

Chronic Equine Wounds: What Is the Role of Infection and Biofilms?

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Abstract: Wound research is an evolving science in the equine species. In particular, interest is growing regarding the role that microorganisms play in delaying both acute and chronic wound healing. Equine wounds, particularly lower limb wounds, frequently display delayed healing and infection is commonly the underlying reason. This review will summarize the current research and knowledge surrounding equine wound healing and wound care. Particular focus is placed on the role that microbes play in chronic equine wounds and the significance of associated bacterial biofilms.

Both acute and chronic wounds are highly prevalent in horses and represent a significant management challenge to veterinary surgeons.¹ Particular concern exists in equine wounds that are at risk from infection. Lower limb wounds are particularly susceptible to colonization from microorganisms and are notoriously problematic. The risk of infection is heightened because horses are exposed to a vast array of microorganisms that have the ability to colonize open wounds.² Maintaining a wound microenvironment that helps to suppress microbial proliferation, particularly at problematic anatomical sites, such as the dorsal tarsocrural region, is of paramount importance. This can usually be achieved by appropriate wound management strategies.³ Specific anatomical reasons for the failure of distal limb healing that have been proposed include a relatively poor blood supply and reduced tissue oxygenation compared to the thoracic region. These are recognized factors that lead to an increase in the microbial bioburden within the wound bed.⁴

Treatment methods that are employed in the management of horse wounds focus on rapid and efficient wound evaluation, scrupulous, aseptic surgical techniques, and conscientious and prolonged aftercare. Appropriate antibiotic treatment regimes are routinely employed when the wound is at risk of becoming infected or is known to be infected.⁵

Wounds can be either traumatic or surgical in origin; both types can fail to heal and become chronic although traumatic wounds are more commonly affected by healing difficulties. The incidence and prevalence of traumatic wounds in horses is considered to be high⁶⁻⁸ and a high percentage become chronic, adding more complexity to wound healing management strategies. Chronic wounds in horses have a similar pathophysiology to human chronic wounds.⁹ Management practices utilized for human chronic wounds are also being employed successfully in the management of equine traumatic and chronic wounds.¹⁰

Wounds become more challenging when bacteria penetrate a synovial structure and result in synovial sepsis. In these cases, appropriate and timely treatment is critical to successful recovery.^{11,12} However, even with appropriate treatment, synovial infections can result in equine mortality.¹³

Postsurgical infections are a serious concern for veterinary surgeons,⁵ and as previously mentioned, indigenous microbiota have the ability to penetrate and colonize the hosts tissue leading to a further delay in wound healing in horses.² The percentage of horses that develop infected surgical wounds is significantly higher than the figure reported in human¹⁴ and small animal studies¹⁵ suggesting that horses may be at an increased risk of infection, possibly as a result of the horses' heavily colonized local environment. However, these differences could be a reflection of variations in the definitions employed to denote an "infected wound."¹⁶

Current guidelines that are used for the prevention of equine postoperative infections include: 1) maintaining tissue health and 2) topical and systemic administration of appropriate antimicrobials.

However, postoperative infections with bacteria (eg, methicillin-resistant *Staphylococcus aureus* [MRSA]) are increasing, implying that treatment of these cases requires continued investigation.¹⁷

Equine Wound Healing

When skin is damaged an inflammatory response is immediately initiated leading to the formation of a localized fibrin/fibronectin clot. This clot is rich in growth factors and is responsible for triggering fibroblast proliferation, migration, and subsequent granulation tissue formation.¹⁸ Clotting and inflammation within the wound bed are transient mechanisms that provide a temporary scaffold. The process of repair terminates when the wound is closed completely with the ultimate formation of a scar,

which creates a permanent barrier to microbial ingress. Re-epithelialization involving keratinocyte migration across the wound surface¹⁹ and concurrent tissue contraction⁹ form the basis of natural wound closure. Inflammatory cells, fibroblasts, and capillaries combine to form contractile tissue, which is responsible for the centripetal contraction of the wound margins. Angiogenesis is then triggered by growth factors²⁰ including fibroblast growth factor 2 (FGF2)^{21,22} and vascular endothelial growth factor (VEGF).¹

In uncomplicated acute wounds such as a surgical incisional wound, the healing process is conventionally completed within 2 weeks with restoration of tissue tensile strength occurring within a few weeks thereafter. However, the type of wound and associated complications can extend the time frame of this process.^{9,23} The chronic wound does not progress sequentially through the "normal" stages of wound healing²⁴ with inhibited stages being the principle pathological processes.

Variations in Healing Rates Between Equine Thoracic and Limb Wounds

Wound healing rates in horses vary in relation to the wound severity and its anatomic site. For example, there is a greater tendency of exuberant granulation tissue growth and excessive scarring on the lower limbs of horses compared to similar wounds in the thoracic region.⁹ Myofibroblasts are less well orientated in the limb when compared to trunk wounds and this is suggested as a cause for the decreased contraction efficiency of wound beds in limb wounds.²³ The relatively poor blood supply to the limbs may play a significant role through decreased oxygen supply and decreased distal limb temperature. These factors are thought to contribute to an imbalance in the growth factor profiles required for effective healing.⁴ In particular, the prolonged presence of tissue growth factor β 1 (TGF- β 1), is blamed, in part at least, for the formation of exuberant granulation tissue.^{4,25} The presence of an increased microbial bio-burden in the lower limb compared to thoracic wounds also significantly delays healing although the precise mechanisms for this are not clear.⁴

Chronic Infection

Chronic wounds are characterized by a stalled healing process that persistently refuses to progress through the normal wound healing stages.^{9,26} Visually, chronic wounds appear red and inflamed²⁷ and often produce fetid wound exudate.⁹ Figure 1 shows a 2-week-old nonheal-



Figure 1. A 2-week-old nonhealing wound that produced a slimy wound exudate and a strong fetid odor. This wound clearly had multiple factors that could affect healing efficiency including infection, tissue deficits, necrotic tissue, and movement within the wound bed—a typical problem encountered in distal limb wounds of horses.

ing wound with a slimy surface layer characteristic of an inherent biofilm, exudate, and recalcitrance to healing. Biofilms are defined as a group of microorganisms that exist in a self-synthesized protective matrix and are encased within a three dimensional matrix of extracellular polymeric substances.²⁸

A number of factors that can lead to wounds becoming chronic have been identified. Wound infection and chronic inflammation are blamed in particular.²⁹ Imbalances in the relative concentrations of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are known to delay wound healing significantly.³⁰ The expression of TIMPs in chronic wounds is reduced when compared to the expression of MMPs. This results in an elevation in overall proteinase activity, elevated protein degradation within the wound bed and consequent delayed wound healing.³¹

Bacteria and Equine Wound Healing

Microflora of the healthy horse. The indigenous microbiota of a healthy horse skin is rich and varied (Table 1) and this community structure is considered significant to horse health. Generally, gram-positive bacteria are reported to comprise the highest proportion of the resident bacteria on the skin of the horse, whilst environmental gram-negative bacteria are reported to colonize horse skin less frequently.³²

Microflora of equine wounds. Historically the term “critical colonization” was used to determine if a wound was at risk of becoming infected. The traditional practice of reporting a wound as infected purely based on a bacterial number greater than 1×10^5 viable organisms per gram is now considered largely out dated.^{33,34} A more

Table 1. Predominate resident microflora of horses as reported in 4 studies.

Reference	Resident equine flora
Galuppo et al. (1999)	<i>Corynebacterium</i> , <i>Streptomyces</i> , and <i>Streptococcus</i> spp.
Rothschild et al. (2004)	<i>Helcococcus ovis</i>
Matsuo et al. (2001)	<i>S sciuri</i> *, <i>S cohnii</i> , <i>S hominis</i> , <i>S haemolyticus</i> , <i>S gallinarum</i> , <i>S lentus</i> , <i>S simulans</i>
Cochrane et al. (2009)	<i>P aeruginosa</i> *, <i>S epidermidis</i> , <i>Serratia marcescens</i> , <i>E faecalis</i> *, <i>Providencia rettgeri</i>
Orsini et al. (2004)	<i>Bacillus</i> sp., nonhemolytic <i>Staphylococcus</i> sp., <i>Micrococcus</i> sp., <i>Corynebacterium</i> sp.
Hague et al. (1997)	<i>Bacillus</i> , <i>micrococcus</i> , and non-hemolytic <i>Staphylococcus</i> spp.

*S = *Staphylococcus*

*P = *Pseudomonas*

*E = *Enterococcus*

meaningful classification of the microbial status of wound infection is the point when the host's immune response is triggered.³⁵ An impaired host immune response is not able to decrease the bacterial level within a wound sufficiently to prevent clinically significant infections from developing. Reduced effectiveness of immunity and antibiotic treatments may be indicative of the presence of a bacterial biofilm.^{36,37}

In some circumstances, the inhabiting isolates are environmental pathogens such as *Actinobacillus equuli*, a species usually associated with septicemia in foals.³⁸ More often, however, wound infections result from commensal flora acting as opportunistic pathogens. *Streptococcus zooepidemicus*, *Staphylococcus aureus*, *alpha Streptococcus* species, *E coli*, and *P aeruginosa* have been isolated from a variety of clean and clean-contaminated orthopedic procedures.¹⁶

The strategies used by bacteria to cause and induce persistent infections in wounds are not fully understood, however they are likely to be multifactorial. In human studies, *S aureus* and *E coli* have been reported to act via specific adherence proteins to suppress the influx of inflammatory cells³⁹ and to enhance bacterial attachment to human cells,⁴⁰ respectively. *Pseudomonas aeruginosa* has been widely reported as an opportunistic wound pathogen in human studies.⁴¹ By residing deep within the wound *P aeruginosa* manages to keep the wound in a prolonged inflammatory state.⁴² Synergistic interaction with other bacteria such as *Burkholderia cepacia*,

results in a total increase in pathogenicity of both organisms.⁴³ While this synergy has yet to be reported in equine wounds, the ability of *P aeruginosa* to form and survive within protective biofilms specifically in chronic equine wounds has been reported.⁴⁴

Successful comprehensive culture of the microbial species involved in chronic wounds is not always possible because either they do not grow under standard culture conditions, eg, anaerobic species such as *Bacteroides* require specific anaerobic transport medium,⁴⁵ or because they are protected within the wound by a bacterial biofilm where they exist in a viable but “non-culturable” state.⁴⁶

Biofilms

It has been suggested that chronic, non healing wounds may result from bacteria that survive within an antimicrobial “barrier” known as a biofilm.⁴⁶ Biofilms are diverse, dynamic, and unique, and reflect their local environment and inhabiting species.⁴⁷ Single species bacteria can form biofilms (Figure 2),⁴⁸ but more commonly, biofilms result from complex combinations of multiple species of aerobes and anaerobes⁴⁹ that interact via intracellular communication.^{50,51} Biofilms have been reported to exist deep in the wound bed where they can significantly prevent their isolation and identification.⁵² Although some biofilms are naturally beneficial,^{53,54} unwanted biofilms have been linked to a number of human disorders including cystic fibrosis,^{43,55-57} endocarditis,^{58,59} catheter-related infections,⁶⁰⁻⁶³ surgical site infections,⁶⁴ and chronic wounds. The latter include pressure sores, diabetic ulcers, venous ulcers, and ulcers that are secondary to ischemia.⁶⁵ These human wounds share certain common characteristics with chronic equine wounds, and like the human wounds described above, equine wounds have shown evidence of biofilms.^{36,44}

Biofilms and resistance. Human leukocytes can kill planktonic *S aureus* but killing the same bacteria within a biofilm requires up to 1000-fold increase in cell numbers. Leid et al⁶⁶ demonstrated that in comparison to the equivalent planktonic bacteria, biofilm residing bacteria have an increased resistance to the host’s immune response as well as to antimicrobial drugs.³⁴ Furthermore, bacteria within a biofilm can demonstrate resistance in the absence of standard resistance mechanisms such as efflux pumps and target mutations.⁶⁷ One explanation for the decreased susceptibility to treatment is that antimicrobial agents and phagocytes are unable to penetrate the exopolymer matrix and reach the bacte-

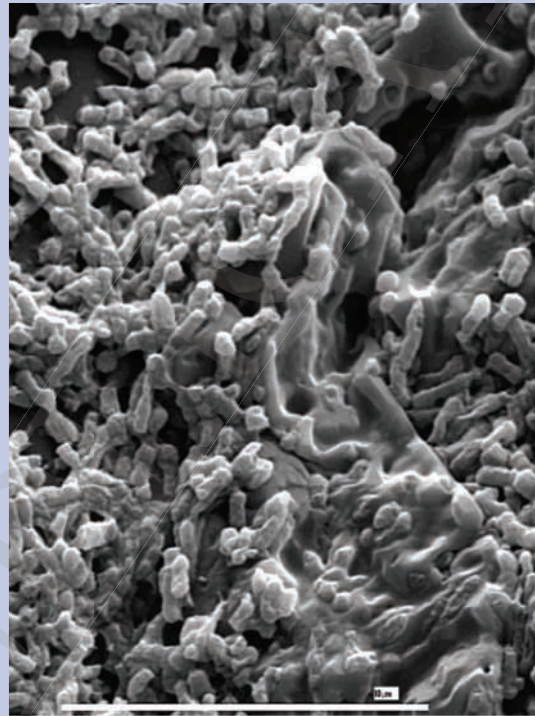


Figure 2. A 24-h *P aeruginosa* biofilm grown *in vitro* on polystyrene pin lid and visualized through scanning electron microscopy (Scale bar = 10 μ m; magnification x3500).

ria.^{68,69} However, antimicrobial penetration into a mature biofilm and an inactivity of antimicrobial agents once they are inside the biofilm has been demonstrated.⁶⁶ These apparent contradictions have been ascribed to the bacterial species responsible for biofilm formation⁷⁰ and to the age of the biofilm. Older mature biofilms develop fluid channels, which provide a transport route for leukocytes and antibiotics into the biofilm, whereas immature biofilms, such as the 2-day-old biofilm used in the Hoyle et al⁶⁸ study, may not yet have become complex enough to acquire such systems. Once inside the biofilm, the inactivity of the antibacterial agents such as penicillin can possibly be explained by a slower growing phenotype, produced in response to low nutrient conditions.⁷¹ The bacteria then remain present in the wound and re-establish the infection once conditions become more favourable.⁷²⁻⁷⁴ A decrease in nutrient availability and/or high levels of waste products can also lower the pH and oxygen levels of the microenvironment, inhibiting the effectiveness of antimicrobial agents such as aminoglycosides.⁷⁵

Treatment of biofilms. Primary closure of the wound is not usually attempted when infection is sus-

pected. Instead, treatment focuses around the removal of the infecting biofilm. Primary closure can then be reconsidered if, following removal of the biofilm, vascular supply and healthy tissue are plentiful.^{34,76} Currently, the most effective management of a wound that is complicated by biofilm formation involves sharp surgical debridement of the biofilm and its inhabiting bacteria.^{44,77,78} Topical treatment without debridement may not adequately remove a biofilm.⁷⁹ Following biofilm removal topical antimicrobials can be applied to the wound to prevent bacterial re-attachment and therefore limit the extent of re-infection.⁸⁰

Conclusion

Equine wound healing studies continue to investigate procedures such as the use of differing techniques of wound closure,⁸¹ varying methods of skin grafts,¹⁰ the role of platelet rich plasma,⁸² and the use of hyperbaric oxygen⁸³ in relation to delayed wound closure.⁸⁴ Whilst work continues on various topical treatments ranging from growth hormones to honey,⁸⁰ these too must be able to penetrate and be effective within the biofilm to be useful in treating chronic equine wounds. Removal of microorganisms is essential for the successful application of any wound treatment procedure. The role of microorganisms within wounds⁸⁵ and their ability to produce and persist within a biofilm remains an important area of study.⁴⁴

In addition to the traditional laboratory methods that establish the minimum inhibitory concentration (MIC) of antimicrobial drugs, the minimum biofilm eradication concentration (MBEC) of the cultured isolates should be considered when antimicrobials are going to be used in wound management. In this way, more appropriate doses of antimicrobial drugs can be employed.⁸⁶

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