



Histomorphological Study of Cutaneous Wound Healing in Rabbits Using Xenogenic Adipose Derived Stem Cells

Sidhartha Sankar Behera^{1*}, Indramani Nath¹, Sadananda Nayak¹, Subash Chandra Parija², Uma Kanta Mishra³, Akshya Kumar Kundu⁴, Susen Kumar Panda⁵, Smruti Ranjan Mishra⁴ and Monalisa Behera⁵

¹Department of Veterinary Surgery and Radiology, C.V.Sc. and A.H., OUAT, Bhubaneswar, Odisha, INDIA

²Department of Veterinary Pharmacology and Toxicology, C.V.Sc. and A.H., OUAT, Bhubaneswar, Odisha, INDIA

³Department of Veterinary Anatomy and Histology, C.V.Sc. and A.H., OUAT, Bhubaneswar, Odisha, INDIA

⁴Department of Veterinary Physiology, C.V.Sc. and A.H., OUAT, Bhubaneswar, Odisha, INDIA

⁵Department of Veterinary Pathology, C.V.Sc. and A.H., OUAT, Bhubaneswar, Odisha, INDIA

*Corresponding author: SS Behera; Email: drsidharthavety2006@gmail.com

Received: 10 Aug., 2019

Revised: 17 Sept., 2019

Accepted: 25 Sept., 2019

ABSTRACT

The present study was carried out to evaluate the potential of adipose-derived mesenchymal stem cell (AD-MSCs) to enhance the rate of healing of full-thickness excisional skin wounds in rabbits. Six healthy adult New Zealand white rabbits and five healthy Swiss Albino mice were used for the study. Two, 2 × 2 cm full-thickness skin (thoracolumbar region) excisional wounds were created; one on each side of the dorsal midline in each animal. Adipose tissue was collected from the abdomen of the mice and processed for isolation of AD-MSCs. The wounds were randomly assigned to either injection of adipose-derived mesenchymal stem cell into the wound margins (AD-MSCs), or topical application of Povidone iodine (5%) solution (PI) as positive control. The wound healing was assessed by evaluation of granulation tissue formation, epithelisation and histomorphological study on 7th, 14th, 21st and 28th postoperative days. Better epithelisation was seen histologically in AD-MSCs treated wounds than in PI-treated wounds. Histomorphological examination of the healing tissue showed early disappearance of inflammatory reaction, significantly more neovascularisation, and more fibroplasias and early lay down and histological maturation of collagen in AD-MSCs treated wounds than in PI treated wounds. Hence the application of xenogenic stem cells can be used for tissue engineering and regenerative medicine in animals.

Keywords: Adipose-derived mesenchymal stem cells, histopathology, wound healing

Normal wound healing proceeds in four phases which includes hemostasis, inflammation, proliferation and remodeling. In hemostasis phase there will be formation of clot, and this phase starts within few seconds of tissue injury. In inflammatory phase all the defensive cells such as, neutrophils, macrophages, monocytes migrate to the site of injury. Proliferation phase occurs when collagen secretion, re-epithelialization, and scar formation occurs. At last, in remodeling phase, the scar undergoes changes that may last as long as one year (Collawn and Patel, 2014). Despite the advancements of research in wound healing, researchers are still aiming at early wound healing without any complications. All wound healing agents aim

at providing a pathogen-free, protected and moist area for wound healing to occur (Murphy and Evans, 2012). Thus, there is a need to identify a viable and effective approach for enhancement of wound healing. Now-a-days stem cells have emerged as a viable alternative for the treatment of this complex pathology for enhancement of tissue regeneration (Mester *et al.*, 2017). These mesenchymal stem cells can be isolated from developing embryo, bone marrow, peripheral blood, adipose tissue, liver and fetal adnexa (cord blood, amniotic fluid, amniotic sac and

How to cite this article: Behera, S.S., Nath, I., Nayak, S., Parija, S.C., Mishra, U.K., Kundu, A.K., Panda, S.K., Mishra, S.R. and Behera, M. (2019). Histomorphological study of cutaneous wound healing in rabbits using xenogenic adipose derived stem cells. *J. Anim. Res.*, 9(5): 645-652.



Wharton's jelly) in different species (Dhenge *et al.*, 2018). Out of which, adipose tissue can be obtained with less invasive procedures than other tissues. More importantly, adipose tissue-derived stem cells (AD-MSCs) can be recovered in high quantities because adipose tissues are abundant reservoirs of mesenchymal stem cells approximately 100 times fold higher than bone marrow. Therefore, adipose tissue represents an abundant, practical and appealing stem cell source for regenerative medicine. Autologous AD-MSC therapy has been proved safe and therapeutically successful and extensively used in clinical practice. However, in recent year's use of xenogenic stem cells are of more concern due to its wide clinical application. For this reason in the present study attempts were made to study the efficacy of xenogenic adipose derived stem cells from mice (Swiss Albino) in wound healing of rabbits through histo-morphological study.

MATERIALS AND METHODS

Experimental design

Six healthy adult New Zealand white rabbits and five healthy Swiss Albino mice were used for the study which was approved by the Institutional Animal Ethical Committee (21/IAEC) of College of Veterinary Science and Animal Husbandry. A total of 12 wounds were created, two wounds on dorsum of the body of each rabbit. Out of which six were treated by application of xenogenic adipose derived mesenchymal stem cells (AD-MSCs) and rest six were treated using Povidone iodine (5%) solution (PI) as positive control.

Adipose tissue collection and processing

Swiss Albino mice were used for collection of abdominal adipose tissue samples for isolation of adipose derived stem cells. The mice were anaesthetized individually using an intraperitoneal injection of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (80 mg/kg) mixture and adipose tissue was collected from the abdomen. The adipose tissue was processed and cultured inside a humidified CO₂ (5%) incubator at 37°C as per Reich *et al.* (2012) up to 70-80% confluence for isolation and application of AD-MSCs on wound bed.

Wound creation

For wound creation rabbits were anaesthetized by intramuscular administration of xylazine hydrochloride (6 mg/kg body weight) and ketamine hydrochloride (60 mg/kg body weight). Lumbosacral region was prepared aseptically and two full-thickness skin excision wounds (2 × 2 cm square) were created one on each side of the dorsum of the animal. All wounds were cleaned and treated on the same day of wound creation. Application of AD-MSCs and povidone iodine (5%) solution (PI) were assigned randomly. The AD-MSCs were injected subcutaneously around the wound edges and applied over the wound. In the wounds designated as PI group, povidone iodine (5%) solution was applied topically using a piece of sterile cotton gauze. Then both the wounds of each animal were covered by a paraffin wet bandage. Post-operatively enrofloxacin (5 mg/kg) and meloxicam (0.2 mg/kg) were administered intramuscularly for 5 and 3 days respectively in all the animals.

Histomorphological study of wound

Full-thickness skin tissue samples, including normal skin around the healing wounds from both treatments, were collected on 7th, 14th, 21st and 28th postoperative days. After collection of tissues, these wounds were repaired by suturing and the animals were excluded from the study. The tissues were fixed in 10% buffered formalin and routine paraffin embedding haematoxylin and eosin (H&E) staining was done. Special staining for collagen fiber was done by using Masson's trichrome staining (Bancroft *et al.*, 1996). The tissue sections were evaluated microscopically by using histological scoring system as per Sarode *et al.* (2014) (Table 1).

RESULTS AND DISCUSSION

The average histological scoring was recorded on 7th, 14th, 21st and 28th post-operative days and the detailed results were presented in Table 2. On histological examination, it was observed that by day 7 after wounding there was no evidence of epithelialization in PI group wounds; whereas regenerating epithelium was detected in AD-MSCs treatment group, which was slight to moderately thicker than normal skin. There was mild to moderate inflammatory reaction and the inflammation was

Table 1: Showing different histological parameters and their scores as per Sarode *et al.* (2014).

Sl. No.	Parameters	Score			
		1	2	3	4
1	Epithelialization	Resembling normal skin	Slightly thick to normal skin	Moderately thick to normal skin	Thicker than normal skin
2	Inflammation	Resembling normal skin	Mild	Moderate	Severe
3	Fibroblast	Resembling normal skin	Mild	Moderate	Severe
4	Neovascularization	Resembling normal skin (0-1 new blood vessels)	Mild (2-5 new blood vessels)	Moderate (6-10 new blood vessels)	Severe (> 10 new blood vessels)
5	Collagen fibre density	Denser	Dense	Less dense	—
6	Collagen fibre thickness	Thicker	Thick	Thin	—
7	Collagen fibre arrangement	Best arranged	Better arrangement	Worst arrangement	Worst arrangement

Table 2: Mean \pm SE values of histomorphological parameters of excisional wounds in two groups during different days

Parameters	Day 7		Day 14		Day 21		Day 28	
	PI	AD-MSCs	PI	AD-MSCs	PI	AD-MSCs	PI	AD-MSCs
Epithelization	3.33 \pm 0.19	2.92 \pm 0.23	3.08 \pm 0.19	2.50 \pm 0.19	3.00 \pm 0.17	2.17 \pm 0.24	2.83 \pm 0.21 ^a	1.17 \pm 0.11 ^b
Inflammation	2.75 \pm 0.22 ^a	1.83 \pm 0.27 ^b	2.58 \pm 0.15 ^a	1.58 \pm 0.23 ^b	2.25 \pm 0.13 ^a	1.50 \pm 0.15 ^b	2.00 \pm 0.12 ^a	1.25 \pm 0.13 ^b
Fibroblast	2.42 \pm 0.19 ^a	3.25 \pm 0.18 ^b	2.75 \pm 0.18 ^a	3.92 \pm 0.08 ^b	3.75 \pm 0.13	3.42 \pm 0.19	3.00 \pm 0.25	2.75 \pm 0.25
Neovascularisation	2.50 \pm 0.15 ^a	3.25 \pm 0.18 ^b	3.00 \pm 0.17	3.50 \pm 0.15	3.17 \pm 0.24 ^a	2.75 \pm 0.13 ^b	3.50 \pm 0.19	2.17 \pm 0.17
Collagen fibre density	3.00 \pm 0.17	2.58 \pm 0.15	3.00 \pm 0.17	2.25 \pm 0.25	2.75 \pm 0.13	2.08 \pm 0.26	2.50 \pm 0.15 ^a	1.75 \pm 0.22 ^b
Collagen fibre thickness	3.00 \pm 0.12	2.75 \pm 0.13	2.83 \pm 0.11	2.50 \pm 0.19	2.75 \pm 0.22 ^a	2.33 \pm 0.19 ^b	2.50 \pm 0.19 ^a	2.00 \pm 0.12 ^b
Collagen fibre arrangement	3.25 \pm 0.18	2.75 \pm 0.18	2.75 \pm 0.18	2.50 \pm 0.19	2.50 \pm 0.19 ^a	1.75 \pm 0.18 ^b	2.50 \pm 0.19 ^a	1.00 \pm 0.00 ^b

N.B. Values of the two groups differ significantly, if possess different superscripts within the same row at the specific day of observation ($P < 0.05$).

significantly higher ($P < 0.05$) in PI group than AD-MSCs treatment group. In PI treated wound there was presence of large amount of neutrophilic infiltration throughout the wound bed with mild interstitial oedema at certain places on the wound surface and the surface of wound was overlaid by a thin layer of necrotic tissue which was intact (Fig. 1A). In AD-MSCs treated wound the necrotic tissue was in the process of detachment. Below the necrotic zone the epidermal layer showed proliferation of squamous epithelium at some places that showed discontinuity in many places. Mild infiltration of neutrophils and lymphocytes was observed (Fig. 1B). The mean fibroblast score was significantly higher ($P < 0.05$) in AD-MSCs treatment group than PI. Significant differences ($P < 0.05$) in the mean scores for neovascularisation were recorded, where more number of blood vessels were recorded in AD-MSCs treatment groups than PI. In AD-MSCs

treated wound there was proliferation of fibroblasts with moderate amount of thin collagen, where as in PI treated wounds the amount of collagen was scanty (Fig. 1C, 1D). However there was no significant difference observed in the mean scores for collagen fibre density, thickness and arrangement between the groups.

By 14th postoperative day, epithelialization was evident in both the treatment groups; however no significant difference in the mean score of epithelialization was noticed. No initiation of hair follicle and gland formation was noticed in PI treated wound (Fig. 2A), but in AD-MSCs hair follicles and glandular structures were observed with marked proliferation of capillaries (Fig. 2B). The infiltration of inflammatory cells was seen in both the treatment groups but was significantly higher ($P < 0.05$) in PI group. The wounds treated with AD-

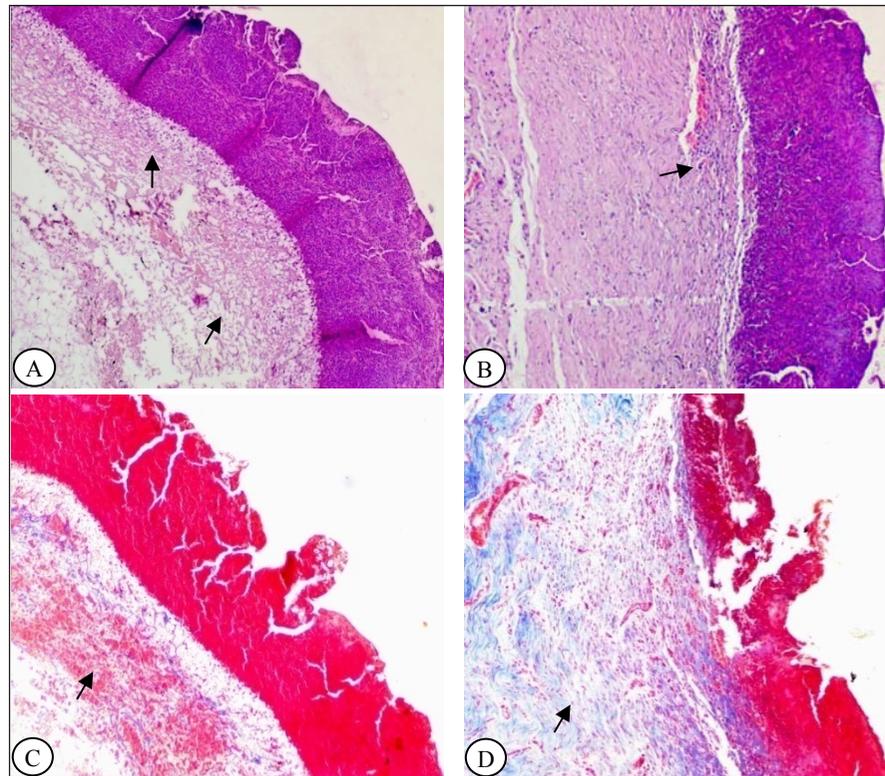


Fig. 1: Photomicrographs of PI and AD-MSCs treated groups on day 7 (A) Photomicrograph of PI wound tissue showing neutrophilic infiltration with loose connective tissue below scab. H&E×100 (B) AD-MSCs wound tissue showing mild neutrophilic infiltration and moderate connective tissue below scab. H&E×100 (C) Photomicrograph of PI wound tissue showing haemorrhage beneath the scab and less thin collagen in dermis. MT×100 (D) Photomicrograph of AD-MSCs wound tissue showing proliferation of fibroblasts with moderate amount of thin collagen. MT×100

MSCs showed early disappearance of inflammation in comparison to PI treated wounds. The mean score of fibroblasts was significantly higher ($P<0.05$) in AD-MSCs treatment group indicating moderate to severe fibroblastic proliferation. No significant difference in the mean scores for neovascularisation observed between PI and AD-MSCs treatment group. There were less dense fibres indicating poor collagen formation in PI compared to AD-MSCs (Fig. 2C). There was no significant difference recorded between PI and AD-MSCs treatment groups in the mean score for collagen fibre arrangement; however the fibres were thick and less wavy in AD-MSCs indicating better arrangement compared to PI and the amount of collagen was moderate to high in AD-MSCs treatment group (Fig. 2D).

On 21st day post wounding there was no significant difference recorded in the mean epithelialization score indicating complete epithelialization in both the groups. The mean score suggested that the epithelium in PI

group was moderately thick to normal skin and in AD-MSCs group it was slightly thick to normal skin. (Fig. 3A, 3B). AD-MSCs treatment group showed very little amount of inflammatory reaction, while in PI treatment there was mild to moderate inflammation. The fibroblast arrangement was better in AD-MSCs. The mean score for neovascularisation showed significant difference ($P<0.05$) between PI and AD-MSCs group. The vascularisation was moderate in PI group. There was a significant difference recorded between PI and AD-MSCs groups in mean fibre thickness and arrangement score that indicates thick and better arranged collagen fibres in AD-MSCs treatment than PI. Concentrations of collagen were thin, less dense, running in some part of section in PI treatment (Fig. 3C), where as in AD-MSCs collagen fibres run in all throughout the section but they were dense and robust (Fig. 3D).

On 28th day, mean score for epithelialization, inflammation, collagen fibre density, thickness and arrangement

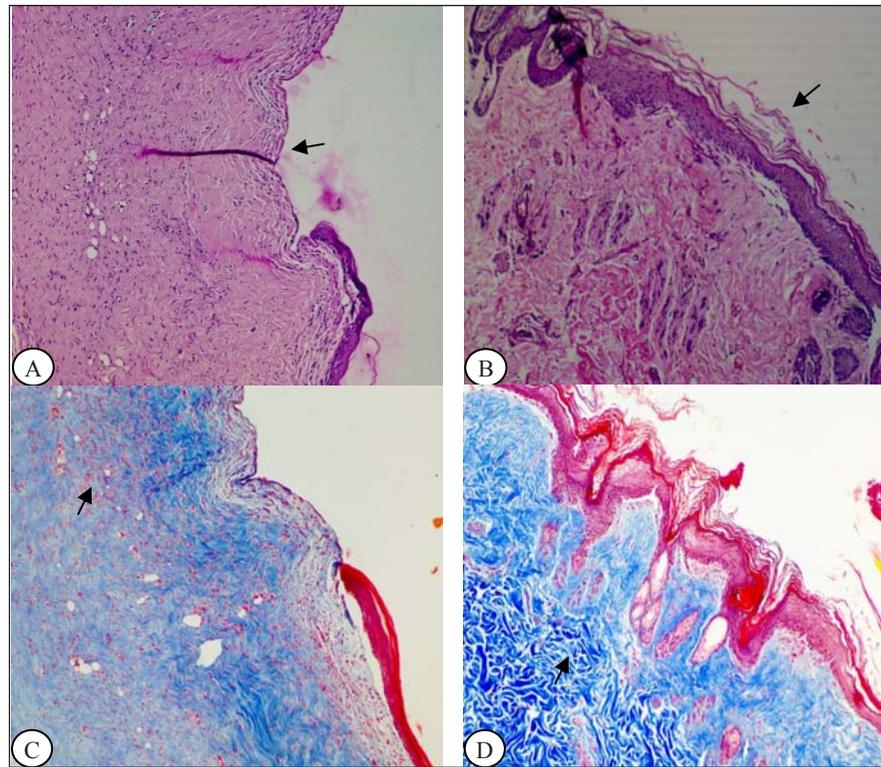


Fig. 2: Photomicrographs of PI and AD-MSCs treated groups on day 14 (A) Incomplete epithelialization and lack of hair follicles and glands in PI treated wound. H&E×100 (B) Complete epidermis with stratum corneum and initiation of hair follicles in AD-MSCs treated wound. H&E×100 (C) Poor collagen formation and fibroblastic proliferation in PI treated wound. MT×100 (D) Thick and less wavy collagen deposition in the stroma in AD-MSCs treated wound. MT×100

were significantly different ($P < 0.05$) in PI and AD-MSCs treatment groups. The AD-MSCs treated group showed better epithelialization, inflammatory changes, neovascularisation and fibroblast proliferation resembling normal skin. In PI treated wound there was formation of scar tissue in the epidermis and absence of glands in the dermis (Fig. 4A), where as the epidermal layer in AD-MSCs treatment showed a large number of hyperplastic epithelial regeneration and dermis revealed presence of sebaceous glands and hair follicles at the periphery of the healed skin (Fig. 4B). Below epidermis, connective tissue proliferation and blood vascularization were moderate to normal indicating a proper healing process in AD-MSCs treatment. Collagen fibre in PI treatment were almost flat, short and tortuous (Fig. 4C), where as fibres were thick, robust and wavy in AD-MSCs treatment (Fig. 4D). Compared to PI treatment group AD-MSCs treatment had better arranged, thicker and denser collagen fibres indicating better healing in AD-MSCs treatment than PI.

It has been suggested that histomorphological assessment of healing in open wounds give a better way of evaluation than clinical examination (Abramo *et al.*, 2004; Hu *et al.*, 2015; Singh *et al.*, 2017). Hence, in this study, tissue biopsies were collected at different stages of wound healing like on days 7th, 14th, 21st and 28th post-operative days which helped in providing sequential evaluation of wound healing.

Wound healing is a complex process involving inflammation, epithelialization, neoangiogenesis, proliferation, and collagen matrix formation. This complex process is carried out and regulated by numerous growth factors, cytokines, and chemokines. A reduction in the cytokines released by local inflammatory cells and decreased neovascularization impairs healing processes. ASCs have been shown to be effective in wound healing by modulating the immune response, secreting paracrine factors, and promoting therapeutic angiogenesis leading wound regeneration (Bertozzi *et*

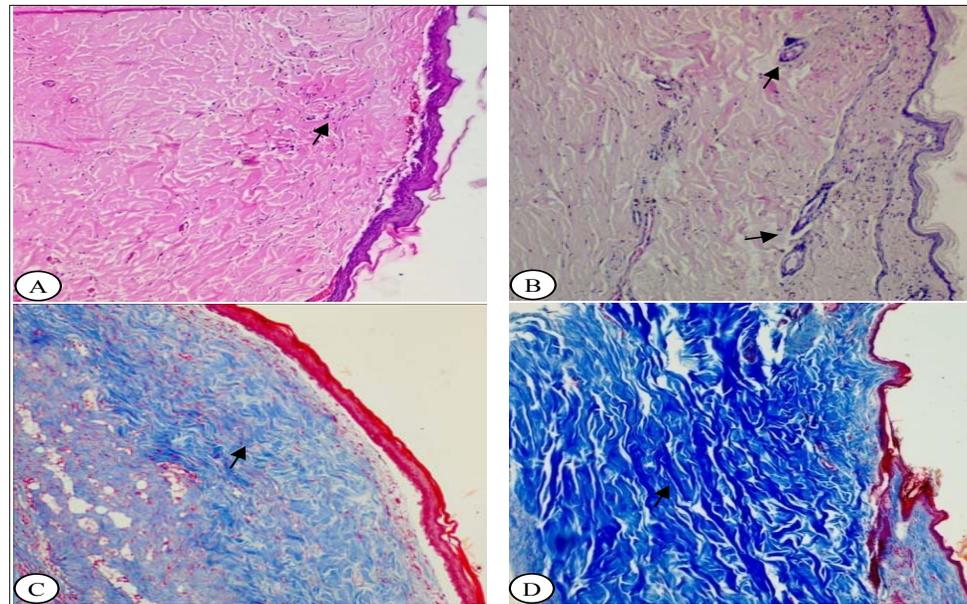


Fig. 3: Photomicrographs of PI and AD-MSCs treated groups on day 21. **(A)** Complete epidermal layer and scanty inflammatory cells in PI treated wound. H&E×100 **(B)** Complete epidermal layer and formation of hair follicles in AD-MSCs treated wound. H&E×100 **(C)** Thin and less dense collagen fibres arranged in an irregular manner in PI treated wound. MT×100 **(D)** Dense and robust collagen fibres running all throughout the section in AD-MSCs treated wound. MT×100

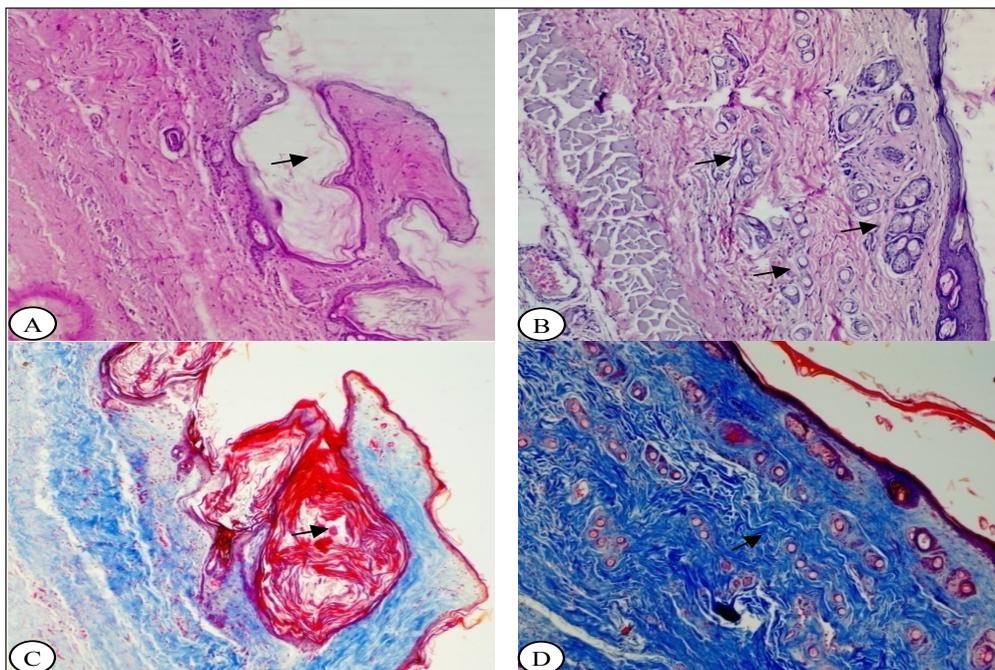


Fig. 4: Photomicrographs of PI and AD-MSCs treated groups on day 28 **(A)** Complete epidermis, formation of scar tissue and lack of glands in PI treated wound. H&E×100 **(B)** Complete epidermis, multiple hair follicle and glands in AD-MSCs treated wound. H&E×100 **(C)** Complete epidermis, formation of scar tissue and lack of glands in PI treated wound. MT×100 **(D)** Thick, robust and wavy collagen fibres in AD-MSCs treated wound. MT×100.

al., 2017). In the present study, AD-MSCs treated group showed better epithelialization, inflammatory changes, neovascularisation and fibroblast proliferation resembling normal skin. According to Kuo *et al.* (2016) ASCs enhances wound epithelialization and neovascularization by increasing expression of growth factors such as EGF and VEGF and via the induction of epithelialization in the peri-wound zone. ASCs play important role in wound healing by acting in different phases: inflammatory, proliferative and remodeling phase (Vidor and Contesini, 2018). ASCs enhance tissue regeneration through two main mechanisms, either by differentiating into skin cells, or by secretion of paracrine factors which down regulate the inflammatory response.

The main mechanism of the MSCs, is the paracrine signaling to the cells from the injury site that reduces the inflammation, stimulating the angiogenesis and inducing the cell migration and proliferation (Proding *et al.*, 2017). However, according to Uysal *et al.* (2014) the differentiation of the MSCs in keratinocytes and endothelial cells have the same function as the paracrine signaling to accelerate the neovascularization and the re-epithelialization of wounds.

Adipose derived stem cells (ASCs) are mesodermal in origin, but they can be differentiated into several lineages of osteogenic, chondrogenic, adipogenic, cardiomyogenic, myogenic, and neurogenic cells. They also have potential to differentiate into tissues of endo- and ectodermal lineages such as hepatocytes, pancreatic islet cells, endothelial cells, neural cells and epithelial cells (Mehrabani *et al.*, 2013).

According to Li and Guo (2018) ASCs differentiate into fibroblasts, keratinocytes, and endothelial cells and also secrete some cytokines that can promote their proliferation and migration. ASCs participate in the formation of vascular-like structures, also enhance the neovascularization of ischemic tissue. As per Hong *et al.* (2013) ASC can activate the fibroblast phenotype, increase the recruitment of endothelial cells and macrophages, and promote the granulation tissue formation. Granulation tissue of the wound is mainly composed of fibroblasts, collagen fibres and small new blood vessels. It is required for wound healing, as it is extremely resistant to infection and serves as a barrier against systemic infection, provides a surface over which epithelium is able to migrate, plays

a vital role in wound contraction and also contains the fibroblasts that produce the collagen for wound healing (Hu *et al.*, 2015). In our study, granulation tissue appeared early and at faster rate in AD-MSCs treatment group than PI treatment.

ASCs promotes the proliferation and differentiation of fibroblasts in injured wounds, also inhibits the excessive proliferation and migration of hypertrophic scar fibroblasts and reduce the expression of related cytokines (Deng *et al.*, 2018). According to Bertozzi *et al.* (2017) ASCs are able to secrete a variety of cytokines, growth factors, and chemokines to regulate angiogenesis and immune responses through paracrine mechanisms, ultimately promoting the repair of damaged tissues. These stem cells express and secrete cytokines which are important for angiogenesis, such as stromal cell-derived factor 1 (SDF-1), VEGF, PDGF-BB, basic FGF, Ang-1, IGF-1, matrix metalloproteinases, IL-6 and IL-8. Out of these, VEGF is the most effective and specific growth factor that regulates angiogenic processes, and it can stimulate the mobilization, recruitment, and migration of progenitor endothelial cells, accelerating the onset of angiogenesis. The transplantation of ASCs to skin graft enhances survival through the secretion of growth factors such as VEGF and TGF- β 3, which inhibits scarring and promotes better collagen organization in vivo, regulating wound re-epithelialization.

Inflammation is necessary for healing as it plays a role in combating infection and inducing the proliferation phase, but healing progresses only after inflammation is controlled (Borena *et al.*, 2009). Hence, early disappearance of inflammation in AD-MSCs -treated wounds in the present study might have facilitated the progress to the next phase of wound healing. Neovascularisation, denser, thicker and better arranged collagen fibres were also recorded in AD-MSCs treatment group than PI treatment. Histological observations revealed that the AD-MSCs augmented wound healing activity significantly by increasing cellular proliferation, formation of granulation tissue, neovascularisation, synthesis of collagen, epithelialization and early histological maturation in excisional wounds.

CONCLUSION

Histomorphological study showed that the adipose tissue-derived mesenchymal stem cells augmented wound healing



activity significantly by increasing cellular proliferation, formation of granulation tissue, neovascularisation, synthesis of collagen, epithelisation and early histological maturation in excisional wounds. Hence, it can be used widely in routine clinical cases for wound healing.

REFERENCES

- Abramo, F., Martins, P., Lloyd, D.H., Auxilia, S.T., Noli, C., Leotta, R. and Pfeiffer, D. 2004. An evaluation of methods for the assessment of healing of open wounds in the dog. *Vet. Dermatol.*, **15**(1): 13.
- Bancroft, J.D., Stevens, A. and Turner, D.R. 1996. Theory and practice of histological techniques. 4th Edn. Churchill Livingstone, Edinburgh, pp. 129.
- Bertozi, N., Simonacci, F., Grieco, M.P., Grignaffini, E. and Raposio, E. 2017. The biological and clinical basis for the use of adipose-derived stem cells in the field of wound healing. *Ann. Med. Surg.*, **20**: 41-48.
- Borena, B.M., Pawde, A.M., Amarpal, Aithal, H.P., Kinjavdekar, P., Singh, R. and Kumar, D. 2009. Evaluation of healing potential of autologous bone marrow derived nucleated cells on incisional wounds in dogs. *Indian J. Vet. Surg.*, **30**(2): 85-89.
- Collawn, S.S. and Patel, S. 2014. Adipose-derived stem cells, their secretome, and wound healing. *J. Cell Sci. Ther.*, **5**(3): 1-3.
- Deng, J., Shi, Y., Gao, Z., Zhang, W., Wu, X., Cao, W. and Liu, W. 2018. Inhibition of Pathological Phenotype of Hypertrophic Scar Fibroblasts Via Coculture with Adipose-Derived Stem Cells. *Tissue Eng.*, **24**(5-6): 382-393.
- Dhenge, S., Gade, N., Mishra, O. and Kumar, V. 2018. Dose-dependent In Vitro Cytotoxic Effects of Phosphorus Doped Graphene Oxide Titanium Oxide Nanocomposite (P-GO-TiO₂ NC) on Caprine Wharton's Jelly Derived Mesenchymal Stem Cells (WJ-MSCs) and Erythrocytes. *J. Ani. Res.*, **8**(6): 959-966.
- Hong, S.J., Jia, S.X., Xie, P., Xu, W., Leung, K.P., Mustoe, T.A. and Galiano, R.D. 2013. Topically delivered adipose derived stem cells show an activated-fibroblast phenotype and enhance granulation tissue formation in skin wounds. *PLoS one*, **8**(1): 1-11.
- Hu, M.S., Leavitt, T., Malhotra, S., Duscher, D., Pollhammer, M.S., Walmsley, G.G. and Longaker, M.T. 2015. Stem cell-based therapeutics to improve wound healing. *Plast. Surg. Int.*, pp. 1-7.
- Kuo, Y.R., Wang, C.T., Cheng, J.T., Kao, G.S., Chiang, Y.C. and Wang, C.J. 2016. Adipose-derived stem cells accelerate diabetic wound healing through the induction of autocrine and paracrine effects. *Cell Transplant.*, **25**(1): 71-81.
- Li, P. and Guo, X. 2018. A review: therapeutic potential of adipose-derived stem cells in cutaneous wound healing and regeneration. *Stem Cell Res. Ther.*, **9**(302): 1-7.
- Mehrabani, D., Mehrabani, G., Zare, S. and Manafi, A. 2013. Adipose-derived stem cells (ADSC) and aesthetic surgery: a mini review. *World J. Plast. Surg.*, **2**(2): 65-70.
- Mester, A., Opincariu, D., Benedek, I. and Benedek, I. 2017. Stem cell therapy in wound healing. *J. Interdiscip. Med.*, **2**(s4): 20-24.
- Murphy, P.S. and Evans, G.R.D. 2012. Advances in wound healing: A review of current wound healing products. *Plast. Surg. Int.*, 190436: 1-8.
- Prodinger, C.M., Reichelt, J., Bauer, J.W. and Laimer, M. 2017. Current and future perspectives of stem cell therapy in dermatology. *Ann. Dermatol.*, **29**(6): 667-687.
- Reich, C.M., Raabe, O., Wenisch, S., Bridger, P.S., Kramer, M. and Armhold, S. 2012. Isolation, culture and chondrogenic differentiation of canine adipose tissue- and bone marrow-derived mesenchymal stem cells—a comparative study. *Vet. Res. Commun.*, **36**(2): 139-148.
- Sarode, I.P., Pawde, A.M., Amarpal, Kinjavdekar, P., Aithal, H.P., Madhu, D.N., Monsang, S.W. and Sivanarayanan, T.B. 2014. Evaluation of the aqueous extract of *Tridaxprocumbens* Linn. in the healing of full thickness skin wounds in rabbits. *Indian J. Vet. Surg.*, **35**(2): 103-106.
- Singh, R.K., Saxena, A., Singh, R. and Bag, S. 2017. Xenotransplantation of caprinemesenchymal stem cells into rabbits for full thickness skin wound healing. *Int. J. Eng. Tech. Sci. Res.*, **4**(8): 1010-1025.
- Uysal, C.A., Tobita, M., Hyakusoku, H. and Mizuno, H. 2014. The effect of bone-marrow-derived stem cells and adipose-derived stem cells on wound contraction and epithelization. *Adv. Wound Care.*, **3**(6): 405-413.
- Vidor, S.B. and Contesini, E.A. 2018. Stem cells to improve the wound healing. *J. Head Neck Spine Surg.*, **3**(1): 1-5.