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Xenogeneic Grafts Using Porcine Small Intestinal Submucosa in the Repair of Skin Defects in 4 Birds

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Abstract: Trauma and skin defects are common presentations in companion and wild birds. Two barn owls (*Tyto alba*), an umbrella cockatoo (*Cacatua alba*), and an American crow (*Corvus brachyrhynchos*) were presented for varied skin wounds. Porcine small intestinal submucosa was used as a xenogeneic graft to repair wound defects in each bird. The wounds healed within 6 weeks in all 4 birds, with less intensive wound management than that required for healing by second intention. All birds returned to normal function.

Key words: porcine small intestinal submucosa, xenogeneic graft, wound management, skin, birds, barn owl, cockatoo, crow, *Tyto alba, Cacatua alba, Corvus brachyrhynchos*

Introduction

The structure of bird skin, characterized by epidermis, dermis, subcutis, and feathers, has been extensively reviewed and documented,[1] and several sources exist on the topics of avian dermatology, dermatologic diseases, and treatment.[2–6] Captive birds are often presented for infectious and noninfectious dermatoses that require surgical treatment. Common avian traumatic skin conditions include mutilation, keel injuries, gunshot wounds, leg-hold trap injuries, and entanglement. Skin trauma in the keel region of psittacine birds is often associated with feather plucking, automutilation, and traumatic injury after severe feather clips.[2,3,5,7,8] Keel damage is one of the most commonly observed traumas in captive bustards in the United Arab Emirates.[7] Many keel wounds become chronic, necrotic, and granulomatous lesions, and some can involve the underlying keel bone. In raptors, the skin and underlying soft tissues of the tarsometatarsus can be traumatized from poorly fitted or damaged jesses. Similar wounds are seen in psittacine birds with tight leg bands.[2] In free-ranging birds, trauma associated with vehicular accidents, predation by domestic animals, and electrocution[9] commonly results in skin lacerations, soft-tissue damage, and bone fractures.

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Current techniques for the repair of keel injuries include conservative care and surgery. One report describing 15 cases of keel injuries caused by automutilation in psittacine birds indicated that extensive surgery was more effective (100%) than was prolonged conservative treatment (20%).[8] Surgery involved resecting the necrotic tissue, elevating and resuturing the superficial pectoral muscles and skin, bandaging the wing or wings, and placing a collar for 6 weeks.

The progression of wound healing and the management of soft-tissue wounds on the wings of birds have been described.[2] Wound-management techniques include preventing further contamination, cleaning the wound, surgically debriding necrotic tissue, using topical and systemic antimicrobial therapy, protecting new granulation tissue, and managing delayed wound closure. Additionally, wing injuries often require immobilizing the wing to facilitate bandaging and to prevent excessive movement that might delay healing.[2] Because of the lack of soft tissue on wings, the thin skin of birds, and the importance of tendons such as the tensor propatagialis,[10] wing injuries in free-ranging birds intended for release must be managed with utmost care.

Various wound management techniques have been used in birds with large skin defects. Cutaneous autografts have been used successfully in ostriches (*Struthio camelus*)[11] and great horned owls (*Bubo virginianus*),[12] whereas skin flaps have been used in ring-necked pheasants (*Phasianus colchicus*), a rock dove (*Columba livia*), a red-tailed hawk (*Buteo ja-*

maicensis),[13] and other raptors.[6] Allografts have been used frequently in chickens (to determine inbreeding coefficients), with graft survival positively correlated with genetic homogeneity.[14] A recent publication details the use of skin flaps and grafts in raptors.[15]

Vet Biosist (Global Veterinary Products Inc, New Buffalo, MI, USA) is derived from porcine small intestinal submucosa (SIS) that has been dehydrated and sterilized. This collagen-rich biological mesh contains fibronectin, decorin, hyaluronic acid, chrondriotin sulphate A, and various growth factors. Although only a single case report on the use of porcine SIS in a raptor exists,[15] it has been successfully used in dogs, cats, rats (*Rattus norvegicus*), and rabbits (*Oryctolagus cuniculus*) for applications including bladder, muscle, tendon, blood vessel, cornea, and skin repair.[16–23]

In this case series, we describe the successful use of porcine SIS as a xenogeneic graft in repairing severe skin defects in 2 barn owls (*Tyto alba*), an umbrella cockatoo (*Cacatua alba*), and an American crow (*Corvus brachyrhynchos*).

Case Reports

Cases 1 and 2

Two captive adult barn owls, both 3-year-old males that weighed 495 g and 512 g, were presented for examination because of severe left pelvic limb lameness. General husbandry and feeding practices were considered appropriate for this species. However, in both owls, physical examination revealed localized swelling of the tarsometatarsal region with a serosanguineous discharge. These injuries were associated with very tight jesses on the left pelvic limbs of both owls (Fig 1). The owner reportedly had placed new jesses on both owls 2 weeks earlier, but the poor-quality leather had shrunk to cause the current condition. The owls were otherwise clinically normal.

Both owls were managed similarly. Each was premedicated with butorphanol (1 mg/kg IM; Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA, USA) 15 minutes before induction with 4% isoflurane administered by face mask. A 3-mm inside-diameter endotracheal tube was placed, and anesthesia was maintained at 1–3% isoflurane with intermittent positive pressure ventilation every 4 seconds (Small Animal Ventilator, Vetronics BAS, West Lafayette, IN, USA). Anesthetic monitoring included assessment of reflexes, esophageal pulse oximetry, and ultrasonic doppler over the superficial ulnar artery. Lactacted Ringer's solution (10 ml/kg per hour) was given intravenously during surgery for each owl.

Figure 1. Initial presentation of wounds on the tarsometatarsus of a barn owl (case 1). The left jess has been removed to reveal a necrotic and ulcerated wound.

The feathers around the jess and in the immediate tarsometatarsal region were plucked, and the jesses were carefully cut away from both limbs. In both owls, the left tarsometatarsal region was necrotic with complete circumferential involvement underneath the jess. The affected limb was prepared for aseptic surgery and draped in a standard manner. All devitalized and necrotic tissue was carefully and sharply dissected. Hemostasis was maintained by bipolar radiosurgery (Ellman EMC Surgitron 3.8 MHz, Ellman International, Hewlett, NY, USA). The only noteworthy difference between the 2 owls was that, because of the soft-tissue damage, the flexor tendon sheath of digits 2 and 3 was visible in case 1. However, the tendon itself was not disrupted. Once the surgical site was ready for grafting, a single sheet of porcine SIS was placed in sterile saline for 2 minutes to rehydrate the graft. The material was then cut in half to form a double layer and laid over the debrided area. The porcine SIS was cut to an approximate size that would permit complete circumferential enclosure of the skin defect. The graft was applied with the rough side

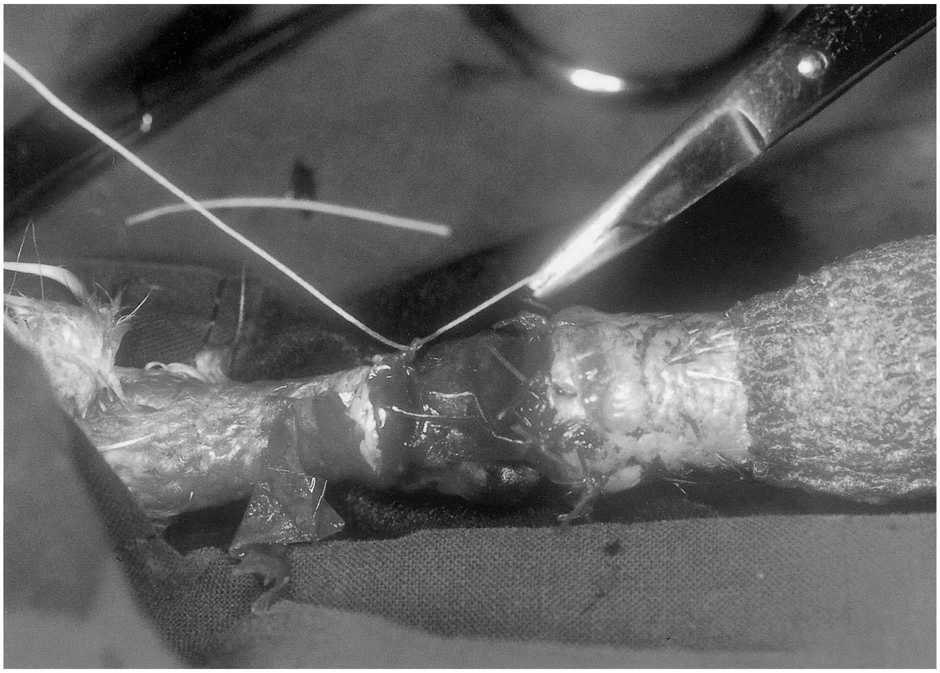

Figure 2. Suturing the porcine small intestinal submucosa graft to a barn owl (case 2) with a wound similar to that described in Figure 1. The graft has been secured below the epidermal margin of the distal skin border with absorbable suture in a simple interrupted pattern.

against the wound, as recommended by the manufacturer. Starting distally, the graft was tucked under and sutured to the skin with polyglactin 910 (4-0 Coated Vicryl, Ethicon, Somerville, NJ, USA) in a simple interrupted pattern. The porcine SIS was then cut precisely to reach the proximal skin margin and similarly sutured to the skin below the epidermis (Fig 2). Once suturing was complete, the graft and wound were inspected for integrity (Fig 3) before the graft was covered with silver sulfadiazine

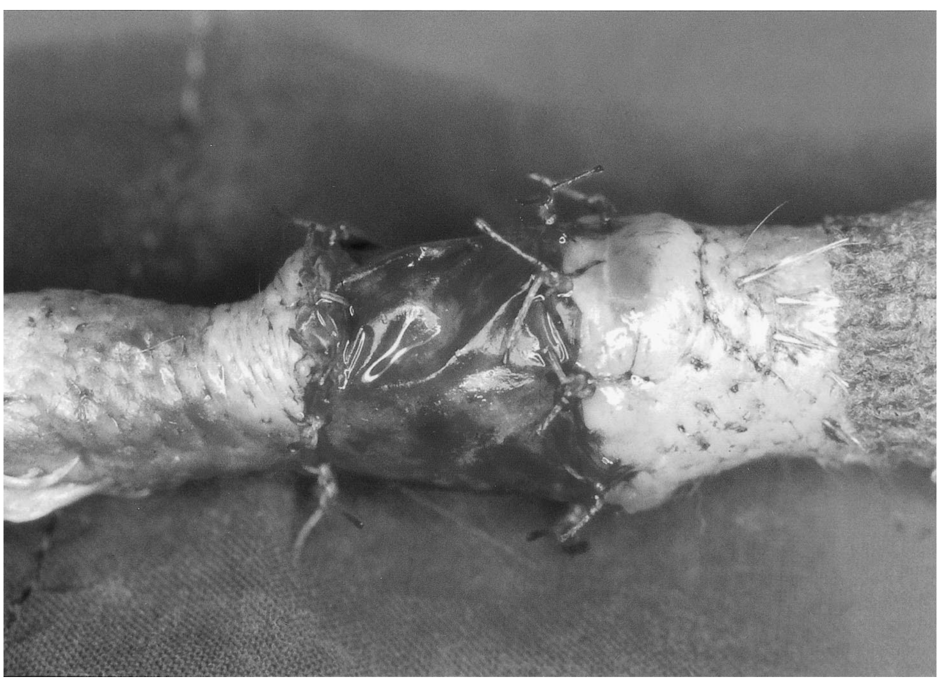

Figure 3. Surgical site of the barn owl (case 2) in Figure 2 immediately after applying the graft. The small intestinal submucosa graft has been sutured around the circumferential skin defect in the tarsometatarsal region.

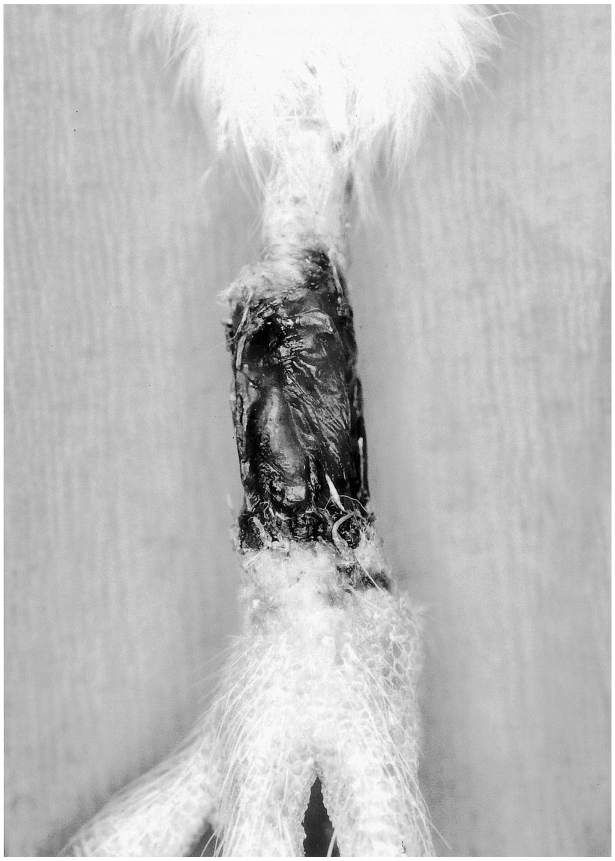

Figure 4. Surgical site of the barn owl (case 2) 1 week after surgery. The dark coloration is caused by coagulated blood and serum within the graft matrix.

Figure 5. Surgical site of the barn owl (case 2) 2 weeks after surgery. The sutures have started to be absorbed.

cream (Silvadene Cream 1%, Hoechst Marion Roussel, Kansas City, MO, USA), a hydrocolloid dressing (ULTEC Pro Alginate Hydrocolloid Dressing, Kendall, Mansfield, MA, USA), and self-adhering elastic bandaging tape. Both owls recovered from anesthesia uneventfully and were fed that night. A second dose of butorphanol (2 mg/kg IM) was administered 8 hours after the initial dose. The owls were discharged the next day, and the owner was asked to maintain them in a small aviary (2 m × 2 m × 2 m).

For 3 days after surgery, the wounds were examined and the dressings were changed daily. At each bandage change, silver sulfadiazene cream was reapplied to maintain the porcine SIS in a moist environment as directed by the manufacturer. No evidence of lameness was observed, and both owls could flex and extend all digits normally. For the next 8 weeks, the owls were re-evaluated once weekly. At 1 week after surgery, the grafts were intact and uninfected (Fig 4). Coagulated blood had accumulated to form a dark scab involving the graft. The bandages were permanently removed at this time. After 2 weeks, the grafts were still intact, and scab involved most of the graft matrix. The area remained uninfected and the sutures started to be shed (Fig 5). By 3 weeks, all suture material was lost and feather regrowth had begun (Fig 6). At 6 weeks, the scabs had been shed to reveal intact skin with minimal scar tissue and progressive feather regrowth (Fig 7). The wounds were considered healed at this time. At 10 weeks, the owner reported continued feather regrowth and said that little evidence of the previous injuries was visible.

Case 3

A 6-year-old male umbrella cockatoo, weighing 782 g, was presented with a keel injury of 4-months duration. The initial injury had been caused by a traumatic landing onto a concrete floor after the bird's wings were clipped. Previous conservative treatment including collars and topical medications had failed. The cockatoo was frequently observed self-mutilating the area and had caused a second lesion distal to the original wound. Husbandry and feeding practices were considered appropriate for this species.

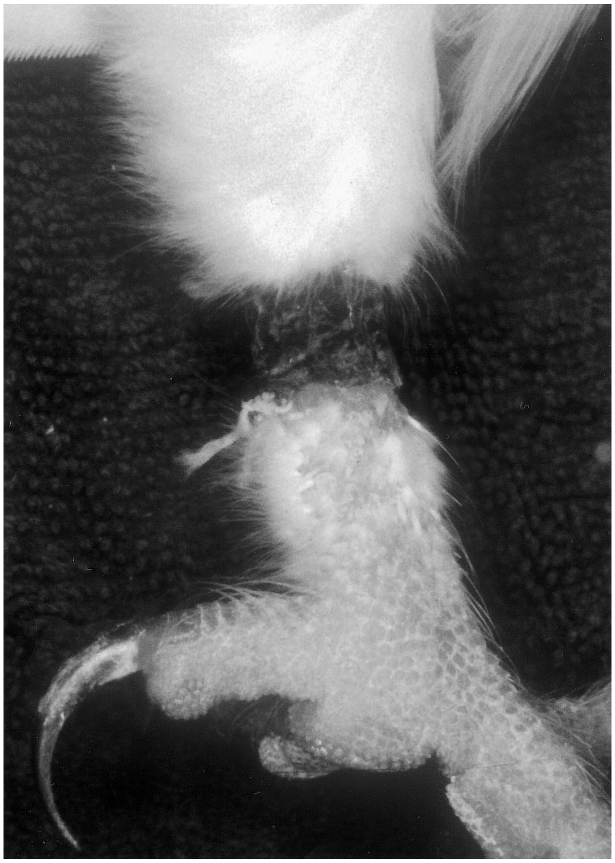

Figure 6. Surgical site of the barn owl (case 1) 4 weeks after surgery. All the sutures have been lost, and feather regrowth distal to the graft site is evident.

Figure 7. Surgical site of the barn owl (case 2) 6 weeks after surgery. The scab has been lost, and regenerated skin with minimal scarring, along with prominent feather regrowth on the graft site, is present.

On physical examination, a 3-cm × 2-cm granulomatous lesion was visible on the ventral midline, approximately 2 cm caudal to the thoracic inlet. The second lesion, 2 cm × 1 cm, was located on the midventral border of the right pectoral region (Fig 8). Both lesions were well circumscribed and ulcerated, with dry exudate. The surrounding area was devoid of feathers because of self-plucking. The cockatoo was otherwise clinically normal. Results of routine hematologic and plasma biochemical testing, as well as dorsoventral and lateral radiographs, did not indicate any other abnormalities.

The cockatoo was premedicated with butorphanol (1 mg/kg IM) and anesthetized in a similar manner as previously described. The entire keel region was prepared and draped for surgery. The primary lesion, including a small area of damaged keel bone, was excised with a #15 scalpel blade. A 2-mm margin of normal tissue was removed to ensure complete excision. Hemorrhage was controlled by bipolar radiosurgery. The excision created a large tissue defect that could not be closed by conventional means. A single layer of porcine SIS was cut to the shape and size of the defect and rehydrated in sterile saline. The graft was then positioned over the defect, rough side toward the wound, and sutured to the skin below the epidermal border with 4-0 polyglactin 910 absorbable suture (Vicryl, Ethicon) in a simple continuous pattern. The graft was covered with silver sulfadiazine cream, and a moisture-permeable adhesive film dressing (OpSite, Smith and Nephew, Largo, FL, USA) was applied to the area. The second lesion, which involved only the skin and subcutis, was surgically resected and the skin was closed routinely (Fig 9). The cockatoo recovered uneventfully from anesthesia and began eating within 1 hour. A collar was placed around its neck to prevent self-trauma of the surgical sites. A second dose of butorphanol (2 mg/kg IM) was administered 8 hours after the initial dose. The cockatoo was discharged the day after surgery.

The cockatoo was examined 2 and 4 days after surgery to inspect the wound and to change the dressing. On each occasion, the dressing and antibiotic cream were carefully removed and replaced. This procedure was completed without sedation or

Figure 8. Umbrella cockatoo (case 3) with a primary keel injury and a smaller self-inflicted wound.

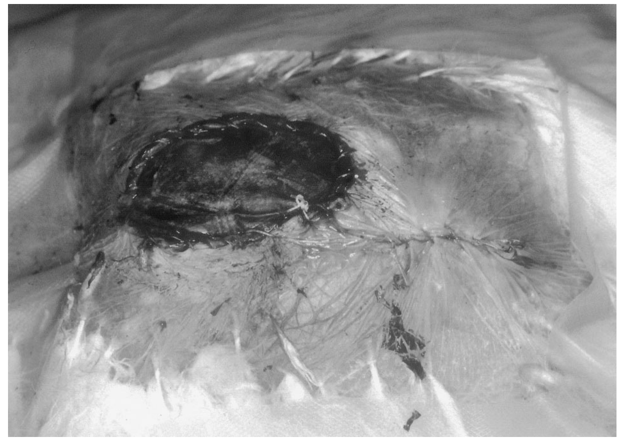

Figure 9. Surgical site of the umbrella cockatoo (case 3) described in Figure 8 immediately after applying the graft. The graft was secured, and the surgical wound created by excising the second smaller lesion was closed with absorbable suture in a continuous pattern.

anesthesia. The graft became progressively darker over the first week after surgery because of accumulated coagulated serum and blood within and under the graft matrix (Fig 10). The bandage was permanently removed at day 6, but the owner was instructed to apply the silver sulfadiazine cream on the wound twice daily for another week. By 2 weeks after surgery, the graft was intact and uninfected and all wound treatment ceased. At 6 weeks, the scab was shed to reveal healed integument with no visible scarring, and the collar was removed. The cockatoo resisted feather picking and self-mutilation of the site and was considered clinically normal at 10 weeks after surgery.

Case 4

An American crow in good body condition (500 g) was presented for evaluation of a traumatic wing injury presumably sustained during a collision with a vehicle. On examination, the crow was recumbent and depressed and had poor peripheral pulses and pale mucous membranes. A 5-cm long, 2-cm wide skin defect was present on the ventral aspect of the left wing, and the metacarpal bones were exposed (Fig 11). Small particles of soil and a hemorrhagic discharge covered the wound. Several other superficial abrasions and contusions were present on the head, pectoral region, and opposite wing. No bone fractures or joint abnormalities were found. The crow was treated symptomatically with warmed lactated Ringer's solution (90 ml/kg IV administered over 20 minutes), dexamethasone (2 mg/kg IM), trimethoprim-sulfa (60 mg/kg IM), and butorphanol (1 mg/kg IM). The wound was gently flushed with a dilute chlorhexidine solution and rinsed with physiologic saline. A light wet-to-dry dressing was applied and overlaid by a figure-of-eight bandage. Lactated Ringer's solution was administered subcutaneously 6 hours after admission (60 ml/kg). By 8 hours after admission, the crow was standing and was gavage fed with a nutritional supplement. The next morning, the crow was bright, alert, perching, and eating. Treatment with trimethoprim-sulfa and butorphanol was continued.

Anesthesia was induced with 3% isoflurane administered by face mask. The crow was then intubated with a 3-mm endotracheal tube and ventilated manually every 5 seconds during surgery. The bandage was removed and the wound was aseptically prepared. Feathers were plucked from surrounding tissues while avoiding primary feathers, which were held away from the surgical site with self-adhesive bandaging tape. Any foreign material was removed from the wound. The skin edges of the defect were identified, and edges that did not appear viable were trimmed until hemorrhage occurred. A single layer of porcine SIS was cut to approximate the size of the wound and rehydrated in saline. Beginning with the dorsal edge of the wound, the porcine SIS was tucked under the epidermal edges of the entire wound and sutured in place, rough side toward the wound, with 4-0 absorbable monofilament polydioxanone suture (Ethicon) in a simple interrupted pattern. The porcine SIS was trimmed as needed to overlap approximately 1 mm under the epidermal edges (Fig 12). The graft was covered with triple antibiotic ointment and a nonadherent dressing. A figure-of-eight bandage was applied to immobilize the wing and maintain the dressing in place. Flunixin meglumine (1 mg/kg IM; Banamine, Schering Plough Animal Health) was administered before recovery. The crow recovered from anesthesia un-

Figure 10. Surgical site of the umbrella cockatoo (case 3) 1 week after surgery. The dark discoloration at the graft site is caused by coagulated serum and blood within the graft matrix.

eventfully. After surgery, treatment with trimethoprim-sulfa (60 mg/kg PO q12h for 10 days) and flunixin meglumine (1 mg/kg IM q48h for 6 days) was continued.

The crow was re-evaluated daily for the first week and every 2 days thereafter. The bandage was removed every other day to inspect the graft and reapply triple antibiotic ointment. The graft appeared intact and uninfected throughout the first week. At the end of the first week, triple antibiotic

Figure 11. Ventral aspect of the traumatized left wing of a crow (case 4) after debriding and cleaning the wound. Note the exposed metacarpal bones.

Figure 12. Ventral aspect of the distal left wing of the crow (case 4) described in Figure 11 after applying the graft.

was discontinued and a sterile surgical lubricant was applied to prevent graft desiccation. At the end of the third week, the graft appeared dark and adhered to underlying granulation tissue. Some sutures on the ventral aspect had been shed. At this time, the dressing and figure-of-eight bandage were discontinued. The crow was discharged to a licensed wildlife rehabilitator with instructions to inspect the graft 3 times weekly and to maintain the crow in a small enclosure, which would restrict wing movement. By week 7, the rehabilitator reported that the scab had shed and the new epithelium overlying the previous defect appeared normal. The crow was placed in a large aviary intended for flight reconditioning. Three weeks later, the crow was flying normally and was released into a similar environment from which it came.

Discussion

In this case report series, we present the details of the successful use of a xenogeneic graft material in 4 birds. Skin grafting is often difficult in birds because of the lack of easily harvested skin. Porcine SIS offers a valuable alternative that has been shown to be both practical and effective. Skin reconstruction techniques currently used in veterinary medicine include long-term wound care and surgery.[24,25] In avian medicine, wound management and healing by second intention is more commonly used than surgical repair.[2] The medical management of avian wounds is similar in many ways to that of other animals and centers on accurate assessment, wound preparation, and the appropriate use of topical medications and dressings.

Common surgical practice for wound repair in birds has largely been restricted to debridement and primary closure by standard suturing techniques.[2] However, some reports have offered more advanced approaches to repairing skin defects that involve the cranium, tarsus, and keel regions.[8,11,13] Keel injuries have been repaired by surgically excising diseased skin, pectoral muscle, and keel bone, then closing the wound to allow healing by primary intention.[8] Postoperative management consists of collars, wing bandages, and examinations every 5–6 days for 6 weeks. Dorsal cervical pedicle advancement flaps have been successfully used in a ring-necked pheasant, a rock dove, a red-tailed hawk, and other raptors.[6,11] Postoperative care was minimal and healing was complete within 2 weeks. Free skin grafts have been used in poultry for genetic research and in 2 clinical cases involving an ostrich with a tarsal wound and 2 great horned owls.[11,12] The ostrich received a full-thickness meshed skin autograft (taken from under the left wing) to cover a skin defect left by the excision of a large tarsal granuloma. In the ostrich, postsurgical wound management was intensive, with dressings changed daily for 3 weeks. The graft procedure was successful and, despite a localized area of rejection caused by focal tenosynovitis of the long digital extensor, complete healing was reported by 90 days after surgery.[11] In the report of the 2 great horned owls, the skin graft was harvested from the inguinal area and the midpropatagium. In both owls, several sites were needed to harvest sufficient skin, and in 1 owl, an external fixator was needed to immobilize the graft site on the foot.[12]

Skin defects in all species can be repaired with advancement flaps or free grafts (auto-, allo-, or xenografts). Advancement flaps are typically considered the most successful of the 3 procedures because they are easier to apply and an intact vascular supply to the graft is maintained.[24,26] However, because avian skin is thin, skin flaps can be problematic in birds; skin is tight over most areas of the body and thus cannot be easily manipulated for flaps without disrupting vascularity.

Grafts of any kind may fail because of infection, movement between the graft and the tissue bed, fluid accumulation under the graft, or poor vascularization, usually associated with immunogenic rejection.[14,24,25] The use of autografts minimizes this risk. In birds, the main disadvantage of autografts is the poor availability of excess skin required for harvesting. In large species, such as the ostrich, full-thickness grafts of sufficient size can be harvested from several areas. However, in smaller birds, the surgeon may be restricted to areas of freely movable skin such as the neck or the inguinal areas.

The use of grafts from other animals may alleviate the problem of graft availability, but the necessity of a surgical procedure on a second animal may be impractical, unethical, or illegal depending on circumstances and jurisdiction. In addition, host rejection of the graft is an overriding concern. Allografts (grafts from genetically distinct individuals of the same species) have been used in poultry research to assess inbreeding, as increasing genetic diversity increases the likelihood and speed of graft rejection.[14] For this reason, xenografts (from animals of a different species) have not been tried clinically for fear of immediate rejection.

Porcine SIS has been studied, both in vitro and in vivo, as a multipurpose tissue graft in several species.[27–30] Results have indicated that 80% of porcine SIS venous grafts remain successful and patent after 6 months, compared with 88% success for autografts.[27] The success of porcine SIS xenografts can be related to several characteristics of this bioscaffold material. The low immunogenicity of porcine SIS is attributed to glutaraldehyde sterilization and stabilization which removes surface antigens during the manufacturing process.[28] The typical disadvantage of such treatment is calcification leading to poor host-tissue incorporation and mechanical failure of the graft. However, studies using a rat subcutaneous model have shown porcine SIS to cause no implant mineralization and little peri-implant fibrosis.[28] Porcine SIS also contains various extracellular and basement membrane factors that are considered key for successful implantation. Glycosaminoglycans (hyaluronic acid, heparin, heparan sulfate, chondroitin sulfate A, and dermatan sulfate) are important components of the extracellular matrix that have been isolated from porcine SIS. These factors, along with fibronectin, have been linked to antithrombosis and anticoagulation, angiogenesis, rapid neovascularization, cell differentiation and proliferation, organized deposition of collagen, and tissue regeneration.[29,30] Although the process is only poorly understood, in mammals the graft can be remodeled and replaced by the host tissue within 90 days.[27–30] This phenomenon, which depends on maintaining a moist graft environment for the first 7–10 days, would explain the new avian integument, including feathers, observed at the graft sites in the cases we describe.

Conservative wound management is often prolonged and is prone to failure or scarring of the area.[2,8] In addition, management is often long term and intensive, requiring frequent handling of patients. Although habituated pet birds typically tolerate intensive wound management, owners and keepers may be frustrated by frequent visits to the clinic. The barn owls we describe were habituated to perching on a gloved hand; however, they were not accustomed to additional restraint. The crow was unaccustomed to any form of human contact. The stress associated with frequent handling may not be tolerated by some species of free-ranging birds, or it may lead to negative sequelae such as delayed healing, secondary bacterial or fungal infection, or trauma from capture or restraint. With the crow, conservative management was not an appropriate option because the exposed metacarpal bones necessitated rapid closure of the wound to prevent the bones from desiccating or becoming infected. In this case, the porcine SIS graft not only provided a suitable network for wound healing but also protected viable underlying structures during the healing process.

By using porcine SIS, wound healing may be reduced to 2–6 weeks, depending on the species of bird, wound characteristics, and technique.[8–10] In the 2 barn owls and the crow, skin from surrounding areas was not available for primary closure or a skin flap. In the cockatoo, the pectoral musculature could have been undermined and closed after surgical repair of keel wounds.[8] However, that technique requires wing bandaging for 6 weeks, which was deemed unsuitable for this cockatoo. Healing occurred within 6 weeks in the birds we describe, without the need for wing immobilization which is in accordance with observations made in other species.[16–23,27] All 4 birds returned to normal function.

When used correctly, porcine SIS grafts require careful management with topical antibacterial medication and bandaging for the first 7–10 days to prevent graft desiccation. After that time, bandaging does not appear to be crucial. In contrast, healing by secondary intention in the cases described would have required at least 6 weeks of bandaging and sustained wound management.[8] No restraint devices were necessary for the owls or crow after surgery, but a collar was deemed essential for the cockatoo to prevent self-trauma.

Porcine SIS is commercially available as a single lyophilized sterile sheet, 70 mm × 100 mm, with a shelf life of 12 months (Vet Biosist). It is convenient to use and, with little practice, is easy to fashion to suit surgical requirements. The main disadvantage of this product is expense; however, when considered against the alternative costs of long-term conservative management, the expense of the graft is acceptable. Our success in using porcine SIS in the 4 cases we describe indicates that this product should be considered a potentially valuable xenograft material in avian wound management and reconstructive surgery.

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