

# The Effect of Using Bone Marrow Mesenchymal Stem Cells Versus Platelet Rich Plasma on the Healing of Induced Oral Ulcer in Albino Rats

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**Background and Objectives:** Oral ulceration is one of the most common debilitating condition that affects the oral cavity. In this study, the effect of locally injected platelet rich plasma (PRP) and bone marrow-derived mesenchymal stem cells (BMSCs) on the healing of oral ulcer was investigated.

**Methods and Results:** An ulcer was induced in buccal mucosa of rats by using 5mm biopsy punch followed by application of cotton swab soaked with formocresol for 60sec. The ulcer was left untreated in the control group, treated with intralesional injection of PRP, or isolated cultured BMSCs. Data were analyzed clinically, histologically and immunohistologically on day 3, 5, 7 and 10. BMSCs group showed smaller ulcer area throughout the whole experimental period than the other groups with complete resolution of the ulcer on day 10, unlike the control group. However, there was no significant difference with PRP, on day 5, 7 and 10, regarding clinical ulcer size. BMSCs group showed better histological results regarding the rate of epithelial cell migration, the number of inflammatory cells, thickness and organization of collagen fibres and the number of blood vessels, with complete re-epithelization on day 10. BMSCs group showed a greater number of anti-PCNA positive nuclei throughout the whole experimental period than the other groups except on day 5, PRP had higher mean numbers of anti-PCNA positive nuclei in both tissues.

**Conclusions:** Both PRP and BMSCs accelerate wound healing and enhance the quality of the healing tissue with the latter being slightly more effective and faster.

**Keywords:** Formocresol, Induced oral ulcer, Mesenchymal stem cells, Platelet rich plasma, Rats, Wound healing

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## Introduction

Oral ulceration is one of the most common debilitating and widespread conditions that affect the oral cavity. It is a major concern for public health, as it affects the patient's quality of life dramatically. An oral ulcer occurs consequently to epithelial damage that is a result of a wide range of injuries. They can be caused by trauma, infection, immune imbalance, allergic reactions, burns, use of chemical drugs, chemotherapy and radiotherapy (1). Owing to the rich innervations of the oral mucosa, the prolonged and painful ulceration may present significant problems to the patient, such as difficulty in eating, speaking, swallowing and maintaining good oral hygiene. On the other

hand, due to the good vascularity of the oral tissues, most oral ulcers heal relatively quickly. However, in case of severe, recurrent or chronic ulcers, general treatment modalities are needed to restore the function and physiology of the injured tissue, this can include the application of steroids, tetracycline, analgesics, and antiseptic mouthwashes, among others (2). These therapies were recommended for the symptomatic management to reduce pain and aid healing of lesions; however, long-term frequent use of these medications have many side effects, including acne, rashes, sweating, weight gain and increased blood glucose levels. In addition, steroids may affect bones and cause existing infections to get worse or allow new infections to occur, or cause allergic and hypersensitivity reactions (3).

Regenerative medicine concept is emerging as an alternative to the conventional line of treatment in wound healing. Scientific advances in understanding the molecular mechanism of wound healing helped in developing the concept of administration of growth factors directly to the wound as part of its care (4). Platelet rich plasma (PRP) and mesenchymal stem cells (MSCs) represent a biological pool of wide range of growth factors and cytokines that are essential for optimum wound healing (5, 6).

PRP is an autologous concentration of human platelets above baseline in a small volume of plasma. The properties of PRP are based on the production and release of multiple growth and differentiation factors (7). PRP gel is used widely in promoting the healing of chronic cutaneous non healing wounds (8). Also, it is used the field of surgery for the treatment of a variety of hard tissue defects, as it accelerates bone formation (9).

On the other hand, mesenchymal stem cells is emerging as new potential clinical application in wound healing. BMSCs are fibroblast-like cells that are characterized by combinations of cell surface markers and by their ability to differentiate into diverse cell types (10). BMSCs has been widely used in chronic wounds healing extensively (11).

Accordingly, we hypothesized that using both PRP and BMSCs will enhance oral ulcer healing, however, we aimed to compare the healing effect of both remedies through clinical, histological and immunohistochemical evaluation.

## Materials and Methods

### Experimental animals and ulcer induction

Male albino rats 8~10 weeks of age, weighing 250~300 g were obtained and housed in the animal house of faculty of medicine, physiology department, Alexandria Univer-

sity. They were maintained in plastic cages with sawdust-covered floors at a temperature of 24°C in a dark-light cycle of 12 hours with free access to water and commercial food. The experimental protocol was designed in accordance with the guidelines for the responsible use of laboratory animals in research (12). All experimental procedures were approved by the ethical committee at Tanta University.

Rats were anaesthetized with an intramuscular injection of ketamine hydrochloride 10% and xylazine hydrochloride 2% (AMOUN, Egypt) at a dosage of 0.06 ml/100 g and 0.03 ml/100 g, respectively. A homogeneous standardized circular oral cheek ulcer was obtained using 5mm biopsy punch (13), followed by perpendicular application of a flexible swab with a cotton tip soaked in formocresol for 60s (14).

Sixty rats were randomly divided into three groups (n=20).

*I) Control group:* ulcers were left without any treatment.

*II) PRP group:* ulcers were treated with local injection of 0.1 ml of freshly prepared PRP solution at five points, four points circumferentially around the margins and the fifth point in the ulcer centre, using a 27-gauge syringe, immediately after ulcer induction. The injection was 2~3 mm deep and was done slowly to allow proper diffusion of solution in the loose connective tissue in the submucosa of cheeks.

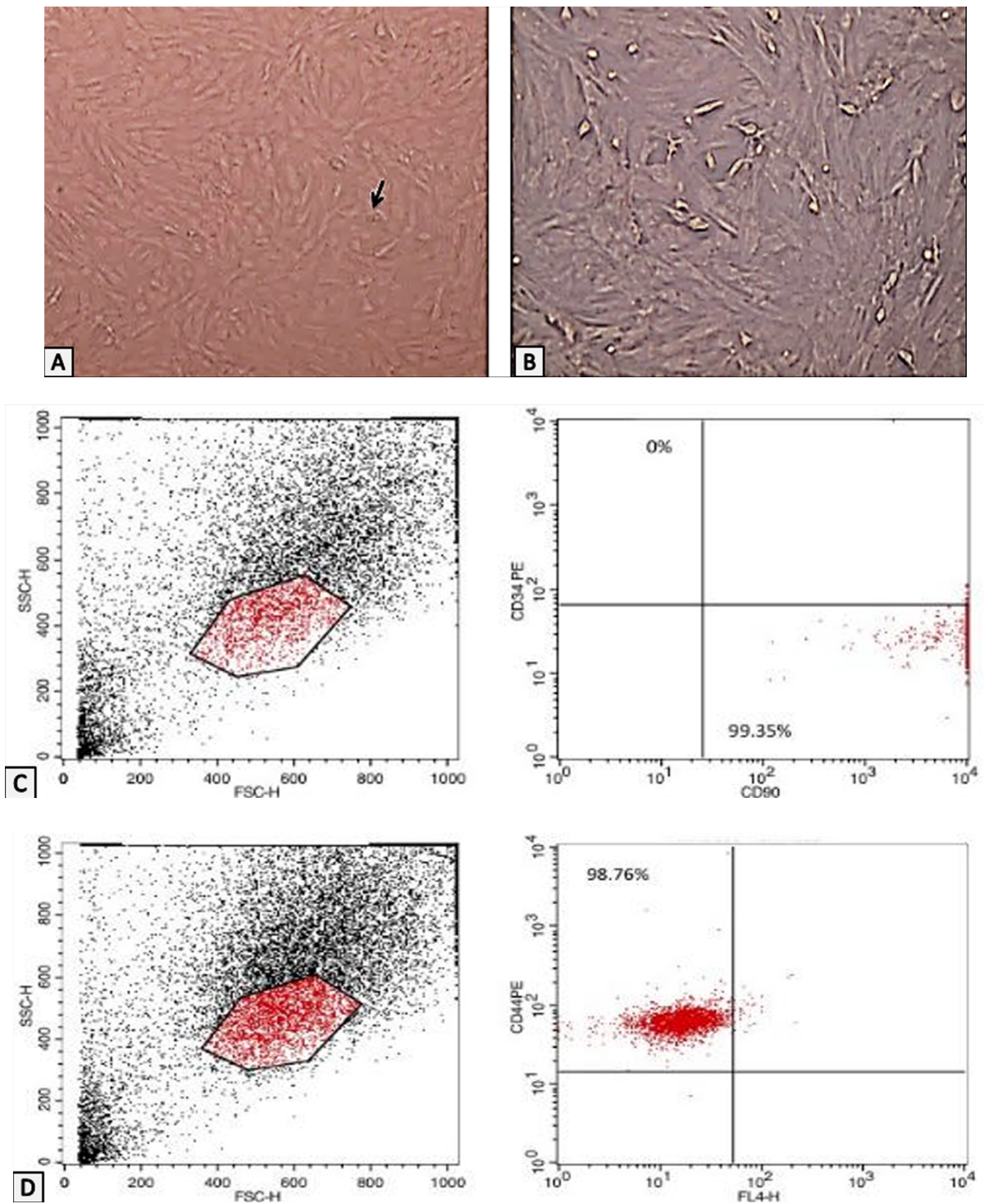
*III) BMSCs group:* ulcers were treated with local injection of the isolated BMSCs solution ( $1 \times 10^6$  cells suspended in 0.1 ml of complete media) (15) at five points, four points circumferentially around the margins and the fifth point in the ulcer centre, using a 27-gauge syringe, immediately after ulcer induction. The injection was 2~3 mm deep and was done slowly to allow proper diffusion of solution in the loose connective tissue in the submucosa of cheeks.

### PRP preparation

Blood was drawn directly from heavily anaesthetized rat by cardiac puncture and injected in a sodium citrate tube 3.2% for separation of PRP then centrifuged at 800 RPM for 15 min. The average platelet count was  $647 \times 10^3 / \mu\text{L}$ .

### Culture, harvesting, and characterization of BMSCs

Bone marrow was flushed from both femurs of a rat after its euthanization with complete culture medium. Complete medium was composed of DMEM-LG, 10% FBS, 1% L-Glutamine & 1% pen/strept (Lonza Bioproducts, Belgium). Flushed bone marrow was centrifuged at 1200 RPM for 8 min. Cell pellets were resuspended in 5 ml



**Fig. 1.** Morphological characteristics of BMSCs using Phase-contrast inverted microscope. (A) Passage 2, 80% confluent showing homogeneously fibroblastic like cell monolayer ( $\times 100$ ). (Black arrows) showing cells undergoing mitosis. (B) Higher magnification of cells ( $\times 400$ ) passage 2 showing spindle shaped fibroblast like cells with processes forming a monolayer. (C & D) FACS analysis for BMSCs at passage 3 demonstrated that 98.76% and 99.35% of the cultured cells expressed the mesenchymal CD44 and CD90 markers respectively, while they were negative for CD34 hematopoietic marker. These results indicated that relatively purified MSCs were isolated.

complete media, transferred into T-25 flask and cultured in humidified 5% CO<sub>2</sub> incubator at 37°C. At passage 3 cells were harvested by trypsinization then counted for transplantation.

Cells were characterized using cell surface markers by fluorescence-activated cell sorting (FACS) analysis on a FACS caliber flow cytometer equipped with Cell Quest software (Becton Dickinson). The cells were stained with different fluorescently labelled monoclonal antibodies (mAb). MSCs were characterized for CD44, CD90 (Abcam, UK) as a positive markers, in addition to CD34 (Abcam, UK) (hematopoietic surface markers).

### Evaluation of ulcer healing clinically, histologically and Immunohistochemically

Clinical analysis of the healing process was assessed through standardized photographs at day 3, 5, 7 and 10 after ulcer induction and ulcer area was measured in mm<sup>2</sup> using the free hand selection tool ImageJ software (ImageJ 1.48s, Wayne Rasband, National Institute of Health, USA).

Tissue sections were stained using H&E stain (for general examination) and Gomori trichrome stain (for demonstration of collagenous components).

Finally, Images of the immunostained sections were analyzed for expression of anti-PCNA antibodies using the ImageJ software. The areas showing the brown DAB immunostaining were manually counted using the multi-point tool. The number of anti-PCNA-positive in epithelial and connective tissue cells were counted using a magnification of ×400 in 5 measuring fields in each specimen, and mean value was calculated. Immunohistochemical control (IHC) was stained without the primary antibody.

### Statistical evaluation

Statistical analysis was performed blindly using SPSS version 16.0 (SPSS, Chicago, IL, USA). Quantitative data from imageJ analysis were described using mean and standard deviation. Data of clinical and immunohistochemical staining of different studied groups were compared and analyzed using analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparisons. Significance of the obtained results was judged at  $p \leq 0.05$ .

## Results

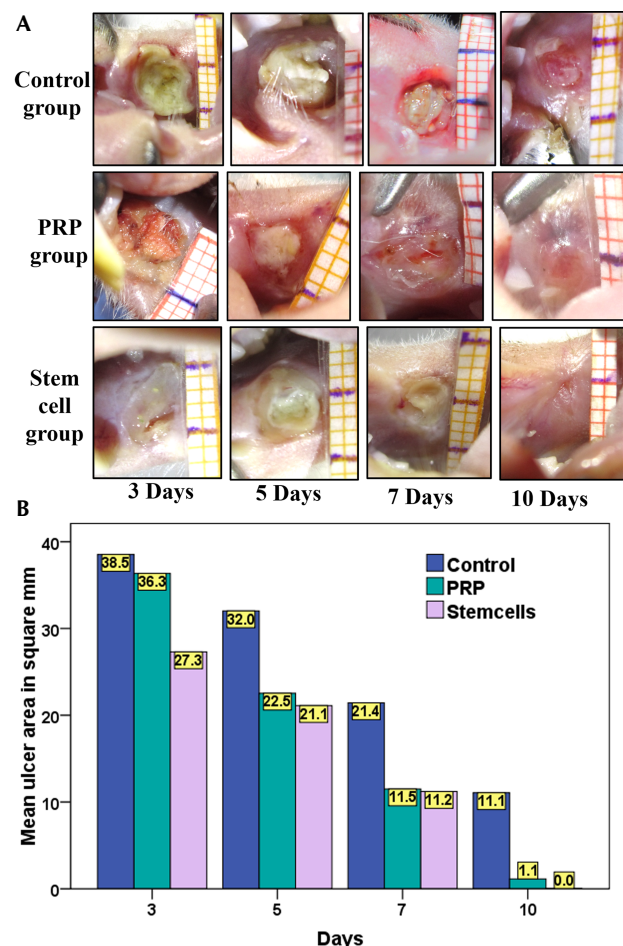
### Flow-cytometry characterization

FACS analysis for BMSCs at passage 3 demonstrated that 98.76% and 99.35% of the cultured cells expressed the mesenchymal CD44 and CD90 markers respectively, while

they were negative for CD34 hematopoietic marker (Fig. 1).

### Clinical/macroscopic analysis

Clinically the rats of both PRP and stem cell groups showed substantial extra-oral swelling after administration of intralesional injection of PRP and stem cells. This swelling began to decrease gradually after day 3 compared to the control group which showed minimal swelling from the beginning. In all groups, on day 0, the presence of ulceration covered with a whitish-yellow necrotic membrane was clinically observed. During the first 3 days, the injuries in the cheek mucosa of the animals were clinically visible and were crateriform and red in all groups. From days 5 to 7, the size of the injuries decreased in all groups; however, in the control group, the ulcer had irregular bor-



**Fig. 2.** (A) Clinical view of buccal ulcer of control group, PRP group, stem cell group at day 3, 5, 7 and 10 after ulcer induction. (B) Column chart representing the mean of ulcer surface area in mm<sup>2</sup> in the control, PRP and stem cell group in the different time intervals (PRP, Platelet rich plasma).

ders with a whitish halo surrounding the reminiscent injury. On the 10<sup>th</sup> day, the ulcer of the BMSCs was healed. Also, in the PRP group the ulcer was considered healed; however the epithelial covering of the ulcer was thin. On the other hand the ulcer of the control group was still clinically present (Fig. 2).

There was a significant difference between groups regarding the reduction in the ulcer surface area in all intervals. However, there was no significant difference between control and PRP groups on days 3. Although, there was no significant difference between PRP and BMSCs groups on days 5, 7 and 10, there was a significant reduction of the ulcer area of BMSCs group in comparison with the PRP group. Similarly, BMSCs group showed a significantly smaller ulcer area than the control group throughout the whole experimental period (Fig. 2, Table 1).

### H&E histological results

On day 3, wounds of the control group were displaying extensive ulcerations. The epithelial cells at the wound margin were oedematous. The wounds of the PRP group exhibited deep epithelial cells migration at the wound margin. Also, in the BMSCs group, there was epithelial proliferation and migration at the wound margin evidenced by increased mitotic figures in the epithelial cells.

On day 5 and 7, the wounds of the control group displayed delayed epithelization and migration above the thick underlying inflamed granulation tissue. In the RPR group, the migration of the wound epithelium had progressed across the wound bed, but the centre of the wound space was still open. Also, BMSCs group displayed epithelial proliferation and migration toward the wound.

On day 10, reepithelization was incomplete in the control group. In the PRP group, the wound area was covered

by an atrophic, thin layer of stratified squamous epithelium with thin keratin layer and flat rete ridges. However, the BMSCs group showed wound closure with almost complete reepithelization, the epithelium displayed maturation and moderate thickness of keratin layer (Fig. 3).

### Trichrome histological results

In the control group at day 3 after ulcer induction, collagen fibres at the centre of the lesion were thin and distributed in a haphazard manner. They were surrounded by numerous inflammatory cells. The PRP group displayed a discrete deposition of thin, disorganized collagen fibres was observed, at the centre of the lesion. Whereas in the BMSCs the granulation tissue underneath the epithelial wound margin displayed moderate amount of collagen fibres. It was infiltrated by a moderate intensity of inflammatory cells and numerous variable sized blood vessels.

On day 5 and 7, the control group displayed the granulation tissue with inflammatory cell infiltrate, some blood vessels and collagen fibres that were moderately thick and randomly distributed. In the PRP group, a thick granulation tissue with inflammatory cells infiltration at the surface of the wound. While the deeper layers showed less inflammation with fine interlacing collagen fibres, numerous dilated blood vessels. In the BMSCs group, deeply stained collagen bundles were seen arranged parallel to epithelial surface beneath the moderately inflamed granulation tissue. Well organized collagen bundles with variable numbers of fibroblast cells and the new blood vessels were clearly seen.

On day 10, the control group displayed moderate inflammatory cell infiltration, some blood vessels and colla-

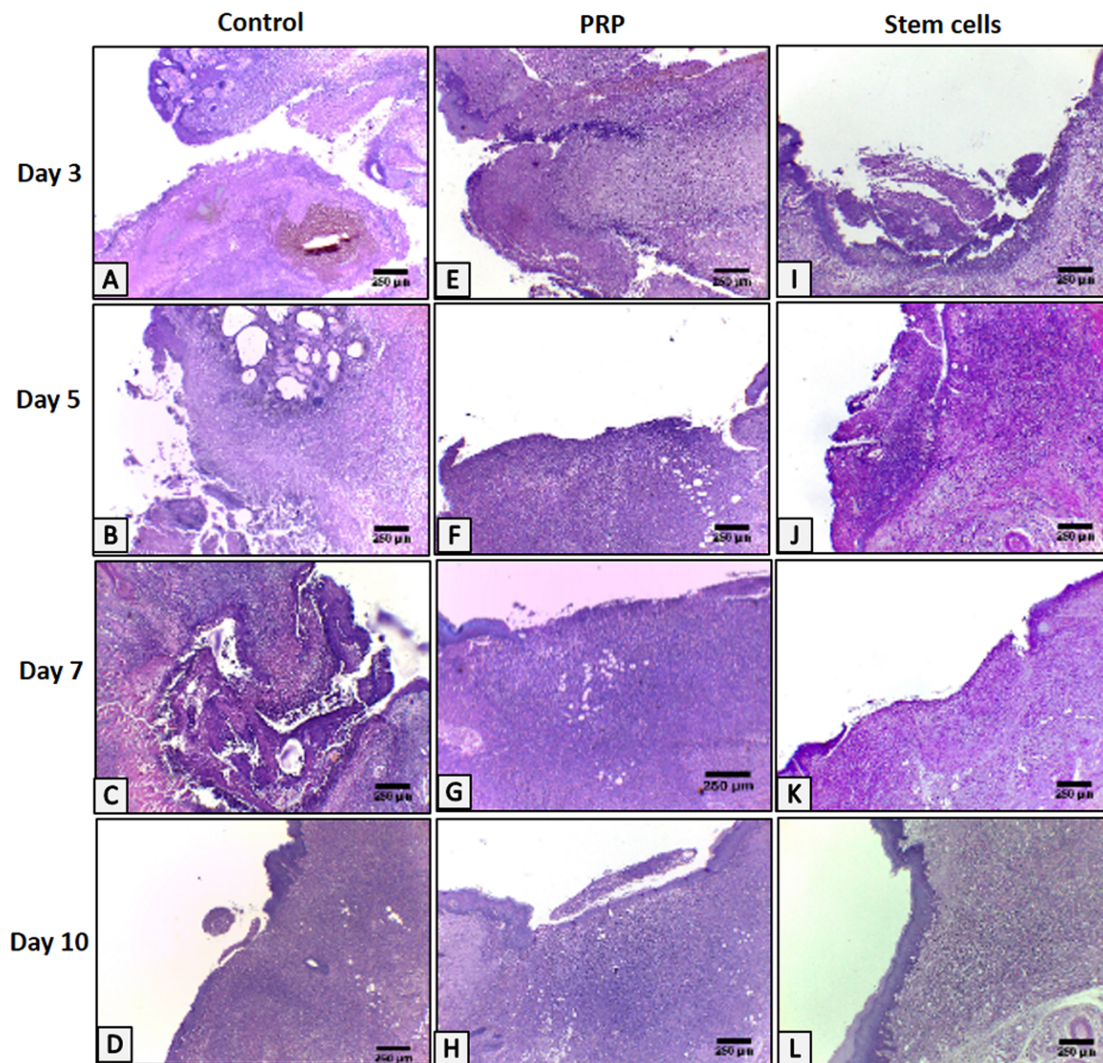
**Table 1.** The mean surface area of ulcer in mm<sup>2</sup> in each time interval within each group

		Mean ulcer area/mm <sup>2</sup>				
		0 days	3 days	5 days	7 days	10 days
Control		25.01±0.0	38.52±4.22 (154%)	32.01±2.80 (128%)	21.43±3.25 (85%)	11.06±5.79 (44.24)
PRP		25.01±0.0	36.33±2.96 (145%)	22.54±6.41 (90%)	11.50±5.69 (46%)	1.14±0.71 (4%)
BMSCs		25.01±0.0	27.29±4.78 (109%)	21.11±3.09 (84%)	11.21±2.21 (44%)	0.00±0.00 (0%)
ANOVA	F	0.16	10.72	8.98	10.59	15.58
	p-value	0.85	0.002	0.004	0.002	0.00
<i>Tukey HSD</i>			(Control, PRP)	(Control, PRP) <sup>a</sup>	(Control, PRP) <sup>a</sup>	(Control, PRP) <sup>a</sup>
			(Control, BMSCs) <sup>a</sup>	(Control, BMSCs) <sup>a</sup>	(Control, BMSCs) <sup>a</sup>	(Control, BMSCs) <sup>a</sup>
			(PRP, Stem cells) <sup>a</sup>	(PRP, BMSCs)	(PRP, BMSCs)	(PRP, BMSCs)

<sup>a</sup>The mean difference is significant at p≤0.05 level.

Values are given as mean±SD, in mm<sup>2</sup>.

PRP: Platelet rich plasma, BMSCs: Bone marrow mesenchymal stem cells.



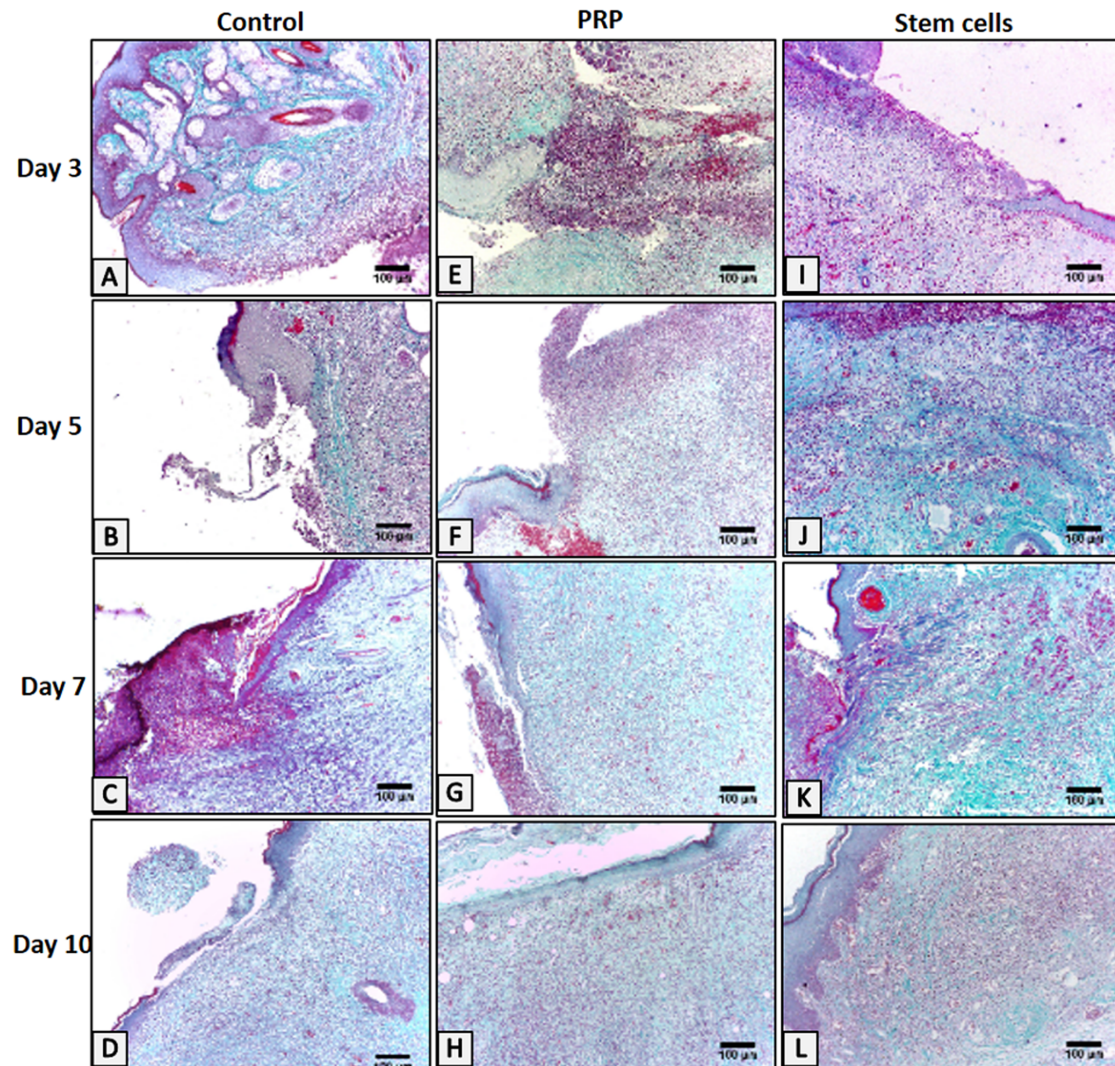
**Fig. 3.** Representative histological images of ulcer healing in the control group (A~D), the PRP group (E~H) and the BMSCs group (I~L). Control group: Minimal epithelial migration above the dense granulation tissue on days 3 (A), 5 (B) and 7 (C). Persistence of a small ulcer area in epithelium with moderately inflamed underlying connective tissue on day 10 (D). PRP group: Deep epithelial cells migration at the wound margin above the dense granulation tissue on days 3 (E), 5 (F) and 7 (G). Complete wound closure on day 10 (H), the wound area is covered by thin layer of stratified squamous epithelium with thin keratin layer and flat rete ridges and the underlying connective still showing signs of moderate inflammation. BMSCs group: Epithelial proliferation and migration at the wound margin with moderate density of granulation tissue filling the wound core and has inflammatory cells infiltration on days 3 (I), 5 (J) and 7 (K). Full wound closure with complete re-epithelization on day 10 (L), the epithelium at the wound area showing maturation with moderate thickness of keratin layer and the underlying connective still showing signs of moderate inflammation. Haematoxylin-eosin staining; magnification, 40 $\times$ . Scale bar=250  $\mu$ m (PRP, Platelet rich plasma).

gen fibres that were moderately thick and randomly distributed. In the PRP group, thick collagen fibres perpendicular to the surface epithelium and some inflammatory cells were seen. It also contained many blood vessels with extravasated red blood cells were seen. While in the BMSCs group the connective tissue started to regain its normal architecture with more organized collagen fibres, many fibroblasts, high vascularity and mild inflam-

matory cells infiltration (Fig. 4).

#### Immunohistochemical results

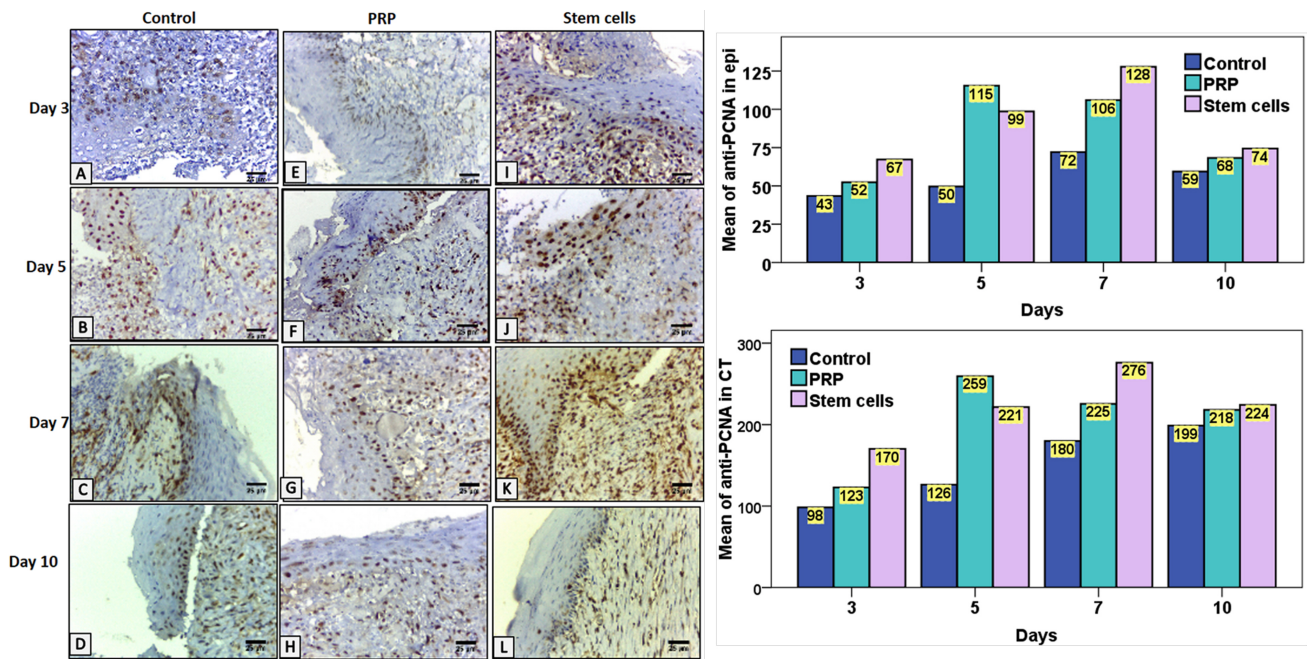
On day 5 and 7, the number of anti-PCNA positive nuclei in epithelial tissue of PRP group was significantly higher than the control group. Interestingly, BMSCs group showed a greater number of anti-PCNA positive nuclei in epithelial cells throughout the whole experimental period



**Fig. 4.** Representative histological images of ulcer healing in the control group (A~D), the PRP group (E~H) and the BMSCs group (I~L). Control group: Collagen fibres at the wound area are thin and distributed in haphazard manner surrounded by numerous inflammatory cells, small new blood vessels and extravasated red blood cells on days 3 (A) and 5 (B). Combination of fine and moderately coarse collagen fibres that are randomly distributed with moderate inflammatory cells infiltration in addition to numerous small blood vessels on days 7 (C) and day 10 (D). PRP group: The central granulation tissue consists of fine interlacing collagen fibres, numerous dilated blood vessels and extravasated red blood cells on days 3 (E) and 5 (F). The connective tissue exhibiting thick collagen fibres with variable number of fibroblasts and numerous blood vessels on days 7 (G) and 10 (H). BMSCs group: moderately arranged interlacing collagen fibres running somewhat parallel to the surface epithelium, neovascularization and discrete inflammatory infiltration on days 3 (I) and 5 (J). The connective tissue starting to regain its normal architecture with more organized collagen fibres, many fibroblasts, high vascularity and mild inflammatory cell infiltration on days 7 (K) and 10 (L). Trichrome staining; magnification, 100 $\times$ . Scale bar=100  $\mu$ m (PRP, Platelet rich plasma).

compared to other groups except on day 5 where PRP group had higher mean numbers of anti-PCNA positive nuclei. Regarding PCNA expression in the underlying lamina propria cells, there was a significant increase of the marker expression between the control and PRP groups in all intervals except on day 3. There was a significantly higher expression of PCNA between PRP and BMSCs

groups on day 3 and 7. On the other hand, there was no significant difference between PRP and BMSCs groups on day 5 and 10 although PRP scored higher levels on day 5. However, BMSCs group showed a greater number of anti-PCNA positive nuclei throughout the whole experimental period than the control (Fig. 5, 6, Table 2, 3).



**Fig. 5.** Left image, representative images of immunolocalization at wound area in the control group (A~D), the PRP group (E~H) and the BMSCs group (I~L). In the control group, relatively smaller number of nuclei showed immunoreactivity, in all the days compared to the PRP and BMSCs group. The BMSCs group showed a slightly higher number of positive nuclei than PRP group at the basal and suprabasal cells of epithelium and in some keratinocytes in migrating epithelium at ulcer surface. Similarly, the connective tissue cells, as well as endothelial cells lining the blood vessels of the BMSCs group, expressed a greater number of nuclear positive cells compared to the PRP and the control group. Anti-PCNA antibodies; magnification, 400×. Scale bar=25 μm (PRP, Platelet rich plasma). Right image, Column chart representing the mean of anti-PCNA antibodies positive nuclei in epithelial tissue (epi) in the control, PRP and stem cell groups in the different time intervals. Column chart representing the mean of anti-PCNA antibodies positive nuclei in connective tissue (CT) in the control, PRP and stem cell group in the different time intervals.

**Table 2.** The mean number of anti-PCNA positive nuclei in epithelial tissue in each group within same time interval

		Mean of anti-PCNA positive nuclei in epithelial tissue			
		3 days	5 days	7 days	10 days
Control		43.40±7.82	49.60±8.26	67.80±3.96	73.00±4.52
PRP		52.40±8.79	115.40±15.12	106.00±11.06	75.40±5.17
BMSCs		67.20±7.72	98.60±7.82	127.80±13.08	86.40±5.50
ANOVA	F	10.92	48.91	44.71	9.86
	p-value	0.002	≤0.001	≤0.001	0.003
Tukey HSD		(Control, PRP) (Control, BMSCs) <sup>a</sup> (PRP, BMSCs) <sup>a</sup>	(Control, PRP) <sup>a</sup> (Control, BMSCs) <sup>a</sup> (PRP, BMSCs) <sup>a</sup>	(Control, PRP) <sup>a</sup> (Control, BMSCs) <sup>a</sup> (PRP, BMSCs) <sup>a</sup>	(Control, PRP) (Control, BMSCs) <sup>a</sup> (PRP, BMSCs) <sup>a</sup>

<sup>a</sup>The mean difference is significant at p≤0.05 level.

Values are given as mean±SD.

PRP: Platelet rich plasma, BMSCs: Bone marrow mesenchymal stem cells.

## Discussion

The process of ulcer healing is a genetically programmed series of biologic events that normally integrates complex biological and molecular mechanisms, such as cell migration and proliferation, along with extracellular ma-

trix deposition, angiogenesis, and remodelling (16). However, chronic non-healing ulcers are trapped in an inflammatory cycle and fail to proceed to normal stages of wound healing, as the ulcer bed is phenotypically altered as a result of prolonged exposure to inflammatory cytokines, and bacterial toxins. These all contribute to cell



**Table 3.** The mean number of anti-PCNA positive nuclei in connective tissue in each group within same time interval

		Mean of anti-PCNA positive nuclei in connective tissue			
		3 days	5 days	7 days	10 days
Control		102.20±8.167	126.20±32.561	179.80±14.601	198.80±4.76
PRP		122.80±14.37	259.40±26.26	225.40±13.44	218.00±5.00
BMSCs		170.20±24.66	221.40±35.82	276.00±19.42	224.20±10.18
ANOVA	F	20.68	23.28	45.02	17.37
	p-value	≤0.001	≤0.001	≤0.001	≤0.001
Tukey HSD		(Control, PRP) (Control, BMSCs) <sup>a</sup> (PRP, Stem cells) <sup>a</sup>	(Control, PRP) <sup>a</sup> (Control, BMSCs) <sup>a</sup> (PRP, Stem cells)	(Control, PRP) <sup>a</sup> (Control, BMSCs) <sup>a</sup> (PRP, BMSCs) <sup>a</sup>	(Control, PRP) <sup>a</sup> (Control, BMSCs) <sup>a</sup> (PRP, BMSCs)

<sup>a</sup>The mean difference is significant at  $p \leq 0.05$  level.

Values are given as mean±SD, in mm<sup>2</sup>.

PRP: Platelet rich plasma, BMSCs: Bone marrow mesenchymal stem cells.

senescence and transform the normal progression of healing into a self-sustaining cycle of inflammation and injury (10).

In this study we induced chronic ulcers formation. As from a histopathologic view, acute ulcers are shallow ulcers or erosions. while, chronic ulcers are defined as deep tissue defects penetrating even beyond proper muscle layers, to which we did using the punch and further using the formocresol which provided chronic microenvironment to the wound (13, 14, 17).

Researchers have been seeking novel alternative treatments that aim to accelerate the rate of healing, to improve its quality and achieve regenerative process rather than a repair orientated one, through replacing senescent resident cells and to re-establish the normal healing cascade (10). These alternative therapies include PRP and BMSCs, which both of them represent a biological pool of wide range of growth factors and cytokines that are essential for optimum wound healing (5, 6). In this study, we hypothesized that both remedies will have a beneficial effect on healing induced oral ulcers in rats and aimed to compare their effect.

Platelets is well known to contain growth factors, coagulation proteins, adhesion molecules, cell-activating molecules, cytokines, integrins, inflammatory molecules which are pre-synthesized and packaged into the  $\alpha$ -granules (18). Thus using PRP allowed for the releasing of a pool of highly concentrated different biologically active substances and signals derived from platelets (19, 20). For instance, using enzyme-linked immunosorbent assay, there was a 7-fold increase in TGF- $\beta$ , 18-fold increase in PDGF, a 1.9-fold increase in IGF-1 and 4.6-fold increase in EGF in PRP compared with whole blood (21).

We found that using PRP accelerated clinical resolution

of oral ulcers in comparison to control in all intervals except at the beginning and the end of the experiment. Although there was a gradual decrease in the ulcer size of the PRP group over the investigated intervals, the only statistical significance was reported between 5 to 7 days. This may be partially explained by the significantly increased proliferation rate of both epithelial and connective tissue cells observed at day 5 (22).

Histologically, we found that unlike the control the wounds of the PRP group exhibited signs of epithelial cells migration at the wound margin from day 3, until the wound was completely covered by a thin layer of stratified squamous epithelium with a thin keratin layer and flat rete ridges. This enhancement of proliferation and migration of basal and suprabasal epithelial cell was achieved by expanding the action of PDGF, TGF- $\beta$ , EGF and KGF (19).

On day 5 and 7, the lamina propria of the PRP group displayed fine interlacing collagen fibres which were thickened by day 10. PRP contains elevated levels of FGF, PDGF and EGF that stimulate fibroblast proliferation (22), and high amount of TGF- $\beta$ 1 that up-regulate expression of type I collagen, MMP-1 in fibroblasts and increases fibronectin and glycosaminoglycan synthesis (23). These combined factors were found to quicken the maturation of collagen, that can lead to an increase in the strength of wound tension (24).

Also, we observed numerous dilated blood vessels from the early stages of oral ulcer healing, to which may be attributed to the strong stimulant effect PRP has on capillary regeneration in wound healing (25). This effect is mediated via growth factors, such as VEGF, PDGF and IGF, to which that induce angiogenesis, and therefore increase the nutrition supply to the area and decrease the

healing time (26). Furthermore, platelets release histamine and serotonin, which both function to increase capillary permeability, thus significantly allowing inflammatory cells to access the wound site (27).

All these released growth factors accelerate wound healing by various overlapping mechanisms such as stimulating angiogenesis, granulation tissue formation and epithelialization (25).

Interestingly, PRP was found to boost the inflammatory phase of wound healing and hasten tissue debridement, by increasing the concentration of PDGF and TGF- $\beta$ , to which are a chemotactic factor for neutrophils and monocytes (28). In addition, it contains soluble fibrinogen that modulates the activity of monocytes and macrophages and therefore mediates the transition between inflammatory and regenerative stages of injury response, breaking the viscous cycle of continue inflammation in non-healing ulcers (29).

Clinically, we found that unlike PRP group, there was a significant reduction in ulcer size between BMSCs and the control groups in all intervals. Also, we found that BMSCs group yielded better clinical resolution at the beginning and at the end of the intervals. However, there was no significant reduction between both groups in day 5 or 7. This is may be attributed to the higher cellular proliferation rate of both epithelial and connective tissue cells scored on day 5 by the PRP group. Although there is no clear explanation of such finding, we speculate that this may be due to the fact that PRP acts as a reservoir for concentrated amount of growth factors that are released at the same time. However, once this robust amount of factors is exhausted, there is no renewed or sustained source for them (30).

On histological examination, we found that the ulcer of BMSCs group progressively healed in a favourable manner as epithelial proliferation and migration at the wound margin was evidenced, by increased mitotic figures in the epithelial cells from day 3. This migration continued until ulcer closure with almost complete reepithelization. The epithelium displayed maturation and moderate thickness of keratinisation. These findings are consistent with the steady increase of epithelial cells proliferation noticed from the beginning of the experimental period till its end. This is may be explained by the following *first*; the paracrine effect of the transplanted stem cells through growth factors and cytokines, which they release to increase epithelial cells proliferation (31, 32) and to make the micro-environment more appropriate for the repair of injury (33). *Second*; by transdifferentiating into keratinocytes directly (34, 35). *Third*; MSCs were found to be immunor-

eactive to fibronectin, which suggested that MSCs may interact with keratinocytes to accelerate wound healing (36).

Also, we observed that from day 3, the underlying lamina propria displayed moderate amount of collagen fibres and numerous variable sized blood vessels. The organization and maturation of collagen fibres proceeded through time intervals rapidly, until by the end of the experiment. The connective tissue started to regain its normal architecture with more organized and mature collagen fibres, many fibroblasts, high vascularity and mild inflammatory cells infiltration. This is in consistent with other studies that have found that MSCs was more effective in treating oral mucositis and ulcers by promoting the organization and deposition of collagen (17, 37). The high vasculature seen in the BMSCs group throughout the experimental period, compared to control group could be explained through previous studies, which state that MSCs can acquire phenotypic characteristics of vascular endothelial cells (36). In addition to this, it was found that MSCs express mRNAs for various angiogenic factors such as VEGF, which increase angiogenesis and contributes to the observed clinical improvement (17).

Collectively, we found that at the end of our experimental period, stem cell group yielded better results than PRP group. This was presented clinically in the faster rate of ulcer healing. Histologically, the stem cells group showed better tissue organization and maturation and histochemically, by a significantly higher cellular proliferation rate in most intervals. This is can be explained as although they both share the following characteristics; i) the boosting of inflammatory response, ii) Antimicrobial effect, iii) releasing growth factor, iv) increasing both cellular and capillary proliferation. MSCs uniquely have more beneficial properties that evokes faster tissue regeneration and not just simple wound repair (11). First; MSCs possess a high expression of several pro-healing genes that ensure growth factors and cytokines production for a more extended period of time (38). Secondly; they can transdifferentiation into different cell types needed for tissue regeneration. Thirdly; MSCs revealed a powerful chemotactic migration toward epidermal keratinocytes, dermal fibroblasts and endothelial cells (39). Finally; it is noteworthy that MSCs increase production of type III collagen needed specifically in tissue repair (40).

Several limitations are inherent in this study, we used healthy rats even though oral ulcers are more prevalent in local or systemic immunosuppressed patients. Moreover, this study was limited to male rats only, therefore it didn't show the impact of sex hormones on the wound healing process and fluctuations, concerning their levels during

the reproductive cycle of female rats. In addition, we used 8~10 weeks old rat, that are fairly young rats and have faster healing abilities. This might explain the short time intervals that we used in the experiment based on our pilot study, therefore proving it necessary to demonstrate differences as rats advances in age. Furthermore, it would have been beneficial to quantitatively assess histological data, despite present study focusing on what influences the rate and quality of clean wound repair.

Another limitation in the possibility of locally administered stem cell dying before being significantly differentiate due to oxygen limitation, this was overcome by injecting large number of cells ( $1 \times 10^6$ ) that was injected at multiple points around the ulcer. We do not know whether the transplanted MSCs differentiated into functional cells that replaced the damaged cells in the tissue or that they remained as they were. Further study would be needed to fully clarify the fate of the MSCs in the mucosa. More studies should aim to create a gold standard protocol for PRP generation and characterization, optimal concentration of PRP, optimal timing of the injection, its persistence over time and on the consequent effects on the final outcome for each specific application. Also, further studies should focus on the impact of local wound micro-environment on MSCs engraftment, in addition long-term systemic effects of MSC-based therapy.

In conclusion, this study suggests that both PRP and BMSCs can accelerate the healing of induced oral ulcers in rats, however BMSCs yielded improved clinical and histological results. These findings suggest that one day MSCs can be a fast alternative therapy for non healed chronic oral ulcers that standard remedies cannot heal. Hopefully with the emergence of new MSCs subpopulations, (that are effortlessly isolated in a quicker, less intrusive way and are better characterized) to be selectively applied to specific disorder which may result in an enhanced therapeutic effect.

#### Potential Conflict of Interest

The authors have no conflicting financial interest.

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