Simple Techniques of Skin Grafting Horses

Jim Schumacher

The most common indication for skin grafting horses is to promote healing of a wound so large that it cannot heal by second intention (Fig. 1.), but nearly any open wound can be grafted. The more rapid healing achieved with a skin graft may make grafting more economical than a lengthy period of bandaging, and it may allow the horse to return to athletic performance sooner. Skin grafting should not be regarded as an option of last resort but instead should be considered when treating a horse for any open wound that cannot be closed primarily, especially if the wound is in an area where healing by second intention is likely to be prolonged or result in unsightly scarring.

The two basic types of skin grafting are pedicle grafting and free grafting. A pedicle graft is created adjacent to the wound and remains connected to the donor site by a vascular pedicle, ensuring its viability. It imparts a good cosmetic appearance because the graft is composed of all components of skin. Pedicle grafts are rarely used to cover wounds of horses because mobilizing skin of horses to advance a pedicle graft to cover the adjacent wound is difficult.

A free skin graft is a segment of skin that has no vascular connections because it has been detached completely from its donor site and relocated to a wound at a remote site. An autograft, or isograft, is a graft transferred from one site to another on the same individual and is the most practical type of graft applied to wounds of horses because the graft provokes no immune response. An allograft, or homograft, is a graft transferred between two members of the same species, and a xenograft, or heterograft, is a graft transferred from a member of one species to a member of another species. An allograft or xenograft is sometimes applied to a wound as a biological dressing. Free skin grafts can be also classified as full-thickness or split-thickness. Full- or split-thickness skin grafts can be applied to the surface of wounds as sheets (i.e., sheet grafts) or implanted within the wound (i.e., island or seed grafts). Each technique of skin grafting has its advantages and disadvantages, and so, the technique selected depends on the circumstances, such as the size and location of the wound, the necessity for a good cosmetic appearance, available instrumentation, experience of the surgeon, and the financial resources of the owner.

A free graft can be accepted by nearly any type of tissue, except bone devoid of periosteum, tendon devoid of paratenon, and cartilage devoid of perichondrium, but to accept a free graft, the wound should be vascular and free of infection and necrotic tissue. The graft attaches to the wound by fibrin within minutes after being applied and is nourished initially by imbibing plasma by capillary action into the lumen of the graft’s vessels, a process referred to as plasmatic imbibition. Blood vessels and fibroblasts from the wound invade the fibrin within 48 to 72 hours. After several days, new capillaries from the wound cross the fibrin matrix to anastomose with capillaries in the graft, a process called inosculatation. New capillaries from the wound invade other vessels within the graft and cut new vascular channels into its dermis, a process referred to as neovascularization. The graft is revascularized by day 5 and is attached firmly to wound by day 10.

Infection is, by far, the most common cause of failure of skin grafts to be accepted, but other causes are excessive inflammation of the wound, accumulation of fluid beneath the graft, and motion. The
wound becomes infected when the tissue concentration of bacteria surpasses $10^5$ organisms per gram of tissue, but the concentration necessary to infect a wound can be much less than $10^5$ per gram of tissue for some bacteria, particularly $\beta$-hemolytic streptococci and pseudomonads. $^{3,5}$ Wounds of the distal portion of limbs of horses that heal by second intention are inherently inflamed, and therefore, grafts applied to wounds of horses may be more prone to failure than are grafts applied to wounds of other species. $^{7,8}$ Blood, serum, or exudate accumulated beneath a graft prevents fibrin from attaching the graft to the wound and obstructs influx of capillaries from the wound into the graft. $^9$ Shearing forces between the graft and the wound, caused by movement of the bandage, disrupt the fibrin attachment and impair re-vascularization.

A fresh wound accepts a graft more readily than does a granulating wound, and a wound filled with immature granulation tissue accepts a graft more readily than does a wound filled with mature granulation tissue. Survival of a skin graft is better correlated to the concentration of bacteria in the wound than to any other factor. $^{10}$ The bacterial status of a wound is usually assessed qualitatively, however, rather than quantitatively. A wound should be assumed to be infected if it shows signs of inflammation, but even a wound showing no signs of inflammation may still be infected. $^{10}$

Infection is most often caused by streptococcal or pseudomonas spp. Streptococcae are nearly always susceptible to a $\beta$-lactam antibiotic, $^{11}$ and pseudomonads are usually sensitive to aminoglycoside antibiotics. $^5$ A $\beta$-lactam antibiotic may become ineffective in resolving a streptococcal infection if other bacteria in the wound secrete $\beta$-lactamase, an enzyme that inactivates $\beta$-lactam antibiotics. $^{12}$ The efficacy of the $\beta$-lactam antibiotic against streptococci is preserved if the $\beta$-lactam antibiotic applied to the wound is effective against $\beta$-lactamase producing bacteria also present in the wound. The efficacy of the $\beta$-lactam antibiotic can also be preserved by concomitant administration of clavulanic acid, a potent inhibitor of $\beta$-lactamase. Clavulanic acid is combined most commonly with amoxicillin or ticarcillin.

Infection of a granulating wound is best resolved by administering the appropriate antibiotic topically, rather than systemically, because an antibiotic administered systemically often fails to reach a therapeutic concentration in the granulation tissue. $^{3,13}$ Most bacteria and inflammatory cells can be removed from an infected, granulating wound by excising the granulation tissue to the level of or slightly below the margin of the surrounding skin.

**Island Grafting**

The simplest method of grafting is island grafting, which is a technique in which a small disc or strip of full-thickness or partial thickness skin is implanted into a granulating wound for the purpose of increasing the area of epidermis from which epithelialization can proceed. Types of island grafts applied to wounds of horses are punch grafts, pinch grafts, and tunnel grafts.

Punch grafts are full-thickness plugs of skin harvested and implanted into granulation tissue using skin biopsy punches. Punch grafts are harvested with a punch directly from an inconspicuous site on the horse, such as the portion of the neck that lies beneath the mane. After preparing the donor site, grafts are harvested about 1 cm apart using a 6- to 8-mm diameter skin biopsy punch. The small wounds created by the punch can be closed with a suture or staple but are usually left unsutured to heal by second intention. Subcutaneous fascia is excised from each graft to expose dermal vasculature to permit plasmatic imbibition and inosculation. $^{14}$ One technique used to remove subcutaneous fascia from the graft is to penetrate the skin with the skin biopsy punch, and then, using thumb forceps, lift the edge of the graft to expose subcutaneous tissue, which is excised from the dermis. Removing subcutaneous tissue from each plug is tedious (Fig. 2), and therefore, some clinicians prefer to harvest punch grafts from a section of skin excised from the cranial pectoral region. $^{15}$ The donor site is closed...
in one or two layers. Subcutaneous fascia is excised from the section of skin to expose the dermal vasculature, and plugs are harvested from the excised section of skin by using the biopsy punch.

The recipient holes in the wound are created about 5 or 6 mm apart and should be created before the grafts are harvested to ensure that haemorrhage has ceased before the grafts are implanted. Creation of the holes should begin distally and proceed proximally. The recipient holes are created with a slightly smaller punch than that used to harvest the grafts to allow for contraction of the graft. A cotton-tipped applicator inserted into the holes prevents a blood clot from forming within the hole and facilitates locating the holes for inserting the grafts (Fig. 3). The grafts are inserted into recipient holes, using a hemostat or tissue forceps, with the graft's epidermis oriented toward the surface of the wound.

Pinch grafts are small discs of skin, harvested by tenting skin to create a cone of skin and then excising this cone to create a small disc, which is implanted into granulation tissue. Pinch grafts can be harvested directly from an inconspicuous site, such as the horse's perineum, neck, ventrolateral aspect of the abdomen, or pectoral region. Hair is clipped from the donor site, the site is cleaned, and desensitised by injecting local anaesthetic solution subcutaneously. Skin is most easily tented by using a 20-gauge, hypodermic needle with a bent point (Fig. 4). The cone of skin is excised with a #11 scalpel blade to create a 3-mm diameter disc. Discs 3 mm or less in diameter are split-thickness, whereas the center of larger grafts tends to be full-thickness. The graft is placed on the wound, with its epidermal side up, and a pocket into which the disc is implanted is created in the granulation tissue immediately distal to the graft by using a #15 scalpel blade inserted into the granulation tissue at an acute angle to create a shallow pocket. The disc, which adheres to the wound, is pushed into the pocket using the scalpel blade used to make the pocket. By using this technique, the surgeon can create and implant the pockets without averting his or her eyes from the wound. No consideration need be given to the direction of hair growth as the graft inserted because orienting the hair in its proper direction has little effect on the cosmetic outcome and is tedious. Implantation should begin distally and proceed proximally so that the haemorrhage from the sites of implantation does not obscure that portion of the wound that has yet to be implanted.

The thin layer of granulation tissue overlying each pinch graft sloughs, usually between the first and second week. The superficial, pigmented portion of pinch and punch grafts frequently sloughs at about this time exposing pale dermis, which may be difficult to distinguish from surrounding granulation tissue, giving the impression that all of the graft has sloughed. A red ring of advancing epithelium is seen surrounding each pinch or punch graft by three weeks, and these rings of epithelium increasingly expand until they converge to cover the entire wound (Fig. 5). Half or more of pinch and punch grafts
can be expected to survive. The time required for the wound to epithelialize is inversely proportional to the amount pinch or punch grafts applied.

Tunnel grafts are thin strips of full-thickness or split-thickness skin implanted into tunnels created in granulation tissue.¹⁷-¹⁹ Tunnel grafts can be harvested, using a variety of techniques, from many sites and can be implanted with the horse sedated or anaesthetized. They can be harvested from the ventral aspect of the flank with the horse anaesthetized or from the neck or cranial pectoral region with the horse sedated.

One technique of harvesting strips of skin is, after preparing the donor site, to create linear wheals, 2- to 3-cm wide and slightly longer than the width of the wound to be grafted by subcutaneously injecting isotonic saline solution (if the grafts are harvested with the horse anaesthetized) or local anaesthetic solution (if the grafts are harvested with the horse sedated).¹⁷-¹⁹ A straight intestinal forceps is applied to the base of the wheal so that skin protrudes from the jaws of the forceps, and the protruding skin is excised with a scalpel blade. The width and thickness of the graft is determined by the amount of skin protruding above the jaws. If a full-thickness strip of skin is harvested, the donor site should be closed with sutures or staples. If a split-thickness strip is harvested, the partial-thickness wound created is left unsutured. Subcutaneous fascia must be excised from full-thickness tunnel grafts to expose the dermis. A method of harvesting full-thickness strips of skin is to excise a 2- to 3-cm wide, full-thickness, elliptical section of skin from the ventrolateral aspect of the flank or cranial pectoral region, and, after sharply excising subcutaneous tissue from the graft, to divide this skin into 2- to 3-mm wide strips. The wound is closed in one or two layers. Split-thickness strips of skin can be cut from a split-thickness section of skin harvested from the ventrolateral aspect of the thorax or the ventral aspect of the abdomen (see split-thickness skin grafting below).

To implant a tunnel graft, the shaft of a long, thin, alligator forceps is inserted through the granulation tissue at the edge of the wound, perpendicular to the long axis of the limb, at a depth of 2 to 3 mm until the end of the forceps emerges at the other side of the wound tissue. One end of the graft is grasped with the forceps, and the forceps and graft are pulled back through tunnel created by the forceps, taking care that the graft’s epidermis is oriented toward the surface of the wound. The protruding ends of the graft are fixed to the skin surrounding the wound with a suture, staple, or cyanoacrylate glue. The graft is implanted in two steps if the wound is too convex to be spanned by the alligator forceps.¹⁷-¹⁹ In this situation, the end of the forceps is inserted at the margin of the wound and exited in the centre of the wound. The graft is grasped by the jaws of the forceps and pull through the tunnel. The end of the forceps is inserted into the margin of the opposite side of the wound and exited at or close to the site of entry of the graft in the center of the wound. The end of the graft is grasped by the jaws of the forceps and pulled back through the second tunnel. Grafts are implanted about 2 cm apart. If the graft was implanted shallowly (i.e., at ≤ 2 mm), the granulation tissue overlying the graft is likely to slough within a week exposing the re-vascularized graft. If the graft was implanted deeply (i.e., at 4 to 5 mm), granulation tissue overlying the grafts must be excised six to 10 days later, with the horse sedated or anaesthetized.¹⁷-¹⁹

Island grafting requires no expensive equipment and little expertise and can usually be performed with the horse sedated. Island grafts are often accepted by wounds that have little chance of accepting a sheet graft, such as inflamed wounds or wounds in areas of high motion. Rejection of one graft has no effect on acceptance of other grafts. Island grafting is usually reserved for small wounds and for circumstances where epithelial scarring is unimportant to the owner. Island grafting is tedious, and the
grafts can be applied only to granulating wounds. Complications of tunnel grafting include inadvertent dislodging of a graft while attempting to expose it and failure to locate buried grafts.

**Full-Thickness Sheet Grafting**
A full-thickness graft is most easily harvested from the cranial pectoral region - one of the few places on a horse where the skin is relatively mobile. The graft is usually harvested with the horse sedated, after desensitising the donor site with local anaesthetic solution. The donor site is sutured in one or two layers. Subcutaneous tissue is sharply excised to expose the dermal vasculature to permit plasmatic imbibition and inosculation. Hair follicles in the dermis are visible when the subcutaneous tissue has been adequately removed. The graft is attached to the recipient site with staples, sutures, or cyanoacrylate glue. When attaching the graft with staples or sutures, the recipient site must be desensitised with local or regional anaesthesia. Attaching the graft to the recipient site with slight tension opens the small dermal vessels for plasmatic imbibition and inosculation.\(^2^0\)

Full-thickness grafting is usually reserved for fresh, uncontaminated wounds because full-thickness grafts are not as readily accepted as split-thickness grafts because full-thickness grafts require more nourishment and because they have fewer exposed blood vessels for inosculation and revascularization.\(^9^,2^1\) Full-thickness sheet grafting can be performed with the horse sedated without using expensive instruments. Wounds healed with a full-thickness skin graft resist trauma better and are more cosmetic than are wounds healed with any other type of grafts. Full-thickness grafts can be used to cover only small wounds because the horse’s lack of redundant skin limits the availability of donor skin. The largest graft that can be harvested from the chest, while allowing the donor site to be easily closed with sutures, is an ellipse usually not wider than 8 cm. The owner should be cautioned that the sutured donor site might dehisce but reassured that an open wound on the chest heals rapidly and cosmetically, almost entirely by contraction.

**Split-Thickness Sheet Grafting**
A split-thickness sheet graft is composed of epidermis and a portion of dermis. A split-thickness graft is harvested by splitting the dermis with a power-driven dermatome, a drum dermatome, or a free-hand knife. Power-driven dermatomes and drum dermatomes allow harvesting of grafts of precise width and thickness, and they can be operated with little experience, but they are expensive, require skilled maintenance and may fail to operate properly when proper functioning is critical. Drum dermatomes are seldom, if ever, used to harvest sheet grafts from horses. Although they are not as expensive as power-driven dermatomes, their major disadvantage for harvesting grafts from horses is that the length of the graft is limited by the circumference of the drum.

Split-thickness grafts large enough to cover extensive wounds of horses can be harvested economically with any one of a assortment of free-hand dermatomes designed for harvesting skin of human beings. A free-hand knife is far less expensive than is a power-driven dermatome or a drum dermatome, requires no skilled maintenance, does not malfunction, is easily cleaned and sterilized, and can be transported easily. Wider grafts can be harvested with the free-hand knife than with most power-driven dermatomes, and a great advantage of a free-hand knife over a power-driven dermatome is that using a free-hand knife, a split-thickness sheet graft can be harvested from the ventral aspect of the abdomen, which is a difficult feat using most power-driven dermatomes.

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**Fig. 6. A hand-held skin grafting knife should have an adjustable roller and a disposable blade.**
The most easily used hand-held knives have an adjustable roller in front of a disposable blade. An example is the Watson skin grafting knife (Watson Skin Grafting Knife; Padgett Instruments, Inc., 1520 Grand, Kansas City, MO) (Fig. 6). Only a moderate amount of practice is required to become proficient in the use of this knife because the adjustable roller controls the depth of cut. The position of the adjustable roller in relation to the blade is controlled by a calibrated knob at one end of the roller and a lock at the other end. The correlation between each calibration on the knob and the thickness of the graft is learned by experience. The depth of cut depends on the distance of the roller from the blade, the pressure applied to the knife during the harvest, and the angle of incidence at which the knife is held.\(^{11}\) By applying greater pressure to the knife or by increasing the knife's angle of incidence, the thickness of the graft can be increased without changing the position of the roller.

A split-thickness sheet graft can be harvested safely only with the horse anaesthetized. The donor site of a split-thickness graft heals leaving a large epithelial scar, and, therefore, a split-thickness graft should be harvested from an inconspicuous site. The ventral aspect of the abdomen is the least conspicuous site from which to harvest a split-thickness graft on a horse, but harvesting from this site using a power-driven dermatome is difficult to impossible. A surface firmer than the ventral aspect of the abdomen, such as the ventrolateral aspect of the thorax, is usually required to harvest a split-thickness graft using a power dermatome. A free-hand knife is generally required to harvest split-thickness skin from the ventral aspect of the abdomen.

To harvest skin from the ventral aspect of the abdomen using a free-hand knife, the horse is positioned in lateral recumbency with the ventral portion of the abdomen protruding over the edge of the table so that the movement of the knife is not restricted by the table. The donor site is prepared for aseptic surgery, but draping is unnecessary. Harvesting usually begins at the umbilicus and proceeds cranially, and so, if the surgeon is right-handed, the horse is best positioned in right lateral recumbency. The knife and donor site should be lubricated with isotonic saline solution to reduce friction between the skin and the knife. The knife is pressed into the abdomen with moderate pressure at an acute angle of 5 to 10 degrees. The dermis is cut by moving the blade up and down and forward while an assistant applies slight tension to the cut end of the graft (Fig. 7). After harvesting several centimeters of graft, the surgeon should stop to examine the graft and donor site to determine if the graft is being harvested at the desired thickness. A deep cut through the dermis transects large but few vessels and creates an opaque graft, whereas a shallow cut creates a semi-transparent graft and transects many small vessels. To change the depth of cut, the adjustable roller can be repositioned, pressure applied to the knife can be changed, or the knife's angle of incidence can changed. A thin, split-thickness skin graft is harvested with much more difficulty than is a thick graft. The graft is separated from the donor site by tilting the knife upward.

The graft is fixed to the margins of the wound with sutures, staples, or cyanoacrylate glue while the horse is anaesthetized, but if the graft is applied after the horse recovers from anaesthesia, the margin of the wound must be desensitised by using local or regional anaesthesia when attaching the graft with sutures or staples. The graft can be attached to the margin of the wound with cyanoacrylate glue without using local or regional anaesthesia. The overlapped and glued margin of the graft eventually desiccates and sloughs. Applying the graft after the horse recovers from anaesthesia removes the risk of damage to the graft that might occur during recovery and decrease the time of anaesthesia.
Split-thickness sheet grafts can be harvested in sheets large enough to cover wounds too large to be covered by a full-thickness graft, and they are accepted more readily than are full-thickness grafts because split-thickness grafts require less nourishment and have more exposed vessels in the dermis for inosculcation and revascularization. The appearance and durability of a wound healed with a split-thickness skin graft are poorer than that of a wound healed with a full-thickness skin graft. Harvesting a split-thickness graft is far less convenient and far more expensive than harvesting a full-thickness graft or island grafts because the horse must be anaesthetized. Unlike island grafts, which are independent of one another, failure of a portion of a sheet graft may result in loss of the entire graft. Epithelial scarring at the donor site of a split-thickness sheet graft can be extensive.

Meshing Sheet Grafts
A split- or full-thickness sheet graft can be applied to a wound as a solid or meshed sheet, but most are meshed before they applied to a horse. The primary reason to mesh, or fenestrate, a sheet graft is to expand the graft to allow it to cover a wound larger than the graft itself. Meshing a sheet graft also prevents exudate or blood from becoming interposed between the graft and the wound and enables a topically applied antimicrobial agent to uniformly contact a large portion wound. Fibrin fills the fenestrations increasing the graft's stability on the wound, and because it can expand, a meshed graft is better able to tolerate motion.22 A graft can be meshed with a scalpel blade or with a meshgraft dermatome, such as the relatively inexpensive, Padgett mechanical skin mesher (Mesh Skin Graft Expander, No. Z-PD-170; Padgett Instruments, Kansas City, MO), which consists of staggered parallel rows of blades, housed in an aluminum block (Fig. 8). The graft is placed on the block, dermal side down, and pressed into the cutting blades with a Teflon-covered rolling pin. The cutting blades fenestrate the graft in a staggered pattern allowing the graft to be expanded to three times its original area.

To manually mesh a sheet graft, the graft is fixed to a sterile piece of cardboard or plastic, and, using a scalpel, staggered, parallel rows of incisions are created in the graft. The longer and more numerous the incisions, the greater is the expansion. Meshing a graft manually is tedious and time-consuming, especially if the graft must be greatly expanded, or if the graft is large. Full-thickness sheet grafts of horses are difficult to mesh on commercial mesh graft dermatomes and so must usually be meshed manually.

Expanding a meshed graft exposes portions of the wound within the fenestrations, and each exposed portion of the wound must heal by contraction and epithelialization. The exposed portions of the wound epithelialize rapidly because of the extensive amount of borders from which epithelial cells can migrate. The healed wound is eventually covered with a uniform pattern of diamond-shaped, epithelial scars within the fenestrations.

Aftercare
The grafted wound is covered with a sterile, non-adherent dressing, to which an appropriate antimicrobial agent has been applied. The dressing is secured to the wound with conforming rolled gauze, and a bandage is applied over the dressing. If the wound is located in a region hard to immobilize, shearing forces can be minimized by securing the dressing to the wound with elastic, adhesive tape instead of rolled gauze, or a cast can be applied to completely immobilize the grafted portion of the limb. A splint applied to the outside of a bandage may suffice if casting the limb is impractical. The bandage is usually not changed for 4 to 5 days after grafting to avoid disrupting the
graft, unless nosocomial infection with virulent bacteria, such as Streptococcus and Pseudomonas spp. is a problem, in which case, the bandage should be changed daily. An antimicrobial agent effective against both β-hemolytic Streptococcus and Pseudomonas spp., such as ticarcillin with clavulanic acid (Timentin; GlaxcoSmithKline, Research Triangle Park, NC) should be applied topically to the wound at each bandage change.23

Storing Split-thickness Sheet Grafts
Skin grafts submerged in isotonic saline solution or lactated Ringer’s solution can be stored in a refrigerator for at least week.8,24 Using a culture medium, such as McCoy’s 5A medium, to which a small volume of serum has been added, extends the life of the stored graft to 3 weeks or longer.25 McCoy’s 5A medium is a balanced electrolyte solution containing amino acids, vitamins, dextrose, and a pH indicator, phenol red. The horse’s own serum or a commercially available, antibody-free, equine serum should be used to avoid antigenic reaction of the serum to the graft.26 The concentration of serum in the McCoy’s 5A medium should be between 10 and 33.27 Stored skin can be used to cover defects created by partial or complete loss of the primary graft. A graft harvested when a horse is anaesthetised for treatment for a wound can be stored until the condition of the wound has improved sufficiently to permit grafting. A stored graft is more readily accepted than a fresh graft because grafts stored for 24 hours or more undergo anaerobic metabolism, which causes release of metabolites that encourage rapid vascularization of the graft.28

Using Grafts as Biological Bandages
A wound can be dressed temporarily with a cutaneous allograft or xenograft to stimulate angiogenesis, speed epithelialization, prevent infection, and retard the formation of exuberant granulation tissue.29-32 A cutaneous allograft or xenograft vascularizes before it is rejected, which enhances its ability to defend the wound against infection, but even a graft that is rejected before it can vascularize helps protect the wound from bacterial infection by trapping bacteria in the fibrin attaching the graft to the underlying wound. Wounds treated with an allograft or xenograft require far fewer bandage changes than do wounds covered with a non-biological dressing.

References