

A REVIEW ON EQUINE WOUND MANAGEMENT AND HEALING PROCESS

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✉ Supporting Information

ABSTRACT: Skin lacerations and other traumatic injuries of the integument are frequently seen in equine practices and range from relatively minor cuts to severe, potentially debilitating injuries. The early stages of wound healing (inflammatory or debridement stage) that are clean or clean contaminated are the best candidates for primary or delayed primary closure. Wounds that are in the later stages of healing or are contaminated or infected heal best by second intention. Management is dictated by the nature and size of the wound, the area of the body on which the wound occurs, and several aspects of wound healing. The age of the wound, integrity of the local blood supply, degree of contamination, location of the injury, skin loss, and local tissue damage must all be considered when deciding on the most appropriate method for managing a particular wound. In addition to biologic factors, the physical size of equine patients and the environment in which they are kept present unique management challenges not encountered in the treatment of soft tissue injuries in other species. Appropriate wound care is always a balance between improving the wound environment and harming the cells that are integral to the healing process. Consequently, the veterinarian must carefully weigh the benefits and the detriments of any particular action, not only for the immediate results but also for the long-term healing process. Understanding the principles and limitations of reconstructive procedures, adhering to the basic principles of equine wound management, and providing appropriate postoperative care all contribute to a successful outcome.

Keywords: Debridement, Equine, Inflammation, Management, Wound

LIST OF ABBREVIATIONS

ATP	Adenotri Phosphate
DACA	Drug Administration and control Authority
ECM	Extracellular Matrix
EGT	Exuberant Granulation Tissue
FAOSTA	Food and Agricultural Statistics
HBO	Hyperbaric Oxygen
L	Liter
MMPs	Matrix Metalloprotenases
PA	Plasminogen Activator
PCV	Packed Cell Volume
PMNs	Polymorphonuclear Leukocytes
PSI	Pounds Per Square Inch
SIS	Small Intestinal Submucosa
SPP	Species
VEGF	Vascular Endothelial Growth Factor

INTRODUCTION

More than 72% of the world's horse population is found in developing countries specifically kept for draft purpose (Swann, 2006). Ethiopia has more than 6 million donkeys, the second largest donkey population in the world next to China, 1.9 million horses and over 350,000 mules (FAOSTAT, 2012). Equines are important animals to the resource-poor communities in rural and urban areas of Ethiopia, providing traction power and transport services at low cost (Dinka et al., 2006).

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In Ethiopia, the rugged terrain characteristics, absence of well-developed modern transport networks and the prevailing low economic status of the community necessitate the use of equines for transportation (Mengistu, 2003). Wound is an open mechanical injury of the skin (epidermis), underlying tissues and organs. It is characterized by pain, gaping bleeding functional disturbance. The most common cause of wounds in working equine are over loading, accidents, improper position of load predisposing to falling, hyena bites, donkey bites, injuries inflicted by horned Zebu (DACA, 2006). Some hobbling methods, inappropriate harnesses or yokes that may be heavy and ragged, long working hours may cause discomfort and inflict wounds (Mekuria et al., 2013).

Wounds are one of the primary welfare concerns of working equids (Sells et al., 2010). The type of wound in working donkeys includes tissue damage with or without blood/exudates/ pus, abscess formation, or any secondary bacterial complication. Bites (lacerated wounds) will be identified by irregular edges with underlying tissues removed as well as hemorrhage (Sevendsen, 2008).

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 equine is considered to be high (Singer et al., 2003) 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 wound (Cochrane et al., 2003). Treatment methods that are employed in the management of wounds focus on rapid and efficient evaluation, scrupulous, aseptic surgical techniques, and conscientious and prolonged aftercare. Appropriate antibiotic treatment regimens are routinely employed when the wound is at risk of becoming infected or is known to be infected (Griffiths et al., 2003). There for, the aim of this review paper is to clarify the types of wound in equine and what are the processes and the phase to heal it.

GENERAL OVER VIEW ABOUT WOUND

Initial preparation

After the equine has been restrained appropriately and the area has been desensitized with local anesthetic, the clinician decides whether to treat the wound with aseptic technique or if a simple clean technique is adequate. If there is a possibility of cross-contaminating a wound while preparing and exploring, aseptic technique should be used. The next step is to clip the hair over and around the wound site. To minimize the amount of clipped hair that gets into the wound, it can be covered with water-soluble, sterile, lubricating gel prior to clipping. It is best to put on a pair of examination or sterile gloves, apply the gel onto the fingers, and rub the gel into place. This technique generally ensures that the gel will remain in contact with the wound bed. As the hair is clipped, it is trapped in the gel. The gel with the trapped hair can simply be rinsed from the wound with water or saline. Only when the entire wounds area has been clipped and prepared can the clinician begin to fully appreciate the complexity of the wound. In many cases, seemingly insignificant wounds had invaded synovial structures, and because appropriate aggressive therapy was not instituted, the horse was eventually euthanized (Auer and Stick, 2006).

Wound assessment

Wound assessment is of the most important steps in wound management. More repaired wounds fail because of improper preparation and assessment than because of improper therapy. Properly preparing the wound provides an accurate assessment of the wound and deeper structures. Wound preparation begins with placing the animal in allocation and environment that will allow the clinician to best determine the status of the wound (Auer and Stick, 2006).

Wound classification

Wounds are typically classified on the basis of degree of contamination. Clean wounds, usually seen only in surgical situations, are not infected and do not involve the respiratory, alimentary, or urogenital tract. Clean contaminated wounds, generally seen in surgical situations, involve the lumen of the respiratory, alimentary, or urogenital tract. Contaminated wounds are generally traumatic in nature and may have gross contamination and necrotic debris. Infected wounds generally involve large numbers of bacteria, inflammation, edema, and suppuration (Knottenbelt et al., 1998).

Debridement

Debridement is the process of removing dead (necrotic) tissue or foreign material from and around a wound to expose healthy tissue. Necrotic tissue and bacterial infection are major roadblocks to effective and cosmetic wound healing. An open wound or ulcer cannot be properly evaluated until the dead tissue or foreign matter is removed. Wounds that contain necrotic and ischemic (low oxygen content) tissue take longer to close and heal. This is because necrotic tissue provides an ideal growth medium for bacteria, especially for *Bacteroides* spp. and *Clostridium* perfringens that causes the gas gangrene so feared in military medical practice. Though a wound may not necessarily

be infected, the bacteria can cause inflammation and strain the body's ability to fight infection. Debridement is also used to treat pockets of pus called abscesses. Abscesses can develop into a general infection that may invade the bloodstream (sepsis) and lead to amputation and even death. Burned tissue or tissue exposed to corrosive substances tends to form a hard black crust, called an eschar, while deeper tissue remains moist and white, yellow and soft, or flimsy and inflamed. Eschars may also require debridement to promote healing. The most common types of debridement are sharp, mechanical, chemical, and autolytic (Falanga and Harding, 2002).

Sharp debridement. Sharp debridement refers to the use of forceps, scissors, or a scalpel to remove devitalized tissue, debris or other foreign materials from a wound bed (Ayello et al., 2008). After surgical debridement, sharp debridement is the most aggressive form of debridement available to clinicians, and also the most rapid. Vigorous and repeated sharp debridement of necrotic tissue and debris is considered the standard of care for many patients with open wounds. The technique is indicated in wounds presenting with significant necrosis, callus, advancing cellulitis or sepsis, or thick adherent eschar. Chronic wounds tend to require repeated debridement because of the likely re-emergence of necrotic tissue and bio burden in the wound bed. Sharp debridement has a number of contraindications, and should not be used when area cannot be adequately visualized or when the material to be debrided is unidentified. The technique should be used with caution in patients who are immunosuppressed, thrombocytopenic, or receiving anticoagulant therapy. As sharp debridement can be stressful for the patient, the procedure should be halted if the patient experiences uncontrolled pain or if there is extensive bleeding. Importantly, the procedure should not proceed if the clinician fatigues, and should only be undertaken if sufficient time is available to both clinician and patient (Myers, 2008).

Mechanical debridement. Mechanical debridement is more traumatic, and unfortunately it is probably the most commonly used method in equine. It can be performed using woven gauze, lavage, wet-to-wet, or wet-to-dry dressings. Mechanical debridement can be a useful tool, and it can be beneficial in the healing process, but it can be very traumatic. Often, when using gauze, too much pressure is applied and trauma to the wound bed ensues. Only gentle pressure should be used. If more aggressive debridement is necessary, sharp debridement should be performed. Woven gauze provides mechanical debridement characteristics superior to those of nonwoven gauze. Lavage can be used successfully for mechanical debridement. It involves two critical components, the selection of a non-cytotoxic cleansing solution and the delivery of that solution to the wound surface with appropriate pressure and volume to wash away the necrotic debris without pushing it further into the tissue planes of the wound (Ovington, 2001). The pressure should be between 10 and 15 pounds per square inch (psi). One way to achieve this pressure is to attach a 19-gauge needle or catheter to a 35-ml syringe (Rodeheaver, 2001). Many devices are available. Showerheads are safer and more effective than single-jet lavage systems. Therefore, devices that produce high pressures (e.g., the Water Pik) should not be used. A simple, gentle, low-pressure lavage system can be made by using a 16-gauge needle to punch four to eight holes in the cap of a 1-L bottle and then squeezing saline out. The fluids most commonly used in veterinary medicine are dilute antiseptics. Saline has been shown to be effective in reducing bacterial counts in an infected wound but to have no effect on wound healing in clean wounds (Hollander et al., 1998). In one study comparing saline and water in an infected wound in a rat model, saline reduced the bacterial counts by 81.6% whereas tap water reduced bacterial counts by 82% (Muscati et al., 1998). However, work by Buffa and coworkers suggests that tap water is very toxic to fibroblasts. When exudate is present, surfactant-based wound cleansers are more effective than saline or poly ionic fluids (Rodeheaver, 2001).

Chemical debridement. Chemical debridement has been used in many different forms in medicine. Dakin's solution, a diluted sodium hypochlorite (bleach) solution, was originally used during World War I. Other chemical agents are hydrogen peroxide, acetic acid, and, more recently, hypertonic saline. Chemical debridement is nonselective and should be reserved for very contaminated wounds. However, although hypertonic saline is good in selected cases, sharp debridement is probably a better technique for removing large amounts of necrotic debris. Hypertonic dressings provide an effective chemical debridement with minimal damage to the wound in the early stages of wound healing (Auer and Stick, 2006).

Autolytic debridement. Autolytic debridement takes advantage of the body's own ability to dissolve dead tissue. The key to the technique is keeping the wound moist, which can be accomplished with a variety of dressings. These dressings help to trap wound fluid that contains growth factors, enzymes, and immune cells that promote wound healing. Autolytic debridement is more selective than any other debridement method, but it also takes the longest to work. It is inappropriate for wounds that have become infected. Autolytic debridement is the least traumatic of these techniques which is achieved by leaving wound fluid (containing white blood cells and enzymes released from dead white blood cells) in contact with the wound bed. The white blood cells and enzymes affect only the dead and necrotic tissue, leaving healthy cells intact for wound healing. This can occur only in moist wounds. When wounds are allowed to dry, autolytic debridement is slowed significantly. Autolytic debridement reduces the bacterial count by allowing access of white blood cells to the wound bed without causing continued trauma to the wound bed. However, sharp debridement should be performed before using autolytic debridement, as the latter technique is ineffective in the presence of large volumes of necrotic material (Harper and Michael, 2001).

1) Non antimicrobial dressings

Collagens, Maltodextrins. Topical dressings such as collagens and maltodextrins are designed for use in the granulating stage of wound repair. They are both hydrophilic and should maintain a moist wound bed. They are available in powder or gel form. If the wound does not have a lot of exudate, it is probably best to use the gel form to prevent drying out of the wound bed. Maltodextrins may provide nutrition to the wound bed (Purna and Babu, 2000).

Biologic Dressings. Various substances have been used recently as biologic dressings. These dressings are intended to provide a framework over which other cells migrate, and as a stimulant to those cells to form the tissue that is desired. Some of the more commonly used biologic dressings include porcine small intestinal sub mucosa (SIS), porcine bladder basement membrane, equine amnion, and various skin products. The porcine small intestinal sub mucosa and bladder products are not rejected by the host as other xenografts. From clinical experience, they appear to be best if they are kept moist. In a recent study, SIS dressing was applied to fresh and chronic wounds. Compared with similar wounds treated without SIS, exuberant granulation tissue was reduced and drainage was facilitated, which reduced wound exudation and improved epithelialization. Overall, wound healing was faster and costs for bandages and hospitalization were reduced (Dressel et al., 2004). Other studies have shown that the use of equine amnion reduces wound retraction and granulation tissue formation while improving epithelialization. Amnion has been shown to be beneficial as a non-adherent dressing in skin grafting (Goodrich et al., 2000).

Gauze. The use of gauze to dress and bandage wounds has its origins in ancient times, having been firmly established by the fifth century and is still in use today. The term 'gauze' represents two types of bandaging material: woven gauze is the 100% natural cotton cloth that we are most familiar with. Non-woven gauze refers to more modern, synthetic dressings made of rayon or synthetic fiber blends (Jones, 2006). Woven gauze is problematic in dressing and packing wounds as it sheds fibers when cut and may leave debris in the wound bed when removed. It is also absorbent and tends to stick to the wound, resulting in trauma upon removal as it quickly dries out the wound, becoming trapped within the eschar. Until recently, this was considered advantageous as a dry wound was considered optimal for healing and the removal of the embedded dry eschar was seen as a form of physical debridement. The perception that a wound was best healed under dry conditions persisted from the times of Hippocrates until relatively recent research promoted the maintenance of a moist wound environment (Eglasein, 2001). Gauze strips soaked in antibiotics such as EUSOL, proflavin or chlorhexidine were used to pack wounds to prevent closure and promote granulation from the wound base. This was subsequently changed to saline-soaked gauze because the use of antibiotics in this fashion was thought to be potentially cytotoxic. Saline was employed as a hypertonic solution but quickly dried out, resulting in painful removal (Queen and Steed, 2004). Today, woven gauze is seen as a 'wet to dry' dressing and utilized in a range of wound care strategies. Despite its non-selective mode of physical debridement, trauma to the wound bed and resultant pain, it is still the most utilized wound dressing in the world (Steed, 2004). It is also utilized as a vehicle for antimicrobial agents but presents complications such as degradation or inactivation of the antimicrobial agent upon exposure to the high protein levels within wound fluid and lateral bacterial migration into the wound bed within the moist environment. Factors such as cost, education and the ability to follow best practice are thought to determine selection of wound dressings and may account for continued use of this ancient product, seemingly surpassed by modern dressings (Boateng et al., 2001). Although gauze is commonly used, a range of more appropriate dressings has been available for a number of years. These dressings employ many technological advancements and exhibit qualities considered to be essential in the 'perfect' wound dressing such as: the ability to maintain a moist wound environment while preventing maceration of surrounding tissue; non-adherent and a traumatic; thermally insulating and gas-permeable; antibiotic and non-toxic (Martineau and Shek, 2006).



Figure 1. Bandaging

Hydrocolloids: Hydrocolloid dressings, first developed in 1982, consist of an inner layer of hydrophilic gel made from gelatin, pectin, sodium carboxy methylcellulose and poly isobutylene, backed by a film, forming a flexible wafer dressing (Dumville et al., 2011). These occlusive dressings have been shown to provide a moist, hypoxic wound environment which promotes autolytic debridement. They also allow gas exchange and are semipermeable to vapor but may cause maceration if used on heavily exuding wounds, although it's been suggested that the application of a hydrocolloid dressing may reduce the amount of exudate produced by a wound. Various specific types of hydrocolloid dressings have come to market, but while they differ in size, shape, exudate absorption and intended use, their basic mode of action remains the same (Davies and Rippon, 2006).

Hydrogels: Hydrogels consist of cross-linked polymers such as starch, cellulose or other plant- or animal-derived polysaccharides and contain up to 96% water (Juris et al., 2011). They can provide moisture to dry wounds as well as absorb excess exudate, depending on moisture levels at the wound and are a traumatic when used correctly (Boateng et al., 2001). Hydrogels also facilitate the autolysis of necrotic tissue, and do not support bacterial growth but their use is limited to dry and low exuding wounds as they can cause maceration to surrounding tissues when higher volumes of wound exudate are present (Dumville et al., 2011). There is also a risk that when used on exuding ischaemic ulcers their use can result in a shift from dry to wet gangrene within the wound. Interestingly, one group has recently reported some active antimicrobial and antifungal properties in a hydrogel dressing based on polyvinyl pyrrolidone/polyethylene glycol polymers (Biazar et al., 2012). Hydrogels are available in a variety of formats such as sheets, gels and beads which enable a tailored application to individual wounds. Hydrogels are utilized for the treatment of burns, chronic ulcers, and surgical wounds and even injected into the spinal column (Macaya and Spector, 2012).

Foams. Polyurethane foam dressings are easy to use and customize as they can be cut to shape and come in a range of absorbencies. They are designed to absorb excess wound exudate while maintaining a moist wound interface and providing thermal insulation. They also prevent maceration of surrounding healthy tissue and facilitate the removal of slough (Varma et al., 2008). A range of foam dressings exists with some incorporating other components to enhance absorbance, control infection or ensure a traumatic removal. These impregnated foams are used for a wide range of applications in both acute and chronic wounds, including post-surgical dressing, application on heavily exuding wounds or for packing deep cavity wounds (Dinah and Adhikari, 2006).

Silicone dressings. Silicone is used either as a contact dressing or as the contact layer within a dressing, for example, Mepilex, a polyurethane foam membrane coated with a soft silicone layer (White, 2005). It is also used as a coating on materials like non-woven polyester nets. In negative pressure therapy, silicone-coated polyester enhances healing rates in sheep models and may assist in the prevention of hypertrophic scarring when combined with pressure therapy. Dressings incorporating soft silicone contact layers adhere to dry skin while remaining non-adherent to the wound site, resulting in a traumatic removal and a decreased risk of damage to the wound site upon dressing changes. Silicone can be used on a range of acute and chronic wounds as it is incorporated in many different bandaging strategies (Losi et al., 2012).

Capillary action dressings. Capillary action dressings incorporate an absorbent pad of hydrophilic fibers, typically comprising 80% polyester, 20% cotton fibers between two layers of perforated, permeable, non-woven polyester. Exudate is removed from the wound by capillary action and the excess is spread laterally through the absorbent pad along a capillary pressure gradient, preventing tissue maceration. These dressings decrease bacterial load on the wound surface, assist in debridement and desloughing, but may adhere to wounds with low levels of exudate, resulting in traumatic removal. They are best used in conjunction with a non-adherent contact layer and are not recommended for arterial or heavily bleeding wounds (Deeth et al., 2012).

Odour-absorbent dressings. Odour in a wound is primarily produced from anaerobic bacteria, and whilst the initial line of management should be infection control, it is often advantageous to incorporate an odour-absorbing dressing into the treatment protocol. These dressings use charcoal or activated carbon to absorb odour from the wound and are often used in conjunction with absorbent secondary dressings. The odour-causing molecules are retained by the carbon and charcoal is shown to retain bacteria; when incorporated with antimicrobial agents like silver, antibacterial activity is attained. Efficacy in retaining odour and absorbing wound exudate varies considerably across products (Thomas et al., 2012).

2) Antibacterial dressings

Honey dressings. The use of honey as an antibacterial is well established in modern wound care, with medical-grade honey used in a variety of commercially available dressings. These dressings provide antimicrobial and anti-inflammatory properties through autolytic debridement and maintenance of a moist wound environment while inhibiting bacterial growth, stimulating wound healing and deodorizing the wound, although research trends are mixed in regard to their overall efficacy (Molan, 2006). Honey is bactericidal and antifungal against approximately (Zhong et al., 2010) bacterial strains, both gram-positive and gram-negative, and some yeasts and is often used to control bacterial strains resistant to conventional antibiotics (Cooper, 2008). Antimicrobial action is both mechanical

and enzymatic. Like sugar pastes, honey can inhibit bacterial growth through its osmolality, where the high concentration of sugars causes water to be drawn from the local wound environment. This also maintains a moist wound environment by stimulating fluid transfer from surrounding tissues. Whilst this action dilutes the honey, its antibacterial effects remain (Kwankman, 2012). Honey is applied topically to a wide range of wounds in the form of an ointment, for packing cavities, or impregnated within a hydrogel or alginate dressing. When used as an ointment, the honey will rapidly dilute due to absorption of wound exudate as well as increase in fluidity upon warming to body temperature and may, therefore, require frequent dressing changes in order to maintain efficacy (Molan, 2001).

Iodine dressings. Iodine, a natural halogen, is an antiseptic and available in a range of topical applications. Like all antiseptics, it targets a broad spectrum of bacteria and other pathogens such as fungi, viruses, protozoa and prions through non-specific action (Sibbald et al., 2011). Iodine has been used to prevent and treat infection since the fourth century BCE and debate over its use remains. Although antiseptic use has declined due to the rise of resistant bacterial strains, modern preparations of iodine in managing infection are being explored, but results are conflicting and general consensus remains to be reached (Angel et al., 2008). Iodophors, one such modern formulation, were developed in the 1950s by complexing elemental iodine to a surfactant to improve solubility and reduce cytotoxicity effects. Elemental iodine is cytotoxic against fibroblasts, keratinocytes and leukocytes, thus impeding wound healing. The use of iodophors in modern wound dressings ensures release of lower concentrations of free iodine into the wound exudate. The most widely utilized formulations are povidone-iodine and cadexomer-iodine (Chaikof et al., 2002). The former, while being the most commonly utilized form of iodine in the clinical setting, is not recommended for long-term use or for complex wounds. Indeed, the use of current formulations of povidone-iodine is still contentious. Previous research has shown that clinical concentrations of as little as 1% are cytotoxic to granulocytes and monocytes in vitro and systemic iodine toxicity can occur with povidone-iodine dressings, which typically contain concentrations of 7.5% (Burks, 1998). Studies exploring cadexomer-iodine formulations as a topical application found them to be effective in controlling bacterial load. Subsequent studies in humans and porcine models showed an acceleration of epidermal migration and re-epithelialisation, through upregulation of cytokines like vascular endothelial growth factor (VEGF) (Ohtani et al., 2007). Cadexomeriodine was also found to positively affect healing rates in chronic wounds. Cadexomer-iodine formulations are available as ointments, powders or dressings (hydrogels, ointments gauze, knitted viscose, beads and paste) (Flores and Kingsley, 2007).

Silver dressings. Although the antibacterial action of silver is well established, with silver dressings used in a wide range of infected wounds, their potential cytotoxicity remains an issue (Kim et al., 2012). Ionic, metallic and nanocrystalline forms of silver have been employed as foams, hydrofibres and hydrocolloids. The amount of free silver available to action upon the wound varies from product to product, which impacts upon the effectiveness of the dressing (Jude et al., 2007). Silver ions act upon bacteria by binding and disrupting proteins and nucleic acids through interaction with their negatively charged groups such as thiol groups, carboxylates, phosphates, hydroxyls, imidazoles, indoles and amines as well as stimulating the generation of reactive oxygen species. As a result, cellular changes rapidly occur through a number of mechanisms that result in loss of viability. Investigations have begun only recently on the systemic toxicity of silver nanoparticles. Asharani et al., 2009 investigated the cytotoxicity of silver nanoparticles on human glioblastoma and lung fibroblast cells in vitro. The nanoparticles were found to penetrate into mitochondria and nuclei, interrupting ATP synthesis and resulting in DNA damage. Others have noted the cytotoxic effects of silver nanoparticles on keratinocytes in vitro (Zanette et al., 2011).

Other antibacterial dressings. Antibacterial agents such as chlorhexidine have been incorporated into a wide range of commercially available dressings and washes (Aramwit et al., 2010). Chlorhexidine has been utilized in infection control since the 1950s but has been mostly limited to irrigation and wound cleansing protocols with limited evidence to support efficacy (Cooper, 2004). Chitosan acetate is currently used as a haemostatic dressing in the form of a bandage, but some investigations into its antimicrobial action have shown it can prevent fatal systemic sepsis and control the growth of *Pseudomonas aeruginosa* and *Pseudomonas mirabilis* (Dutta et al., 2011).

WOUND CLOSURE TECHNIQUES

After the wound has been successfully débrided, cleaned, and examined, it needs to be closed. The options are suture closure, healing by second intention, skin grafting, or some combination of these to provide a continuous epithelial surface over the wound. The type of closure technique to use depends on what caused the wound, the time from injury, the degree of contamination, the extent of the injury, and potential dead space. That there is a “golden period” of 6 hours from the wounding, after which the wound is considered to be infected, is no longer deemed correct, and it behooves the clinician to examine the wound carefully to determine which of the following techniques to use for wound closure.

1) Primary closure

Primary closure is a technique whereby the wound is closed immediately and completely, using strict aseptic technique. This is the technique most likely to provide the best cosmetic result. Unfortunately, primary closure is

acceptable only in wounds with minimal tissue loss, minimal bacterial contamination, and minimal tension on the wound edges after closure. Regardless of whether primary closure or delayed primary closure is performed, the wound needs to be cleaned and prepared for closure, because excess bacteria in the wound increase the possibility of wound dehiscence. There are many suturing techniques for wound closure and many reviews of suture types for primary closure (Lott-Crumpler and Chaudhry, 2001). For areas of tension, complex suture patterns such as the near-far-far-near, vertical mattress, and horizontal mattress patterns provide more tension reduction than simple patterns such as a simple interrupted or simple continuous pattern. The near-far-far-near suture pattern is most satisfactory, as it provides apposition of the skin edge at the same time as tension relief. Additionally, the mattress patterns can be used with stents to reduce pull-through at the skin-suture interface. A large-diameter suture material should be used if tension exists. One or a combination of four techniques can be used to manage dead space in a wound: suture, meshing, passive or active drains, and pressure bandages. Each technique has benefits and risks that need to be weighed. Suture material, although very useful in wound closure, can also act as a foreign body. Excess suture use (too much suture, too large a diameter, or too many knots) can potentiate infection. Consequently, the clinician should use the smallest-diameter suture material possible, use monofilament, absorbable suture material, and use only surgeon's knots (or interrupted sutures if absolutely necessary when the suture material must be buried). Good surgical technique also benefits the patient, as it reduces trauma to the wound site. If dead space cannot be managed by suture placement, meshing or suction drains (passive or closed) should be employed. However, drains not only allow evacuation of dead space but can also act as a conduit for bacteria to enter the wound. Drains should be left in place only as long as necessary to reduce the possibility of infection. Pressure bandages can and should be used whenever possible in addition to sutures, meshing, or drains, or sometime in place of them to reduce dead space. A properly applied bandage closes dead space without adding any foreign material. However, if a bandage is too tight, the blood supply to the wound may be compromised, leading to wound failure.

2) Delayed primary closure

In a delayed primary closure, the wound is initially treated as an open wound to allow debridement and reduce bacterial contamination. Next, the wound is closed primarily. In some cases, only a portion of the wound can be completely closed. Delayed primary closure is reserved for wounds that have mild to moderate bacterial contamination, minimal tissue loss, and minimal tension on the wound edges after closure. Drains may be placed to evacuate fluid after closure. Delayed wound closure is very useful in the management of abdominal incisions after colonic rupture (Bender, 2003). These studies showed as much as a twofold increase in incisional infection with primary closure of contaminated abdominal wounds. Delayed primary closure after 3 to 5 days did not result in an increased hospital stay. Wounds destined for delayed primary closure should be debrided and cleaned to reduce the bacterial burden. Hypertonic saline dressings, topical antimicrobials, and systemic antimicrobials can be very useful in treating wounds prior to closure.

3) Second-intention healing

Second-intention healing occurs when primary or delayed primary closure cannot be accomplished. In most cases, these wounds have gross contamination and moderate to severe tissue loss that would make closure difficult. They must heal completely through the process of contraction, granulation, and epithelialization (Hohn, 1977).

PHASES OF WOUND REPAIR

1) Acute inflammation

Inflammation prepares the wound for the subsequent reparative phases. It encompasses vascular and cellular responses whose intensity is strongly correlated to the severity of trauma. The injured endothelial cell membrane releases phospholipids that are transformed into arachidonic acid and its metabolites, which mediate vascular tone and permeability as well as platelet aggregation. The first response of the damaged blood vessel is vasoconstriction, lasting 5 to 10 minutes, after which vasodilation ensues and promotes diapedesis of cells, fluid, and protein across the vessel wall into the extravascular space. Coagulated blood and aggregated platelets together form a clot within the defect that, despite providing limited strength to the wound, seals off the injury and prevents further bleeding. The clot also functions as a scaffold through the presence of a large number of binding sites on blood proteins that are recognized by special surface receptors found on migratory inflammatory and mesenchymal cells. Activated platelets are among the earliest promoters of inflammation, via the release of potent chemo attractants and mitogens from their storage granules. These serve as signals to initiate and amplify the reparative phases of healing and are detailed later. Over time, the surface clot desiccates to form a scab that protects the wound from infection. This scab is in turn lysed by plasmin and sloughs along with dead inflammatory cells and bacteria as healing proceeds underneath. The provisional extracellular matrix (ECM) will be replaced by granulation tissue in the next phase of repair. Leukocytes are recruited from the circulating blood pool to the site of injury by the numerous vasoactive mediators and chemo attractants supplied by the coagulation and activated complement pathways, by platelets, by

mast cells, and by injured or activated mesenchymal cells (Singer and Clark, 1999). These signals initiate the processes of rolling, activation, tight adhesion, and finally transmigration of inflammatory cells through the microvascular endothelium.

Chemo attractants additionally stimulate the release of enzymes by the activated neutrophils; these enzymes facilitate the penetration of the inflammatory cells as they migrate through vascular basement membranes. Cellular influx begins early, and neutrophil numbers progressively increase to reach a peak 1 to 2 days after the injury. The neutrophils act as a first line of defense in contaminated wounds by destroying debris and bacteria through phagocytosis and subsequent enzymatic and oxygen-radical mechanisms. The principal degradative proteinases released by the neutrophils to rid the site of denatured ECM components are neutrophil-specific interstitial collagenase, neutrophil elastase, and cathepsin G. Neutrophil migration and phagocytosis cease when contaminating particles are cleared from the site of injury. Most cells then become entrapped within the clot, which is sloughed during later phases of repair. The neutrophils remaining within viable tissue die in a few days and are phagocytized by the tissue macrophages or the modified wound fibroblasts. This marks the termination of the early inflammatory phase of repair. Although the neutrophils help create a favorable wound environment and serve as a source of pro-inflammatory cytokines, they are not essential to repair in uninfected wounds (Simpson and Ross, 2009).

The rapid increase in macrophage numbers under inflammatory conditions is predominantly caused by the emigration of monocytes from the vasculature, which then differentiates into macrophages to assist resident tissue macrophages at the wound site for a period lasting from days to weeks. In this manner, the responsive and adaptable pluripotent monocytes can differentiate into macrophages, whose functional properties are determined by the conditions they encounter at the site of mobilization. Like the neutrophils, the macrophages are phagocytes and thus carry out débridement and microbial killing. Unlike the neutrophils, the wound macrophages play a key role in the reparative phases of healing. Indeed, adherence to the ECM (which consists of a cross-linked supporting framework of collagen fibrils and elastin fibers, which is saturated with proteoglycans and other glycoproteins) stimulates monocytes to transform into phenotypes that have the ability to continually synthesize and express the various cytokines necessary for their survival, as well as for the initiation and propagation of new tissue formation in wounds. On arrival at the site of inflammation, macrophages participate in bacterial killing via mechanisms that parallel those of the neutrophils. Three inducible, secreted, neutral proteinases have been identified in macrophages: elastase, collagenase, and plasminogen activator (PA). These proteinases aid in degradation of damaged tissue and debris, which must be cleared before repair can proceed. Despite the new data gleaned from the study on mice without macrophages (Martinet *et al.*, 2003) acute inflammation is still considered crucial to the normal outcome of wound repair. Indeed, macrophages are regarded as the major inflammatory cell responsible not only for debridement but also for recruitment of other inflammatory and mesenchymal cells, and for subsequent induction of angiogenesis, fibroplasia, and epithelialization. Thus, a general approach for improving wound repair may be to recruit or possibly activate monocytes. For example, it has recently been shown that priming a planned incision site with recombinant pro-inflammatory cytokines nearly doubles the breaking strength of an acute wound (Smith *et al.*, 2000). Likewise, honey and sugar applied to open wounds have been shown to enhance fibroplasia and epithelialization, possibly via their chemo attractant and stimulatory activity on the tissue macrophages (Molan, 1999) and (Swaim and Bohling, 2003). Extensive scarring or fibrosis of any organ may cause catastrophic loss of function of that organ. In the horse, a comparable condition is the development of exuberant granulation tissue in skin wounds. Wilmink and colleagues believe this is related to a deficient but protracted inflammatory response in the horse when compared with ponies, especially when wounds are located at the distal aspect of the limb. They found that the number of poly-morpho-nuclear leukocytes (PMNs) was high in ponies during the first 3 weeks after experimental full- thickness wounding, but it subsequently decreased rapidly, whereas in the horse the initial number of PMNs was lower, but it remained persistently elevated during the entire 6- week study (Wilmink *et al.*, 1999). Furthermore, peripheral blood leukocytes from ponies produce more reactive oxygen species essential to bacterial killing than do those of horses (Wilmink *et al.*, 2003), which corresponds to the more pronounced initial inflammatory response and to the better local defense against wound infection clinically apparent in the pony. A handful of equine studies have been undertaken with the intent of encouraging a powerful yet brief acute inflammatory response and thus limiting the subsequent fibrosis that appears in response to injury to the distal portion of the limb in horses. Wilson and colleagues found that although an activated macrophage supernate effectively restrained proliferation of equine fibroblasts *in vitro*, no significant *in vivo* effects were found on distal limb wounds. Another study found that a protein-free dialysate of calf blood (Solcoseryl, Solco Basle Ltd., Birsfelden, Switzerland) provoked a greater inflammatory response, with faster formation and contraction of granulation tissue within deep wounds (Wilmink *et al.*, 2000). Subsequently, it inhibited repair by causing protracted inflammation and delaying epithelialization. Finally, a field study was recently performed to determine the efficacy of Vulketan gel (Janssen Animal Health, Beerse, Belgium) in preventing exuberant formation of granulation tissue in equine lower limb wounds (Engelen *et al.*, 2004). The active ingredient appears to antagonize serotonin-induced suppression of wound macrophages, thus allowing a strong, effective inflammatory response to occur.

2) Cellular proliferation

Fibroplasia. The proliferative phase of repair comes about as inflammation subsides and is characterized by the eventual appearance of red, fleshy granulation tissue, which ultimately fills the defect. Although the earliest part of this phase is very active at the cellular level, this does not immediately translate into a gain in wound strength. Indeed, during the first 3 to 5 days after injury, mesenchymal cells such as fibroblasts and endothelial and epithelial cells are rapidly invading the wound in preparation for matrix synthesis and maturation; however, these latter reinforcing mechanisms lag somewhat. Granulation tissue is formed by three elements that move into the wound space simultaneously: macrophages debride and produce cytokines and growth factors, which stimulate angiogenesis and fibroplasia; fibroblasts proliferate and synthesize new ECM components; and new blood vessels carry oxygen and nutrients necessary for the metabolism and growth of mesenchymal cells, and confer to the granulation tissue (Singer and Clark, 1999). This stroma of which fibronectin and hyaluronan are major components, replaces the fibrin containing clot to provide a physical barrier to infection and, importantly, to proffer a surface across which mesenchymal cells can then migrate. A number of matrix molecules, as well as chemo attractants, cytokines, and growth factors released by inflammatory cells, are believed to stimulate fibroblasts from adjacent uninjured skin to proliferate and express integrin receptors to assist migration into the wound space. Integrins are trans membrane proteins that act as the major cell-surface receptors for ECM molecules and thus mediate interactions and transduce signals between cells and their environment. They are particularly critical to the migratory movements exhibited by wound-healing cells. Migration immediately precedes advancing capillary endothelial buds but follows macrophages, which have cleared a path by phagocytizing debris. Fibroblasts themselves also possess an active proteolytic system to aid migration into the cross-linked fibrin blood clot; proteinases include PA, various collagenases, gelatinase, and stromelysin. Once fibroblasts have arrived within the wound space, they proliferate and then switch their major function to protein synthesis and commence the gradual replacement of provisional matrix by a collagenous one, probably under the influence of various cytokines and growth factors. As the wound matures, there is a marked increase in the ratio of type I (mature) to type III (immature) collagen; proteoglycans also become abundant within the mature matrix. The greatest rate of connective tissue accumulation within the wound occurs 7 to 14 days after injury, and thus this is the period with most rapid gain in tensile strength. Thereafter, collagen content levels off as fibroblasts retract their synthetic machinery; this corresponds to a much slower gain in wound strength, which occurs as the wound remodels. The fibroblast-rich granulation tissue is then replaced by a relatively avascular and acellular scar as the capillary content regresses and fibroblasts either undergo apoptosis or acquire smooth-muscle characteristics and transform into myofibroblasts that participate in wound contraction. The latter phenomena are regulated by the physiologic needs and/or the micro-environmental stimuli present at the wound site. It appears that if the signal to down regulate fibroblast activity is delayed beyond a specific time point, apoptosis is permanently impaired, which ultimately leads to an imbalance between collagen synthesis and degradation and the formation of excessive scar tissue (Luo et al., 2001). Undeniably, repair of full-thickness wounds is subject to excessive formation of granulation tissue, with subsequent delays in epithelialization and contraction, especially when wounds are located at the distal aspect of the limb. Surprisingly, in vitro fibroblast growth from tissues isolated from the horse limb is significantly less rapid than growth of fibroblasts from the horse trunk (Bacon et al., 2000). In vivo, an elevated and persistent mitotic activity exists in distal metatarsal wounds of horses, compared with the activity present in wounds healing normally on the hindquarters (Leault et al., 2004). In a recent study in wounds of the distal limbs of horses, we found that the silicone dressing surpassed a conventional permeable, non-adherent dressing for preventing the formation of exuberant granulation tissue and improving tissue quality (Ducharme-Desjarlais et al., 2005)

Angiogenesis. Besides initiating the inflammatory response through interaction with leukocytes, microvascular endothelial cells play a key role in the proliferative phase of repair. The formation of new capillary blood vessels from preexisting ones (angiogenesis) is necessary to sustain the granulation tissue newly formed within the wound bed. Angiogenesis, in response to tissue injury and hypoxia, is a complex and dynamic process mediated by diverse soluble factors from both serum and the surrounding ECM environment in particular, angiogenic inducers including growth factors, chemokines, angiogenic enzymes, endothelial cell-specific receptors, and adhesion molecules (Liekenset al., 2003), many of which are released during the previous inflammatory phase of repair. Construction of a vascular network requires sequential steps that include augmented microvascular permeability, the release of proteinases from activated endothelial cells with subsequent local degradation of the basement membrane surrounding the existing vessel, migration and sprouting of endothelial cells into the interstitial space, endothelial cell proliferation and formation of granulation tissue, differentiation into mature blood vessels, and stabilization, eventually followed by regression and involution of the newly formed vasculature as the tissue remodels (Li et al., 2003). Angiogenic stimuli are down regulated or the local concentration of inhibitors increases and most of the recently formed capillary network quickly involutes through the activity of matrix metalloproteinases (MMPs) (Zhu et al., 2000) and apoptosis of endothelial cells. The wound color becomes correspondingly paler as the rich capillary bed disappears from the granulation tissue. Exuberant granulation tissue that develops in wounds of the lower limbs of horses is characterized

microscopically by a great number of microvessels. Although the reason angiogenesis is more prominent in this location remains obscure, it is tempting to speculate that the regional paucity of blood supply may impart an effect via up regulation of various angiogenic factors. Indeed, hypoxia is known to stimulate proliferation and synthetic activity of fibroblasts. In support of this hypothesis, we have recently shown that although a greater number of microvessels are microscopically apparent within the granulation tissue of limb wounds in equines, their lumens are occluded significantly more often than the lumens of microvessels within thoracic wounds, which may corroborate the existence of a hypoxic environment in wounds of the lower limb. Thus, via up regulation of various angiogenic factors, hypoxia may lead to excessive fibrosis. Alternatively, deficient apoptotic signals may lead to persistence of micro capillary endothelial cells and subsequent angiogenic activity.

Epithelialization. All body surfaces are covered by epithelium, which acts as a selective barrier to the environment. Epithelium provides the primary defense against hostile surroundings and is a major factor in maintaining internal homeostasis by limiting fluid and electrolyte loss. The outer region of skin, a multilayered stratified squamous epithelium (the epidermis), interfaces with the musculoskeletal framework by means of a connective tissue layer (the dermis) and a fibrofatty layer (the subcutis). Epidermis is attached to the dermis at the level of the basement membrane, a thin, glycoprotein-rich layer composed primarily of laminin and type IV collagen. This attachment is mediated by hemidesmosomes, which physically attach the basal cells of the epidermis to the underlying dermis, as well as by vertically oriented type VII collagen anchoring fibrils, which bind the cytoskeleton. Although epithelial migration commences 24 to 48 hours after wounding, the characteristic pink rim of new epithelium is not macroscopically visible until 4 to 6 days later, although this is variable because the rate of wound closure depends on the animal species as well as on the wound site, substrate, and size. For example, epithelialization is accelerated in a partial thickness wound, because migrating cells arise not only from the residual epithelium at the wound periphery but also from remaining epidermal appendages. Furthermore, the basement membrane is intact in this type of injury, precluding its lengthy regeneration. On the other hand, during second-intention healing of a full-thickness wound, epithelialization must await the formation of a bed of granulation tissue to proceed. Wounds in the flank area of a horse epithelialize at a rate of 0.2 mm per day, compared with a rate as slow as 0.09 mm per day for wounds in the distal portion of limbs. In preparation for migration; basal epidermal cells at the wound margin undergo phenotypic alterations that favor mobility and phagocytic activity. Additionally, various degradative enzymes necessary for the proteolysis of ECM components are up regulated within cells at the leading edge, facilitating ingestion of the clot and debris found along the migratory route. The migratory route is determined by the array of integrin receptors expressed on the surface of migrating epithelial cells, for various ECM proteins. Indeed, a fundamental reason why migrating epidermis dissects the fibrin eschar from wounds is that normal epithelial cells cannot interact with the fibrinogen and its derivatives found within the clot because they lack the appropriate integrin. Once the wound surface is covered by epithelial cells that contact one another, further migration from the margin of the wound inward is inhibited by the expression within the ECM of laminin, a major cell adhesion factor for epithelial cells. Although initial migration does not require an increase in cellular multiplication, epidermal cells at the wound margin do begin to proliferate 1 to 2 days after injury to replenish the migratory front. This corresponds histologically to epithelial hyperplasia, as cellular mitosis increases 17-fold within 48 to 72 hours. The new cells leapfrog over those at the wound margin to adhere to the substratum, only to be replaced in turn by other cells coming from above and behind. The newly adherent monolayer subsequently restratifies in an attempt to restore the original multilayered epidermis. In full-thickness wounds healing by second intention, such as those commonly managed in equine practice, provisional matrix is eventually replaced by a mature basement membrane zone. Repairing epidermis reassembles its constituents from the margin of the wound inward, in a zipper like fashion. Epidermal cells then revert to a quiescent phenotype and become attached to this new basement membrane through hemidesmosomes and to the underlying dermis through type VII collagen fibrils. This particular aspect of epithelialization is time consuming, occurring long after total wound coverage is apparent, which may explain the continued fragility of neopidermis for extended periods after macroscopically complete repair. This is particularly evident in large wounds of the limb, where epidermis at the center is often thin and easily traumatized. Wounds in horses commonly fail to epithelialize altogether. This occurs in two distinct types of wounds: those in which fibroplasia are excessive and those of an indolent nature. In the former, protruding granulation tissue may act as a physical impediment to epithelial migration and it may inhibit epithelial cell mitosis. The relative absence of epithelial cells could in turn lead to persistent synthesis of fibrogenic growth factors by fibroblasts (Lepoole and Boyce, 1999) and defective apoptosis signaling, thus establishing a vicious cycle culminating in proud flesh formation. Conversely, indolent wounds possess a granulation bed of deficient quantity and quality, thus hindering migratory efforts by epithelial cells. In this case, it is critical to encourage the formation of a healthy granulation bed. Although hydrogel dressings have been advocated for this purpose, a recent study is not supportive (Dart et al., 2002). In the case of limb wounds presenting delays in epithelialization but possessing a healthy bed of granulation tissue, the value of skin grafting is undisputed. Grafting exerts a significant inhibitory effect on both endothelial cell and fibroblast growth

while enhancing proliferation and migration of epithelial cells. It is, however, critical that the graft be obtained from a site that normally heals well and in which contraction is a prominent feature (e.g., from the lateral cervical, abdominal, or pectoral regions). The inhibitory effect of grafts on fibroblast proliferation and collagen synthesis may be regulated by a soluble epithelial cell-derived product (Lepoole and Boyce, 1999) possibly a cytokine or a growth factor such as epidermal growth factor (EGF), which enhances epithelialization via positive effects on epithelial cell migration, proliferation, and differentiation (Burling et al., 2000). To encourage ingrowth of mesenchymal cells in indolent wounds during the proliferative phase, biomaterials such as collagen membranes and sponges have been developed and are appraised as improving rate and quality of repair. Collagen may function as a substrate for hemostasis; as a template for cellular attachment, migration, and proliferation; and as a scaffold for more rapid transition to mature collagen. A porous bovine collagen membrane was shown to generate a strong inflammatory response in full-thickness limb wounds of equines, which may augment the cytokine or growth factor content of wound tissues, although it did not significantly alter the total wound, or the epithelialization or contraction process. A commercially available collagen matrix derived from porcine small intestinal submucosa (Vet BioSIS, Cook Veterinary Products, Inc, Spencer, Ind.) and containing a plethora of proteins and growth factors, has been designed as a scaffold for tissue ingrowth and is promoted as reducing scarring. Regrettably, a recent study determined that it offers no apparent advantage over a nonbiologic, nonadherent synthetic dressing for treatment of small, granulating wounds of the distal limb of horses (Gomez et al., 2004)

Matrix Synthesis and Remodeling. In addition to epithelialization, contraction contributes to the successful closure of full-thickness wounds. Contraction is defined as a process whereby both dermis and epidermis bordering a full-thickness skin deficit are drawn from all sides centripetally over the exposed wound bed. Wound contraction is divided into three phases. An initial lag phase (wherein skin edges retract and the wound area increases temporarily for 5 to 10 days) occurs because significant fibroblastic invasion into the wound is a prerequisite for contraction. Subsequently, a period of rapid contraction is followed by a period of slow contraction as the wound approaches complete closure. The number of myofibroblasts found in a wound appears to be proportional to the need for contraction; thus, as repair progresses and the rate of contraction slows, this number decreases. During wound contraction, the surrounding skin stretches by intossusceptive growth, and the wound takes on a stellate appearance. Contraction ceases in response to one of three events: the wound edges meet and contact inhibition halts the processes of both epithelialization and contraction; tension in the surrounding skin becomes equal to or greater than the contractile force of the myofibroblasts; or, in the case of chronic wounds, a low myofibroblast count in the granulation tissue may result in failure of wound contraction despite laxity in the surrounding skin. In this case, the granulation tissue is pale and consists primarily of collagen and ground substance. Wound contraction is greater in regions of the body with loose skin than in regions where skin is under tension, such as the distal aspect of the limb. Although it has been speculated that the shape of the wound may influence the process of contraction, this does not appear relevant in wounds at the distal extremities where skin is tightly stretched and not easily moved. Skin grafts have been reported to inhibit contraction by preventing formation of myofibroblasts or by accelerating the myofibroblast life cycle. As contraction concludes, myofibroblasts disappear, either by reverting to a quiescent fibroblast phenotype or by apoptosis, primarily in response to reduced tension within the ECM (Grinell et al., 1999). The myofibroblast persists in fibrotic lesions, where it may be involved in further ECM accumulation and pathologic contracture but leading to significant morbidity particularly when it involves joints or body orifices. Significant differences exist with regard to contraction between horses and ponies and between distinct areas of the body. Wound contraction is clearly more pronounced in ponies than in horses (Wilmink et al., 1999) and the rate of contraction of limb wounds is at best 25% that of flank wounds. The conversion of ECM from granulation to scar tissue constitutes the final phase of wound repair and consists of connective tissue synthesis, lysis, and remodeling, also referred to as maturation. Collagen macromolecules provide the wound tissue with tensile strength as their deposition peaks within the first week in primary wound repair, and between 7 and 14 days in second-intention healing. Although this corresponds to the period of most rapid gain in strength, only 20% of the final strength of the wound is achieved in the first 3 weeks of repair. At this time, collagen synthesis is balanced by collagenolysis, which normally prevents accretion of excessive amounts of collagen and formation of pathologic scars. It appears that during the development of exuberant granulation tissue in horses, collagen synthesis continues unabated (Schwartz et al., 2002). The balance between synthesis and degradation determines the overall strength of a healing wound at a particular time. The first newly deposited collagen tends to be oriented randomly and therefore provides little tensile strength, whereas during remodeling the fibers re-form along lines of stress and therefore resist dehiscence more effectively. Crosslinking in the later formed collagen is also more effective, although never to the same extent as in the original tissue. A recent study has shown that newly accumulated collagen fibrils are disorganized in wounds at the distal aspect of the forelimb of horses but more normally organized in thoracic wounds (Schwartz et al., 2002).

1) Patient Factors

Age and Physical Status: The patient's age and physical status may influence the rate of wound healing. They consequently also appear more susceptible to infection (Stotts and Wipke-Tevis, 2001). Although there currently is no similar data for the horse, in the author's experience it appears that young horses heal more readily and with fewer complications than do older horses. As Theoret points out, since horses, compared to companion animals, are less commonly affected by these diseases, they are generally not a concern (Theoret, 2006). An exception may be horses suffering from Cushing's disease (Pars intermedia dysfunction) in which high endogenous cortisol can suppress inflammation sufficiently to delay healing (Knottenbelt, 2007). Also, since high concentrations of glucocorticoids are known to be immunosuppressive, it is logical that horses suffering from this malady may be more susceptible to wound infection.

Anemia/Blood Supply/Oxygen Tension: Because most of the oxygen in blood is carried by hemoglobin, it is intuitive that anemia should be an important factor in reduced oxygen delivery and impaired wound healing; this concept however, is unfounded. Data suggest that normovolemic anemia with a packed cell volume (PCV) >20, unrelated to malnutrition, cancer, or chronic infection, does not appear to affect wound healing. However, hypovolemia associated with hemorrhage and anemia or shock can greatly impair healing if not corrected (Hunt et al., 2000). Decreased perfusion of the wound appears to be the cause of altered healing. Local tissue hypoxia that results from insufficient blood volume in hypovolemic patients inhibits many of the responses that initiate healing. An oxygen gradient exists between the nearest functioning capillary and the wound edge. The oxygen tension near a wound capillary is between 60 and 90 mm Hg; however, near the advancing edge of granulation tissue the oxygen tension approaches 0 mm Hg. This decrease is caused by the diffusion gradient and the consumption of oxygen by cells at the wound margin. Since the activities of the new fibroblasts (migration, proliferation, and protein synthesis) rely on the rate at which new capillaries are formed, the wound tensile strength is limited by perfusion and tissue oxygen tension. The maturity and fragility of the new blood vessels forming in an acute wound appear to be affected by oxygen tension. New vessels forming in a hypoxic environment (13% inspired oxygen) are immature and bleed easily. Conversely, new vessels forming in a hyperoxic environment (50% inspired oxygen) are mature and form at a more rapid rate than do vessels in either a normoxic (21% inspired oxygen) or hypoxic environment (Hopf and Rollins, 2007). Reduced oxygen tension, besides inhibiting fibroblastic replication and migration, development of collagen, and tensile strength, also renders the wound more susceptible to infection by altering cellular phagocytic mechanisms (Hunt et al., 2000). When leukocytes ingest organisms and wound debris, more oxygen is consumed. Lack of sufficient oxygen slows the activity of leukocytes and decreases superoxide release, making the wound more susceptible to infection (Hopf and Rollins, 2007). So correction of hypovolemia, and possibly the use of hyperbaric oxygen (HBO) therapy, should reduce the incidence of wound infection and allow healing to progress normally.

Malnutrition and Protein Deficiency. Wound healing is impaired with mild to moderate short or long-term protein energy malnutrition. It appears that the direction of patient is moving toward metabolically (positive or negative) at the time of injury or surgery is most important, since the adverse effect of protein energy malnutrition occurs well in advance of the external evidence of weight loss. Impaired nutrition can alter growth factor synthesis and fibroblastic proliferation, and limit hydroxy proline and collagen deposition, as well as impair immune functions and oxygen transport in healing patients. Insufficient lipid levels, important to inflammation and membrane stabilization, have also been shown to adversely affect wound healing (Stotts et al., 2001). The impairment in wound healing is easily reversed by providing adequate nutrition. Although this has not been proven in horses, it seems logical that there would be an effect of inadequate nutrition on wound healing in this species as well. Hypoproteinemia alone adversely affects wound healing primarily by altering fibroplasia, angiogenesis, remodeling, and gain in tensile strength, consequently prolonging the repair phase of healing. The impairment in wound healing from hypoproteinemia is seen well in advance of alterations in the plasma protein levels. As an example, albumin fractions are depleted almost immediately following withdrawal of protein from the diet. Since albumin is the major oncotic pressure stabilizer in the intravascular compartment, it is not surprising that a decreased serum concentration is associated with poor healing outcomes (Stotts et al., 2001). Even though the alteration in healing is not strongly correlated to plasma protein levels, when these levels fall to 6 g/dl, healing is retarded. Below 5.5 g/dl, a 70% incidence of wound disruption is expected, and below 2 g/dl, wound healing is disrupted, edema occurs, and death ensues. Because fats can be synthesized from carbohydrates and carbohydrates can be synthesized from protein but protein can only be produced from protein or its digested byproducts (amino acids and peptides) a protein rich diet is required to counteract the adverse effects on wound healing. Feeding D-L methionine to protein-deficient animals reverses the retardation in wound healing (Plumb, 2005).

Dehydration: Dehydration of the patient as well as the wound can negatively affect wound healing. The poor perfusion of peripheral tissues in the dehydrated patient is thought to be the reason that healing is delayed in these subjects (Peacock, 1984). This problem can easily be rectified by hydrating the patient. Wound dehydration will be discussed later under “nature of the wound.”

2) Wound Factors

Trauma. Excessive trauma, associated with the wound or at a site or sites remote from the wound (e.g., multiple lacerations or multiple fractures) can negatively affect repair and make the wound more susceptible to infection. When the effects of simultaneous trauma from either fracture or muscle contusion elsewhere on the body were examined, a delay in wound tensile strength was observed out to 15 days post-trauma. A delay in gain of wound tensile strength was also observed at the other sites when a second wound was made within 14 days of the first, and the degree and loss in gain of wound tensile strength was proportional to the severity of trauma (Peacock, 1984).



Figure 2. Excessive trauma to the distal metacarpal region

Age of Wound: A chronic wound (slow or non-healing) is often associated with an underlying problem that has exerted a negative impact on the normal progress in repair. Chronic inflammation from foreign bodies, necrotic tissue, repetitive mechanical trauma, and the application of caustic agents is a common cause. The goal in treating a chronic wound is to eliminate the causal agent/agents and convert the wound environment to one that closely resembles that of the acute wound; this is best done by wound debridement. In a case in which repeated mechanical trauma is the underlying cause, the use of appropriate bandaging/splinting techniques to immobilize the region is most important to ensure a successful outcome (Kane, 2001).

Location: Wounds of the distal extremities (carpus/tarsus and below) of horses heal more slowly and are more problematic than wounds of the upper body and head regions. Specifically, delays in epithelialization and contraction, as well as the propensity to develop exuberant granulation tissue (EGT), commonly afflict full-thickness wounds of the distal limbs (Theoret et al., 2001). Although the causes of problematic healing in the horse's distal limbs have yet to be completely clarified, several have been proposed. Better blood supply, a greater amount of adnexal structures, and the thinner epidermis covering the head and neck contribute to the more rapid and cosmetic repair occurring in these regions. Wounds of the distal extremities have an absence of underlying musculature, may be near highly mobile joints and bony prominences, and are often more contaminated than are body wounds (Cochrane et al., 2003). Differences between growth characteristics of trunk and limb fibroblasts and a tardy and prolonged inflammatory response are believed to contribute to the development of EGT, especially in limb wounds, in the horse. A study showed that fibroblasts isolated from the horse distal limb grow significantly more slowly than those of the trunk. The horse displays a deficient and protracted inflammatory response compared to the pony, especially in wounds involving the distal extremities (Wilmink, 1999). Furthermore, leukocytes from horses appear to be poorly equipped to kill bacteria compared to those of ponies (Wilmink, 2003). Moreover, their local cytokine profile is skewed in favor of fibrogenic mediators (Schwartz et al., 2002). Deficient contraction of limb wounds compared to body wounds may be due to a poor arrangement of myofibroblasts, precluding an ordered contractile activity.

Cytokine profiles may also negatively affect contractility (Wilmink, 2001). Wounds subjected to excessive movement, such as those located over highly moveable joints or those oriented perpendicular to lines of skin tension (e.g., perpendicular to the limb's long axis), are often slow to heal, and they usually form a disproportionate amount of scar tissue. Movement can also occur between healing skin and underlying tendon or muscle or between the heel bulbs, causing the wound edges to gape during weight bearing (Knottenbelt, 1997).

Nature of the Wound

A) Type: Degloving injuries that damage (strip off) the periosteum and the paratendon are more susceptible to infection and subsequent osteomyelitis or septic tendonitis because of loss of blood supply. In these cases, soft tissue coverage of the site should be achieved as soon as possible because of the increased risk of bony sequestration, tendon degeneration, and uncontrollable bone and soft tissue infection if the blood supply is not quickly re-established. Wounds in which a flap of tissue is at odds with the distribution of blood vessels in the extremities often experience delays in healing and are more susceptible to infection. In most cases it is best to delay suturing these wounds until a healthy bed of granulation tissue forms; this will ensure a good blood supply to support the healing of the skin flap following suturing. Stabilizing the flap into a somewhat normal position using a few large sutures, while awaiting definitive treatment, will prevent skin flap retraction (Stashak, 2003).



Figure 3. Wounds in which movement between the healing skin and underlying tendon will either delay or prevent healing.

B) Degree of Contamination. Wounds may be classified according to contamination and increasing risk of infection. A clean wound is one created surgically under aseptic conditions in situations where a contaminated site is not entered. Wounds are considered clean, clean contaminated, contaminated, or dirty contaminated/infected. As would be expected, the greater the contamination the greater the risk for infection. Dirty wounds have a 25-fold greater infection rate than do clean wounds. Wounds contaminated with fecal material and dirt run a high risk of infection despite therapeutic intervention; indeed, feces may contain up to 1011 microorganisms per gram (Stashak, 2006). Specific infection potentiating fractions (IPFs) found in the organic and inorganic components of soil increase the wound's susceptibility to infection. These highly charged fractions reduce the effects of white blood cells, decrease humoral factors, and neutralize antibodies. Consequently, as few as 100 microorganisms can cause infection in wounds contaminated with soil. The metabolic impact of contaminating bacteria (bioburden) on tissues can significantly impair healing (Robson, 1997).

C) Wound Fluids. Wounds with pockets that allow exudate or seroma/hematoma to accumulate are often slower to heal and are susceptible to infection. While persistent contact with inflammatory products present in the exudate during the repair phase is believed to cause the delay in repair, accumulation of seroma/hematoma further provides an excellent medium for bacterial growth, thus making the wound more susceptible to infection. Expanding fluid pressure from the exudate/seroma/hematoma may also be great enough to alter the local blood supply (Stashak, 2003). Drainage of the fluid-filled pocket followed by bandaging, where applicable, is usually all that is needed. Experiments have identified differences between acute and chronic wound fluids in humans (Rao et al., 1995). Metalloproteinases, essential in the various phases of healing but detrimental in the case of persistent up-regulation, were found to be 5 to 10 times higher in chronic wound fluid. Serine proteinases that degrade fibronectin and impede

collagen synthesis and epithelialization were also found to be increased in chronic wound fluids. Interestingly, when a chronic wound reverts to active healing, the levels of metalloproteinase activity decrease significantly, which parallels the processes observed in normally healing acute wounds (Tren Grove et al., 1999).

D) Infection: Infection is considered to be a major cause of delayed wound healing, reduced gain of tissue tensile strength, as well as dehiscence following wound closure. Potential pathogenic bacteria bind to extracellular matrix proteins (e.g., fibronectin), which may limit the latter's availability for promoting migration of mesenchymal cells and consequently bear a direct negative effect on wound healing. Additionally, bacteria that produce exotoxins (e.g., *Clostridium* spp, *S. pyogenes*, *S. aureus*) cause more tissue damage, creating a microenvironment conducive to their survival. Those with thick capsules (e.g., *S. pyogenes*, *S. aureus*, and *Klebsiella pneumoniae*) are more resistant to phagocytosis by leukocytes. During the process of bacterial degradation, released endotoxins can activate coagulation pathways which may cause thrombosis of the microvasculature or systemic organ or immune dysfunction, and activate macrophages to release more inflammatory mediators (Brumbaugh, 2005).

CONCLUSION AND RECOMMENDATION

A vital trait of living organisms continually subjected to insults from the environment is their capacity for self-repairs. Whether the injury is a deliberate act of surgery or accidental, it generates an attempt by the host to restore tissue continuity. The two processes involved in wound healing are repair and regeneration. Regeneration entails the replacement of damaged tissue with normal cells of the type lost, and this is possible only in tissues with a sustained population of cells capable of mitosis, such as epithelium, bone, and liver. Repair is a stopgap reaction designed to reestablish the continuity of interrupted tissues (Theoret, 2005). The combination of skin loss and the amount of redundant or loose skin surrounding a wound has an obvious influence on the clinician's ability to close a wound and the extent to which wound contraction will contribute to the final result. The effects of the location of the wound on the body as well as breed differences on wound healing in horses are well recognized (Jacobs, 1984) as is the influence of surrounding skin tension on wound contraction (Walton, 1972). A large retrospective study revealed that primary closure was successful in only 24% of horse wounds and 39% of pony wounds, more than half of which were located on the limb. Thus, a significant number of wounds must heal by second intention. Unfortunately, this type of repair leads to formation of a much larger scar tissue than that formed after successful primary closure, and function and appearance may be adversely affected. Wound healing is part of normal body maintenance and depends on the patient's general state of health, blood supply/anemia/local oxygen gradient, location, vitamins (A, E, and C) and minerals (Zinc).

From this review the following recommendations are forwarded:

- Financial and time constraints are often imposed on the practitioner, so it is advisable to spend time discussing the owner's expectations, the possible complications, and the costs associated with these procedures before beginning wound management.
- The extent to which some reconstructive and skin mobilization techniques can be used depends on the presence of adequate tissue surrounding the wound.
 - The practitioner should consider location, nature, cleanness of wound before starting management.
 - Animals should offered balanced nutrition in adequate amounts prior to surgery and/or elective surgery.
 - Therefore, additional investigation should conduct to find out a cost effective management of wound with locally available materials.

DECLARATIONS

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Authors' contributions

MB conceived the review, coordinated the overall activity, and reviewed the manuscript. AM supervising all in all activities

Availability of data and materials

Data will be made available upon request of the primary author

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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