Third Edition

EQUINE WOUND MANAGEMENT

Edited by

Christine Theoret

Jim Schumacher

WILEY Blackwell

Equine Wound Management

To our families and friends.

To Aotearoa, for a warm welcome during the preparation of this book – Mauruuru koutou.

Equine Wound Management

Third Edition

Edited by

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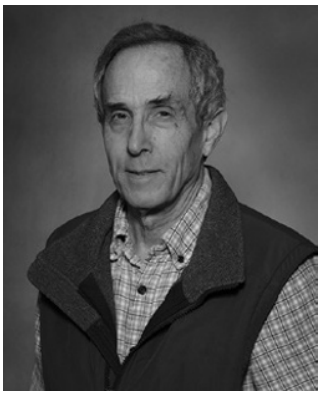
Dr. Christine Theoret is a full professor at the University of Montreal where she teaches veterinary anatomy and surgery in the DVM program. She received a DVM degree from the University of Montreal (1991) and completed an internship in equine medicine/surgery at the same institution in 1992. She then undertook a joint residency/MSc program in surgery at the Western College of Veterinary Medicine (1992–1995). She became a diplomate of the American College of Veterinary Surgeons in 1997. In 2000, Dr. Theoret received her PhD degree, studying the molecular mechanisms that govern healing and scarring, from the University of Saskatchewan.

Dr. Theoret founded the Comparative Veterinary Tissue Healing Laboratory in 2002, where she has since trained more than 30 highly qualified personnel, mostly MSc and PhD students. Her research has led to the publication of numerous articles in peer-reviewed, scientific journals. In 2008 she co-edited the second edition of the textbook *Equine Wound Management*. Dr. Theoret has served on the advisory boards of various national and international associations, including a term as President of the Veterinary Wound Management Society.

Jim Schumacher



Jim Schumacher graduated from Kansas State University in 1973. He worked in equine and food animal practices in Texas, California, and Kansas for 5 years after graduation. Most of this time in private practice was spent working in feedyards in Kansas. He completed an MSc degree and a residency in large animal surgery at Texas A&M University in 1980.

He was a member of the faculty at Texas A&M University until 1997. Since then he has worked at the University of London, Auburn University, the University College Dublin, and the University of Tennessee, where he has been a member of the faculty of the Department of Veterinary Clinical Sciences since 2003. He is a diplomate of the American College of Veterinary Surgeons and a member of the Royal College of Veterinary Surgeons. He has lectured students about surgery for the past 35 years.

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Preface

Wounds are among the most common medical conditions seen by veterinarians in their equine patients and one of the topics least addressed during the veterinary curriculum or at continuing education meetings. Because the horse's response to wounding differs from that of man, laboratory animals or even other veterinary patients, wound-management textbooks used in the human healthcare field or in small animal practice cannot be relied on to provide appropriate/specific therapeutic guidelines. Moreover, the general-purpose textbook covering equine medicine and surgery cannot possibly address the topic with the required depth because of the abundance of information on wound physiology and management now available. Consequently, the Equine Wound Management textbook is an essential reference for equine veterinarians because it provides readers with state-of-the-art theoretical and practical information, enhanced by an abundance of helpful tables, line drawings, and color figures.

With Dr. Ted Stashak firmly embracing a well-earned retirement, I (Dr. Theoret) was faced with the choice of a new co-editor willing to fill big shoes. Dr. Jim Schumacher courageously accepted the challenge and, due to his vast clinical experience and his familiarity with editorship, he has been an inestimable asset to the smooth execution of the task at hand. Together, we have striven to create a well-balanced book that addresses the needs of students, practitioners, postgraduate veterinarians in training programs (research- or clinically-oriented), and specialists (surgeons). Moreover, with the aim to make the book more reader-friendly, practical information has been highlighted in the text in easy-to-spot, quick-to-read "tips", and a companion interactive website posts text, questions/answers, figures, case series, "how to" videos, etc.

Since the second edition of this textbook was published in 2008, hundreds of new, relevant studies have been performed, and summaries of these findings and practical applications thereof have been included in the third edition. The wound-care market for human patients has grown in leaps and bounds over the past few years; consequently, countless new topical medications and interactive dressings have appeared on the market. In many cases, the use of these products in horses has not yet been thoroughly investigated. Despite the aforementioned differences in healing of wounds of horses, veterinarians may be tempted to extrapolate data from human or lab animal trials to the horse. Consequently, Chapters 5 and 6 have been thoroughly updated, and the author presents evidence for the effects of selected products specifically on healing tissues of the horse. Another important change since the previous edition is the increased awareness of antibiotic resistance. Accordingly, a concerted effort has been made by all contributing authors to promote responsible antimicrobial stewardship by better describing the infection continuum and reviewing the premise of antibiotic resistance and biofilms. These topics are particularly well addressed in chapters 3, 4, and 19. Finally, it seemed appropriate to add a section on dog-bite wounds and gunshot wounds to Chapter 20 and to add an entire chapter on innovative adjunctive therapies, discussing the most recent developments.

In closing we wish to express our gratitude to the authors for their willingness in bringing to this textbook all their valuable experience. We are pleased that we were able to include many world-renowned specialists to produce the highest-quality material. We are indebted to these people who generously contributed their clinical insight and current research data.

Acknowledgment

The third edition of *Equine Wound Management* is here today thanks to Ted Stashak's vision and commitment to serve the equine veterinary community.

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/theoret/wound

The website includes:

- a webliography from chapter 17 in the book;
- case studies;
- figures from the book as PowerPoint slides, to download;
- interactive multiple choice questions and answers;
- videos.

CHAPTER 1

Physiology of Wound Healing

Christine Theoret, DMV, PhD, Diplomate ACVS

Chapter Contents

Summary

Prior to undertaking the management of a wound, the underlying biology of wound healing must be understood so that the best approach at the correct time can be selected, and so that problems with healing, if they arise, are recognized.

This chapter aims to provide an update on the physiologic, cellular, biochemical, and molecular aspects of wound repair.

Introduction

A vital trait of living organisms, continually subjected to insults from the environment, is their capacity for self repair. Whether the injury is from surgery or accidental, it generates an attempt by the host to restore continuity to tissue. Two processes are involved in healing: regeneration and repair. Regeneration entails the replacement of damaged tissue with normal cells of the type lost and is only possible in tissues with a sustained population of cells capable of mitosis, such as epithelium, bone, and liver. Conversely, repair is a "stop-gap" reaction designed to re-establish the continuity of interrupted tissues with undifferentiated scar tissue. Repair is, therefore, an inferior method of healing, producing scar tissue that is less biologically useful than the tissue it replaced, and that may adversely affect adjacent normal tissues. When complications of wound healing arise, the final result is even worse.

Accidental wounds occur commonly in horses and exert a significant welfare concern and financial burden on the equine industry. A large study by the United States Department of Agriculture's National Animal Health Monitoring System found, in 2006, that injury/wound/trauma was the most common medical condition affecting horses, with a prevalence of 4.7% in equids 6 months of age and older.[2] Injury/wound/trauma was the leading cause of death of foals less than 6 months old, accounting for 24% of deaths, while for horses at least 6 months old, it accounted for 16% of deaths and was the leading cause of mortality, after old age.[2] A study in Mexico conducted specifically on a population of working equids found a prevalence of 20.6% for cutaneous pathologic conditions; among these, skin wounds (abrasions, lacerations, abscesses) were the most prevalent (6.8%).[3]

Figures are also concerning in Europe. A study conducted in the UK found that wounds were the most common type of injury reported by horse owners, accounting for roughly half of all injuries occurring over a 12-month period.[4] Another study found that wounds accounted for 21.6% of veterinary

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Companion website: www.wiley.com/go/theoret/wound

treatments of injured polo ponies in the UK.[5] Horses in the southern hemisphere do not seem to fare any better: wounds ranked as the third most common medical condition encountered by equine practitioners in Australia and New Zealand and ranked second, after colic, as the most common cause of death or euthanasia.[6,7]

Finally, skin trauma/wounds are a frequent cause of morbidity in athletic horses. A study in Thoroughbred racehorses has shown that 70% of injuries leading to early retirement are the result of a musculoskeletal injury, of which 7% are associated with wounds or lacerations.[8]

The objective of repair is to re-establish an epithelial cover and to recover the integrity, strength, and function of the skin. Partial-thickness cutaneous wounds (e.g., abrasions and erosions) heal primarily by migration and proliferation of epidermal cells from the remaining underlying epithelium, as well as from the adnexal structures (i.e., hair follicles and sweat and sebaceous glands), with little participation of inflammatory or stromal cells. In contrast, second-intention repair of full-thickness cutaneous wounds hinges on four coordinated and interrelated phases (Figure 1.1). Partitioning the process into discrete phases suggests simplicity while, in reality, healing is exquisitely complex. The phases rely on interactions between multiple cellular types, their surrounding matrix, and the soluble mediators that govern the numerous activities required to rebuild the tissue. Moreover, the interactions are not static but rather in a state of constant flux, resulting in a microenvironment that is continually evolving as the wound heals.[10]

Before veterinarians can positively influence wound healing, they must understand its mechanisms so that they select the appropriate techniques of wound management. In fact, Hippocrates once said, "Healing is a matter of time, but it is sometimes also a matter of opportunity."[11]

Skin anatomy

The skin is the largest organ and serves key functions including physical protection, sensation, temperature regulation, and insulation. It is composed of two compartments – the epidermis and the dermis (Figure 1.2a). In the horse, the epidermis consists of five layers of keratinocytes: the *stratum basale*, *stratum spinosum*, *stratum granulosum*, *stratum lucidum*, and *stratum corneum* (Figure 1.2b). Additional epidermal components, referred to as skin appendages, include hair follicles, sweat glands, sebaceous glands, and hooves/nails. Although 90–95% of the cells populating the epidermis are keratinocytes, this compartment also includes melanocytes, Langerhans cells, and Merkel cells. 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 through 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.[12]

The dermal compartment consists of two regions, the papillary dermis and the reticular dermis. This compartment is composed of dense, fibroelastic connective tissue and constitutes the bulk of the skin. The epidermis projects into this underlying connective tissue via extensions known as rete pegs or ridges. A network of collagen fibers provides tensile strength to the dermis, and elastin and glycosaminoglycans (GAGs) ensure resilience. Collagen type I is the major collagen of the dermis (~62%) whereas collagen type III comprises ~15% of the dermis.[13] The fibroblast is the principal type of cell found in the dermis; perivascular mast cells and tissue macrophages are also found within the dermis. The connective tissue supports these cells and also a network of nerves, epithelial glands, keratinizing

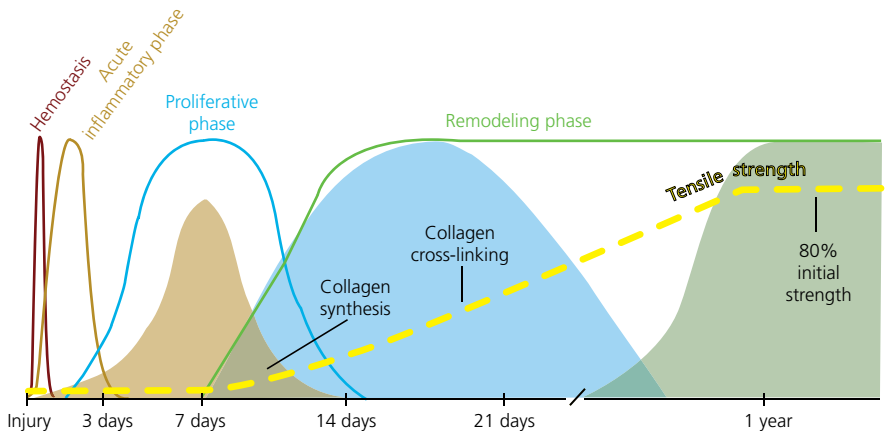


Figure 1.1 Temporal profile of synchronized phases and gain in tensile strength of healing cutaneous wounds. Solid lines show the healing profile of laboratory animals while superimposed shaded areas show the profile of healing full-thickness wounds on the limb of horses. It should be noted that the timescale is suggestive and depends on the size and extent of the wound. Source: Modified by Marco Langlois (Faculté de médecine vétérinaire, Université de Montréal) from Stashak & Theoret 2014.[9] Reproduced with permission of Elsevier.

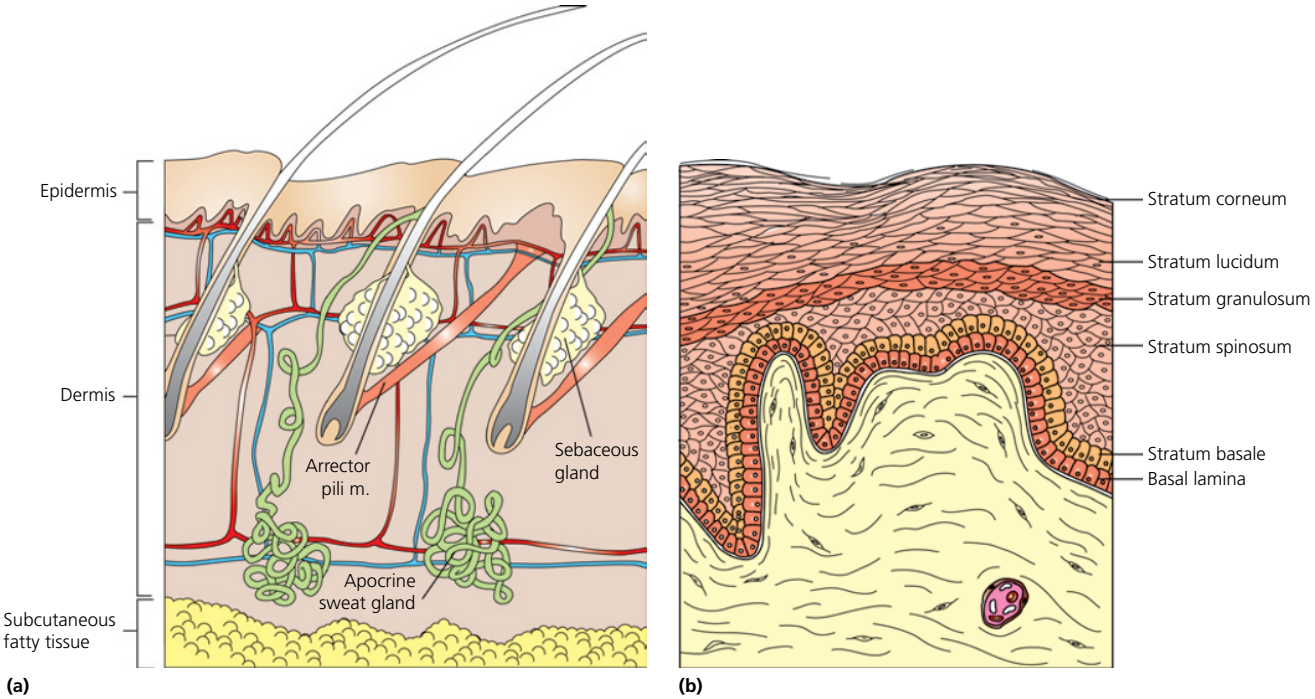


Figure 1.2 **(a)** Diagram of a cross-section of skin, showing the epidermal and dermal compartments. **(b)** Diagram of the layers of the epidermis of horse skin. Source: Stashak & Theoret 2014.[9] Reproduced with permission of Elsevier.

appendages, and a microvascular and lymphatic system. Indeed, only the dermal compartment is vascularized; nutrients reach the epidermis by diffusion.

In the horse, the thickness of the skin varies according to body site. For example, in the Dutch warmblood, the skin on the head, neck, and ventral abdomen is relatively thin, measuring between 1.73 mm (±0.16) and 2.03 mm (±0.2) whereas the skin on the limbs is slightly thicker, measuring an average of 2.83 mm (±0.27) for the forelimb and 2.89 mm (±0.24) for the hindlimb, depending on the specific anatomic location (e.g., the skin over the dorsal coffin joint is particularly thick, measuring 4.54 mm [±0.39]).[14]

The subcutaneous tissue (i.e., tissue just deep to the skin) is also known as the hypodermis or superficial fascia and is not considered part of the skin. It is comprised of loose connective tissue; approximately half of the body's fat stores are located in this region. The hypodermis anchors the skin to the underlying organs and allows the skin to move relatively freely. It also acts as a shock absorber and insulates the deeper body tissues from heat loss.

Phases of wound repair

Hemostasis/coagulation

The first phase of wound healing begins immediately upon injury, is completed within hours, and is dedicated to hemostasis and the formation of a provisional wound matrix. Hemostasis was long considered to be a component of the inflammatory phase, and only recently has its individual significance to wound healing been recognized. Many of the processes that occur during the ensuing phases rely on the normal execution of this short, but critical, initial phase.

Wounding traumatizes blood vessels, which results in hemorrhage. The injured endothelial cellular membrane releases phospholipids that are transformed into arachidonic acid and its metabolites that mediate vascular tone and permeability. Peripheral vasoconstriction, lasting 5–10 minutes, limits bleeding but simultaneously starves the surrounding tissues of oxygen and nutrients normally carried by the blood. The resulting transitory hypoxia and increased glycolysis, as well as pH changes, are beneficial because, along with the effects of the original vascular trauma, they enhance the activation, adhesion and aggregation of platelets, thereby initiating the intrinsic coagulation cascade, ultimately leading to the formation of a blood clot that seals the vessel.[15]

This clot, besides providing a small amount of strength to the wound, has multiple roles. It forms a provisional matrix, rich in fibrin, fibronectin, vitronectin, and thrombospondin, that fills the space created by the wound and serves as a scaffold for migrating cells. Special surface receptors (integrins) on inflammatory and stromal cells recognize binding sites on the proteins within the scaffold, ensuring ingrowth of cells involved in healing. This cellular influx is mediated by chemoattractants released by degranulating platelets, among other cells, present in the scaffold. Indeed, activated platelets are amongst the earliest

promoters of inflammation, via the release of potent chemoattractants and mitogens from their storage granules.[16] Mediators released not only by platelets but also by mast cells modify vascular tone and permeability, which increase within 5–10 minutes of wounding (apparent clinically as a localized redness, heat and swelling of the wound), and thereby facilitate the aforementioned cellular migration and the diffusion of nutrients and oxygen required to sustain these newly arriving cells.

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.

Inflammation

The inflammatory phase of the wound healing cascade (also referred to clinically as the debridement phase) is activated during the hemostasis/coagulation phase. It can roughly be divided into an early phase, characterized by recruitment of neutrophils, and a late phase, characterized by the appearance and transformation of monocytes. Inflammation prepares the wound for the subsequent phases of healing. It purges the body of alien substances and disposes of dead tissue, while the participating cellular populations liberate mediators to amplify and sustain the events to follow. The intensity of the inflammatory response is strongly correlated to the severity of trauma and determines the extent of scarring.

Leukocytes are recruited from blood circulating to the site of injury by the numerous vasoactive mediators and chemoattractants supplied by the coagulation and activated complement pathways, by platelets, by mast cells,[10] and by injured or activated stromal cells.[17] These signals initiate the processes of rolling, activation, tight adhesion, and, finally, transmigration of inflammatory cells through the microvascular endothelium. Chemoattractants also stimulate the release of enzymes by the activated neutrophils, expediting their penetration through vascular basement membranes. Diapedesis of neutrophils is further facilitated by increased capillary permeability brought about by the release of a spectrum of vasodilatory agents. Cellular influx begins within minutes and the concentration of neutrophils at the wound progressively increases to reach a peak 1–2 days after injury. 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. 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 the later phases of repair. The neutrophils remaining within viable tissue die in a few days and are phagocytized by the tissue macrophages or modified wound fibroblasts, marking the termination of the early inflammatory phase of repair.[17] Although neutrophils help create a favorable environment within the wound and serve as a source of pro-inflammatory cytokines, they are not essential to repair of non-infected wounds. Indeed, a classic series of experiments in the 1970s showed that, as long as conditions were kept sterile, depletion of neutrophils in a guinea pig wound model seemed not to perturb tissue repair.[18] More recent knockdown experiments in mice support the depletion studies of the 1970s and go further, showing that repair is even more rapid than in wild-type sibling mice so long as conditions are sterile.[19]

The rapid increase in the number of macrophages in inflamed tissue is predominantly caused by the emigration of monocytes from the vasculature, followed by differentiation of the monocytes into macrophages to assist resident tissue macrophages at the wound site for a period of days to weeks. In this manner, the responsive and adaptable pluripotent monocytes can morph into macrophages whose functional properties are determined by the conditions they encounter at the site of mobilization and that change during healing. Macrophages play a central role in all phases of wound healing and orchestrate the overall process. During the early inflammatory phase, macrophages exert pro-inflammatory functions, such as antigen presentation, phagocytosis, and the production of inflammatory cytokines and growth factors that facilitate wound healing (Figure 1.3). The phenotype of wound macrophages during this phase is probably the classically activated or so-called "M1 phenotype." Later, during the proliferative phase of healing, macrophages stimulate proliferation of dermal, endothelial, and epithelial tissue to complete formation of the extracellular matrix (ECM), angiogenesis, and epithelialization. Macrophages can then change the composition of the ECM during the remodeling phase by releasing degradative enzymes (Figure 1.4). This suggests an important role for alternatively activated macrophages (also known as "M2 phenotype") in this phase of wound healing.[20,21]

More recent studies using genetically modified neonatal mice have shown that, like neutrophils, macrophages might not be essential for tissue repair.[22] Nevertheless, they probably play an important role in the regulation of fibrosis and scarring by degrading matrix.[23]

In spite of this new, somewhat conflicting evidence, acute inflammation is still considered crucial to the normal repair of wounds in adult mammals exposed to infective agents. When inflammation fails to resolve, however, and a chronic inflammatory response is established, the process can become dysregulated, resulting in pathologic wound repair and the accumulation of permanent fibrotic scar tissue at the site of injury. This fibrosis is characterized by the excessive accumulation of ECM components, including collagens, fibronectin, and hyaluronic acid at the site of injury, leading to a decrease in organ function and, in some cases, organ failure and death. In humans, an estimated 45% of deaths in the western world are now attributed to diseases in which fibrosis plays a major etiologic role.[24] One such "fibroproliferative disorder" encountered in full-thickness cutaneous wounds of the horse and that leads to increased scarring is the development of exuberant granulation tissue (the reader is referred to Chapter 15).

After injury, once infection has been countered and repair completed, all the inflammatory cells disperse from the wound.

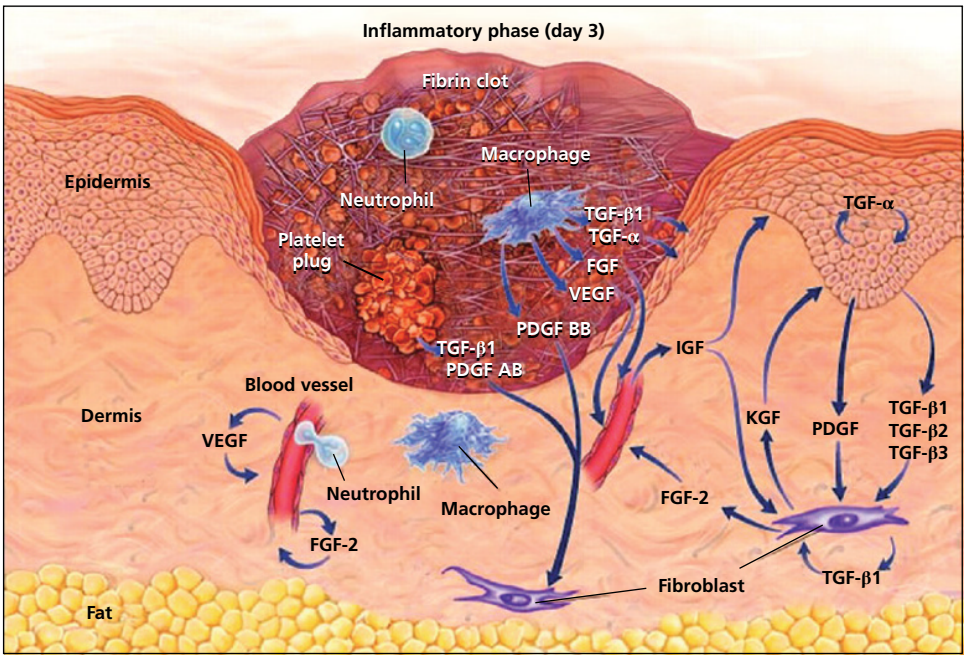


Figure 1.3 Illustration of a full-thickness cutaneous wound showing the cellular and molecular components present 3 days after injury. FGF, basic fibroblast growth factor; IGF, insulin-like growth factor; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor. Source: Singer 1999.[17] Reproduced with permission of NEJM.

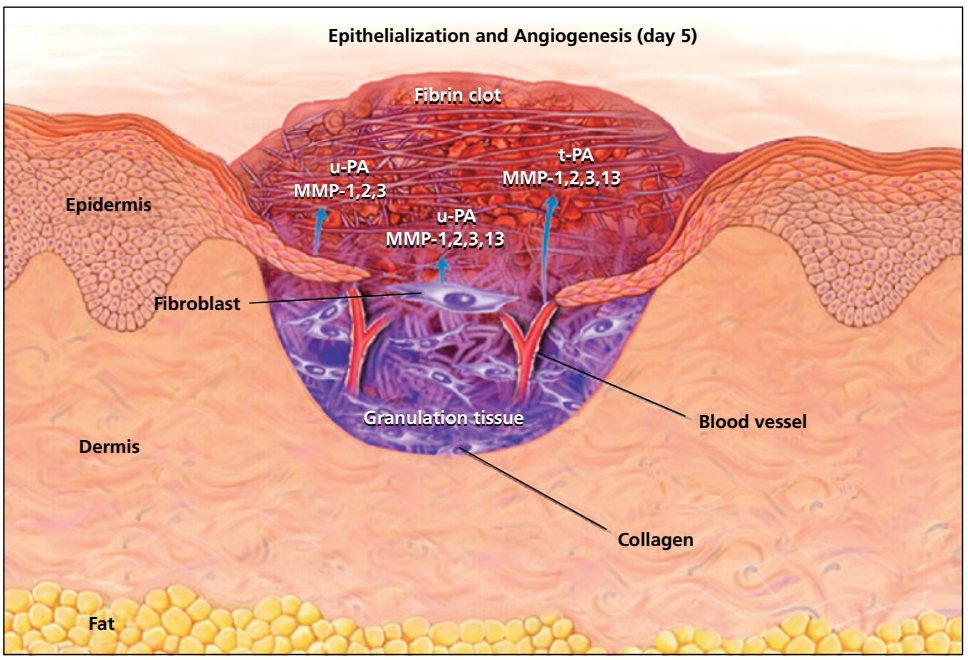


Figure 1.4 Illustration of a full-thickness cutaneous wound 5 days after injury showing angiogenesis, fibroplasia, and epithelialization. uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; MMP, matrix metalloproteinase. Source: Singer 1999.[17] Reproduced with permission of NEJM.

For inflammation to resolve, each of the events that initiated it must be halted or even reversed. Apoptosis, or programmed cellular death, is the universal pathway for eliminating unneeded cells in a phagocytic process that does not elicit additional inflammation.[25] This mechanism prevails during all phases of wound repair because each phase relies on rapid increases in specific cellular populations that prepare the wound for repair (inflammatory cells) or deposit new matrices and mature the

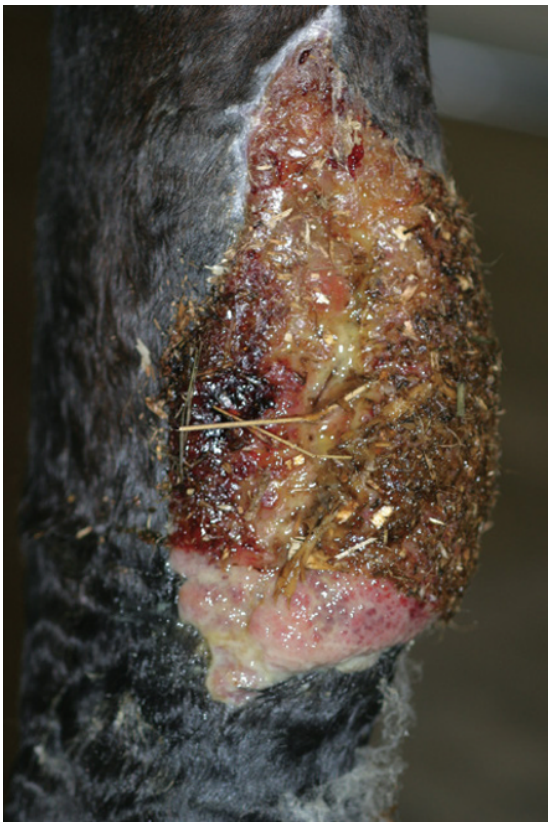


Figure 1.5 This metatarsal wound failed to heal for 7 months as a result of chronic low-grade inflammation due to exposure as well as superficial and deep infection. The wound in fact became larger rather than smaller, illustrating suspension of the healing process. Courtesy of Dr. Derek Knottenbelt.

wound (stromal cells), but then must be eliminated prior to progression to the next phase of repair. Indeed, a mature scar is typically acellular. There are several steps at which the process of resolution could go astray, leading to suppuration, chronic inflammation (Figure 1.5), and/or excessive fibrosis.

Tip

- Clinicians have the greatest influence on the acute inflammatory phase of healing: proper surgical debridement and irrigation, good hemostasis, and adequate drainage can greatly hasten wound healing.

Cellular proliferation

The main objective of the proliferative phase (also referred to clinically as the repair phase) is to achieve protection of the wound's surface via the formation of granulation tissue and a new epithelial cover and to restore the vascular network to nourish the new tissues.

Fibroplasia

The proliferative phase of repair comes about as inflammation subsides and is characterized by the appearance of red, fleshy granulation tissue, which ultimately fills the defect. Although the earliest part of this phase is very active on a cellular level, this activity does not immediately translate into a gain in the wound's strength. Indeed, during the first 3–5 days following injury, fibroblasts and endothelial and epithelial cells rapidly invade the wound in preparation for synthesis and maturation of the matrix or for wound coverage; however, these latter reinforcing mechanisms lag somewhat. Granulation tissue is formed by three elements that simultaneously move into the defect created by the wound: *macrophages*, which debride and produce mediators, such as cytokines and growth factors, that stimulate angiogenesis and fibroplasia; *fibroblasts*, which proliferate and synthesize new components of the ECM; and *new blood vessels*, which carry oxygen and nutrients necessary for the metabolism and growth of cells, and confer to the granulation tissue its characteristic red, granular appearance.

This stroma, rich in fibronectin and hyaluronan, replaces the fibrin clot to provide a physical barrier to infection and, importantly, provides a surface across which cells can migrate. A number of matrix molecules as well as cytokines and growth factors released by inflammatory cells are believed to stimulate fibroblasts from adjacent uninjured dermis and subcutaneous tissue to proliferate and express integrin receptors to assist their migration into the defect. Integrins are transmembrane proteins that act as the major cell-surface receptors for ECM molecules and thus mediate interactions between cells and their environment. They are particularly critical to the migratory movements exhibited by cells involved in wound healing, such as epithelial and endothelial cells and fibroblasts.[26] Migration of fibroblasts immediately precedes advancing capillary endothelial buds but follows macrophages that have cleared a path by phagocytizing debris. Fibroblasts themselves also possess an active proteolytic system, comprising proteinases, such as plasminogen activator (PA), various collagenases, gelatinase, and stromelysin,[27] to aid their migration into the fibrin blood clot.

After fibroblasts have arrived within the defect created by the wound, they proliferate then switch their function to protein synthesis and commence to gradually replace the provisional matrix with a collagen-rich one, probably under the influence of various cytokines and growth factors. As the wound matures, the ratio of type I (mature) to type III (immature) collagen markedly increases; proteoglycans also become abundant within the mature matrix. The greatest rate of accumulation of connective tissue within the wound occurs 7–14 days after injury, at least in the laboratory rodent, which translates into the period of most rapid gain in tensile strength (see Figure 1.1). Thereafter, the collagen content within the wound levels off as fibroblasts down-regulate their synthetic activities; this corresponds to a much slower gain in tensile strength as the wound remodels. The fibroblast-rich granulation tissue is subsequently replaced by relatively avascular and acellular scar tissue, as the capillaries within the wound regress and fibroblasts either undergo apoptosis[28] or acquire characteristics of smooth muscle and transform into myofibroblasts that participate in wound contraction. The latter phenomena are regulated by the physiologic needs and/or the

microenvironmental stimuli present at the wound. It appears that if the signal to down-regulate fibroblast activity is delayed beyond a specific time point, apoptosis is permanently impaired, ultimately leading to an imbalance between collagen synthesis and degradation[29] and the formation of excessive scar tissue.

Angiogenesis

Besides initiating the inflammatory response through interactions with leukocytes, microvascular endothelial cells play a key role in the proliferative phase of repair. The formation of new capillaries from pre-existing ones (angiogenesis) is necessary to restore oxygenation and to provide required nutrients to the granulation tissue newly formed within the wound. Angiogenesis, which occurs in response to tissue injury and hypoxia, is a complex and dynamic process mediated by diverse soluble factors provided by the serum and the surrounding ECM. These factors are, in particular, angiogenic inducers, including growth factors [most notably vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, platelet-derived growth factor (PDGF), and members of the transforming growth factor (TGF)-β family],[30] chemokines and angiogenic enzymes (notably the serine proteinase thrombin), endothelial-specific receptors, and adhesion molecules, such as integrins,[31] many of which are released during the 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 cellular proliferation and differentiation into mature blood vessels (i.e., arterioles and venules), eventually followed by regression and involution of the newly formed vasculature as tissue remodels.[32] Angiogenesis depends not only on the cells and cytokines present, but also on the production and organization of components of the ECM that provide a scaffold through which endothelial cells can migrate and a reservoir and modulator for growth factors. Thus, endothelial cells at the tip of capillaries begin their migration into the wound in response to angiogenic stimuli and the absence of neighboring cells on the second day after injury. Cytoplasmic pseudopodia extend through fragmented basement membranes; subsequently, the entire endothelial cell migrates into the perivascular space. Cells remaining in the parent vessel near the tip of the angiogenic sprouts begin to proliferate, providing a continuous source of microvascular endothelial cells for angiogenesis. A new capillary sprout has no lumen when it first develops; after it fuses with a neighboring sprout to form an arcade, it canalizes, allowing erythrocytes to pass into and through it. Formation of a lumen probably involves the joining of plasma membranes of individual and/or adjacent cells, as well as extensive intracellular vacuolization followed by fusion of the vacuoles to form "ring cells," which ultimately fuse to form seamless capillaries. Capillaries then become stable as endothelial cells interact with the new basement membrane within 24 hours of new vessel formation and via the recruitment of pericytes and smooth muscle cells. In healing wounds, this vigorous angiogenic response results in a density of vessels that far exceeds that of capillaries in normal, uninjured tissue[33] and provides the granulation tissue with its red, granular appearance.

After the stroma has been completely reconstituted, a rich vascular supply to the wound is no longer needed. Pro-angiogenic stimuli are down-regulated and/or the local concentration of anti-angiogenic factors (thrombospondin, interferon gamma-induced protein 10/CXC motif chemokine 10, and Sprouty-2)[34,35] increases, and most of the recently formed capillary network quickly involutes through the activity of matrix metalloproteinases (MMPs),[36] in particular MMP-1 and MMP-10,[37] and selective apoptosis of endothelial cells. The color of the wound pales as the rich capillary bed disappears from the granulation tissue.

Epithelialization

Epithelialization is defined as the process of covering denuded epithelial surfaces and is essential for successful closure of the wound. In addition to the aforementioned hemostatic activities that establish a temporary barrier, the residual epithelium beneath the clot moves centripetally to participate in closure of the wound. Even though epithelial migration commences 24–48 hours after wounding, the characteristic pink rim of new epithelium (Figure 1.6) is not macroscopically visible until some time later. This "lag" varies according to the species of animal and the site, size, and substrate of the wound. For example, epithelialization is accelerated in a partial-thickness wound because migrating cells arise not only from the residual epithelium at the wound's periphery but also from remaining epidermal appendages. Furthermore, the basement membrane is intact in this type of injury, precluding a lengthy regeneration. On the other hand, during second-intention healing of a full-thickness wound,

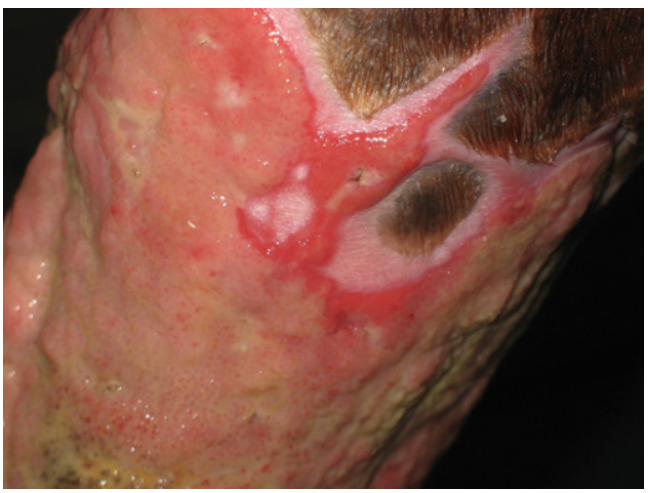


Figure 1.6 Large full-thickness metatarsal wound that healed partially by second intention and was subsequently grafted successfully. The wound showed excellent epithelialization from the healing margin of the wound. The healthy epithelial tissue is characterized by an area of hyperemia adjacent to it. Courtesy of Dr. Derek Knottenbelt.

epithelialization cannot proceed until a bed of granulation tissue has formed. In full-thickness, 7–9 cm^2 wounds created experimentally in horses and ponies and left to heal by second intention, after an initial lag phase, epithelialization progressed over the wound surface at a rate of 0.63 mm/week for metatarsal wounds in ponies, 0.48 mm/week for metatarsal wounds in horses, 0.75 mm/week for buttock wounds in ponies, and 0.62 mm/week for buttock wounds in horses between the third and the seventh week of healing.[38]

The regenerative capacity of the epidermis relies on keratinocyte stem cells (KSC) that reside within specific microenvironments referred to as stem cell niches. The following three niches have been identified: the bulge of the hair follicle (HF), the base of the sebaceous gland, and the basal layer of the interfollicular epidermis (IFE).[39] In response to epidermal injury, the HF and IFE niches participate in epithelialization of the defect.[40]

To close the defect in the epidermis, keratinocytes at the wound's edge must first loosen their adhesions to each other (desmosomes) and to the basal lamina (hemidesmosomes) and develop the flexibility required to migrate over the new matrix. Numerous regulators play a critical role in modulating the proliferation and migration of keratinocytes during epithelialization; these include chemokines, cytokines, integrins, keratins, ECM molecules, and MMPs, among others. A discussion of these is beyond the scope of the text; the reader is referred to a recent review of the subject.[41]

Additionally, various degradative enzymes necessary for the proteolysis of components of the ECM are up-regulated within cells at the leading edge of neo-epithelium, facilitating ingestion of the clot and debris found along the migratory route. This route is determined by the array of integrin receptors for various ECM proteins, expressed on the surface of migrating epithelial cells. Once the surface of the wound is covered by epithelial cells contacting one another, further migration is inhibited by the expression, within the ECM, of laminin, a major factor responsible for adhesion of epithelial cells.

Although initial cellular migration does not require an increase in cellular multiplication, basal keratinocytes at the wound's margin do begin to proliferate 1–2 days after injury to replenish the migratory front; this corresponds histologically to epithelial hyperplasia (Figure 1.7). The new cells leap-frog over those at the wound margin and adhere to the substratum, only to be replaced in turn by other cells coming from above and behind. The newly adherent monolayer subsequently restratifies to restore the original multi-layered 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. Repairing epidermis reassembles its constituents from the margin towards the center of the wound.[17] Epidermal cells then revert to a quiescent phenotype and become attached to this new basement membrane through hemidesmosomes and to the underlying neodermis through type VII collagen fibrils. This particular aspect of epithelialization is time consuming, occurring long after total coverage of the wound by epithelium is apparent, which may explain the continued fragility of neoepidermis for extended periods after repair is macroscopically complete. This is particularly evident in large wounds of the limb, where new epidermis is often thin and easily traumatized (Figure 1.8).

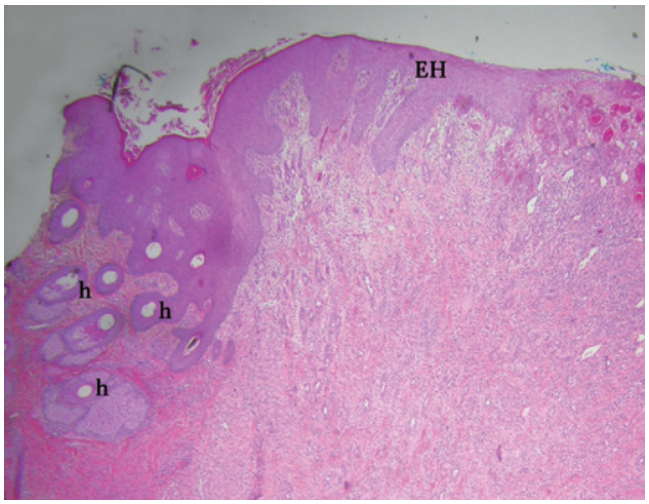


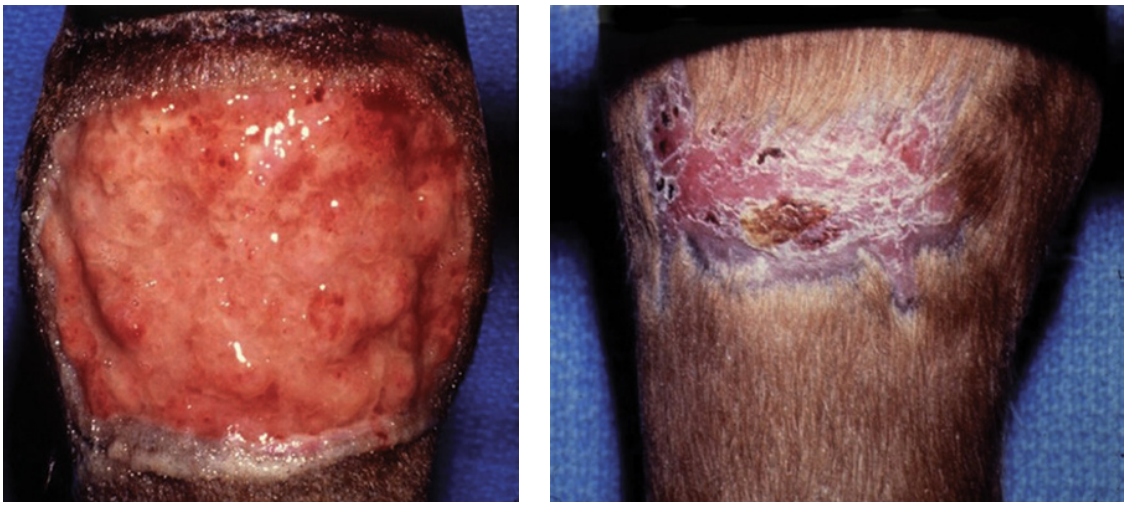
Figure 1.7 Photomicrograph of a wound edge sample of tissue. Normal unwounded skin to the left demonstrating epidermal appendages (h, hair follicles); new hyperplastic epithelium (EH) to the right, overlying granulation tissue bed. Source: Theoret 2004.[42] Reproduced with permission of Elsevier.

Matrix synthesis and remodeling (also referred to clinically as the maturation phase)

Mature ECM is a non-cellular scaffold composed of proteins, glycosaminoglycans, polysaccharides, and water, that facilitates bidirectional communication between cells and their biochemical/biophysical environment.[43] During the remodeling phase (occurring from 3 weeks to up to 1 year after injury), the components of the ECM undergo certain changes to ensure strength, integrity, and function of the replacement tissue.

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.[44] This usually occurs during the second week after injury. Wound contraction not only accelerates closure, it also enhances the cosmetic appearance and strength of the scar because proportionally less area of the wound must be covered by newly formed epithelium of inferior quality, which is fragile and lacks normal nervous, glandular, follicular, and vascular components (Figure 1.8b). For this reason, a high degree of contraction is a desired feature of wound repair, at least in the horse.

Wound contraction is thought to result from a combination of tractional forces generated by migrating fibroblasts and the action of a specialized fibroblast phenotype, the myofibroblast. As fibroblasts migrate into the provisional matrix of the wound under the influence of various cytokines, tension within the wound reaches the threshold required, along with the action of TGF-β1 and the ED-A splice variant of fibronectin,[45] to trigger fibroblasts to differentiate into myofibroblasts.[46] The latter are the most abundant cellular elements of healthy granulation



(a) (b)

Figure 1.8 **(a)** A 5-day-old, full-thickness, experimentally created wound over the dorsal surface of the fetlock. Granulation tissue is beginning to fill the wound. **(b)** The same wound, 75 days after creation. Neoepidermis is thin, dry, and hairless, and could be easily traumatized. Courtesy of Dr. Ted Stashak.

tissue and are aligned within the wound along the lines of contraction. The most striking feature of the myofibroblast is a well-developed alpha smooth muscle actin (α-SMA) microfilamentous system, arranged parallel to the cell's long axis and in continuity with the components of the ECM via various integrins. In addition to these cell–substratum links, intercellular connections, such as gap junctions and hemidesmosomes, ensure that neighboring cells exert tension on one another.

Wound contraction is divided into three phases. An initial lag phase (wherein skin edges retract, and the area of the wound increases temporarily for 1–2 weeks, depending on its anatomic location) occurs prior to substantial fibroblastic invasion of the wound, as a prerequisite for contraction. Subsequently, a period of rapid contraction is followed by one of slow contraction as the wound approaches complete closure. The number of myofibroblasts found in a wound appears proportional to the need for contraction, and thus, as repair progresses and the rate of contraction slows, this number decreases accordingly. During wound contraction, the surrounding skin stretches by intussusceptive growth, and the wound takes on a stellate appearance.

Contraction ceases in response to one of three events: the wound's edges meet, causing contact inhibition to halt the processes of epithelialization and contraction; tension in the surrounding skin becomes equal to or greater than the contractile force generated by the α-SMA of the myofibroblasts; or, in the case of chronic wounds, a low number of myofibroblasts in the granulation tissue may result in failure of the wound to contract, despite laxity in the surrounding skin. In the latter 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 extremity of the equine limb. Although the shape of the wound has been speculated to influence the process of contraction, this does not appear relevant in wounds of the distal limb where skin is tightly stretched and not easily moved.[47]

Wound contraction was measured in full-thickness, 7–9 cm^2 experimental wounds left to heal by second intention on the limbs and hindquarters of horses and ponies. The wounds were bandaged for the first 3 weeks then left unbandaged. Following an initial lag phase of 1 week, wound contraction became apparent in buttock wounds of horses and ponies and in metatarsal wounds of ponies. The percentage decrease in wound surface area attributable to wound contraction between the second and the fourth week of healing was 47% for the body wounds of ponies vs. 35% for the limb wounds of ponies, and 38% for the body wounds of horses vs. 0% for the limb wounds of horses. After week 4, the rate of wound contraction slowed to less than 5% per week for these wounds, up to complete healing. The metatarsal wounds of horses showed a different pattern: the lag phase of healing lasted 4 weeks and this was followed by an average rate of contraction that did not exceed 2.5% per week.[38]

As contraction concludes, myofibroblasts disappear, either by reverting to a quiescent fibroblastic phenotype or by apoptosis,[28] primarily in response to reduced tension within the ECM.[48] The myofibroblast persists in fibrotic lesions where it may be involved in accumulation of more ECM and pathologic contracture, a condition leading to substantial morbidity, particularly when it involves joints or orifices, but rarely encountered in the horse.

The conversion of ECM from granulation to scar tissue constitutes the final phase of wound repair, also referred to as maturation, and consists of synthesis of connective tissue, lysis, and remodeling. Proteoglycans replace hyaluronan during the second week of repair, support the deposition and aggregation of collagen fibers, and make the mature matrix more resilient. Collagen macromolecules provide the tensile strength to the wound as their deposition peaks within the initial week, when healing occurs by first intention, and between 7 and 14 days, when healing occurs by second intention, in the laboratory rodent.

Although this corresponds to the period of most rapid gain in strength, only 20% of the final strength of the skin wound is

achieved within the first 3 weeks of repair. At this time, collagen synthesis is balanced by collagen lysis, which normally prevents accumulation of excessive amounts of collagen and formation of a pathologic scar. 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 reform along lines of stress and, therefore, resist dehiscence more effectively. Cross-linking of the later-formed collagen is also more effective, but never occurs to the same extent as in the original tissue. The new collagen weaves into that which pre-existed and also appears to bond to the ends of old collagen fibers. These welds are points of weakness, which may rupture when stressed.

Remodeling of ECM within a wound depends on the presence of various proteolytic enzymes (proteinases) released from inflammatory and mesenchymal cells, such as MMPs and serine and cysteine proteinases (cathepsins). Those of the MMP family are collectively capable of degrading virtually all components of the ECM. Although MMPs are not constitutively expressed in skin, up-regulation occurs whenever proteolysis is required, such as during cellular migration and remodeling of the matrix. Inactive precursors of the MMPs (pro MMPs) are cleaved in the extracellular space by proteinases, such as plasmin and trypsin, left over from the inflammatory and proliferative phases, but also by other MMPs. To date, two dozen different MMPs, all distinct gene products, have been characterized.[27]

Based on domain organization and preference for substrate, MMPs may be divided into the following groups: 1 – collagenases; 2 – gelatinases; 3 – stromelysins; 4 – matrilysins; 5 – metalloelastases; 6 – membrane-type MMPs; 7 – other MMPs.[49] Although the major function of most MMPs is probably to process bioactive molecules, such as chemokines and cytokines, as well as their respective receptors, their ability to degrade ECM proteins, as demonstrated by some members of the MMP family (MMP1, MMP3, MMP13, and MMP14 are capable of cleaving collagen; MMP7 can process syndecan-1 and elastin), suggests they have a role in remodeling during wound healing.[27] Comprehensive lists of MMPs, including their physiologic and *in vitro* substrates, can be found in proteinase databases.[50,51]

Homeostasis between collagen synthesis and degradation during the remodeling phase depends on the simultaneous presence of MMPs and non-specific inhibitors, such as α2-macroglobulin and α1-antiprotease, as well as natural specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). TIMPs are a gene family of four structurally related members, TIMP-1 through -4, that inhibit conversion of MMPs from a zymogen to an activated state and that irreversibly bind to the catalytic site of active MMPs.[49]

Under most circumstances, an imbalance between MMPs and TIMPs leads to abnormal resolution and delayed repair. Indeed, although the presence of MMPs is essential for the wound to mature, it may also be responsible for the inability of chronic wounds to heal. For example, fluid found in chronic wounds is characterized by elevated concentrations of proteinases, particularly MMP-9 and serine proteinases, which lead to excessive degradation of proteins and the inactivation of critical growth factors. Chronic wounds also contain reduced concentrations of TIMPs, in particular TIMP-1.[52]

Wound remodeling continues for up to 2 years, during which time there is no net increase in collagen content, but instead, the collagen fibers rearrange into a more organized lattice-like structure, under the influence of local mechanical factors, progressively increasing the tensile strength of scar tissue. The majority of type III collagen fibers laid down early in healing are replaced by collagen type I, fibers become increasingly cross-linked, and the normal skin ratio of 4:1 type I to type III collagen is re-established. Glycosaminoglycans are steadily degraded until they reach concentrations found in normal dermis. The duration of the maturation phase depends on a variety of factors, including the patient's genetic makeup, age, location of the wound, type of injury, and duration of inflammation.

At maximum strength, cutaneous wounds in mice remain 15–20% weaker than the normal surrounding tissue,[53] but a study in horses showed that cutaneous wounds, fully healed by second intention, withstood a maximum breaking load equivalent to only 60% of the breaking force of normal, intact skin.[54] Importantly, skin appendages are usually not regenerated after wounding, resulting in a loss of other functions of skin.

Non-invasive methods to monitor healing

There are objective, non-invasive methods to monitor the progress and assess the quality of healing of cutaneous wounds, focusing on anatomic, mechanical, physiologic, and esthetic properties.[55] The goal of these technologies is to provide detailed information regarding skin components imperceptible to visual inspection and that enable the clinician to assess the severity of a wound and its healing potential, thereby guiding therapeutic decisions. Optical technologies that have, to date, been applied to wound assessment in humans include: near infrared imaging, thermal imaging, optical coherence tomography, orthogonal polarization spectral imaging, fluorescence imaging, laser Doppler imaging, microscopy, spatial frequency domain imaging, photoacoustic detection, and spectral/hyperspectral imaging.[56] Both infrared thermography (IRT) and near infrared spectroscopy (NIRS) have been used in the author's laboratory to study the healing of experimental wounds in horses.[57,58] Near infrared spectroscopy provides quantitative information on the structural and chemical components of cutaneous tissue, specifically oxygen saturation, hemoglobin, and water content. This tool enabled the identification of hypoxia in limb wounds relative to body wounds during the early phase of healing in horses.[57] Concomitantly, cutaneous wound temperature, as measured by IRT, and by extension skin blood flow, was found to be significantly lower in limb wounds than in body wounds.[58] Because low oxygen levels may promote a feeble yet prolonged inflammatory response to wounding, which could interfere with and retard the subsequent phases of healing, these experimental data aid our understanding of the impaired healing that commonly afflicts horses, particularly where limb wounds are concerned.

Other tools allow the measurement of wound dimensions to improve objective monitoring during healing, in view of adjusting the treatment plan, if required, and facilitating communication with the horse owner. A couple of recent studies have evaluated the accuracy and reliability of some of these methods when used in horse wounds.[59,60] Two-dimensional (2-D) measurements of the area of the scar/wound can be taken using manual planimetry, digital planimetry or digital imaging combined with computer-aided analysis. With manual planimetry, the borders of the wound/scar are traced on to sterile acetate film placed over the wound

and used to approximate measurements of area by counting squares of known size within the boundaries of the tracing on a superimposed reference grid (i.e., graph paper). With digital planimetry, acetate tracings of the wound are digitized by retracing them on to a tablet, allowing automated calculations.[59] Digital imaging and computer-aided analysis are based on the concurrent viewing of a reference scale/ruler on images of the wound (placed for the purpose of calibration) and calculation of measurements of area from tracings of the image using a commercial software, such as the open-source image processing program, Image J.[61] Although 2-D planimetry provides a reproducible and inexpensive way of measuring the surface area of a scar/wound,[62] useful to quantitate epithelialization and contraction of a healing wound, it does not capture the specific characteristics of a granulation bed, nor does it allow measurements of the wound's volume. Quantitative topographic methods allowing 3-D assessments have been tested in wounds of horses, and based on these tests, investigators found that stereophotogrammetry can be applied for 3-D reconstructions and analyses of equine wounds using a self-developing camera system and commercially available software (Photomodeler Scanner).[59] A laser beam wound camera (SilhouetteStar, ARANZ Medical) was also shown to be capable of accurately determining the area and depth of experimental wounds on horse cadavers.[60]

Mediators of wound repair

Wound repair relies on a complex amalgamation of interactive processes involving formed elements of blood (e.g., erythrocytes, platelets, leukocytes), ECM, and mesenchymal cells. Although histologic and morphometric observations have lead to a detailed description of the kinetics of cellular and macromolecular components involved in repair, much remains to be learned about the regulation of such activities. Restoration of structural integrity and partial functional properties appears to rely on soluble mediators, synthesized by cells present within the wound or within the surrounding tissue, that coordinate migration, proliferation, and synthesis of proteins by the various cellular populations involved in the repair process.

Cytokines, defined as 4–60 kDa signaling glycoproteins released by most nucleated cells, are among the most important soluble mediators regulating wound repair. They act in concentrations of 10^{9}–10^{12} M in an autocrine (same cell), paracrine (adjacent cell), or endocrine (distant cell) fashion, via cell surface receptors. Receptors are proteins with an extracellular site to bind the cytokine and a transmembranous component to transmit the signal to the intracellular site where it must reach nuclear DNA for a specific response to occur. Cells may have different numbers of receptors for different factors; the concentration of factors in the area and the number of receptors that are bound determine the response generated. Growth factors are cytokines that exert primarily mitogenic influences. Both *in vivo* and *in vitro* studies analyzing non-healing wounds have shown deregulation of various cytokines, suggesting a potential target for therapy that has led to a robust interest in using exogenous cytokines in the clinical setting to improve outcomes of healing wounds.[63]

The cytokines that play a significant role in wound repair are summarized in Table 1.1. For more detail, the reader is referred to a comprehensive review of the topic.[64]

Table 1.1 Cytokines involved in wound repair.

Name	Abbreviation	Source	Major function
Granulocyte macrophage colony stimulating factor	GM-CSF	Macrophage, lymphocyte, fibroblast, endothelial cell	Differentiation and maturation of hematopoietic stem cells; recruitment of inflammatory cells; mediation of epidermal proliferation; promotion of myofibroblast differentiation and wound contraction
Interferon	IFN	Monocyte and macrophage, lymphocyte, mesenchymal cell	Proinflammatory; release of other cytokines; inhibits fibrosis
Interleukin	IL	All nucleated cells, in particular macrophage and lymphocyte	Proinflammatory; enhances epithelialization, angiogenesis, and remodeling
Tumor necrosis factor	TNF	Macrophage, lymphocyte, mast cell	Proinflammatory; enhances angiogenesis, epithelialization, and remodeling
Connective tissue growth factor	CTGF	Fibroblast	Mediator of TGF-β activity (cell proliferation and ECM accumulation)
Epidermal growth factor	EGF	Platelet, saliva	Epithelialization; chemotactic and mitogenic to fibroblast
Transforming growth factor-α	TGF-α	Macrophage, epithelial cell	MMP synthesis (remodeling); angiogenesis
Fibroblast growth factor (basic)	FGF-2	Inflammatory cell, fibroblast, endothelial cell, keratinocyte	Chemotactic and mitogenic to fibroblast and epithelial cell; protein synthesis; angiogenesis
Insulin-like growth factor	IGF	Liver, platelet	Chemotatic and mitogenic to endothelial cell; migration of epithelial cell; fibroblast proliferation; protein and GAG synthesis
Keratinocyte growth factor	KGF (FGF-7)	Fibroblast	Chemotactic and mitogenic to epithelial cell; mitogenic to endothelial cell
Platelet-derived growth factor	PDGF	Platelet	Chemotactic to inflammatory cell and smooth muscle cell; increases keratinocyte motility; mitogenic to fibroblasts; enhances protein synthesis; induction of myofibroblast phenotype
Transforming growth factor-β	TGF-β	Platelet, lymphocyte, mast cell, monocyte and macrophage, endothelial cell, epithelial cell, fibroblast	Chemotactic to inflammatory and mesenchymal cell; fibroblast proliferation; protein synthesis; ECM deposition (inhibition of MMP; induction of TIMP); wound contraction
Vascular endothelial growth factor	VEGF	Macrophage; fibroblast; endothelial cell; epithelial cell	Angiogenesis

Conclusion

The equine practitioner, when presented with a wounded horse, should fully understand the physiologic mechanisms underlying repair to enable design of an appropriate treatment plan. In the following chapters of this book, experienced authors share their opinion on how to best manage specific injuries. The reader benefits from a detailed understanding of the different phases of healing, as well as thorough knowledge of the mediators governing them, because these dictate the approach to follow, particularly when the wound is complicated by chronic inflammation and/or an excessive proliferative response.

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CHAPTER 2

Differences in Wound Healing between Horses and Ponies

Jacintha M. Wilmink, DVM, PhD

Chapter Contents

Summary

Observing differences in wound healing between horses and ponies has provided valuable information about the intrinsic process of wound healing and the common complications encountered when managing traumatic wounds in the equid. Ponies heal faster and with fewer complications than do horses. To a large extent, these differences can be explained by disparity in the local inflammatory response, which, in turn, relates to differences in the functional capacity of leukocytes. Research data indicate that a maximal effect of treatment will be obtained in clinical practice if a differential approach is used, optimizing conditions for each successive phase of wound healing. In particular, the effect of treatment on the inflammatory response is of paramount importance to the other phases of healing and should, therefore, always be considered when managing a wound. When treating wounds healing by second intention, inflammation should be stimulated until the wound has filled with granulation tissue, and thereafter it should be inhibited.

Horses and ponies: same species, different healing characteristics

The horse evolved over the course of millions of years from a small forest dweller to a large ungulate that inhabited the vast open plains of the temperate zone. It became a "flight" animal whose instinctive reaction to danger is to run.[1] Evolution took place as a response to various environmental and climatic challenges, and the development of special features resulted from selection by mankind. Both evolution and selection led to the large variety of breeds known today.

The equine species can be roughly subdivided into horses and ponies, and this division is determined by the height of an adult at the withers (ponies are <1.48 m). Whether ponies are just small horses has been debated for decades. The discussion of this topic, with respect to wound healing, started in the 1980s when Bertone *et al.*[2] found, in a study examining second-intention healing, that wounds of ponies heal faster than those of horses and without the formation of exuberant granulation tissue (EGT). Other authors, however, reported that wounds of ponies do develop EGT,[3,4] and the faster healing of wounds in ponies could not be confirmed.[4] Because a difference in wound healing between horses and ponies might provide information about the basic biology of equine wound healing and about the complications commonly associated with wound repair in this species, Wilmink *et al.* performed a series of experiments to investigate differences in wound healing between horses and ponies.[5–7] They proved that skin wounds in ponies heal faster and with fewer complications than do similar wounds in horses and demonstrated that these differences were based on the

Equine Wound Management, Third Edition. Edited by Christine Theoret and Jim Schumacher.

Companion website: www.wiley.com/go/theoret/wound

efficiency and capacity of the leukocyte to produce various mediators.[8,9] Differences in healing are, however, apparently not related to body size. This is confirmed by a study in donkeys and Caspian miniature horses, equine subspecies of similar size, which showed that wound healing in donkeys is faster than healing in Caspian miniature horses.[10]

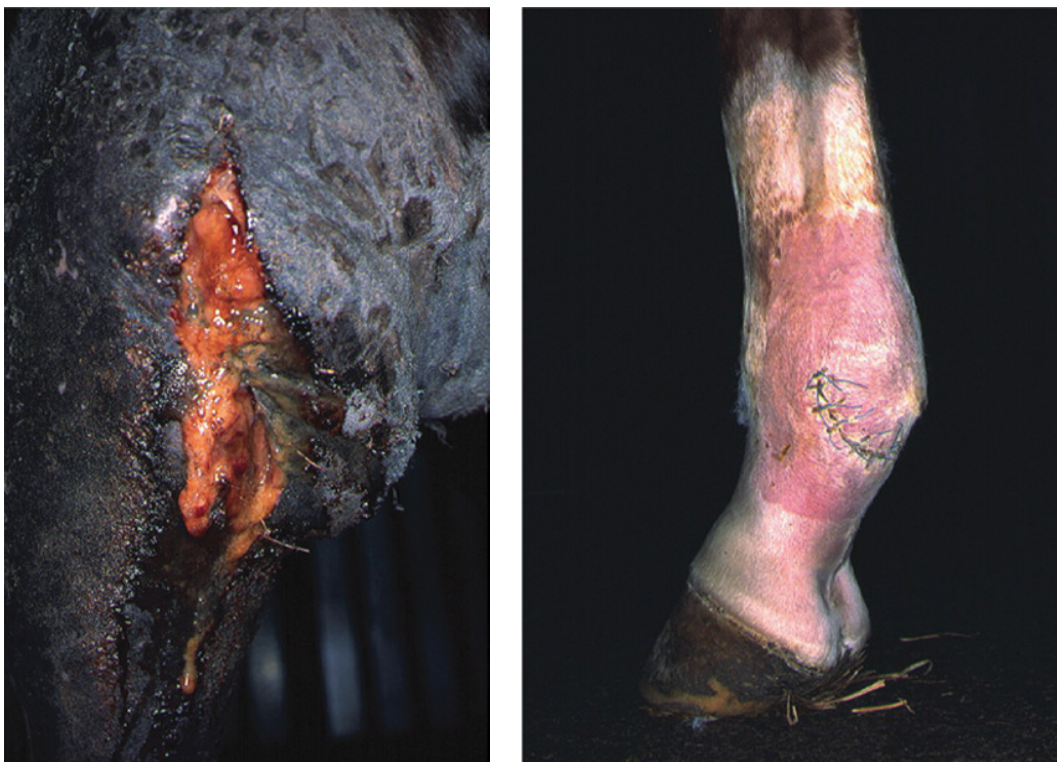
What remains to be elucidated is the cause of the observed differences in healing between horses and ponies. The longer period of domestication of the horse may have precluded natural selection against poor healing, because the wounded horse was/is tended to by man. Additionally, the artificial selection of various features, such as height, athletic capacity, and appearance, might have favored the development of some diseases and undesirable characteristics, including the reduced efficiency of leukocytes. Moreover, horses with wounds so poorly healed that athletic ability is impaired are often used for breeding, thus perpetuating a hereditary tendency toward poor healing. In contrast, ponies have been domesticated for a much shorter time, and, consequently, a poor capacity for healing may have been eliminated by natural selection. Moreover, artificial selection within pony breeds has been less intense, and many pony breeds maintain subpopulations (and genetic reserves) in the wild. The hypothesis that natural and artificial selection have an influence on wound healing is supported by the faster healing of donkeys compared to Caspian miniature horses,[10] the latter being selected based on a special feature, namely their speed.

The physiologic and pathophysiologic differences in healing observed between horses and ponies with experimentally induced wounds have since been corroborated by other research[7–9,11,12] and by clinical observation. These observations have resulted in improvements in guiding the management of wounds of equids and led to faster healing and the prevention of complications.

First-intention healing (primary wound closure)

Primary closure of traumatic wounds of horses is preferred over healing by second intention because healing by primary intention is usually faster, and the cosmetic and functional results are better. Unfortunately, either partial or complete wound dehiscence may ensue after primary closure. Whether or not a wound dehisces depends on many factors, including those relating to the wound itself, to the horse, to the treatment, and to the environment.[13,14]

The success of primary closure of traumatic wounds was evaluated retrospectively by Wilmink *et al*. in more than 500 equine patients admitted to a referral clinic.[7] The study revealed that primary closure is significantly more often successful in ponies than in horses (Figure 2.1), and that bone sequestra form significantly less often in ponies when bone is exposed during



(a) (b)

Figure 2.1 **(a)** Wound on the elbow of a horse, which suffered dehiscence following primary closure, as a result of infection. **(b)** Wound on the distal aspect of the limb of a 4-year-old pony with an open fetlock joint and damage to the lateral collateral ligament. The wound was sutured approximately 8 hours after it occurred and it healed successfully, without dehiscence. Source: (b) Wilmink 2004.[15] Reproduced with permission of Elsevier.

injury. The predominant cause of wound dehiscence and of sequestration of bone is infection.[7,16,17] Other factors contributing to dehiscence are tension on the wound's margins, excessive movement of the sutured region, and involvement of certain structures, such as tendons, ligaments, and synovial cavities. Other factors besides infection contributing to formation of a sequestrum include exposure of cortical bone and extensive trauma.[18] The risk of infection is influenced by many factors, including the time elapsed since injury, the degree of contamination, the degree of tissue damage, and the thoroughness of debridement and irrigation. Wound debridement determines the concentration of bacteria left in the wound and is one factor that can be controlled clinically.[13,14,17] The concentration of bacteria, in combination with factors at the wound that facilitate bacterial colonization, such as the presence of dead space, a hematoma, and devitalized tissue, is critical to whether or not a wound becomes infected (the reader is referred to Chapter 4 for more information about factors involved in the development of infection). Although bacterial colonization and development of infection are greatly influenced by the administration of antibiotics, the effectiveness of the patient's own local defense, its acute inflammatory response, is as least as important to the prevention of infection.[19]

In the aforementioned retrospective study by Wilmink *et al.*, all these factors relating to wound infection were evaluated and compared between horses and ponies.[7] Ponies and horses did not differ with respect to age and gender, nor with respect to wound characteristics, such as location, duration, contamination, and treatment. The wounds of ponies, however, were generally deeper, and ponies were more apt to suffer laceration of an extensor tendon, to incur damage to the periosteum, to have exposed bone, and to have their wound closed while standing, rather than while anesthetized. Additionally, ponies were significantly less likely to receive antimicrobial therapy and to be administered a non-steroidal anti-inflammatory drug (NSAID). Ponies were, therefore, at greater risk of bacterial challenge than were horses because their wounds were deeper and debridement was less complete since it was performed with the ponies standing, and because they were less likely to receive antimicrobial therapy. In spite of this greater risk, wound infection occurred less often, implying that the acute inflammatory response in ponies is more effective than that of horses at reducing bacterial contamination. The more frequent use of NSAIDs in horses, however, may have contributed to a reduced effectiveness of the inflammatory response.

Second-intention healing

Wounds are allowed to heal by second intention when closure is not feasible or affordable, or when a wound dehisces after being closed primarily. Second-intention healing was investigated in horses and ponies by creating excisional wounds on the metatarsi and buttocks that extended through the periosteum of the metatarsal bone or 18 mm into the muscle on the buttocks, in imitation of clinical wounds.[5,6] This investigation found that wounds of ponies heal significantly faster than do wounds of horses (Figure 2.2) and that the speed and efficiency of healing seem related to remarkable differences between horses and ponies in the phases of healing.[5,6] Subsequent studies showed that these differences can be attributed to variations in the function of leukocytes.[8,9,11]

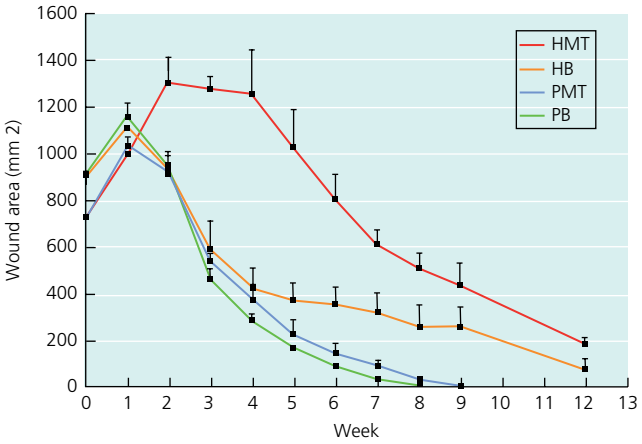


Figure 2.2 Wound area as a function of time (mean + s.e.m.). HMT, metatarsal wounds of horses; HB, body wounds of horses; PMT, metatarsal wounds of ponies; PB, body wounds of ponies. Source: Wilmink 1999.[5] Reproduced with permission of John Wiley & Sons.

Clinically apparent phases during wound healing

Wound healing is often divided into general phases of hemostasis, inflammation, proliferation, and synthesis and remodeling of matrix (see Chapter 1). Because these phases overlap and occur simultaneously in all tissue components, distinguishing them from one another is difficult. Consequently, this division is somewhat academic. In a clinical setting, dividing healing into the following macroscopically apparent events may be more practical: inflammation, formation of granulation tissue, wound contraction, and epithelialization. Although these events also overlap, they largely succeed one another and occur more or less chronologically. Moreover, they are clearly visible to the practitioner observing second-intention healing.

Inflammation

The inflammatory response to wounding is more prompt in ponies, as demonstrated by comparing the leukocytic infiltration of experimental wounds of ponies and horses.[6] High numbers of polymorphonuclear cells (PMNs) are found during the first 3 weeks of healing, after which the PMNs disappear rapidly from the wounds of ponies. In contrast, the influx of PMNs is sluggish and weak in horses, but PMNs persist so that, beginning 4 weeks after wounding, the number of PMNs exceeds those in the wounds of ponies.[6]

The leukocytes of ponies are more efficient than those of horses. An *in vitro* study showed that the leukocytes of ponies produce more reactive oxygen species, such as H_2O_2

or O^- radicals, necessary for bacterial killing after phagocytosis.[9] Within tissue cages implanted subcutaneously and in newly formed granulation tissue, the leukocytes of ponies also produce higher concentrations of other inflammatory mediators, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, and transforming growth factor (TGF)-β, essential to the reinforcement of the inflammatory response, to the induction of fibroplasia, and to wound contraction.[8,9] The superior production of these mediators can thus explain the higher initial influx of leukocytes into wounds of ponies.

Migrated leukocytes, in turn, release more biologically active substances, thus creating a positive feedback that enhances the inflammatory response.[19,20] This feedback may account for the faster debridement of non-viable tissue and fibrin in wounds of ponies, leading to the faster development of a healthy bed of granulation tissue. In contrast, granulation tissue in the wounds of horses retains an irregular and purulent appearance, along with persistent deposits of fibrin.[5,6] An enhanced inflammatory response observed in ponies can, likewise, translate into a more efficient local defense against contaminating bacteria, leading to a superior control of wound infection.[7] A stronger acute inflammatory response, thus, averts the development of chronic, low-grade inflammation and leads to a faster and more thorough preparation of the wound for repair. Indeed, chronic inflammation, such as that seen in horses,[6] perpetuates the release of tissue-damaging lysosomal enzymes, as well as mediators, such as TGF-β, which in turn, over-stimulate fibroplasia and lead to the formation of EGT, which inhibits contraction.[19,21–23]

In summary, the inflammatory response to wounding in ponies is stronger and more succinct; this response appears to be more efficient for healing. In contrast, the inflammatory response in horses is weak in onset but persists over time, and this poor but persistent response may be due to the lower initial production of inflammatory mediators.

Formation of granulation tissue

In a study by Wilmink *et al.*, granulation tissue formed faster in experimental wounds of horses than in those of ponies.[5] The abundant granulation tissue in the wounds of the limbs of horses was observed to push the edges of the wound apart, which may explain why experimental wounds on the limbs of horses enlarged so dramatically during the first 2 weeks after they were created (Figure 2.2). Between weeks 2 and 3 of healing, the granulation tissue of all wounds on the limbs and buttocks of horses and ponies protruded above the level of the surrounding skin and was characterized as excessive (clinically referred to as EGT or "proud flesh"); protrusion of granulation tissue was most prominent in wounds on the limbs of horses (Figure 2.3). The wounds were left unbandaged after week 3, and during week 4 EGT disappeared spontaneously from wounds, except those on the limbs of horses, where the excess tissue had to be trimmed to promote healing.[5] The granulation tissue in horses was traversed by grooves and clefts for a much longer period, and presented a yellowish purulent surface up to week 5 after wounding. The appearance of the granulation tissue on the limbs may have been due to the weak and delayed onset followed by prolongation of the inflammatory phase. In contrast, the granulation tissue in the wounds of the ponies was smooth, regular, and achieved a healthy pink color significantly sooner (Figure 2.4).[5] It was microscopically apparent that fibroblasts continued to proliferate in horse wounds even after granulation tissue filled the wound bed, whereas fibroblasts in pony wounds ceased proliferating at this time. Granulation tissue in wounds of horses presented a chaotic cellular pattern and appeared persistently inflamed, whereas it was organized regularly in pony wounds (Figure 2.5).[6]

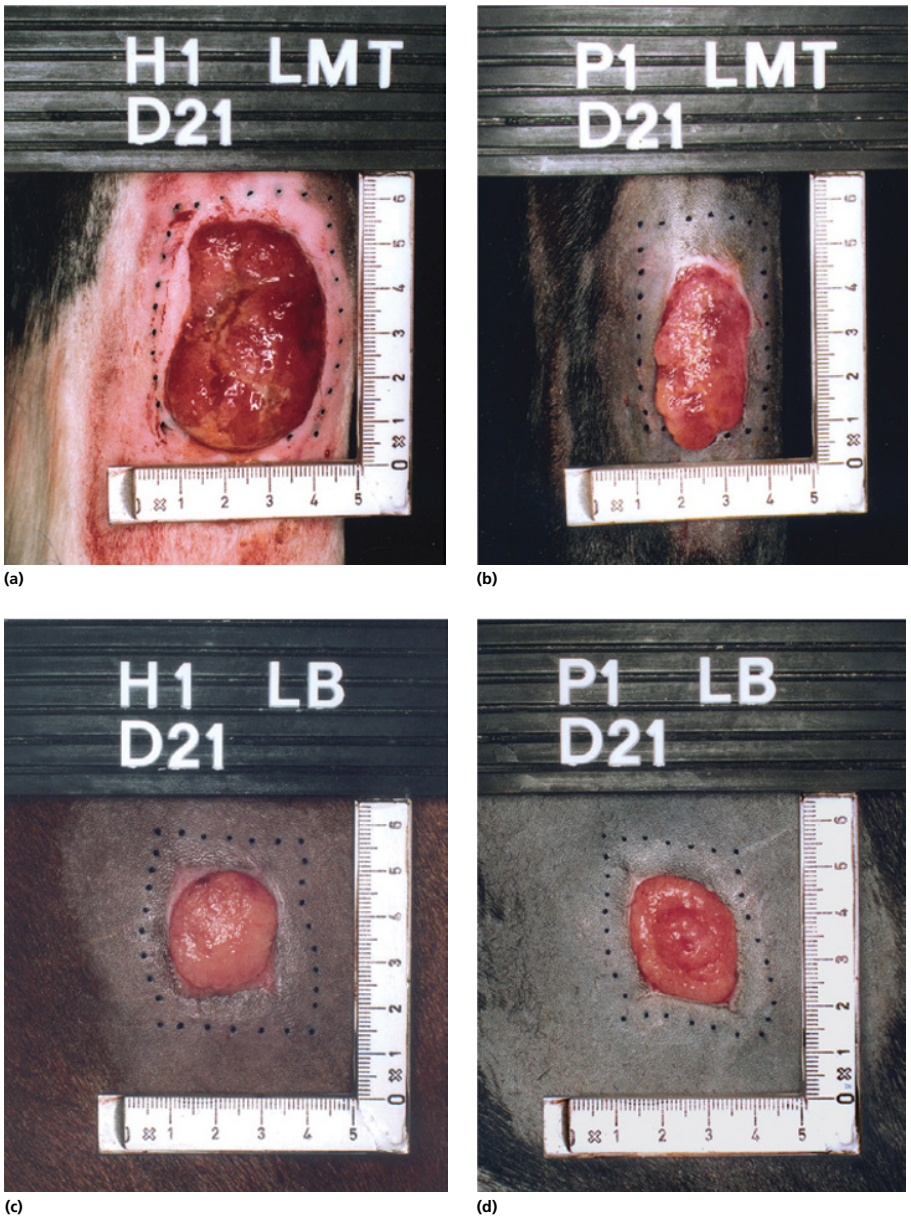
There may be a causal relationship between persistent inflammation and the continuous proliferation of fibroblasts and the synthesis of granulation tissue, via the activity of mediators, such as TNFα, IL-1, IL-6, platelet-derived growth factor (PDGF), TGF-β, and basic fibroblast growth factor (bFGF), which are known to induce fibrosis.[22–24] However, the formation of granulation tissue was less extensive in the wounds of ponies than in those of horses, particularly those located on the limb, even though the granulation tissue of ponies initially contained higher concentrations of TNFα, IL-1, and TGF-β,[5,8,9] which mediate the migration and proliferation of fibroblasts and endothelial cells.[22] Furthermore, fibroblasts from limbs of ponies are known to proliferate faster *in vitro* than those of horses,[11,25] even though fibroplasia seems more rapid, *in vivo*, in horses. Apparently, the balance of mediators, the interaction of mediators with other factors, and time-scale of the presence of mediators *in vivo* are more important than the absolute concentration of mediators in determining the rate of cellular growth, stressing once more the significance of the overall course of the inflammatory response.

The formation of granulation tissue in horses is excessively fast, compared to that of other species[26] and to what is observed in ponies.[5] The fast formation and persistent proliferation of granulation tissue, caused by an unrelenting inflammatory response, clearly contribute to the formation of EGT.[5,6]

Wound contraction

Contraction of wounds of ponies is faster and significantly more pronounced than is contraction of wounds of horses, and contraction of wounds on the body is faster and significantly more pronounced than is contraction of wounds on the limb (Figure 2.6).[5] As a result, second-intention healing is significantly faster in ponies than in horses and significantly faster in wounds on the body than in wounds on the limb (Figure 2.2).[5]

Histologic examination of wounds showed that the myofibroblasts in newly formed granulation tissue of wounds of ponies are organized into a regular pattern within 2 weeks after wounding; the cells are oriented perpendicular to the vessels and parallel to the wound's surface. This pattern is thought to enhance contraction because a good bond between fibroblasts and the surrounding ECM is required for the contractile forces exerted by smooth muscle actin filaments within the fibroblasts



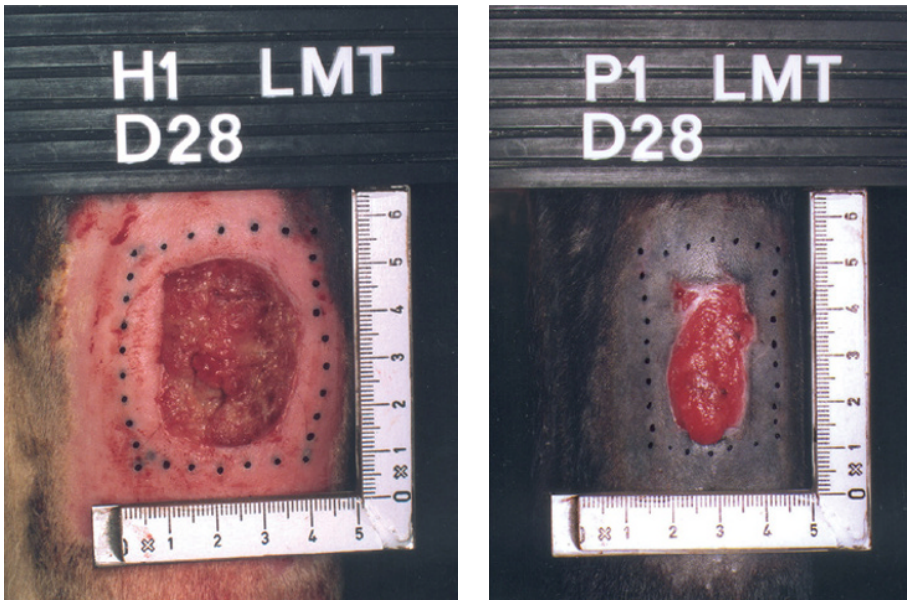
(a) (b) (c) (d)

Figure 2.3 After 3 weeks, the limb wounds of horses **(a)** contained more exuberant granulation tissue than the limb wounds of ponies **(b)**. In the same timeframe, the wounds on the buttocks of horses **(c)** and ponies **(d)** had formed some exuberant granulation tissue protruding above all wound margins. Source: (a and b) Wilmink 2004.[15] Reproduced with permission of Elsevier.

to pull the margin of the wound centripetally (i.e., towards the center of the wound).[27,28] Although the number of fibroblasts and the amounts of smooth muscle actin and collagen do not differ between wounds of horses and those of ponies, the alignment of myofibroblasts of horses is delayed (Figure 2.5).[6]

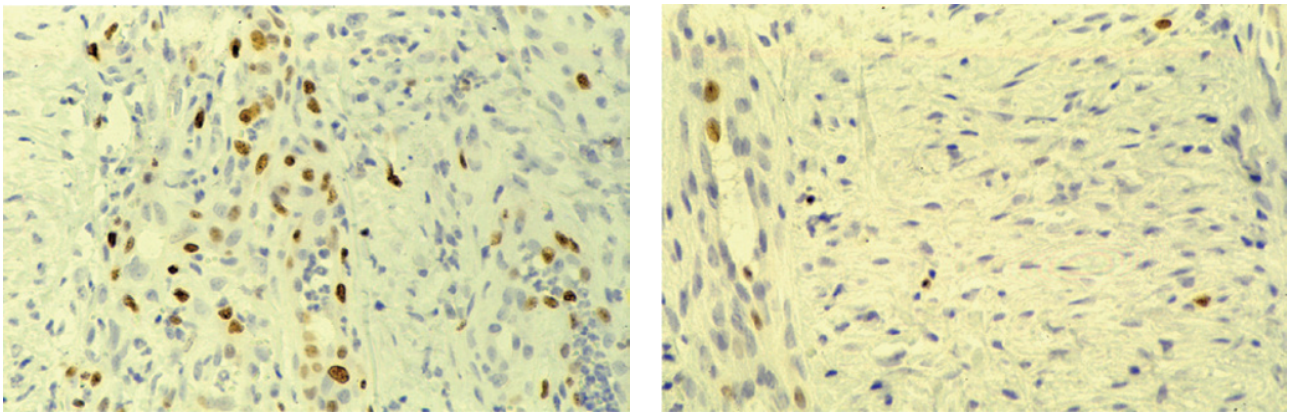
Wound contraction occurs when the forces exerted by myofibroblasts exceed centrifugal (i.e., outward) forces and the local resistance to displacement. Centrifugal forces present in the skin of horses and ponies are similar, as evidenced by the identical enlargement that occurs immediately after the creation of experimental wounds (Figure 2.2). Moreover, there is no reason to believe that the local resistance to contraction in horses and ponies should differ. Variations in wound contraction are, therefore, most likely related to differences in the contractile force generated by myofibroblasts within the granulation tissue.

Surprisingly, the inherent capacity of fibroblasts of ponies to contract, *in vitro*, is similar to that of horses,[11] suggesting that factors in the wound, such as the presence of inflammatory mediators, determine the contractile forces exerted by myofibroblasts and hence the extent of contraction. Indeed, inflammatory mediators, in particular TGF-β, wield major effects on contraction. Interestingly, the concentration of TGF-β



(a) (b)

Figure 2.4 **(a)** By 4 weeks after wounding, the granulation tissue of the limb wounds of horses was traversed by grooves and clefts, and on its surface was a purulent exudate. **(b)** The granulation tissue of pony wounds, on the other hand, was smooth, regular, and had a healthy pink color. Source: Wilmink 2004.[15] Reproduced with permission of Elsevier.



(a) (b)

Figure 2.5 Typical microscopic appearance of the granulation tissue of metatarsal wounds 6 weeks after wounding (DAP-filter, MIB). **(a)** In horses, many brown-colored cells, actively synthesizing DNA and preparing for mitosis, were present. Note the irregular arrangement of the fibroblasts in the tissue and the presence of polymorphonuclear leukocytes. **(b)** In ponies, only a few brown-stained cells are seen in the regularly organized granulation tissue. Source: Wilmink 1999.[6] Reproduced with permission of John Wiley & Sons.

is significantly higher in the newly formed granulation tissue of experimental wounds of ponies.[8] This may explain the faster organization of myofibroblasts and the more extensive contraction observed in wounds of ponies because TGF-β stimulates the differentiation of fibroblasts into myofibroblasts;[29] induces α-smooth muscle actin, α1β1 and α1β2 integrins, collagen, and fibronectin, all factors necessary to wound contraction;[30] and enhances contractile forces.[31] Furthermore, because other inflammatory mediators, such as prostaglandin (PG)E_1, PGE_2, TNFα, IL-1, IL-6, and interferon (IFN)-γ inhibit contractility,[32] a chronic inflammatory response, characteristic of wounds of horses, may exacerbate the deficiency of contraction noted in wounds of horses.

In summary, wounds of ponies heal faster by second intention than do wounds of horses because contraction contributes more to closure of wounds of ponies than to wounds of horses (Figures 2.2 and 2.6). The differences in contraction are not caused by disparity in the innate contractile capacity of the fibroblasts but by the balance of mediators in the wound's environment. The poorer contribution of contraction to closure of wounds on the limb of horses can be attributed to the initial low production of TGF-β and the frequent occurrence of chronic inflammation.[5,6,8]

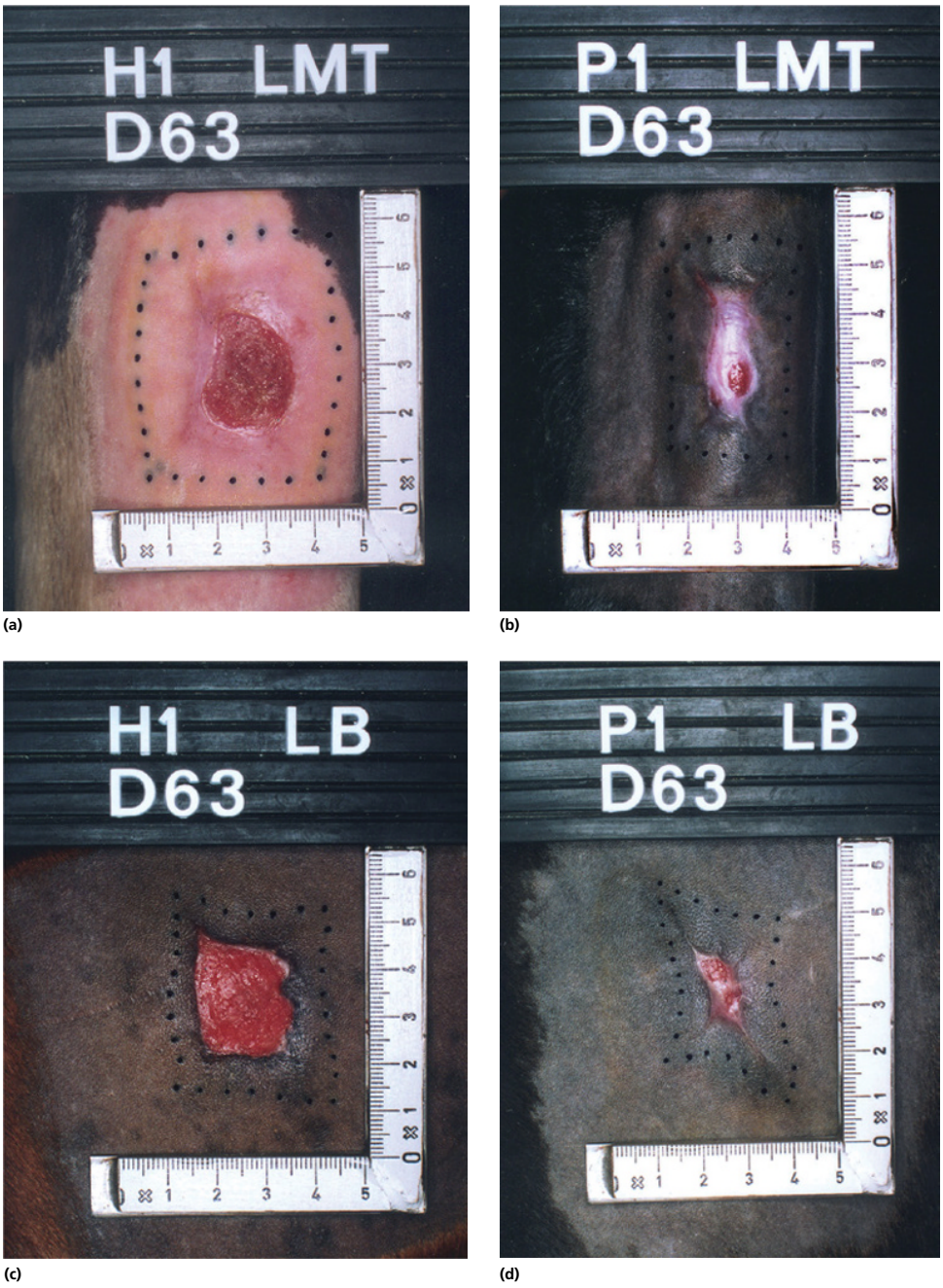


Figure 2.6 After 9 weeks of healing, the contribution of contraction to wound closure can be appreciated by observing the tattoo patterns close to the original margin of the wound. **(a)** The metatarsal wounds of horses have decreased in size. The tattoos show that the wounds have contracted minimally, whereas pronounced epithelialization is visible. **(b)** Closure of the metatarsal wounds of ponies was, to a large extent, the result of contraction and some epithelialization. **(c)** The buttock wounds of horses also healed mainly by contraction, with a small amount of epithelialization. **(d)** The buttock wounds of ponies healed, mainly by contraction, with very little epithelialization. The scar of this pony was unfortunately superficially damaged while shaving the hairs around the scar for the photographs. Source: (a and b) Wilmink 2004.[15] Reproduced with permission of Elsevier.

Epithelialization

Proliferation of epithelial cells in experimental wounds of horses was similar to that of epithelial cells in experimental wound of ponies during the first weeks of healing. Cellular proliferation was temporarily reduced when EGT developed at 3 weeks in wounds on the limbs and the buttocks of horses and ponies.[6] After the third week of healing, an inverse correlation developed between the area of epithelialization and wound contraction, so that wounds contracting the most epithelialized the least (pony versus horse wounds, buttock versus limb wounds) (Figure 2.6, Figure 2.7).[5] This inverse correlation likely developed because the circumference of the wound margin

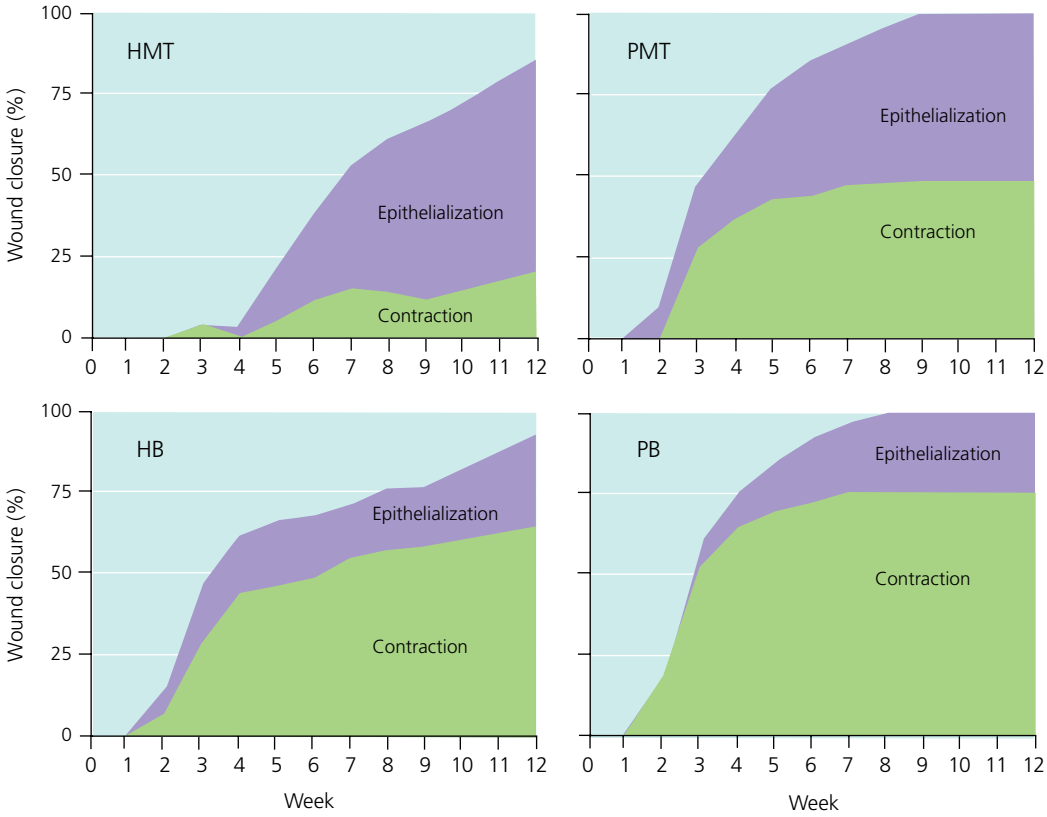


Figure 2.7 Relative contribution of contraction and epithelialization to wound closure. For abbreviations see Figure 2.2. The correlation between the epithelialized area and wound contraction is inversed, so that wounds demonstrating the most contraction show the least amount of epithelialization (pony versus horse wounds, body versus limb wounds). Source: Wilmink 1999.[5] Reproduced with permission of John Wiley & Sons.

furnishing migrating and proliferating epithelial cells decreased. Thus, more epithelialization was seen when wound contraction was poor, such as occurred in wounds on the limb of horses (Figure 2.6).[5] Epithelialization of these poorly contracting wounds was significantly faster than was epithelialization of rapidly contracting wounds from 6 weeks onward, resulting in the largest area of newly formed, inferior-quality epithelium and the most pronounced scars (Figure 2.8).[5]

Epithelialization is also hindered by persistent inflammation, which promotes release of toxic products and lysosomal enzymes from leukocytes. These toxic products not only damage tissue but also inhibit epithelial mitosis by altering the critical balance of cytokines and growth factors upon which epithelial cells depend.[33] Additionally, epithelial mitosis appears to be negatively influenced by the presence of EGT and/or factors inducing EGT,[34] as suggested by the finding that the mitotic activity of epithelial cells in all wounds temporarily diminishes when EGT is present.[6]

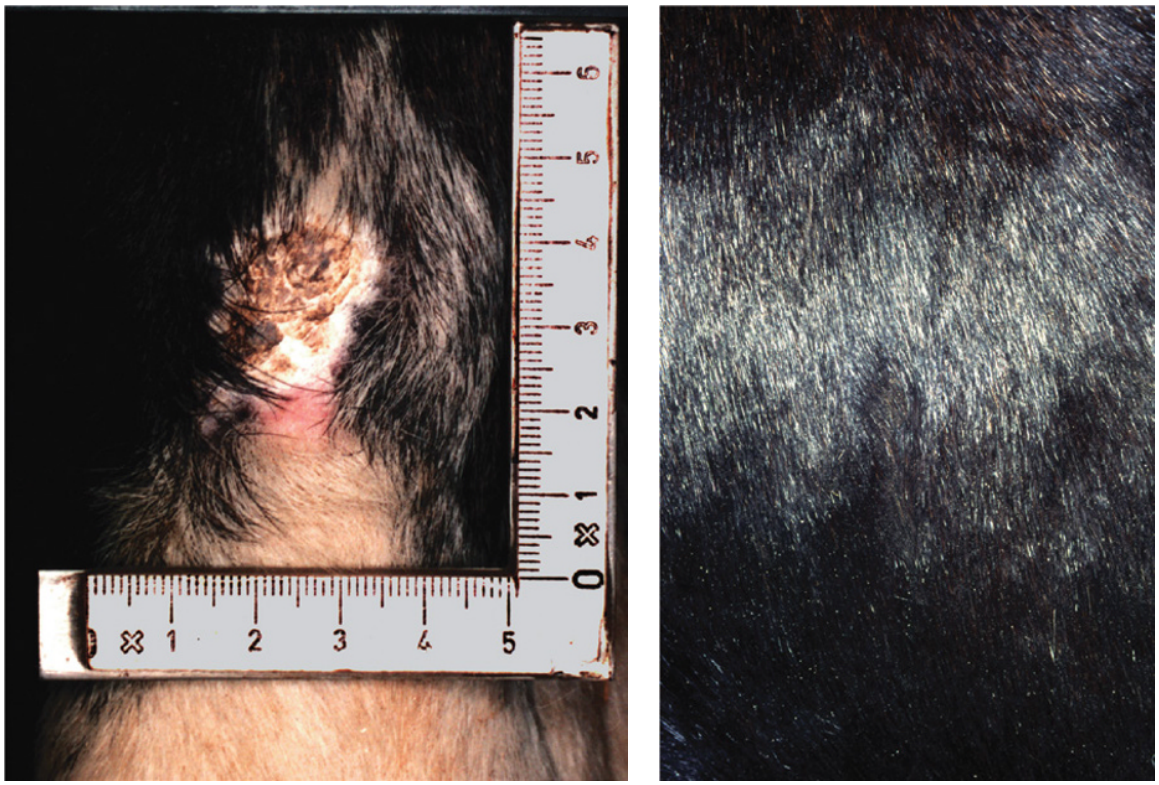
In other words, epithelialization slows when contraction is rapid, as seen in ponies. An increased rate of epithelialization, as seen in wounds on the limb of horses, however, has little effect on the speed of healing because epithelialization is inherently slow. A more extensive scar, characterized by fragility, poor attachment to the underlying basement membrane, and the absence of skin appendages, is formed when epithelialization is the primary mode of wound closure (Figure 2.8).[35]

Clinical application of the results of research

Traumatic wounds of ponies are more apt to heal with a favorable cosmetic and functional outcome than are similar wounds of horses. This usually translates into lower costs of treatment for ponies than for horses. A pony's better prognosis for favorable healing may justify treatment even when a wounded pony has such a low economic value that the owner is reluctant to treat the pony.

First-intention healing (primary wound closure)

The results of the study by Wilmink *et al.* examining primary-intention healing and formation of sequestra in horses and ponies[7] illustrate the detrimental effect of infection in the process of healing and the substantial role of the inflammatory response in local defense. As yet, there are no proven ways to stimulate the inflammatory response in sutured wounds to improve local defense mechanisms, signifying that preventing infection is of paramount importance, particularly for horses.



(a) (b)

Figure 2.8 Scar of a metatarsal wound of a horse **(a)** and a buttock wound of a pony **(b)** 1.5 years after healing. The limb wound in the horse healed mainly by epithelialization, leaving an unsightly scar. The buttock wound of the pony closed mainly by wound contraction, leaving no visible scar. Source: Wilmink 2004.[15] Reproduced with permission of Elsevier.

To prevent infection, as many bacteria as possible should be removed by debridement and irrigation, and, therefore, because surgical debridement is more difficult when the patient is standing, general anesthesia should be the preferred method of restraint, especially when the wound to be debrided is extensive. Debridement of exposed cortical bone appears to protect the bone from infection and subsequent formation of a bone sequestrum. Proliferation and invasion of bacteria remaining after debridement can be prevented by appropriate antimicrobial prophylaxis. Equids suffering from an acute extensive traumatic wound should be administered a broad-spectrum antibiotic as soon as possible after wounding, even if the patient is to be referred to a hospital better equipped to treat it. Antimicrobial therapy should be administered intravenously to ensure an immediate and adequate concentration of the antimicrobial drug within the wounded tissue (the reader is referred to Chapter 19 for more information about antibiotic therapy).

Sutured wounds on a limb should be protected with a bandage or cast to reduce edema and to increase the local temperature, thereby facilitating perfusion of the wound with an adequate supply of leukocytes and oxygen. Bandaging also stimulates biologic processes, such as the production of endothelial and epithelial cells and fibroblasts as well as the synthesis of components of the extracellular matrix (the reader is referred to Chapter 6 for more information about the products used to dress and bandage wounds and Chapter 7 for information about the techniques of bandaging, splinting, and casting).

The fact that the inflammatory response should initially be stimulated, rather than inhibited, implies that the wounded equid should not be administered a corticosteroid, topically or systemically. Furthermore, the routine use of NSAIDs should be avoided, because NSAIDs have been reported to exert adverse effects on wound healing.[36–40] High doses and prolonged use of a NSAID have been shown to reduce inflammation in surgical wounds of horses and ponies;[36,37] consequently, administration of a NSAID may increase the likelihood of infection in a traumatic wound. Additionally, certain NSAIDs have been shown to exert an adverse effect on skin flap survival.[39] Administration of a NSAID may be warranted, however, if the equid is severely lame or if the wounded region is so swollen that local circulation could be compromised. In these instances, a NSAID should be administered, but at the lowest effective dose and for as short a duration as possible. Topical use of disinfectants and antibiotics should also be avoided as many products are toxic to wound cells or inhibit leukocyte function.[41] Similarly, local anesthetics should not be infiltrated close to the wound, as these agents are also toxic for leukocytes

and reduce their mobility and metabolism.[42–44] Consequently, regional (perineural) anesthesia or a line block performed distant to the wound is preferred.[13]

In conclusion, measures should be taken to reduce contamination and to minimize the detrimental effects of treatment on the normal inflammatory response, particularly when the wounded equid is a horse.

What to do

- Consider using general anesthesia, rather than sedation and regional or local anesthesia, to debride and suture wounds on the limbs of horses. Debridement is usually more thorough when the horse is anesthetized.
- Limit the use of NSAID therapy when treating a horse for an acute wound to avoid hindering the natural inflammatory response to injury. NSAID therapy may increase the risk of dehiscence after primary closure.

What to avoid

- Avoid infiltrating a local anesthetic agent near a wound or applying a disinfectant to a wound because local anesthetic agents and disinfectants weaken the inflammatory response by harming leukocytes.

Second-intention healing

Modulating the inflammatory response

The inflammatory response orchestrates the entire healing process. The acute inflammatory response is a requisite for launching healing,[27] whereas chronic or persistent inflammation hinders wound contraction and epithelialization.[45] The inflammatory response in wounds of horses is weak initially and fails to resolve appropriately, and therefore, inflammation initially should be stimulated and later inhibited.[15,46] The primary aim of initial treatment of a wound on a horse is to modulate the inflammatory response so that it more closely resembles that of wounds of ponies (Figure 2.9). This means that inflammation should be encouraged until the wound has filled with granulation tissue and that it should be inhibited from that point onward to reduce the formation of EGT and facilitate contraction and epithelialization.[15,46] The inflammatory response can be controlled during second-intention healing, because the wounded tissues are accessible, whereas it is difficult to control during primary-intention healing. When the inflammatory phase of healing is modulated, the successive phases of the healing process are simultaneously influenced. Thus, the initial enhancement of inflammation boosts the formation of granulation tissue, which in turn hastens wound contraction; precluding chronic inflammation benefits both wound contraction and epithelialization. Treatment of equine wounds healing by second intention, therefore, can be improved by modulating the inflammatory phase, which can be either stimulated or inhibited.

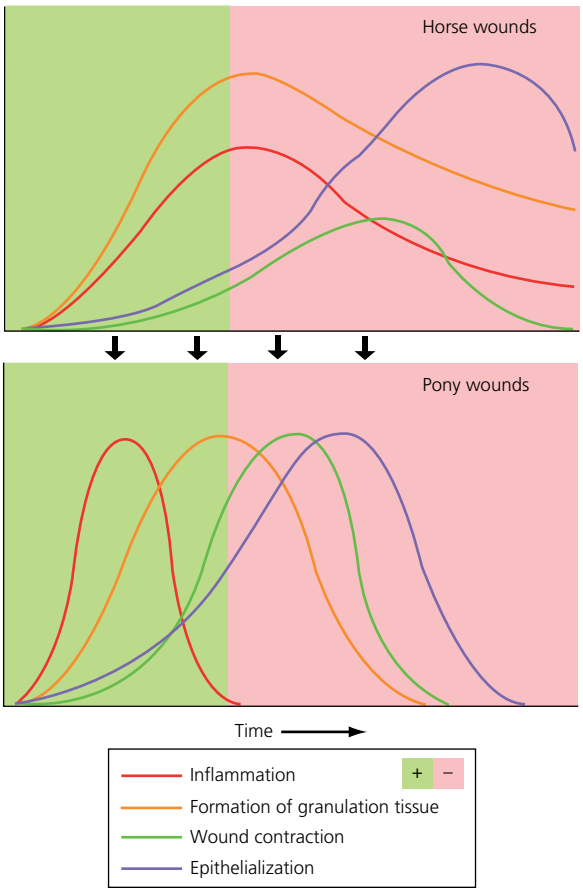


Figure 2.9 Schematic representation of the healing phases of wounds of equids. Inflammation should initially be stimulated (green shaded area) until the wound has filled with granulation tissue, and, thereafter, it should be arrested (pink shaded area) to reduce the formation of Exuberant Granulation Tissue and facilitate contraction and epithelialization. In fact, horse wounds should be treated so that the pattern of healing resembles that of ponies.

What to do

- Stimulate inflammation until the wound has filled with granulation tissue.
- Inhibit inflammation after the wound has filled with granulation tissue.

Stimulating the acute inflammatory response

The initial inflammatory response in wounds of horses should be encouraged, and in no manner limited, until the cavity of the wound has filled with granulation tissue. The inflammatory response can be facilitated, and the risk of infection reduced by surgical debridement, regardless of whether or not a wound is to be sutured. Although surgical debridement is necessary when contamination, necrosis, exposed bone or a frayed tendon are present within the wound of an equid, surgical debridement is especially important when the wound is that of a horse because cellular debridement of wounds of horses by the inflammatory response is slow.

The wound of a horse should be covered with an interactive dressing or topical gel that increases inflammation and thereby stimulates healing.[47] Solcoseryl® (Valeant Pharmaceuticals) has been shown to stimulate inflammation in experimental wounds of horses.[12] Alginates also enhance inflammation, by stimulating macrophages to release inflammatory mediators;[48,49] the increase in inflammation provided by these products can be recognized clinically by the production of more exudate and accelerated autolytic debridement, which lead to more rapid development of healthy and contracting granulation tissue (Figure 2.10). Products containing activated platelets also have the same effect,[50,51] as would hydrogel dressings containing acemannan, Iodosorb® dressings, honey, and sugar. All these products have the potential to activate macrophages but, aside from activated platelets and honey, have not specifically been tested in equine wounds.[51,52]

Bandages themselves favor the inflammatory response by increasing local temperature and by lowering pH. Increased temperature speeds biologic processes in general, and angiogenesis in particular, which in turn speeds migration of leukocytes to the site of injury. Decreased pH causes the hemoglobin dissociation curve to shift, increasing availability of oxygen for the leukocytes, required for the oxidative burst used to kill bacteria after phagocytosis. Bandages consequently enhance the formation of granulation tissue, most importantly by increasing the oxygen gradient between the granulation tissue and the wound's surface.[3]

Stimulating the initial inflammatory response in wounds of horses to a greater degree and in a more specific way is not yet possible, although this might be beneficial to the progression of healing. Interestingly, Solcoseryl® (Valeant Pharmaceuticals), a topical gel found to stimulate the acute inflammatory response in horses, did not exert comparable effects in ponies.[12] Apparently, the suboptimal, initial inflammatory response in horses can be stimulated more effectively than the already adequate one in ponies. Although this might suggest that the choice for special dressings and topical products that enhance the initial inflammatory response is less critical for ponies than for horses, the benefits are certainly seen in clinical practice when using interactive dressings for extensive wounds on the limbs of ponies (Figure 2.11).

Inhibition of the early inflammatory response should also be avoided when healing is occurring by second intention. The use of corticosteroids is, therefore, contraindicated, and the prolonged administration of a NSAID, including a selective cyclooxygenase-2 (COX-2) inhibitor, is questionable.[36–40] Agents toxic to leukocytes, such as many antiseptics, caustics, and astringents, should be avoided. Antiseptics, even when sufficiently diluted, should not be used routinely but only in cases of a bacterial threat and only for a short time. Additionally, wet bandages and hydrotherapy using cold water can be detrimental because they transiently decrease the temperature in local tissue, causing vasoconstriction. A decrease in temperature slows biologic processes, and vasoconstriction limits the influx of leukocytes, nutrients, and oxygen to the wound. For optimal healing, the wetting agent for a dressing and the fluid used for irrigation should be warm (~38 °C) to avoid transient vasoconstriction.[53]

What to do

- Surgically debride the wound, when necessary, even if a wound is not to be sutured, because autolytic debridement of wounds of horses is slow.
- Bandage a wound and apply interactive dressings that stimulate inflammation until the wound has filled with granulation tissue.

What to avoid

- Do not use any product that reduces inflammation, such as a NSAID or a corticosteroid, or that is toxic to inflammatory cells or fibroblasts, such as an antiseptic.

Inhibiting the inflammatory response

As soon as the cavity of a wound has filled in with granulation tissue, the inflammatory response should no longer be encouraged but instead inhibited. Chronic inflammation, inherent to wounds on limbs of horses, enhances the formation of EGT and delays contraction, through persistently elevated concentrations of several mediators (see Chapter 15), and inhibits epithelialization. A direct link between persistent inflammation and delayed wound healing has also been demonstrated in experimental wounds of rabbits.[45]

First, stimuli causing a chronic inflammatory response, such as bone sequestra, foreign bodies or necrotic tissue, should be identified and eliminated from the wound. Additionally, the wound must be protected from environmental microorganisms and foreign material, which can be achieved by covering the wound's surface with inert semi-occlusive absorbing dressings (e.g., Tegaderm foam adhesive, 3M). The number of bacteria on the wound's surface must be controlled because bacteria are strongly chemoattractive to leukocytes, the presence of which perpetuates inflammation. Reduction of surface contamination can be achieved by excising the superficial layer of granulation tissue, thereby removing clefts in which bacteria reside and eliminating many leukocytes and mediators of inflammation, which accumulate in the superficial layers of the granulation tissue. Surface contamination may be further decreased by dressing the wound for 1 or 2 weeks with an antiseptic wound dressing that absorbs exudate (the reader is referred to Chapter 6 for more information about dressings). Systemic antimicrobial therapy is ineffective in reducing the concentration of bacteria on a wound because antibiotics administered systemically often fail to reach a therapeutic concentration within granulation tissue and at its surface.[54] This failure occurs because, in spite of the good blood supply to granulation tissue, fibrin accumulation at the base of granulation tissue prevents adequate penetration of drugs.[54]

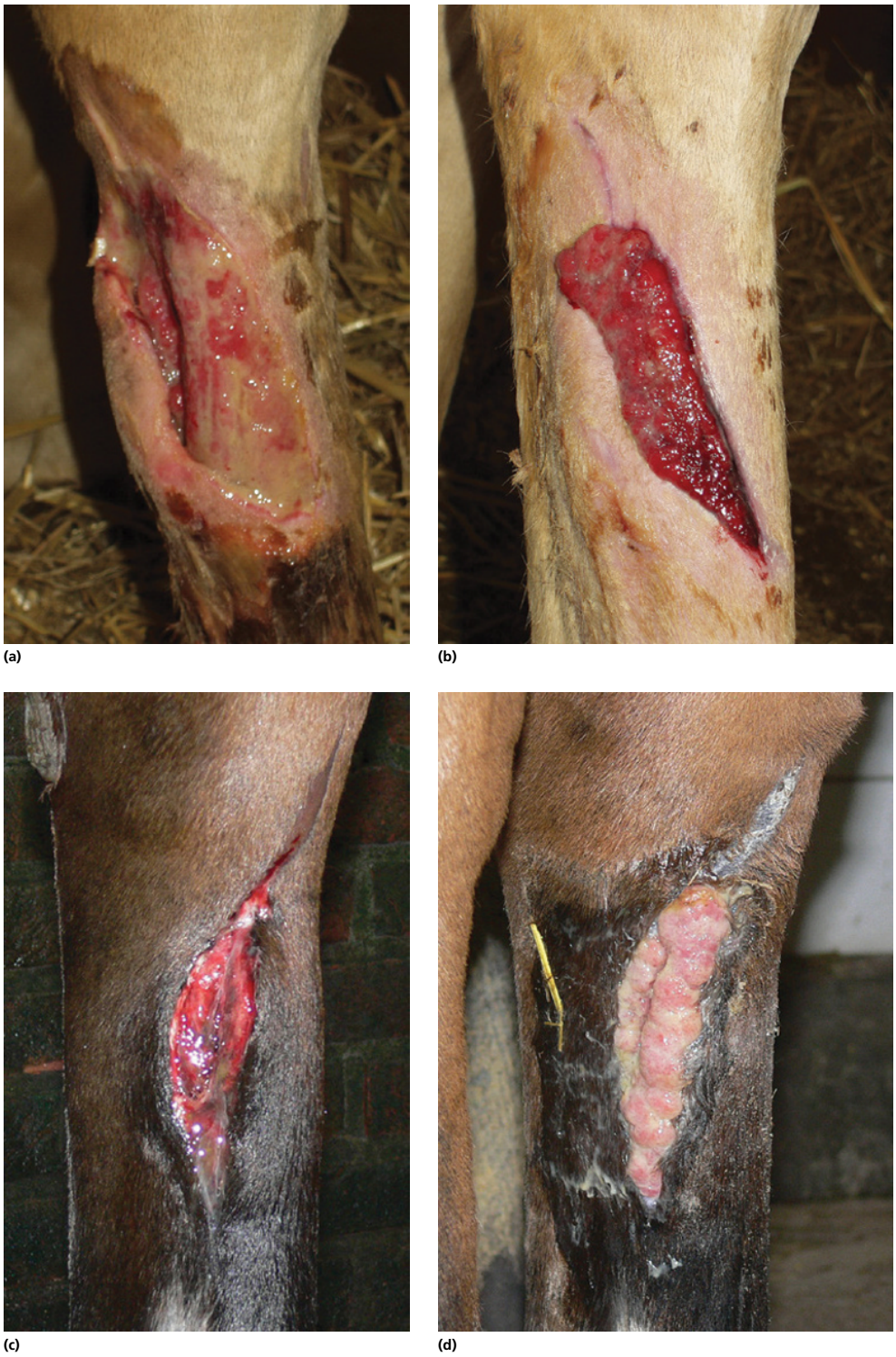
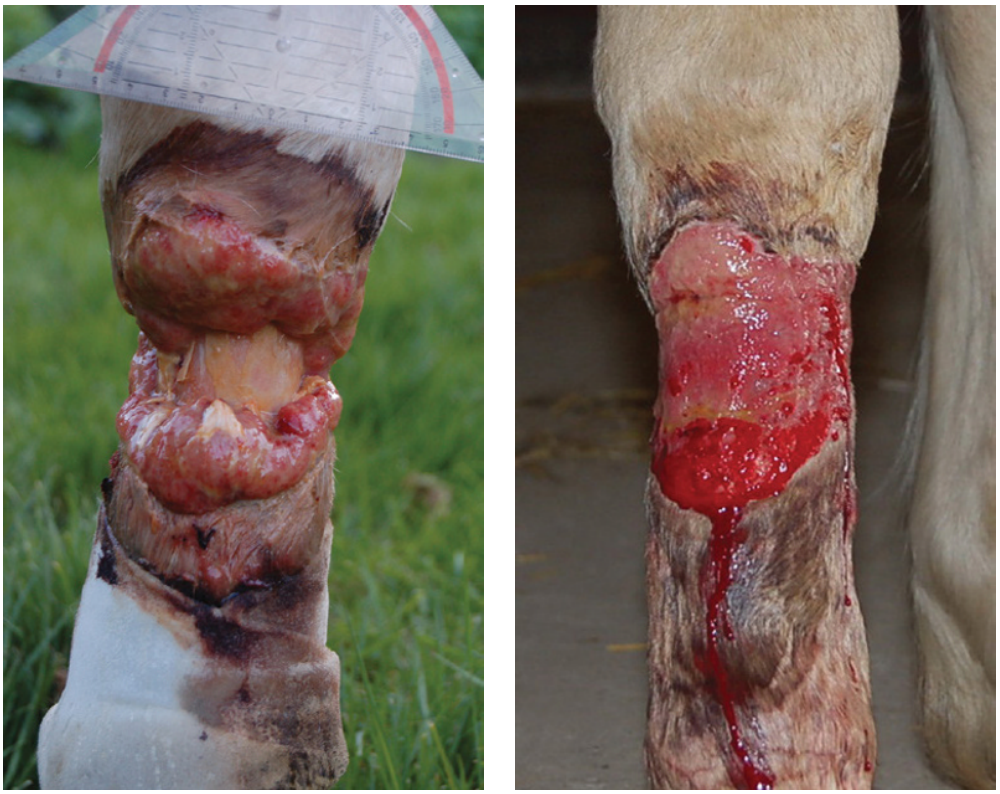


Figure 2.10 **(a)** This traumatic wound on the dorsal surface of the metatarsus of a horse, with extensive undermining, was treated with alginate dressings to stimulate the acute inflammatory response. **(b)** After 13 days, the wound was filled with healthy and contracting granulation tissue. **(c)** A different traumatic wound at a similar location in a horse, but with less undermining and no involvement of the metatarsal bone. The wound was treated with several types of silver foam dressing that do not modulate inflammation. **(d)** After 20 days, the wound was filled with EGT characterized by an irregular surface, riddled by a deep cleft. This wound failed to contract.



(a) (b)

Figure 2.11 **(a)** A large degloving injury on the dorsal surface of the metacarpus of a pony, with exposed bone and lacerated extensor tendons. The wound was debrided and treated with alginate dressings to stimulate inflammation. **(b)** After 15 days, the exposed bone was covered and the wound was filled by granulation tissue. Treatment was continued with a foam dressing. A wound of these dimensions and depth may require at least twice the time to achieve a similar outcome when treated with a non-adherent dressing that is not interactive.

Chronic inflammation can be directly controlled by the topical application of a corticosteroid. A corticosteroid should be applied infrequently, and preferably only once, however, because its effects are not limited to just the inflammatory phase of healing. Theoretically, a similar inflammation-reducing effect could be expected from administering a NSAID, but such an effect has only been shown when parenteral administration is long-term, the dose is high, and the wound is acute.[36,37] Whether or not a clinically apparent effect could be achieved if the wound is chronic is questionable because, as is the case for antimicrobials, fibrin deposits may prevent the NSAID from penetrating the granulation tissue.[54] The effect of a topically applied NSAID on chronic equine wounds is unknown, but topical application of a non-steroidal, non-selective cyclooxygenase (COX) 1-2 inhibitor to experimental wounds of rabbits suffering chronic inflammation did not affect the number of leukocytes in the wounds and the NSAID delayed wound closure.[45] In conclusion, chronic inflammation is most effectively reduced by topical application of a corticosteroid.

Treatments that focus on reducing surface contamination and chronic inflammation concomitantly favor contraction and epithelialization. First, such treatments decrease the concentration of leukocytes on the wound, which in turn reduces the toxic effect of leukocytes on epithelialization, and they reduce the concentration of contraction-inhibiting inflammatory mediators. Second, such treatments decrease the risk of the wound developing EGT, the presence of which has a detrimental influence on contraction and epithelialization. Specific therapies to directly stimulate contraction and epithelialization are not yet available; consequently, contraction and epithelialization can be stimulated only indirectly, by modulating the inflammatory response.

That there is a need to specifically target wound contraction, particularly that of horses, is clear because the rate of contraction determines the speed of second-intention healing and the final cosmetic outcome (Figures 2.6 and 2.8).[5] Because the differences in wound contraction between horses and ponies appear to be caused by local tissue factors, rather than by the innate contractile capacity of fibroblasts, influencing the process may indeed be feasible.[11] Wound contraction is stimulated by TGF-β[31] but inhibited by many other inflammatory mediators abundant in a chronic inflammatory milieu.[32] It might be expected that TGF-β applied to wounds of horses would stimulate contraction, but this was not found in an experimental study,[55] possibly because the formulation of TGF-β or the timing of application was suboptimal. Interestingly, the rate of wound contraction is increased when partial-thickness skin

grafts are applied (i.e., Meek micrografts).[56] Certain dermal factors present in the grafts likely stimulate contraction, but covering the wound surface with grafts also reduces inflammation, which indirectly stimulates contraction (the reader is referred to Chapter 18 for more information about skin grafting).

Epithelialization requires a smooth, healthy bed of granulation tissue that does not protrude above the level of the adjacent skin, along with a moist environment. Epithelialization benefits, therefore, from the measures taken to reduce surface contamination and chronic inflammation because it is inhibited by EGT and by toxic products produced by leukocytes.[19] The environment can be made moist by applying a semi-occlusive dressing; fully occlusive dressings, with the exception of silicone gel dressings,[57] should be avoided in the management of equine wounds because they prolong healing time by providing an environment that encourages bacterial growth, which in turn stimulates chronic inflammation, resulting in excess exudate and EGT.[58] Semi-occlusive foam dressings have the capacity to absorb exudate and bacteria and limit the influence of toxic products released from degenerated leukocytes within exudate. Epithelialization of large wounds is dramatically enhanced by skin grafting, which increases the margins from which epithelial cells proliferate and migrate. Measures to optimize the conditions for epithelialization are more critical in horses than in ponies because epithelialization is the predominant mechanism of closure of wounds on the limbs of horses, whereas epithelialization and contraction contribute equally to closure of wounds on the limbs of ponies.

What to do

- Limit the number of bacteria at the wound's surface, and protect the wound from contamination by bandaging.
- Be gentle with the tissues by avoiding application of irritating substances because these sustain chronic inflammation.
- Debride the wound when its surface has an unhealthy appearance or when healing is delayed, to reduce the number of bacteria and inflammatory cells.
- Cover the wound with an inert semi-occlusive dressing that efficiently absorbs exudate, including bacteria and toxic products released from degenerated leukocytes.
- Apply a corticosteroid topically but only when chronic inflammation delays healing and only to the frequency necessary to keep granulation tissue from becoming exuberant.
- Use skin grafts on extensive wounds of the limb to promote contraction and epithelialization.

Conclusion

Observing the differences in wound healing between horses and ponies has provided valuable information about the intrinsic process of wound healing and the common complications encountered when managing traumatic wounds of equids. Ponies heal faster and with fewer complications than do horses. These differences can, to a large extent, be explained by the disparity in the local inflammatory response, which, in turn, relates to differences in the functional capacity of leukocytes. Research data indicate that, in clinical practice, a maximal effect of treatment can be obtained by using a differential approach to wound healing that optimizes conditions for each successive phase of wound healing. The effect of treatment on the inflammatory response is of paramount importance to the other phases of healing and, therefore, modulation of the inflammatory phase of healing should always be considered when managing a wound. During treatment of wounds healing by second intention, inflammation should be stimulated until the wound has filled with granulation tissue, and thereafter it should be retarded.

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CHAPTER 3

Selected Factors that Negatively Impact Healing

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Chapter Contents

Summary

Wound healing is achieved through orchestrated phases that must occur in the proper sequence and time. Many factors impair normal healing by interfering with one or more of its phases. This chapter reviews the most important factors known to negatively affect healing and describes the mechanisms whereby they exert their detrimental effects. The factors discussed include patient-related factors (horse versus pony, age, nutritional status, disease, and tissue perfusion) and wound-related factors (causes and types of wounds, age and location of the wound, involvement of structures other than skin, nature of the wound, previous treatment, neoplastic transformation, and bioburden). A better understanding of the influence of these factors on repair may lead to a therapeutic approach that negates or diminishes their effects, thereby improving healing and resolving non-healing wounds.

Introduction

Wound healing is a carefully orchestrated series of events that are temporally and spatially linked in a process leading, ultimately, to repair. Many generic factors are known to influence healing, irrespective of species. Systemic and local factors can influence a wound's microenvironment and thereby influence the progression of healing. By identifying and, when possible, manipulating these factors, wounds should proceed to complete healing. Whereas some factors, such as the type and location of the injury, cannot be manipulated, their impact on healing should be considered prior to designing and implementing a plan to manage a wound and providing a prognosis.

Patient factors

Horse versus pony

To optimize wound healing in equids, the clinician should recognize that the horse's response to injury is different to that seen in other species and even to that seen in ponies. The initial inflammatory response of horses to trauma is sluggish and less intense, which, in turn, delays the development of a healthy bed of granulation tissue, resulting in slower and sometimes problematic healing.[1–5] This type of response is more commonly seen in wounds of the distal aspect of the limb (i.e., up to and including the carpus and tarsus) of horses compared to wounds

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of the body. One outcome is that wounds of horses, particularly those on the distal aspect of the limb, are more subject to infection, formation of exuberant granulation tissue (EGT), and delayed healing. This observation correlates with clinical studies that report that ponies are less susceptible to wound dehiscence, less likely to form a bony sequestrum, less likely to develop EGT, and less likely to suffer from delayed wound healing compared to horses.[1] For more information on the differences in healing between horses and ponies, the reader is referred to Chapter 2.

Age of the patient

Advanced age of the patient is known to influence healing in humans, and this has also been documented in experimental animal models.[6] In laboratory animal models, old animals suffer from delayed formation of granulation tissue with reduced tensile strength of the wound early in the healing process, as well as delayed epithelialization and delayed wound closure.[7] Tissue ischemia has been implicated as the cause of these delays, but studies investigating the influence of age on wound healing vary widely, and the anatomic, physiologic, and biologic processes differ substantially between species, making translation of results from one species to another inaccurate.[7] The wound-healing process in healthy, elderly humans appears to resemble that of younger patients, in terms of reaching a similar endpoint, and is usually protracted rather than impaired.[6,8] In aged human skin, the function of macrophages is impaired, proliferation of keratinocytes is decreased, and the dermis is atrophied, all of which when combined, likely lead to a reduced ability of skin wounds of aged patients to progress promptly through all phases of healing.[7] Clinical features apparent during wound healing in human patients over 60 years old include a sluggish inflammatory reaction, a less effective immune response, and a reduction in the capacity of cellular replication, leading to delayed angiogenesis, collagen synthesis, and epithelialization.[8,9]

The effect of advanced age on healing has not been investigated in horses. The impact of aging on wound healing in animals is likely to be less important than in humans because of the relatively lower proportion of geriatric patients in veterinary practice. But, as the standard of veterinary care improves and the emotional value of pets increases, domestic animals are expected to live longer, such that an effect of advanced age on healing may become apparent. The appearance of age-onset diseases probably bears a greater impact on wound healing in animals than does advanced age itself.[7,10] Because equids seem to be more resistant to age-related diseases than do smaller companion animals, advanced age is unlikely to be a significant detriment to wound healing in horses.

Nutritional status and disease

Suitable nutrition is required to sustain cellular repair during wound healing because nutrients are essential to the many biologic processes occurring in the skin. Suitable nutrition is particularly important when cellular division, movement, and differentiation are up-regulated in response to injury.[11] Consequently, in severely malnourished patients, the progression of healing is impeded, and the scar tissue that eventually fills the wound has a decreased tensile strength and is, therefore, susceptible to reinjury.[11,12] The metabolic status (positive or negative) at the time of injury is also important because the energy requirements of healing represent an additional burden to the patient. The new demands imposed by healing cannot be met when the patient is in a negative metabolic state.

Macronutrients (i.e., proteins, carbohydrates, and fats) and micronutrients (e.g., vitamins A, B complex, C, E, and K, and minerals, such as copper, iron, and zinc) are known to play important roles in wound healing.[11] Proteins provide major building blocks for cellular renewal and tissue growth after injury, and adequate protein is needed to maintain a positive nitrogen balance. Protein deficiency may affect hemostasis, inflammation, immunity, formation of granulation tissue, remodeling, and epthelialization.[11,12] Deficiencies of the amino acids cysteine, proline, arginine, tyrosine, and histidine have been shown to adversely affect wound healing by negatively affecting angiogenesis, formation of collagen, and wound remodeling.[11–13] Carbohydrates are the principal source of energy that sustains the high metabolic demands of tissue regeneration.[11] Fats provide energy and contribute to inflammation and synthesis of cell membranes and intracellular matrix.[11]

Various human and animal studies have incriminated a vitamin or mineral deficiency in situations of impaired healing.[11,14] A deficiency of vitamin A is associated with impaired synthesis and stability of collagen, impaired contraction and epithelialization of wounds, and an increase in the patient's susceptibility to infection.[11,15] Deficiency of vitamin B complex is associated with several skin disorders and anemia, each of which may affect wound healing.[11] Deficiency of vitamin C has been implicated in impaired synthesis of collagen and scarring, as well as reduced responsiveness and function of the immune system.[11] Vitamin E is important for stabilization of the cell membrane, and vitamin K plays a key role in hemostasis.[11] Iron is essential for production of red blood cells and synthesis of collagen.[16] Copper deficiency is associated with formation of defective collagen and elastic tissue, leading to reduced tensile strength of the scar.[17] Zinc is a component of key enzyme systems needed for cellular replication and synthesis of protein (e.g., the metalloproteinases that play an important role in the proteolytic remodeling of extracellular matrix during tissue repair).[18] However, a recent study found that wounds of rats treated topically with zinc gluconate healed similarly and contained equivalent bacterial loads as wounds treated with the carrier solution (isotonic saline solution or chlorhexidine) alone.[19]

Concurrent disease may affect wound healing. For example, Cushing's disease (*pars intermedia* dysfunction), which affects mainly older horses, is characterized by high serum concentrations of endogenous cortisol that might suppress inflammation to such an extent that healing could be impaired.[20] High serum

concentrations of glucocorticoids may also be found in horses in response to stress. Protracted periods of hyperglucocorticoidemia may decrease the expression of pro-inflammatory cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor-α, and some chemoattractants required for the inflammatory phase of wound healing and for supporting the migration of immune cells.[21,22] Whereas Cushing's disease and consistently elevated production of glucocorticoids are associated with an increased risk of infection and impaired healing of wounds of humans, their clinical impact on healing of wounds of horses is less clear. More importantly, the stress of a severe injury may decrease appetite, thereby leading to an intake of energy and nutrients insufficient to meet the demands of healing tissues.

Tissue perfusion/oxygen tension

Effective wound healing requires adequate circulation, itself dependent on sufficient hydration, to ensure optimal tissue perfusion and oxygenation. Oxygen is essential to several critical mechanisms underlying wound healing, including bacterial killing, collagen synthesis, and epithelialization. Oxygen plays a pivotal role in eliminating anaerobic bacteria and in reducing formation of biofilm and colonization by microorganisms resistant to antimicrobial drugs. The guideline of debriding a wound back to healthy bleeding tissue is based on the principle that healing progresses more quickly and efficiently under conditions where the granulation bed is well perfused and free from avascular and necrotic debris.[23]

Because most of the oxygen in blood is carried by hemoglobin, anemia might be expected to impair healing. Normovolemic anemia, with a hematocrit above 20%, however, in the presence of adequate blood flow and tissue perfusion, does not seem to be detrimental to wound healing.[24,25] Conversely, hypovolemia can compromise tissue perfusion, thereby negatively impacting healing, mostly by a decrease in activity of leukocytes and production of collagen.[26,27]

Wounding disrupts the local blood vessels, leading to acute hypoxia in the wounded tissues. Hypoxia favors angiogenesis, and the newly formed, temporary capillaries carry much needed oxygen into the wound's microenvironment. Oxygen tension of tissue near capillaries at a wound's margin is between 60 and 90 mmHg compared to oxygen tensions of 30–50 mmHg in normal, uninjured tissue. Cells consume oxygen thereby creating an oxygen gradient between the edge of the wound and the center of the wound.

In humans, the concentration of oxygen in tissue is closely correlated to the progression of wound healing.[25] Chronic, non-healing wounds are hypoxic relative to normally healing acute wounds, with tissue oxygen tensions ranging from 5–20 mmHg.[28] Studies in horses have shown that perfusion of chronic wounds of the limb is significantly inferior to perfusion of non-chronic body wounds.[29,30] Tissue perfusion is even worse in limb wounds that go on to form EGT.[29,30] Metabolic disturbances suggestive of a deficient oxygen supply have been identified in equine limb wounds that have developed EGT,[30] and a relative state of hypoxia during the inflammatory phase of repair of limb wounds, possibly due to occlusion of microvessels, secondary to endothelial hypertrophy, has also been identified.[31,32]

New blood vessels forming in an hypoxic environment are immature and bleed easily,[33] which may explain the friable and hemorrhagic nature of equine EGT observed clinically. Because the phagocytic ability of leukocytes is oxygen dependent,[34] the presence of hypoxia may explain the inefficient inflammatory response documented in limb wounds of horses thought to contribute to the development of EGT.[4,5] Furthermore, low oxygen concentrations encourage angiogenesis, the growth of fibroblasts, and the synthesis by fibroblasts of components of the extracellular matrix, such as collagen, another mechanism whereby the hypoxia present in limb wounds might favor the development of unhealthy EGT.

Tip

- Effective wound healing requires adequate circulation to ensure optimal tissue perfusion and oxygenation. Oxygen is essential for the elimination of bacteria, collagen synthesis, and epithelialization, which are all critical to healing.

What to do

- Wound healing can be optimized by debriding the wound back to healthy bleeding tissue, using appropriate dressings, addressing hydration, and providing effective analgesia. Healing progresses more quickly and efficiently under conditions where the granulation bed is well perfused and free from avascular and necrotic debris.

What to avoid

- Excessive granulation tissue is associated with poor oxygen tension within the wound and will delay healing. Once excessive granulation tissue forms it should be excised back to healthy bleeding tissue flush with the wound edges.

Wound factors

Causes and types of wounds

Surgical wound

Surgical wounds are often classified, based on their degree of intrinsic microbial contamination, into clean, clean–contaminated, contaminated, and dirty or infected (Table 3.1).

Wound healing complications develop less frequently after elective surgical procedures than they do after emergency procedures in horses.[36–38] Stabilizing or improving the physiologic status of a patient before performing an emergency procedure, as well as addressing systemic infection prior to surgery, is likely to reduce the incidence of incisional complications. Careful

surgical planning and sound anatomic knowledge of the region to be exposed, combined with good surgical technique while maintaining tissue perfusion and oxygenation with appropriate fluid therapy, optimize healing. Sharp dissection with a scalpel, where possible, is preferred to blunt dissection or the use of surgical scissors. Hemostasis should be effective, tissue should be preserved and handled carefully, and closure, using appropriate suture material, should be accurate. Application of a suitable antimicrobial regimen may be warranted (the reader is referred to Chapter 4 for more detail). After surgery, the wound may require support by a bandage, after applying an appropriate dressing (the reader is referred to Chapter 6 for more information regarding dressings).

Table 3.1 National Research Council Operative Wound Classifications.[35]

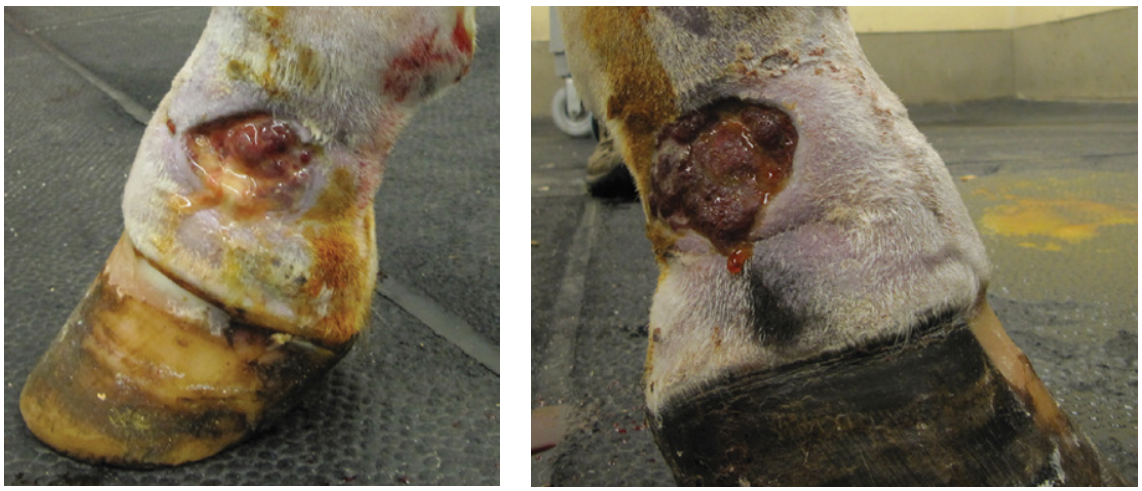
Category	Description
Clean	Elective, primary closure, no drains, non-traumatic, no infection, no inflammation, aseptic technique maintained
Clean–contaminated	Gastrointestinal, respiratory, urogenital tracts entered under controlled conditions and with expected amount of contamination or minor break in aseptic technique
Contaminated	Open, fresh traumatic wound; gross contamination from gastrointestinal tract, opening of urogenital or biliary tract with infected bile or urine; incisions in which purulent material is encountered or major break in aseptic technique
Dirty or infected	Traumatic wound with retained devitalized tissue and/or foreign bodies, and/or fecal contamination or delayed treatment or from a dirty source; perforated viscus encountered or acute bacterial inflammation with purulent exudate encountered

The duration of surgery influences the likelihood of infection and subsequent complications of healing. For example, the incidence of infection in the horse after orthopedic surgery increases 3.6-fold as surgery time extends beyond 90 minutes,[36] whereas the incidence of incisional complications after abdominal surgery doubles when the procedure extends beyond 120 minutes.[37] The association between complications of healing and duration of surgery are probably multifactorial but are likely to reflect additional tissue trauma, drying of tissues, poor tissue perfusion, and increased opportunity for bacterial contamination.[39]

Accidental wound

Accidental wounds may be categorized as closed (e.g., bruise, hematoma, contusion) or open (e.g., abrasion, puncture, laceration, avulsion, degloving, burn), the latter constituting the majority of accidental wounds encountered in equine practice. The greater the inciting trauma, the more severe is the soft-tissue damage and the greater is the risk of subsequent infection. For example, sharp lacerations caused by metal or glass are unlikely to become infected because they are rarely associated with contamination, necrosis, or alteration of the skin's vascular supply.

Conversely, crush injuries and injuries characterized by avulsion and tearing of tissue are at greatest risk for infection because of extensive thrombosis of associated vessels and subsequent loss of blood supply. These types of wounds are almost 100-fold more likely to become infected than those caused by shearing forces.[40] The blood supply to the distal extremity of a horse's limb is commonly compromised, for instance, when wire encircles the limb, thereby creating a tourniquet-like effect. Wounds caused by entrapment of the limb between fixed objects (e.g., a cattle guard or a stall door and wall) or by impact from collision with a solid object or from a kick, are especially susceptible to infection because of the magnitude of soft-tissue injury (Figure 3.1).



(a) **(b)**

Figure 3.1 Example of extensive trauma to the distal aspect of the limb. The wounds were incurred several days prior to presentation and were caused by entrapment of the foot between panels. The wounds' size and the degree of lameness had increased despite antimicrobial and anti-inflammatory therapy. Both wounds communicated with the pastern joint, and a stress radiograph confirmed rupture of the lateral collateral ligament of the proximal interphalangeal joint. **(a)** Lateral view. **(b)** Medial view.

Puncture wounds are susceptible to infection because the penetrating tract often closes, trapping bacteria deep within the tissues. Deep puncture wounds, where the point of perforation is small, may not allow sufficient drainage of contaminated fluids. Accumulation of these fluids within the wound provides an ideal environment for bacterial multiplication, particularly of anaerobic microorganisms. Opening the tract to ensure drainage, irrigating the tract, and packing it with saline-soaked sterile gauze, reduce the risk of infection becoming established.

Tip

- The contamination of a synovial structure (joint, bursa, tendon sheath), as a result of a small puncture wound, may go unnoticed unless contamination of the structure is accompanied by lameness, effusion of the synovial structure, heat over the wounded region, or signs of pain when the affected area is palpated.

What to do

- If perforation of a synovial structure is suspected, thorough physical and lameness examinations should be performed, along with ancillary diagnostic tests, which might include radiographic and/or ultrasonographic examinations and synoviocentesis to obtain fluid for cytologic analysis and bacterial culture and antimicrobial sensitivity testing. Synoviocentesis should be performed at a site remote from the wound, and after synovial fluid is collected, a sterile irrigation solution should be injected into the synovial cavity, under pressure. Egress of fluid from the wound after injection indicates that the wound communicates with the synovial structure.

What to avoid

- Synoviocentesis prior to acquiring radiographic or ultrasonographic images should be avoided to prevent introduction of air into the synovial space because air in a synovial space complicates interpretation of radiographic and ultrasonographic images.

For more information regarding the diagnosis and management of wounds involving synovial structures, the reader is referred to Chapter 16.

Age of the wound (time elapsed since injury)

A wound, either surgical or accidental, is a disruption in the integrity of the skin, which renders underlying tissues susceptible to contamination. A properly managed, clean or minimally contaminated wound should progress through the normal phases of repair. The probability that healing becomes delayed is related, at least in part, to the degree of contamination, the extent of tissue trauma, and the host's response. These factors are particularly important in accidental wounds of horses, because accidental wounds are often contaminated with foreign material and large numbers of various microorganisms.

Historically, an accidental wound, if prepared properly, was considered suitable for primary closure, with little risk of infection, if it was less than 6–8 hours old. This time, referred to as the "golden period," was based on research conducted in laboratory animals and was related to the time required for multiplying bacteria in a closed surgical wound to reach an infective concentration, considered to be more than 10^5 organisms per gram of tissue or milliliter of exudate.[41] This concept of a golden period is largely outdated.[42] Although an open wound often can tolerate a greater bioburden (e.g., 10^6 bacteria) without showing signs of deterioration,[43] the wound's outcome depends predominantly on the adequacy of the host's immune response, the virulence of contaminating bacteria, and local environmental factors (e.g., the presence of devitalized tissue or foreign material) that can potentiate the virulence of bacteria.[44]

Although the duration since injury may be given some consideration, the approach to wound management should be selected in light of other factors, such as the degree and type of contamination, the location and type of wound, including the extent of the trauma to the blood supply and to other nearby or underlying structures, the management of the wound prior to presentation, and the patient's overall physical condition and immune status. Many veterinarians become very astute at visually assessing wounds and at determining if the wound is suitable for primary closure or whether a different approach to wound management is more appropriate. The reader is referred to Chapters 4 and 8 for more information regarding the selection of appropriate methods to manage wounds.

Location of the wound

Specific healing limitations characterize different anatomic regions. For example, wounds on the distal aspect of the limb of horses, left to heal by second intention, expand during the first 2 weeks after trauma, in contrast to those on the body, which change little in size.[45] The increase in size of wounds on the distal aspect of the limb can be substantial, with the surface area of the wound almost doubling over a 2-week period. This time of expansion is referred to as the "lag phase" of healing. After a healthy bed of granulation tissue has formed, the wound starts to contract. The percentage decrease in the surface area of the wound attributable to contraction is greatest between the second and fourth weeks of healing. The capacity of body wounds to heal through contraction far exceeds that of wounds on the distal aspect of the limb. In a well-designed study, the rates of contraction and epithelialization of experimental, full-thickness wounds (7–9 cm^2) on the body and limbs of horses and ponies, left to heal by second intention, were measured. The percentage decrease in wound surface area attributable to wound contraction between the second and the fourth weeks of healing was 47% for the body wounds of ponies vs. 38% for the body wounds of horses and 35% for the limb wounds of ponies vs. 0% for the limb wounds of horses. After week 4, the rate of

wound contraction slowed to less than 5% per week for the wounds of ponies and the body wounds of horses, up to complete healing. The metatarsal wounds of horses showed a different pattern: the lag phase of healing lasted 4 weeks and this was followed by an average rate of contraction that did not exceed 2.5% per week.[46] These differences in rates of contraction between wounds on the body and those on the limb have been attributed to the poorly oriented fibroblasts and myofibroblasts within the immature granulation tissue of limb wounds of horses.[47] In these same experimental limb and body wounds, epithelialization progressed over the wound's surface at a rate of 0.75 mm/week between weeks 3 and 7 of healing for body wounds of ponies, 0.62 mm/week for body wounds of horses, 0.63 mm/week for limb wounds of ponies, and 0.48 mm/week for limb wounds of horses.[46]

Moreover, wounds to the distal aspect of the limb of horses are often heavily contaminated with foreign material, including feces. Whereas short exposure to feces has been shown to improve healing of experimental wounds on the distal aspect of the limb of horses, perhaps by stimulating the acute inflammatory response,[48] wounds contaminated by feces, which may contain up to 1×10^{11} organisms/gram, are likely to become infected if not treated properly. For more information regarding the management of wounds on the distal aspect of the limb, the reader is referred to Chapters 8 and 13.

Finally, wounds on the distal aspect of the limb of horses are more susceptible to the formation of EGT than are similar wounds located elsewhere on the body.[49] The exact location of the wound on the limb further influences healing and formation of EGT; wounds over the dorsal surface of the metacarpo/metatarsophalangeal joint heal more slowly than similar wounds located over the dorsal surface of the metacarpus/metatarsus. Additionally, limb wounds located on the extensor and flexor surfaces of joints and the heel bulbs appear prone to the development of EGT. The reader is referred to Chapter 15 for more information regarding EGT.

Joints, tendons, and ligaments, all of which are vital to normal musculoskeletal function, are not protected by muscle at the distal aspect of the limb of horses. Consequently, a full-thickness skin wound in this location is likely to damage one or all of these structures, causing a substantial increase in the cost of treatment and a guarded or poor prognosis for return to function. Large wounds over bony prominences, particularly those involving the distal aspect of the limb, heal more slowly than those in other regions because they are subjected to excessive movement and high tension. Movement disrupts capillary buds, collagen deposits, and fragile new epithelium, thereby perpetuating the inflammatory response, which, in turn, favors the production of EGT. Although primary closure or delayed closure (primary or secondary) of wounds in these regions may assist healing, it can also be problematic because tension on the suture line might compromise blood supply to the skin. For this reason, many wounds over bony prominences are allowed to heal by second intention.

What to do

- External coaptation (i.e., bandages, splints, or casts) should be used to stabilize a sutured or non-sutured wound in a highly mobile region.

Tip

- If the wound is located in a region where it is subjected to pressure when the horse is recumbent (e.g., *tuber coxae*), preventing the horse from lying down (e.g., by cross-tying the horse or by attaching the horse's lead to an overhead wire) often allows the wound to heal by second intention.

Involvement of structures other than the skin

When assessing a wound in close proximity to a critical anatomic structure, the possibility of damage to that structure should be investigated. Critical structures that may be involved in a wound include synovial cavities, bone, tendon or ligament, pleural or abdominal cavities, eyes, and the paranasal sinuses. The reader is referred to Chapters 16, 14, 17, 12, and 11, respectively, for more information about the management of wounds involving one or more of these structures.

Synovial cavities (joints, tendon sheaths, or bursae) in close proximity to the wound should be examined for involvement, after cleansing the wound, by exploring the wound digitally with a gloved hand or with a sterile probe, by using radiography with a sterile metallic probe inserted in the wound or contrast material injected into the wound, or by using synovial distension. When using synovial distension to verify communication between a synovial cavity and a wound, the needle should be inserted into the synovial cavity at a site remote to the wound (Figure 3.2). The reader is referred to Chapter 4 for a more complete discussion of approaches to wound exploration and to Chapter 16 for more information regarding the diagnosis of synovial involvement and management of wounds involving a synovial structure. Timely identification of synovial involvement and the use of aseptic techniques minimize the risk of more contamination and subsequent infection, thereby optimizing the outcome of a horse with a wound involving a synovial cavity.

Injuries that expose tendons may heal with difficulty by second intention because movement of the tendon within a wound often results in the formation of two separate beds of granulation tissue, one on the tendon and the other on the tissue surrounding the tendon (Figure 3.3).

Tip

- Immobilizing the limb with a splint or a cast restricts movement of the tendon, allowing the separate granulation beds to become confluent, thereby enabling the wound to contract effectively.

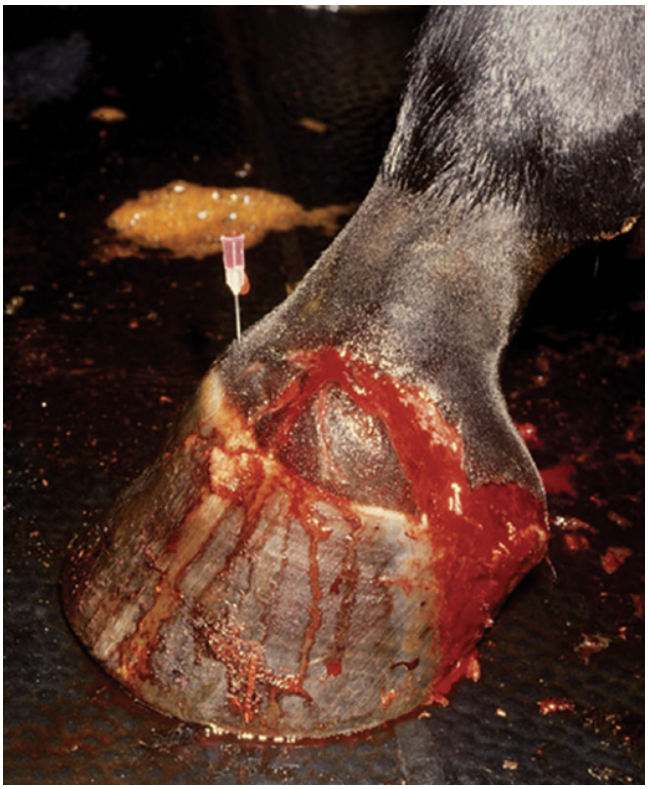


Figure 3.2 A sterile needle has been placed into the distal interphalangeal (coffin) joint at a site remote to the wound. Source: Stashak TS. Wound infection: contributing factors and selected techniques for prevention. *Proc Am Assoc Eq Pract* 2006; **52**: 270. Reproduced with permission of American Association of Equine Practitioners.

Exposure of bone in degloving wounds at the distal aspect of the limb is common, and damage to the periosteum is likely to lead to the formation of a bone sequestrum. Damage to or exposure of the periosteum and surrounding soft tissues may compromise the blood supply to the cortex of the bone. Collateral cortical blood supply may be insufficient to maintain the viability of the traumatized bone. During the first week after injury, exposed bone with adequate blood supply should start producing granulation tissue. Conversely, avascular bone, which usually appears off-white, develops no granulation tissue. Nevertheless, avascular bone may be masked by the gradual ingrowth of granulation tissue from surrounding soft tissues. Healthy cortical bone beneath a sequestrum may produce granulation tissue that may expel the sequestrum from the wound, but more commonly, granulation tissue emanating from the surrounding soft tissues traps the sequestrum within the wound (Figure 3.4). A bone sequestrum acts as a foreign body and harbors bacteria, causing chronic inflammation and, subsequently, the formation of EGT. The bone sequestrum usually makes a radiographic appearance 10–14 days after periosteal injury. A small sequestrum may be resorbed within the healing wound, but in most cases, the sequestrum must be removed surgically. The reader is referred to Chapter 14 for more information regarding the management of bone sequestra.

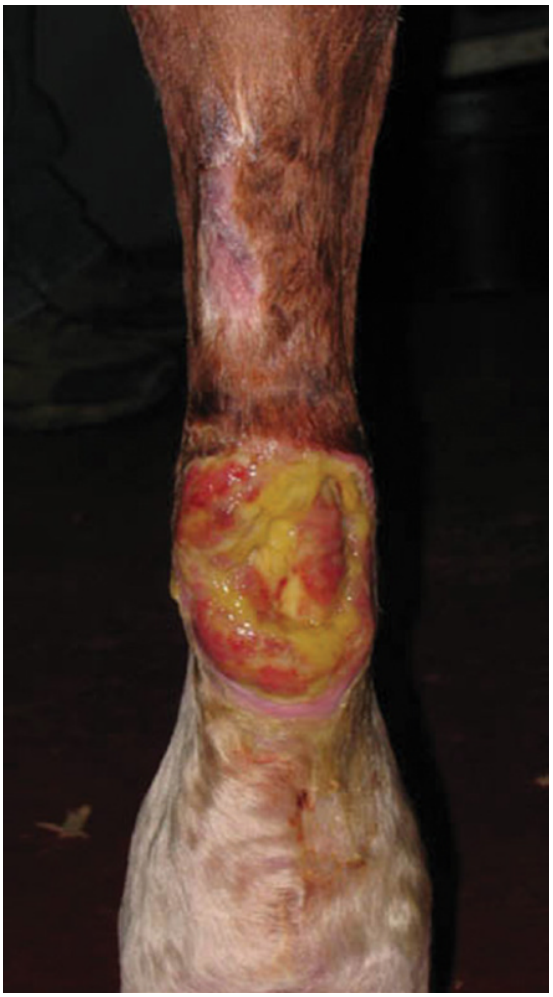


Figure 3.3 Granulating cast sore 3 weeks after the removal of a cast used to manage a laceration over the dorsal surface of the fetlock. Concentric rings of granulation tissue have formed as a result of differential movement between the skin, subcutaneous tissues, paratenon, and the underlying common digital extensor tendon. A portion of the tendon is visible distally with the proximal portion of the tendon covered by granulation tissue.

Wounds to the chest may involve the pleural cavity and are often associated with pneumothorax, hemothorax, or septic pleuritis. If involvement of the pleural cavity is suspected, thorough physical, radiographic, and ultrasonographic examinations are warranted. Hematologic examination can be useful in determining the extent of blood loss and/or the presence of infection. Injuries to the abdomen may be associated with septic peritonitis and damage to or herniation of viscera. Ultrasonographic examination of the viscera and cytologic examination of peritoneal fluid, obtained by paracentesis, are useful aids in diagnosing septic peritonitis. Surgical treatment may be required. Wounds that extend to body cavities may result in systemic complications such as blood loss and poor perfusion, hypoxia or sepsis that may cause chronic debilitation and negatively impact wound healing.

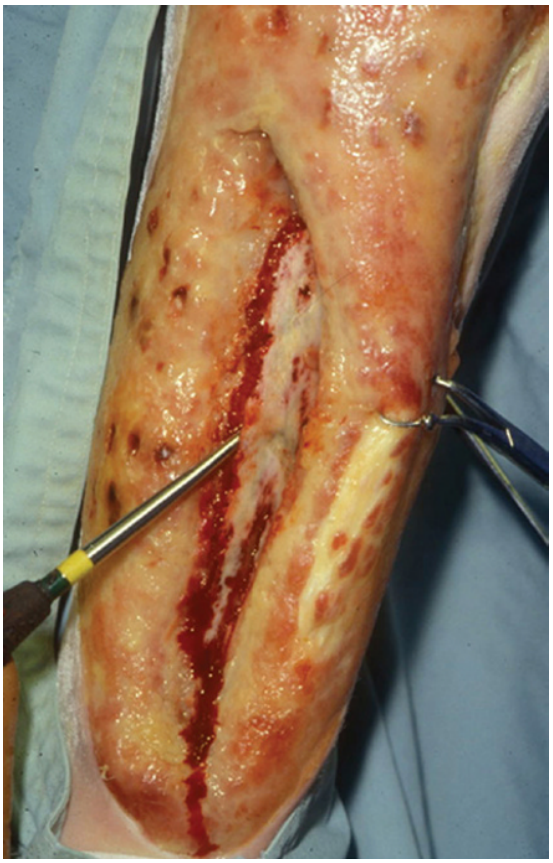


Figure 3.4 Degloving injury of 3 months duration involving the dorsal surface of the metatarsus. A linear defect in the unhealthy-looking granulation tissue was associated with wound drainage as well as stalled wound contraction, signs indicating the presence of a sequestrum. The sequestrum was removed from the sedated, standing horse using local anesthesia.

The reader is referred to Chapter 12 for more information regarding the management of wounds involving the chest or abdomen.

Nature of the wound

The shape, size, and depth of a wound can affect the rate of healing. Wounds on the extremities in which the orientation of a flap of skin is at odds with the distribution of blood vessels often experience a delay in healing and are more susceptible to infection. Arteries in the limbs run from proximad to distad so that large flaps of skin presenting as an inverted "V" suffer compromise to their blood supply (Figure 3.5). For healing to be successful, blood supply must be provided by underlying tissues, or, when the wound is sutured, from vessels that traverse the suture line to revascularize the flap. Because the flap is susceptible to avascular necrosis that inevitably leads to dehiscence after primary closure, delaying suturing this type of wound until a healthy bed of granulation tissue forms may be best. This approach is referred to as "delayed secondary closure" and is described at greater length in Chapter 8.

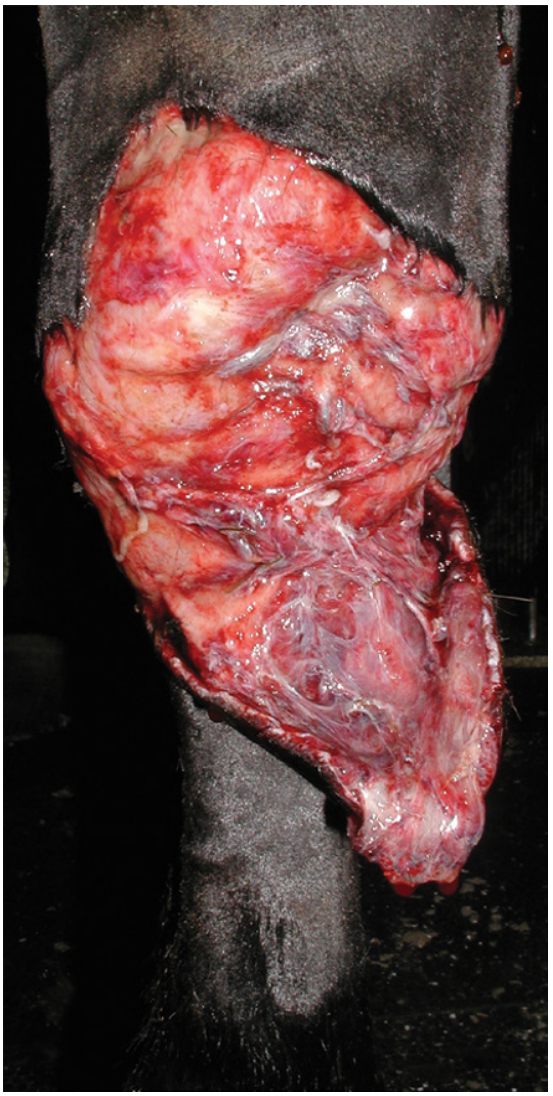
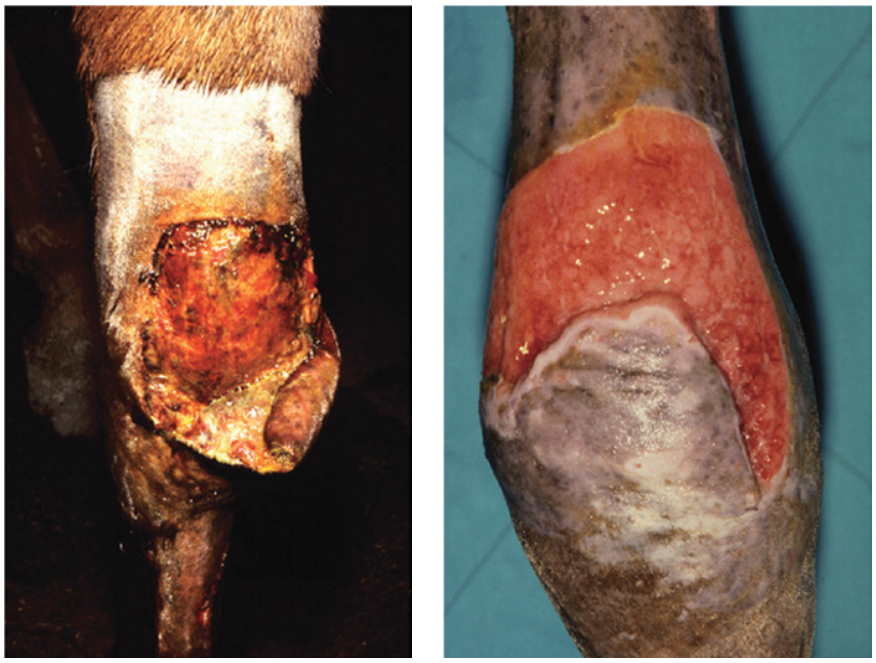


Figure 3.5 Example of an inverted "V"-shaped wound over the dorsal surface of the carpal region. This configuration compromises blood supply to the skin and subcutaneous tissues, resulting in poor wound healing. Courtesy of Dr. Christophe Celeste.

What to do

- The flap should be stabilized in a somewhat normal position with a few large tension sutures, while awaiting definitive treatment, to preserve it from recoil/retraction and to protect the underlying tissues, such as bone and/or tendon, from further injury or dessication (Figure 3.6).

Seromas, hematomas, and edema impede healing and increase the risk of infection by distracting the wound's edge and by reducing capillary perfusion from the pressure they exert on the local blood supply. Large, superficial hematomas that develop from trauma are best left for 5–7 days before being drained to allow for adequate hemostasis. Early and ineffective drainage may lead to recurrence of the hematoma. A drain may be



(a) (b)

Figure 3.6 Example of a flap wound over the dorsal surface of the carpal region. The flap is positioned such that it is at odds with the limb's major distribution of blood vessels. **(a)** At presentation, ~6 hours after injury; note the absence of subcutaneous tissue at the proximal limit of the flap. The tip of the flap felt cool. After cleansing the wound, the skin flap was elevated into its normal position, after which a bandage splint was applied. **(b)** Five days after injury. The skin flap had retracted, and a healthy bed of granulation tissue had formed. This flap should have been stabilized, in as normal a position as possible, using a few large tension sutures. This would have prevented retraction of the skin flap, which complicates delayed closure of the wound.

inserted into a hematoma to ensure drainage, but to do so poses a risk of ascending infection and may be ineffective in draining large amounts of clotted blood. The reader is referred to Chapter 9 for more information about the use of drains in managing wounds.

What to do

- Edema can be controlled by applying cold therapy immediately after injury and subsequently applying a bandage and initiating exercise.

Tip

- A wounded limb that has been bandaged for an extended time often swells within a few hours after the bandage has been removed, especially if the horse is inactive or is confined. Swelling can be minimized by scheduling bandage removal to coincide with an increase in exercise brought about by moving the horse from a stall to a small paddock.

Previous treatments (used by owner)

Various topical and/or systemic treatments may negatively influence healing by slowing cellular proliferation and hindering the immune response of the patient. For more information regarding the effects of various systemically administered drugs, such as antimicrobial drugs, glucocorticosteroids, and non-steroidal anti-inflammatory drugs, the reader is referred to Chapter 4.

Many topical treatments, particularly those for which only anecdotal evidence exists, have been shown to be, at best, unreliable in promoting healing. At worst, some topical treatments, such as application of a caustic agent, may exert a negative effect. Topical agents should be applied to wounds to serve a specific purpose; this highlights the importance of understanding the properties of the active ingredients of the selected product and their proposed effects on healing tissues. For more information about topical wound-care products, the reader is referred to Chapter 5.

What to avoid

- Over-the-counter products that fail to provide a list of ingredients should not be used on wounds.

Neoplastic transformation and cutaneous habronemiasis

Neoplastic transformation at a wound site, although uncommon, should be considered when examining any non-healing, chronic, granulating wound. Transformation of a wound into sarcoid or squamous cell carcinoma has been reported in horses.[20] Apparently, the transformation can occur at a wound at any site on the body. Whereas transformation of a wound into

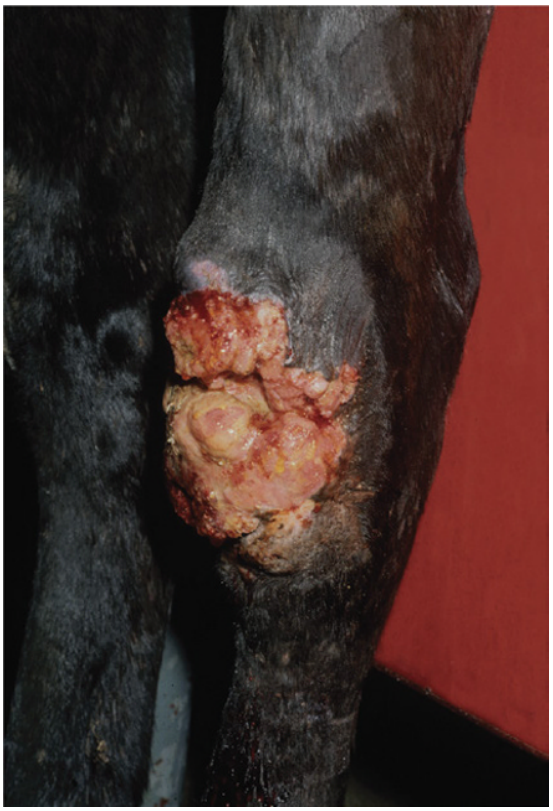


Figure 3.7 Example of a wound that would not heal after repeated attempts to excise the granulation tissue. Histologic examination of the tissue revealed a squamous cell carcinoma.

sarcoid is more likely to occur in a horse with a sarcoid at another site on its body, no such relationship has been established for transformation of a wound into a squamous cell carcinoma. A typical history for neoplastic transformation includes failure of a granulating wound to heal after repeated attempts to debride the granulation tissue or dehiscence of a sutured wound in the absence of an identifiable cause (Figure 3.7). For more information about sarcoid transformation at wound sites, the reader is referred to Chapter 21.

Cutaneous habronemiasis is a common cause of granulomas in horses in some parts of the world. It is caused, in part, by the larvae of the spirurida stomach worms, including *Habronema musca*, *Habronema majus*, and *Draschia microstoma*. Larvae feeding on pre-existing wounds cause a chronic granulomatous reaction as they migrate, halting the healing process. Lesions caused by habronemiasis are chronic and are most commonly seen around the eyes (particularly adjacent to the medial canthus), on the male genitalia, on the distal aspect of the limbs, and at the commissures of the lips (Figure 3.8).[50] The wounds typically contain yellow, calcified material the size of rice grains, and histologic examination of tissue from the wound reveals necrotic foci in a dense fibrous stroma infiltrated with eosinophils and macrophages. The incidence of yearly recurrence is high in certain individuals. Conversely, some horses are resolutely resistant.

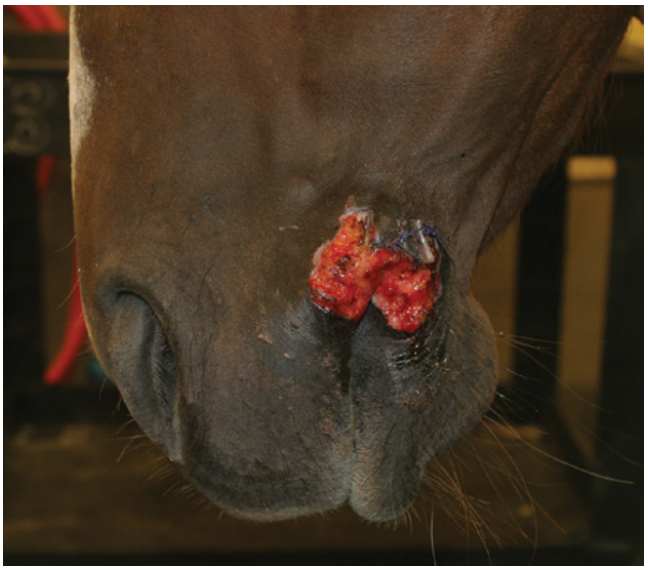


Figure 3.8 A chronic, non-healing wound at the commissure of the mouth. Despite repeated sharp debridement and excision of the granulation tissue, followed by primary closure of the defect, the wound would not heal. Histologic examination of the granulation tissue revealed habronemiasis.

Tips

- Habronemiasis can be easily confused with neoplastic and other non-neoplastic cutaneous masses in horses. Seasonality is an important issue, with the majority of cases occurring in the summer and early fall. Moreover, histologic examination of a wound sample (acquired by biopsy or by resection of the lesion) helps confirm the diagnosis, thereby enabling initiation of appropriate therapy.
- Fly control and regular deworming with ivermectin may reduce the incidence of habronemiasis.

What to do

- Treatment with ivermectin (200 µg/kg), administered intramuscularly may be effective, but extensive surgical debridement or excision of the affected wound may be required if the horse fails to respond to medical therapy.
- Where complete surgical resection of the affected area is not possible, topical application or intralesional injection of a corticosteroid, to reduce the inflammatory response associated with habronemiasis, may be used to complement surgical therapy.

Wound bioburden

Although wounds cease to heal for a number of reasons, perhaps the most common is associated with the effects of the wound's bioburden, which are influenced by the sheer quantity of colonizing microbes, the mixture of species within the wound, and the effects of microbial toxins.[51]

Contamination and infection of accidental wounds left to heal by second intention should be considered separately from surgical site infection, which has been well characterized.[52] The

majority of this section addresses the effects of the bioburden on accidental wounds healing by second intention.

Wound infection can be defined as multiplication of bacteria to such an extent that the host's defenses are overwhelmed, resulting in disruption of healing and damage to the wound.[53] Infection is the endpoint of a continuum of events that reflects the microorganisms' impact on the wound and the host's reaction to this impact.

Bacterial impact continuum

Several classification systems and many definitions have been used to categorize the impact of bacteria on a wound and on the patient. The classical definition is that more than 10^5 microorganisms per gram of tissue or per milliliter of exudate constitutes infection.[41] This number serves as a reasonable threshold for infection in a closed surgical wound that tends toward predictability.[51] An open wound left to heal by second intention, however, often can tolerate a higher concentration of bacteria (e.g., up to 10^6) without showing signs of infection.[41] The outcome is predominantly dependent on the adequacy of the host's immune response and the virulence of contaminating bacteria, as well as local environmental factors (such as the presence of foreign or necrotic material) that might potentiate the virulence of bacteria.[44]

In many clinical settings, accurate determination of the concentration and types of bacteria may not be possible. Consequently, clinicians must rely on clinical signs exhibited by the patient to establish the position of the wound along the bacterial impact continuum.[52] Quantitative and qualitative microbiologic testing of tissue or fluid from the wound, when available, may be used to confirm the diagnosis and guide the selection of appropriate antimicrobial therapy.[43] Importantly, even if the results of microbiologic testing do not indicate infection, a wound should be considered infected if clinical signs of infection, such as heat, swelling, and pain caused by palpation of the wounded region, are present.

A simple classification system that can be used to assist practitioners in determining how bacteria might be impacting a wound healing by second intention is outlined in Table 3.2. This system of classification is not absolutely related to increasing concentrations of bacteria but, in many instances, larger numbers and an increasing variety of bacteria, in particular, anaerobic bacteria, will lead to greater impairment to healing and sometimes to local and/or systemic effects.[52] This progression may also be influenced by the presence of a maturing bacterial biofilm in the wound.[53]

Microorganisms do not replicate in a *contaminated* wound, and a contaminated wound shows no clinical signs of infection. All accidental wounds in horses are at least contaminated. A wound is considered *colonized* when the contaminating bacteria, often resident skin microorganisms, have adhered to the wound and proliferate, thereby competing with healing tissue for nutrients and oxygen. The microbial by-products may be deleterious to the normal physiologic processes of healing;[9] if the growth and death of microbes is kept in balance by the host's immune system, however, the wound does not become infected and heals uneventfully.

There is a point along the continuum of bacterial impact where *critical colonization* occurs. At this point, replicating microorganisms can no longer be managed by the immune system and invade the wound's surface causing a *topical infection* that delays wound healing. When contamination changes to this state of critical colonization (infection), the wound's bioburden becomes the most important factor impeding healing.[54] Many of the classical signs of infection are absent, but the wound bed may appear unhealthy.[55] Specifically, an infected wound may be dull looking and may contain a copious amount of exudate and exuberant, friable granulation tissue, which may appear to be sloughing. The size of the wound may fail to decrease and may even increase.[52]

Further along the continuum, *local infection* arises, which is characterized by bacterial invasion of the local tissues (i.e., those

Table 3.2 Clinical manifestations of the increasing bacterial impact on wound healing.[52]

Level of bacterial impairment	Bacterial activity	Degree of impairment to wound healing and clinical signs
Contamination	Bacteria are on the wound's surface. No division is occurring	No impairment to healing No obvious clinical signs of infection
Colonization	Bacteria are dividing	No impairment to healing. No obvious clinical signs of infection. (Clinical wound appearance does not usually differ from contamination)
Topical infection (critical colonization)	Bacteria are dividing. Bacteria and/or their products have invaded the wound's surface. There might be an increasing variety of bacteria present. Biofilm may be present	No obvious clinical signs of infection. (Clinical wound appearance does not usually differ from contamination)
Local infection	Bacteria and/or their products have invaded the local tissues	Impairment to healing Usually obvious clinical signs of infection localized to wound environment and immediate peri-wound tissue
Regional/spreading infection/cellulitis	Bacteria and/or their products have invaded surrounding tissues	Impairment to healing. Usually obvious clinical signs of infection. May have systemic clinical signs
Sepsis	Bacteria and/or their products have entered the bloodstream and may spread to distant sites or organs	Impairment to healing. Usually obvious systemic clinical signs: patient usually acutely unwell. Damage to organs may occur

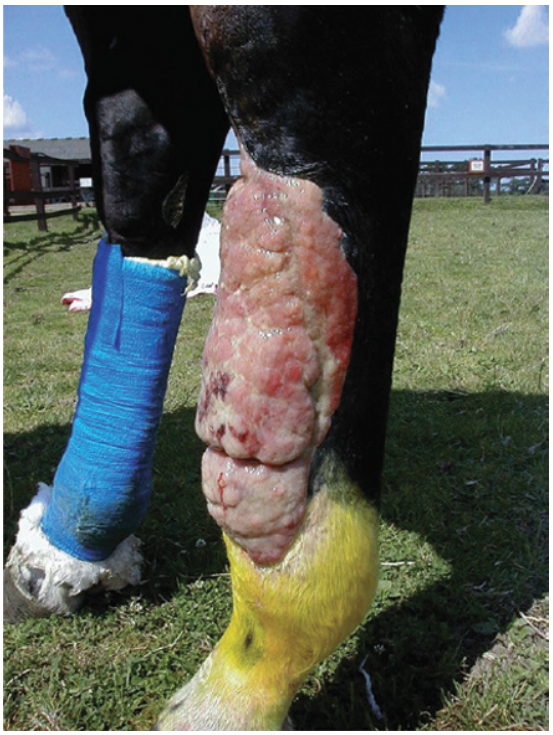


Figure 3.9 Example of a chronic wound showing local signs of infection: the granulation tissue has an irregular surface (clefts and tunnels), has become exuberant, and is covered by unhealthy exudate. Courtesy of Dr. D. Knottenbelt.

beyond the wound's surface), usually accompanied by obvious clinical signs of infection localized to the wound's immediate vicinity. At this point in the continuum, the wound usually increases in size, its granulating surface may exhibit irregularities (e.g., clefts and tunneling), and its exudate is often purulent and malodorous (Figure 3.9).

Regional or spreading infection/cellulitis occurs when the replicating microorganisms spread beyond the wound to injure surrounding tissues, thereby eliciting an immune response from the host.[56] This stage in the continuum is recognized clinically by heat, induration, signs of pain, and swelling, and sometimes by fever.

Wound infection might finally result in *sepsis*, although this is rare in horses because the immune response is usually functional and not easily overwhelmed.

Risk factors

Whether or not infection develops in a contaminated wound depends on many factors, including but not limited to: (1) dose of microorganisms; (2) virulence of microorganisms; (3) wound environment; (4) mechanism of injury; and (5) host response.

The virulence of a microorganism is related to its ability to adhere to eukaryotic cell surfaces, multiply, and evade the host's immune response. The virulence of different microorganisms varies and influences the dose of bacteria required to induce infection. The concentrations of bacteria per gram of tissue or milliliter of exudate necessary to cause infection in a closed surgical wound (10^5 bacteria) or an open wound (10^6 bacteria) have been established, but beta-hemolytic streptococci, notably *S. pyogenes*, are pathogenic at substantially lower concentrations than are many other bacterial species.[57] Moreover, polymicrobial interaction, where two or more microorganisms act synergistically, is likely to decrease the bacterial dose required for infection to result from contamination. For example, co-infection of *Staphylococcus aureus* and *Pseudomonas aeruginosa* is more virulent than infection caused by either species alone.[58] Bacteria found in open wounds are commonly considered to be aerobic, but anaerobic species are now also thought to play a role in infection because the frequency at which they can be isolated from chronically infected wounds has increased.[59]

Although chronic human and equine wounds share many pathophysiologic features,[60] the microbiologic burden of chronic wounds of equids has not been studied to the same extent as that of chronic wounds of humans. Two recent investigations, nevertheless, provide evidence for polymicrobial infection of chronic wounds of equids and show that the microbial genera found in chronic wounds of equids are very similar to those found in chronic wounds of humans, with commensal organisms acting as opportunistic pathogens when the wound's environment is favorable to their replication.[61,62] In the first study, the most common bacteria isolated and cultured from wound swabs were *P. aeruginosa*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Enterococcus faecalis*, and *Providencia rettgeri*. Slight differences were found between isolates cultured from swabs of the wound's surface and those cultured from wound tissues harvested by biopsy.[61] The second study confirmed the polymicrobial community harbored in the wound environment of equids. The average number of bacterial species identified from swabs of the wound's surface was 3.02 ± 1.65 (range 0–8). The species most often isolated from chronic wounds was *P. aeruginosa*, whereas the most commonly isolated genus was *Staphylococcus*. Obligate anaerobic bacteria made up 6.8% of the total isolates.[62]

Wounds on the distal aspect of limbs of horses are often contaminated with feces and soil, making them highly susceptible to infection. This relates, at least partially, to the role of components of soil in potentiating infection. Specific infection-potentiating factors have been identified in soil, in its organic components as well as in its fractions of inorganic clay.[63,64] The ability of these factors to incite infection seems related to damage to the host's defenses, in particular to a reduction in the efficiency of leukocytes to ingest and kill bacteria and to a decrease in specific humoral factors and neutralizing antibodies, thereby eliminating the bactericidal activity of serum. Consequently, for wounds contaminated by these fractions, only 100 bacteria per gram of tissue or milliliter of exudate may be necessary to elicit infection.[63] Other components of the wound's environment that may drive the wound along the bacterial continuum include foreign material (wood, metal, gloving powder,[65] suture, orthopedic implants, drains, etc.),

necrotic tissue (including bone sequestra), hematoma or seroma, or compromised vascular supply.[51] For example, a large comprehensive study reviewing the epidemiology of wound infections of human patients, found that formation of a hematoma or seroma was a leading factor in decreasing a wound's resistance to infection.[66] For information regarding approaches to the management of a hematoma or seroma, the reader is referred to Chapters 4 and 8.

A deficient response by the host is a relatively common cause of infection of wounds of humans who suffer from diseases such as diabetes, obesity, or a vascular disorder. Fortunately, equine patients usually have a functional immune system and, unless suffering from polytrauma or malnutrition, should mount an appropriate immune response to wounding.

Effects on healing

The wound offers bacteria an attractive environment in which they can flourish and, if able to overcome the host's response, cause substantial damage locally and systemically.

Infection is a major cause of delayed wound healing, reduced gain of tensile strength, and dehiscence after closure. The mechanism whereby these detrimental effects of infection occurs is probably the release of bacterial enzymes and metalloproteinases that degrade fibrin (required for migration of fibroblasts and for phagocytic activity of macrophages) and inhibit cytokines responsible for orchestrating healing. Accumulating exudate, in addition, physically forces the wound's edges apart.

Colonizing bacteria consume local oxygen required for healing, release free radicals damaging to tissues, and produce endotoxins that can activate coagulation, causing thrombosis of the microvasculature nourishing the wound,[52] or cause systemic organ or immune dysfunction. Endotoxins also favor the release of more inflammatory mediators, thereby leading to a chronic inflammatory state,[52] as is commonly seen in wounds on the limbs of horses.[1,4]

The overall effect of wound infection is increased costs, increased use of resources due to delayed healing, lost productivity, and pain.

Biofilms

In the previous sections of this chapter, bacteria were viewed from the perspective of planktonic (free-floating) pathogens proliferating and exerting their virulence, mostly as individual organisms. In the case of chronic wounds, however, microbes often exist as sessile (attached), multicellular consortiums known as biofilms.[67] The microbes in biofilms adhere to each other and/or surfaces or interfaces by a self-secreted, three-dimensional, extracellular polysaccharide (EPS) matrix.[68]

Formation of biofilm on a surface can be divided very broadly into three stages: microbial attachment, growth, and detachment.[69] Under natural conditions, planktonic bacteria reversibly adhere to a surface within minutes (stage 1).[70,71] In stage 2, biofilm-growing bacteria bind irreversibly to the surface and become sessile within 2–4 hours, then multiply and differentiate, changing patterns of gene expression in ways that promote survival (usually the result of a type of communication between bacteria known as quorum sensing). After 6–12 hours of firm attachment, the bacteria begin secreting a slimy substance (EPS) around the micro-colonies (stage 3). Once a biofilm has reached maturity (usually within 24 hours),[72] focal areas of the biofilm dissolve to shed planktonic cells, microcolonies and fragments of biofilm, which can disperse and attach to other parts of the wound bed, thereby forming metastatic daughter biofilms in the surrounding area; this occurs within 2–4 days, depending on the species and growth conditions.[71,73]

Biofilms cannot be seen with the naked eye. Rather, confocal or scanning electron microscopic evidence of microbial aggregation/EPS from wound samples is required to confirm the presence of a biofilm. Evidence of the presence of biofilms in wounds of human patients has been provided using scanning electron and confocal microscopy.[74] Biofilms tend to occur more frequently in chronic wounds (60%) than in acute wounds (6%), suggesting a possible role of biofilms in the chronicity of wounds.[74] Although less research has been done on biofilms in animals, biofilms are believed to be involved in many diseases of animals, including wound infections.[75] Two studies recently found evidence of the presence of biofilms in the wounds of horses.[61,62] One study detected polymer-embedded aggregates of bacteria using light microscopy of Gram-stained tissue harvested from the edge of chronic wounds on the limb of eight horses,[61] and the other found that tissue from eight of 13 chronic wounds of horses, examined microscopically after Gram staining, showed evidence that bacterial biofilms were present.[62] Furthermore, bacteria cultured from chronic and acute wounds and examined by microtiter plate assay showed significantly higher potential to form biofilm than did bacteria isolated from intact skin from the same horse.[62]

Single species of bacteria can make up a biofilm, but, more often, biofilms are comprised of multiple species of aerobic and anaerobic bacteria, and, occasionally, yeast, fungi, or protozoa. These organisms interact and develop stable, symbiotic relationships through complex intercellular communications, or quorum sensing, deep within the wound where they can be difficult to isolate and identify.[76,77] Traditional culturing techniques may be inadequate in establishing a comprehensive composition of a biofilm[78] but have identified *Staphylococcus*, *Pseudomonas*, and *Enterococcus* as the predominant organisms in the biofilm of chronic wounds in humans. Molecular analyses of chronic wound samples revealed diverse polymicrobial communities and the presence of bacteria, including strictly anaerobic bacteria, not revealed by culture.[74] In fact, a recent profiling study using 16S RNA sequencing demonstrated an average of 17 genera per wound, most of which were anaerobic.[79] Biofilms found in wounds of horses can be caused by environmental organisms such as *P. aeruginosa*, commonly found in infected wounds. Species of bacteria that constitute part of the normal

microflora of the skin have also been incriminated in the formation of biofilms. These normally harmless commensal organisms may become pathogenic through a combination of endogenous and exogenous factors.

The presence of biofilms has important implications for the management of chronic wounds in horses because the infection resulting from biofilms is more resistant to traditional means of therapy. This is because biofilms protect microorganisms embedded within them, improving their tolerance to the host's immune system, environmental stresses, and antimicrobial therapy, whether it is administered topically (antiseptics) or systemically (antibiotics).[80] Additionally, community living allows easier transfer of genes leading to sharing of advantageous attributes, such as increased virulence.[81] For example, human leukocytes easily kill planktonic *S. aureus*, but when the same microorganism resides in a biofilm, its elimination may require a 1000-fold increase in leukocytes.[75] Likewise, *S. aureus*, when growing within a biofilm, is reported to be up to 100-fold more resistant to antimicrobial therapy when compared to its planktonic counterparts.[82] Neither phagocytes nor antimicrobial agents may be able to readily penetrate the slimy exopolymer, and if antimicrobial agents do succeed in penetrating it, they may be rendered inactive.[83,84]

Although not all wounds with biofilm exhibit typical signs of infection, biofilms may, nevertheless, impair healing by competing for metabolic resources and by interfering with the inflammatory response to wounding. Indeed, biofilms have been shown to induce a chronic non-healing inflammatory phase of healing.[85] Furthermore, biofilms have been shown to delay epithelialization, at least in a murine model.[86]

The ability of the host to control pathogenic organisms decreases as the biofilm matures.[73] Consequently, efforts to prevent or slow formation of a biofilm should be implemented as soon as possible. A biofilm-based wound-care regimen should be multifaceted, with debridement as its most essential component (the reader is referred to Chapter 4 for a thorough description of methods of wound debridement). In addition to removing devitalized tissues that serve as a fertile environment for bacteria, debridement physically disrupts the biofilm structure. The result is a period of reassembly during which organisms are more susceptible to antimicrobial therapies that may prevent bacterial reattachment and limit or prevent reinfection.[87]

The best sample for bacterial culture and antimicrobial sensitivity testing is a deep tissue biopsy specimen, or, if such a sample cannot be obtained, a swab of the deepest tissues, or both. Traditional methods of culture may not be adequate to establish the bacterial composition of biofilm in chronic wounds, complicating the selection of appropriate antimicrobial therapy.[78] In fact, biofilms of chronic wounds are characteristically polymicrobial, and sophisticated molecular techniques are required to identify the bacterial components of the biofilm. If antimicrobial drugs are administered systemically as part of the treatment, the minimum inhibitory concentrations (MIC) established by antimicrobial sensitivity testing may provide suboptimal dosing regimens and should be replaced with minimum biofilm eradication concentrations (MBEC).[75] Antiseptics, in particular the two biofilm-active antiseptics, octenidine and polyhexanide,[73] and silver-based wound dressings are used concurrently to combat biofilms in wounds of human patients.[88] The reader is referred to Chapter 5 for more information about topical wound-care products and to Chapter 6 for more information about wound dressings.

Unfortunately, a situation may arise wherein microorganisms referred to as "persisters" survive a recommended course of antimicrobial therapy because they are protected within the exopolymer matrix of the biofilm, which limits antibiotic penetration.[89] Withdrawal of the antimicrobial drug allows these "persisters" to replicate and, when conditions are appropriate, undergo a phenotypic change to become planktonic and free of the biofilm but with an increased ability to resist antimicrobial drugs and the host's immune response. Antibiotic chemotherapy is becoming increasingly ineffective in the treatment of biofilm infections because of antibiotic resistance.[80] This discovery has led to new strategies to deal with some diseases caused by biofilms. One of these consists of a pulsed dosing regimen wherein low doses of antimicrobial drugs are administered for an extended time while cycling with medication-free periods. This replaces the more classic short-term, continuous high-dose regimen. Using a low dose of antimicrobial drug, or alternatively, concurrently using low doses of two or three antimicrobial drugs targeting the biofilm, is associated with less risk of antibiotic resistance. This approach is postulated to be more effective in achieving a 100% kill and, therefore, less likely to leave behind microorganisms capable of developing antimicrobial resistance.[89] The reader is referred to Chapter 19 for more information regarding the therapeutic approach to eliminating bacterial biofilms.

It is not possible to categorically state when a wound is biofilm-free, because definitive clinical signs of a biofilm and available laboratory tests to detect a biofilm are lacking. Consequently, the most likely clinical indicator of a biofilm-free wound is progression of healing, with reduced formation of exudate and tissue slough.[88]

Conclusion

The rate and success of wound healing is determined by many factors, some of which cannot be manipulated because they already exist when the wounded horse is presented for treatment. Wound bioburden is, without a doubt, the most important impediment to healing but, fortunately, one that can be positively influenced.

Recognizing the factors that negatively impact healing and preventing or correcting these factors are key to promoting repair and returning the horse to its normal function as soon as possible, while achieving a cosmetic outcome.

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