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Allogeneic mesenchymal stem cells therapy along with collagen granules on wound healing in mice

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Abstract
The study was conducted on Swiss albino mice to determine the efficacy of adipose-derived mesenchyme stem cells (AD-MSCs) on wounds along with collagen granules. The adipose tissues were collected from the abdominal cavity and processed following standard procedure, and then seeded in a six well cell culture plate, which was incubated at 37 ºC in a humidified CO_2 (5%) incubator till 70–80% confluence. Then the cells were injected subcutaneously along the margin of the wound, and collagen granules were applied in addition. On average, 13.3±0.33 days were required for complete healing, and the wound contraction area was 3.21±0.04 mm, 1.88±0.02 mm, and 1.14±0.05 mm on days 3rd, 7th, and 14th, respectively. Wounds heal faster because of their individual properties. And this combination showed a promising usefulness in treating wounds by inducing the healing process.

Keywords: AD-MSCs, collagen granules, wound, mice

Introduction
Stem cells have always been an area of interest for researchers in the field of tissue engineering or regenerative medicine. Taking care of either an acute or chronic wound (breakage in the continuity of skin by any means) is challenging for veterinarians because of their self-mutilation habit, which may lead to further secondary infection and delay wound healing. Therefore, finding a potential alternate method to cure the wound quicker than usual is crucial. Stem cells are known for their capability to self-renew through replication and differentiate into different cell lineages. Again, collagen is the most abundant and important extracellular protein produced by fibroblasts. Hence, synthetic collagen substances induce the healing process faster, as they are applied directly and mend wounds and aid in the formation of new tissues. Thus, the objective of this study was to see the efficacy of the combination of adipose-derived mesenchyme stem cells (ADMSCs) and collagen granules for wound healing in mice.

Materials and Method
The work was carried out in the Department of Veterinary Surgery and Radiology, C.V.Sc. & A.H., OUAT, Odisha with the permission from the Institutional Animal Ethical Committee (IAEC) of College of Veterinary Science and Animal Husbandry. Swiss albino mice of either sex were used for isolation of stem cells from adipose tissue after anaesthetising individually with a mixture of injection xylazine hydrochloride @ 12.5 mg/kg and ketamine hydrochloride @ 80 mg/kg through intraperitoneal route (Tranquilli *et al.,* 2007) [8]. After following proper standard aseptic procedure, mid-ventral incision was made and adipose tissues were collected & kept in phosphate buffer saline. The collected tissues were washed with PBS and minced manually. Later it was treated with 5 ml of 0.05% Trypsin-EDTA (Gibco, Thermo Scientific, USA) in 10 ml PBS and incubated at 37 °C for 15 min. The entire content was filtered through a 70µm cell strainer (SPL, Life Sciences, and India) and 1 ml of FBS was added. The filtrate was centrifuged at 1200 rpm for 10 minutes. The pellet was suspended in 5 mL of RBC lysis buffer (Himedia) and incubated at room temperature for 5 minutes before centrifugation. The cell pellet was re-suspended in fresh media containing DMEM Nutrient Mix F12, 20% FBS, and 1% antibiotic antimitotic solution (Gibco, Thermo Scientific, USA). Finally, the filtered cells were seeded in a six-well cell culture plate (final volume of 1 ml containing DMEM Nutrient Mix F12 + 20% FBS + 1% antibiotic-antimitotic solution) in a humidified CO_2 (5%) incubator at 37 °C.

The media were replaced every three days until confluence reached 70-80%. The cells were passaged multiple times to enhance cell population by trypsinization (0.05% Trypsin-EDTA, Gibco, Thermo Scientific, USA).

For application, animals were anaesthetized same as the procedure used for collection of tissue sample. On dorsal area, wound was made with the help of 5mm biopsy punch. The cultured stem cells were injected subcutaneously along the borders of the wound and then were covered by collagen granules. Time required for complete healing was recorded (Bigbie *et al.*, 1991) [3] and wound contraction was evaluated on 3rd, 7th and 14th postoperative days (Parameshwaraiah and Shivakumar, 1998) [5] and was measured by Image J software and average was taken.

Results and Discussion

The development of new connective tissue matrix, collagen deposition, and cellular migration are all aided by a number of different growth factors that are involved in wound healing (Singer and Clark, 1999). Lesions were seen to be completely healed within 13.3±0.33 days without any presence of exudates or any secondary infection. On day 0, the average wound area was 5.73±0.06 mm. By the 3rd, 7th, and 14th days, the wound area was 3.21±0.04 mm, 1.88±0.02 mm, and 1.14±0.05 mm, respectively. Stem cells have significant prospective to mature into different cell types throughout early growth and life (Bethesda, 2016) [2]. Mesenchymal stem cells not only attributed their ability to differentiate but also to produce various antigenic factors like vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (BFGF) (Al- Khalidi *et al.*, 2003) [1]. Stem cells can differentiate into any new cells, producing anti-inflammatory cytokines that reduce the duration of the inflammatory phase, thereby enhancing the healing process. Stem cells themselves have bactericidal properties, which secrete antimicrobial factors, increasing the phagocytosis process in wound healing (Mei *et al.*, 2010) [4] and in addition, collagens are proline-rich proteins (Stadelmann *et al.*, 1998) [7], which enhance the healing process by promoting the formation of early granulation tissue and wound contraction (Writte and Barbul, 1997) [9]; therefore, their synergistic effect might enhance a wound's ability to heal faster.

Conclusions

Mesenchyme stem cells produced from adipose tissue are a reliable source of stem cells and can develop into a wide variety of cell types. It is much easier to collect the stem cells from adipose tissue than from bone marrow, and it causes less discomfort in animals. Stem cells show a promising future in the section of tissue engineering and turn a new leaf in the world of veterinary medicine. The synergistic effect of adipose-derived mesenchyme stem cells and collagen granules speeds wound healing by increasing the formation of granulation tissue. The future use of this combination to treat acute or chronic wounds more effectively and quickly may be beneficial.

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