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Skin Grafting in Horses

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Skin grafting is one of the most common reconstructive techniques employed in the horse, and the results usually offer a more cosmetic and functional scar than would occur with second-intention healing. In most cases, convalescent times are shorter, giving an earlier return to performance. This issue of *Large Animal Veterinary Rounds* offers a detailed account of autogenous skin-grafting techniques, including an in-depth discussion of skin-graft physiology.

Autogenous skin grafting involves the transfer of skin components harvested from an uninvolved anatomic area to a wounded area on the same animal. Grafting techniques commonly employed include:

- Pinch or punch grafts
- Tunnel grafts
- Split-thickness sheet grafts
- Full-thickness sheet grafts

Irrespective of the technique employed, management of the recipient wound bed is probably the single most important determinant of success. A comprehensive understanding of skin-graft physiology allows rational decision-making during the operative and immediate postoperative period.

Traditionally, skin grafting was reserved for the management of granulating wounds; however, immediate application of skin grafts to fresh wound beds has been used successfully.¹ In fact, the fresh wound environment (surgical wound or recent accidental wound) is consistently friendlier for grafted skin than even the most optimally managed chronic granulating wound. In essence, the recipient bed needs to be vascular and free of infection and devitalized or necrotic tissue. Avascular areas, such as bone, paratenon, or infected tissue (chronic wounds), are poor graft recipients, and grafting should be delayed until the wound bed is covered with healthy neogranulation tissue.

Skin-graft physiology

“Graft take” is defined as acceptance of the graft and involves revascularization of the grafted skin; this is the incorporation process of grafted tissue into the recipient tissue bed. Possibly the most important prerequisite for successful skin grafting in chronic wounds is the formation of an infection-free granulation tissue bed. Although the incidence of deep-seated infection in proliferative granulation tissue is low, a layer of necrotic collagen coagulum and superficial bacterial contamination is common, and should be removed prior to grafting. Graft acceptance is a complex stepwise process involving vascular, mechanical, and cellular components.^{2,3}

Adherence

Graft adherence is the mechanism through which the graft is attached to the granulation tissue bed within a few minutes of placement. Fibrinogen, contained in the wound bed, is converted into fibrin and essentially functions as tissue glue holding the graft in place. This adhesion is the only



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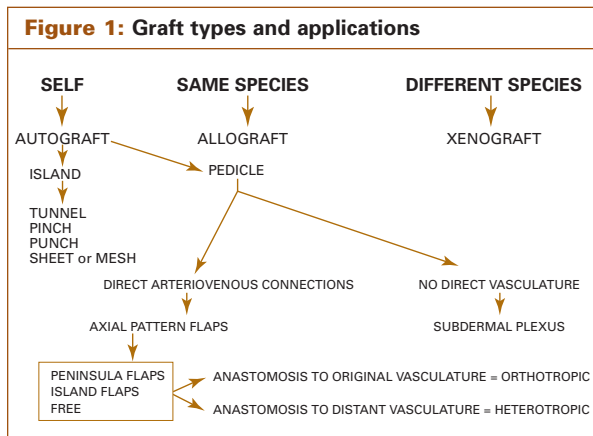
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method of attachment in the first few days; it is extremely delicate and easily disrupted, thus careful bandaging is required to maintain graft integrity (Figure 1).

Early graft nutrition: serum imbibition

Free grafts are, by definition, avascular and therefore ischemic. Nutrition of the graft prior to revascularization occurs through a process of serum imbibition. Initially, the grafted tissue absorbs fluid and erythrocytes by capillary action. This process is thought to provide nutrition for the first 36–48 hours, during which time anaerobic metabolism within the graft results in lactic acid production and the release of vasoactive substances enhancing revascularization. Passive fluid transfer leads to graft edema and a clinical appearance of cyanosis in the grafted tissue due to the presence of erythrocytes. Weight gains associated with fluid accumulation peak at 2–3 days (up to 40% of the increase in weight) after which time revascularization removes the fluid and the graft returns to its original weight by Day 9. The importance of this nutritive function is illustrated by the fact that thin split-thickness grafts are more tolerant of long imbibition periods than thick grafts; further, degeneration of the graft follows a gradient causing the superficial layers to suffer the most damage.

Graft revascularization

Revascularization of the skin graft occurs by two processes:

- inosculation (connection of existing vessels in the graft to vessels in the recipient bed)
- neovascularization (ingrowth of new vessels).

The relative contribution of each process is a point of disagreement among graft researchers and may depend on graft thickness, the quality of graft adherence, and the type of wound bed (fresh tissue or granulation tissue).

Primary revascularization occurs by inosculation and the anastomosis of capillary buds with the graft vasculature. This occurs without the definition of veins and arteries, thus initially the circulation is sluggish. By days 4 to 7, true circulation is restored and a large number of obsolete anastomotic connections regress.

Secondary vascularization or definitive vascularization occurs by the ingrowth of vessels from the recipient granulation tissue bed. While inosculation is important for initial nutrition, the final vascular arrangement of the graft is a result of this secondary process. In rats, dermal penetration can occur within 12 hours and the dermal-epidermal junction can be reached within 48 hours. Delayed vascularization occurs in situations of bacterial contamination, poor adherence, and increased distance from graft to bed or insufficient recipient bed vascularization.

The development of lymphatic drainage parallels vascularization of the graft. It begins from days 2 to 7; however, lymphatic flow is slightly delayed compared with the vascular component (Figure 1).

Graft reinnervation

Reinnervation usually begins within the first few days of graft placement. This occurs with the use of pre-existing neurolemmal sheaths as scaffolds or by direct penetration of the graft. The presence of nerve fibres does not parallel the return of sensation, which may be delayed, incomplete, or absent.

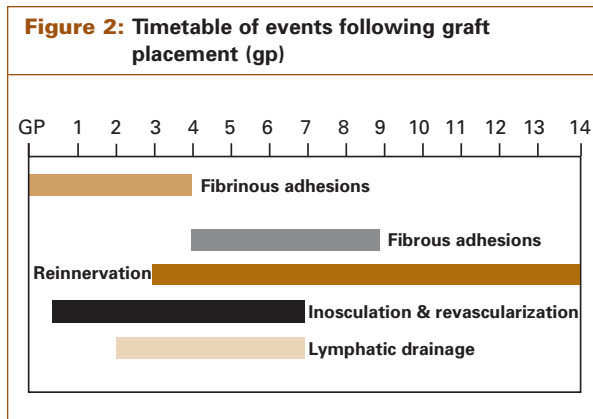
Graft organization

Graft organization occurs at the same time as revascularization. Fibroblasts infiltrate the fibrin layer beginning on Day 4, with a firm connection established by Day 9. If the processes of vascularization and collagenous adhesion occur in good time, there is little loss of epidermal basement membrane, dermal collagen, or elastin; within 5 months, up to 85% of graft collagen has been replaced.

Graft failure

Graft failure can occur for myriad reasons; however; the major determinant of failure is the inability to maintain good adherence and the subsequent failure of the “take” process. Fluid accumulation underneath the graft results in the elevation of the graft from the wound bed, which limits adherence and reduces or prevents revascularization. Either hemorrhage and serum extravasation between the graft and the wound bed will result in fluid accumulation and graft separation.

Infection results in inflammation and exudation; in the horse, the most common surface contaminants are *Streptococcus* species, *Escherichia coli*, *Pseudomonas* species and *Staphylococcus* species. True infection (10^5 or greater organisms/cm³) is associated with almost universal graft failure.⁴ When bacteria concentrations are lower, some graft success may be achieved, except with wounds containing *Streptococcus* species. The presence of *Streptococcus* species at any concentration level is a contraindication for grafting. These organisms produce proteolytic enzymes that convert plasminogen to plasmin, resulting in fibrinolysis and a complete loss of early graft adhesion to the wound bed.⁵ Some bacterial strains (β -hemolytic *Streptococcus* spp.) directly produce toxins and cause a significant problem in equine species primarily due to the ubiquitous nature of this organism.



Motion between the graft and the wound bed will shear the fragile capillary buds. To an extent, this is a greater concern with sheet or mesh grafts than in the common punch, pinch, and tunnels grafts.

Graft types and applications

Grafts from the same animal are termed autografts, grafts from another animal of the same species are allografts, and from another species, xenografts (Figure 2).⁶ The choice of graft depends on the time it will remain on the wound, as well as the availability of material. In short-term applications, xenografts or allografts can be used as wound-management tools because they are readily available and can be stored for extended periods of time. Autografts represent a permanent solution to wound management; although it is a rare complication in the horse, obtaining graft material from the same wounded animal can result in donor site morbidity and, possibly, eschar formation at a site remote from the initial wound.

Tunnel, pinch, and punch grafts are examples of island grafts, which by definition are devoid of blood supply. Portions of skin and subcutaneous tissue with an intact vascular supply are termed pedicle grafts and are commonly used in small animal and human reconstructive surgery. These are versatile grafts because they can be used to cover areas with a poor blood supply, in areas that are difficult to immobilize, or to provide immediate covering for subcutaneous structures that suffer from exposure injury (eg, nerves, blood vessels, and tendons). Pedicle grafts can be classified according to their circulation, location, or composition. Those containing a direct cutaneous artery and vein are axial pattern flaps. Grafts elevated without the inclusion of direct vasculature and reliant on the circulation from deep or subdermal plexuses are termed subdermal plexus flaps. Axial pattern flaps have an excellent blood supply and enable the surgeon to create large flaps for use in humans and small animals. They are termed peninsula flaps if they remain attached to the body by skin, island flaps if they remain attached only by their underlying vasculature, and free flaps if this pedicle is severed. A free flap anastomosed to the original vasculature is an orthotrophic flap, whereas a

free flap reattached to a vascular trunk distant from the site of origin is termed a heterotrophic flap.

Unfortunately, the use of axial pattern flaps in the horse has been a universal failure. Lees et al⁷ identified and described the vascular supply of a large myocutaneous flap in horses, which was vascularized by a superficial branch from the caudal branch of the deep circumflex artery and vein. These same authors described the transfer of axial pattern flaps from the flank region, to the dorsum of the tarsus and the face of a horse.⁸ In their report, the island grafts were universally successful, but all orthotrophic grafts failed due to technical difficulties and, other than one heterotrophic graft that lasted 21 days, all other heterotrophic grafts failed. The case that survived to Day 21 was attributed to neovascularization from the wound bed and not to the patency of the anastomotic vasculature, since dissection post mortem indicated tortuosity of the arterial and venous anastomoses. The authors postulated that the remainder of the heterotrophic grafts failed despite adequate microvascular anastomosis due to a phenomenon known as no-reflow. This results when the microcirculation to ischemic tissue is not restored after vascular patency has been re-established. This may be caused by a slow, progressive or complete failure of circulation. The pathogenesis is unclear, but may be due to altered blood constituents, intrinsic resistance of static blood, or ischemic damage to the microcirculation. The no-reflow phenomenon and Virchow's classic triad of vascular failure (blood vessel wall, blood flow, and blood constituents) may explain early failure of technically adequate anastomotic flaps. Failure of free-flap transfer is rare in other species and almost unheard of in the human literature. The authors suggest that flap failure in the horse may be due to the no-reflow phenomenon or to unusual behaviour of equine blood.⁸

In addition to defining skin grafts by their vascular supply, grafts are further subdivided into full-thickness (includes epidermis and dermis) or split-thickness (contains epidermis and only a portion of the dermis). There are varying degrees of split-thickness grafts (thin or thick).

Graft expansion is a method using multiple small incisions through the donor skin to enlarge it and cover more recipient granulation tissue bed;⁶ if the initial graft is a sheet, this converts it into a mesh graft. In most cases, mesh grafts are the ideal choice because they provide the best functional and cosmetic appearance. However, they are expensive to perform, they typically require general anesthesia, and failure of the entire graft is likely once any part of the graft begins to fail. Mesh grafts are often not a viable option in the horse because most injuries occur in areas of high motion (eg, carpus and tarsus) or in areas difficult to bandage.

Tunnel, pinch, and punch grafts involve the transfer of skin to the granulating wound. These techniques do not require advanced surgical expertise and the instrumentation needed is readily available in most practices. With proper case selection, these techniques can be readily applied in the practice setting.

Figure 3: Skin-graft harvest: punch grafts are cut from donor site



Skin grafting: a step-by-step approach to punch grafting in the horse

Preparing the recipient site

Preparation of the graft recipient site is critical to the overall success of the skin-grafting process. The primary goal is a well-vascularized bed of granulation tissue that is free of both deep and superficial infection.

- Topical medications, including steroid-based medications, used to retard proud-flesh proliferation should be discontinued 7 to 10 days prior to grafting.
- Exuberant granulation tissue should be cutback to the level of the surrounding skin to obtain a flat, fissure-free graft bed.
- The author follows these debridements with a topical antibiotic medication placed on a nonadherent dressing immediately following the procedure in an effort to further reduce the bacterial load.
- Most wounds can be grafted 48 hours after recipient-bed debridement.

Obtaining the graft

Identify the donor site; this is usually the neck (under the mane fold) in the standing horse or the caudo-lateral belly wall in the anesthetized animal. The hair is clipped and the region is aseptically prepared.

- Full-thickness skin grafts are obtained using a 6-mm skin punch. It is important to remember that skin will retract (up to 60%) immediately following harvesting; therefore, donor sites should be larger than recipient sites. Significant attention should be given to ensuring that there is no subcutaneous tissue attached to the bottom of the graft (Figure 3). This fascial or fatty tissue impedes imbibition and inosculation, and may result in a failure of the graft to “take.”
- Grafts are placed on a saline- or blood-soaked gauze after trimming to ensure that they do not dry out.
- The donor sites do not need to be closed with sutures.

Graft placement

Preparation of the wound bed for the placement of the punch grafts involves using a 4-mm biopsy punch to create holes in the graft bed (approximately

Figure 4: Skin-biopsy punch is used to create graft sites in wound bed



1 graft/cm²). The result is a series of circular holes cut in the flat, coagulum and fissure-free granulation tissue bed (Figures 4 and 5).

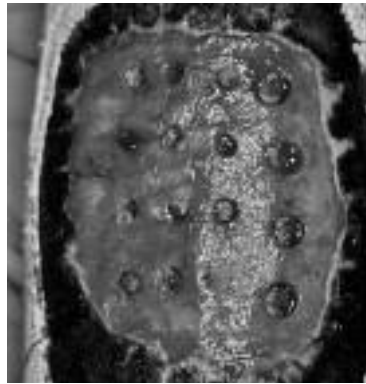
Hemorrhage is controlled either by using sterile cotton buds, placed in the holes, or by a pre-placed tourniquet proximal to the affected region. Hemorrhage makes placing the grafts difficult, since they tend to “float” out of the recipient holes. Tourniquet use allows graft placement, as well as placement of a pressure bandage prior to release that prevents graft displacement. Some hemorrhage difficulties can be avoided if the wound bed holes are made before the skin grafts are collected, allowing time for hemorrhage to cease.

- Punch holes must be deep enough to situate the grafts flush with the surface (Figure 6), but not too deep because granulation tissue will cover the epidermal components of the graft and require further surgery to remove.
- Nonadherent pads should be placed over the grafted region and held in place with a conforming primary bandage layer. Reduced or complete immobility of the region is achieved with the use of either a thick stable bandage or possibly a cast.

Figure 5: Granulation tissue plugs are removed from wound bed



Figure 6: Wound with punch grafts in place



- This bandage should **not** be removed or changed for 7 days!
- The bandage layer will develop a strong odour and, potentially, strike-through of the bandage will occur. Removal of the bandage prior to fibrinous adhesions becoming fibrous runs a significant risk of graft movement and microsheading of the neovascularization.
- Bandage removal at 7 days should be performed with care. The author usually soaks the primary layer with saline prior to removal, which aids in reducing adhesion to the underlying graft and granulation tissue. At this stage, grafts may appear blue-grey; this is a function of graft edema, as well as deoxygenated erythrocytes within the tissue. These tissues may not be necrotic and, as such, should not be removed. Subsequent to the first bandage change above, bandages can be changed every 48 to 72 hours, with careful observance of aseptic technique.
- If the limb was placed in a cast to reduce movement, cast removal can occur at 3 weeks, assuming that the horse is using the cast appropriately.

Advances in skin grafting: grafting extensive burns

The prompt closure of wounds is of major importance in the human burn patient to prevent or control further fluid loss and support thermoregulation, as well as to prevent superinfection. In these cases, the autograft is the “gold standard” that results in the best long-term functional and cosmetic outcomes. However, in those patients with >50% of the body burned, the preservation of sufficient donor sites to accomplish an autograft is limited. As a result, various allo- and xenografts have been used as temporary dressings prior to treatment; for example, these may include porcine skin and human amniotic membrane. In the late 1980s, an artificial skin was developed for use in these situations. Artificial skin should be: free of bacteria, nontoxic, noninflammatory, nonantigenic, and serve as a barrier to microorganisms, yet be permeable to vapor while contributing to thermoregulation. It must

adhere to the wound bed, and support the local defense mechanisms and wound healing. It must also be elastic and durable in the long-term, demonstrating a growth potential similar to human skin with good aesthetic qualities.

The presence or absence of a dermal layer in burn wounds is of primary importance, since various investigators have indicated that the dermal layer provides a durable scaffold offering adequate long-term graft stability. The presence of a dermal layer is also essential for growth regulation and differentiation of cultured keratinocytes. In burn patients, full-thickness autografts would be needed to provide sufficient dermal structures for the support of keratinocyte differentiation and maturation.

Recently, the development of a bilayer artificial skin (Integra® Dermal Regeneration Template [DRT]) fulfilled all of the requirements for successful use. It is composed of an upper silicone membrane and a lower spongy sheet of highly purified bovine type 1 collagen (harvested from bovine tendon) cross-linked with glycosaminoglycans (GAGs). The optimal pore size, 50 µm (±20) allows the infiltration of cellular tufts of fibroblasts and capillaries when it is placed on the wound bed. This gradually becomes a biosynthetic neodermis and minimizes the need for autologous dermis. However, it still requires the use of a second procedure 10–14 days after placement, to cover the neodermis with split-thickness autografts. These are most often taken from the axilla or pubic regions, since they tend to be spared from injury.^{9–11}

Another method of covering large burn wounds is the use of a hyaluronic acid-based 3-dimensional fleece, onto which are placed autologous fibroblasts.¹² These fibroblasts are taken by biopsy, replicated in a static or perfusion culture system, and then integrated onto the scaffold. The combined fibroblast scaffolding is placed over the wound (like Integra® DRT) until it is incorporated. Subsequently, this is covered in another hyaluronic acid-based membrane (20 µm thick) with laser-drilled microperforations (Laserskin®) onto which autologous keratinocytes have been seeded. As a result, dermal and epidermal components are restored, but this still requires a two-step procedure.¹³

Improvements in these procedures for human patients involve the use of Integra® DRT and the immediate removal of the overlying silicone membrane. This membrane is replaced by an ultra-thin split-thickness graft, which turns a two-step process into a single procedure.

Most recently, biopsy-harvested autologous keratinocytes have been seeded onto Integra® DRT in a continuous-perfusion culture medium and transplanted onto skin defects in rats. In this manner, the dermis is replaced and acts as the required scaffolding for the development and differentiation of keratinocyte-based epidermis. If this proves successful in

human patients, then donor-site morbidity will soon be only a historical concern and burn victims will receive full-thickness autologous grafts from a single innocuous skin biopsy.^{14,15}

James L. Carmalt, is an Associate Professor of Large Animal Surgery in the Department of Large Animal Clinical Sciences at the Western College of Veterinary Medicine. His research interests are in the areas of equine-dentistry and equine orthopedic disease.

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Abstract of Interest

Preservation of skin by refrigeration for autogenous grafting in the horse

SCHUMACHER J, CHAMBERS M, HANSELKA DV, MORTON LD.

Eighteen stored split thickness meshed skin grafts were applied to surgically created lesions on the metacarpal and metatarsal regions of six horses. Donor skin was harvested from the sternal region,

meshed and stored at 4 degrees C in a cell culture medium containing 10% serum. Stored grafts were applied to the wounds at 1, 2, and 3 week intervals. Acceptance of the grafts stored for 1 week was generally poor (1 of 6 grafts), whereas that of the 2 and 3 week old grafts was generally excellent (10 of 12 grafts). Poor acceptance of the 1 week old grafts was attributed to streptococcal infection of the recipient wounds. Using the storage medium and grafting technique described, excellent acceptance can be expected after graft storage of up to 3 weeks.

Vet Surg. 1987;16(5):358-361.

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