Local anti-microbial delivery systems for therapy of orthopaedic infection in the horse

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This thesis is presented for the degree of Research Masters with Training at Murdoch University

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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Dr Peter G Harding BVSC (Hons) MACVSc
I would like to dedicate this document to my family, particularly my parents, Alan and Jane, who without their love, support and encouragement I would not have achieved as highly as I have today.
Abstract:

Orthopaedic disease makes up a significant proportion of the caseload for the equine veterinary surgeon. Presentation of a patient with a septic synovial structure or osteomyelitis is one of the most serious disease processes in the spectrum of orthopaedic disease in the equine patient. The complexities of achieving effective and successful therapy and minimising the long term complications are not only challenging but stressful for the attending clinicians.

Traditional therapy of such conditions has involved surgical debridement of devitalised and infected tissue along with systemic antibiotic therapy. More recently improved outcomes have been achieved with the addition of local antimicrobial therapy to treatment regimes.

Newer modes of antimicrobial delivery are currently sought after; ideally implants which will sustain local antimicrobial at therapeutic concentrations; are biodegradable; do not incite host inflammation; and do not inactivate antimicrobials during assembly. This manuscript reviews orthopaedic infections in the horse, the modalities of therapy available to the equine veterinarian, specifically newer modes of providing antimicrobial therapy, and efficacy of their use. Currently the ideal method of local antimicrobial delivery for orthopaedic infection in the horse has not been developed.
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**Pathogenesis of orthopaedic infection**

By definition osteomyelitis refers to an inflammatory process accompanied by bone destruction and caused by an infecting micro-organism.\(^1\) Infection and the resulting bone destruction can involve several regions of the bone – such as the cortex and the marrow (osteomyelitis) or cortex and periosteum only (more correctly termed osteitis). Osteomyelitis in the horse is relatively uncommon per se, however osteitis, involving the cortical bone, its periosteum and the surrounding soft tissue, is not an uncommon occurrence in the horse. In the adult orthopaedic infections include septic synovial structures, septic osteitis and osteomyelitis with the inciting cause being classified as haematogenous or post traumatic.

Orthopaedic infection in the foal is almost exclusively joint associated occurring via haematogenous localisation of bacteria and has been classified into three categories based on the anatomic location of the infection.\(^2\) As amended from Firth 1983:

- **S type** – presence of serofibrinous or fibrinopurulent arthritis with no macroscopic evidence of osteomyelitis at necropsy
- **E type** – osteomyelitis of the epiphysis at the subchondral bone junction
- **P type** – osteomyelitis directly adjacent to the physis

A number of host factors that have been identified that may predispose the development of septicaemia and haematogenously localising infections. Inadequate transfer of colostral immunoglobulins,\(^3\) or the use of corticosteroids suppressing the immune response,\(^4\) increasing the susceptibility for the development of pneumonia, diarrhoea, and umbilical infections. Bacteraemia can then follow seeding from these primary sites of infection.\(^2,4,5\)

The anatomy of the vasculature within the neonatal metaphysis is thought to promote the transmural localisation of bacteria.\(^6\) In human infants minor trauma is thought to lead to
hematoma formation, vascular obstruction, and subsequent bone necrosis that is susceptible
to inoculation from transient bacteraemia, which can occur due to minor trauma from
routine tooth brushing - which leads to bacteraemia 25% of the time. In rabbit models of
acute haematogenous osteomyelitis it has been shown that trauma increases the chance of
infection in presence of concurrent bacteraemia. A similar disease process may occur in
the horse however the exact pathogenesis remains unknown in the horse.

Synovial sepsis results when the size of the inoculum (dependant on the individual micro-
organism virulence and pathogenicity) overcomes the inherent synovial defence
mechanisms. Colonisation of synovial spaces leads to a marked inflammatory response,
initiated by the release of enzymes, free radicals and inflammatory mediators from
synoviocytes. The inflammatory cascade leads to the production and release of a
multitude of destructive enzymes and mediators, such as interleukin-1, tumour necrosis
factor and free radicals, which all act to disrupt the normal synovial homeostasis, cell
metabolism, and synovial membrane permeability. This culminates in a severe imbalance
in cartilage homeostasis with activation and release of metallomatrix proteinases (zinc
dependant endopeptidases) which play a key role in cartilage degradation, and joint
pathology.

In the adult equid most cases of cases of synovial sepsis and osteomyelitis can be classed as
post traumatic, secondary to external or surgical trauma and contamination of the wound.
Aside from direct inoculation of contaminants into synovial cavities and bone, trauma
contributes to the development of infection by damaging adjacent tissue, reducing blood
supply to the affected area, which can lead to the formation of necrotic regions of inert
tissue. Bacteria are then able to adhere to this tissue and infection then follows. Trauma to
the local tissues and wound formation has been shown to depress the local immune and
inflammatory defences to bacterial infection, with an increasing degree of severity of tissue
injury having an increased risk of infection. Interestingly the presence of bacteria in a wound alone has been shown to be insufficient to cause osteomyelitis.

Osteomyelitis may result when overwhelming contamination is combined with severe trauma, dead bone or metallic implants; especially when bone fragments or implants are unstable. Surgical site infection is reported to occur in 10% of orthopaedic surgical cases in the horse - of which 50% have been classed as clean contaminated surgeries. This is a significantly larger proportion of post-operative infections compared to our counterparts in the human field of orthopaedics, where surgical site infection is reported to occur at 1.3% in total hip arthroplasties, otherwise at rates less than 1% for all other procedures. Various risk factors for the development of post-operative infection have been identified in humans and in the horse:

- Patient related factors – obesity, prolonged hospitalisation, malnutrition, colonisation with methicillin resistant *Staphylococcus aureus*.

- Surgical factors:
  - Procedure (clean, clean contaminated, contaminated and dirty, surgical preparation (scrub solutions, hair removal, draping and surgical field barriers), surgical facility (airflow, drapes, gowns, masks), surgical technique (atraumatic, aseptic, instrumentation), surgery duration, post-operative care (incision care, peri surgical antimicrobial therapy).
  - Duration of surgery – the longer the duration of surgery the greater the exposure of the tissue to environmental contaminants.
  - Lack of debridement of contaminated or devitalised soft tissue and bone – avascular bone acts as nidus for chronic inflammation and infection. The
decision for removal of bone fragments should be based on fragment size, importance to stability, and amount of soft tissue attachments.\textsuperscript{19}

\begin{itemize}
  \item Unstable implants.\textsuperscript{19}
\end{itemize}

- Environmental factors – method of recovery, cleanliness of recovery facility, dressing of the surgical site for recovery.

The development of biofilms challenges to our ability to treat orthopaedic infections in the horse, as they promote attachment of bacteria through synergistic tropism; they are inpenetratable by inflammatory cells and antibiotics; they inhibit host inflammatory cells and phagocytosis; persistent host inflammation pleads to more tissue damage and allowing for ease of spread of bacteria, exposure of new surfaces for adherence and an abundance of nutrients that can be taken up by bacterial colonies for growth.\textsuperscript{1,20,21} The inherent resistance of biofilms to antimicrobial factors seems to be mediated by several factors including reduced metabolic rate, adaptive stress responses and down regulation of cell division of the deeply embedded microbes.\textsuperscript{1} Within biofilms bacteria can multiply and differentiate in multiple species. Antibiotics conventionally are selected for their efficacy against exponentially growing planktonic cells, not against biofilm cells, which largely explains there lack of effectiveness in treating biofilm related infections.\textsuperscript{22}

Chronically the hallmark of osteomyelitis is the presence of an involucrum – a region of live, encasing bone that surrounds infected dead bone within a compromised soft tissue envelope.\textsuperscript{23} This challenges the therapy of orthopaedic infection due to the dead bone acting as a nonliving surface for the attachment of bacteria and the formation of a biofilm.\textsuperscript{13}

It has been shown in animal models that some form of bone damage – mechanical, chemical or insertion of a foreign body is required to reliably establish infection.\textsuperscript{24} In humans the risk of subsequent infection is highly correlated to the degree of soft tissue injury associated with
the open fracture. Traumatised soft tissue and bone leads to exposure of potential binding sites for bacteria to attach. Tissue trauma compromises perfusion leading to tissue and bone necrosis – which can act like a foreign body in this setting, posing as a nidus for bacterial infection. Trauma has been reported to alter the host response to infection by suppressing the acute inflammatory response to the presence bacteria and depressing cell mediated immunity. Trauma impairs the function of polymorphonuclear leukocytes, including chemotaxis, superoxide production, and microbial killing.
Diagnostic modalities

Osseous infection in the horse is characterised by lameness which can vary according to the severity and duration of the infection. Acutely heat, swelling, and pain on digital palpation of a wound or surgical incision may be noted. Sinus tract formation and exudate drainage from the site develop. Systemically the animal remains otherwise unchanged apart from a variable fever. In cases of septic osteitis the animal may present with a mild lameness and a non-healing wound. Osteomyelitis in the horse is nearly always associated with moderate to severe lameness as do cases with synovial sepsis. Open drainage of infected synovial structures, intra-synovial medication with corticosteroids, or administration of systemic anti-inflammatories may lessen the degree of lameness at the time of examination. On physical examination vital parameters are usually within normal limits, with variable fever present. Synovial effusion, heat, swelling and palpation of the structure and surround soft tissues are key findings for localisation of the infected structure. Alternatively a wound adjacent a synovial structure may identified.

Synovial contamination and sepsis associated with wounds can be confirmed by observing communication of the synovial structure and the wound. Gross examination of synovial fluid at the time of collection, assessing the colour and turbidity can give some very useful information. Sepsis usually results in turbid, flocculent and non-viscous synovial fluid due to increased cellularity and total solid content of the fluid. Synovial fluid cytology is the gold standard for confirmation of synovial sepsis. Normal synovial cytology yields a predominantly mononuclear cell population, <10% neutrophils with a total white blood cell count <5x10^9 cells/L, and total protein <20mg/L. Findings of >80% neutrophils with variable toxicity, a total white blood cell count > 10x10^9 cells/L and a total protein > 40mg/L are consistent with synovial sepsis. Only 25% of cases will have bacteria identified on cytology.
In most cases of equine orthopaedic infection peripheral blood samples most consistently display a hyperfibrinogenaemia with or without a leucocytosis (marked leucocytosis characterised by a neutrophilia is an expected finding in the foal). Blood samples should be submitted for aerobic and anaerobic culture and sensitivity if the animal has evidence of systemic disease.

In cases of osteomyelitis deep aspiration of fluid accumulations should be examined cytologically to determine their nature and the presence of infectious agents along with submission of a sample for culture and sensitivity testing. Cytology consistent with infection usually contains a predominance of neutrophils and a low number will also contain the offending pathogen also. Samples for culture should be inoculated directly into commercial broth to enhance pathogen growth and reduce sample turn-around time.

Many imaging techniques are available for diagnosing equine orthopaedic infections including radiography, magnetic resonance imaging (MRI), computed tomography (CT), Scintigraphy and more recently biomarker analysis. The accuracy of diagnostic imaging modalities for detecting orthopaedic infection in humans is approximately 80-90%. Radiography remains the most commonly used modality for diagnosis of osseous involvement in synovial sepsis and osteomyelitis in equine practice; however it is a very insensitive method for diagnosis due to the requirement of 50-70% demineralisation of the affected bone before being radiographically identifiable as lysis on plain films. This can lead to a significant lag period between onset of infection and identifiable changes present on radiographs of up to 21 days. Contrast radiography can be implemented to determine communication of a draining sinus or wound with neighbouring joints and bone, along with identification of cartilage defects not observed on plain radiographs.

Ultrasonography in conjunction with other imaging modalities, such as radiology, also provides invaluable information – the extent of subtle periosteal irregularity, the presence of
radiolucent foreign bodies with in wounds, and contributes to accurate collection of samples for diagnostic purposes. Ultrasound can allow examination of anatomic regions not easily assessed by physical or radiographic examination, such as the shoulder joint. Ultrasonography however can lead to patient discomfort, only allows assessment of periosteal surface, and hence cannot identify sclerosis or lysis of bones.

Computed tomography is being used more frequently in the diagnosis of orthopaedic infections in the horse, especially in anatomic regions which have been previously hard to interpret with radiographic studies. Computed tomography not only provides clear anatomic detail of osseous structures, but reveals changes earlier in the disease process compared to conventional radiography. Computed tomography is capable of identifying, and is superior to magnetic resonance imaging, in detection of sequestra, cloaca’s, involucra and intraosseous gas. Contrast enhanced computed tomography enables distinction between necrotic tissues from surrounding normal tissue. The presence of metal in close proximity to osteomyelitis leads to a significant reduction in image resolution due to beam hardening artefact thereby complicating interpretation of changes at the bone-implant interface. CT in horse is not widely available at this point in time; carries a significantly greater cost than more traditional forms of imaging; and also carries the inherent risks of general anaesthesia to undertake the study.

Magnetic resonance imaging allows for early detection of osteomyelitis and an accurate assessment of the extent of the disease with excellent structural definition and spatial resolution. Magnetic resonance imaging has been found to have the highest sensitivity in detection of lysis in the order of 3-5 days following the onset of infection. The combination of STIR and T1 spinecho sequences shows a high specificity and sensitivity for detection of osteomyelitis. Contrast enhanced magnetic resonance imaging with gadolinium allows for more accurate detection of sequestra and involucra. Due to the magnetic fields involved in developing an image, metal implants cannot be used with this imaging modality.
development of standing units for imaging the distal limbs of the horse avoids the risks associated with general anaesthesia in the horse, however low availability and high cost have limited the common day use of this modality in diagnosis of equine orthopaedic infections.

Nuclear medicine is being utilised more frequently in combination with other methods of imaging to for localisation and diagnosis of orthopaedic infections. The extremely high sensitivity of this modality of imaging is coupled with a very low specificity for the diagnosis of osteomyelitis hence requires of other imaging modalities to differentiate osteomyelitis from fractures, arthropathies, neoplasia, or cellulitis.\(^{39}\)

Serum biochemical markers have been investigated as a non-invasive means of specifically detecting changes in bone metabolism that may indicate post-operative osteomyelitis. Specifically osteocalcin, bone specific alkaline phosphatase and deoxypyridinoline have been examined in the rabbit and were found to have an accuracy of 96% for predicting osteomyelitis at 4 weeks in a rabbit femoral fracture model.\(^{40}\) In this model the biochemical markers predicted osteomyelitis before any changes could be observed on conventional radiographs. The biochemical changes observed with osteomyelitis in the rabbit model has not been validated in horses; and remains a research application only.
Common pathogens implicated in Equine orthopaedic infections:

The spectrum of pathogens implicated in orthopaedic infections can vary with animal signalment; type of osteomyelitis; location of the disease; geographical location; and the presence of an open wound or implant. In cases of haematogenous osteomyelitis in foals gram negative enteric bacteria are predominately isolated with *Escherichia coli* reported as the most frequent isolate. More recently an increased prevalence of isolation of *Streptococcus, Staphylococcus* and *Salmonella* spp in blood cultures from foals with septic arthritis/osteomyelitis has been reported, with *Escherichia coli* more commonly isolated from infected bone samples. The pathogen isolation in equine neonates is in contrast to that of human neonates and infants with septic arthritis and osteomyelitis. In the human field, up until the 1940’s gram positive isolates were the cause for bacteraemia’s however the prevalence reduced markedly with the introduction of antibiotics. More recently gram positive isolates have accounted for bacteraemia in humans with *Staphylococcus aureus* isolated most frequently in cases of haematogenous osteomyelitis with a prevalence of 60 - 90%, followed by *Streptococcus* species at a prevalence of 20-50%. The re-emergence of gram positive isolates has been attributed to antimicrobial regimes that promote resistance and the increased rate of invasive procedures providing the opportunity for nosocomial infection. There have reports of relative increases in the incidence of gram positive isolates in blood cultures from bacteraemia foals; however this appears to be confined to certain hospitals and may reflect increased incidence in neonatal nosocomial infection. Isolates from adult horses with osteomyelitis or septic arthritis in association with a wound contained mixed infections with *Enterobacteriaceae*, non-beta haemolytic *Streptococci*, coagulase positive *Staphylococci*, beta-haemolytic *Streptococci*, and coagulase negative *staphylococci* in order of decreasing frequency respectively. The antibiotic resistance patterns of equine orthopaedic isolates from 1974-1979 were compared to those from 1980-1985 and revealed that there was an increase in percentage of coagulase positive
*Staphylococci* to all antibiotics except oxacillin and amikacin, along with *Escherichia coli* isolates resistant to all antibiotics except amikacin. Mixed isolates are also commonly encountered with osteomyelitis associated with an implant or fracture repair, with *Enterobacter* spp and coagulase positive *Staphylococcus species* the most commonly isolated gram negative and positive pathogens respectively. In contrast, iatrogenic infections of joints following joint medication or surgery commonly isolate a pure growth of *Staphylococcus species*. 
### Table 1. Summary of sensitivity patterns and isolates amended from Moore et al Sneider et al; Synder; Goodrich

<table>
<thead>
<tr>
<th>Population</th>
<th>Problem</th>
<th>Most common organism isolated</th>
<th>Antibiotic most likely to be effective</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foals</td>
<td>Haematogenous osteomyelitis/physitis</td>
<td><em>Enterobacteriaceae</em></td>
<td>Amikacin, Cephotaxime, Moxalactam</td>
<td>Wilson and Madigan 1989; Schneider 1992</td>
</tr>
<tr>
<td>Mature</td>
<td>Iatrogenic septic arthritis</td>
<td><em>Staphylococci</em></td>
<td>Amikacin</td>
<td>Schneider 1992</td>
</tr>
<tr>
<td>Mature</td>
<td>Septic arthritis 2º wound</td>
<td>Mixed:</td>
<td></td>
<td>Schneider et al 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Enterobacteriaceae</em></td>
<td>Amikacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>β <em>Streptococcus</em></td>
<td>Cephalothin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Staphylococcus</em></td>
<td>Amikacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>haemolyticus</td>
<td>Amikacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonhaemolyticus</td>
<td>Amikacin</td>
<td></td>
</tr>
<tr>
<td>Mature</td>
<td>Osteomyelitis 2º wound</td>
<td><em>Enterobacteriaceae</em></td>
<td>Amikacin</td>
<td>Synder 1987</td>
</tr>
<tr>
<td>Mature</td>
<td>Osteomyelitis 2º implant /# repair</td>
<td>Mixed:</td>
<td></td>
<td>Snyder et al 1987</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Enterobacteriaceae</em></td>
<td>Amikacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non β <em>Streptococcus</em></td>
<td>Chloramphenicol TMS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>coagulase positive</td>
<td>TMS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Staphylococcus</em></td>
<td>Amikacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>β streptococcus</td>
<td>Cephalothin</td>
<td></td>
</tr>
</tbody>
</table>
**Treatment:**

**Surgical curettage:**

A combined antimicrobial and surgical approach should be considered in all cases of osteomyelitis. Debridement of the lesion is essential for the removal of avascular bone, purulent material, necrotic debris. Avascular bone acts as a continual source of inflammation; leading to isolation from vascularisation by development of an enveloping layer of granulation tissue there by preventing resorption of the bone (sequestra formation) and impeding bone healing. This not only suppresses healing but also reduces deposition of systemically administered antimicrobials. In cases of haematogenous osteomyelitis in human neonates, and infants, surgical therapy is generally unnecessary, with intense medical therapy adequate for a successful outcome; which is in stark contrast to post traumatic osteomyelitis of fracture repair in which surgical removal of the infected fragments and foreign bodies can lead to a successful outcome with little antimicrobial therapy.

A number of surgical techniques have been described for management of septic synovial structures with all aiming to physically remove bacteria, devitalised tissue, inflammatory products and wound debris. Synovial aspiration, distension-irrigation, through-and-through lavage, arthrotomy, drains, and endoscopy have all been described for use in the horse. Endoscopic lavage and debridement of septic synovial structures carries a number of advantages when compared to other methods of management. These include improved visualisation, allowing accurate evaluation of the structure; identification and debridement of foreign material, devitalised and necrotic tissue; improved access to a greater proportion of the synovial cavity; minimal morbidity; reduced hospitalisation; maximal function recovery; and more accurate prognosis for recovery. The implementation of endoscopic lavage and debridement for septic synovial structures has led to improved success of therapy of synovial sepsis in the horse.
**Antibiotic therapy:**

The introduction of antimicrobial drugs in the 1940’s revolutionised human and veterinary medicine by allowing economic and effective treatment of bacterial disease. The widespread use of antimicrobials in human and veterinary medicine since their discovery has coincided with the simultaneous development of bacterial resistance. The therapeutic benefits (clinical improvement) from the use of antimicrobials occurs due to facilitating the host immune systems removal of microbes via direct bacterial cell death or inhibition of bacterial cell growth. Antimicrobials have been classified into two main categories; those that are concentration dependant where increasing concentrations at the focus of infection improve bacterial kill; or time dependant where exceeding the MIC for a prolonged percentage of the inter dosing interval rather than maximal concentration above MIC correlates with improved efficiency of bacterial kill. Adverse effects from administration of antimicrobials can occur as a result of primary pathology to specific organs or generalised systemic reactions, alternatively, adverse reactions can occur secondarily due to the antimicrobials affecting commensal bacterial populations, promoting overgrowth by pathogenic organisms. The study of the pharmacokinetic and pharmacodynamic properties of antimicrobial agents, and their interactions, has led to proposed outcomes in regards to clinical improvement, growth promotion and adverse reactions.

To reduce the incidence of antimicrobial resistance in bacterial populations, and prevent its ongoing development, it is of extreme importance that the most effective antimicrobials are selected for use, administered by the most optimal route, and at the most effective dose. To reduce the selection pressure on commensal bacterial populations it is implicit that the optimal dosage strategy to remove the offending pathogen delivers the minimal appropriate dosage to remove the target species. Further pharmacokinetic and pharmacodynamic parameters can be determined which minimise the selection window for resistance associated with the target pathogen.
Pharmacokinetics and pharmacodynamics:

Pharmacokinetics is the study of the movement of drugs in the body including the processes of absorption, distribution, localisation in tissues, biotransformation and excretion. The pharmacokinetic data provides a guide as to the concentrations achievable within tissues – from the maximum plasma concentration ($C_{\text{max}}$) and area under the plasma concentration – time curve (AUC).

Pharmacodynamics is the study of the mechanism of action of drugs and other biochemical and physiological effects. Pharmacodynamics provides a guide as to the in vitro interaction of the antimicrobial agent and the micro-organism.

The most important parameters of pharmacokinetics are the area under the plasma concentration time curve from 0 to 24 hours (AUC$_{0-24}$), the maximum plasma concentration ($C_{\text{max}}$) and the time (T) during which concentrations exceed a defined pharmacodynamic threshold. The most useful pharmacodynamic parameter is the minimum inhibitory concentration (MIC) the lowest concentration of antimicrobial which inhibits the growth of the target bacteria. Efficacy however may depend upon achieving concentrations in plasma several fold higher than the MIC of the pathogen, alternatively it may be dependent on maintaining concentrations in plasma just above MIC for a prolonged period of time.

For concentration dependant antimicrobials (aminoglycosides and fluoroquinolones) the pharmacodynamic indices that predict the efficacy of the antimicrobial against an organism are the $C_{\text{max}}$:AUC and the AUC:MIC – maximum efficacy has been reported to occur when the $C_{\text{max}}$:MIC>10 and the AUC:MIC>125. For some pathogens and drugs it is more complex and requires a combination of concentration and time of exposure (Co-dependency), as is the case with glycopeptide antimicrobials such as vancomycin. In the study of pharmacokinetics the site of measurement of the concentration may be an issue as plasma concentrations may not reflect the concentration of the drug at the site of the
pathogen (i.e. CSF, intracellular or intraocular). It must also be taken into consideration that the MIC is determined in culture broth and not in the environment in which the bacteria is growing in vivo – i.e. blood, synovial fluid, pus, intracellular fluid. The pH, aerobic or anaerobic environment, and growth phase, are also important in vivo; particularly in specific anatomical or physiological locations such as the mammary gland or urine, or in pathological situations of severe inflammation or abscessation.\textsuperscript{57}

The in vitro activity of some drugs does not accurately reflect there in vivo activity due to the post antibiotic effect (PAE) and post antibiotic leukocyte enhancement PALE.\textsuperscript{56} PAE is the persistent suppression of bacterial growth following removal of an antimicrobial from the locus of the bacteria,\textsuperscript{68} and is specifically defined as the time required for an organism to demonstrate viable regrowth following removal of an antibiotic.\textsuperscript{69} The occurrence and magnitude of the PAE is dependent on the micro-organism and the type and concentration of the antimicrobial along with the duration of exposure.\textsuperscript{57} The mechanism for PAE vary: for beta lactams - the length of time it takes for the bacteria to synthesis new penicillin binding proteins; for aminoglycosides, the length of time taken for the drug to disassociate from the ribosome and diffuse from its site of action, and then for protein synthesis to recommence.\textsuperscript{57} Beta lactams express PAE for gram positive bacteria only. Antimicrobials that inhibit DNA or protein synthesis tend to impart long PAE for gram negative bacteria.\textsuperscript{56} The PALE describes the increased susceptibility to phagocytosis and intracellular killing demonstrated by bacteria following exposure to an antimicrobial drug. In vivo, the PAE effect for aminoglycoside’s is prolonged by the synergistic effect of host leukocyte activity – it is believed that leukocytes have enhanced phagocytosis and killing activity after exposure to aminoglycoside’s - post antibiotic leukocyte enhancement.\textsuperscript{70}

Repeated exposure to sub-optimal drug concentrations is now recognised as the single most important factor for the emergence of resistance.\textsuperscript{71} Optimal dosing strategies may acquire the appropriate drug concentrations for the appropriate amount of time for target pathogens.
however commensal organisms may express differing sensitivities and therefore be selected upon for resistance, which could transfer resistance genes to pathogen bacterial populations.\textsuperscript{72}

Therapy for equine orthopaedic infections has revolved around the use of beta lactam and aminoglycoside antimicrobials, whilst waiting for specific culture and sensitivity results. Beta lactams are classed as time dependant antimicrobials where by the time that the drug remains above MIC is the greatest determinant of likely efficacy.\textsuperscript{73} Exceeding the MIC by 1-5 times for 40-100\% time of the inter-dosing interval is thought to be highly specific.\textsuperscript{74} In deep-seated infections penetration of the beta lactam to the site of the bacterial locus may depend on the plasma concentration as the process of distribution will depend on local blood flow and simple diffusion down the concentration gradient of the drug. The $\text{AUC}_{0-24}$ and $C_{\text{max}}$ thereby play an integral role in the distribution and activity of time dependant antimicrobials.\textsuperscript{75} Controversy remains as to the effect of the magnitude by which the concentration of time dependant antibiotics, such as cephalosporins, exceeds MIC and its influence on activity.\textsuperscript{76} Some data supports the theory that the $C_{\text{max}}$ achieved with beta lactam antimicrobials influences that kill.\textsuperscript{77}

Aminoglycosides are classed as concentration dependant antimicrobials for the gram negative bacteria for which they are commonly used against;\textsuperscript{78} however they do impart some concentration independent activity when used as an adjunct therapy for gram positive bacteria. The $C_{\text{max}}$:MIC ratio has been shown to be the most useful pharmacokinetic and pharmacodynamic indicator of predicting the efficacy of aminoglycosides, with increasing $C_{\text{max}}$:MIC correlating with clinical response. A $C_{\text{max}}$:MIC$>10$ has been recommended for once daily therapy however care needs to be taken to avoid toxicity. Toxicity of aminoglycosides is related to the trough period below the threshold that would incite toxicity.\textsuperscript{79}
Traditional therapy for osteomyelitis has consisted of systemic antimicrobial therapy and improvement of the wound environment. Systemic antimicrobials were the cornerstone of therapy however use alone inevitably has been inadequate in many cases for the treatment of severe osteomyelitis or synovial sepsis. Administration of intravenous gentamicin at 6.6mg/kg once daily has been reported to reach a $C_{max}$ within 1.4 hours at approximately 5 times the MIC of most pathogens encountered in equine orthopaedic infections. The reported half-life of gentamicin in equine tissue is 1.4 hours, thereby the concentration of gentamicin would be below the reported MIC for most pathogens within 6 hours, however with an estimate of the duration of the post antibiotic effect the expected duration of effective concentrations of gentamicin would be approximately 13 hours which may be an ineffective regime for severe orthopaedic infections in the horse. The combination of gentamicin with a beta lactam antimicrobial may prolong the effective concentration of gentamicin due to the recognised synergism between the two antimicrobials.

Parenteral administration of gentamicin has been shown to reach significantly lower $C_{max}$ in synovial fluid, synovial membrane, joint capsule, and subchondral bone of healthy horses compared to that achieved with local antimicrobial administration. High concentrations of systemic antimicrobial are required to attain sufficient local levels in contaminated tissues with compromised vasculature which not only increases the risk of systemic toxicity and side effects but also promotes the evolution of bacterial resistance. Systemic antibiotic therapy also has the drawback of the significant cost associated with medicating the average weight horse. However in spite of this systemic antimicrobial therapy still plays a role in the management of osteomyelitis in the horse in combination with local delivery techniques. The first line of systemic broad spectrum antibiotic therapy or prophylaxis in equine practice usually comprises a combination of penicillin and gentamicin which has been shown to
provide consistent broad spectrum of coverage, is relatively cheap and has minimal side effects.\textsuperscript{50}

The use of local antibiotic delivery techniques arose in Europe in the 1970’s with the advent of joint arthroplasty techniques in humans. Early reports found that penicillin, erythromycin and gentamicin mixed into the cement used to fix prosthesis to bone lead to high local concentrations of the antimicrobials that persisted for an extended period of time.\textsuperscript{83} The institution of local antimicrobial delivery methods into the prophylaxis for, and treatment of, a number of human orthopaedic conditions has led to significantly improved success rates. The use of local antibiotic delivery methods in the veterinary field has also developed, albeit at a slower rate, however having the same positive impact on successful therapy.
**Local Antimicrobial delivery**

Local antibiotic therapy has led to concentrations of antimicrobials at the bacterial locus that are many times the MIC of the offending organisms for a significantly longer period of time, low systemic concentrations thereby avoiding systemic toxicity and a significant reduction in costs associated with therapy, all of which have resulted in a significant improvement in the successful treatment of horses with orthopaedic infections.27 The modes of local antibiotic delivery that are currently available include:

- Antimicrobial impregnated polymethylmethacrylate
- Intravenous and intra-osseous regional limb perfusion
- Intra-articular/intrathecal medication
- Biodegradable implants:
  - Calcium sulphate based implants
  - Calcium phosphate based implants
  - Chitosan based implants
**Antibiotic Impregnated Polymethylmethacrylate:**

The use of antimicrobial impregnated polymethylmethacrylate (AIPMMA) implants for the prevention and treatment of osteomyelitis in horses has drastically improved the success rate of therapy over the past 30 years. The concept of using AIPMMA is based on the principle that antibiotic will be slowly released from the cement over time, thereby achieving continuous antimicrobial action in situ. Implantation of AIPMMA results in high local antimicrobial concentrations for a prolonged period of time. The slow local release of antimicrobials can result in concentrations within the wound fluid of up to 200 times that which could be achieved with systemic administration of the same drug and maintain antimicrobial concentrations above MIC can last for up to 80 days after implantation.

Variable reports of the efficacy of this local form of therapy for treating osteomyelitis in the horse have been described, with success rates in the vicinity of 80% reported. In the human field prophylactic use of AIPMMA has resulted in a 34% reduction in orthopaedic surgical infection rates.

Antimicrobials diffuse through the cement down the concentration gradient in to the surrounding local tissue in a bimodal elution pattern— with rapid elution over the first 24 hours with subsequent low levels of elution over the following weeks to months. Most researchers agree that the majority of antimicrobials are released from the cement in the first few hours to days, followed by a longer duration of elution at a substantially lower level.

Factors such as molecular weight of the drug, molecular weight and cross linking of the polymer, porosity of the cement, drug solubility in the polymer, surface area of the implant, concentration of antimicrobial in the implant and volume of fluid surrounding the implant all contribute to the elution characteristics of the antimicrobial from the implant.
The elution rate of an antimicrobial from AIPMMA implants is directly proportional to the surface area of the implant—hence small rough spheres are recommended as these will have the greatest surface area to volume ratio. The amount of antimicrobial impregnated within the PMMA is directly proportional to the amount that can be eluted and the maximal concentration of the antimicrobial in the eluent. Early reports found a variation in elution between the use of liquid and powdered antimicrobials in the construction of AIPPMA, more recent reports have failed to attain the same result rather finding that the elution characteristics were unchanged between the use of powdered and liquid formulations of gentamicin and amikacin.

Due to the broad spectrum of activity of gentamicin in the human field, along with its bactericidal, heat stable and water soluble characteristics, much literature has focused on its use in AIPMMA. However in equine clinical practice amikacin has gained more widespread use due to the broader spectrum of activity and efficacy of treatment against pathogens commonly encountered in equine orthopaedics. A range of antibiotics have been identified to have good elution characteristics from PMMA and include the following: amikacin, gentamicin, tobramycin, amoxicillin, ciprofloxacin, imipenem, ticacillin, cephazolin, clindamycin, vancomycin, erythromycin, metronidazole, fluoroquinolones.

The mechanical properties of the PMMA are altered with the addition of antimicrobial; measurable reductions in compressive strength occur even at very low concentrations. Current recommendations in the production of AIPPMA beads is to impregnate less than 10% antibiotic of the weight of the PMMA used to make the implants thereby minimising any reductions in the compressive and tensile strength of the implant when used for plate luting procedures. The ultimate compressive strength has also been shown to be effected to varying degrees depending on the antimicrobial impregnated; cephazolin has been shown to reduce the ultimate compressive strength less than that which occurs with gentamicin.

Gas
sterilisation of the impregnated implants was shown to have a greater reduction in ultimate compressive strength compared to those which were stream sterilised.84

More recent studies have highlighted that implants not used for fixation rather for local deposition of antimicrobials should contain antibiotics at the concentration which would achieve the normal systemic dose of the antibiotic and no greater, thereby avoiding potential systemic toxicity and preventing the potential antimicrobial resistance that could develop when antimicrobial concentration for impregnation to PMMA is based solely on the biomechanical properties of the implant.96

The combination of different groups of antimicrobials for systemic administration is common practice to take advantage of any potential synergy of activity between different antimicrobial groups and to broaden the spectrum of activity of the antimicrobials. The clinical use of a combination of antimicrobials in PMMA for local antibiotic therapy is also used clinically however the combination of antimicrobials within the same implant changes the elution characteristics of the antimicrobials: significantly higher initial elution of antimicrobials occurs due to passive opportunism – increased elution of both antimicrobials due to increased porosity of the cement.97 Numerous combinations of antimicrobials have been studied and include the following: vancomycin/amikacin, cephazolin/amikacin, cephazolin/metronidazole, metronidazole/gentamicin.96,98 When comparing the co-elution of two antimicrobials from PMMA to those containing one antimicrobial only, the total antimicrobial eluted is significantly greater for co-elution, however the concentration of the antimicrobials in the eluent remain above MIC for common pathogens for a significantly shorter period of time compared to PMMA with one antimicrobial impregnated.96 The increased elution may result from increased porosity of the bead – with more antimicrobial added to the PMMA the surface pores enlarge, the bead surface roughens and more channels from the surface to the interior of the bead are formed.88 These changes serve to increase the surface area of the bead resulting in increased contact with the eluent fluid across the bead.
surface and increased elution of the antimicrobial. There is also the potential that combining antimicrobials may result in a chemical reaction that leads to increased elution rates of antimicrobials from the PMMA. These changes in elution may not correlate with increased effectiveness – a greater rate of elution allows for more rapid depletion of antimicrobial stored within the PMMA and subsequent concentrations of the antimicrobial may fall below the appropriate MIC for the offending pathogen. This may be beneficial in prophylaxis of infection with implants however may not be beneficial in the treatment of pre-existing osteomyelitis, preventing resistance, or reducing systemic toxicity. Tobramycin/oxacillin combination has been identified as a combination that has a negative effect on elution.

Despite the significant improvement in success of therapy for osteomyelitis in humans and animals over recent decades with the use of antimicrobial impregnated polymethylmethacrylate, much research is directed toward developing biodegradable vehicles for local antimicrobial delivery. As PMMA is not a biodegradable substance, its use as an antibiotic impregnated bead clinically in humans necessitates a second surgical procedure for removal. In the veterinary field removal is not always required. The pharmacokinetic profile of antimicrobial elution form PMMA is not ideal – with initial peak concentrations followed by a significant reduction in concentration that may not maintain therapeutic concentrations. The trailing low concentrations of antimicrobial following the initial peak has raised concerns for potential propagation of antimicrobial resistance. The exothermic reaction (cement curing heat production) of the of PMMA has also raised concerns in potentially reducing the concentration of active antimicrobial impregnated within the bead (polymyxin B, tetracycline, chloramphenicol), along with local tissue necrosis from thermal, mechanical and chemically induced effects. Further to this PMMA has been shown to have detrimental effects on the innate antibacterial properties of human serum in the normal patient - namely the cascade of proteins that make up what is commonly referred to as complement. It has been postulated that in the diseased patient where the
immune competency has already been compromised, the effect of PMMA could potentially compound the reduction in immune competence and promote infection. The most common clinical complication with the use of AIPMMA beads has been soft tissue damage on removal and the formation of fibrous connective tissue complicating removal. PMMA beads are not recommended for use in joints due to the abrasive damage that is incited on articular cartilage. There have also been limited reports of toxicity associated with its use, that being acute renal failure of a patient following use of gentamicin impregnated PMMA.
**Regional limb perfusion:**

Over the past decade regional limb perfusion has become a common method of for administration of local antimicrobials in the treatment of orthopaedic infection of the equine distal limb. Regional limb perfusion allows clinicians to achieve high concentrations of antimicrobial in the region of infection, which is thought to improve the success of therapy of treating bacterial infections. The technique involves exsanguination and isolation of the distal limb from the systemic circulation by application of an Eschmarchs tourniquet and then injecting the antimicrobial into the isolated vasculature. Infusion of the perfusate leads to distension of the vasculature in the isolated region of the limb, thereby creating high pressure and concentration gradients between the intravascular and extravascular compartments. It is imperative that proper application of the tourniquet is undertaken as isolation of the regional vasculature is essential for this technique to be effective. The infusion of perfusate leads to dilation of the venous capillaries, post capillaries and lymphatics, with relaxation of the contacts between the endothelial cells and pericytes, thereby creating spaces in the vascular wall for further diffusion without cellular damage. This maximises the diffusion of antimicrobials in the perfusate from intravascular compartment in to the surrounding tissue, achieving high concentrations in poorly vascularised area’s of tissue that harbour bacteria which would otherwise remain potentially unaffected by systemically administered antimicrobials. Following removal of the tourniquet the perfused tissues serve as a depot for continued diffusion of the agent locally; maintaining high concentrations of the antimicrobial in the region for a significant period.

The perfusate is either administered via a superficial vein (intravenous regional limb perfusion) or injected directly into the medullary cavity of a bone (intra-osseous regional limb perfusion). The intra-arterial route has been discouraged due to the potential for more frequent and severe toxic effects on the arterial endothelial cells compared to that on venous endothelial cells. Reports of perfusion of the distal limb with radio-opaque dye...
and Indian ink have revealed that the perfusate is distributed via the venous system with either form of administration. 111 There is the potential for perfusate to migrate through the diaphysis and exit to the systemic circulation if via diaphyseal and epiphyseal vessels proximal to the tourniquet. 105 Comparison of perfusate distribution with intravenous and intra-osseous administration of 99m-technetium pertechnetate revealed no difference in the radio nucleotide uptake in the distal limb of horses. 108 However in a study comparing intravenous and intra-osseous administration of amikacin sulphate to the tarsal region in horses has revealed that the amikacin concentration in the tarso-crural joint was consistently higher following intravenous administration. 111 The concentration of antimicrobial agents achieved within synovial fluid when performing regional limb perfusion is not as high as those achieved by direct intra-articular administration, 112 however the concentrations within the joint and the surrounding soft tissue and osseous structures have been reported to achieve 100 times MIC for periods up to 36 hours, which is advantageous when there is infected soft tissues along with synovial sepsis. 105,111,113,114 Systemic administration of antimicrobials has been shown to be far inferior to regional limb perfusion with C_{max}:MIC ratios achieved in the target tissues with the latter form of administration reported to be 3-30 times that achieved with systemic administration. 105,111,113

The dosing frequency of antimicrobials administered via regional limb perfusion is based on the pharmacokinetics/pharmacodynamics of the individual antimicrobial agents. Aminoglycosides are classed as concentration dependant agents as discussed previously, the greater the C_{max}:MIC the greater antimicrobial effect they have, therefore once daily therapy or every second day therapy as a regional limb perfusion can be undertaken with aminoglycosides due to the long post antibiotic effect they exert. As cephalosporins are time dependant agents the proportion of time the concentration of the antimicrobial is above MIC rather then the C_{max} determines the activity of the agent, therefore more frequent administration is required. Ceftiofur, a third generation cephalosporin, time dependant
antimicrobial, has been shown to remain above MIC most common pathogens of septic arthritis for greater than 24 hours in the radiocarpal joint following intravenous regional limb perfusion, indicating once daily administration should be adequate.\textsuperscript{115} Regional limb perfusion with time dependant antimicrobials has been shown to result in therapeutic concentrations in infected ischemic tissue for longer periods than with systemic therapy.\textsuperscript{107} Optimal dosages used for regional perfusion in horses have not yet been determined,\textsuperscript{107} however empirical dosages based on the limited number of studies on the pharmacokinetics of regional limb perfusion in the horse exist.

The use of a single antimicrobial agent in the perfusate is the current recommendation\textsuperscript{107}, despite a report of the successful use of a combination of antimicrobials.\textsuperscript{116} At the end of this chapter a table has been modified from Rubio-Martínez and Cruz, 2006\textsuperscript{107}: “Antimicrobial regional limb perfusion in horses” (Table 2) which summarises commonly used antimicrobials used for regional limb perfusion horses.

The optimal volume of perfusate for regional antimicrobial perfusion has not been determined; the larger the volume of perfusate the greater the intravascular pressure achieved and therefore increased diffusion from the vessel into the surrounding tissue. However as intravascular pressure increases, so too does the risk of perfusate leaking under the tourniquet into the systemic circulation thereby reducing the effectiveness of the treatment.\textsuperscript{107} The anatomic location and horse size have influenced the volume of the perfusate used up to this point with wide variation in volumes reported. A volume of perfusate based on body weight has been suggested – 0.1ml/kg, which may provide a volume of perfusate proportional to the vascular volume of the individuals distal limb.\textsuperscript{117} The tourniquet plays a very important role in ensuring the effectiveness of this form of local antimicrobial therapy. A measure of a tourniquets effectiveness is the maximum venous pressure that can be achieved during administration of the perfusate before perfusate leaks
under the tourniquet.\textsuperscript{118} Tourniquet pressure and width, patient characteristics, site and rate of injection, volume of perfusate, and previous exsanguination all influence the effectiveness of the tourniquet.\textsuperscript{118} It has been recommended that tourniquets should have a width 20\% greater than the diameter of the limb, the limb should be exsanguinated prior to perfusion, and infusion rate should be low at a site distal to the tourniquet to increase the effectiveness of the tourniquet.\textsuperscript{118}

Pneumatic tourniquets are preferred over rubber tourniquets as the pressure applied to the limb is evenly distributed, can be measured and monitored.\textsuperscript{119} Tourniquet complications are reduced when applied over well-muscled regions of the limb where the nerves and vessels are protected with soft tissue, and when the tourniquet pressures are the minimum required to prevent leakage.\textsuperscript{119} The tourniquet pressure required to stop bleeding when used for haemostasis has been reported to be approximately 100\textsuperscript{119,120}mgHg higher than the maximum systolic blood pressure, in most cases 150-170mmHg.\textsuperscript{119,120} In the clinical setting however, when used for regional limb perfusion it is unknown what pressures are achieved with rubber tourniquets, and pressures in the vicinity of 300-500mgHg are used with pneumatic tourniquets without complications.\textsuperscript{111,119} The optimal duration of vascular isolation for regional limb perfusion has not been determined however 30 minutes has been reported to be a clinically efficient duration of tourniquet application for the procedure.\textsuperscript{105,115}

Complications associated with regional limb perfusion occur infrequently. The limitations of intravascular regional limb perfusion lie in difficulty of identification of the digital veins if swelling is present and vascular thrombosis following repeated catheterisation.\textsuperscript{113} Transient non painful soft tissue swelling has been reported to occur with intra-osseous regional limb perfusion; however was found to be self-limiting requiring no further treatment.\textsuperscript{111,113} The use of regional limb perfusion in foals with septic conditions of the tarsi has been associated with development of second foci of infection in one report.\textsuperscript{121} More recently the development of osteomyelitis and osteonecrosis of the proximal phalanx was reported as a
complication of intra-osseous perfusion of the proximal phalanx with gentamicin, which ultimately lead to pathological fracture of the first phalanx and euthanasia of the horse.\textsuperscript{122} The dose of gentamicin used for regional limb perfusion in this report was 5mg/kg, a substantially higher dose than previously reported for clinical use.\textsuperscript{117} It is assumed that this complication resulted from the dose of gentamicin used, which is consistent with reported toxic effects of gentamicin on marrow derived human mesenchymal stem cells at a high concentrations, which could compromise the bone-healing process.\textsuperscript{123}

Antimicrobial regional perfusion is now accepted as part of the standard treatment protocol for severe wounds, septic synovial structures and osteomyelitis in the horse. The institution of this method of antimicrobial delivery is thought to have contributed to the improved success of treatment in equine orthopaedic infections.

Table 2. Reported use of RLP for treatment of horses with orthopaedic infections

<table>
<thead>
<tr>
<th>Site or type of infection</th>
<th>No. horses</th>
<th>Age</th>
<th>No. of RLPs/horse</th>
<th>Antimicrobial (dose range/RLP)</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal portion of the limb</td>
<td>24</td>
<td>30 d–24 y</td>
<td>1–9</td>
<td>Gentamicin (100–300 mg) Amikacin (125 mg–2 g) Ampicillin (9 g)</td>
<td>104,121,124</td>
</tr>
<tr>
<td>Fetlock joint and proximal sesamoid bones</td>
<td>20</td>
<td>3 m–18 y</td>
<td>1–4</td>
<td>Gentamicin (100 mg–1 g) Timentin (125 mg–1 g) Amikacin (1 g)* 13, 15, 27 K+Pen (10 X 106 units)*</td>
<td>121,124</td>
</tr>
<tr>
<td>Proximal portion of the limb</td>
<td>5</td>
<td>10 d–3 mo</td>
<td>1–3</td>
<td>Gentamicin (100 mg–1 g) Amikacin (125–1 g)* K+ Pen (106 units)*</td>
<td>104,116,121</td>
</tr>
<tr>
<td>Miscellaneous (septic arthritis, Gentamicin (1 g) osteomyelitis)</td>
<td>22</td>
<td>Unspecified</td>
<td>1–4</td>
<td>Amikacin (1–2 g) Timentin (1 g)* K+Pen (106 units)*</td>
<td>124,128</td>
</tr>
</tbody>
</table>
Distal portion of the limb includes septic processes in hoof, coronary band, pedal laminae, phalanges, interphalangeal joints and navicular bursa. Proximal portion of the limb includes septic processes in digital flexor tendon sheath, metacarpal bones, metatarsal bones, carpus, and tarsus. Fetlock joint refers to metacarpophalangeal or metatarsophalangeal joints.

*Combined in some cases.

K+ Pen = Potassium penicillin.
**Intrasynovial antimicrobial medication:**

Intrasynovial medication with antimicrobials is used widely in equine practice for prophylaxis and treatment of septic synovial structures. However is not recommended as a sole method treatment for synovial sepsis rather as part of a multimodal approach used in combination with arthroscopic debridement, joint lavage and systemic antibiotic therapy. The ideal agent has to not only be effective in killing bacteria within the joint, but also not infer detrimental effects on the articular cartilage, synovium and surrounding soft tissue structures.

Gentamicin has been extensively studied and found to reach concentrations within the joint that far exceed that of systemically administered gentamicin and also achieves significantly higher synovial concentrations than that achieved with regional limb perfusion - in the order of 800 times the synovial gentamicin concentration.\textsuperscript{126} The concentration of gentamicin within synovial fluid has been shown to be maintained above the MIC for most pathogens encountered in equine synovial sepsis for greater then 24 hrs.\textsuperscript{112} Gentamicin has been shown to reduce the pH of synovial fluid in normal horses following intra-articular medication, and in horses with synovial sepsis, however contrary to \textit{in vitro} negative effects of low pH on antibacterial activity; this has been shown not to reduce the efficacy of gentamicin in \textit{in-vivo} models of joint sepsis.\textsuperscript{112} It has been postulated that the reduction in pH of the synovial fluid following intra-articular medication may exacerbate bacterial killing due to creating an unfavourable environment for bacterial survival.\textsuperscript{112} Intra-articular administration of gentamicin has been shown to consistently produce a mild transient inflammation of the synovial membrane which is self-limiting.\textsuperscript{127} In a canine model gentamicin has been shown to passively diffuse across the capillary membrane of normal and osteomyelitic bone.\textsuperscript{128}

Systemic administration of gentamicin is inadequate for attaining therapeutic concentrations of gentamicin within bone.\textsuperscript{128} Following intra-articular administration of 1g gentamicin the concentration of gentamicin within the bones adjacent to the medicated joints were shown to
be above MIC for the commonly isolated pathogens of equine orthopaedic infections for 8-12 hours.126

Amikacin, also an aminoglycoside, has been reported as the most commonly used antimicrobial for intrasynovial medication in horses in north America.129 The efficacy of amikacin against a wide range of bacteria implicated in equine orthopaedic infections accounts for its widespread use.130 The reported MIC of amikacin for susceptible bacteria has been reported to be 4 micrograms/ml,131 which is not only easily surpassed with intra-articular medication with 500mg of amikacin but is maintained above MIC for 48 hours in inflamed joints.132 Synovial inflammation has been shown to not only reduce the time for which amikacin concentrations remain above the MIC for susceptible isolates, but also reduces the $C_{\text{max}}$ attained, compared with non-inflamed joints.132 This was attributed to the increased vascularity of inflamed joints allowing more rapid removal from the joint.132 In the same study it was reported that unlike gentamicin, amikacin did not incite any cytological evidence of joint inflammation in normal joints.132

Ceftiofur sodium, a broad spectrum, beta lactamase resistant, third generation cephalosporin with bacteriocidal activity has also been investigated for intra-articular use in the horse. Unlike gentamicin and amikacin, ceftiofur sodium is classed as a time dependant antimicrobial. It has been reported that 150mg of ceftiofur administered via intra-articular injection not only achieves $C_{\text{max}}$ more rapidly but also maintains concentrations higher within the synovial compared to intravenous administration of 2.2mg/Kg.133 The concentration within synovial fluid remained above the reported MIC of 2ug/ml of most susceptible pathogens for up to 24 hours, however this study was performed in horses free of musculoskeletal disease therefore this may not be representative of concentrations achieved or maintained in inflamed synovial structures.133 Ceftiofur was found to impart minimal synovial inflammation, and no significant alterations in articular cartilage or synovium morphology.133
Doxycycline, a tetracycline antimicrobial that has less interference with calcium binding than agents in this group, has also been investigated for direct intra-articular administration in a bovine calf model.\textsuperscript{134} Doxycycline has been shown to have anti-inflammatory and chondroprotective properties, thought to be imparted via modulation of inflammatory mediators (PGE\textsubscript{2} and NO) and reduction in metallomatrix proteinase activity.\textsuperscript{135,136} Medication of healthy calf joints with doxycycline at 5mg and 10mg did not induce any adverse effects apart from transient mild synovial inflammation similar to that observed with aminoglycosides and cephalosporins, and was therefore concluded to be compatible with the synovial tissues.\textsuperscript{134} Despite these encouraging findings, no other data is present at this time regarding the pharmacokinetics of doxycycline as an intra-articular medication in the horse.

Direct intra-articular injection of synovial spaces has been reported for daily administration of a number of antimicrobials including gentamicin, amikacin, and ceftiofur.\textsuperscript{126} Despite resulting in high intrasynovial antimicrobial concentrations it requires daily preparation of the overlying skin and repeat trauma to the synovial membrane and capsule.\textsuperscript{69} Numerous methods of direct delivery of gentamicin to the synovial space have been developed apart from direct intra-articular needle deposition; these include continuous infusion catheters, collagen sponges, and fibrin pads. Continuous infusion of antimicrobials to the synovial space yields steady state concentrations in the vicinity of >100 MIC of common isolates of equine orthopaedic infections with the advantage of avoiding repeated articular injections, articular damage resulting from antimicrobial impregnated beads or there retrieval, and the limitations of anatomic locations of regional limb perfusion.\textsuperscript{137} In early reports on normal tarso-crural joints in horses this method of delivery resulted in higher concentrations of gentamicin within the synovial fluid, synovial membrane and subchondral bone then that achieved by intra-articular injection or by regional limb perfusion.\textsuperscript{80} The ability for continuous delivery of antimicrobials to the synovial space may be more relevant for time dependant antimicrobials such as cephalosporins rather then concentration dependant
antimicrobials such as gentamicin. The continuous presence of the infusion catheter within equine joints has been reported to have no effects on histological scores of articular cartilage damage or synovial membrane inflammation. Despite encouraging results with clinical use of this method of delivery it has not been widely instituted in routine management of synovial sepsis due to cost and technical difficulties.
Case series: Outcome of horses with synovial sepsis treated with local antimicrobial therapy at Murdoch University (2007-2010)

Synovial contamination/sepsis is not an uncommon presentation to the equine veterinarian. The condition is recognised, and managed as, a medical emergency due to the potential fatal outcome of the disease. Synovial structures – whether it be diarthrodial joints, tendon sheaths or bursae, have a similar anatomic and physiologic make up, with a synovial lining that produces and maintains the physical, cellular and biochemical environment.\(^5\)

Introduction of micro-organisms into the synovial cavity can occur via a number of means. These include direct deposition from trauma to or penetration of the synovial structure, extension from perisyovial infection, haematogenous localisation or through iatrogenic deposition with surgery or centesis/medication of the structure.\(^5\) The virulence and number of the contaminating organisms along with the presence of devitalised or traumatised tissue and foreign material all predispose the development of infection.\(^5,139\) In young animals immunological incompetence is also recognised as a predisposing factor for the development of synovial infection.\(^2,4\)

The synovium and articular cartilage undergo progressive destruction due to the combined effects of the pathogen toxins and protease release, along with the release and induction of free radicals and cartilage degrading proteases by the host immune response.\(^5\) Historically the development of intrasynovial pannus (fibrocellular conglomerates) has hindered the management of the disease and contributed to poor outcomes in horses as it can surround foreign material and necrotic debris thereby creating a nidus for persistent infection.\(^140\) Synovial membrane diffusion is impeded by pannus development, resulting not only in synovial malnutrition but also impaired delivery of antimicrobials to the synovial structure.\(^140\) The objectives of therapy include debridement of foreign material, debridement of contaminated/infected tissue, removal of micro-organisms, removal of destructive enzymes and radicals, promotion of healing tissue and restoration of a normal synovial
environment. Outcomes from endoscopic surgical management of contaminated and infected synovial structures followed by a combination of systemic and local antimicrobial therapy have been reported by a number of authors. Survival rates of 65%-90% following endoscopic debridement and antibiotic therapy have been reported, with 54-94% of animals returning to preoperative level of performance. Admission to a hospital greater than 24 hours after contamination of a synovial structure has been previously shown to significantly increase the risk of development of synovial sepsis and significantly increase the risk of non-survival. The presence of marked pannus, regional intravenous antimicrobial therapy and systemic antimicrobial therapy for greater than 7 days have also been associated with non-survival and reduced postoperative performance.

Local antimicrobial therapy has been advocated to obtain high local antimicrobial concentrations whilst avoiding systemic toxicity. Both intravenous regional limb perfusion and synovial administration of antimicrobials have been reported for the management of contaminated and infected synovial structures. This case series describes the outcomes of 44 patients with contaminated or infected synovial structures, treated with endoscopic lavage and debridement, along with systemic and local antimicrobial therapy. The hypothesis of this study is that the success of treatment of septic synovial structures with local antimicrobial therapy would be consistent with earlier reports.

**Materials and Methods:**

**Horses:**

Medical records (2007-2010) of horses which received endoscopic lavage and debridement of a synovial structure for management of synovial contamination or infection were reviewed. Synoviocentesis was attempted in all cases. In cases where a sample could not be collected and a wound was present, distension of the synovial cavity was undertaken to detect communication between the wound and the synovial cavity. A synovial cavity was
classed infected if synoviocentesis yielded samples in which the differential neutrophil count was greater than 80% and infected or contaminated if communication of the synovial cavity with a wound could be demonstrated via synovial cavity distension with sterile saline. Patients less than 12 weeks of age were excluded. Only cases where endoscopic lavage and debridement were undertaken were included. Data retrieved included signalment, degree of lameness, time and cause of synovial injury, structures involved, time to referral, if a positive culture was obtained, method of local antimicrobial administration and number of local antimicrobial treatments, number of subsequent synovial lavages following initial endoscopic lavage, synovial culture results, synovial differential cell counts and total solids, synovial cytology, time to discharge.

Outcome:

Short term success was defined as survival to discharge. Long term follow up was obtained via telephone questionnaire of owners, referring veterinarians or trainers at least 6 months. Successful long term outcomes were defined as return to equal or better performance, or they were sound however retired for other reasons. Horses remaining lame were classed as unsuccessful.

Results: Forty four horses met the inclusion criteria. The mean age was 5.5 years STD, (range 3 months – 16 years). There were eight entire males, 21 females, and 15 geldings. The majority of the population was made up of thoroughbreds (23), the rest of the population was comprised of a mixture of breeds; warmblood (5), quarter horse (5), standardbred (4), Arabian (3), crossbred (2), whaler (1) and paint horse (1).

Thirty five horses (79.5%) sustained wounds which resulted in synovial sepsis, 5 horses (11.3%) were classed as iatrogenic synovial sepsis developing synovial sepsis following surgery or intra-articular medication. Three horses (6.8%) developed infection following
local extension from a foot abscess, and in one (2%) horse the origin of infection is unknown.

Forty seven synovial cavities were involved in the 44 horses which met the inclusion criteria. The synovial cavities involved were as follows: metacarpophalangeal joint (9); digital tendon sheath (9); tibiotarsal joint (6); femoropatella joint (5); middle carpal joint (4); distal interphalangeal joint (3); navicula bursa (2); extensor radialis tendon sheath (2); cuboidal joint (1); scapulohumeral joint (1). Of the 44 horses 24 (55%) had a single joint involved; 15 (34%) had a single bursa or sheath involved; and 5 (11%) horses had multiple structures involved. Of the 44 horses, the time to referral was available for 43 horses; with 20 horses (47%) being referred within 24 hours, 8 horses (19%) referred within 1-7 days of onset of disease and 15 horses (34%) being referred for further examination and management greater than 7 days after onset of disease.

Of the forty-four horses, a synovial fluid sample was collected from 37 cases. Cytology was performed on all 37 synovial fluid samples: one horse had a normal percentage of neutrophils (<10%); six horses had a moderate increase in percentage of neutrophils (10-80%); and thirty had a marked increase in percentage of neutrophils (>80%). The average white cell count was recorded for thirty six samples was 56.94 +/- 66.05 x10^9 cells/L (range 0.3-338x10^9 cells/L) of which 6 horses (17%) had a normal total nucleated count (<5x10^9 cells/L). The average total solids of the 36 synovial fluid sample which were recorded was 54.84 +/- 20.56 mg/DL (range 10-112mg/DL), with one horse having normal total solids (<20mg/DL), seven horses having a moderate increase (20-40mg/DL) and 28 having marked increase in total solids (>40mg/DL). Of the thirty seven samples collected, 32 had synovial fluid submitted for culture. Of these only 12 (38%) yielded a positive culture result.

All horses received at least one endoscopic lavage and debridement of the synovial cavity. All endoscope portals were closed with simple interrupted 2-0 polypropylene sutures.
a subcutaneous layer was required wounds were closed with a simple continuous layer of 2-0 polyglactin 910 in the subcutaneous tissue, and all skin wound were closed with a combination of tension relieving near-far-far-near sutures or vertical mattress sutures of no 2 polydioxanone and then apposition of the skin edges with simple interrupted sutures of 2-0 nylon. Twenty-six horses (59%) had one or more subsequent through and through lavage of the synovial cavity via 18 or 16 gauge needles.

All horses received systemic antibiotics, which consisted initially of procaine penicillin and gentamicin and then was modified if culture results indicated alternate sensitivity patterns. All horses that had involvement of structures distal to the crus or gaskin had sterile primary dressings applied over the surgical wound closures and then supported by a heavy cotton wool bandage. In all cases the bandage was changed 24 hours following surgery to assess wound drainage and then bandage changes occurred every 48 hours at which time synoviocentesis followed either synovial medication or intravenous regional limb perfusion were undertaken. Twenty-one of the horses (48%) received intrasynovial antibiotics as a sole method of local antimicrobial delivery every 48 hours, four (9%) received intravenous regional limb perfusion as a sole method of local antimicrobial therapy every 48 hours, and 19 horses (43%) received a combination of intrasynovial medication and intravenous regional limb perfusion with protocol varying between cases and clinician preference. All horses received phenylbutazone 2.2mg/kg orally twice daily initially and then reduced to once daily and then ceased at the discretion of the attending clinician.

Forty two (95%) of the 44 horses were successfully discharged from hospital, two (5%) horses were euthanized prior to discharge as they were unresponsive to therapy. The average duration of hospitalisation was 14.9 days (range 3-90 days). Eleven horses (26%) were discharged less than 7 days after admittance; 16 horses (38%) were discharge between 7 and 15 days following admittance; and 15 horses (36%) were discharged more than 15 days following admittance. Of the 42 horses discharged from hospital, follow up information was
available for 41 horses. Of these, 33 horses were sound (78%) more than six months following discharge, with 54% (18) of these horses returning to their preoperative performance level. Eight horses (19%) remained lame and one horse was lost to follow up.

Of the horses with a single joint involved 19 of 24 (36.5%) were sound six months after discharge; 11 of the 15 (73%) horses with a single bursa or sheath involved were sound six months following discharge; and three of five horses (60%) with multiple structures returned to soundness six months following discharge. Of the horses that received intra-articular administration of antimicrobials alone 15 out of 19 (79%) were sound six months following discharge; those receiving intravenous regional limb perfusion alone 3 of 4 were sound at follow up; and 15 of 19 (79%) of those receiving a combination of intrasynovial and intravenous regional limb perfusion were sound at follow up. Of the 43 horse for which time to referral was available, 16 out of 20 (80%) were referred within 24 hours were sound at six months; 6 out of 8 (75%) that were referred between 1-7 days were sound; and 11 out of 15 (73%) that were referred greater than 7 days after onset of disease were sound more than six months following discharge.

**Discussion:**

The population in this case series reflects that of past reports, with a predominantly Thoroughbred population. The majority of contaminated and infected synovial structures were due to involvement of the structure in a traumatic wound, which is also consistent with the report by Wright in 2003, however the incidence of wounds in the pathogenesis of septic synovial cavities is far greater than that reported by Schneider et al in 1992.

Forty-two of the forty-four horses survived to discharge leading to a short term success rate of 95%, which is higher than the 90% success rate reported by Wright et al 2003 and the 85% short term success rate reported for adult horses by Schneider in 1992 however the
population in the two latter studies was more than three times the size of the present case series. The long term success rate in the present case series was 78%, with 54% of these horses returning to their preoperative performance level. The successful return to preoperative performance levels in the present series is comparable to that reported by Schneider et al 1992 (56.5%), however are far inferior to the 81% return to preoperative performance that was more recently reported by Wright et al 2003. The reason for such a reduced successful return to preoperative performance cannot be determined in this study as factors that have been previously found to heavily influence long term return to soundness, such as the presence of osteochondral lesions and the presence of marked pannus, were not assessed in this case series.

Endoscopic examination, lavage, and debridement were performed in all cases in this series. Endoscopy permits thorough evaluation of the synovial cavity whilst simultaneously allowing for removal of foreign material and infected and devitalised tissue. Visually directed lavage of the synovial cavity can also be undertaken which is thought to aid in removal of free floating debris, debulk micro-organisms and remove destructive radicals and enzymes. As in previous reports all patients were managed in a way that facilitated effective surgical debridement of the synovial cavity and conversion from a contaminated or infected structure to that of a clean-contaminated structure that could be closed safely. This was based on prior reports that endoscopic surgery is capable of thoroughly cleansing the synovial cavity, and that closure of wounds would prevent ongoing contamination of tissue and secondary infection.

The average length of hospitalisation in the current series was 14.9 days (range 3-90 days), which is less the 18 days (range 6-71 days) that was reported by Wright et al in 2003 and the 21 days reported by Schneider et al 1992.
Infected synovial structures as a result of a traumatic wound are likely to have multiple bacterial species involved.\textsuperscript{30} Based on previous reports of bacterial isolates\textsuperscript{30,47,48} and the reported sensitivity patterns\textsuperscript{47,48} with suggested synergism between penicillin and gentamicin all horses were initially started on a course of procaine penicillin and gentamicin. All horses received this antimicrobial regime until the clinically sound and had normal synovial fluid parameters or a culture and sensitivity result indicated resistance of the offending organism was present.

This study has many limitations, with major limitation being that of a small number of cases meeting the inclusion criteria. This restricted the statistical analysis to descriptive statistics alone, as there were too few cases with a variety of synovial structures involved, to allow meaningful statistical analysis of the impact of local antimicrobial therapy on the outcomes of therapy of such cases.

As opposed to previous reports where local antimicrobial therapy was only instituted in selected cases\textsuperscript{10} and carried an association with non-survival and reduced postoperative performance\textsuperscript{10}, all cases in this series underwent some form of local antimicrobial administration and was therefore not associated with a poor prognosis or reduced return to function. The success of therapy in this case series is comparable to previous reports, and supports the use of local antimicrobial therapy in management of septic synovial structures in the horse. A case controlled prospective study would be required to assess the impact the institution of routine local antimicrobial therapy alone on short and long term success for horses with septic synovial structures.
Biodegradable antimicrobial impregnated implants:

Gentamicin impregnated collagen sponge

Commercially available gentamicin impregnated collagen sponge have been used in dogs, cattle and horses.\textsuperscript{150-154} Purified collagen type one has been used as a vehicle for antimicrobial delivery due to its inherent biocompatibility, low antigenicity and biodegradability.\textsuperscript{155,156} The collagen protein is highly conserved between species, such that manufactured carrier biomaterials (films, gels, sponges) from purified animal and recombinant sources of type one collagen impart low immunogenicity.\textsuperscript{155} A study in healthy adult rabbits where gentamicin impregnated collagen sponges were implanted into the medullary canal of the femur revealed local bone gentamicin concentrations remained above MIC and systemic gentamicin concentrations remained below which toxicity occurs for greater then 28 days.\textsuperscript{157} Gentamicin impregnated collagen sponges have been used extensively and successfully for prophylaxis against the development discospondylitis in humans undergoing discectomy procedures.\textsuperscript{158} Achieving therapeutic concentrations of antimicrobial in the disc space is of concern with systemic antimicrobial administration due to disc vasculature declining from birth until the end of the second decade, where it remains that only the periphery of the disc’s remain vascularised.\textsuperscript{158} Gentamicin impregnated collagen sponges have been shown to provide high local antimicrobial concentrations above MIC for more than 7 days following implantation into soft tissue.\textsuperscript{156} Gentamicin impregnated collagen sponges have been reported in the veterinary literature in the successful treatment of discospondylitis in a two year old boxer dog.\textsuperscript{154}

Implantation of gentamicin impregnated collagen sponges in to the tarsocrural joints of horses revealed that gentamicin rapidly eluted from the collagen sponge reaching $C_{\text{max}}$ within 3 hours and then rapidly decreasing, at a rate comparable to that of clearance of gentamicin from direct injection.\textsuperscript{159,160} Rapid elution attaining initial high $C_{\text{max}}$ followed by
suddenly decreasing concentrations of gentamicin was also reported in human trials of gentamicin impregnated collagen sponges in people with chronic osteomyelitis. Only minimal inflammatory changes were observed in horses that underwent implantation of gentamicin impregnated collagen sponges in the tarsocrural joint however these changes could not be differentiated between that induced by gentamicin alone; and that potentially imparted from the collagen sponge. Despite only minor histological inflammatory changes in the synovial fluid an acute onset of lameness and periarticular swelling consistently developed at 12 hours post insertion of the implant. The study of this delivery modality in the tarsocrural joint of the horses concluded that gentamicin impregnated collagen sponges did not offer any advantage over direct intra-articular medication of the joint.
Calcium sulphate

Calcium sulphate, commonly known as Plaster of Paris, has been used since 1892 to fill bone defects and to act as a bone graft substitute.\textsuperscript{162} Calcium sulphate is a biodegradable substance, with relatively rapid resorption characteristics that may result in more complete elution of antimicrobials; along with osteoconductive properties - promoting new bone formation in the contained defects.\textsuperscript{163} Significant resorption of implanted calcium sulphate and replacement with new bone formation has been shown to occur in 6 weeks in a number of animal models of osteomyelitis.\textsuperscript{164,165} Medical grade calcium sulphate is a relatively pure alpha hemihydrate crystal that hardens with hydration.\textsuperscript{166} Water soluble antimicrobials can be incorporated into the crystalline structure of the calcium sulphate hemihydrate matrix, thereby impregnating the implant.\textsuperscript{163} Calcium sulphate has been shown to remain cohesive with up to 12\% weight of antibiotic loading.\textsuperscript{82} The bio-ceramic properties of calcium sulphate imparting full resorption imply that all antimicrobial impregnated in the implant should be delivered to the local tissues.\textsuperscript{163} As the calcium sulphate is resorbed by the body, it does not require a second procedure to remove the implant.\textsuperscript{163} Calcium sulphate is reported to be well tolerated by the body, non-immunogenic, and fully biodegradable.\textsuperscript{166}

The rate of resorption of the calcium sulphate has been found to be proportional to the density of the crystal, thereby imparting slow or rapid resorptive characteristics determined by the method in which it is produced.\textsuperscript{166} Tobramycin impregnated calcium sulphate beads have undergone considerable research in animal models of osteomyelitis\textsuperscript{165,167,168} and have also been used successfully in clinical cases of chronic osteomyelitis in people.\textsuperscript{166,169} Tobramycin has been shown to elute from calcium sulphate beads providing therapeutic local concentrations for up to 28 days whilst maintaining non-toxic serum levels.\textsuperscript{163} Even when used in a canine model to provide 1.8 times the maximum prescribed human dose, serum tobramycin levels fell below detectable levels within 24 hours.\textsuperscript{163,165} Tobramycin impregnated bone pellets have been shown to be safe and effective methods of local

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administration of antimicrobials and dead space management in animal models of osteomyelitis and contaminated open fractures. Calcium sulphate pellets containing 2%, 4% and 10% tobramycin used in a canine humeral model have all revealed similar antimicrobial release profiles, with systemic serum concentrations falling below detectable levels within 24 hours. However local osseous and soft tissue concentrations were maintained within therapeutic range for 7 days. In dogs with clinical osteomyelitis following tibial plateau levelling osteotomy procedures, tobramycin impregnated calcium sulphate beads were used for successful treatment in conjunction with surgical debridement; and were found to be radiographically unapparent within 5 weeks.

In vitro trials of gentamicin impregnated calcium sulphate placed in porcine serum reported elution of 80% of the total gentamicin within the bead in the initial 48 hours, and continued to release gentamicin at lower levels, however still at concentrations that inhibited E.coli growth for the 14 day duration of the study. The persistent low concentration after the initial peak concentration, achieved at 48 hours, may not achieve concentrations that are bactericidal for all pathogens encountered in equine orthopaedic infections and therefore may not be sufficient for use in the horse; rather may be better suited for prophylaxis of infection following orthopaedic surgery in the horse.

Elution pharmacokinetics have been described for gentamicin, vancomycin, teicoplanin and clindamycin from calcium sulphate beads, revealing a high initial release of antimicrobial in the first 24 hours (45% and 80% of the total glycopeptide and gentamicin/clindamycin respectively in the initial 24 hours) and then a sustained lower rate of release over the following 10 days. Doubling the concentration of the antimicrobial contained within the bead resulted in a higher initial antimicrobial release, and a more prolonged two-fold increase in antimicrobial elution in the second phase of elution.
Conventional calcium sulphate implants remain present in the tissue following the initial rapid elution of impregnated antimicrobials; this has been shown to provide a surface for bacterial adhesion and thereby allow establishment of infection. In an effort to prevent musculoskeletal infection following trauma, conventional calcium sulphate implants which resorb at the same rate as new bone forms, have been modified to rapidly resorb; thereby completely eluting impregnated antimicrobials locally leading to high concentrations of antimicrobial over a short period of time. A variation in the crystal structure and therefore surface area of the pellets allow for the rapid dissolution of impregnated antibiotics and pellet resorption. The amikacin, gentamicin and vancomycin elution from rapidly dissolving calcium sulphate pellets has been reported as follows: 50-70% of the antibiotic elutes in the first 4 hours, 85-90% by 8 hours, and over 95% by 12 hours, with pellets completely dissolved within 16 hours. Amikacin impregnated rapidly resorbing calcium sulphate pellets were shown to significantly reduce infection rate distal limb wounds in a goat model.

Calcium sulphate has been reported to incite a transient cytotoxic effect in humans leading to a sterile self-limiting benign inflammatory reaction which responds well to anti-inflammatory medication. The uniformity and shape of the crystalline nature of purified medical grade calcium sulphate imparts the predictable resorption rate in vivo; however this may also allow for accelerated graft resorption and accumulation of calcium rich fluid which is believed to be responsible for the inflammatory response. Others have postulated that the occasional serous drainage associated with the calcium sulphate implants is due to an osmotic effect of the implant.
In vitro elution characteristics of amikacin from commercially available calcium sulphate beads

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Objective: To describe the\textit{ in vitro} elution characteristics of amikacin from commercially available antibiotic-impregnated calcium sulphate hemihydrate and dextran sulphate beads placed in phosphate-buffered saline or equine plasma, and to observe the rate of implant dissolution in saline or plasma.

Study Design: Experimental study

Methods: Commercially available calcium sulphate hemihydrate-dextran sulphate beads impregnated with amikacin sulphate and clindamycin hydrochloride were incubated at 37°C in plastic test tubes containing sterile phosphate-buffered saline (PBS) or equine plasma. The eluent surrounding the bead was collected and completely refreshed with drug free sterile phosphate buffered saline or equine plasma at predetermined time points. Antimicrobial levels were measured in the eluent using liquid chromatography-mass spectrometry.

Results: Antimicrobial release from the beads followed a biphasic elution pattern with an initial rapid release followed by a slower phase. In saline the antimicrobial concentration fell below the lowest measurable concentration in saline (0.5\textmu g/ml) within 12 hours, whereas in equine plasma the
antimicrobial concentration fell below the lowest measurable concentration in plasma (2ug/ml) within 6 hours. The beads had completely dissolved in PBS by 72 hours whereas in plasma only partial dissolution of the beads was noted throughout the 28 days of the experiment.

**Conclusion:** Amikacin eluted rapidly from the commercially available antibiotic-impregnated calcium sulphate hemihydrate–dextran sulphate beads in phosphate buffered saline and fell below 4 ug/ml within 6 hours. The rate of bio-implant dissolution was consistently slower in equine plasma compared to that in phosphate-buffered saline.

**Key words:** local antimicrobial therapy; calcium sulphate; amikacin

**Abbreviations**

PBS Phosphate buffered saline; PMMA polymethylmethacrylate; LC-MS/MS liquid chromatography tandem mass spectrometry; MIC minimum inhibitory concentration; $C_{\text{max}}$ maximum serum concentration;

**Introduction**

The development of antimicrobial resistance is a major concern in human and in veterinary orthopaedic surgery and wound management.\textsuperscript{17,48,178} Contaminated wounds involving trauma to deep soft tissue structures and exposed bone surfaces occur commonly in equine practice often resulting in deep soft tissue infections, local osteitis, and rarely osteomyelitis.\textsuperscript{27,179} Therapy usually involves a combination of surgical and medical therapy aimed at removal of gross contamination, devitalised tissue and micro-organisms, the promotion of soft tissue healing, and restoring function of the soft tissues and bone.\textsuperscript{50,180} Where bone and deep soft tissue structures are infected systemic antimicrobial therapy alone is often ineffective at
obtaining a satisfactory outcome due to the inability to achieve high concentrations of antimicrobials at the site of infection.²⁷

Following the successful use of bio-implants in human orthopaedic infections, similar therapy is now beginning to be utilized in equine practice. These implants provide a means of delivering high concentrations of antimicrobials at the site of infection while avoiding possible toxic side effects associated with high systemic concentrations.²⁷,¹⁸¹ Antimicrobial impregnated polymethylmethacrylate (PMMA) has been, and remains the most widely used antimicrobial impregnated implant in equine practice.²⁷ These implants have resulted in improved outcomes for horses with osteomyelitis when compared to the use of systemic antimicrobials alone.⁸⁸,¹⁸² The prophylactic use of antimicrobial impregnated PMMA in human joint prosthesis has resulted in as much as a 34% decrease in infection rates.⁸⁸,⁸⁹

Despite the success of PMMA as a carrier for delivery of local antimicrobials there are potential problems associated with this chemical compound. PMMA is poorly biodegradable often necessitating an additional surgery to remove the implants¹⁵³ and therefore creating unwanted dead space.⁹⁰ If the beads are left in situ there is risk of persistent antimicrobial residues for months to years that may potentiate antimicrobial resistance.¹⁸³ Ironically if left in place the PMMA may act as a nidus for infection after antimicrobial elution has reduced below therapeutic levels.¹⁸⁴ Finally the construction process of PMMA involves an exothermic reaction that has detrimental effects on heat sensitive antimicrobials and produces toxic substances.¹⁸⁵,¹⁸⁶,¹⁸⁷

The development of the ideal antimicrobial delivery system for use in therapy of orthopaedic sepsis continues to be the focus of on-going research. The aim has been to develop a system that is biodegradable, does not evoke an inflammatory response, has no detrimental effects on the antimicrobials during the construction process, and is capable of sustaining release of the antimicrobials at appropriate therapeutic concentrations for a sustained period.⁶⁹,¹⁸¹
Alternatives to PMMA have included antimicrobial impregnated collagen sponges, which despite good success in an experimental equine septic arthritis model\textsuperscript{152}, was demonstrated to rapidly elute gentamicin in the tarsocrural joint in the horse, dropping well below therapeutic levels within 48 hours.\textsuperscript{159} Ferric-hyaluronate loaded with amikacin has also been assessed \textit{in vitro} and \textit{in vivo} in equine joints and also failed to provide prolonged antimicrobial release.\textsuperscript{188} Calcium sulphate hemihydrate (plaster of Paris) has also been used as a carrier for antimicrobials and could be an ideal local delivery system suitable for both soft tissue and osseous infections.\textsuperscript{166,167,189,190} The compound is absorbable, osteoconductive in the presence of osseous tissue but evokes no bone formation in the absence of periosteum or bone. Reports of the \textit{in vitro} elution profiles of antimicrobial from antimicrobial impregnated calcium sulphate hemihydrate beads have provided consistent results of initial rapid elution of antimicrobials followed by a prolonged slower phase of drug release.\textsuperscript{171,191,192}

The objective of this study was to examine the elution characteristics of a commercially available pre-prepared antimicrobial impregnated calcium sulphate hemihydrate – dextran sulphate bead. Our hypotheses were: (1) amikacin elution would result in concentrations that remain above 4 \textmu g/ml, the reported MIC for common equine pathogens, for a prolonged period; (2) there would be no difference in elution of amikacin from beads placed in saline and in equine plasma; and (3) there would be no difference in the rate of dissolution of the calcium sulphate hemihydrate – dextran sulphate when the beads were placed in phosphate buffered saline compared to those placed in plasma.

\textbf{Materials and Methods}

Amikacin concentrations were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS). The lower limit for detection of concentrations of amikacin in phosphate buffered saline was 0.5 \textmu g/ml. The method was less efficient in detecting
amikacin in plasma when low concentrations were present. The lowest concentration that amikacin could be detected accurately in plasma by the LC-MS method was 2.0 ug/ml.

Six commercially available calcium sulphate hemihydrate-dextran sulphate beads impregnated with amikacin and clindamycin (Matrix III Antibiotic Beads™, Royer Animal Health, Frederick, Maryland) were placed individually in to sterile plastic test tubes. The manufacturer label claims a mean bead weight was 23mg, containing a standardised 3.2% amikacin (0.736 mg amikacin/bead) and 1.6% clindamycin (0.368 mg clindamycin/bead) with a mean sphere diameter of 3mm. The weight and diameter of the individual beads were not measured prior to undertaking the experiment. Two series of assays, one series in PBS and one series in equine plasma, were undertaken simultaneously over the following 28 days to establish the amikacin elution profiles from the beads. Three calcium sulphate hemihydrate-dextran sulphate beads were each placed individually in 3ml of PBS, and three beads were placed individually in 3mls of commercial equine plasma and incubated at 37°C. At predetermined sample times (3hrs, 6hrs, 12hrs, 24 hours, 48hrs, 72hrs, 5days, 7days, 14days and 28 days) the PBS and plasma samples were inspected grossly to determine if the beads had completely eroded. Following visual inspection if the bead had not completely eroded then the entire volume of eluent was collected from the tube and refreshed with 3 ml of the same drug free fluid, either PBS or plasma. Following refreshment of the eluent, the tubes containing the beads were inverted 15 times before replacement in the incubator at 37°C. The eluent samples were then labelled and stored at -20 degree Celsius until antimicrobial assay using LC-MS.

Amikacin in saline sample preparation

Saline samples and calibration standards were diluted 1:1 with 1% formic acid in acetonitrile and filtered at 0.45 um. Quality control spikes were prepared by spiking ultra pure water with amikacin at 0.5, 1, 2, 10 ug/ml, which were then processed as for the samples.
In order to determine sample concentrations where calculated concentrations exceeded the calibration range of 0.5 to 10 µg/ml, samples were diluted a further 1:10 with 50% acetonitrile/1% formic acid in ultra pure water. Further quality control spikes were prepared at 10, 50, 20 and 100µg/ml, and were diluted a further 1:10 with 50% acetonitrile/formic acid as for diluted samples. Calibration standards at 0.5, 0.8, 1, 1.5, 2, 5, 10, 20 µg/ml amikacin were diluted 1:1 in 50% acetonitrile/1% formic acid in ultra pure water.

Amikacin in plasma sample preparation

Plasma samples were protein-precipitated with 4 volumes of acetonitrile/1% formic acid and centrifuged. A portion (portion A) of the supernatant was evaporated to dryness under nitrogen, reconstituted in one-fifth volume of 50% acetonitrile/1% formic acid.

A second portion (portion B) of the supernatant was diluted with one volume of 50% acetonitrile/1% formic acid. Portion B was used to determine amikacin content for samples exceeding the calibration range for analysis of Portion A preparations. Matrix-matched calibration standards were prepared from blank plasma extracts spiked at 0.5, 0.8, 1, 1.5, 2, 5, 10, 20 µg/ml amikacin.

Instrumental methods

Sample volumes of 40 µL were chromatographically separated on an Agilent 1200 liquid chromatography system with an Atlantis HILIC Silica 4.6 x 150 mm column of 5 um particle size. The mobile phase consisted of 5 mM ammonium formate at pH 2.5 (A) and 1% formic acid in acetonitrile (B), pumped at a gradient of 10% to 50% A buffer at 0.5 ml/min. Instrument responses for transitions m/z = 586.4 to 163.1 and m/z = 586.4 to 264.2 were monitored on an AB SCIEX 4000 QTRAP tandem mass spectrometry system used in the positive electrospray ionization mode.
Liquid chromatography-tandem mass spectrometry (LC-MS/MS) data for saline samples were processed using Anlayst 1.5 (AB SCIEX) by integrating the peak area for the m/z = 586.4 to 163.1 against calibration standards.

**Results**

Amikacin eluted rapidly from the beads in PBS falling below the lower limit of detection within 12 hours, and remained below the lower limit of detection thereafter until complete bead dissolution occurred between 48 and 72 hours. This finding was consistent across all three assays. Amikacin eluted rapidly from the beads in plasma and fell below the lower limit of detection in plasma within 6 hours. The calcium sulphate hemihydrate- dextran sulphate beads eroded at a far slower rate, with no complete bead dissolution in equine plasma occurring throughout the 28 days of the study.
Table 1. Amount of amikacin eluted (µg/ml) in phosphate buffered saline

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Table 2. Amount of amikacin eluted per bead (µg/ml) in equine plasma

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**Discussion**

The results of this study have shown that amikacin elutes rapidly from the calcium sulphate hemihydrate dextran sulphate beads in PBS, consistently falling reported MIC of most pathogens encountered in equine orthopaedic infections of 4ug/ml\(^47\) within 6 hours. There by disproving our first hypothesis that amikacin elution would result in concentrations that remain above 4 ug/ml, the reported MIC for common equine pathogens, for a prolonged period.

Amikacin is an aminoglycoside antimicrobial, the rate and extent of bacterial kill is related to attainment of a high maximum serum concentration (\(C_{\text{max}}\)) in relation to the minimum inhibitory concentration (MIC), which ideally should be \(\geq 8-10\) ug/ml.\(^{193}\) The reported MIC for most pathogens encountered in equine orthopaedic infection is 4 ug/ml,\(^{47}\) hence it would be desirable to achieve initial local concentrations of amikacin of 32-40 ug/ml. In phosphate buffered saline the concentration of amikacin initially peaked at (average \(C_{\text{(max)}} = 100\)ug/ml) at 1hr in phosphate buffered saline, approximately 25 times the reported MIC for equine pathogens however elution slowed quickly to produce concentrations that were below MIC at 6 hours. The elution characteristics of the amikacin from this bio-implant in phosphate buffered saline is in agreement with earlier *in vitro* reports of antimicrobial elution from calcium sulphate based implants, where an initial rapid elution occurred followed by a prolonged slower rate of elution.\(^{191,192,194}\) If the elution of amikacin from the bio-implant occurs in a similar fashion *in vivo*, then it offers no advantage in the clinical setting over other local delivery systems such as intra-articular or intravenous regional limb perfusion, which have been reported to achieve initial high concentrations and also maintain local osseous concentrations of gentamicin sulphate above MIC for 8 hours in the horse, along with a relative ease of administration and lower cost.\(^{126}\)
Local antimicrobial therapy is commonly used in equine practice for musculoskeletal wounds and infections. When used prophylactically with orthopaedic implants or used therapeutically in the presence of infection, local antimicrobial therapy aims to deliver active antimicrobial to the site of infection, at concentrations that exceed the MIC for offending pathogens for a therapeutically relevant period of time.\textsuperscript{195} This not only alleviates the risk of systemic toxicity, but results in local concentrations far superior to that which can be achieved from systemic dosing at a reduced cost and without adversely impacting dead space management. Given the growing concerns in human and veterinary medicine of antimicrobial resistance, avoidance of sub-therapeutic antimicrobial concentrations in the management of infection is a priority.

Using the methodology as described we were unable to account for the total amount of drug that was reportedly present in the bead. Possible explanations could include problems with the experimental design, analytical technique, or inadequate drug within the bead. Preliminary studies using the LC-MS/MS technique revealed that this methodology was highly accurate in detecting amikacin in spiked PBS samples to a lower limit of detection of 0.5\,ug/ml. As these beads were manufactured from an external commercial source we were unable to verify the amount of drug present in each bead.

Our finding that amikacin eluted rapidly from the calcium sulphate hemihydrate dextran sulphate bead in PBS is consistent with the findings of others investigating the elution characteristics of antimicrobials from calcium sulphate based implants.\textsuperscript{192,196,197} Manufacturers of the implant used in the current study claim that the inorganic/polymer composite provides an innovative drug delivery matrix which has a highly-controllable release profile and dissolution rate. The findings in this study would suggest that this is not the case in PBS, however the dissolution of the implant was noted to be substantially slower in plasma and controlled drug delivery and bead dissolution may therefore hold true \textit{in vivo}. Contrary to \textit{in vitro} findings, it has been recognised \textit{in vivo} that elution of tobramycin from
calcium sulphate based bio-implants for the treatment of experimentally induced osteomyelitis in rabbits has resulted in significantly slower and more prolonged elution of the antimicrobial thereby maintaining a therapeutic concentrations within local tissues for 7 days,\textsuperscript{165} further to this tobramycin impregnated calcium sulphate based bio-implants have been successfully used in combination with surgical debridement clinically in chronic osteomyelitis in humans.\textsuperscript{167} The difference in findings between \textit{in vitro} and \textit{in vivo} findings could indicate a problem in experimental design, and that the PBS model may not be ideal for the \textit{in vitro} assessment of bio-implant antimicrobial elution characteristics. We chose this model because it has been widely used, and is an accepted model, to characterise the elution patterns of antimicrobials from implants.\textsuperscript{82,94,96,192,196,197} Whilst it may not allow accurate assessment of antimicrobial elution \textit{in vivo}, it does allow comparison of antimicrobial elution from implants with differing formulations and substances.

A media that better reflects the \textit{in vivo} environment may provide a more accurate representation of the \textit{in vivo} elution characteristics when studied in vitro. Elution characteristics within equine plasma may be more reflective of that \textit{in vivo} as compared to the PBS, and may be a more reliable model for \textit{in vitro} assessment of elution characteristics of antimicrobials from bio-implants. In an effort to overcome the shortfalls of using PBS, we chose to undertake a simultaneous elution study in equine plasma to observe for any difference in the elution characteristics of amikacin from this bio-implant. The use of equine plasma as a media for antimicrobial elution studies has not been reported thus far. In validating the analytical methodology we were unable to accurately measure amikacin below 2ug/ml in equine plasma. This reason for the low recovery of amikacin in equine plasma by this method is unknown. The low amikacin recovery impacted on the variability of the data at lower concentrations and therefore, our reporting limits for this method were quite high compared with those for the saline method. The elution characteristics of amikacin were therefore not determined in equine plasma; hence our second hypothesis cannot be proved or
disproved. A previous study reported the elution characteristics of gentamicin from a calcium sulphate based delivery vehicle in porcine serum; revealing the elution characteristics were comparable to studies using phosphate buffered saline as the eluent.\textsuperscript{171}

The rate of elution of antimicrobials from calcium sulphate based implants is influenced by the crystal density, shape of the implant (surface area to volume ratio), amount of antimicrobial impregnated within the particular implant, and the volume and rate of refreshment of the wound fluid. The first three factors in this study were controlled by the manufacturer of the product, whereas the final factor was controlled by the study design. The method of sampling the eluent influences the rate at which antimicrobials elute from calcium sulphate based bio-implants; with complete refreshment of eluent leading to much more rapid of elution of antimicrobial, compared with the slower rates of elution that occur with only partial refreshment of the eluent at sampling times.\textsuperscript{198} As continuous agitation was not conducted throughout the study, we elected to completely refreshment the eluent in case the amikacin was not evenly distributed throughout the eluent, thereby not providing an accurate amikacin elution concentration. The complete refreshment of eluent should be taken into consideration when contemplating the use of this bio-implant clinically where the rate of refreshment of wound fluid may not be as high. The elution characteristics reported in this study may be more reflective of the amikacin elution characteristics from the implant in a highly exudative wound environment.

Our third hypothesis that there would be no difference in the rate of degradation of the implant in phosphate buffered saline was not proven. The calcium sulphate hemihydrate-dextran sulphate beads were consistently completely degraded in phosphate buffered saline by 72 hours, whereas they remained only partially degraded by 28 days in the equine plasma. It was noted that the beads developed a biofilm around them in the equine plasma, but the nature of the film was not determined in this study. Given amikacin is highly dissolvable in water and has less than 5% protein binding, the poor recovery of amikacin
from the bio-implant in equine plasma may have been due to the biofilm creating a barrier for diffusion of the amikacin out of the implant. However if the elution characteristics of amikacin from the bead were assumed to be similar to that in phosphate buffered saline then it brings in to question the propensity for this implant to promote antimicrobial resistance during the prolonged slow phase of elution of sub-therapeutic concentrations of antimicrobial. Furthermore, the persistence of the implant in equine plasma raises concerns regarding the implant acting as a foreign body or nidus for infection during the second slow phase of elution.

**Conclusion**

Amikacin eluted rapidly from the commercially available amikacin impregnated calcium sulphate hemihydrate– dextran sulphate beads in phosphate buffered saline. If this is a true reflection of elution *in vivo* then it calls to question the appropriateness of this bio-implant for *in vivo* use in the management of orthopaedic infections in the horse. Alternatively, the elution observed in phosphate buffered saline raises the question of the appropriateness of this model for assessment of *in vivo* elution of such implants. The slower dissolution of this bio implant in plasma compared to that in phosphate buffered saline also raises concerns about the potential for the implant to not only promote antimicrobial resistance due to prolonged low level antimicrobial elution, but also the propensity for the implant to act as a nidus for continued infection.

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Calcium phosphate based biomaterials

Bone consists of 70% inorganic mass (apatite) and 30% organic mass (collagen), with the predominant ions being that of calcium and phosphate. Bio-ceramics have a compatible composition of calcium and phosphate to bone and this has resulted in extensive study for their use as vehicles for local antimicrobial release and bone substitutes. Bio-ceramics are classified into three categories: Bioinert ceramics (Sintered alumina and zirconia), bioactive ceramics (bioactive glass) and bioresorbable ceramics (hydroxyapatite, sintered beta tricalcium phosphate). Bio-inert ceramics incite a low grade inflammatory reaction resulting in a thin fibrous tissue capsule that walls the implant off from the body where the implant persists.

Hydroxyapatite

Hydroxyapatite constitutes the inorganic part of bone and has imparted added benefits of local drug delivery in that it is biocompatible, bioactive encouraging new bone formation, and has high binding affinity for a variety of bioactive molecules. Hydroxyapatite is capable of releasing bioactive molecules for a prolonged period, along with aiding in space filling for reconstruction and regeneration of living tissue in the local area. A number of characteristics of fabrication of the hydroxyapatite have been shown to influence the effectiveness as a local drug delivery agent. The pore size of the hydroxyapatite has been shown to influence not only the elution of contained drugs but also the migration of osteoblasts and mesenchymal cells and matrix deposition in the empty space. The elution of antimicrobials from hydroxyapatite with lower pore percentage and majority micro pores has been found to be superior to constructs with higher pore percentage in vivo and in vitro; however it is thought that the ideal construct has a mixture of large pores to facilitate osteoblast ingrowth and small pores to sustain a release of antimicrobials at therapeutic concentrations over a protracted period of time. The pore interconnection of
hydroxyapatite also influences the performance as it imparts the channelling for cell
distribution and migration, thereby allowing for efficient blood vessel formation. A bi-
model distribution of pore sizes has been proposed to be ideal; as large pore size encourages
bone ingrowth whilst the small pores facilitate the slow prolonged release of the
impregnated drug. A negative surface charge of hydroxyapatite scaffold has been shown
to improve drug attachment, accelerate mineral deposition, reduce the time for cell
attachment and improve the tissue ingrowth. The size of the antimicrobial molecules also
has an influence on the rate of elution, as does the solubility of the antimicrobials.

The size, solubility and binding capacity of the antimicrobial to the biomaterial all influence
the elution characteristics of the implant as a method for local antimicrobial delivery. The
smaller the size of the antimicrobial molecule the faster the molecule can diffuse through the
implant; the more soluble the antimicrobial the faster it will elute from the implant. The
binding affinity of antimicrobials is determined by the number of available carboxyl groups,
the more carboxyl groups the more intensely bound the molecule to the bio-ceramic and
therefore the slower the release into the local tissue. A number of antimicrobials have
been reported in research models to elute efficiently at therapeutic levels for a prolonged
period, including gentamicin, fosfomycin, imipenem, amphotericin B and combined
ceftriaxone sodium/sulbactam sodium.
**Chitosan:**

Chitin, a modified polysaccharide, is the structural component of arthropod exoskeletons, fungi cell walls, and cephalopod beaks, can be modified by removal of acetyl groups to form chitosan. Chitosan is a biodegradable and biocompatible polymer, which has various medical uses including artificial skin, absorbable suture material, wound healing accelerator.\(^{207}\) Immuno-enhancing, antitumor, antibacterial and hypocholesterol properties have also been identified and are thought to be dependent on the molecular size and chemical structure of the chitosan derivatives.\(^{307}\) Derivatives of chitosan have been chemically modified such that they differ in the arrangement and number of hydroxy and amino groups, which is thought to impart the physiological activity of the molecule.\(^{207}\) Chitosan has been shown to reduce blood clot time in a dose dependant manner and increase the release of platelet derived growth factor AB and transforming growth factor B1.\(^{207}\) The mechanism of action by which chitosan and its derivatives cause haemostasis is thought to be independent of the classical path of coagulation, with no direct effects on the extrinsic or intrinsic pathways identified, rather it is thought to induce haemostasis enhancement of adhesion and aggregation of platelets.\(^{207,208}\) Much research has been directed towards combining chitin and chitosan with calcium based products such as calcium phosphate and hydroxyapatite to form composite materials that have the qualities of toughness and flexibility of the polymer, and strength and hardness of the calcium products, thus creating a bio-implant that would provide the scaffold for new bone growth (osteogenic potential) with the binder qualities of the polymer preventing implant migration and a suitable means of local drug delivery.\(^{209,210}\) These products have been studied as cements for bone defect filling and also engineered in-to microspheres for filling bone defects and local drug delivery.\(^{211-213}\) *In vitro* studies have revealed release of gentamicin sulphate from pure chitosan scaffolds to have a similar bimodal elution pattern, as with PMMA and calcium sulphate, in that there is a high initial burst release of gentamicin followed by prolonged release at a lower level.\(^{214}\)
The magnitude of release of gentamicin from the chitosan films is reported to be directly proportional to concentration of gentamicin incorporated into the film and to be inversely proportional to the level of cross linking of the chitosan. Composite materials have been shown to reduce the magnitude of the initial gentamicin release and maintain release at higher concentration for a more prolonged period when compared to pure chitosan films. In vivo studies of the use of chitosan composites for local drug delivery have been promising. In a rabbit model of osteomyelitis treated with gentamicin impregnated chitosan bar it was found that approximately 11% of the total gentamicin was eluted in the first 24 hours and then continued to elute and maintain local bone concentrations above MIC for over 8 weeks. A report of clinical use of gentamicin impregnated chitosan bars in the management of human chronic osteomyelitis has also yielded good results with gentamicin concentration in wound fluid several hundred times MIC for Staphylococcus aureus, achieving complete resolution of disease in 16 of 18 patients, and no systemic side effects, leading to the conclusion that gentamicin impregnated chitosan bars are a cheap, safe and effective local drug delivery device for treatment of chronic osteomyelitis. Research and clinical use of chitosan in the horse has so far not been documented, however holds much promise as a vehicle for local antimicrobial release.
Concluding comments:

Management of orthopaedic infection in the horse has been vastly improved over the past 20 years. This can be attributed to improved surgical and medical management of such conditions; and the institution of local antimicrobial therapy. Antimicrobial impregnated polymethylmethacrylate has not only contributed immensely to the improved success of management of orthopaedic infection in the horse, but has improved our understanding of pharmacokinetics and pharmacodynamics of local antimicrobial therapy. As not all properties of polymethylmethacrylate are ideal for impregnation with antimicrobials and for implantation into tissue for local antimicrobial release, alternate local delivery techniques have been developed.

Clinically, intravenous antimicrobial regional limb perfusion and direct intra-synovial administration of antimicrobials has become common day practice in the management of orthopaedic infection in the horse. Previous reports of septic synovial structures have only instituted local antimicrobial therapy in selected cases and carried an association with non-survival and reduced postoperative performance, this however may have been biased due to only instituting local antimicrobial therapy in severe cases or those unresponsive to initial therapy. In the review of the use of local antimicrobial therapy for septic synovial structures at Murdoch University, all cases in the series underwent some form of local antimicrobial administration. The success of therapy in this case series is comparable to previous reports, and supports the use of local antimicrobial therapy in management of septic synovial structures in the horse.

Bioresorbable implants have been less widely used in the horse and remain predominantly a research method of local antimicrobial administration. Calcium sulphate has been identified as a promising vehicle for local antimicrobial delivery; with positive attributes of bio-absorption and osteoconductivity in the presence of osseous tissue but evokes no bone
formation in the absence of periosteum or bone. However an antimicrobial impregnated calcium sulphate implant that can provide therapeutic concentrations of antimicrobials for therapeutically relevant periods of time remains to be found. Closer examination of one commercially available amikacin impregnated calcium sulphate bead undertaken in this project revealed amikacin eluted rapidly from the beads in phosphate buffered saline. If this is a true reflection of elution in vivo then it calls to question the appropriateness of this bio-implant for in vivo use in the management of orthopaedic infections in the horse due to the short period of antimicrobial elution. Alternatively, the elution observed in phosphate buffered saline raises the question of the appropriateness of this model for assessment of in vivo elution of such implants. The slower dissolution of this bio implant in plasma compared to that in phosphate buffered saline also raises concerns about the potential for the implant to not only promote antimicrobial resistance due to prolonged low level antimicrobial elution, but also the propensity for the implant to act as a nidus for continued infection. Many alternative calcium sulphate bio-implants have been studied, however to date none of these implants have been ideal. Research into alternate calcium sulphate composites, calcium phosphate bio-ceramics and chitosan compounds hold promise for development of the ideal implantable vehicle for local antimicrobial delivery.
Bibliography:


