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Clinical application of both amniotic membranes and adipose derived mesenchymal stem cells in a cat with a large skin defect

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Abstract: Surgical procedures on large skin defects can be challenging in the short term due to the size of the lesion, infection, and tissue defect. A regenerative therapy for skin wounds has been applied to promote the healing process. An 8-month-old, Korean domestic short-haired female cat, weighing 3 kg, was rescued with extensive defects on the right flank to right inguinal region caused by bite wounds. In this case, amniotic membranes and adipose-derived mesenchymal stem cells were used as the regenerative therapy to treat the large skin defect rather than a surgical intervention alone. To the best of our knowledge, this is the first report of a case with of a large skin defect treated by applying allogeneic amniotic membranes and allogeneic mesenchymal stem cells to a cat.

Keywords: feline; healing process; regenerative therapy; skin wound

Treatment of large skin defects due to traumatic events in small animals can be difficult. It requires the accurate removal of the damaged or necrotic tissue, intensive care with protection and regeneration to the injured skin tissue, and local or systemic infection control (Zubin et al. 2015). Furthermore, the structure of the skin in cats is different to that in dogs. Cats have a low density of tertiary and higher-order vessels; the rate of contraction, epithelialisation, and total wound healing are all significantly slower than those in dogs.

Skin wound therapy can be categorised in two ways. First, conventional wound management is a procedure including the debridement of necrotised tissues, reconstruction of the damaged tissues (e.g., skin grafting) and medical treatment, in order to normally recover damaged tissues and reduce venerable complications and pain (Zubin

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et al. 2015). Second, regenerative skin wound therapy aims to accelerate wound healing, repair damaged tissues, and reduce inflammation. However, it should not be considered as a primary procedure when essential conventional treatments are needed, but as a supplementary treatment (Borena et al. 2015). Regenerative therapy includes two main options: growth factor-based therapies (e.g., platelet-rich plasma) and cell-based therapies (e.g., mesenchymal stem cells, amniotic membranes) (Borena et al. 2015). Mesenchymal stem cells are a promising option in regenerative therapy and have been a considerable cell-based therapy in human medicine for decades. A number of studies describe the effectiveness of mesenchymal stem cells on a variety of diseases (Markoski 2016; Quimby and Borjesson 2018). In addition, one case has been reported to effectively treat skin defects using autologous platelet-rich plasma and autologous mesenchymal stem cells in a dog (Zubin et al. 2015).

Likewise, an amniotic membrane can also be used for the regenerative skin wound therapy (Duarte and Duval-Araujo 2014; Campelo et al. 2018). The amniotic membrane is the innermost layer of the embryonic membrane that covers the foetus. It has a variety of anti-inflammatory effects and contains many growth factors, cytokines, and signalling molecules (ElHeneidy et al. 2016). Moreover, the amniotic membrane has also served as a biomaterial for use in aiding wounds in human medicine. A number of studies have described the potential of amniotic membranes to accelerate wound healing and have the potential to decrease exudates and pain (Mermet et al. 2007; Loeffelbein et al. 2014). In veterinary medicine, however, there are only a few studies on this due to difficulties in obtaining a supply and providing for the demand.

We aimed to report the treatment of a large skin defect by applying an allogenic amniotic membrane and allogenic mesenchymal stem cells to a cat for the first time.

Case description

An 8-month-old, female, Korean domestic cat, weighing 3 kg, was rescued with severe skin wounds and referred to the Animal Emergency Trauma Centre, Seoul National University. The patient was injured by severe bite wounds and had extensive tissue defects of the right flank to the right inguinal region (approximately 20 × 15 cm, width × length). The physical examination revealed that the wound was severely contaminated, and inflammation along with a haemorrhagic purulent exudate were observed. Remarkable findings were observed in terms of mild hypothermia (37.5 °C) and cachexia (body condition score 3/9). Capillary refill time was within 1–2 s and the mucous membrane colour was pale pink with mild dryness. The owner who intended to adopt the cat fully agreed to all the wound treatment processes. A high white blood cell level (41.92 × 10^9/l; reference range, 5.2–17 × 10^9/l) was observed in the complete blood cell count with a low normal range of haematocrit (31.8%; reference range, 30.3–52.3%). In the serum chemistry, the phosphate was slightly high (2.5 mmol/l; reference range, 1.0–2.4 mmol/l), with a high level of aspartate aminotransferase (AST) (1.46 μkat/l; reference range, 0–0.8 μkat/l) and an electrolyte imbalance with a low sodium concentration (143 mmol/l; reference range, 150–165 mmol/l) was observed. In the venous blood gas analysis, a high lactate concentration was detected (2.6 mmol/l; reference range, 0.5–2.0 mmol/l). Subsequently, an invasive treatment, such as irrigation and wound debridement, were considered. A surgical debridement and open wound management were performed due to the contamination and the size of the lesions (Figure 1A). Sugar therapy and a wet-to-dry bandage were performed. An antibiotic therapy of cefazolin (Cefazoline injection; Chong Kun Dang, Seoul, Republic of Korea) 25 mg/kg, intravenous (i.v.), analgesia with tramadol (Trodon injection; Aju Pharm Co, Ltd, Gyeonggi-do, Republic of Korea) 3 mg/kg, i.v., and sedation with medetomidine (Domitor; Orion Pharma, Turku, Finland) 0.1 mg/kg, intramuscular (i.m.), were administered before all the procedures. The postoperative medication included two antibiotics: cefazolin (Cefazoline injection; Chong Kun Dang, Seoul, Republic of Korea) 25 mg/kg, i.v., metronidazole (Metrynal injection; Dai Han Pharm Co, Ltd, Gyeonggi-do, Republic of Korea) 15 mg/kg, i.v., analgesic tramadol (Trodon injection; Aju Pharm Co, Ltd, Gyeonggi-do, Republic of Korea) 3 mg/kg, i.v., and gastrointestinal agent famotidine (Gaster injection; Dong-A ST, Seoul, Republic of Korea) 0.5 mg/kg, i.v. The following day, the treatment yielded a significant decrease in exudates, and the patient showed good appetite and vitality. The lactate concentration was within the normal range (1.0 mmol/l; reference range,

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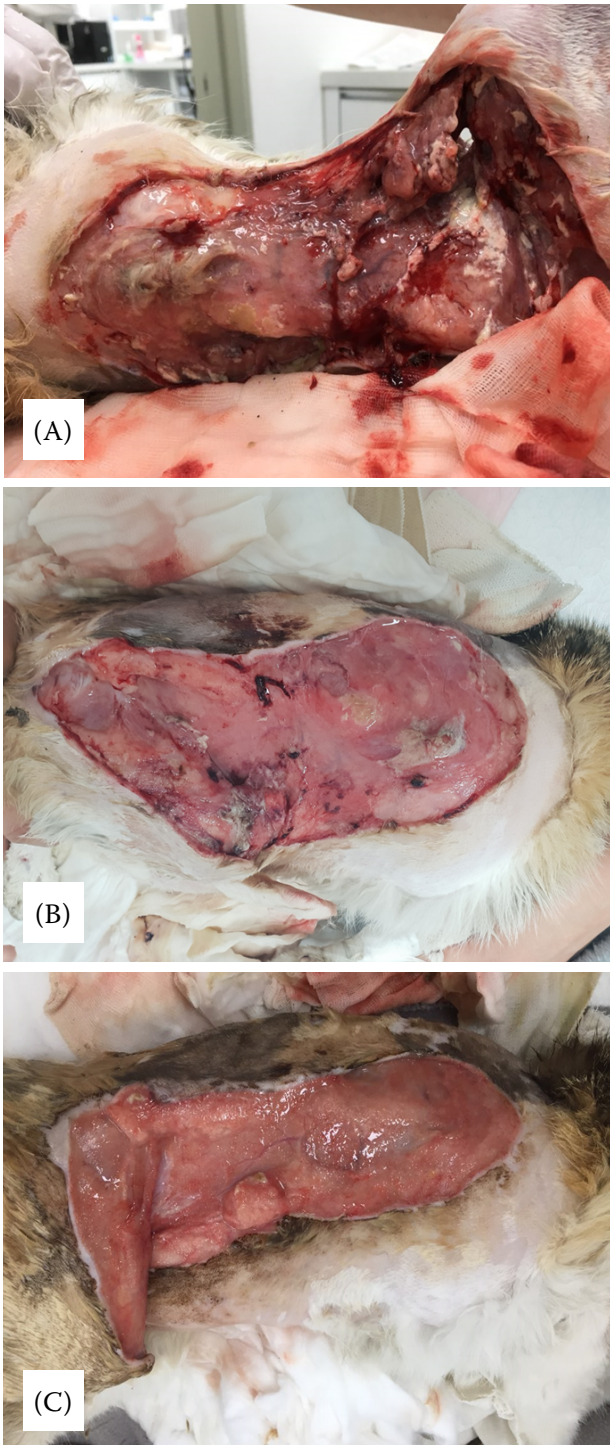

Figure 1. Large tissue defect on the right flank to right inguinal regions, after debridement on day 1 (A), day 3 (B) and day 14 (C)

0.5–2.0 mmol/l) in the venous blood gas analysis. There were no abnormal signs on physical examination, and the body temperature had increased to 38.4 °C. The treatment was repeated two times per day. By the third day of treatment, the amount of inflammation and exudation had progressively decreased (Figure 1B). The complete blood cell count showed that the white blood cell level was still high ((48.15 × 10^9/l; reference range, 5.2–1 × 10^9/l) and the haematocrit was decreased (21.8%; reference range, 30.3–52.3%). Wet-to-dry bandaging was performed along with a sugar therapy once a day, and the medication were administered twice a day. Sedation was performed prior to bandaging. Soft canned food was provided, and fluid therapy was maintained due to loss of the patient's appetite and dehydration. On day 6 of the treatment, the sugar therapy was stopped after confirmation of the minimal necrotic tissues and exudates. On day 9, most of the blood parameters were within the normal ranges except for a high number of white blood cells (32.92 × 10^9/l; reference range, 5.2–1 × 10^9/l) and a low range of haematocrit (29.3%; reference range, 30.3–52.3%). The same treatment was continued. On day 14, the inflammation was noticeably reduced, but the other treatments, such as regenerative therapy, were considered because the defect area was still too large for surgical correction (Figure 1C). On day 16, the patient was well maintained with no remarkable changes in its condition. On the same day, another cat severely injured by a falling trauma was brought to the centre. In the radiographic images, the patient was pregnant and suspected to have five gestational sacs. Despite the intensive care and treatment, the cat died from shock and severe trauma right away. The gestational sacs were removed immediately in order to use their amniotic membranes (Figure 2).

In addition, adipose tissue was extracted from the abdomen and handed to the Department of Internal Medicine at Seoul National University to cultivate the mesenchymal stem cells. The separated amniotic membranes were washed with sterile 0.9% normal saline and antibiotics aseptically. The membranes were applied to the skin defect area of the first cat, and a skin releasing incision was performed. Foam dressings were used to remove the inflammatory mediators and to maintain moist conditions on the wound surface. On day 20, from the adipose tissue, mesenchymal stem cells were cultivated with the help of the Department of Internal Medicine at Seoul National University. Detailed information related to the mesenchymal stem cells can be found through a previously published article (Park et al. 2021). The mesenchymal stem cells were intradermally injected in the wound lesions

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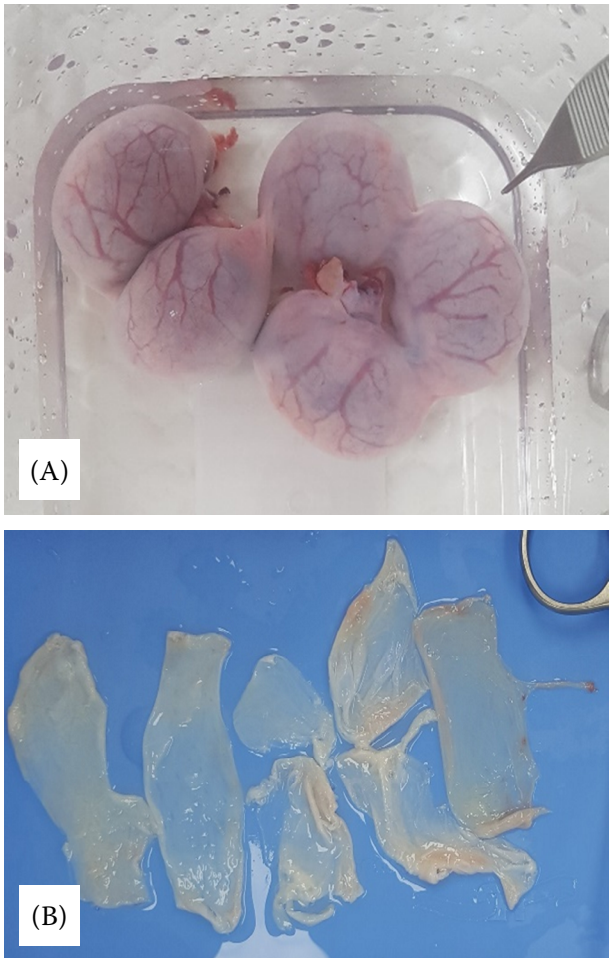

Figure 2. The five sacs and amniotic membranes from the cat

(A) The gestational sacs extracted from the uterus after caesarean section. (B) Amniotic membranes after being stripped away from the sac

after being premedicated with dexamethasone (Jeil Dexamethasone injection, Jeil Pharmaceutical Co, Ltd, Yongin, Republic of Korea) 0.2 mg/kg, i.v., and chlorpheniramine (Chlorpheniramine Maleate injection; Huons, Co, Ltd, Seoul, Republic of Korea) 0.5 mg/kg, i.v., four times every other day (Figure 3A). No immune response or other abnormalities were found in the blood work and physical examination after the injections. The bandaging and medical treatment were routinely performed. On days 22 to 28, a rapid rate of recovery was observed along with a remarkable granulation of the cell tissues at the wound site. Healthy granulation tissue filled in the defect in which new blood vessels were embedded (Figure 3B,C). The patient was well maintained and gained weight. On day 30, the damaged tissues were well repaired along with the re-

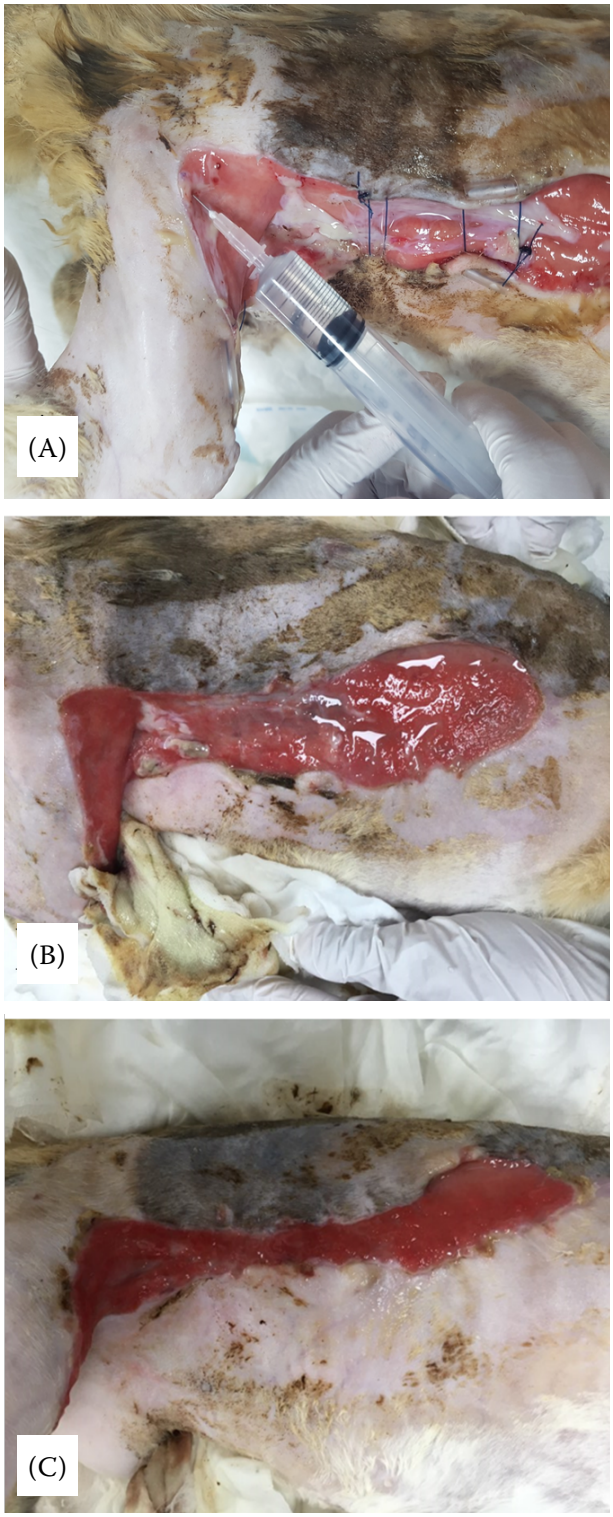

Figure 3. Images of the healing process

Mesenchymal stem cells were injected and amniotic membranes were applied on the wound edges on days 20 (A), 30 (B), and 37 (C)

epithelisation on the skin margins. The skin release technique was repeated to help reconstruct the skin

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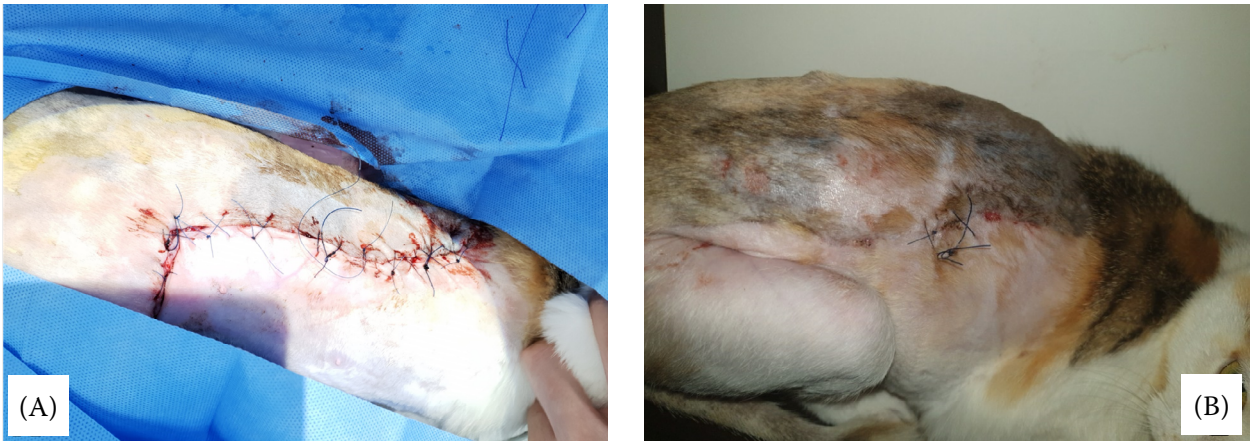

Figure 4. Image after the skin reconstruction on day 38 (A). The stitches were removed on day 50 (B)

the next week. On day 37, surgical reconstruction was possible, and a skin reconstruction was performed (Figure 4A). The patient was adopted after the end of the treatment without any complications or infection for 50 days (Figure 4B). After two years of follow-up, it was confirmed that the cat was doing well with no problems in the wound area.

DISCUSSION

Decision-making is very important in wound management. When looking at the wound, the gross appearance of the stages should be well understood. A full physical examination and blood tests must be performed, and diagnostic imaging can also be performed when needed.

The patient, in this case, had heavily contaminated and infected wounds with extensive tissue loss. The open wound had to be managed for as long as it was necessary to control the infection. Conventional wound management along with the help of regenerative skin wound management was determined since surgical closure was not suitable for the wound.

Regenerative wound management helps to reduce the inflammation, accelerate wound healing, and recover damaged tissues (Borena et al. 2015). In addition, cell-based therapies have been studied in animals to prove their potential effectiveness in regeneration. Although cell-based therapies in veterinary clinical trials are still needed to encourage more studies, many basic studies on stem cells in veterinary medicine can provide the potency of clinical benefits in a variety of areas (Volk and Theoret 2013).

In one case report, the use of cell-based therapy on a large skin defect in a dog was successfully treated over 480 days (Zubin et al. 2015). The method applied, in this case, was inspired by the study mentioned before, presenting that the injection of mesenchymal stem cells boosts the healing and regeneration of skin defects. In human medicine, the use of mesenchymal stem cells in wound management promotes angiogenesis, reduces inflammation, and accelerates wound closure through paracrine interactions (Rodriguez et al. 2015; Lee et al. 2016). In addition, adipose-derived mesenchymal stem cells (which were used in the author's case) have advantages in extraction compared to other stem cells by minimally invasive procedures with large quantities (bone marrow stem cells), having the potential to differentiate into multiple cell lineage pathways (adipogenic, chondrogenic, osteogenic), and can be transplanted to an autologous or allogenic host without immune rejection (Mizuno 2009; Konno et al. 2013).

Application of an amniotic membrane in wound management has been used in human medicine for decades (Barski et al. 2018). It has several characteristics that make it particularly suitable for wound healing. Not only does it contain many cytokines and essential growth factors, it also relieves pain, reduces inflammation, and accelerates the wound healing process (Skardal et al. 2012; ElHeneidy et al. 2016). It is also antibacterial, non-immune, and provides biological barriers (ElHeneidy et al. 2016). Moreover, an amniotic membrane has been used as a biomaterial dressing in the treatment of cornea reconstruction, acute or chronic skin wounds, and burns in human medicine. Many studies suggest that an amniotic membrane appears

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to serve as a safe substance that promotes proper epithelialisation while suppressing excessive fibrosis (Loeffelbein et al. 2014; Favaron at al. 2015). There are limited studies and clinical trials using amniotic membranes in veterinary medicine, especially in feline cases. However, one original research paper suggested that cat amniotic membranes are non-tumorigenic and are safe to use in cell-based therapies (Vidane et al. 2014).

Despite the necessity of additional studies on the use of mesenchymal stem cells and amniotic membranes in felines, a successful treatment was made by applying mesenchymal stem cells and amniotic membranes on large skin defects on a cat for the first time. As the size of the defect could not be treated by conventional wound management, the application of a regenerative therapy led to promising results in the wound healing. Mesenchymal stem cells and amniotic membranes may be an effective therapeutic option for large skin defects, especially when secondary intention healing is suggested. The use of cell-based therapies may help to improve the healing process in wounds and regeneration much quicker. Although the cost of the treatment and dedicated care of the patient may be considered in clinical veterinary medicine, these treatments can lead to a patient having a successful quality of life.

Conflict of interest

The authors declare no conflict of interest.

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