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Preethi K
Ph. D Scholar, Department of Veterinary Surgery and Radiology, College of Veterinary Science, PVNRTVU, Rajendranagar, Hyderabad, Telangana, India

Gireesh kumar V
Professor, Department of Veterinary Surgery and Radiology, College of Veterinary Science, PVNRTVU, Rajendranagar, Hyderabad, Telangana, India

Raghavender KB
Professor & University Head, Department of Veterinary Surgery and Radiology, College of Veterinary Science, PVNRTVU, Rajendranagar, Hyderabad, Telangana, India

Correspondence
Preethi K
Ph D Scholar, Department of Veterinary Surgery and Radiology, College of Veterinary Science, PVNRTVU, Rajendranagar, Hyderabad, Telangana, India

Free skin grafts in dogs-A review

Preethi K, Gireesh kumar V and Raghavender KBP

Abstract
Skin grafting aims at early healing of a wide skin defect with better cosmetic appearance and proper hair growth. Though some wounds heal after routine skin care, proper debridement and suture, (Cockbill and Turner, 1995) wide wounds of extremities usually require additional measures like plastic surgery. While the development of new techniques and devices has significantly improved the functional as well as the aesthetic results from skin grafting, the fundamentals of skin grafting have remained the same, a healthy vascular granulating wound bed free of infection. Adherence to the recipient bed is the most important factor in skin graft survival and research continues introducing new techniques that promote this process. Biological and synthetic skin substitutes have also provided better treatment option with the use of growth factors in Platelet Rich Plasma. A variety of skin grafting techniques have been used for treatment of large extensive wounds in extremities. Among these pinch grafts, free full thickness grafts, mesh grafts, partial thickness grafts, strip grafts, pedicle grafts and skin flaps etc have been treated in clinical and experimental animals (Vishwasrao and Mantri, 1990; Anjaiah *et al.,* 2001; Wani and kulkarni, 1995; Segfried *et al.,* 2005; Makady 1991 and Slatter, 2003). However, there is paucity of literature on skin grafting in animals. Even today, skin grafts remain the most common and least invasive procedure for the closure of soft tissue defects but the quest for perfection continues.

Keywords: skin grafts, granulation bed, platelet rich plasma, partial thickness mesh graafts

Introduction
Treatment of wounds with extensive loss of skin is a challenging task and the task on the distal part of the limb is complicated by paucity of tissues (Ijaz *et al.*, 2012 and Zubin *et al.*, 2015) [13]. Use of skin grafting techniques offer a practically alternative to reconstruct such defects. Skin grafts are valuable armamentarium for reconstruction of larger superficial wounds after establishing a bed of granulation tissue or for immediate reconstruction of clean wounds overlying healthy muscle (Bristol, 2005) [6]. A skin graft is a segment of free dermis and epidermis transferred to a distant recipient site. They may be full thickness (epidermis and entire dermis) or partial thickness (epidermis and a variable portion of dermis). They are used for defects that cannot be reconstructed by direct apposition or skin flaps (usually limb and large trunk defects). The survival of a graft depends on the absorption of tissue fluid and development of new blood supply (Fossum, 2007) [12]. In dogs, skin grafting is primarily indicated for injuries to the skin of the extremities where skin immobility precludes tissue shifting and the construction of local advancement flaps for repair (Jensen, 1957; McKeever and Braden, 1978 and Pope, 1988) [16, 18, 28].
Skin grafting aims at early healing of a wide skin defect with better cosmetic appearance and proper hair growth. A variety of skin grafting techniques have been used for treatment of large extensive wounds in extremities. Among these pinch grafts, free full thickness grafts, mesh grafts, partial thickness grafts, strip grafts, pedicle grafts and skin flaps etc have been treated in clinical and experimental animals (Viswasrao and Mantri, 1990; Anjaiah *et al.,* 2001; Wani and Kulkarni, 1995; Siegfried *et al.,* 2005; Makady 1991 and Slatter, 2003) [40, 1, 41, 31, 23, 32].

Anatomy of Canine Skin
Integument is the largest organ of the body and serves as the body's first line of defense against microorganisms. It comprises 24% of body weight of the puppy whereas only 12% of that of an adult dog. Skin of dogs and cats is quite different from human skin. Skin thickness, hair growth and circulation vary regionally between species and between dog and cat breeds to some degree. Sub dermal plexus in dogs and cats is therefore of major importance in reconstructive skin surgery and should always be preserved when undermining skin for local flaps, especially when no direct superficial arteries can be incorporated into the proposed flap. Subdermal plexus flaps receive their blood supply from small vessels derived from the

subdermal (deep) plexus which consists of the terminal branches of the direct cutaneous vessels and runs parallel to the skin surface (Karayannopoulou *et al.* 2014) [17]. Direct cutaneous arteries are responsible for supplying large areas of skin in dogs and cats (Swaim and Henderson, 1997) [38]. They run parallel to the skin in the hypodermis and arise from perforator arteries. Musculocutaneous vessels play only a minor role in dogs and cats, rendering certain skin grafting techniques commonly used in man of limited use (Pavletic, 2010) [26].

Indications of Skin Grafting

Traumatic shearing or degloving wounds of the distal extremities are a common injury for which skin grafting may be indicated. Due to the relative immobility of the skin on the extremities, development of local flaps is generally impracticable (Swaim, 2003) [36]. Free flaps and full thickness skin grafts can be used for big defects involving the forelimb as second intention healing will render delayed wound contracture and in some cases can lead to functional limb deformity due to certain circumstances (Loqman *et al.,* 2004) [21]. Skin grafts assist wound healing by replacing dermal collagen and providing biological occlusion and protection of the wound. Skin grafting is one of the reconstructive techniques employed in horses as it provides a more cosmetic and functional scar, and in most cases, convalescent times are shorter, giving a early return to performance (Carmalt, 2008) [7].

Donor Site

The donor site should be areas of abundant, thin skin with a hair coat that resembles the recipient site. Skin was usually harvested from the side of the trunk as the skin was abundant in that region (Krahwinkel, 2005) [20]. The ventral thorax, lateral thigh, back, and neck regions were best donor sites for split-thickness graft in dogs. (Swaim, 1990) [34]. The donor site may be allowed to heal as an open wound by epithelialization however often heals with little or no hair growth (Keever and Barden, 1978) [18].

Recipient Site

Grafts will not take on recipient beds that have stratified squamous epithelial surfaces. Bones, Cartilage tendon or nerve denuded of overlying connective tissue cannot support a skin graft. (Converse, 1977) [9]. Contaminated recipient bed is, it should be cleaned, irrigated thoroughly, debrided and grafted at the same time and an allowance is made for drainage (Swaim, 1980) [33]. The granulation tissue is not necessary before a graft is applied. Healthy muscle, periosteum, and peritenon which is vascularized can support a skin graft (Pavletic, 2010) [26].

Granulation Tissue

Unhealthy granulation tissue due to poor vascular supply appears pale pink or blanched to dull, dusky red. Usually the first layer of granulation tissue to be laid down in the wound is pale pink; as the granulation tissue deepens and thickens, the color becomes bright, beefy red. (Barbara *et al.*, 2007) [3]. Chronic granulation tissue is completely excised if it is present and grafting is delayed until a healthy granulation bed forms, usually 4 or 5 days. In the presence of healthy granulation tissue, the epithelium at the wound edge is removed and the top of healthy granulation tissue may be scraped or superficial 0.5-2 mm of granulation tissue may be excised with a sharp blade. Alternatively, the surface of the granulation tissue may be wiped with a gauze sponge to prepare for grafting (Fossum *et al.*, 2007) [12]. Granulation tissue will begin to form rapidly if wound environment is conductive to healing. Once the wound is covered with granulation tissue, the edges will start to epithelialize. Healthy granulation tissue is recognized as, being red, being moist, and having a slightly irregular surface, bleeding actively when manipulated, producing a low volume of serous exudates and having a rim of advancing epithelium at the edge (Yool, 2012) [42].

Free Skin Grafts

Skin grafts are a segment of free dermis and epidermis transferred to a distant recipient site. Punch grafts, pinch grafts, strip grafts, stamp grafts and mesh grafts are commonly used free grafts as partial coverage grafts to increase the total recipient surface area that a small graft harvest can cover (Pavletic, 2010) [26]**.** They may be Full thickness with epidermis and entire dermis or Partial thickness with epidermis and a variable portion of the dermis. They are used for defects that cannot be reconstructed by direct apposition or skin flaps (Fossum, 2007) [12]. In full thickness skin graft, the entire dermis and epidermis must be harvested and the subcuticular fat may be trimmed to achieve appropriate thickness. They also opined that full thickness skin grafts have a slower revascularization rate and higher graft failure rate (Triana *et al.*, 2011) [39].

Advantages of Partial Thickness Skin Grafts

Meshed, thin partial-thickness grafts (0.063cm); meshed, medium partial-thickness grafts (0.127 cm); and meshed, full-thickness grafts in dogs suggested that thin partial-thickness grafts had the highest survival rate (89%), with full-thickness grafts (58%) and medium-thickness grafts (47%) having lower success rates (Keever, 1978) [18]. Split thickness graft is thin and is less likely to die from lack of nutrition during the first few days after transplanting, and the donor area regenerates by itself; in fact, in the human some areas have been used 4-5 times at intervals of 4-5 weeks. Jensen (1957) [16]. Meshed partial-thickness skin grafts possess the characteristics of rapid donor site re-epithelialization and multiple harvests of partial thickness skin from areas of thick skin. (Probst *et al.*, 1983) [29]. The advantage of partial-thickness skin grafts in dogs is in the reconstruction of large full-thickness wounds without sacrificing full-thickness skin from donor sites. Donor sites heal as partial thickness wounds and have the advantage of minimal tissue injury because the wound is made under aseptic conditions with minimal trauma (Pavletic, 1999) [25]. Split thickness skin grafts are thinner and have lower metabolic requirements than full thickness grafts and therefore they exhibit better ‘take’, or survival and are preferable if the wound bed is not well vascularised. Split thickness grafts contract more than full thickness skin grafts and in general produce a less favourable cosmetic result because they are less able to correct a greater contour defect, exhibit poorer texture, have unpredictable pigmentation, lower durability and also a more visible donor site (Kingsnorth and Majid, 2001) [19]. Partial-thickness skin grafts had also been shown to survive more readily than full-thickness skin grafts because of the denser capillary network in the more superficial dermal layers, which more readily allowed capillary linkup (Swaim, 2003) [36].

Disadvantages of Partial Thickness Skin Grafts

Split thickness graft cosmetic appearance is not as good as that of the full thickness graft as there is some shrinkage. The graft will be dry and desquamate for several months during which time it will have to be softened with lotions. Due to it being rather thin, it does not resist trauma very well. Hair will not grow from this type of graft (Jensen, 1957) [16]. Keever *et al.* (1978) [18], Probst *et al.* (1983) [29], Swaim (2003) [36] observed that the disadvantages of partial-thickness skin grafts include technical difficulty while harvesting, initial low durability of partial-thickness skin that is susceptible to trauma, sparse or absent hair growth on both the grafted and donor sites, and a scaly appearance resulting from the lack of sebaceous glands. Partial thickness donor sites managed as open wounds tend to be more painful than if they were excised and primarily closed (Swaim, 2003) [36].

Mesh Grafts

Meshing allowed drainage and expansion of the graft, facilitating its adjustment into irregular surfaces (Swaim *et al.*, 1985) [37] and reduces the chance of haematoma collection and allows good draping within a concavity. These qualities ensure good contact with the graft bed and hence good graft take. It allows fluid and blood to pass easily through the graft via multiple fine perforations, enabling earlier intra-operative application to the bed without haematoma collection (McCulley *et al.*, 1999) [24]. Meshing both a partial- and full-thickness skin graft decreases graft contracture by increasing the number of edges from which epithelialization can occur. Additional advantages of meshed skin grafts include the reconstruction of irregular surfaces (concave and convex) and application in a graft bed with exudate (blood or serum) (Aragon *et al.* 2004) [2].

Blood Supply to Graft

Pope (1985) [27] described the take of the graft as a success when revascularization occurs. Initially the graft was nourished by absorption of plasma-like fluid, known as plasmatic imbibation. During first 24 hours, the graft vessels gradually become dilated and filled with static blood. Revascularization of the skin grafts occurs by direct connection of graft and host vessels and by ingrowth of new vessels along preexisting graft vessels (Probst *et al.*, 1983 and Pope, 1988) [29, 28]. The rate of ingrowth of new capillaries was approximately 0.5 mm/day in free skin grafts. (Eriksson and Zarem, 1977) [11]. Two or three days after grafting, the new blood flow from the granulation bed into the graft is established. A graft that is applied on healthy granulation tissue is generally revascularised within 48 to 72 hours (Pope, 1988) [28]. Successful graft healing, or graft 'take', was dependent on the establishment of arterial connections and adequate drainage. This must occur by the 7th or 8th post-operative day, or the graft will die. The graft bed must supply adequate vasculature for the graft (Macphail, 2013) [22].

Failure of Skin Graft

The gross appearance of a rejected skin graft looks cyanotic, hemorrhagic, and edematous with an area of surrounding erythema and induration in the recipient site. The ultimate fate of the rejected graft is a dry eschar which is sloughed as host epithelium infiltrates the subeschar surface (Rapaport, 1965) [30]. Usually failure of partial-thickness skin graft survival can be caused by motion of the graft over the wound bed, infection, or hematoma and seroma formation beneath the graft, which prevent adhesion and capillary linkup (Pavletic, 1999) [25]. The grafts are pale when they are initially placed on a wound, they may appear cyanotic during the next 48 hours. The darker color takes on a light reddish tinge by 72-96 hours. By post-operative day 7 or day 8, the entire graft is red if survival is complete. A normal color gradually returns by day 14. Avascular necrosis is indicated by persistently pale areas, these eventually necrose and slough. Dry ischaemic necrosis may appear as black discoloration (Fossum, 2007) [12]. A graft 'take' is a successful skin transplantation in which the transplant heals in its new location; grafts 'do not take' on bone, tendon, cartilage, nerve or areas of movement i.e. joints.

Appearance of The Graft After Grafting

In dogs with complete success, the 1st bandage change on third day revealed an edematous graft as expected to a normal tissue response (Bowler *et al.*, 2001) [5]. The graft was seen to be revascularized by ingrowth of new vessels from the bed into the graft by ninth day. Newly formed vessels are dilated for some time and later on mature as the differentiation process continues until a new system of vessel network is developed (Ercan *et al.*, 2005) [10]. On sixth day, the grafts were bluish black in color. The healing process associated with random vascular anastomosis of vessels with veins rather than with arteries gives bluish black tinge to the graft (Jennifer *et al.*, 2006) [15]. Split thickness autogenous skin graft is devoid of blood vessels when harvested from donor site and applied on the wound. The transudate is produced on the recipient bed in such circumstances and is absorbed by plasmatic imbibitions and ultimately leads to edema.

Complications of Skin Grafting

Post-operative infection can have devastating effects. Infection between the graft and recipient bed may result in dissolution of the fibrin seal, or the graft may be physically elevated from the graft bed by the exudates produced. Care must be taken not to contaminate the graft when the bandages are changed frequently (Bojrab, 1998) [4]. Use of NSAIDs adversely affect the surgical outcome by interfering with hemostasis, wound healing and hence necessitate discontinuation. (Jeffrey, 2000) [14]. Infection is detrimental because bacteria may cause dissolution of fibrin attachments. Proteolytic enzymes and plasminogen activators released by bacteria, disrupt the fibrin bonds. Beta haemolytic streptococci and pseudomonas species produce large amounts of plasmin and proteolytic enzymes. Pseudomonas also produces elactase that breaks down elastin. Elastin adheres to fibrin, which facilitates graft adhesion (Swaim, 2000) [35]. Successful grafting requires asepsis, an adequately prepared recipient bed consisting of healthy granulation tissue, proper harvesting and preparation of the graft, meticulous surgical technique and strict post operative care. Factors that are essential for the survival of skin grafts include good contact between the graft and the recipient bed, normal tension on the sutured graft, strict immobilization after grafting and prevention of accumulation of blood or serum under the graft (Siegfried *et al.*, 2005) [31]. Complete contact between the dermal surface and the graft bed is necessary for ingrowth of capillaries from granulation tissue where there is no contact or if there is constant movement between the graft and the recipient, the graft will not take (Siegfried *et al.*, 2005) [31].

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