propellant)
    - The projectile as a steel sphere encased in a frangible 3D printed sabot to eliminate the uncontrolled effects associated with bullet tumbling, breakup and/or distortion
    - Use freshly harvested porcine organs immediately or after refrigeration. Re-heating the organs to the core body temperature of 37°C is not necessary.
2. The current tested simulants cannot represent the bio-physical properties of the lungs. Manufacturing of a new organ simulant or testing of other existing simulants that could represent the bio-physical properties of the lungs and testing using the above methodology is required.

3. Accurate analysis of the characteristics of skeletal wounds caused by projectiles can assist in determining the manner of death.

Future Directions

1. Conduct experiments with low velocity projectiles (~200m/s) using the developed methods and compare to the results of high and medium velocity projectiles.
2. Using the methods established, identify or develop organ simulants that could represent the bio-physical properties of all the organs and tissues of the human body.
3. Hospital data bases of digital CT scans and MR images (i.e. non-pathological or abnormal), with adherence to ethical protocols, can be used to determine the dimensions of the internal organs and aid in the construction of 3D moulds of these organs.
4. Construct 3D physical anatomical models and/or computer programmes (e.g. Finite Element Analysis) with the data obtained from the organ and simulant experiments, that could be used in future ballistic research to benefit:
    - manufacturing of new ammunitions and firearms
    - manufacture of body armour and other armour used in warfare
    - investigations into behind body armour injuries
    - planning of battle strategies to minimise injuries to the armed forces and maximise injuries to the enemy
    - planning of procedures for the treatment of injuries caused by different types of ammunition and firearms.

Appendices

Appendix A: Chapter 1 Manuscript

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Ballistics and anatomical modelling – A review

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ABSTRACT

Ballistics is the study of a projectiles motion and can be broken down into four stages: internal, intermediate, external and terminal ballistics. The study of the effects a projectile has on a living tissue is referred to as wound ballistics and falls within terminal ballistics. To understand the effects a projectile has on living tissues the mechanisms of wounding need to be understood. These include the permanent and temporary cavities, energy, yawing, tumbling and fragmenting. Much ballistics research has been conducted including using cadavers, animal models and simulants such as ballistics ordnance gelatine. Further research is being conducted into developing anatomical, 3D, experimental and computational models. However, these models need to accurately represent the human body and its heterogeneous nature which involves understanding the biomechanical properties of the different tissues and organs. Further research is needed to accurately represent the human tissues with simulants and is slowly being conducted.

Contents

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1. Introduction

Ballistics is the study of projectiles in motion. This includes the investigation of the projectile and the changes occurring during its motion from the barrel to its target. This motion can be divided into four stages.

i) Internal ballistics – the study of the projectile during the acceleration phase within the barrel of the firearm
ii) Intermediate ballistics – the study of the projectile within the first few centimetres after the projectile leaves the barrel
iii) External ballistics – the study of the projectile's flight from the first few centimetres after leaving the barrel to the target
iv) Terminal ballistics – the study of the projectile during the penetration of the target (i.e. solid material) [1–4]. If the target is a living animal (i.e. including humans), the study is referred to as wound ballistics and investigates the effects of interaction of the projectile and the tissue [3,5–8].

Ballistics related research has been conducted to investigate the flight patterns of different bullets, the lethality of ammunition types and their effects on targets and to develop body armour and protection methods against different types of ammunition.

2. Overview of firearms and ammunition

Thousands of different types of firearms have been manufactured, but they are broadly grouped into three classifications: rifles, handguns and shotguns.

2.1. Rifles

Rifles have a distinctively longer barrel and imparts high energy and velocity to a bullet, thus has a greater wounding potential at long distances than the other two categories [1]. Rifling, which involves cutting of spiral parallel grooves into the inner surface of the barrel (i.e. the bore), is an important feature. This feature, causes a rotational motion of the bullet along its longitudinal axis, creating a gyroscopically stabilised spin on the bullet during its flight. Thus, increases the range and accuracy and also reduces the tendency of the bullet to tumble in flight [3,5,9–12].

2.2. Handguns

Handguns have short barrel lengths, are concealable and are commonly used at close ranges [13]. The amount of energy generated is low and causes minor temporary cavitation, thus the wounding is limited to the permanent wound cavity caused by the bullet. However, there is a wide variety of expanding and deforming bullet types, which maximises the wounding potential within the permanent cavity [13].

2.3. Shotguns

Shotguns generally do not have riffled barrels and are called smooth bore firearms. They are designed to fire multiple shot-shell pellets instead of a single bullet, thus are commonly used for bird hunting or firing at fast moving targets. When shot-shell pellets are fired, they spread out as the distance from the barrel increases. This spread of pellets could be reduced by installing a choke mechanism at the end of the barrel to reduce the diameter of the nozzle. The wounding characteristics of shot-pellets are unique due to the type of ammunition used. At close range, the pellets stay grouped and act as one single mass and create a significant and distinct wound. Most often, the pellets will not exit the body, as they lose their kinetic energy and momentum rapidly. At a greater distance, the pellets spread and cause multiple injuries as they enter the body. However, each pellet will lose kinetic energy due to air drag and thus may not penetrate tissue at extreme ranges [13].

3. Mechanisms of bullet wounding

The mechanisms by which a bullet causes trauma is determined by the shape, construction, mass and velocity of the bullet, and the bio-mechanical properties of tissues, such as elasticity and density [11,14] (Fig. 1). Mass and velocity of a bullet determine

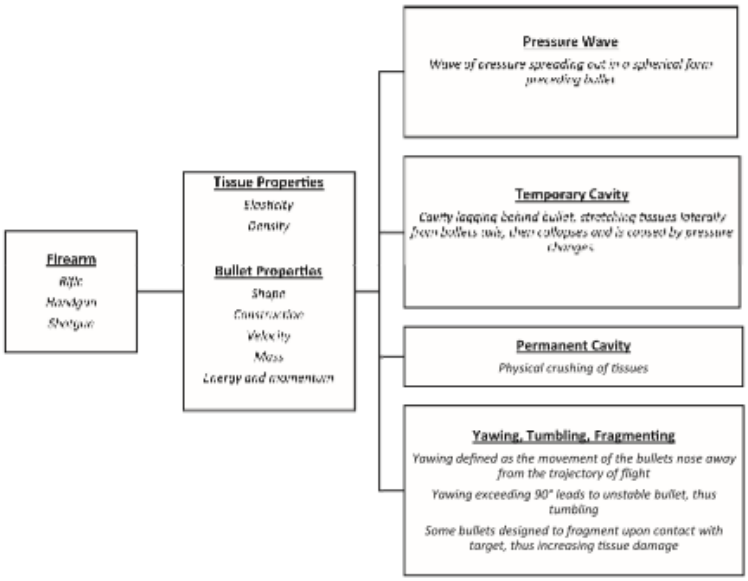

Fig. 1. Flow chart of the factors determining the wounding in tissues.

the potential to destroy tissues, while the shape and construction account for the amount of tissue damage [15,16]. Mechanisms for tissue injury resulting from bullets have been investigated extensively by European and American medical researchers [13,17–23].

The factors that determine the extent of wound damage are as follows:

3.1. Density, viscosity and elasticity of tissues

The density, viscosity and elasticity are the properties of a tissue that will determine the amount of injury that could be caused by a projectile [5,12,24]. The human body is heterogeneous. Stress-strain response of different organs/tissues (i.e. including internal organs) [25]; elastic properties of ribs, sternum, bone replacement materials [26] and vertebral column [27,28] have been determined and they vary among the organs/tissues. Therefore, the amount and the type of injury caused by a projectile of the same shape, size, mass and velocity to an organ/tissue in the body may vary according to the variation in the physical properties of them. The wounding potential of a projectile is also proportional to the amount of energy the projectile delivers to the tissues. However, the net effect of an injury caused by a projectile on an individual may depend on the specific functions lost to the body by the wound caused to the organ/tissue [5,29].

3.2. Energy and momentum of the projectile

A widely accepted determinant of the wounding power of a bullet is the mass and velocity of the bullet, thus its momentum. The latter, is directly proportional to the amount of energy transferred into the tissues by the bullet, during its passage through the tissue [30]. Some research believes it to be the most important factor to cause tissue damage [10,31], however other mechanisms can influence the wounding potential. Momentum is an important factor that could be used to estimate the wounding potential of a bullet, but has been found to underestimate the wounding potential [32]. The amount of energy carried in a bullet is dependent on the amount of gun powder ignited to fire the bullet, and the distance from the muzzle of the firearm to the tissues. A high powered firearm fired closely to the tissues will transfer more energy into the tissue than a bullet that has spent a significant amount of time in flight before interacting with the tissues, i.e. the bullet has lost energy during the flight [5,33]. A bullet could dissipate a small amount of its energy, if it passes directly through the organs/tissues and exits from the other side of the body. On the other hand, a bullet that stops within the body will transfer all its energy into the organs/tissues of the body. A bullet that is designed to fragment on impact will be an example of a projectile that will transfer its energy into the tissues and will most likely stop within the body. This is in comparison to a bullet that is designed to stay intact during its passage through tissues, thus exits the body retaining a large proportion of its energy and continues in flight [5].

Projectiles have been classified into low and high velocity categories [10,34], however, the definitions of the two categories vary among publications. Bellamy and Zajtchuk [35] have defined high velocity as speeds exceeding 600 m/s, while Rozen and Dudkiewicz [10], have described it as speeds greater than that of sound in air (1100 feet/s or 335 m/s). The velocity of projectiles fired from military rifles ranges from 700–950 m/s and that from handguns ranges from 250–370 m/s [5,12]. Using the velocity of bullets, shotguns are generally classified as low velocity weapons [10,36,37]. However, the latter firearms cause major damage to bones, joints and soft tissues, including nerves and vascular structures due to the spread of the shot pellets [10,36,37]. These findings may indicate that both high and low velocity weapons cause severe damage to organs/tissues of the body. The use of impact velocity of a projectile, as the sole indicator of the extent and severity of an injury may be misleading [5,31,38–42]. For example, a large calibre bullet that has a large mass but travelling at a slower velocity will still cause massive tissue damage through the formation of a permanent cavity i.e. the bullet crushes the tissues.

3.3. Permanent cavity

A permanent cavity or wound track is formed as the bullets frontal surface physically crushes the tissues it penetrates. The damage to the tissues is permanent and varies with different calibres of bullets. The path formed is not necessarily a straight line due to differences in tissue densities and contact with bones that causes the bullet to deflect [1,12,14]. The size and shape of the permanent cavity could be affected by three mechanisms; i) projectile yaw, where at the point of strike, the bullet yaws by 90° and travels with its long axis striking the tissues, leading to a cavity that is 10–14 times the size of the diameter of the projectile [3,6,10,14]; ii) bullet deformation, where mushrooming or flattening of the tip increases the bullets diameter and iii) bullet fragmentation, which may occur with high velocity bullets or on contact with bones [14,20,43,44] (described in detail later). A permanent cavity is usually surrounded by an area of cellular and endothelial damage (i.e. in the skeletal muscles), bruising, necrotic tissues or an area of haemorrhage [5,35].

3.4. Pressure wave and temporary cavitation

A pressure wave is formed at the point of impact of the bullet with the organ/tissue and spreads out rapidly in a spherical form preceding the bullet. It has been described to be travelling at approximately the speed of sound in water (~1500 m/s) [1], which is different from the speed of sound in air (335 m/s) [12]. These waves progress through tissues and dissipate the energy they are carrying into the surrounding structures [45,46]. The ability of this wave to cause tissue damage is disputed by some authors, as their duration is short (~2 μsec), thus often deemed to play no part in wounding [14,18,38,47–49]. However, injuries to the capillary endothelium in animal tissues [50]; traumatic brain injury [51], central and peripheral nervous system [50] have been linked to the pressure wave. The magnitude of the pressure wave has been measured to be as high as 60 atm [12]. Evidence is increasing to support the theory of remote injuries by this pressure wave [52,53]. This pressure wave could be closely linked to the temporary cavity, which is generally seen only in high velocity bullets. One study [50], found that the pressure waves come in small bursts and oscillates, moving through the body with a velocity that is similar to that of the sound in water. They also found that the function of endothelial cells in smaller vessels were influenced by these waves, however it is unknown if it is a temporary or permanent effect.

Approximately 1–4 milliseconds after the bullet hits the body, a temporary cavity, lagging behind the bullet, is formed in body tissues. This is due to the movement and interaction of the bullet with the tissue causing changes in pressure, which leads to dislocation of tissues laterally from the bullets axis by stretching [1,5,10,17,29,33,54–56]. It could reach a size of up to 11–12.5 times the diameter of the bullet rapidly and has been described as a significant aspect of the wounding process [22,57,58]. This cavity collapses just as quickly as it is formed and other outward motions occur in elastic tissues and continues even with the bullet

used in ballistic wound research. In such research, controversial findings have been reported in relation to bio-mechanical properties (i.e. particularly tensile strength) of organs/tissues. These findings ranged from no effect on biomechanical properties [75,76], to decrease in tensile strength [77–79], and increase in tensile strength and elastic modulus [80–82]. The latter changes have been linked to dehydration and redistribution of water during freezing [80–82]. Histological changes seen in frozen-thawed tissues are loss of cellular fluid, cell shrinkage, extracellular fractures and haemolysis [74,83–85]. Furthermore, in collagen rich tissues, the collagen fibres became more prominent with freezing and such tissues resisted deterioration better than other soft tissues with low collagen content [85]. In addition to organs/tissues from unfixed cadavers, those from embalmed cadavers have been used in ballistic wound research. Embalming alters the biomechanical properties of both bones and soft tissues [86], thus not suitable for ballistic research. Cadavers used for research are generally from body-donation programmes. Thus, they are usually from elderly people. The elasticity and tensile strength of organs/tissues alter with increasing age [71,74,87], thus such organs/tissues will not be suitable for ballistic wound research. Furthermore, use of human cadavers for ballistic research could generate ethical issues. Taking these into account, the use of human cadavers for ballistic research may be discouraged [88–90].

4.1.2. Animal models

In ballistics research, live and deceased animals including horses, cattle, goats, sheep, dogs and pigs have been used extensively to conduct trauma studies [30,89–91], however are being less used today. The use of pigs as a substitute for human tissue is a common scientific practice as they are anatomically similar to humans. However, the skin and subcutaneous tissue of pigs are thicker than humans [89], thus pigs may not be an ideal substitute for humans in the investigations involving skin and subcutaneous tissues. Furthermore, the use of live large animals in ballistics testing or even other scientific research may cause ethical and moral issues. Therefore, their use must be carefully scrutinised.

4.1.3. Ballistic ordnance gelatine (10% and 20%)

Ordnance gelatine is a product derived from collagen proteins in animal products through a process of hot water acidic extraction and available in consistencies between 50 and 300 Bloom (strength and stiffness measure of gelatine) [89,92–94]. The strength and stiffness is not solely determined by the Bloom Number, but also the concentration and temperature during preparation [89].

Ballistic ordnance gelatine is currently an accepted human soft tissue simulant used in ballistic testings, if it is calibrated to a set standard [59]. Calibration only began in the mid-1980s when it was recognised that uncalibrated gelatine had deficiencies [88,100,101]. Calibration of the gelatine was carried out using a small number of samples, thus a disagreement exists in relation to the results [23,53]. Some tests are being conducted into the resemblance of these simulants to that of porcine tissues [103,104]. It has been found to replicate the mechanical properties of skin, fat, fascia and muscle tissue of a porcine thigh [59]. As porcine tissues are similar to that of human tissues, it has led researchers to accept that the tissue penetration characteristics by projectiles is the same as what would occur in the soft tissue of humans [59,65,88–90,95,101,102]. It is advantageous as it allows the visualisation and photographic representation of the projectiles wound profiles [88,96,97] as well as reproducing elasticity similar to some human tissues [2]. However, it lacks the bio-mechanical properties of other tissues and organs, thus may not accurately represent all soft tissues of the human body [98]. Furthermore, radial cracks occur, when the gelatin is penetrated by a bullet, which is different to the behaviour of the human tissues in response to penetration by a bullet [88]. This could create difficulties in translating the findings generated in ballistics ordnance gelatine to real wounding occurring in the human body [90,99].

The North Atlantic Treaty Organisation (NATO) currently uses ordnance gelatine 20% at 10 °C ± 2 °C with a Bloom Number between 250 and 300, although there is no calibration standard for this concentration [65]. The FBI standard formulation of 10% at 4 °C is calibrated in accordance with the method developed by Fackler and is considered to be a better soft tissue simulant than the NATO formulation [23,90,99,105]. However, both simulants vary and are being used in ballistics research.

Even though ballistic ordnance gelatine allows for the investigation of general tissue trauma resulting from projectiles without the ethical issues arising from the use of animals or cadavers, it only resembles porcine tissues, and in particular porcine thigh muscle. Thus, it does not accurately replicate the biomechanical properties and responses of the heterogeneous tissues and organs within the human body.

4.1.4. Synthetic tissue simulants – old and new

The use of synthetic tissue simulants in wound ballistics research is common. It is preferred over biological tissues as there are no ethical issues, the risk of infection is reduced, they allow photography and observation of how the projectiles cause injuries, particularly when the tissue simulants are transparent [38,88,89]. Soap, wet packs, and clay have commonly been used for research. The non-elastic nature of simulants such as clay and soap allow the maximum size of the temporary cavity to be viewed frozen in place, however the permanent cavity cannot be seen. Therefore, if these materials are solely used for wound ballistics research, invalid conclusions could be made [18,38,89,90,96,106–110].

New homogeneous synthetic simulants are being developed which address the issues occurring with ballistic ordnance gelatine [65]. Perma-Gel is a clear synthetic medium which is stable at room temperature and has similar properties to 10% ordnance gelatine at 4 °C. It can be reconstituted and is gaining acceptance, particularly among researchers in the USA [65]. Physically Associating Gelatine's (PAG) are also being developed in the USA and are derived from triblock copolymers, which exhibit deformation behaviours similar to ballistics ordnance gelatine and therefore, it is an alternative tissue simulant [111,112]. Another suitable soft tissue simulant is transparent gel candle, created from kraton and white paraffin oil and meets FBI calibration standards. This has an additional advantage of allowing improved photography of the permanent and temporary cavities due to its transparency. Additionally, this product is unaffected by bacterial contamination unlike ordnance gelatine [113]. However, these new synthetic simulants are still homogeneous and the findings in these simulants cannot describe all the wounding occurring in the human body and its organs.

4.2. Anatomical models

Simulating all the different organs and tissues within the human body is a difficult task. A lot of research has and is being conducted towards developing anatomical models for various reasons including motor vehicle collision investigations, projectile testings and the development of body armour. Simulating the human body involves producing synthetic bone, blood, organs, skin and muscles and can eventually lead to computational Finite Element Analysis (FEA) models. A summary can be seen in Table 2.

moving a distance away [20,56]. Similar to pressure waves, the tissue damage caused by the temporary cavity varies greatly according to the size, the anatomical location of the cavity and the direction of least resistance taken by the pressure wave in the tissue [19]. The temporary cavity may cause significant injury by exceeding the elastic limit of a tissue, i.e. in tissues with minimal amount of elastic elements e.g. brain, liver, kidneys, spleen, pancreas, capillaries. Tissues that are more elastic (e.g. muscles, lungs) are fairly resistant to damage from this cavitation process [14,18,20,21,55,59,60]. Temporary cavitation could fracture bones that are located some distance away from the path of the projectiles, particularly with high velocity bullets [6,11,12,14,18,57,58,61]. Yawing or tumbling in a projectile increases the size of the temporary cavity and tissue disruption. Vascular damage during temporary cavitation is extensive, small blood vessels such as capillaries are particularly affected [5,33]. Clothing has been found to increase the risk of an indirect fracture and a larger temporary cavity, potentially due to the clothing causing rapid yaw and possibly fragmentation of the projectile [54].

3.5. Yawing, tumbling and fragmenting

A stable bullet in flight is described as travelling nose-on with its central axis close to the trajectory. If the axis deviates in a tangent from the trajectory, it is referred to as yawing [5,14,57]. Rifles and handguns significantly reduce this yawing during flight by having rifling within the barrel of the firearm, thus promoting spin on the bullet to stabilise it during the flight. The stabilising action is overcome when it enters a higher density target, such as the human body [14,29,35,62]. If the yaw is exceeded approximately by 15°, it causes the bullet to become unstable [22]. Once unstable, yawing will continue and eventually exceed 90°, at which point the bullet will begin to tumble [5,35]. Once the gyroscopic stability is lost, drag increases leading to increases in permanent and temporary cavitation's, thus increasing the tissue damage [63–65]. In this situation, in some bullets, the entire length of the projectile acts against the tissues, increasing the amount of injury [1,5,11,13,22,35,38,42,53,66–69]. It is common for some bullets such as 7.62 × 39 mm Soviet to yaw to 180° and travel base forward until it exits the tissues or its energy has dissipated [11,14,18].

Some bullets are designed to fragment or deform upon contact with the target, thus increasing potential tissue damage. If a bullet makes contact with bone, fragments of the bone can become secondary projectiles and increase the tissue damage [63,70]. When a tissue is perforated, it loses its elasticity and tears or becomes detached as it can no longer absorb the stretching caused by the temporary cavity formation [18,44,58]. Thus, an assumption can be made that a fragmenting or deforming bullet will create more tissue damage than one that is designed not to fragment [2,16].

4. Ballistics research

Wound ballistics research has important applications in commercial, military, civilian and medical areas relating to the human body. In this research, many aspects in relation to flight patterns of bullets, wounding potential of different ammunitions, and development of new ammunitions and protective body armour have been investigated. A summary of the current materials and the advantages and disadvantages associated with each material can be seen in Table 1.

4.1. Materials used for research

4.1.1. Cadavers

The aim of many ballistic research is to investigate ballistic wounds or wounding potential of bullets on human body, organs or tissues. In such research, the organs/tissues used should retain the normal/natural biomechanical properties, particularly the tensile strength. Thus, organs and tissues from fresh human cadavers have been used [65,71–74]. Similarly, organs/tissues obtained from frozen and thawed, un-embalmed human cadavers have also been

Table 1
Overview of the advantages and disadvantages associated with currently used materials in ballistics experiments.

Current Material	Advantages	Disadvantages
Human Cadavers	• Actual human organs/bodies	• Alterations in tensile strength, elastic modulus, biomechanical properties [75–82] • Dehydration and redistribution of water during freezing [80–82] • Loss of cellular fluid, cell shrinkage, extracellular fractures, haemolysis [74,83–85] • Elderly and tensile strength and elasticity increase with age [71,74,97] • Ethical issues arise with use of human tissues
Animals	• Wide variety of animals [30,89–91] • Well known pigs closely resemble human tissues	• Skin and subcutaneous tissue of pigs are thicker than humans [89] • Ethical and moral issues may arise
Ballistics Ordnance Gelatine (10%/20%)	• Derived from animal products [89,92–94] • Removes ethical issues • Accepted as a human soft tissue simulant mainly representative of a porcine thigh [59] • Transparent nature allows visualisation and photographic representation [88,96,97] • Elasticity similar to some human tissues [2]. • Calibration is needed to match physical properties of live porcine thigh tissues [88,100,101]	• Lacks bio-mechanical properties of all tissues and organs • Radial cracks occur when penetrated by a bullet [88] • Only represent porcine thigh • Affected by bacterial contamination, decomposition and short storage life [113]
Synthetic simulants	• No ethical issues • Risk of infection reduced • Allows photography and observation due to transparent nature [38,88,89] • Great variety in types [65,111–113] • Some can be reconstituted [65] • Unaffected by bacterial contamination [113]	• Do not replicate the heterogeneous nature of tissues/organs of human body • Some are non-elastic, thus not showing all wounding mechanisms [18,38,89,90,96,106–110]

Table 2
Summary of some anatomical models being used for various purposes.

Model	Reference	Details	Use	Advantages	Disadvantages
Dummy torso	[114,115]	Wood, water, plastic, foam lungs, water filled torso, pressure transduce in airways	Assessing hazards, biodynamics of impact associated with pressure loads	Measures pressure through airways	Not accurate representation of human body and organs
AUSMAN torso	DSTO	Made from polyurethane with a stainless steel rib cage	Blast investigations	Includes rib cage	Not accurate representation of human body and organs
Torso	Naval Research Laboratory (NRL)	Ordnance gelatine with rib cage enclosed Silicone gel for lungs	Non-penetrating ballistics impacts	Includes rib cage	Ordnance gelatine cannot represent all organs of torso
Test dummy	[116]	Ribs, spine, transducers	Non-penetrating ballistic impacts	Transducers	Not usable for penetrating ballistic trauma Excludes soft tissues and elastic and physical properties of organs
Physical surrogate torso model (STM)	[117]	Organs created from silicone gel	Non-penetrating ballistics impacts	Anthropometric values included Bones have tensile properties of cancellous bones	Not accurate representation of human body and heterogeneous nature of tissues/organs
Human torso Finite Element Model (HTFEM)	[118–120]	Finite Element Analysis (FEA) including heart, lungs, liver, stomach, ribs and sternum	Non-penetrating and blast research	Computer model including some properties of tissues and bone Pressure sensors in organs	Does not include all aspects of organs and anthropometric values
Sheep thorax	[122]	Includes lung, small intestine, large intestine	Blast impacts	Viscoelastic properties of the organs modelled	Does not include many critical organs Non-human
FEA model	[123]	Computer model	Frontal impact simulation	Realistic skeletal structure	No internal organs
FEA model	[27]	FEA computer model of thorax, abdomen, shoulder, head-neck of average adult male	Stress analysis under loading	Musculoskeletal structures and internal organs included Validated against post mortem human subjects	Not accurate for use in penetrating ballistics trauma
FEA model	[124]	FEA model of human thorax	Impact loading onto protective body armour from a projectile	Elastic properties for skeleton Viscoelastic properties for muscle Validated against cadaver tests	Non-penetrating ballistics trauma
Human Surrogate Torso Model (HTSM)	[119,120]	Torso model representing 5th male percentile in size	Trauma resulting from impact on ballistic resistant vests	Internal organs matched to density of porcine organs Replicate bone, cartilage, soft tissues and connective tissues	Does not include accurate anthropometric measurements of torso and internal size of organs Soft and hard tissue properties not all included Non-penetrating trauma only
The Visible Human Project	[130]	Thorax and abdomen computer model	Non-penetrating ballistic and blast injury evaluations	Detailed aspects of human body for anatomical study purposes	Non-penetrating trauma only
Frangible Surrogate leg model (FSL)	DSTO [72,125–129]	Constructed from materials that simulate bone, cartilage, soft tissues and connective tissue	Land mind wounds of lower leg	Anatomically accurate leg	Not torso and thorax

4.2.1. 3D modelling/experimental models

Anthropometric dummies of the human torso have been created for various research fields including blast tests, ballistics and car crash investigations. In earlier years, the dummies consisted of wood, water and plastic with foam for the lungs, water filled torso and included pressure transducers in the airways [114,115]. These models have developed over time with the Defence Science and Technology Organisation of Australia (DSTO) creating a torso model referred to as AUSMAN. This was made from polyurethane and a stainless steel rib cage, and used for conducting blast investigations. The Naval Research Laboratory (NRL) created a torso using ordnance gelatine with a rib cage enclosed. This torso used a different material (silicone gel) for the lungs. Crash test dummies have been created and developed for the use in non-penetrating ballistic impact testing [116]. Roberts, Merkle [117] created a physical human surrogate torso model (STM), which incorporated anthropometric values for the design of organs and bones. The bones were created to have tensile properties similar to that of cancellous bone and organs were created from silicone gel. These models have created a basis for anatomical modelling, but do not include all the aspects of the human torso and the bio-mechanical properties of the different organs.

4.2.2. Computational models

A mathematical computational modelling method, namely Finite Element Analysis (FEA), breaks down a problem into a finite number of simple problems using linear and non-linear equations in 2D and 3D computer programs. This technique has advanced medical research and allowed physical surrogates of the human body to be created for use in, but not limited to, non-penetrating

ballistic impact and blast research [118–120], along with modelling traumatic brain injuries [121]. In 1998 a 3D model of a sheep thorax that responded to blast impacts was created and included the lung, small intestine, and large intestine, all of which assumed linear viscoelastic properties [122]. Further research provided finite element models for frontal impact simulation including a realistic skeletal structure, but did not include the internal organs [123]. Eventually, models included both the musculoskeletal structures and internal organs [27,124]. A Human Surrogate Torso Model (HTSM) has been created to investigate trauma resulting from the impact on ballistic resistant vests [119,120] and represents a 5th male percentile in size, where the internal organs were fabricated to have the same density as porcine organs. These have been designed to replicate as accurately as possible, bone, cartilage, soft tissues and connective tissues of the body. However, some of these models lack correct anthropometric measurements with appropriate soft and hard tissue properties, and are designed to study only non-penetrating injuries [119]. There is currently no computational model that is usable for penetrating ballistics trauma. Multiple frangible surrogate leg models (FSL) have been created including one by The Australian Defence Science and Technology Group (DTSG) which are anatomically accurate and constructed from materials which simulate bone, cartilage, soft tissues and connective tissue. These models are being used to study the loading response in landmine lower leg injuries [72,125–129]. Human surrogate models, including the thorax and abdomen, are already being created for non-penetrating ballistic and blast injury evaluations. The Visible Human Project and associated projects using this data has incorporated very detailed aspects of the human body for anatomical study purposes [130]. However, the development of a surrogate model incorporating the bio-mechanical properties of the heterogeneous nature of the internal human organs and tissues has not been created but would be of great benefit to investigate penetrating ballistic trauma.

5. Biomechanical properties of tissues and organs

To create accurate anatomical models of the human body, the biomechanical properties of the tissues and organs need to be known. This is a widely researched area and it is not possible to include all the relevant literature here. However, the important biomechanical properties include tensile strength, strain, breaking strength, viscosity, viscoelasticity, elasticity and density [71–73,87]. Over the year's research has been conducted regarding the elastic properties of the ribs, sternum and the vertebral column [26–28]. Other research has included tests on silicon gel simulants under strain to determine the internal organ properties [25], along with testing various organs under compression loads [131]. Liver, kidney and spleen tissues have been tested under static and dynamic elongation tests [132–138] and shear tests [139–141].

6. Conclusions

Wound ballistics research has a variety of applications, but is limited by currently available simulants. Ballistics ordnance gelatine is the most popular simulant, but has its limitations as outlined. All of these simulants are homogeneous and do not take into account the heterogeneous nature of the human body, where skin, bone and tissues all have different compositions. Three dimensional modelling will need to incorporate modelling the internal organs and their heterogeneous nature, size and location. Although progress has occurred over the years in anatomical modelling, further work needs to occur to develop an accurate model for penetrating ballistics trauma. The use of computed tomography may be applicable in gaining accurate sizing of organs. The addition of bone and skin simulants to form experimental models of the human body is a first step, but more advanced anatomical models are required for research in the field of ballistics. This will in turn lead to the development of computer models and simulations.

Conflicts of interest

No conflicts of interest.

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Appendix B: Chapter 3 Manuscript

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PAPER

ANTHROPOLOGY; PATHOLOGY/BIOLOGY

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Effects of Re-heating Tissue Samples to Core Body Temperature on High-Velocity Ballistic Projectile–tissue Interactions

ABSTRACT: Damage produced by high-speed projectiles on organic tissue will depend on the physical properties of the tissues. Conditioning organic tissue samples to human core body temperature (37°C) prior to conducting ballistic experiments enables their behavior to closely mimic that of living tissues. To minimize autolytic changes after death, the tissues are refrigerated soon after their removal from the body and re-heated to 37°C prior to testing. This research investigates whether heating 50-mm-cube samples of porcine liver, kidney, and heart to 37°C for varying durations (maximum 7 h) can affect the penetration response of a high-speed, steel sphere projectile. Longer conditioning times for heart and liver resulted in a slight loss of velocity/energy of the projectile, but the reverse effect occurred for the kidney. Possible reasons for these trends include autolytic changes causing softening (heart and liver) and dehydration causing an increase in density (kidney).

KEYWORDS: forensic science, ballistics, projectile–tissue interaction, temperature, porcine tissues, biology

The injury effects of high-velocity firearm projectiles, such as rifles (approximately 900 m/s), have been investigated using human cadavers (1,2), synthetic materials, ballistics gelatines (3,4), and animals/animal tissues (5,6). Areas of the body commonly affected by high-velocity projectiles and those fired from low-velocity firearms, such as handguns or low-powered rifles (approximately 300 m/s), have been investigated previously, and a number of different injury scoring systems have been developed (7,8). Porcine organs have been commonly used as substitutes for human organs in ballistic tests.

As the human body is maintained at a constant core temperature of 37°C (9), it is believed that investigations on cadaveric material, including porcine organs, be conducted at living tissue temperature to recreate the physical properties of the living tissue and provide a suitable basis for interpreting the results. This becomes important as the physical properties of an organ, particularly its elasticity and texture, may vary with changes in temperature. Temperature dependence also applies to organ/tissue simulants such as ballistics gelatine, which need to be tested at a particular temperature in order to accurately mimic the behavior of those tissues (10). Furthermore, when human or animal tissues are removed from a recently deceased body, they need to be cooled rapidly to a refrigeration temperature of not more than 4°C, in order to minimize autolytic changes and to preserve the physical properties of the organs (11).

This introduces a dilemma for the wound ballistics investigator who requires the test organs to behave in a manner representative of living tissue, and this requires the organs be conditioned at 37°C for the duration of the testing. It is unknown whether during this time, the samples may become subject to change resulting from autolysis, dehydration, and partial denaturation. This may also affect tissue simulants such as ballistics gelatine that is moisture dependent and subject to drying and polymerization (10,12).

This study describes an experiment that was carried out to investigate the effect of conditioning time of porcine liver, kidney, and heart (without pericardium) tissue samples stored at 37°C on the penetration behavior of 6.35-mm-diameter steel sphere projectiles fired at high velocity (~900 m/s). This experiment is being conducted to determine whether the length of time the tissues are re-conditioned to core body temperature affects their bio-mechanical properties for ballistics studies purposes. The results of this study will be used for further analyses on tissues and their retardation effect on projectiles traveling at high velocity, which will ultimately be used to develop a complex physical human anatomical torso model simulant for use in wound ballistics studies. In a forensic setting, these results may not directly be useful; however, an accurate physical anatomical model will aid in a variety of trauma studies (e.g., sharp, blunt and ballistic trauma) as well as development of body protection systems.

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Materials and Methods

Equipment

Live firing experiments were undertaken at an indoor ballistic test facility located at the Defence Science and Technology Group (DSTG), Edinburgh, South Australia. A purpose built remotely operated 7.62 × 51 mm firearm, fitted to a fixed mount, enabled the firing of specially prepared ammunition. The test projectiles comprised 6.35-mm-diameter steel spheres (mass = 1.043 g) encased within frangible plastic sabots. The cartridge was filled with a single base (nitro-cellulose) gun propellant to provide a projectile launch velocity of approximately 900 m/s. This velocity was chosen as it would provide impact energies analogous to a military 5.56 mm caliber cartridge. Use of steel sphere projectiles eliminated uncontrolled effects associated with bullet tumbling, breakup, or distortion. The gun was set up at a distance of 10 m from the test specimen. This distance would enable the plastic sabot to separate cleanly from the sphere and not impact the test specimen, while providing the highest possible accuracy and impact velocity. A Doppler radar was used to track the spherical projectiles velocity from the firearm muzzle to the test specimen. A pair of optical infrared sighting screens recorded the spherical projectiles residual velocity after having passed through the test specimen. Two high-speed video cameras provided top and side views of the test specimen. High-intensity LED lighting enabled the cameras to be operated at 10,000 frames per second with a shutter speed of 1/150,000th of a second which provided sufficient image resolution and number of frames to undertake a postfiring analysis of the shot line, target thickness and projectile entry and exit velocity.

Materials

Porcine organs including the heart (without pericardium), liver, and kidneys were obtained from freshly slaughtered pigs (approximately 80 kg) at a local abattoir. On the day of slaughter, the organs were cut into approximately 50-mm-sided cubes and individually placed in zip-lock plastic bags (to facilitate mounting of the specimen on to the sample holder and to minimize dehydration of the tissue) and refrigerated at 4°C until they were needed for ballistic experiments. The tissue samples were kept in their zip-lock bags and placed in a temperature conditioning chamber set at 37°C for 1–7 h until such time as they were required for testing. A specimen holder had been specifically constructed to support the tissue sample in its zip-lock bag in an upright position and minimize distortion of its cubic shape. We have fired the projectile through the walls of an empty zip-lock bag situated 10 m from the muzzle and found that the exit velocity of the projectile after passing through the walls of the bag (with no specimen, expanded to 50 mm) was the same as the velocity of the unopposed projectile at the same distance from the muzzle of the firearm. Therefore, there was no noticeable velocity loss of the projectile caused by the plastic walls of the bag. The steel spherical projectiles were discharged from a distance of 10 m. The entry and exit velocities were recorded by the Doppler/IR sight screens (Doppler velocity method), shot line thickness was measured from video recording and entry and exit velocities were also calculated from the high-speed video recording (HSV velocity method).

The projectiles entry velocities were obtained by the two methods—Doppler velocity method and high-speed video calculations—were compared to determine reliability of the test methods. Fig. 1 shows correlations of results obtained by the two

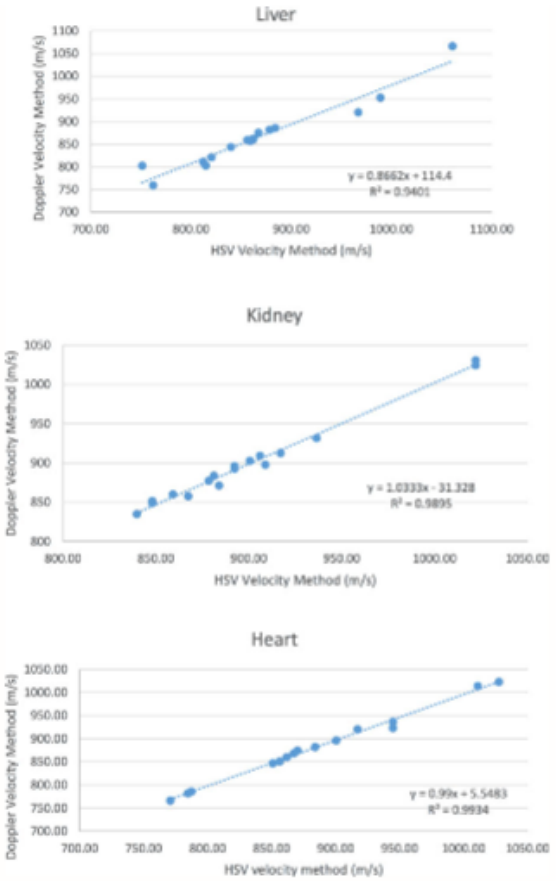

FIG. 1—*Correlation of projectiles entry velocity results obtained using the Doppler method and the high-speed video (HSV) method for the three organs (kidney, liver, and heart).*

methods. Coefficients of determination (r^2) for all three organs indicate that results provided by the two methods were practically identical and assured their accuracy.

Statistical Analysis

Independent sample *t*-tests, assuming differences in standard deviations, were used to compare mean entry and exit velocities and analysis of variance (ANOVA), comparing several means in separate samples at the same time was used to compare velocities in different organs. Linear regressions of time in the conditioning chamber at 37°C and velocity loss (m/s) were applied to determine the relationship between conditioning time and ballistic test results.

Results

Entry and exit velocity means for each organ (Table 1) from both velocity measuring methods are presented, along with mean energy loss (J/m) per tissue thickness. No significant differences were noted between the two methods for the liver, kidney, heart

TABLE 1—*Mean and standard deviation (SD) of entry and exit velocities from both velocity measuring methods and mean energy loss (J/m).*

	HSV					Doppler/Chronograph				
	Entry		Exit			Entry		Exit		
	Mean Velocity (m/s)	SD	Mean Velocity (m/s)	SD	Mean Energy loss (J/m)	Mean Velocity (m/s)	SD	Mean Velocity (m/s)	SD	Mean Energy Loss (J/m)
Liver	867.6	77.5	639.9	60.9	3452.3	865.8	69.2	634.7	57.2	3481.3
Kidney	900.2	52.7	716.0	53.0	3662.3	898.9	54.8	713.2	47.2	3703.1
Heart	885.3	75.8	645.9	58.4	3278.3	882.0	75.3	636.6	58.4	3333.9

TABLE 2—p-*values for comparison of entry velocities using HSV velocity method above diagonal and Doppler method below diagonal. No significant differences seen in entry velocities.*

	Liver	Kidney	Heart
Liver		0.5281	>0.9999
Kidney	0.4650		>0.9999
Heart	>0.9999	>0.9999	

for entry velocities ($p = 0.9461$; 0.9418; 0.9061), or the exit velocities ($p = 0.7987$; 0.8719; 0.6692).

The average entry velocities of the spherical projectiles did not differ significantly between the various organs, permitting a direct comparison of the results (Table 2).

Heart

Slight surface changes occurred in the heart as the time spent in the temperature chamber increased. The color changed from the normal red to a brownish red color.

The median time the heart samples ($n = 15$) spent in the temperature conditioning chamber was two hours (1.0–4.98 h). Mean tissue thickness was 58.97 mm (SD 5.6).

Significant differences were seen between the entry and exit velocities for both methods, HSV $p = <0.0001$, Doppler $p < 0.0001$ (Fig. 2). The average velocity loss was HSV 239.4 m/s (SD 31.4), Doppler 245.3 m/s (SD 27.4).

As the time spent in the conditioning chamber at 37°C increased, the loss in velocity decreased for the heart. Both methods of measuring velocity show this trend (Fig. 3). A partial correlation coefficient between the velocity loss and time with thickness of the tissue samples remaining statistically constant for each test was calculated as −0.618. This is a significant correlation and as it is negative, as time increases the velocity loss decreases.

Liver

The appearance of the surface of the liver tissue altered as the time spent at 37°C increased. As the time increased, the surface color became a darker brown, resembling the appearance of slightly cooked liver.

The median time liver samples ($n = 17$) spent in the temperature conditioning chamber was two hours (0.8–6.78 h). Mean tissue thickness was 52.81 mm (SD 8.7).

Significant differences were seen between the entry and exit velocities for both methods, HSV $p = <0.0001$, Doppler $p < 0.0001$ (Fig. 4). The average velocity loss was HSV 227.7 m/s (SD 42.8), Doppler 231.1 m/s (SD 35.0).

As the time spent in the conditioning chamber at 37°C increased, the loss in velocity decreased for the liver. Both methods of measuring velocity show this trend (Fig. 5). A partial

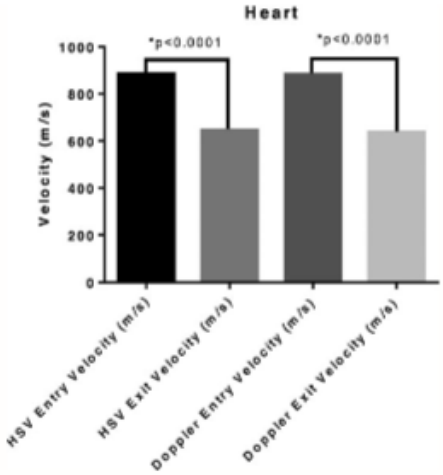

FIG. 2—*Comparison of the mean entry and exit velocities for both methods, showing significant differences between the entry and exit velocity.*

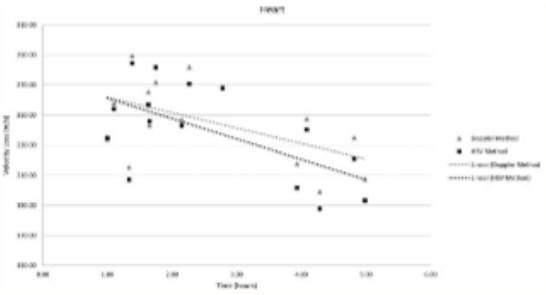

FIG. 3—*Velocity loss of projectile through the heart as time spent in conditioning chamber at 37°C increases, using both methods of velocity measurements.*

correlation coefficient between the velocity loss and time, with thickness of the tissue samples remaining statistically constant for each test, was calculated as −0.502. This is a significant correlation and as it is negative, as time increases the velocity loss decreases.

Kidney

The external surface of the kidney had slight visual changes in the color, resembling that of slightly cooked meat.

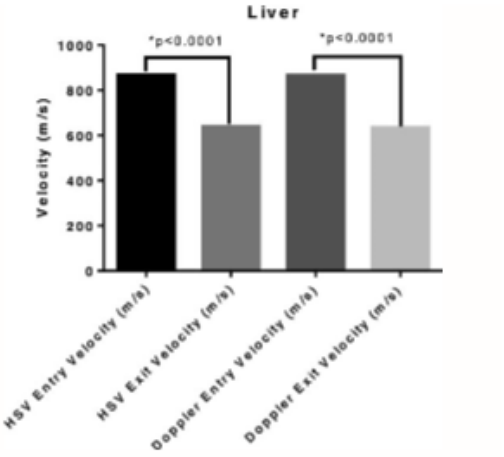

FIG. 4—*Comparison of the mean entry and exit velocities of both methods, showing significant differences between entry and exit velocities.*

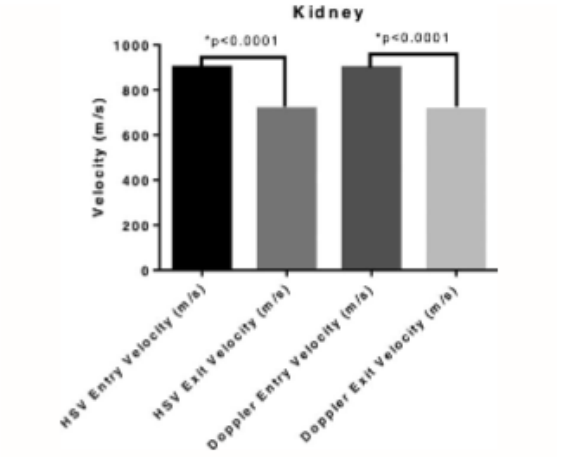

FIG. 6—*Comparison of mean entry and exit velocities for the two methods, showing significant differences between the entry and exit velocity.*

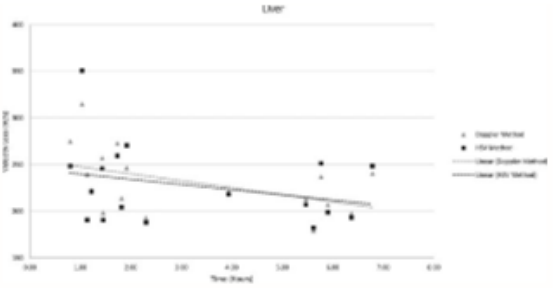

FIG. 5—*Velocity loss of projectile through liver as time spent in conditioning chamber at 37°C increases, using both methods of velocity measurements.*

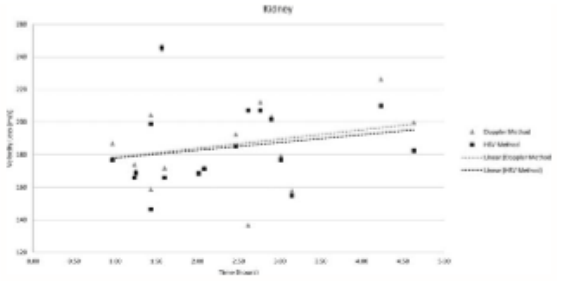

FIG. 7—*Velocity loss of projectile through the kidney as time spent in conditioning chamber at 37°C increases, using both methods of velocity measurements.*

The median time kidney samples (n = 17) spent in the temperature conditioning chamber was two hours (0.97–4.63 h). Mean tissue thickness was 42.64 mm (SD 5.6).

Significant differences were seen between the entry and exit velocities for both methods, HSV p = <0.0001, Doppler $p < 0.0001$ (Fig. 6). The average velocity loss was HSV 184.3 m/s (SD 24.7), Doppler 185.7 m/s (SD 27.1).

As the time spent in the conditioning chamber at 37°C increased, the loss in velocity increased for the kidney. This, however, is not significant increase. Both methods of measuring velocity show this trend (Fig. 7). A partial correlation coefficient between the velocity loss and time, thickness of the tissue samples remaining statistically constant for each test, was calculated as +0.290. This is not a significant correlation, and as it is positive, as time increases the velocity loss also increases.

Discussion

The entry velocities of the projectiles into all three tissues did not vary significantly for either of the velocity measuring methods, indicating that the specially built ammunition maintained the velocity within a range that allows comparisons between tissues. Viewing of the high-speed video recordings showed no sample distortion or movement during impact, thus not affecting the projectiles. A strong correlation is seen between the two methods for measuring velocity; thus, the results of both methods are similar. It is therefore acceptable to use either method for future ballistics testings.

The mean velocity of the projectiles reduced significantly from the entry to exit in the heart, liver, and kidney specimens. This indicates that the projectiles lost velocity and thus energy during their passage through each tissue. Furthermore, the thickness of the tissue specimens proved sufficient to reduce the velocity of ballistic projectiles in adequate magnitude to allow comparisons between tissues.

As a high-velocity projectile passes through a tissue, it loses velocity and thus energy (based on the equation for energy, $K = \frac{1}{2}\ mv^2$). The resultant damage to the tissue occurs ultimately from two sources. The change in pressure occurring on initial penetration and a pressure wave causes the temporary cavity, which stretches the tissues away from the projectile, before collapsing. In the literature, it is suggested that the damage to the tissue is greatest in the least elastic tissues. A permanent cavity occurs as the projectile crushes the tissues leaving a permanent passage through the tissue, while a temporary cavity occurs by stretching the tissues away and then collapsing (13–15).

Organs which are most susceptible to this stretch injury would have low elasticity that is close to water density, such as the liver, while the heart would be less susceptible (16). It would be expected that in tissues which are least elastic, the loss of velocity would be greatest, as these tissues lack the ability to stretch and collapse without damage. However, in this study, the injury to the heart was the greatest and that to the kidney was the least, which disagrees with the elasticity concept. The explanation may lie in the fact that the friction coefficients of these tissues and their densities differ in opposite directions to elasticity.

The rate of decrease in the velocity of a projectile when it is traveling through a tissue depends on the size and mass of the projectile (i.e., cross-sectional area and length), composition and thickness of the tissue (15). In this experiment, the size of the projectile and the thickness of the tissue is relatively constant, and thus, the observed differences in the reduction in the velocities among the tissues resulted from the differences in the composition of the tissues. Heart has densely arranged cardiac muscle fibers surrounded by fibrous connective tissues. In addition, the heart consists of an epicardium that consists of connective tissue containing blood vessels and pericardium. The latter has a dense parietal fibrous pericardium (9). The heart samples used here consisted of myocardium without the pericardium. Liver consists largely of parenchyma and portal triads rich in fibrous tissue. Liver parenchyma consists of cords of hepatocytes surrounded by sinusoids and veins (9). Thus, liver is less dense than the heart, particularly the myocardium. Kidney has a capsule and renal parenchyma and has very much less fibrous connective tissue than the liver (9). Thus, liver could be considered as denser than the kidney, which has been found in density values (17).

The liver and heart showed decreases in velocity loss over the period of time at 37°C; however, the kidney showed a slight increase. With a linear trend for time spent at 37°C and velocity loss, whether negative or positive, the composition of the tissues did not vary significantly over the maximum time in this experiment to alter the velocity loss of the projectile. The time spent in the hot conditioning chamber was not of a length that would show a clear change in the velocity loss of a projectile penetrating it. However, as these samples have been removed from the body and removed of the blood flow, the histology of the samples may vary, but is not visible to the naked eye and future research will investigate this aspect.

Due to no significant differences, heating the tissues to 37°C and retaining them at that temperature in the temperature conditioning chamber until tested did not have an effect on the size of the reduction in the velocity from the point of entry to exit from the tissues. However, organs/tissues of the body undergo autolytic changes and microbial breakdown from the time of an animal's/human's death. Therefore, in this experiment, the tissues were refrigerated immediately after the slaughter of the pigs to minimize and reduce these changes. The pigs were slaughtered under the sterile conditions of an abattoir; thus, the microbial breakdown of the tissues would have been minimal. However, heating of the specimens to 37°C and maintaining them at that temperature in the temperature conditioning chamber accelerates autolytic and microbial breakdown of the organs/tissues. Thus, the length of time the tissues kept in the temperature conditioning chamber determines the degree of autolytic and microbial breakdown. The amount of enzymes present within the cells of a tissue may also contribute to the rate of autolytic degradation. Possible reasons for the decrease in velocity loss over time in the heart and liver are the tissue became softer possibly due to autolytic changes, while the increase in the velocity loss seen in the kidney may be caused by the kidney tissues becoming denser possibly due to dehydration.

In all tissues placed in the temperature conditioning chamber, some discoloration that was visible to the naked eye occurred. This change was limited to the surface of the tissue and may have resulted from the loss of moisture from the surface of the tissues. The temperature of the chamber was maintained at 37°C; thus, the tissues would not have become cooked or burnt, as a much higher temperature would be needed for this to occur. Furthermore, this temperature does not appear to affect the physical properties of tissue components. Therefore, these organs are unlikely to change their resistive characteristics when penetrated by high-velocity projectiles. It is possible, however, that prolonged exposure to a 37°C environment could result in an increase in the activity of the intracellular enzymes leading to autolytic degradation of the tissues. Further studies would be required to determine this.

Conclusion

While the findings of this study reveal no statistical differences in the velocity loss of a high-speed projectile and the duration the organ samples were maintained and tested at 37°C, the slight changes in physical appearance suggest that autolysis and dehydration is possibly occurring. Future investigations will determine the extent of autolytic and dehydration effects; these organs are undergoing during the heating phase. Without further studies determining the extent of enzymatic activity and dehydration, it would be best practice to minimize the length of time organ tissues remain at 37°C after removal from the body; however, a specific time frame cannot be concluded from this study alone.

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ORIGINAL ARTICLE

A Histological Analysis of Visceral Organs to Evaluate the Effect of Duration of Heating From Refrigeration to Core Body Temperature for Ballistics Investigations

Caitlin Humphrey, BHlthSc(Hons) and Jaliya Kumaratilake, PhD

Abstract: Animal organs have been used in ballistics research to investigate the effects on human organs. Such organs are refrigerated until the investigation to minimize autolytic degradation and at times have been reheated to the human core body temperature to simulate the in situ environment. The aim of this investigation was to study the microstructural changes that may occur in fresh chilled visceral organs of the thorax and abdomen (ie, heart, lung, liver, and kidney) during the period of reheating to 37°C. Fifty-millimeter cubes of porcine heart, lung, liver, and kidney were taken rapidly after slaughter, chilled overnight, and the next morning were reheated to core body temperature (37°C). Histological changes occurring in the tissues during the reheating phase were investigated. The findings indicated that no cytoplasmic or nuclear changes occurred in any of the tissues during the period of reheating. Therefore, reheating of animal organs to the human core body temperature is not necessary, if the organs are refrigerated.

Key Words: heart, histology, kidney, liver, lung, projectile-tissue interaction, tissue degeneration

(*Am J Forensic Med Pathol* 2017;38: 326–332)

The manner in which injuries are caused by projectiles passing through human organs/tissues have been investigated using animal tissues[1–4] and/or tissue simulants (eg, ballistic simulants).[5–12] Porcine tissues/organs are the commonly used animal resource in ballistics and other scientific research, as the gross and microstructures are similar to those of the human organs/tissues.[1,13,14] Tissues removed from live animals after anesthesia (ie, including biopsies) or after slaughter undergo degenerative changes, which results from the cutting off of circulation, and the rates of degradation vary among different organs and tissue components within an organ. The changes tissues undergo are aerobic to anaerobic respiration, molecular, ultrastructural, microstructural, and gross anatomical in progressive stages.[15,16] Minimizing these changes are very critical in ultrastructural and light microscopic research investigations, pathological diagnosis, and tissue/organ transplants.[17] The effects of tissues on projectiles passing through, particularly on the retardation of velocity and the loss of energy, depend on hardness and fibroelastic properties of the tissues.[4] The hardness and the fibroelastic properties of tissues depend on the amount of ossification and/or calcification, and the distribution of collagen and elastic tissues, respectively. These tissue components are more resistant to degradation than the cellular components of tissues. Furthermore, to simulate the fibroelastic properties of organs/tissues to those of the living human, the ballistic investigations were traditionally carried out at the core human body temperature, 37°C. Therefore, organs removed from slaughtered animals are immediately chilled to 4°C (ie, to minimize degenerative changes) and reheated to 37°C before ballistic testing. In a recent ballistic investigation, porcine organs cut into 50-mm^3 blocks were maintained at 37°C for 1 to 7 hours before testing. Interestingly, the velocity loss from the projectiles passing through them was not significantly different between the times they spent at 37°C.[4] These findings indicated that the degradation of the tissue components determining the hardness and fibroelastic properties of the tested organs during the period of reheating was not adequate to cause a significant change in reductions of the velocity or energy loss from the projectiles.

The aim of this investigation was to study the microstructural changes that may occur in fresh chilled visceral organs of the thorax and abdomen (ie, heart, lung, liver, and kidney) during the period of reheating to 37°C.

METHODS

Hearts, lungs, livers, and kidneys were obtained from a local abattoir from 4 freshly slaughtered pigs (each 80-kg body weight). Approximately 50-mm cubes were cut from each organ, placed in individual ziplock plastic bags and refrigerated overnight at 4°C. The next morning, each sample was photographed and weighed, and the temperature at the surface and at the center of the block was measured. Furthermore, an approximately 5 × 5 × 10-mm piece of tissue was dissected off and fixed in 10% buffered formalin for histological investigation. Thereafter, the tissues were heated in a temperature-conditioning chamber set at 37°C. Every 10 minutes, the temperature on the surface and at the center of the tissue block was measured until the latter temperature reached 37°C. Then, the tissue was weighed and photographed, and another sample (approximately 5 × 5 × 10-mm piece) was taken and placed in a 10% buffered formalin for histological investigation. Surface temperature was measured using an HP-880EK digital infrared noncontact thermometer (accuracy ±0.5°C) (Zhuhai Jida Huapu Instrument Co., Ltd. China), and the temperature at the center of the tissue block was measured using a LCD Probe Thermometer (accuracy ±1.0°C) (Tech Brands, Australia). The core temperature was measured by inserting the probe toward the center of each block of tissue.

Heart, lung, liver, and kidney samples fixed in 10% buffered formalin were dehydrated through a graded series of ethanol and embedded in paraffin wax as described by Pearse.[18] Five-micron-thick sections were cut, mounted onto glass slides, and stained with hematoxylin-eosin as described by Pearse[18] and examined using an Olympus BX 50 microscope (Olympus Corporation, Tokyo, Japan) coupled to a digital imaging system (consisting of Olympus SC 100 digital camera [Olympus Corporation, Tokyo, Japan] and Stream Essentials imaging software [Olympus Corporation, Tokyo, Japan]).

RESULTS

The mean weights of the organs (±SEM) at the commencement of the experiment (ie, refrigerated samples) were

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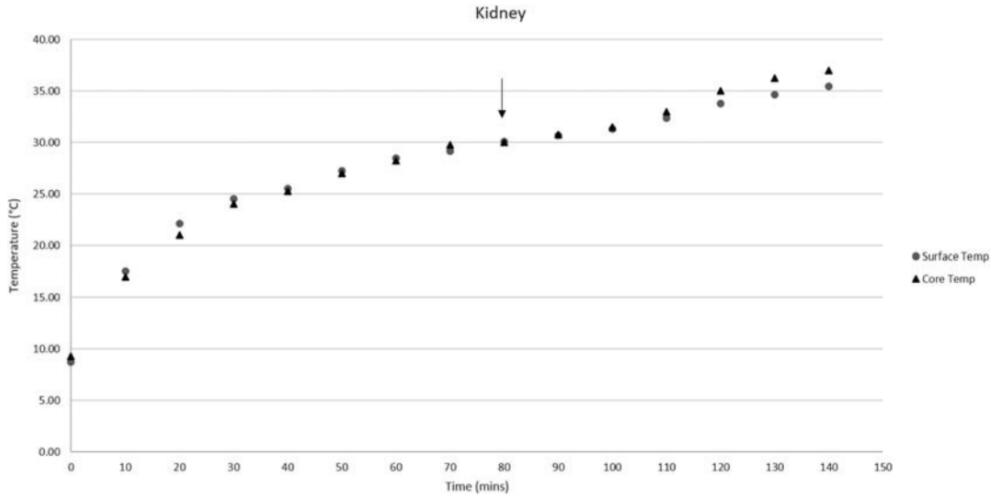

FIGURE 3. The core and surface temperatures of the kidney samples measured at 10-minute intervals during the time taken to reach the core temperature to 37°C in the temperature-conditioning chamber. The arrow indicates the approximate time at which a surface color change occurred.

the experiment (Figures 1–4). In each organ, the temperature increased at an approximate rate of 1°C per 10 minutes, and as the temperature approached 37°C, the rate of increase slowed down (Figures 1–4).

The color of the surface in each organ altered at a different time of heating, and it is indicated in Figures 1 to 4 by arrows. The surface color changed first in the kidney samples, followed by the liver, heart, and lung samples. Figure 5 shows the changes in surface color in the organ samples.

Microstructural (Histological) Changes

Heart. In samples taken either at 4°C or at core 37°C, abnormalities (ie, changes in staining color or the structure) were not seen in the cytoplasm and nuclei of cardiac muscle cells, cells of the connective tissue matrix (ie, tissues among cardiac muscle cells), walls of blood vessels, and red blood cells in the capillaries (Fig. 6, A and B). However, in the cardiac muscle cells that were at and near the cut surface of the tissue block, the cytoplasm was less dense (note that the staining color was similar to the rest of the cardiac muscle cells), cross striations were very prominent, and the nuclei showed varying degrees of chromatin condensation (Fig. 6C). This change was seen in samples taken at both 4°C and 37°C, but the chromatin condensation of the nuclei was seen in more cardiac muscle cells of the samples taken at 37°C.

Lung. In samples taken either at 4°C or at core 37°C, abnormalities (ie, changes in color or structure) were not detected

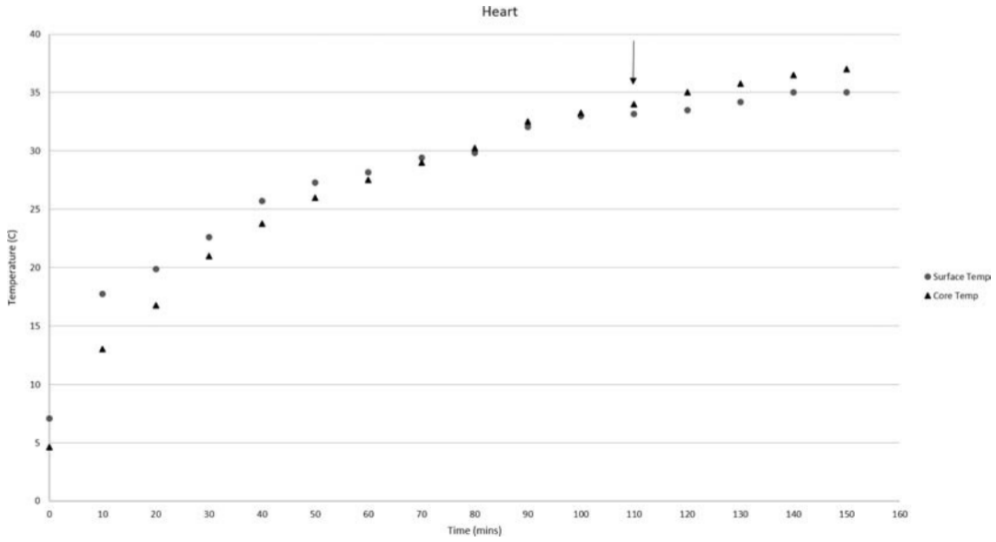

FIGURE 4. The core and surface temperatures of the heart samples measured at 10-minute intervals during the time taken to reach the core temperature to 37°C in the temperature-conditioning chamber. The arrow indicates the approximate time at which a surface color change occurred.

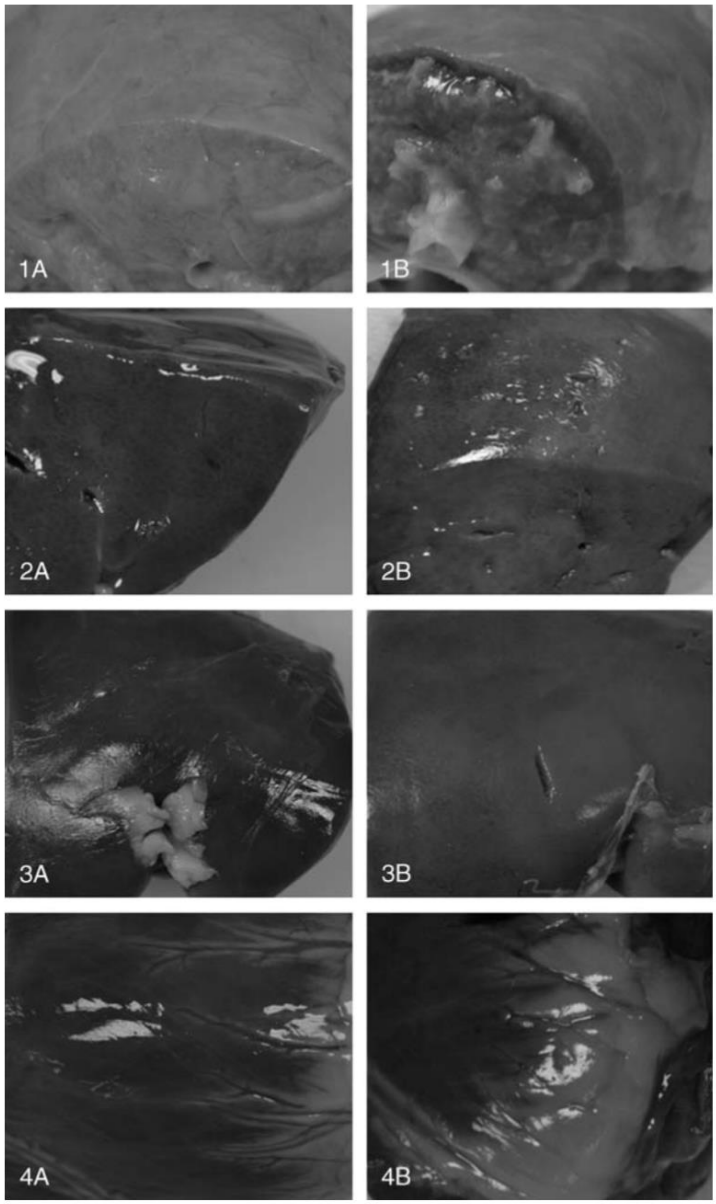

FIGURE 5. Surface color of the lung (1), liver (2), kidney (3), and heart (4) at removal from 4°C (a) and during the heating phase in the conditioning chamber (b).

in the cytoplasm and nuclei of epithelial cells of alveoli, bronchi and bronchioles, and walls of blood vessels, bronchi, and bronchioles (Fig. 7, A and B). In the lung samples taken at 4°C, more alveoli were open and distended compared with the lung samples taken at 37°C. In lung tissues taken at both temperatures, areas of compact cell densities were seen; such regions were more common in the samples taken at 37°C (Fig. 7C).

Liver. Liver samples taken either at 4°C or at core 37°C did not show abnormalities (ie, changes in color or structure) in the cytoplasm and nuclei of hepatocytes, Kupffer cells, walls of sinusoids and central veins, and bile ducts and vascular elements of portal tracts (Fig. 8, A and B).

Kidney. Kidney samples taken either at 4°C or at core 37°C did not show abnormalities (ie, changes in color or structure) in cytoplasm and nuclei of cells of glomeruli (including Bowman capsules), tubules, and walls of blood vessels (Fig. 9, A and B).

DISCUSSION

Cells of organs removed from slaughtered animals or for human organ transplantation are commonly expected to undergo degeneration and necrosis followed by autolysis after the removal. These changes could commence from the time of slaughter of an animal or removal from a live animal or human body.[19,20] This

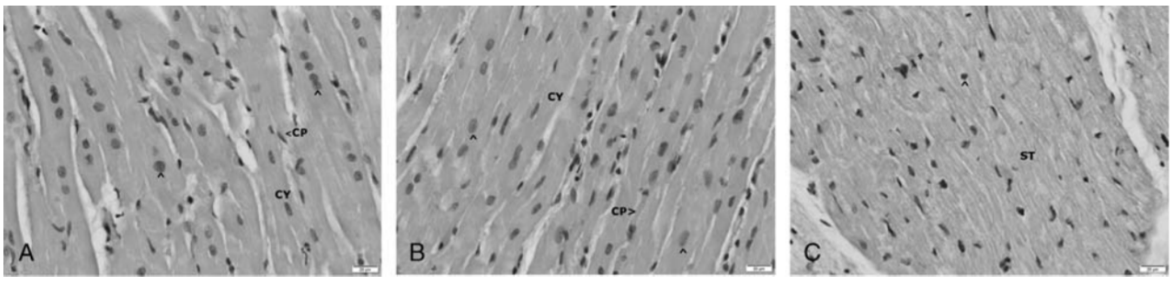

FIGURE 6. Heart tissue viewed at 20× magnification. A, Refrigeration temperature; B, 37°C; C, 37°C. ^ Indicates nuclei; CP, capillary; CY cytoplasm; ST, striations.

is the reason why tissues are fixed in chilled fixatives immediately after removal from the body for ultrastructural investigations. However, cytoplasmic and nuclear changes that indicate cell degeneration, necrosis, or autolysis at light microscopic level was not seen in the heart, lung, liver, and kidney samples removed from the slaughtered pigs, even after warming for 130 to 200 minutes in a temperature-conditioning chamber (Figs. 6–9). These are novel and interesting findings. Complete obstruction to a coronary artery or a segmental artery of the kidney or liver leads to infarction of the respective end-artery areas of the blocked artery in the heart,[21] kidney,[22,23] and liver[24] within a short time. In lungs, such infarctions are less frequent because of the double circulation and possibly diffusion of oxygen into the lining cells of the alveoli. Heart, kidney, and liver are highly functionally active organs; thus, their oxygen demand is very high. Therefore, cutoff of oxygen supply for a short period because of the obstruction of an artery causes infarction in the end-artery area of the obstructed artery. Slaughtering of an animal causes cessation of functioning of the vital organs such as heart, lung, kidney, and liver rapidly; thus, their oxygen demand may drop markedly. Therefore, the cells in these organs could survive for a longer period without an oxygen supply or immersion in a specific buffer.[20] This may be the reason for the cells in the organs of the current investigation to remain unchanged during the period of investigation (Figs. 6–9). Refrigeration (at 4°C) of the 50-mm^3 blocks of the organs could have further reduced the metabolic rate of the cells and protected them until commencement of the experiment and gradual warming up to the core temperature of 37°C. The changes seen in the cardiac muscle cells, at the cut end of the block (Fig. 6), could be the effects of direct physical injury of cutting.

Porcine organs have been used in ballistic research to investigate injury patterns of projectiles passing through human organs.[5] In such experiments, the organs have been warmed to 37°C to simulate the human in situ condition.[4] Projectiles penetrating body organs lose their energies and reduce velocities. The amount of energy loss and the reduction in velocity depend on the fibroelastic properties of each organ. Fibroelastic properties of organs are determined primarily by the connective tissue matrix of the organ.[5] Connective tissues, particularly the fibrous elements, are more resistant to autolytic degradation than are the cellular elements of the organ. Current findings clearly indicate that the cellular elements do not undergo autolytic degradation during the period of warming from refrigeration temperature to core temperature of 37°C (Figs. 6–9). Therefore, the loss in energy and the drop-in velocity of projectiles penetrating fresh refrigerated porcine tissues should not be different from those of projectiles passing through porcine organs warmed to core temperature of 37°C. Therefore, in ballistic research, warming of body organs under investigation to the human core body temperature of 37°C is not necessary.

The areas of cell densities seen in the lung samples (Fig. 7) are regions of alveolar collapse resulting from the escape of air trapped in them. During warming of the lungs, air from numerous alveoli has escaped, causing the increase in the number of areas of cell densities seen in the lung samples warmed to core temperature of 37°C. Heat would have also been lost with the air escaping from the alveoli leading to the observed drop in temperature of the lung samples, before the final rise in temperature (Fig. 1).

The cells of the liver samples warmed to 37°C were like those of the liver samples before warming and did not show any evidence of shrinking (Fig. 8). Therefore, the 6.5 g of fluid that drained out from the liver samples during warming of the tissue may not have come from the liver cells. Liver is the organ that generates approximately 25% to 50% of the lymph drained via the thoracic duct.[25,26] Therefore, the fluid that drained out could be the lymph that was in lymphatic vessels. Furthermore, liver contains a large volume of blood in branches of the portal vein, sinusoids, central veins, and the remainder of the venous tree.

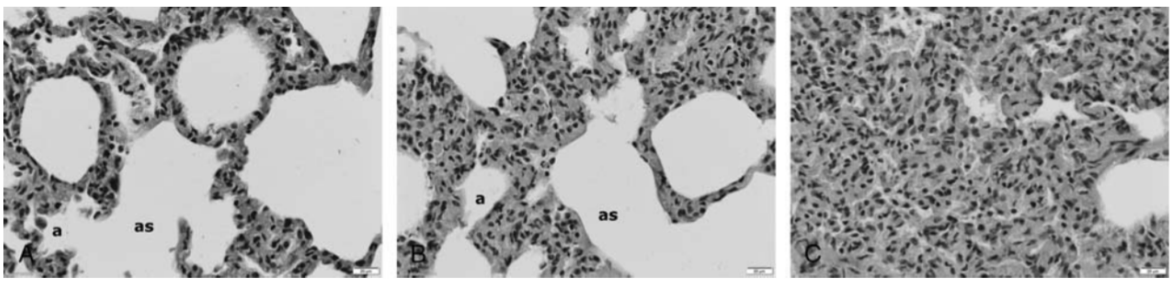

FIGURE 7. Lung tissue viewed at 20× magnification. A, Refrigeration temperature; B, 37°C; C, 37°C. a Indicates alveoli; as, alveoli sac.

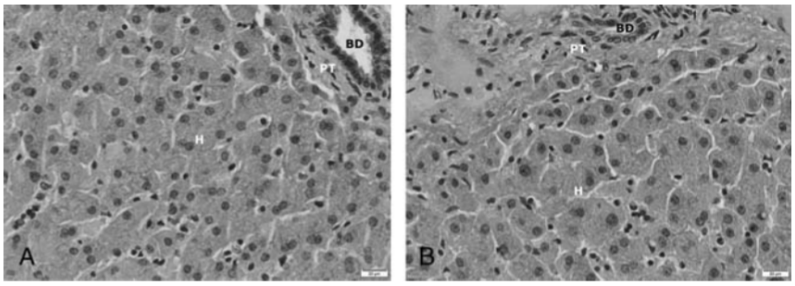

FIGURE 8. Liver tissue viewed at 20× magnification. A, Refrigeration temperature; B, 37°C. H indicates hepatocyte; PT, portal tubule; BD, bile duct.

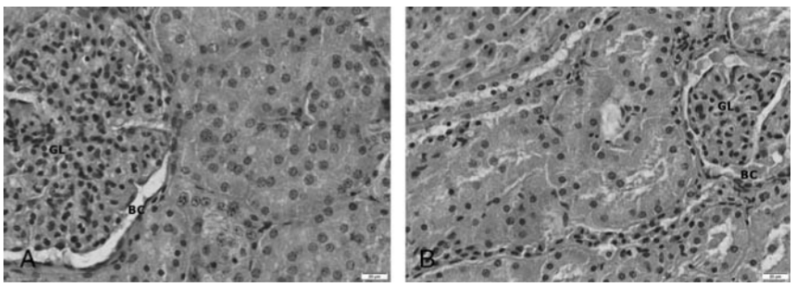

FIGURE 9. Kidney tissue viewed at 20× magnification. A, Refrigeration temperature; B, 37C. GL indicates glomerulus; BC, Bowman capsule.

Therefore, some of the fluid could also be the serum that escapes out (ie, from cut surfaces) after clotting of the blood.

CONCLUSIONS

The degenerative changes in the cells of the organs removed rapidly from the body and chilled quickly are minimal. During reheating to core body temperature (37°C) after refrigeration, the cells remain unchanged during the period of reheating. The time taken for reheating of the organ to human core body temperature, 37°C, depends on the specific organ. In ballistic research, heating of the chilled organ to human core body temperature is not necessary.

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Appendix D: Chapter 9 Manuscript

ANTHROPOLOGICAL REVIEW • Vol. 80(2), 207–218 (2017)

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Anthropological analysis of projectile trauma to the bony regions of the trunk

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ABSTRACT: Ballistics literature often focuses on soft tissue injures and projectile trauma to the cranium. Minimal details on the bony characteristics of projectile trauma to the thorax/abdomen regions have been published. This study aims to analyse projectile trauma to the bony trunk region including the ribs, vertebrae, scapula, sternum and the hip bone to form a better understanding of the characteristics and biomechanics of skeletal trauma caused by a projectile and contribute to the existing database on skeletal trauma caused by projectiles. Fourteen cases of documented projectile trauma to the bony regions of the trunk from the Hamman-Todd Human Osteological Collection at the Cleveland Natural History Museum, Ohio were analysed. Of the 14 individuals with gunshot wounds examined, 40 wounds occurred to the bones. Twenty-four injuries to the ribs, 1 ilium, 11 vertebrae, 3 scapulae, and 1 sternum. Fracture patterns, heaving and bevelling can be used to determine the direction of travel of the projectile which can be evident on the ribs, sternum, scapula and ilium. It is critical to understand the wounding patterns associated with projectile trauma to the torso region as this is often targeted, due to being the centre of mass.

KEY WORDS: thorax; bullet trauma; fracture; bone trauma; ribs; ballistics injury

Introduction

Much research in ballistics has focused on soft tissue injuries including autopsy features and experimental aspects utilising animal models, ballistics simulants and dummy models (Bir et al. 2016; Humphrey et al. 2017; Humphrey and Kumaratilake 2016; Jönsson et al. 1988; Mabbott et al. 2016; Schantz 1978). When it comes to bony injuries from projectiles, a lot of research has been conducted into maxillofacial ballistics trauma (Berryman et al. 1995; Lahren et al. 1987; Stefanopoulos et al. 2015; Viel et al. 2009). The literature has been able to document the characteristics of projectile skeletal trauma to the skull as well as soft tissue characteristics, however minimal details on the thorax/abdomen bony regions in particular the scapula, ribs, sternum and vertebrae have been published (Langley 2007).

In 1995, Ubelaker (1995), published an anthropological analysis of an individual who was assassinated sustaining gunshot trauma. The analysis produced

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evidence for projectile path and thus direction of fire based on the displacement of bone fragments, fractures, bevelling and appearance of the fractures. More recently, Langley (2007), produced an anthropological analysis of gunshot wounds to the chest region with the focus on assessing the usefulness of the characteristics of the wounds in determining the direction of fire. Using 54 documented cases of gunshot wounds to the thorax, Langley (2007), found that due to the ribs occupying a significant portion of the thorax, they were commonly hit by a projectile and that the bullets leave distinctive marks on ribs which are able to determine the direction of fire. Langley concluded that further analyses of the bony structures of the thorax are needed to get a better understanding of the biomechanics associated with bony projectile trauma to the thorax. Most forensic anthropology text books also describe bone trauma from projectiles (Byers 2015; DiMaio 2015; Dirkmaat 2014). Many other researchers have investigated the biomechanics of gunshot wounding, characteristics of wounds caused by different projectiles, how low velocity projectiles cause injury, prediction of the calibre of the weapon and manner in which death occurred (Berryman et al. 2012; Berryman and Symes 1998; de la Grandmaison et al. 2001; DiMaio 2015; Lahren et al. 1987; Langley 2007; Smith and Wheatley 1984; Spitz and Spitz 2006). However, misinterpretations have been noted, thus there is a need to understand the relationships between soft and hard tissue trauma injuries (Fackler 1987; Fackler 1988). Symes et al. (2012), have suggested that the interpretation and assessment of high velocity impact to osseous tissue is only in its early stages and therefore more research is necessary to properly understand and accurately interpret the bone injuries resulting from projectiles.

This study aims to analyse projectile trauma to the bony trunk region including the ribs, vertebrae, scapula, sternum and the hip bone to form a better understanding of the characteristics and biomechanics of skeletal trauma caused by a projectile. This study will contribute to the existing literature on skeletal trauma caused by projectiles.

Materials and Methods

The Hamman-Todd Human Osteological Collection at the Cleveland Natural History Museum, Ohio, contains 44 documented cases of gunshot trauma. Two cases had been returned to their respective families; twelve had unknown location of wound or not visible wound (i.e. soft tissue trauma only); 3 had wounds to the limbs; thirteen had wounds to the cranium and fourteen had wounds to the torso region, that were sustained in real life situations. The fourteen torso region cases are discussed here. Each case was documented as gunshot wound being the cause of death, and where known, the manner of death was also documented (4 homicide, 10 unknown). The age and sex were noted from the Museums records (mean age 29.86 (SD 7.57); sex: female 14.2%, male 85.7%). Causative weapon was not known in the cases; however, no shotgun injuries were present due to the unique wounding properties of these types of weapons. Shots expelled from such a firearm form a large cloud of pellets that expands in diameter as distance increases. Due to the small size of each pellet, its kinetic energy is low and therefore one could expect multiple low impact injuries.

A visual examination of the entire skeleton (where present) occurred to determine the location of the wounds. A Canon 5D Mark III with macro lenses camera was used to photograph the wounds. No autopsy records or pathologists evaluations were available to assess soft tissue details, thus only anthropological analysis of the wounds occurred.

Results

Of the 14 individuals with gunshot wounds examined in this study, 40 wounds occurred to the bones. Twenty-four injuries to the ribs, 1 ilium, 11 vertebrae, 3 scapulae, and 1 sternum.

Fig. 1. Vertebrae of Individual HTH 524, displaying 7th, 8th and 9th vertebral body shattering

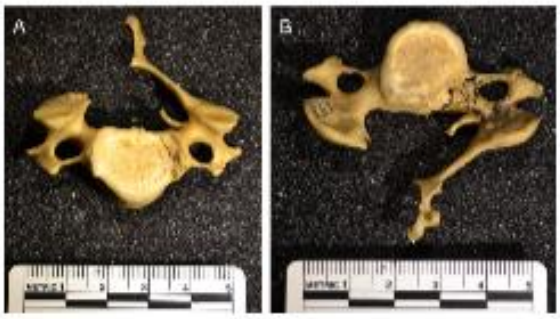

Fig. 2. Vertebra of Individual HTH 1430, displaying clear fracture and missing segment of cervical vertebra (A, B)

Vertebrae

In the vertebrae, the injuries were often a shattering of the vertebral body, small clear fractures, and missing segments of bone including the pedicle. This occurs in the cervical, thoracic and lumbar vertebrae (Figures 1–4).

Fig. 3. Vertebrae of Individual HTH 1812, displaying fracture and missing segments of Lumbar 4

Fig. 4. Vertebrae of Individual 659, displaying vertebral shattering of thoracic 3, 4, 5 and 6 (A, B)

Sternum

The sternum, particularly the corpus sterni (Figure 5), showed both an entry and exit wound which were distinguishable from each other, thus the ability to determine the projectile path. The entry wound was smaller with radiating fractures. The exit wound, larger and more irregular in shape, also produced radiating fractures, however these heaved outwards.

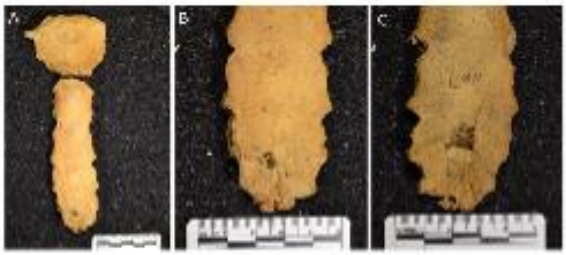

Fig. 5. Sternum of Individual HTH 1163. Full sternum displaying entry wound (A), entry wound (B), and exit wound (C), with small radiating fractures

Ribs

The ribs showed varying types of wounds. The first being small nicks in the bone as the projectile passed and minimally made contact with the bone. These present as semi-circular/oval defects on either the caudal or cranial edge of the ribs (Figures 6, 7, 8, 9, 10, 11). The diameter of the wound may reflect the calibre of the

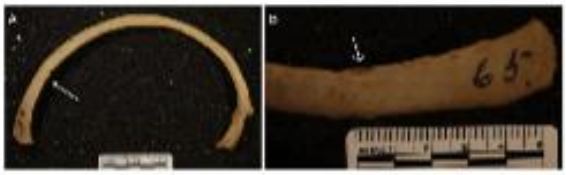

Fig. 6. Superior view of Individual HTH 65 right 4th rib (A) with arrow indicating wound on cranial edge of the sternal end. Close view of the wound (B), indicated by arrow

Fig. 7. Inferior view of Individual HTH 65, left 4th and 5th rib (A), with arrow indicating wound on caudal edge of sternal end of 4th rib. Close view of wound (B)

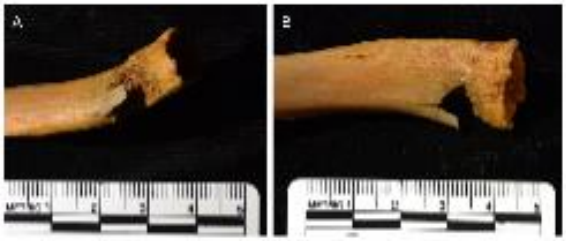

Fig. 8. Individual HTH 1662, displaying circular defect in sternal end, caudal edge with flaking inwards (A, B)

Fig. 9. Right 8th rib of individual HTH 596 (A). Wound located on cranial edge on articulating end of rib. Fractures running along length of rib in spiral pattern (B), and the clear circular entry wound (C)

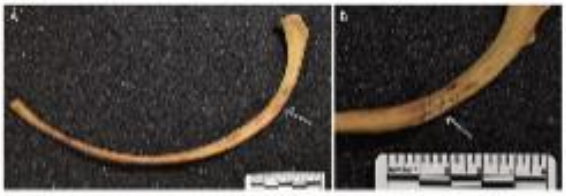

Fig. 10. Individual HTH 1238 left 9th rib displaying bone nick on cranial edge with depressed bone (A, B), and small radiating fractures lifting outwards (B)

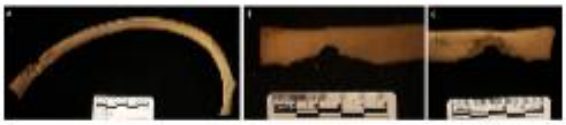

Fig. 11. Individual HTH 1361. Left 3rd rib displaying circular defect on sternal end, caudal edge (A). Clear circular entry with missing segments (A), external bevelling and flaking of exit wound (B)

bullet, being large or small, however, such evidence is not definitive. The second type are fractures on either the sternal or vertebral end of the rib. These fractures run along the length of the rib (Figures 12, 13).

Fig. 12. Individual 524 displaying fracture running along length of rib on caudal edge of articulating end

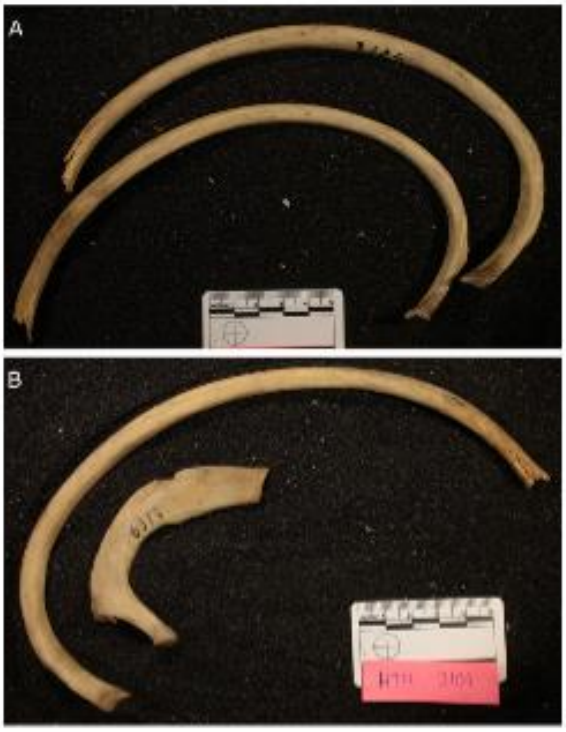

Fig. 13. Individual HTH 2104. Right 5th and 6th ribs with fractures running along cranial surface edge of sternal end (A). Left 1st rib displaying small notch, and other rib with damage to sternal end

Fig. 14. Individual HTH 1361. Right 6th rib displaying fracture through shaft bending laterally

Fig. 15. Individual 1238 right 3rd rib. Caudal edge on articulating end displaying oval defect with radiating fractures similar to a butterfly pattern seen in long bone fractures

Other fractures can occur mid-shaft, often fracturing a rib completely into separate pieces, two or more which can be reconstructed (Figure 14). The third is a combination of a small circular nick in the bone, with fractures that run along the edge of the rib, some appearing similar to spiral fracture patterns (Figures 8, 9). What has been referred to as butterfly fractures in long bones, may also occur in these types of rib fractures (Figure 15). When the circular defect is present, the entry and exit side may be distinguished with the use of bevelling on the exit wound, entry wound is circular and smaller than the exit, depressed or heaving fractures (Figure 8, 9, 11). Multiple fractures may appear in the same individual, which can be difficult to distinguish as peri- or post-mortem (Figure 16, 17). These fractures can be simple transverse fractures across the width of the rib, or run along the rib, as in oblique

fractures. If the bullet arrests in the rib, it may form a wound similar to Individual HTH 985 (Figure 18), where the bone remodels around the bullet (as would occur if the bullet was not removed and the patient survived).

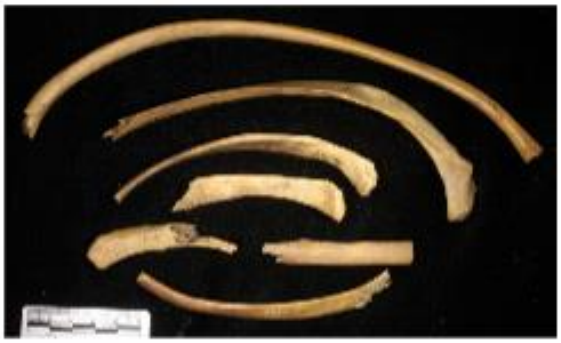

Fig. 16. Individual HTH 1163 displaying commingled ribs with various fractures with no clear entry or exit wounds

Fig. 17. Individual HTH 659. Fracture of left 3rd rib through shaft (A, B). Right 2nd, 3rd, 4th ribs with no distinct entry or exit wounds but fractures through shaft (C). Left 2nd rib with fracture through and along shaft (D)

Fig. 18. Individual HTH 985. Wound on right 7th rib. Potentially due to bullet arresting in rib and bone displaying signs of remodelling around bullet

Scapulae

Due to the irregularity and non-uniform thickness of the scapula, the wounds vary. The three injuries to the scapulae

Fig. 19. Individual HTH 659 with circular wound in subscapular fossa with fracturing

Fig. 20. Individual HTH 1709. Left scapula with damage to infraspinous fossa (A), while right scapula shows damage to infraspinous fossa and vertebral border of scapula spine and missing segments (B)

in this study showed that when a bullet penetrates the subscapular fossa (Figure 19) a clear circular wound is visible but fracturing into segments occurs. In individual HTH 1709 (Figure 20), both the left and right scapulae were injured. The fracturing pattern on the left scapula shows in the infraspinous fossa fractured fragments heaving in the dorsal direction. The right scapula shows similar fracturing of the fragile and thin infraspinous fossa as well as the vertebral border of the scapular spine. In this individual, no clear entry or exit wound was visible. Most likely the projectile entered the thorax anteriorly and caused cavitation that produced injuries to both scapulae.

Ilium

The ilium also showed distinct entry and exit wounds. The entry was oval in shape with depressed bone fragments and small radiating fractures. The exit wound, had significant external bevelling.

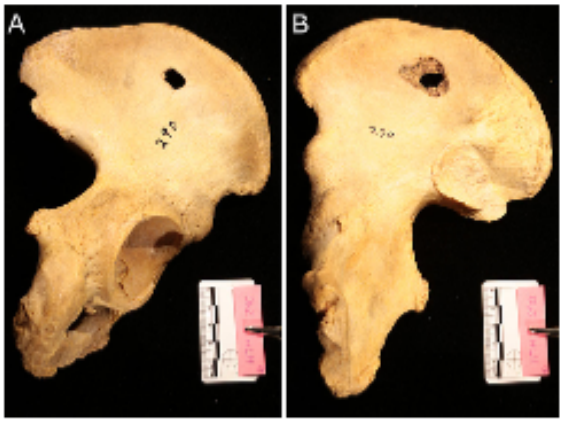

Fig. 21. Individual HTH 290 with wound on left ilium displaying circular wound (A), and clear exit wound with external bevelling (B)

Discussion

When firing a weapon at an individual, the majority of people will aim for the centre of mass, this being the thorax and abdomen region or torso. In classical training of military and police, the trainees are instructed to aim at the centre of mass of the body, which is located in the torso region. Within the torso are major organs and thus death is highly likely. However, along with the soft tissues, there are also bony structures including the sternum, vertebrae, ribs, scapula, clavicle and lower down the pelvis. The projectile is therefore often to produce skeletal trauma to these regions in the victim. However, it has been found that a bullet can pass through the intercostal spaces without leaving evidence on the bone (Langley, 2007). The analysis of soft tissue, when available, can determine many details about an individual's death, however, the analysis of the skeletal elements by a forensic anthropologist can provide additional support and is critical when only skeletal material is available.

Unlike soft tissues, bone offers more resistance against penetration by a bullet due to its composition, hardness, density and strength (Bartlett 2003; Janzon et al. 1997; Stefanopoulos et al. 2015). Due to its nature, bone tissue cannot absorb the energy transferred from a bullet to the extent in which soft tissues do. Instead, bones act like a brittle material (Kieser et al. 2014; Stefanopoulos et al. 2015), when the stress/strain is beyond that they can rebound from, fractures occur. Ballistics fractures fall within sudden force and high speed category (Chapman 2007; Hamblen et al. 2007), and such fractures could result from direct impact of projectiles with excessive speed

or a projectile at a vulnerable point in the bone (Byers 2015; Chapman 2007). The force exerted must be greater than what the bone can withstand and often occurs with projectiles moving at velocities ranging between 61 and 171 metres per second. When this occurs, the bone will fracture (DiMaio 2015; Harvey et al. 1962; Sellier and Kneubuehl 1995). Huelke et al. (1968) found that for visible damage of the bone, a velocity of 213.36 m/s or more was required.

The skeletal wounding potential of a projectile will depend on numerous factors associated with the projectile itself as well as the bone which it makes contact with. These will affect the characteristics of the injury. The type of bone which the bullet contacts will affect the characteristics of that injury. Bone consists of, on a molecular level, collagen and a calcium phosphate (hydroxyapatite) which give it its flexibility, strength and rigidity. Bone can be categorised according to the shape (i.e. long, short, flat, irregular). Projectile injuries to the flat bones, such as the cranial vault, have the distinguishing feature of bevelling which can determine the entry and exit wound (Chapman 2007; DiMaio 2015; Quatrehomme and İşcan 1997, 1998a,b, 1999). The ribs have an oval elongated cross-section, and contain some tubular properties, although the entire inside of the rib is comprised of trabecular spongy bone. Wounds in the ribs may also show bevelling or flaking in the direction of bullet travel, as seen in (Figures 8, 11, 14). This bevelling appearance of wounds is critical in determining the direction of travel of a bullet, and as seen in this study, can be seen in the ribs (Figure 8, 11, 14), sternum (Figure 5), ilium (Figure 21). It is less seen in the scapula (Figure 19, 20), potentially due to the thin nature of this bone compared to the thicker sternum which has a greater trabecular bone content (Tersigni-Tarrant and Shirley 2012).

The direction in which the force of the bullet penetrates the bone will also determine the characteristics of the wounds. If a bullet penetrates the thorax cavity, travelling through the intercostal spaces, no bone defects may appear. However, if the bullet contains a significant amount of energy which is deposited into the surrounding tissues by Newtons Laws, a temporary cavity will occur which may cause fractures in the midshaft of the ribs (Figures 14, 16, 17). Unlike in soft tissues, the temporary cavity in bone tissue is not followed by the collapse of the cavity, rather the lack of elasticity causing a pulverisation effect to the bone (i.e. fracture) (Huelke et al. 1968; Janzon et al. 1997; Stefanopoulos et al. 2015). The transfer of energy to bone in ballistic injuries is less understood (Kieser et al. 2014; Molde and Gray 1995; Stefanopoulos et al. 2015), in comparison to that in soft tissues. The soft tissues, due to their elastic properties, are able to absorb the energy transferred and revert back to their normal state, unless their elasticity is overcome. In bones, this elasticity value is less than that in soft tissues i.e. less energy is required to fracture the bone and it acts in a brittle manner. The amount of energy transferred to bone is influenced by the amount of contact time between the bullet and the bone, and this is inversely proportional to the velocity. Thus, a bullet travelling with low velocity will have more contact with the bone compared to a high velocity bullet and therefore it is possible for these slow bullets to cause more damage (Rothschild 2011; Stefanopoulos et al. 2015). However, high velocity bullets can have an explosive effect where when penetrating

soft tissues indirect fractures are caused to nearby bones (e.g. ribs or long bones) due to the expansion of the temporary cavity and the transfer of high energy (Clasper 2001; Hollerman et al. 1990; Humphrey and Kumaratilake 2016; Janzon 1983; Mellor 1994; Stefanopoulos et al. 2015).

A bullet may also contact the bone at an angle, such as with the scapula (Figure 20), and the bone will fracture in a comminuted way. When this occurs, the way the bone fractures (e.g. heaves outwards) can be used to determine the direction in which the bullet travelled. When the bullet penetrates any bone perpendicular to the surface, it is highly likely that the bullet will completely penetrate the bone, and a clear entry/exit wound will appear. This could occur here in the sternum (Figure 5), scapula (Figure 19), ilium (Figure 21). When the bullet penetrates the ribs, perpendicular, it may not make contact with the whole rib and therefore produce a nick in the rib. This nick will be oval/circular in shape, mimicking the shape of the bullet (Figures 6–11). If high energy, the rib may also fracture along the length of the rib (Figure 9, 12, 13, 15).

Fractures in the ribs, and also the vertebrae, may also be due to the passing of a bullet in close proximity to the bones, however not directly penetrating them. This would possibly only occur if the energy of the bullet is high enough to transfer the energy to the surrounding tissues and overcome the strength of the bone. With the vertebrae, as also found by Langley (2007), there is no clear entry or exit wounds, and the vertebral body often is comminuted (Figures 1–4). Secondary missiles occur when minute fragments from the impacted bone cause their own permanent cavity. This can also occur with fragmenting bullets. This causes further trauma to other portions of the body that can magnify the damage beyond that of the simple drilling effect of the bullet itself (Harger and Huelke 1970). These are most often seen through radiographic studies (Amato et al. 1989). It has been found through experimental studies that the secondary fragments (e.g. bullet fragments, jacketing and bone shards) have the same possibility of lethality as the original bullet. The amount of damage increases if the impact velocities are great (i.e. 243 m/s) (Harger and Huelke 1970). It could be presented as jagged edges, blown out fracture edges, large quantities of bone loss (Robens and Küsswetter 1982).

The fractures to the ribs may also be able to be described by the general types of fracture terminology based on the pattern of the fracture which reflects the type of force acting on the bone. For example, transverse fractures where the bone fractures perpendicular to its long axis under tension occur often (Figure 16, 17), oblique fractures where a 45 degree angle to its long axis under bending and compression occurs (Figure 17), spiral fractures, often occurring in long bones, have been noted in the rib cases (Figure 9). As ribs and shafts of long bones have a shape of oval tube, the force acting on these bones in torsion will create an oblique-like fracture which encircles the axis of the ribs. An interesting find was in one case (Figure 15) where a fracture similar to a butterfly fracture occurred. The bone is penetrated by a bullet, where the force is a combination of tension, compression and bending creating a nick in the bone directly from the bullet and a triangular fragment and two segmented pieces (Figure 15). This often occurs in long bones (Symes et al. 2012) and in

blunt force trauma. The force acting in this type of wound produces angulation fractures (Ubelaker 1995).

As with gunshot wounds to the cranium, bevelling is a distinguishing feature of entrance and exit wounds. This characteristic has been seen here on some of the ribs (Figure 11) as well as on the ilium (Figure 21). The heaving of a fracture in a particular direction or displaced fragments of bone can also determine the direction of travel (e.g. Figure 14).

Conclusion

It is critical to understand the wounding patterns associated with projectile trauma to the torso region as this is the centre of the mass and is often targeted. The fracture patterns on the ribs can be used to determine the direction of travel of the projectile. Further analysis of more specimens will provide a greater understanding of these wounding patterns and controlled experimental studies may lead to the development of a bone simulant which is able to be used in these experiments.

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Authors' contributions

CH collection of data, analysis and interpretation of wound trauma, concept, writing and finalising article, approval of final article version; MH interpretation of wound trauma, critical revision and drafting of article, approval of final article version.

Conflict of interest

There are no conflicting interests.

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