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PAPER

Seed skin grafts for reconstruction of distal limb defects in 15 dogs

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Objectives: **To report the surgical technique of seed skin grafting and clinical application for reconstruction of wounds on the distal limb of client-owned dogs.**

Materials and Methods: **Medical records from The Animal Hospital at Murdoch University were retrospectively reviewed for dogs requiring reconstruction using seed grafting for distal limb skin defects between January 2009 and May 2020.**

Results: **Fifteen dogs were included. Grafting was performed on distal limb wounds at or below the carpus or tarsus, following trauma (n = 12) or neoplasia excision (n = 3). Complete epithelialisation with minimal contracture was recorded at a median of 4 weeks (range 3 to 8 weeks) after implantation. Median follow-up was 37 months (range 3 to 55 months) after grafting. Postoperative complications included epidermal inclusion cyst in two dogs. Good functional outcome with acceptable cosmesis despite sparse hair growth was achieved in all cases.**

Clinical Significance: **Seed grafting is a simple technique that can be used reliably to reconstruct wounds on the distal limb in dogs where other reconstructive techniques are not suitable. Complete epithelialisation with sparse hair growth, good long-term functional outcome and minimal complications can be expected.**

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INTRODUCTION

Closure of wounds on the distal limb of dogs and cats is made difficult by the limited available local skin. Various techniques have been described to reconstruct distal limb wounds, including primary closure, local advancement or rotational flaps, axial pattern flaps, distant pedicle or pouch flaps (Swaim 1987, Miller *et al.* 1991, Lemarié *et al.* 1995) and free skin grafts (Fowler *et al.* 1987, Gregory & Gourley 1990, Pope 1990, Probst 1990, Swaim 1990, Miller *et al.* 1991, Tong & Simpson 2012), including those with concurrent application of negative pressure wound therapy (NPWT) (Demaria *et al.* 2011, Stanley *et al.* 2013, Nolff & Meyer-Lindenberg 2015, Miller *et al.* 2016). Alternatively, distal limb skin defects can be managed as open wounds and left to heal by second intention (Miller *et al.* 1991, Fowler 2006, Prpich *et al.* 2014). However, the time to heal by second intention is variable and wound complications are frequent, including wound contracture that can restrict movement.

Free skin grafting in dogs was first described by Jensen (1959) and later by Alexander & Hoffer (1976) and Swaim (1990). This technique requires a piece of skin harvested from a distant site, devoid of attachment, to be placed on a vascular wound bed and then become incorporated and vascularised at that site. Full thickness skin grafts include dermis and epidermis and split thickness grafts include epidermis and varying partial thicknesses of dermis. Further categorisation of free skin grafting includes sheet, mesh, strip, stamp and seed (punch/pinch) grafts (Alexander & Hoffer 1976, Swaim 1990). There are multiple reports of outcomes after mesh grafting, and more recently, with application of NPWT, but there is a paucity of data on clinical application and outcome of seed grafts. It has been suggested that the results for seed grafts are less cosmetic and less robust than other free grafts (Begum *et al.* 2019), which may have limited their use (Scharf 2017). However, seed grafts are an alternative to second intention healing when other reconstructive techniques such as skin flaps, pouch flaps and free skin grafts are less applicable, including small wounds, isolated peripheral locations (distal limb below the carpus or tarsus, nose, *etc.*), areas of high movement, in sites of low grade infection, inconsistent granulation tissue and graft bed irregularity.

The objectives of this case series were to describe the surgical technique of seed graft harvest and implantation and report the application of this technique and clinical outcome in a case series of dogs.

MATERIALS AND METHODS

Medical records of dogs that underwent seed grafting for a distal limb skin defect at The Animal Hospital at Murdoch University between January 2009 and May 2020 were reviewed and all identified cases were included. Information extracted from the records included signalment, history, wound location and indication for seed grafting (including histologic diagnosis where appropriate), surgical findings, results of microbial wound tissue culture, duration of postoperative hospitalisation, bandaging protocol, complications and postoperative outcome. Human ethics approval (project number: 2019/023) was obtained before contacting owners by telephone to determine postoperative limb function, cosmetic appearance and mass recurrence (where applicable).

Surgical technique

For cases with traumatic injury, the distal limb wound defect was managed until a vascular granulation tissue wound bed was evident. The entire wound surface was not always smooth or confluent. Deep tissue microbial cultures were performed before grafting in all cases, and at the time of grafting when deemed necessary. A negative wound tissue culture before grafting was not essential but assisted in ongoing case management. Concurrent orthopaedic injuries were managed as deemed appropriate by the attending clinician. For cases with neoplasia excision, surgical margins were confirmed to be free from neoplastic cells before seed grafting.

The wound and donor sites (lateral thorax/abdomen) were prepared aseptically with 2% chlorhexidine gluconate solution. The lateral thorax and/or abdomen were chosen as the donor site due to the thin readily available skin at a location suitable for tension-free primary closure of the resultant defect. The dog was positioned in lateral recumbency, with the affected limb orientated as required, based on location of the wound to be grafted. A template of the recipient bed was created with a sterile piece of paper, glove packet or similar, to determine the maximum amount of skin required. The donor skin was sharply excised deep to the myocutaneous (panniculus) muscle using a number 10 scalpel blade. The panniculus muscle was included in the donor skin harvest as ease of dissection underneath this tissue plane facilitates speed of harvest and ease of closure. The donor site was closed with simple continuous subcutaneous (3-0 poliglecaprone) and Ford-interlocking skin (3-0 nylon) suture patterns.

The graft was stretched across a sterile piece of cardboard or plastic using sutures with the subcutaneous tissue facing uppermost. The panniculus muscle and gross subcutaneous fat was first trimmed from the graft using Metzenbaum scissors. Then, using a number 15 scalpel blade, or fine dissecting scissors, the remaining fat was scraped or cut from the surface of the skin such that the cobblestone appearance created by exposure of the hair follicle bulbs in the dermis was visible. Care was taken to avoid damage to the hair follicles.

A 4-mm diameter skin punch biopsy (Biopunch, Henry Schein) was used to cut small pieces of skin, each piece becoming a seed graft. The punch was angled parallel to the direction of hair growth to maximise the number of follicles *per* graft. An approximately 5-mm deep pocket was made in the granulation tissue using a number 15 blade, at an angle of ~30°, and the bottom half of the graft inserted, with the haired surface uppermost, protruding at the level of the wound bed. The seed grafts were placed approximately 2 to 3 mm apart in a staggered fashion to cover the entire wound bed. No sutures were placed. We recommend beginning seed graft insertion from the dependent surface then moving proximally to avoid haemorrhage obscuring further grafting.

The graft site was covered with one to two layers of non-adherent petrolatum-impregnated gauze (Jelonet, Smith & Nephew), followed by antimicrobial-impregnated gauze (Kerlix, Covidien), an absorbent layer (Soffban, BSN medical) and a tertiary cohesive layer (Fun-Flex Pet Bandage, Kruuse). Care was taken to apply the bandage smoothly and evenly, and to avoid torqueing the bandage (which might cause shearing of the seed grafts away from the host site). Any repeat bandaging was performed in the same manner, with bandage changes delayed for as long as possible but no later than 5 to 7 days apart.

All dogs received perioperative prophylactic intravenous antibiotics (cefazolin 22 mg/kg IV at induction and every 90 minutes of surgical time unless guided by preoperative wound culture results). Peri and intraoperative opioid analgesia and postoperative non-steroidal anti-inflammatory medications (meloxicam 0.1 mg/kg orally every 24 hours or carprofen 2 mg/kg orally every 12 hours) were also administered, both at the discretion of the attending clinician.

RESULTS

Signalment

Fifteen dogs (11 male, four female) were included (Table 1). There were eight mixed-breed dogs, and one each of German shepherd, kelpie, Great Dane, German short-haired pointer, golden retriever, whippet and Dalmation. Median age of all dogs was 4 years (range 4 months to 10.5 years) with a median weight of 23.5 kg (range 6.0 to 62.3 kg).

Seed grafting

A board-certified surgeon (GH) performed all surgical procedures. All grafts were placed on distal limb wounds, at or below the carpus or tarsus. Grafting occurred following trauma due to various inciting causes in 12 dogs or following neoplasia excision, specifically Grade 1 soft tissue sarcoma in three dogs. Five dogs with trauma had concurrent metacarpal/metatarsal fractures or carpal/tarsal instability requiring repair.

Grafting occurred at a median of 3 weeks (range 1 to 12 weeks) after trauma in 12 dogs, depending on orthopaedic and recon-

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Table 1. Signalment, clinical features and outcomes of 15 dogs that underwent seed grafting for reconstruction of skin defects on the distal limb

Dog	Age	Breed	Weight (kg)	Location	Indication	Concurrent injuries	Wound dimensions	Seed grafts	Post-op seed count (% survival)	Time to epithelialisation (weeks)	Long-term follow up (months)	Limb function	Hair growth	Complications
1	4 months	Border collie cross	16.2	Right metatarsus	Trauma	None	–	15	–	3	29	Good	Good	None
2	1 years 9 months	German shepherd	38	Right metacarpus	Trauma	Right metatarsal trauma	–	20	–	5	22	Good	Sporadic	None
3	10 years	Labradoodle	36.3	Left tarsus	STS	None	5×6 cm	–	–	8	21	Good	Good	None
4	1 year	Border collie cross	27.8	Left and right tarsus	Trauma	Left tarsal external skeletal fixation	7×2 cm	–	–	6	44	Good	Sporadic	None
5	4 years 6 months	Kelpie	20	Right metatarsus	Trauma	Internal fixation of multiple right metacarpal fractures	–	–	–	6	44	Good	Good	None
6	1 year	Great Dane	62.3	Right carpus	Trauma	None	–	60	–	5	45	Good	Minimal	Epidermal inclusion cyst
7	9 years	Maltese cross	8.5	Right metatarsus	Trauma	None	–	10	–	4	37	Good	Sporadic	None
8	1 year	German short-haired pointer	33	Right metacarpus	Trauma	Right pancarpal arthrodesis	5×3 cm	25	–	4	37	Good	Sporadic	Licking causing abrasion
9	4 years 4 months	beagle cross	16.9	All four pes	Trauma	None	–	50	–	3	25	Good	Sporadic	Licking
10	6 years 9 months	Greyhound cross	37.5	Left carpus	STS	None	–	30	26 (87%)	8	17	Good	Minimal	None
11	4 years	Chihuahua cross	6	Right metatarsus	Trauma	None	5×3 cm	9	–	3	46	Good	Sporadic	None
12	5 years	Golden retriever	29.6	Left metacarpus	STS	None	–	20	18 (90%)	4	40	Good	Minimal	Epidermal inclusion cyst
13	3 years	Whippet	12.7	Right tarsus	Trauma	Right tarsal external skeletal fixation	–	31	–	4	36	Good	Sporadic	None
14	7 years 6 months	Dalmatian	23	Left tarsus	Trauma	Multiple left metatarsal fractures	–	26	24 (92%)	4	55	Good	Sporadic	None
15	2 years 6 months	Shar-pei cross	23.5	Right metacarpus	Trauma	Scapula fracture	–	40	–	3	3	Good	Sporadic	None

Hair growth was subjectively graded (sporadic: isolated hair tufts; minimal: interspersed hair tufts across entire surface; good: coalescing hair growth across entire surface).

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structive procedures performed beforehand. For the three dogs with mass excision, grafting occurred 7 days after excision in one dog and after 18 days in two dogs. In all three dogs surgical margins were confirmed to be free from neoplastic cells before seed grafting.

The number of seed grafts implanted was recorded in 12 dogs, with a median of 25.5 seed grafts (range 9 to 60) required in accordance with the size of the wound bed. Wound dimensions were recorded in four dogs (Table 1).

Microbial wound cultures

Microbial cultures of deep tissue from the wound bed before grafting from 11 of 12 dogs with trauma had growth of one or more bacteria: Non-haemolytic *E. coli* (n = 2), *Serratia* sp. (n = 3), *Staphylococcus pseudintermedius* (n = 5), *Enterococcus* sp. (n = 6) and *Pseudomonas* sp. (n = 2). Cultures were negative in two of three dogs following mass excision and culture from one grew *S. pseudintermedius*. Deep tissue culture performed at the time of grafting in two of the 15 dogs grew multi-resistant *Enterococcus* sp. Follow-up deep tissue cultures, 3 and 4 weeks after grafting, in a further two dogs with self-trauma, grew multi-resistant *S. pseudintermedius* and *S. aureus*, respectively. Targeted antimicrobial treatment was instituted in all dogs with a positive culture and discontinued either when the wound developed a granulating surface, was free from exudate, or had a subsequent negative repeat tissue culture.

Short-medium term outcomes

Bandages were first changed at a median of 4.5 days after grafting (range 1 to 7 days). Two dogs required repeat bandaging having chewed their bandage at 1 and 2 days, respectively, after grafting. The contact layer was soaked with sterile saline to facilitate removal and avoid seed graft disruption. All dogs were hospitalised until at least the first bandage change before being discharged with instruction for strict confinement. Subsequently bandages were changed with or without sedation according to the animal's temperament. The frequency of ongoing bandage changes depended on the appearance of the graft as well as the condition of the bandage. Bandaging continued until the wound had epithelialised and was sufficiently robust to obviate abrasion. No bandage-related complications were encountered.

We observed the surface of the seed graft to become confluent with the wound bed and any overlying granulation tissue appeared to slough or regress. The superficial epidermis of the graft was the last to be revascularised; therefore, the layer dies, producing a slough. Seed counts were performed in three of 15 dogs at follow-up bandage changes, with 87%, 90% and 92% of seeds identified in these three dogs. Complete epithelialisation was noted at recheck examinations at a median of 4 weeks (range 3 to 8 weeks) after graft implantation (Figs 1 and 2).

Long-term outcomes and complications

Median long-term follow-up was 37 months (range 3 to 55 months) after grafting. At the time of writing, 14 of the 15 dogs were still alive. One dog was euthanised for reasons unrelated to the grafting at 12 months following Grade 1 soft tissue sarcoma excision and seed grafting. The owner of this dog reported good use of the operated limb without tumour recurrence up to the time of euthanasia. At long-term follow-up by telephone and submission of photographs, all other owners reported that their dogs had good limb function, with no lameness or reduced range of motion, and complete epithelialisation with minimal restrictive contracture of the wound. Sporadic tufts of hair were noted on most grafts (Fig 3). The area of epithelialisation had become pigmented in dark-skinned dogs (Figs 1D, 2E and 3B). Neither of the remaining two dogs that had tumours excised suffered recurrence. Two dogs developed epidermal inclusion cysts that required surgical excision. One of these dogs had further cyst formation and required repeat excision followed by open-wound management. Two dogs were

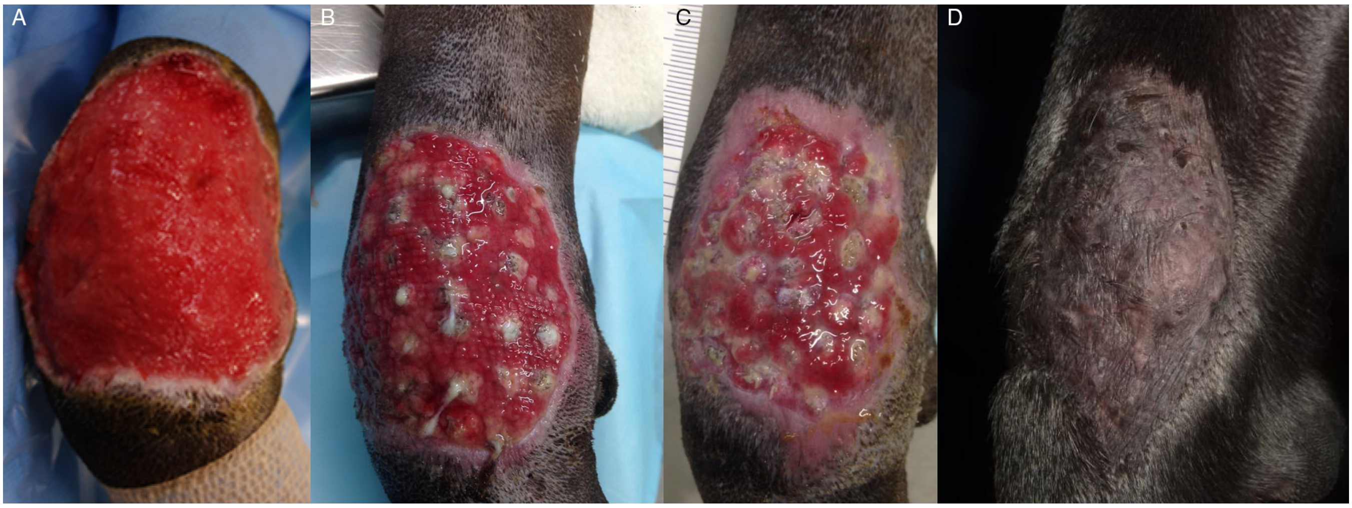

FIG 1. Photographic series of seed grafting on the dorsal carpus in a 1-year-old male neutered Great Dane. (A) Healthy granulation bed ready for seed grafting. (B) Viable seed grafts with mild epithelial surface sloughing 1 week after seed grafting. (C) Epithelialisation between seed grafts and at the periphery of the wound 2 weeks after seed grafting. (D) Complete epithelialisation with pigmentation 5 weeks after seed grafting

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FIG 2. Photographic series of seed grafting on the palmar metacarpus in a 2½-year-old male neutered shar pei cross. (A) Open wound with exposed tendons. Note, metacarpal and carpal pads subsequently sloughed. (B) Healthy granulation bed ready for seed grafting. (C) Viable seed grafts embedded in wound bed 1 week after grafting. Note, some mild epithelial surface sloughing on grafts. (D) Epithelialisation between seed grafts and at the periphery of the wound 2 weeks after seed grafting. (E) Complete epithelialisation with pigmentation 3 weeks after seed grafting

FIG 3. Appearance of seed grafted wounds at long-term follow up in: (A) 10-year-old Labradoodle (21 months), (B) 1-year-old Border collie cross (44 months) and, (C) 4½-year-old kelpie (44 months)

reported to lick at the grafted site. One of these dogs caused abrasion of the area and went on to require removal of orthopaedic implants that had been applied for pancarpal arthrodesis. Implant removal alleviated self-trauma in this dog. All owners were satisfied with the cosmetic outcome but stated the graft site was noticeable due to lack of hair growth.

DISCUSSION

This case series describes the use of seed grafts for reconstruction of skin defects on the distal limb at or below the level of the carpus or tarsus in 15 dogs. Seed grafting should be considered for such wounds when other full-thickness skin reconstructive

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techniques are impossible because it offers an alternative to reliance on second-intention healing. Our report documents good functional outcomes in all dogs, even those complicated by orthopaedic injury. Complications were manageable, although repeat intervention was required in one dog with epidermal inclusion cyst formation. In our case series, the seed grafts were robust and resulted in epithelial coverage of wounds with irregular surfaces, even when most had documented infection before grafting. While the healed grafted site was obvious and hair growth was sparse, it was cosmetically acceptable, and owners were satisfied with the outcome.

A vascular wound bed is required for any free skin graft, with acute grafting on muscle, or delayed grafting on granulation tissue recommended (Swaim 1990). All our grafts were placed on a granulation tissue bed; however, the entire wound surface was not always smooth or confluent and granulation tissue was not always consistent in depth or vascularity across the wound bed. Protracted open wound management to achieve an ideal smooth surface, as for a free sheet graft, was not necessary, given the ability to place the seeds across an irregular surface and still maintain graft/wound bed contact. On average, grafting was performed within a week of wounding for acute cases, which also allowed time for confirmation of clean margins after tumour excision. Grafting was timed according to management of other comorbidities in traumatic cases.

Infection has been reported as detrimental to free skin graft revascularisation (Scharf 2017). In our cases, grafting did not occur until the wounds were clinically healthy, but a positive tissue culture from the wound before (12 of 15 dogs) and at the time of grafting (two of 15) was not prohibitive and any ongoing antibiotic treatment was targeted based on microbial culture and sensitivity testing. Further, non-commensal bacterial species were identified for the four dogs with positive cultures at the time of and after grafting. These findings suggest that absence of infection was not necessary for a successful outcome.

For wounds of the distal limbs, particularly at or below the level of the carpus or tarsus, reconstructive techniques are limited, and second-intention healing becomes a default management strategy. Prpich *et al.* (2014) described second-intention healing of large wounds in the distal limb following excision of low-grade soft tissue sarcoma in 31 dogs. Twenty-nine (94%) wounds healed completely by second intention, but at a protracted median time of 53 days, with the longest being 179 days. In comparison, seed-grafted wounds in our cohort were recorded to have epithelialised in half the time, at a median of 4 weeks (28 days) after grafting. Since our assessment is retrospective, and timing only corresponds to recheck appointments, the time taken for complete epithelialisation is likely over-estimated. Although Prpich *et al.* (2014) reported tumour recurrence was uncommon (1/31; 3%), wound-associated complications were noted in eight of 31 (26%) of dogs, including intermittent epidermal disruption and wound contracture. The process of epithelialisation in second-intention healing requires epithelial cells to migrate across the wound surface from the epithelial edge (Pastar *et al.* 2014). This continued cell migration results in a thin, sometimes one cell-layer thick, friable layer of epithelium. While it can stratify over time, it is often easily abraded. In our case series, the seed grafted wounds resulted in epithelium that appeared thick, as indicated by the owners and evidenced by the photographs, without abrasion of the skin surface and with limited contracture. We anticipate that the new epithelium is thicker since the defects between seed grafts are small, allowing epithelial migration and stratification to occur quickly and reliably (Swaim 1990). Rapid epithelialisation in a wound will result in less contracture because, although the two events are independent, a wound stops contracting when the epithelial edges contact. Since seed grafting promotes epithelialisation, it obviates wound contraction, which is an important factor in wounds on areas of motion such as the distal limbs (Pastar *et al.* 2014).

Complications are frequent with free skin grafts in small animals, with early experimental studies reporting percentage graft take of 59% (McKeever & Braden 1978), 81% (Bauer & Pope 1986), and 90% (Pope 1985) of the surface area for full-thickness grafts. A retrospective study of 20 cats and 32 dogs with full-thickness skin grafts for distal limb wounds reported 77% and 38% graft take, respectively (Riggs *et al.* 2015). Partial-thickness grafts and meshing of full-thickness grafts have historically improved graft viability (McKeever & Braden 1978). More recent reports with the application of NPWT over free mesh grafts describe take up to 95% (Demaria *et al.* 2011, Stanley *et al.* 2013, Nolff & Meyer-Lindenberg 2015, Stanley 2017, Nolff *et al.* 2018). In our series, graft take was reliable with few grafts lost or dislodged according to visual assessment. Seed grafting proved robust and reliable for irregular, sometimes complicated, distal limb wounds where other techniques such as free skin grafts, that rely on entire graft take, may be less suitable.

The most concerning complication was epidermal inclusion cyst formation noted in two dogs. This was managed by excision of the affected area in one dog but further cyst formation in the other dog necessitated repeat excision and open-wound management. We hypothesise that epithelialisation occurred over the seed grafts, trapping an epithelial surface underneath, resulting in accumulation of sebum and epithelial debris, and a foreign body-like reaction. The wounds ultimately healed and the dogs achieved a good functional outcome. Two dogs were reported to lick their grafted sites. It is not possible to discern whether this was due to the technique, the original injury or whether it was pain-, behaviour- or compliance-related.

We hospitalised all dogs until the first bandage change (~5 days) to enhance compliance and enforce strict cage rest. Postoperative bandaging protects the graft from trauma and contamination and maintains contact between the seed grafts and the wound bed (Scharf 2017). In our experience, the application of a non-adherent primary dressing is essential. Several non-adherent dressings with variable hydrophilic and occlusive properties are available. While hydrophilic dressings facilitate a moist wound surface, which will enhance epithelialisation, protracted application can cause maceration of the wound and surrounding tissue (Davidson 2015, Rippon *et al.* 2016). Petrolatum-impregnated dressings are non-occlusive, contain some moisture and do not result in tissue maceration after protracted application for 5 to 7 days. In addition, they allow absorption of exudate and bac-

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teria into the secondary overlying bandages (Lee *et al.* 1987). In particular, the petrolatum-impregnated gauze conforms to the irregular wound surface without sliding over the surface. There are conflicting reports on the effect of petrolatum on epithelialisation. Commander *et al.* (2016) reported petrolatum to increase epithelialisation and decrease scarring in people, while an experimental study in dogs reported that wounds dressed with petrolatum-containing dressings contracted more quickly in the first 7 days and had less epithelialisation than wounds dressed with cotton non-adherent dressings (Lee *et al.* 1987). An alternative dressing could be hydrophilic foam, but this would require application of wound gel to provide exogenous wound moisture. In our experience, hydrophilic foam dressings cause drying and adhesion to the wound after protracted application because the moisture is drawn away from the wound (Jones *et al.* 2006, Rippon *et al.* 2016). In addition, they are difficult to conform to the wound surface and often slide, potentially dislodging the grafts. Thus, there may not be an ideal contact layer, but regardless of the specific product chosen, a non-adherent dressing that maintains moisture at the wound surface, does not desiccate and become adhesive, does not disrupt graft placement or promote maceration is recommended.

Seed grafting followed mass excision in three dogs in our case series. All masses were Grade 1 soft tissue sarcomas that were excised with clean margins and had not recurred at long-term follow up. Tumour excision and seed grafting were performed as staged procedures, in part, so histologic confirmation of complete excision could be verified before investing in wound reconstruction. Tong & Simpson (2012) recommended immediate meshed skin grafting as a single staged method following curative intent tumour excision in the distal limb of dogs as an alternative to second-intention healing and other wound reconstruction techniques. However, this could be premature in the case of incomplete excision and relies on a vascular wound bed, which is less reliably achieved in the very distal limb where tendons are often exposed.

This case series reports on the outcomes of seed grafting for distal limb wounds at or below the level of the carpus or tarsus that were deemed not amenable to other full-thickness skin reconstruction techniques. Seed grafting is a straightforward technique that offers a reconstruction option for irregular, sometimes complicated, wounds on the distal limbs, where other techniques are prohibitive. In the cases presented herein, long-term functional outcome was good, cosmetic appearance was acceptable and complications were manageable.

Conflict of interest

No conflicts of interest are declared by the authors.

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