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Modelling for conflict: the legacy of ballistic research and current extremity in vivo modelling

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ABSTRACT

Extremity ballistic injury is unique and the literature intended to guide its management is commonly misinterpreted. In order to care for those injured in conflict and conduct appropriate research, clinicians must be able to identify key in vivo studies, understand their weaknesses and desist the propagation of miscited and misunderstood ballistic dogma. This review provides the only inclusive critical overview of key studies of relevance to military extremity injury. In addition, the non-ballistic studies of limb injury, stabilisation and contamination that will form the basis from which future small animal extremity studies are constructed are presented. With an awareness of the legacy of military wound models and an insight into available generic models of extremity injury and contamination, research teams are well placed to optimise future military extremity injury management.

INTRODUCTION

Combat wounding is characterised by disabling extremity injury, and 70% of war wounds involve the limbs.^{1–2} Mechanism is key: short duration, high-energy transfer explosions with fragmentation cause 75% of extremity war wounds³ and infection, a source of significant morbidity in survivors of combat,^{4–7} is associated with these injuries.

While the Lower Extremity Assessment Project, a multicentre prospective outcome study of significant limb injury, reports outcomes for limb salvage and amputation,⁸ it is an observational civilian study uncontrolled for initial injury management. Overall, ballistic trauma is notable for the *absence* of its clinical evidence base. Inability to control and stratify injuries, treatment or follow-up compromises study methods supporting the need for in vivo experimentation to further the understanding of war wounding.

This review provides the only critique of key models in the study of combat relevant extremity injury; it highlights inadequacies in existing animal studies that limit their application to combat extremity injury and will guide future model design.

METHOD

Combat wounding is rarely isolated to a single tissue and compound injury of bone and soft tissues predominates. Similarly, seldom are such wounds free of debris or organisms. In order to represent this and present the findings of this review in a structured fashion, in vivo models are categorised according to animal type and then discussed further with regard to fracture, soft tissue injury and infection.

An electronic search of the Medline database using the PubMed search engine limited to

Key messages

- ▶ Extremity ballistic injury is unique and demands individual care, based on clinical and in vivo research.
- ▶ Ballistic injury literature is often misinterpreted: a situation which propagates an often dogmatic approach to war surgery.
- ▶ Large animal models of simple projectile injury must be placed in context with and contrasted to modern combat injury patterns.
- ▶ Civilian small animal models of injury and infection have application to military injury modeling although are limited in their exploitation of contaminated soft tissue injury.
- ▶ A need exists to further develop military specific, contaminated soft tissue small animal soft tissue models.
- ▶ With an awareness of both large and small animal ballistic and extremity trauma models, military surgeons are in a better position to accurately interpret pertinent literature and optimize patient care.

manuscripts published in English until July 2012 was performed. Medical Subject Headings of Wounds, Penetrating, Forensic Ballistics, Models, Animal, Fractures, Bone and Extremities and Boolean operators, were used to construct a search strategy⁹ and although the nature of the studies reviewed preclude systematic review or meta-analysis, the outcome of retrieved abstracts is presented (Figure 1). Retrieved abstracts were analysed for relevance and full text papers obtained. Articles initially missed in this search were obtained from manual searching of the bibliography of retrieved studies.

In order to ensure only articles relevant to the representation and investigation of ballistic extremity trauma were reviewed, a considerable number were excluded from further analysis according to the exclusion criteria in Box 1.

PORCINE MODELS OF BALLISTIC INJURY

The animal of choice for *physiological* resuscitation studies,¹⁰ porcine models, are used to investigate management options in the blast injured casualty.^{11–21} To study gunshot wounding (GSW), however, *anatomical* projectile interaction with tissues is key in the choice of model. Again, swine are historically the most popular choice due to similarity in tissue architecture and scale to the human. The impact of muzzle velocity, the stimulus for much porcine modelling to

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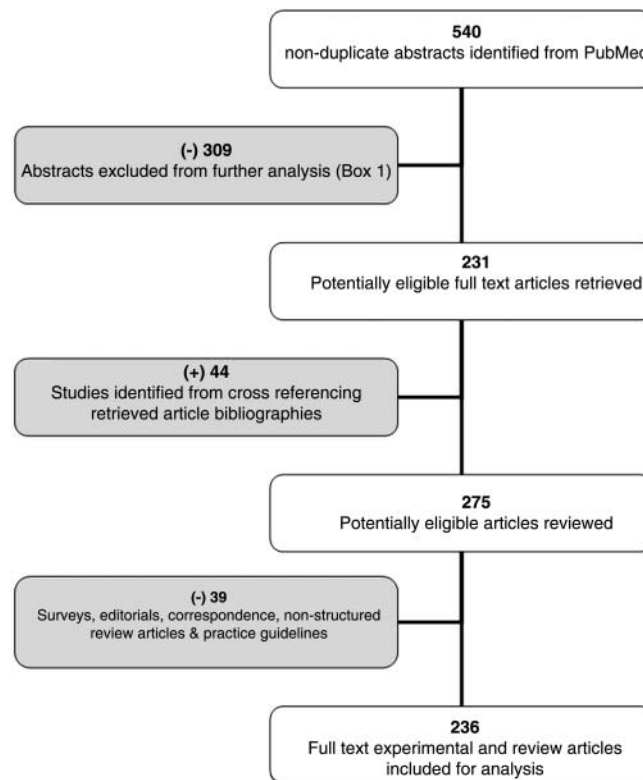


Figure 1 Flowchart of article identification.

follow, was highlighted in a series of clinical papers by DeMuth,^{22–26} attesting that ‘velocity makes the difference’²³ in GSW and that ‘killing power is determined by the velocity at impact’²² based on changes in wounding patterns from the Vietnam War following the issue of a new rifle to the US troops.^{27–28} The 7.62 mm round fired from the M14 rifle was replaced by the lighter, smaller and faster 5.56 mm ammunition for the M16. Increased muzzle velocity was *presumed* to result in greater tissue trauma although it has, in many wounds, the opposite effect. In wounds with short tracts, the smaller, faster bullets were likely to pass through tissue intact. Only in those cases where the bullet was retarded or had significant tract length were devastating injuries seen²⁹ although investigators of the post-Vietnam era, however, made muzzle velocity central to their *in vivo* porcine wounding hypotheses.

Velocity as a variable in wounding is not new, as demonstrated by Kocher’s theory³⁰ of hydrodynamic projectile–tissue interaction: the pressure wave created when a projectile contacts a medium of different density.³¹ This underlies many animal models of GSW, including the Swedish Missile Trauma model (SMTM),³² a series of experiments on the legs of Swedish

Landrace pigs. In earlier versions,³³ standard weaponry are used although later, smooth spheres fired into the pigs legs^{34–35} were correlated with simulant media on a hypothesis that it is solely the conversion of *deposited kinetic energy* which has potential and therefore may be responsible for tissue damage. This philosophy, concentrating only on energy deposition generated through muzzle velocity, disregards the effects of the projectile physically passing through and damaging soft tissue and bone.

This SMTM method is used to investigate local mechanical^{33–36} and metabolic^{37–41} effects of projectiles, the influence of trajectory length,⁴² timing and extent of debridement³⁴ and the influence of antibiotics.³⁵

The SMTM has limitations, which have come to signify the divide in the scientific community over the concepts of muzzle velocity and energy transfer and the impact of both on wounding. First, little attention is paid in the SMTM to fragmentation and its effect on tissues. Berlin *et al*³³ report that damaged bullets resulted in ‘complicated’ or high severity wounds *regardless* of initial velocity. *Despite* this, the authors hypothesise that it is actually the impact of projectile *velocity* that influences the amount of non-viable tissue. Second, the use of simulant media further threatens their findings. A non-elastic (gelatin) tissue stimulant is used rendering it impossible to distinguish a *temporary* cavity (of lesser injurious extent) from the crushing tissue loss of the *permanent* cavity. The blocks are not calibrated following preparation and thus any variance introduced through non-standardised heating of the gelatin will affect the results, further preventing generalisation of kinetic energy transfer results to live tissue.⁴³

Further limitations include the use of steel spheres which do not yaw, nutate or fragment and hence the non-reproducible, non-uniform impact surface and the ‘secondary projectile’ effect is neglected. Animals in which projectiles hit bone were excluded from analysis,^{34–41} despite the relationship among

Box 1 Exclusion criteria for further analysis of retrieved abstracts

- ▶ Non-extremity military ballistic injury articles
- ▶ Exclusively *in vitro* studies
- ▶ Clinical studies of extremity injury management
- ▶ *In vivo* models of simple skin wounding and healing
- ▶ Non-extremity *in vivo* models

fragmentation, fracture and wound severity identified at the outset of the model.³³ These studies therefore have limited similarity to current ballistic injury patterns. Following criticism of the SMTM, the characterisation of wounds by muzzle velocity^{44 45} and the practice of basing debridement on energy transfer came into question as investigators brought into consideration the effect of bullets and other projectiles physically interacting with tissues.⁴⁶

Fackler and his colleagues reported a series of combined in vivo and tissue substitute studies investigating standard munitions in porcine models. In contrast to the SMTM, the models of Fackler *et al*^{47–49} are focused on the effect of bullet deformation on the wound profile. Using a porcine model they demonstrated that the wounding profile was strongly correlated to the design and behaviour of the projectile within tissue, related to its trajectory,^{49 50} the elasticity of the organs in its proximity⁴⁷ and ultimately to its fragmentation.^{51 52} Assessment of the tract through tissue and gelatin blocks revealed that disruption from fragmenting bullets is significant and that fascial decompression often occurs at wounding. In the presence of antibiotic cover, wound excision did not improve time to healing in simple, short tract wounds.⁵³

In contrast to guiding debridement by kinetic energy,³³ Fackler *et al* proposed that projectile fragmentation should guide tissue excision, a hypothesis corroborated by Tikka *et al*.⁵⁴ In contrast to other Scandinavian studies, Tikka's group used standard weaponry and of the munitions fired, the 5.56 mm round underwent greater fragmentation, was associated with greater energy transfer and necessitated the resection of up to twice as much tissue than the larger calibre projectiles. This was achieved by a bullet of less than half the mass (3.6 g) of the two greater calibre rounds (8 g). The lower mass of the 5.56 mm round contributed to its initial *impact* energy being 20% less than the M62 or AK47 ammunition, although its *transferred* energy was 276% greater. The key finding of these models, therefore, in contrast to the SMTM, is that the influence of bullet mass and velocity is of little consequence in comparison to the terminal behaviour of the round.

While Fackler *et al* present evidence more generalisable to the clinical setting than that of the SMTM, the use of smooth projectiles and 'simple' low energy transfer wound models should not be discounted. Although acknowledged to be less representative of compound tissue trauma, they do have relevance to injury by multiple small fragments. In the models of both Bowyer *et al*⁵⁵ and Mellor *et al*,⁵⁶ uniform projectiles are used in a low-energy transfer wounding, avoiding bone and vessels, producing short projectile tracts. These wounds are then used to assess the impact of antibiotic administration, demonstrating that for the selected wound profile, surgical intervention is less likely to be required.^{57–59}

More recently, porcine modelling has moved towards examining the effects of injury distant to the projectile. As with other areas of ballistics, misunderstanding of shock waves leads to confusion. The shock wave is sonic pressure that results from the bullet striking tissue. Travelling at the speed of sound (ie, faster than the speed of the bullet), it passes through tissue *ahead of the projectile*. Cavitation is a separate entity caused by the bullet physically striking tissue and occurs in its wake.³¹ Suneson *et al* demonstrated in vivo and in vitro shock wave injury thus highlighting local, regional and distant injuries resulting from sonic pressure waves. Stigmata of microscopic damage to central and peripheral nerves occurred at a distance and was therefore not attributable to the effects of either the permanent or temporary cavities of the projectile.^{60 61}

Attribution of tissue damage to the sonic wave as opposed to the effects of temporary cavitation has attracted considerable debate. Fackler and Peters are vociferous in their critique and both in correspondence with Suneson *et al*⁶² and in a later published review dismiss injury due to the effects of the sonic wave as myth.⁶³ The arguments used against acceptance of injury by the sonic wave are suspect. Fackler cites comparison with a previously reported animal model⁵³ as proof that distant injury does not occur but the methodology of the two experiments however is disparate and it is impossible to generalise from the work of Fackler *et al*⁵³ to that of Suneson *et al*^{60 61} who make the point that distant injury occurred only at a microscopic level.

Fackler *et al*⁵³ state that in one of their studies 'an area of the thigh with the gunshot wound was sectioned for histological study'. This was in essence a study of local (thigh) tissue effects in which no evidence of distant injury was sought. It is difficult therefore to accept this as experimental proof that the work of Suneson *et al* is flawed. Similarly, Fackler and Peters cite the absence of distant injury seen in the Vietnam conflict. Using data from 1400 GSWs they remark that 'there were no cases of bones being broken, or major vessels torn, that were not hit by the penetrating bullet.'⁶² Again, comparing such macroscopic, local wound evaluation to a controlled animal experiment performed at a distant microscopic level is questionable. In addition, subsequent interrogation of the Vietnam data⁶⁴ reveals Fackler's interpretation to be biased as 'the database contains at least one possible example of this phenomenon'⁶⁴ referring to the influence of pressure waves on nerve tissue injury.

Thus, it can be seen that far from refuting the possibility of distant injury occurring secondary to GSW, these data suggest that such a mechanism may exist. While often contradictory, a large body of experimental evidence exists for the interaction among projectiles, tissue or its substitute.

In addition, increasing evidence exists for trauma distant to the projectile in extremity models not attributable to cavitation phenomena. This is of particular relevance in combat injuries seen as a result of explosion.

NON-PORCINE MODELS OF GSW

The damaging potential of high-velocity bullets was assumed, as with the SMTM, to be so great that they exploded on contact with tissues⁶⁵ and the teaching that widespread debridement should be carried out for high-velocity gunshot wounds resulted. Often incorrectly cited, this erroneous dogma should actually be attributed to Rybeck⁶⁶ who proposed that high-velocity GSWs resulted in a temporary cavitation effect 30 times the diameter of the bullet entering the tissue, but more importantly that tissue within this zone would not survive. He used a canine model in which a smooth spherical projectile was shot through the medial hind leg of dogs and concluded that:

...the temporary cavity following the high velocity missile appeared to affect the tissue as markedly as the contusion trauma. These findings can explain the clinical experience that tissues which have been subjected to the formation of the temporary cavity after a high velocity missile will not survive.

This is the paragraph of text which erroneously has most influenced the management of wounding by gunshot with such a debridement equating to the removal of a mass of tissue some 23 cm in diameter for all presumed high-velocity wounds.⁶³ The concept that huge debridement is mandatory sprang from this canine model and continues to influence surgical doctrine. Of note, Rybeck's is a non-recovery model so that none of the dogs were allowed to recover from injury to provide clinical

evidence to support the histological analysis that temporary cavitation led to such marked tissue destruction.

Contrary to the method of Rybeck,⁶⁶ evidence can be found for *lack* of significant tissue loss and apparent healing from high-velocity wounding in non-porcine recovery models. In an ovine GSW model, Hopkinson and Watts⁶⁷ found that damage to the tissues was confined to a small area around the projectile path. No surgery was performed and the animals recovered and ceased limping by the fourth day. The authors concluded that 'it would appear from the results presented here that the less severe wounds of skeletal muscle, particularly if there is no damage to major blood vessels, might heal spontaneously.' This finding that simple GSW, regardless of muzzle velocity, follow an uncomplicated path to healing is corroborated elsewhere in a caprine model⁶⁸ and also in a development^{69–71} of the ovine model of Hopkinson and Watts.⁶⁷

Of note, however, is that these studies^{69–71} then go on to introduce the deliberate infection of such simple wounds. In contrast to the recovery of the health of animals without surgical intervention or antibiotics, in animals with wounds complicated by gas gangrene, no untreated animals survived and surgery had no impact on outcome. The administration of intramuscular penicillin, however, prevented gangrene and was more effective than either wound incision or excision.

Irrefutable evidence of recovery from simple high-velocity GSW in animal models thus exists and it is latterly acknowledged that the requirement for widespread tissue excision is a myth.^{72–73} It is concerning therefore that permanent tissue damage 30 times the size of the projectile and radical debridement remains quoted in established literature.^{74–77}

Debridement based solely on muzzle velocity is flawed, but it is important to note the lack of contamination in the majority of studies and the clinician must balance excision of tissue due to projectile passage with that required to reduce the burden of contaminating organisms. It is the combination of these processes that guide debridement, not purely the speed at which the bullet travels prior to injury.

NON-PROJECTILE LARGE ANIMAL EXTREMITY INJURY MODELS

The porcine models involve injury to the extremity through passage of a projectile. Other militarily relevant large animal models have investigated extremity trauma in the *absence* of projectile injury. External fixator pin contamination and the impact on subsequent intramedullary nailing are the subject of a series of experiments by Clasper *et al*^{78–80} in an ovine model of importance due to the preponderance of temporising external fixation followed by late definitive stabilisation in this population. In a study of pin sites contaminated with *Staphylococcus aureus*, all tracts became infected in addition to all of the contiguously sited but uncontaminated control pins. This model was subsequently used to demonstrate the role in infection of fluid accumulation at the pin–bone interface⁸⁰ and intramedullary nailing in the presence of infected external fixator pin tracts.⁷⁹ Lack of fracture in the model of Clasper *et al*⁸⁰ was addressed in a modification by Hill and colleagues demonstrating the morbidity associated with early intramedullary nailing of heavily contaminated tibial fractures despite debridement and antibiotic use.⁸¹

Osteomyelitis is also addressed in caprine models and the goat is chosen by Curtis *et al*⁸² for their recovery study of open, *S aureus* contaminated, tibial fracture management. The results of this work corroborate those from the ovine models of both Clasper's and Hill's groups with regard to infection risk in early intramedullary nailing of open fractures and also illustrate the

benefits of increasing animal size in extremity trauma research. Standard surgical approaches and implants are used with only slight modifications.

The limitation of this study is its short time period with the inability to draw conclusions regarding long-term outcome of the different fixation strategies, especially in terms of clinically apparent infection or the impact this may have on fracture union.

Further exploring treatment modalities for open fractures, Svoboda *et al*⁸³ created a militarily relevant bioluminescent musculoskeletal wound model to perform a comparison of bulb syringe and pulsed lavage irrigation. This model represented a true experimental compound extremity injury and is the basis for a number of further studies using *Pseudomonas* species and *S aureus*^{84–88} including the experimental evaluation of topical negative pressure wound therapy augmented with silver dressings.^{89–90}

While allowing for experimental manipulation of variables affecting early operative interventions in limb trauma, these studies are all of short duration and it would be beneficial to extend the study period to assess longer term outcomes, particularly with regard to the establishment of chronic osteomyelitis or the effect of model manipulation on fracture union.

Where the caprine studies benefit most compared to small animal models as highlighted by Salgado *et al*⁹¹ and Curtis *et al*⁸² is their comparative size in terms of osteology and ability to use standard osteosynthesis techniques and instruments. This comparison is not limited to internal fixation and a number of studies have used goats as experimental models for the study of external fixation.^{92–96}

Internal and external fixation for fracture management, debridement and wound care and wound profiling have therefore all been investigated *in vivo* in large animals. Emphasis on muzzle velocity and the implication of energy transfer and debridement on this premise are highlighted in early porcine models alongside more compelling evidence of injury far distant to the projectile's path. Infection as a key component in extremity wounding is similarly noted.

An awareness of these studies and an understanding of those that have erroneously influenced modern wound care is essential to military surgeons and in particular those designing contemporary experimental models of extremity wounding.

SMALL ANIMAL MODELS OF RELEVANCE TO MILITARY EXTREMITY INJURY

Large animals have advantages in terms of similarity of physiology, surgical approach, bone structure and ease of implant instrumentation over smaller animals. Inherent in the use of such larger animals are significant husbandry and welfare issues associated with using more sentient animals for scientific research. Researchers are beholden to use as few animals, of least sentience, as possible in a responsible manner in accordance with the principles of replacement, refinement and reduction.^{97–98} Whereas complete replacement of experimental models with non-animal alternatives may not be plausible, the use of smaller animals of least sentience must be the aim.

Traditionally, models of fracture healing and instrumentation involved large animals and, in particular, the sheep tibia.^{99–101} The advent of gene targeting and significant advances in the evolution of osteosynthesis implants for small animals however has occasioned a move away from large animal models towards lesser sentient, easier housed, genetically manipulated rodents and rabbits.

Murine models of extremity injury and infection

Examples of fracture initiation, stabilisation and healing are available in isolation in rats and mice.^{102–125} Muscle trauma in murine models is less well investigated and where performed is dominated by closed injury^{126–137} with limited relevance to military extremity trauma. While a minority of controlled, reproducible open muscle injury models are reported,^{138–139} the majority of methods use uncontrolled manual application of crushing forceps^{140–145} to the muscle belly with considerable variability. Infection modelling in the murine extremity models is also limited in scope, detailing primarily osseous critical defect or implant related infection in the rat.^{146–163} Similarly, soft tissue contamination models are limited.^{164–165}

Guinea pig models of extremity injury and infection

The guinea pig is notably the animal used by Koch to establish the causative link between organism and disease.^{166–167} In addition, susceptibility to pyogenic staphylococcal infection¹⁶⁸ makes it of relevance to extremity studies both of wounding and prosthesis infection. Although the guinea pig demonstrates advantages of increased size over murine species while sharing their benefits in terms of economy of husbandry, there are drawbacks to their use. They are more expensive and do not offer the same potential for gene deletion technology as is available in the mouse.

In addition, as with the murine models, options for osteosynthesis and fracture modelling are less well established and impart considerable technical and logistic demands on the researcher. Unlike the murine models, fracture studies are limited with only one contemporary biomechanical study of fracture healing with rudimentary intramedullary stabilisation.¹⁶⁹ Similarly, the animal is not routinely used to investigate osteomyelitis, with only one experimental assessment of a contaminated, open fracture.¹⁷⁰

Although fracture and osteomyelitis models are limited in the animal, the experimental investigation of soft tissue infection and wounding is well established in guinea pigs. As the landmark study of Koch influenced the approach to disease causation, similarly the guinea pig contaminated wound model of Friedrich has had significant impact on the approach to extremity wounding and is the historical basis for an emergent approach to open fracture management.¹⁷¹

While numerous such guinea pig models of wounding and infection exist, they are not representative of contaminated extremity injury. Models involving incisional or lacerated wounds created on the flanks or paraspinal region of the animal with or without additional uncontrolled muscle crush through application of artery forceps characterise these studies.^{168–180}

Reproducible, controlled tissue trauma does not feature in these models and none involve the limbs.

Rabbit models of extremity injury and infection

Benefiting from increased osseous dimensions while maintaining the relative ease of husbandry, the rabbit is an established animal model for fracture healing and orthopaedic implant investigation. Compared with the rat or mouse, however, soft tissue trauma is less well modelled in this animal.

The rabbit tibia has been extensively modelled in both open and closed fracture initiation.^{181–187} Stabilisation of fractures has also been reported using relative stability fixation and more advanced osteosynthesis. Early, rotationally unstable Kirschner wire intramedullary fracture fixation^{188–190} has been surpassed by rotationally stable interlocked nails.¹⁹¹ The dynamic

compression plate, synonymous with fracture osteosynthesis, has also been modelled on the rabbit and of note is reported in an early study documenting primary bone healing in rabbit tibiae.¹⁸⁵ This study of Rahn *et al* is significant in demonstrating the process of haversian remodelling in rabbit fracture healing similar to that in adult human bone. This contrasts the rabbit as a bone healing model to the available murine models, which demonstrate limited similarity to adult primary bone healing.

Infection of rabbit bone has been modelled more extensively than any other. It is also notable for rudimentary investigation of wound infection in the Vietnam conflict and is thus explored in greater detail. Simple osteomyelitis models were first developed in the rabbit although they were characterised by failure to produce progressive disease and, in addition, were associated with significant mortality.^{192–194} Norden and Kennedy¹⁹⁵ identified the lack of long term osteomyelitis models in their modification of the work of Scheman *et al*.¹⁹² Percutaneously administering sclerosant and *S aureus* into the proximal tibial metaphysis of New Zealand white rabbits, they introduced the 'gold standard' model of reproducible osteomyelitis capable of experimental manipulation for up to 6 months. Thus established as a standard for the study of bone infection, the model of Norden and Kennedy is used or modified by others.^{196–205}

Careful interrogation of studies citing Norden and Kennedy reveal that a number incorrectly interpret the fundamental aspects of the design and thus caution must be adopted in the interpretation of their results.^{189–207} In an additional example of how miscitations are propagated through scientific literature, Moriarty *et al*²⁰⁸ incorrectly identify their methodology as that of Norden and Kennedy due in part to the earlier miscitation of Melcher *et al*. It is imperative that designers of future rabbit bone infection models are cognisant of these errors in citations in order to allow for successful comparison of experimental findings.

Andriole *et al*^{183–209} developed the work of Norden and Kennedy by extending the study period and incorporating the use of intramedullary nails in the study arms thus using the foreign body effect and enhancing its clinical applicability. Of note, the investigators found that in control animals exposed to inoculation with *S aureus* only, no osteomyelitis developed. In contrast, both groups of animals with either fracture or intramedullary nail or nailing alone developed osteomyelitis with the same dose as the controls. In addition, a second study²⁰⁹ demonstrated that the dose required to produce osteomyelitis in the intervention groups was 100 times less than that required in controls. The value of these studies is the length of the clinical course and the demonstration of the impact of intramedullary devices on osteomyelitis. Of note, no sclerosant is used and the fractures are produced by a three-point bending clamp in a closed fashion. Both the work of Norden and Kennedy and Andriole *et al* thus advanced the animal modelling of osteomyelitis and informed the methodology of subsequent studies. Both designs however have inherent limitations in application to osteomyelitis research due to the absence of soft tissue trauma, which is a key feature and prognostic indicator with significant limb injury.

A study that at first seems to address these limitations is that of Friedrich and Klaue.¹⁸² Assessing the impact of fixation rigidity on the incidence of osteomyelitis following open fracture, Friedrich and Klaue used compression plates and intramedullary nails to stabilise contaminated fractures. Their results indicate that contaminated fractures stabilised rigidly demonstrate a clinical path similar to that of non-contaminated fractures similarly fixed and that contaminated fractures fixed with relative stability

have a greater risk of developing osteomyelitis. Their study design however is poor and allocation to groups and detail of end points are lacking, leading to the questioning of the validity of their findings, despite their apparent clinical application.

The concerns over the lack of applicability to open fractures of the models of Norden and Kennedy¹⁹⁵ and Andriole *et al*^{183 209} are highlighted by Worlock *et al*¹⁸⁸ in the introduction to their model of post-traumatic osteomyelitis where the use of sclerosant and percutaneous inoculum with no soft tissue trauma and fracture is likened more to a model of haematogenous osteomyelitis than that of traumatic origin. While the model of Andriole *et al* does incorporate fixation and fracture in its design, soft tissue trauma does not feature and the inoculation point is distant to the site of fracture.

Using the fracture model of Ashhurst's group,¹⁸⁶ Worlock *et al*¹⁸⁸ performed an open fracture followed by intramedullary nailing of the tibia. In addition to the use of an open fracture by osteotomy, this study advanced the modelling of open fracture by inoculating the fracture site directly, a direct contrast to previous work. They conclude that their model uses animals which are relatively cheap and easily available and the instrumentation required is easily adapted from standard surgical practice. In addition, they were able to reproducibly induce osteomyelitis with no systemic side effects and in contrast to many previous studies, all animals survived. This model benefits from its ability to more closely replicate bacterial contamination at a fracture site and it forms the basis for a number of subsequent studies of therapeutic manipulation of fixation devices for potentially infected fractures^{210–212} and also for studies in which no fracture occurs but direct device infection is enabled by the technique described.^{213–215} In addition, Worlock *et al*²¹⁶ used their model to assess the impact of antibiotics on subsequent infection in open fractures and also carried out a key study relating the stability of fracture fixation to ultimate complication by infection—essentially a more robust investigation than that performed earlier by Friedrich and Klaue¹⁸² but yielding similar findings of decreased post-traumatic osteomyelitis in contaminated fractures that are rigidly stabilised.

Further to the establishment of post-traumatic osteomyelitis as detailed by Worlock *et al*,¹⁸⁸ the impact of implants, bone cement and bone wax in osteomyelitis models is also addressed in the rabbit.^{217–229} Current researchers planning study designs should be aware that as with Norden and Kennedy, the model of Nijhof's group has also been incorrectly cited by Jia *et al*²³⁰ in their study of the prophylactic effects of platelet-leucocyte gel in osteomyelitis by omitting cement from their method, obviously an inaccurate model citation.

In contrast to murine modelling, the investigation of soft tissue trauma in rabbits is sparse. There are no controlled models of open muscle trauma in the rabbit and only one closed study is reported. Zhang *et al*²³¹ used MRI to quantify muscle damage following closed crush injury in New Zealand white rabbits. Their description of injury mechanism is limited, however: 'Then, their right hind limbs were fixed with wooden splints and crushed with 25 kg heavy weight'.²³¹ The only series describing experimental open muscle trauma in the rabbit are military studies from the Vietnam War period. Rutherford *et al*²³² describe the use of a compound soft tissue injury of the buttock region of rabbits in establishing a model of experimental clostridial wounds. Following excision of a full thickness skin flap, non-standardised lacerations were created in the gluteal musculature, down to bone and the incised muscle strips then crushed sequentially with artery forceps. The wounds were then contaminated by soil sourced from the under surface of a motor

car. These uncontrolled experiments resulted in significant mortality both through extensive debridement, including disarticulation at the hip, and also from the effects of gas gangrene. Of note from these experiments however is the effect of early debridement in decreasing mortality and, of particular interest, the increased mortality associated with late, as opposed to simply delayed, debridement. In addition, the administration of topical antibiotics was shown to extend the window in which debridement may influence mortality.

The rudimentary model used by Rutherford *et al*²³² was subsequently chosen by Matsumoto *et al*²³³ to study the effect of various soil contaminants and the impact of topical antibiotics. In a geographical study, Matsumoto *et al*²³³ collected soil samples from swamplands, fertilised farmlands and more arid beach areas around Vietnam during the conflict. The rabbits were prepared as in the model of Rutherford *et al* and a sample of soil applied to the wound. While the methodology is poor and outcomes unclear, the difference in mortality of the rabbits is marked. Reflecting experience from current conflicts²³⁴ and that seen in the original description of war wound bacteriological profiles,²³⁵ the nature of soil influences the bacterial burden of the war wound with wounds occurring in jungle, swamp and fertile farmland associated with greater morbidity than those from more arid climates.

Having established the model of soil samples from conflict zones, Matsumoto *et al*^{236–240} went on to investigate the application of topical and systemic antibiotics to these wounds in a series of subsequent studies. Refining the methodology slightly, they introduced soil samples and also a known quantity of polymicrobial bacterial suspension to the wounds. Of interest, in these studies guinea pigs were also used for the model; however, no justification for this is made. While suffering from considerable inadequacies in method and animal welfare, these papers demonstrate the proposed benefit of early application of topical antibiotic to combat wounds, which is in contrast to clinical observation from World War II.²⁴¹ The model allowed early experimental contamination with one or more organisms associated with conflict wounds and while its methodological flaws limit its use for modern extremity trauma research, it has value as a basis from which to design animal models of standardised injury and contamination.

SUMMARY

The conflicts in Iraq and Afghanistan have refocused attention on combat casualty care and in particular the impact of far-forward resuscitation, prompt evacuation, haemorrhage control and time limited surgery on patient outcome.

Despite these improvements, a burden of significant extremity trauma remains in those surviving combat injury. This patient cohort is a result, in part, of increasing survival but is also due to the nature of current combat wounding. Injury by gunshot has been eclipsed by explosive devices with its late extremity complications of skin coverage, fracture stabilisation and infection and there remains a need to optimise through animal modelling the management of these disabling injuries. Simple, soft tissue wounding based on velocity and studies of smooth projectile passage through muscle and gelatin have limited relevance to modern conflict wounding patterns.

The concept of tissue injury, albeit at a microscopic level, distant to the passage of a projectile raised in swine models following the Vietnam conflict remains unanswered. This is particularly of relevance to the nerve injuries seen from recent conflict and further in vivo work would be of value to investigate this phenomenon.

Non-porcine large animal GSW studies also have flaws in their design that have influenced current military surgical doctrine, particularly notable in terms of erroneous recommendations for debridement extent. Such flaws limit the ability to generalise many of the most cited models to contemporary conflict injury. Models of short duration, high-energy, non-projectile, contaminated extremity injury most representative of current wounding are not seen in large animals.

This lack of complex, contaminated wound models in combination with adherence to the principles of replacement, refinement and reduction has led to the use of small animals such as rats and mice to construct future models of complex military extremity injury. Although murine models are widely used in musculoskeletal research, this review has highlighted limitations in available murine models applicable to military complex extremity trauma.

Murine bone morphology and remodelling raise concerns regarding generalisation to clinical practice both in terms of bone healing and regarding methods of osteosynthesis. Controlled models investigating isolated aspects of fracture initiation, healing and management of contaminated bony defects are available in rats and mice. Studies of compound bone and soft tissue trauma are, by contrast, sparse and where reported are characterised by uncontrolled muscle crushing injury. Controlled soft tissue damage and contamination have not been carried out in rats or mice. The murine models therefore are limited by scale and, to date, fail to generate a model of sufficient compound injury or contamination to have military relevance.

In contrast to the murine models, there is limited evidence for suitability of the guinea pig in the investigation of fracture initiation or management. In addition, while associated with experiments that link bacterial contamination to infection and subsequently influenced the rationale for open fracture management, these are characterised by a lack of control of wounding. Also, the majority of guinea pig studies use the paraspinal musculature in their models, not the extremity, and so generalisation of tissue contamination and healing from these models to modern complex extremity injuries is threatened.

The largest of the small animal models used for the investigation of fracture initiation, healing, contamination and osteomyelitis is the rabbit. As with the murine and guinea pig models, rabbit musculoskeletal experimentation is widespread and the New Zealand white rabbits in particular are accepted throughout healthcare research as a disease and injury model. In comparison to the murine and guinea pig models, the rabbit benefits from increased size and, in particular, more clinically relevant bone healing and ease of osteosynthesis. Rabbit fracture models of the tibia, radius and ulna are abundant and contamination to produce infection either as a percutaneously introduced haematogenous model or one reflective of post-traumatic osteomyelitis is available. These models all use *S aureus* which is the predominant organism associated with late battlefield wound infection sequelae.

Initial fracture fixation options in the rabbit were rudimentary but currently interlocked intramedullary nails, dynamic compression plates and external fixators are available for fracture stabilisation, although the performance of these devices in the presence of contamination is not reported. In addition, where rabbit models are lacking is in the controlled delivery of a high-energy, short duration muscle injury and its subsequent contamination.

Extremity ballistic injury is unique and the literature intended to guide its management is commonly misinterpreted. In order

to care for those injured in conflict, allow debate and the design of preclinical studies, clinicians must be able to identify key in vivo studies, understand their weaknesses and resist the propagation of miscited and misunderstood ballistic dogma.

In order to thus inform military clinicians, we have provided the only inclusive critical overview of key studies of relevance to military extremity injury. In addition, the non-ballistic studies of limb injury, stabilisation and contamination that will form the basis from which future small animal extremity studies are constructed are presented. With an awareness of the legacy of military wound models and an insight into available generic models of extremity injury and contamination, research teams are well placed to optimise future military extremity injury management.

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