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Efficiency of systemic versus intralesional bone marrow-derived stem cells in regeneration of oral mucosa after induction of formocresol induced ulcers in dogs

Lobna A. Aly[1], Hala El- Menoufy,[2] Hesham S. Sadeq,[3] Alyaa Ragae,[4] Dina Sabry[5]

[1]Departments of Oral and Maxillofacial Surgery, [4]General Histology, Faculty of Dentistry, Future University, New Cairo, [2]Department of Periodontology, Oral Medicine and Oral Diagnosis, Faculty of Dentistry, Misr University for Science and Technology, Cairo, [3]Department of Periodontology, Oral Medicine and Oral Diagnosis, Faculty of Dentistry, [5]Department of Medical Biochemistry, Faculty of Medicine, Cairo University, Giza, Egypt

ABSTRACT

Background: Bone marrow mesenchymal stem cells (BMSCs) are the key to regenerative wound healing. MSCs have spatial memory and respond to local environment. The goal of this study was to evaluate the use of systemic and intralesional transplantation of BMSCs for regeneration of oral mucosa in an *in vivo* dog model.

Materials and Methods: Transplantation of undifferentiated green fluorescent protein (GFP)-labeled autologous BMSCs systemically, submucosally or vehicle (saline) was injected around the chemically induced oral ulcer in each group of 18 adult dogs. The healing process of the ulcer was monitored clinically and histopathologically. Gene expression of vascular endothelial growth factor (VEGF) and collagen genes was detected in biopsies from all ulcers. One way ANOVA was used to compare between means of the three groups. Results were considered significant at $P < 0.05$.

Results: Flow cytometric analysis of the MSCs at the passage 3 showed that these cells were negative for CD45 (2.39%). They expressed high levels of CD29 (98.34%). Frozen fluorescence microscopy of sections of the cell-treated oral tissue of all groups indicated that the GFP-transduced implanted cells were integrated within the transplanted tissues. The treatment resulted in dramatic wound edge activation and resurfacing of oral mucosa wound.

Conclusion: Our results revealed that BMSCs may be labeled with (GFP), in order to know the distribution of these cells after administration, and suggest that intralesional administration is an appropriate procedure to achieve acceptable regeneration of the previously injured oral mucosa more than systemic route.

Key Words: BMSCs, intralesional, oral ulcer, systemic

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Address for correspondence:
Dr. Lobna A. Aly
Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Future University, New Cairo, Egypt
E mail: lobna72@gmail.com

INTRODUCTION

Extending the hypothesis that cell therapy may be required to recondition chronic wounds and accelerate their healing leads to the conclusion that stem cells may offer even greater advantages. A rational strategy for the effective use of advanced products in chronic wound healing is likely to require greater understanding of the clinical factors involved, as well as the pathophysiological components that underlie impaired healing.

Optimum healing of a cutaneous wound requires a well-orchestrated integration of the complex biological and molecular events of cell migration and proliferation, extracellular matrix (ECM) deposition, angiogenesis and remodeling. Chronic wounds are common diseases and difficult to heal. The best treatments available can only achieve 50% of wound closure which is often temporary.[1-3]

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Previous studies demonstrated that re-epithelialized mucosa of grossly "healed" experimental ulcers has prominent histological and ultrastructural abnormalities: Reduced height, increased connective tissue and a disorganized microvascular network. These prominent abnormalities may interfere with the mucosal defense and cause ulcer recurrence when ulcerogenic factors are present. Therefore, the quality of mucosal structural restoration may be the most important factor in determining the future ulcer recurrence.[4,5] In some situations, such as for chronic non-healing wounds, the objective of cellular therapy is to reverse those cellular and vascular events that compromise repair.[6,7]

Cellular therapy using mesenchymal stem cells (MSCs) has the potential to address the underlying pathogenesis of impaired wound healing and accelerate tissue repair with more durable tissue integrity. This strategy may result in a more regenerative form of wound repair, with obvious implications for cutaneous wound healing, and any disease characterized by the increased fibroplasia, such as intra-abdominal adhesions, keloids, scleroderma, pulmonary/renal fibrosis, and hepatic cirrhosis.[8-10]

The efforts of researchers to establish the safety of MSC infusion and their effects *in vivo* have led to the application of MSCs for the treatment of various tissue injuries in humans. Thus far, most of the procedures involve local administration or direct injection. However, for conditions such as Alzheimer's, Parkinson's, liver disease, renal failure, and autoimmune diseases, the delivery of MSCs by systemic infusion can be minimally invasive and convenient. Recently, in order to improve the efficacy of stem cell transplantation, committed stem cells are isolated and purified, and single lineage stem cell transplantation is then performed.[11-14]

To make a systemic infusion efficacious, more MSCs are needed by comparison to local delivery. Therefore the aim of our study was directed toward the comparison of the efficacy of systemic versus intralesional bone marrow stem cells in regeneration of oral mucosa after induction of formocresol-induced ulcers in dogs.

MATERIALS AND METHODS

Experimental animals

Eighteen clinically healthy dogs (1- to 3-year-old, weighing 8 to 10.1 kg) were used in this study. These dogs were treated in accordance with the guidelines approved by the Institutional Animal Care and Use Committee of Cairo University.

Bone marrow mesenchymal stem cell (BMSC) isolation and culture

Under general anesthesia with isoflurane inhalation, bone marrow was obtained. A 13-gauge needle was used to penetrate the cortex of the iliac crest of each dog, and about 10 ml of bone marrow was drawn in a syringe containing 1,500 units of heparin. The isolation of MSCs was performed using the methods of Johnstone *et al*.[15] and Kadiyala *et al*.[16] In brief, the bone marrow aspirate was layered onto Histopaque-1077 (Sigma, St. Louis, MO, U.S.A.) and centrifuged at 400 g for 30 min. The collected buffy coat was mixed with 20 ml of Dulbecco's phosphate-buffered saline (DPBS) and centrifuged at 300 g for 5 min. The supernatant was discarded, and the cells were washed two more times with D-PBS. After determination of the cell viability and the number of viable cells by trypan blue staining, the cells were resuspended in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% fetal bovine serum (FBS, Gibco) and antibiotics (penicillin 10,000 U/ml, streptomycin 10,000 µg/ml, amphotericin B 25 µg/ml). The nucleated cells were plated in tissue culture flask at 2.5 × 105/cm2 and incubated at 37°C in a humidified atmosphere containing 5% CO2. On day 4 of culture, the non-adherent cells were removed along with the change of medium. On day 14, the adherent colonies of cells were trypsinized and counted. Cells were identified as being MSCs by their morphology, adherence and their power to differentiate into osteocytes[17] and neurocytes.[18] Differentiation into osteocytes was achieved by adding 1 to 1000 nM dexamethasone, 0.25 mM ascorbic acid, and 1 to 10 mM beta-glycerophosphate to the medium. Kinetic quantitative determination of alkaline phosphatase (ALP) was carried out in the medium of differentiated cells using a commercial kit provided by Stanbio laboratory, Boerne, Texas, USA.

Differentiation into neurocytes was achieved by adding beta-mercaptoethanol, dimethyl sulfoxide and conditioned medium for neuron induction. Differentiation was confirmed by detection of nerve growth factor (NGF) gene expression in the cell homogenate. MSCs were used in this study upon reaching 70 to 80% confluence.[19]

Labeling of MSCs

Undifferentiated MSCs were harvested and were labeled with green fluorescent protein (GFP) using monster green fluorescent protein vector and lipofectamine transfast transfection reagent kit (Promega, Madison, WI, USA). Before transfection, cells were seeded into individual wells of 6 well-plates. After 24 h incubation in growth medium, the cells were exposed to 2 μg GFP plasmid/ well of cells. GFP plasmid was incubated with lipofectamine for 10-15 minutes before subjection to the cells. Following transfection, the cells were incubated at 37°C in humidified air (5% CO2) for 2 h. The transfection medium was then removed and the cells were incubated for an additional 48 h in the complete medium (2 ml per well). For imaging GFP autofluorescence of MSCs, unstained slides were directly analyzed and green autofluorescence detected by inverted fluorescence microscopy (Leica, Germany).[20,21]

In vivo transplantation of undifferentiated GFP-labeled MSCs in induced oral ulcer

Autologous undifferentiated GFP-labeled MSCs were injected in the experimental canines following chemically induced oral ulcer in both treated groups as mentioned in the study design.

RNA extraction and cDNA conversion

Total RNA was isolated from oral canine tissues (frozen in liquid nitrogen) by a single step method using TRIzol® Reagent (Invitrogen, CA, USA). The RNA samples were treated with RNase free DNase at 37°C for 20 min and stored at –80°C for further use. The purity (A260/A280 ratio) and the concentration of RNA were obtained using dual spectrophotometry (Beckman, USA). RNA quality was confirmed by gel electrophoresis. The total RNA (0.5 – 2 μg) was used for cDNA conversion using high capacity cDNA reverse transcription kit (#K1621, Fermentas, USA). The reaction was carried out according to the manufacturer's instruction, using a thermocycler (Biometra Tpersonal, Germany). The converted cDNA was stored at –20°C.

Real-time qPCR using SYBR Green I

Real-time qPCR amplification and analysis were performed using StepOne (Applied Biosystem, USA) instrument. The qPCR assay with the primer sets [Table 1] were optimized at the annealing temperature. Canine GAPDH was amplified as an internal control housekeeping gene for PCR. Each 25 μL of reaction mixture contained 12.5 μL of SYBR Green Maxima (Fermentas, USA), 1 μL of each primers (10 μmol/L), and 1 μL of template cDNA. To confirm the absence of DNA contamination in the reaction mixture, water as a non-template control, was included. The reaction was initiated by activation of Taq polymerase at 95°C for 5 min, followed by 40 two-step amplification cycles: 10 s denaturizing at 95°C, 50 s annealing at 55°C (VEGF) or 60°C (Collagen). After the amplification, melting curve analysis with temperature gradient from 65 to 95°C was recorded every 0.5°C (hold for 5 s). This was performed to confirm that only the specific products were amplified.[20,22]

Table 1: Sequences of primers for conventional PCR

Target gene	Primers (5'→3') Length (nt)	Product size (bp)
VEGF	F:ATGAACTTTCTGCTCTCTTGG R: TCACCGCCTCGGCTTGTC	441
Collagen	F: AGTGCTGTCCCATCTGCTCA R:GCCTTCTCATCAAATCCTCCA	322
GAPDH	F:AACATCATCCCTGCTTCCAC R:TCCTTGGAGGCCATGTAGAC	229

F and R indicate forward and reverse primers, respectively

Induction of oral ulcers

Chemically induced oral ulcers were done in eighteen adult dogs by topical application of pellets soaked in a full strength formocresol, and applied to the buccal mucosa in all animals.

Cell transplantation

Dogs were randomly divided into three equal groups each of six dogs. Six dogs were treated by submucosal injection of autologous bone marrow BMSCs ($2x10^7$) suspended in 200 μl phosphate buffered saline (PBS), another six dogs were intravenously injected with BMSCs at a dose of $2x10^7$ MSCs/ 200 μl PBS, and finally six dogs received PBS only (as a control group). Dogs were injected with either autologous BMSCs or PBS after 3 days of ulcer induction and this was considered day 0.

Clinical and histopathological assessment

The clinical assessment parameters of the oral ulcers in all groups were documented on the Wound Assessment Parameter Scoring Tool (WAPS).[22] This clinically validated method uses sequential scoring that correlates to the actual process of wound healing. Progress reporting is streamlined, concise, and truly shows objective, measurable data. All the dogs were followed up clinically at 0, 3, 7, 10 and 15 days, then tissue biopsies were taken for histopathological study.

Statistical analysis

Data are expressed as mean ± SD. One way ANOVA (Analysis of Variance) was used to compare between means of the three groups. Duncan's test for pair-wise comparisons was used to determine significant differences between means when ANOVA test is significant. Results were considered significant at $P < 0.05$. Statistical analysis was performed using SPSS 16.0® (Statistical Package for Scientific Studies) for Windows.

RESULTS

Morphological, phenotype characteristics and GFP-labeling identification of expanded undifferentiated BMSCs

Under an inverted microscope (Leica, Germany), undifferentiated MSCs were typical of adherent spindle and fibrocyte-like at one week culture and reached 80-90% confluence at 2 weeks culture [Figure 1]. After plastic adherence selection, MSCs were cultured over three passages. Flow cytometric analysis of the MSCs at the passage 3 showed that these cells were negative for CD45 (2.39%). They expressed high levels of CD29 (98.34%). These results indicated that relatively purified MSCs were isolated. Before cells transplantation, GFP- labeled MSCs were analyzed and confirmed for their green auto fluorescence for in vivo cells tracing after transplantation.

MSCs homing and florescence assessment

Frozen fluorescence microscopy of sections of the cell-treated oral tissue of all canine groups indicated that the GFP-transduced implanted cells were integrated with in the transplanted tissues [Figure 2].

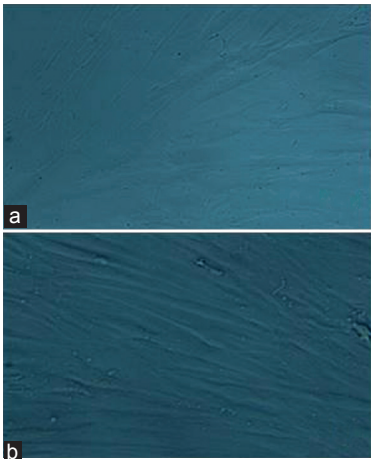

Figure 1: Isolated and cultured undifferentiated MSCs (a) MSCs propagated for 7 days and (b) MSCs reached 70-80% confluence at 14 days. They were identified by their fusiform fibroblast like-structure (Original magnification x10).

Clinical and histopathological assessment

It seems that BMSCs accelerate wound healing without an abnormal wound healing process. Subsequent objective wound assessments provide evidence of tissue response, with decreasing Wound Assessment Parameter Scores, an indicator of wound healing. At day 0, there was no statistically significant difference between the three groups. After 7 and 10 days, control group showed the statistically significantly highest mean score. There was no statistically significant difference between the groups 2 and 3, which showed the statistically significantly lowest mean scores. Groups 2 and 3 showed clinical improvement in their wounds within 7 days following administration of MSCs, and the wounds showed a steady overall decrease in wound size. While after 15 days, there was statistically significant difference between Group 3 and groups 1 and 2 [Figure 3 and Table 2].

The trend analysis of tissue type demonstrates wound improvement clinically as evidenced by a decrease in non-viable tissue with a corresponding increase in the viable tissue [Figure 4]. Ulcers receiving BMSCs showed better healing by histopathologic examination of oral tissue biopsies 2 weeks after induction of the ulcers compared to the control group. In submucosal BMSCs group, complete healing of surface epithelium with signs of increased epithelial proliferation was demonstrated accompanied with increased thickness of surface epithelium and mild subepithelial inflammatory infiltrate. While in the systemic BMSCs group, epithelial tissue showed irregular form of degenerated area

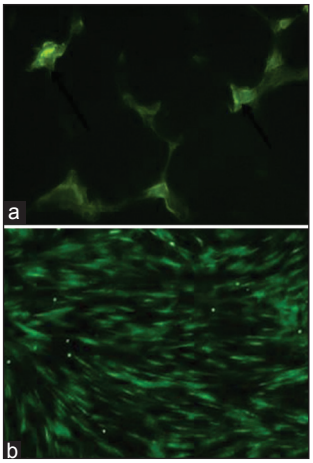

Figure 2: Bone marrow GFP-labeled MSCs were injected a) systemically and b) submucosally

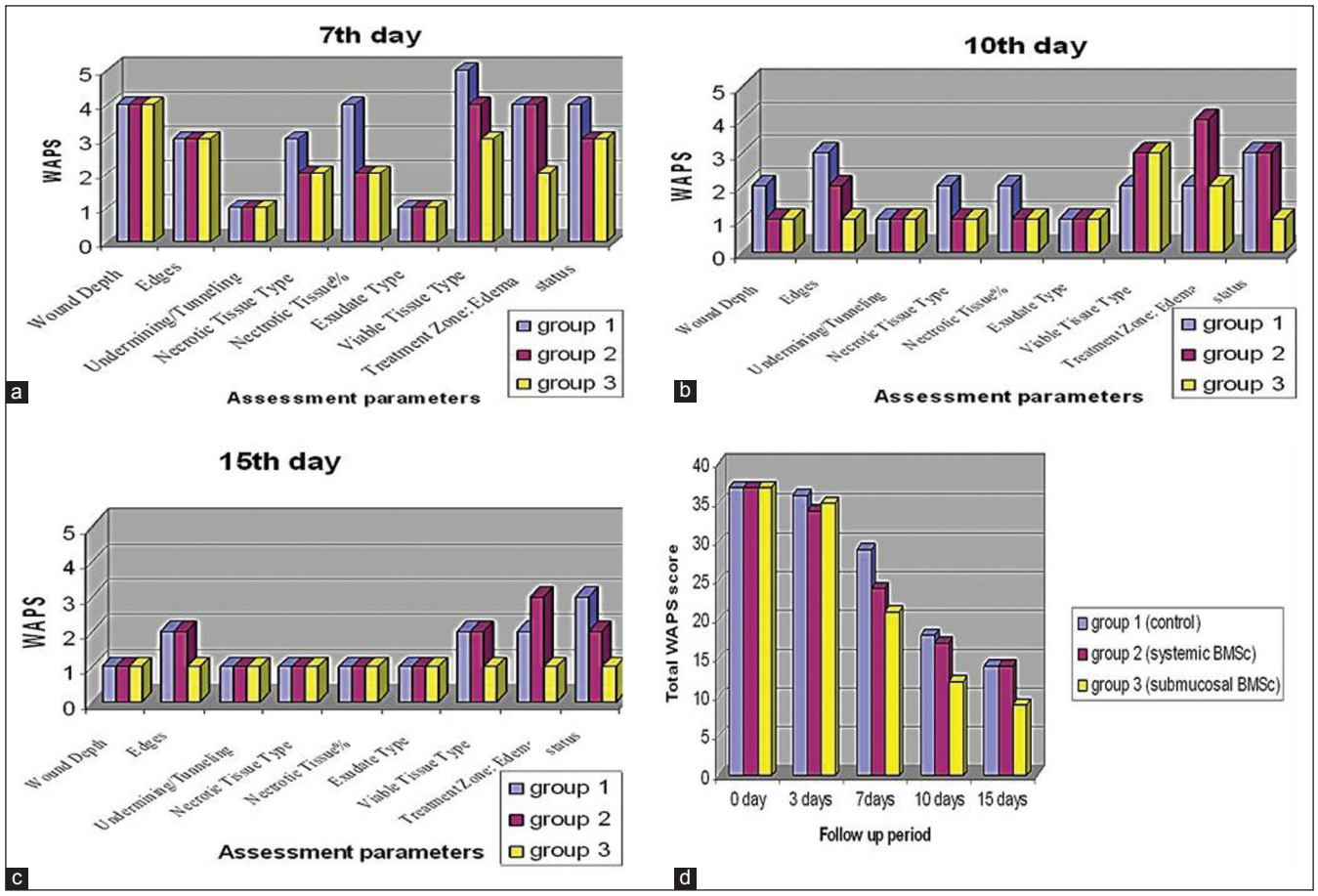

Figure 3: (a-c) The clinical assessment parameters of the oral ulcers in all groups at follow-up periods, (d) comparison between total WAPS score in the three groups

Table 2: The comparison between total WAPS score in the three groups

Period	Group 1 (Control)		Group 2 (Systemic BDSC)		Group 3 (Submucosal BMSC)		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Day 0	36	3.6	34	2.9	35	4.2	1.000
3 days	36	4.2	34	3.2	35	4.1	1.000
7 days	29 [a]	4.1	24 [b]	2.8	21 [b]	2.8	<0.001*
10 days	18 [a]	3.7	17 [b]	3.1	12 [b]	2.3	<0.001*
15 days	14 [a]	2.6	14 [a]	2.9	9 [b]	2.1	<0.001*

**Significant at P < 0.05, (a, b) Means with different letters are statistically significantly different according to Duncan's test*

with abnormal cells arrangement, the irregular histological development of the epithelial tissue showed a remarkable acellularity of the wound edge as compared to other sample of submucosal stem cells but fibroblastic activity was restored [Figure 5].

Expression of VEGF gene was detected in MSC homogenate by RT-PCR Expression of VEGF and collagen gene was more in MSCs-treated group compared with the control group [Table 3].

DISCUSSION

The ability to regenerate the damaged tissues is a common characteristic of multicellular organisms. A cycle of apoptosis and tissue regeneration exists in organisms, and stem cells in and around damaged tissues play among the most critical roles in the wound healing and tissue regeneration. It was generally assumed that factors released upon the tissue damage or apoptosis mobilize and recruit stem and progenitor cells to the damaged site, where they

Table 3: Semiquantitative of PCR products of collagen and VEGF gene expression in oral tissue

	Control ($n = 6$)	Systemic BMSC ($n = 6$)	Submucosal BMSC ($n = 6$)	*P*-value
Collagen DNA NA concentration (μg/mL)	742.7±61.5[a]	1803.4±121.2[b]	1873.4±131.2[b]	<0.001*
VEGF DNA concentration (μg/mL)	878.6±91.762[a]	927.3±158.8[a]	1702.9±209.58[b]	<0.001*

**Significant at P < 0.05. (a, b) values with different letters are statistically significantly different*

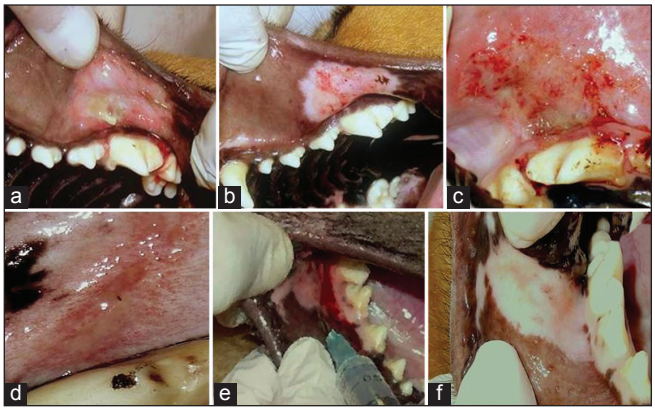

Figure 4: Clinical follow-up: (I) control group: a) Oral ulcer at zero time and submucosal injection of saline alone. b) At 15 days. (II) Systemic BMSCs group: c) Oral ulcer at zero time and intravenous injection of BMSCs. d) At 15 days. (III) Submucosal BMSCs group: e) Oral ulcer at zero time and submucosal injection of BMSCs. f) At 15 days

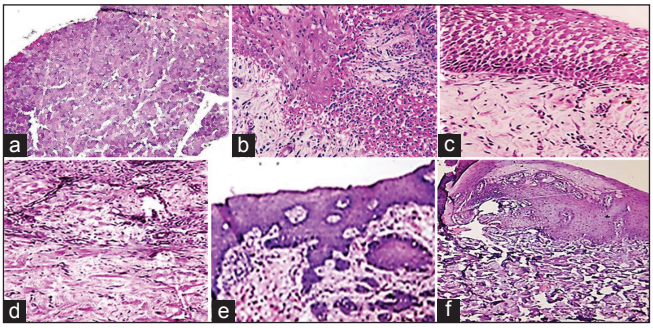

Figure 5: H and E stain of (I) control group: a) Abnormal cell arrangement of epithelium with highly congested area (x100). b) Inflammatory infiltration with degenerated collagenous fibers of the submucosa (x400). (II) systemic BMSCs group: c) Epithelial tissue shows irregular form of degenerated area with abnormal cell arrangement accompanied by remarkable acellularity of the wound edge. d) Fibroblastic activity was restored. (III) submucosal BMSCs group: e) Complete healing of surface epithelium (HEx40). f) Mild subepithelial inflammatory infiltration and normal appearance of submucosa (HEx200)

proliferate and differentiate, eventually replacing the damaged tissues.[23,24] However, a lack of data exists concerning the mechanisms driving MSC trafficking after intravenous, intraarterial, or local intra-tissue application compared with the relatively well-characterized leukocyte homing cascade.[25]

Chronic wounds are common diseases and difficult to heal. The best treatments available can only achieve 50% of wound closure which is often temporary. In our previous study, we found that implantation of BMSCs enhanced wound healing of oral ulcers;[26] a current problem is the development of strategies that ensure that these cells reach wound beds in a timely fashion and in sufficient numbers to maximize their therapeutic benefits. Currently, there are two basic delivery methods: Systemic infusion of cells into the vascular circulation and direct application of therapeutic cells to wound sites. Most therapeutic applications of MSCs to wound/ischemic targets dictate that exogenous (for example, culture-expanded) populations be delivered using either systemic or direct/topical approaches.[27,28] It was hypothesized that applying cells topically may promote revascularization from inside the wound versus only from the periphery.[29]

Systemic delivery mimics the route of endogenous MSCs via the circulatory system with final homing to target sites. During vascular transit, MSCs risk being taken out of circulation, on either a temporary or a permanent basis, in organs such as the lungs, spleen, and liver. This may either delay their transit or reduce the numbers of cells that finally appear at target sites. Upon reaching their target site (or sites), MSCs must exit the vasculature to enter the connective tissue stromal region where their principal functions occur.[30]

Although there may be a plateau between the number of delivered cells and improvement of clinical outcome,[21] a higher number of infused MSCs are expected to give rise to a higher number of engrafted MSCs and better functional outcomes.[31] Several clinical applications of MSCs would theoretically involve the administration of MSCs by the intravenous route. However, limited data are available regarding the ultimate fate of systemically infused MSCs. Studies in rodents suggests a broad initial biodistribution followed by a limited capacity for sustained engraftment.[32,33]

Regarding the homing capability of MSCs, numerous studies have confirmed that systemically infused MSCs can migrate to injured, inflamed tissues and exert therapeutic effects.[34,35] Collectively, studies using different methods for tracking MSCs have shown an initial concentration of MSCs in the lung after transfusion, after which most of the MSCs moved gradually to injured sites or to the liver, spleen, kidney, and bone marrow.[36,37]

In the intravenous transplantation of stem cells, it is very important to observe the survival and curative effects of transplanted stem cells. Traditional immunohistochemical method can easily identify the transplanted cells with specific morphology and tissue-specific antigens. However, transplanted MSCs in targeted tissues present normal cell morphology and may be absent of specific markers. Thus, it is difficult to determine the implanted allogeneic cells at injured sites. Therefore, cells should be labeled *in vitro*. An ideal labeling method *in vitro* must possess high sensitivity and specificity, and long half-life. At present, there are a lot of labeling methods including the GFP labeling, Lacz labeling, BrdU labeling, Y chromosome labeling and DiI labeling. GFP protein is stable. GFP gene can be transfected into MSCs through adenovirus vector, resulting in stable GFP expression in MSCs.[20,22] Although the half-life of GFP is relatively short (4-6 weeks), it is enough to trace the migration of implanted cells during the process of bone formation. In the present study, the authors observed that GFP expressing MSCs were identified at the injured site. These findings suggest that MSCs can target and be incorporated into the oral mucosa, although they were systemically injected.

An alternate method for the delivery MSCs to wound/ischemic sites is through direct or topical delivery. This method is fundamentally different from systemic delivery in that applied MSCs either migrate into the wound bed via non-vascular routes or release bioactive factors from a bandage or other type of carrier at the surface of the wound. A limitation of direct/topical delivery is the accessibility of the target site. Topical administration of MSCs is generally inapplicable for internal organs.[38,39] However, direct injection of concentrated cells has been used to deliver cells to internal organs.[40] Nonetheless, this is an invasive procedure with attendant risks. Systemic delivery of stem cell results in cells being taken from the circulation in the lungs, spleen and liver and not reaching the wound. The high prevalence of peripheral vascular disease in the people with disease also inhibits the intravascular delivery of cell to the affected foot ulcer. The topical delivery of cells allows for concentrated doses of cells to be delivered to a wound and not become trapped in other sites in the body.[41]

For direct/topical delivery to succeed, a highly concentrated population of cells must be either placed onto the surface of the wound or injected immediately adjacent to the wound. The timing of this administration may also be important in that applied MSCs must functionally interact with wound cells at critical stages process. Exactly what these interactions are and when they occur are currently areas of research interest. The importance of delivering a critical number of cells has been described by Falanga *et al*.[38] The mechanisms by which MSCs are concentrated at wound sites vary among studies. Hanson *et al*.[39] have summarized delivery methods that have been employed in small-scale clinical studies. These methods include injection into the wound, inclusion in a topical fibrin spray, and incorporation in a collagen sponge.[38,42] These studies all report improvement in wound healing and these was proved in our study clinically and histopathologically. In short, our study also approved that scarring was reduced and there was a consequent increase in tissue function. Wounds treated with BMSCs healed significantly faster and displayed a more mature histology than did wounds in which cells were not applied. A strong correlation was observed in the number of applied cells and regeneration of the oral mucosa, as there was an advanced granulation tissue formation and re-epithelialization with applied cells as compared with controls. We speculate that in the present study the growth factors might have stimulated the transplanted MSCs to target the oral mucosa and incorporate effectively into the injured lesion. In the MSCs treated groups the expression of VEGF (as proved in culture of MSCs) represents another source of angiogenesis which contributes to observed clinical improvement in the viable tissue type and necrotic tissue percentage. Although there was no significant difference ($P > 0.05$) between the three groups clinically at 15 days, histopathological examination showed better healing in the groups treated with MSCs compared to the control group. These results could be due to higher expression of VEGF and collagen gene in MSCs treated group compared to the control group. This indicates that

MSCs treatment improves the quality of mucosal structural restoration which is the most important factor in determining future ulcer recurrence. However, the variations in delivery methods make it difficult to critically assess cellular and molecular function of MSCs at wound sites. The optimal cellular delivery method for a preclinical or clinical study will likely depend on the type and location of the wound.

In this respect the results of our study support the possible implementation of a cell-based strategy using MSCs for potential wound healing modulation in injured oral mucosa. To date, experimental studies have not presented definite answers regarding the role of implanted BMSCs and related mechanisms. However, several hypotheses have been proposed: 1) Transplanted stem cells play the same role as true MSCs, replace the stem cell group in the specific tissue, and form the new connective tissue. Then, growth is achieved in a similar way as in normal tissue. 2) MSCs or their progenitor cells can be transformed and differentiated under specific microenvironments. Therefore, they can form endodermal or ectodermal tissue as well as mesenchymal tissue. 3) Although seen in particular situations, heterozygotes can be formed between the transplanted BMSCs and the specific cells of the recipient tissue. 4) The transplanted MSCs release paracrine factors and thereby induce the proliferation of specific cells in the transplanted tissue or contribute to making the microenvironment more appropriate for the repair of injury by maximally inhibiting the tissue injury.[43] If paracrine activity is their primary function in wound repair, their presence in wounds would be expected to be transitory. However, if they differentiate into structural tissue cells such as fibroblasts, vascular endothelial cells, or pericytes, their presence would be expected to be longstanding. Current information on MSC longevity in wounds varies. For example, Falanga *et al.*[38] found that most of their topically applied MSCs had exited the wound by 25 days. In contrast, injection of MSCs into the pericardiac region revealed the continuous presence of MSCs for up to 1 year.[44] These variations may depend on the type of tissue, type of wound, the method of labeling cells, or the degree of MSC heterogeneity.

Although our results showed intravenously implanted allogeneic MSCs could directionally migrate to injured oral mucosa, and survive especially in the necrotic sites, the mechanisms underlying the directional migration of MSCs should be further studied. Besides, the efficacy of intravenous transplantation of MSCs in the treatment of oral ulcers should also be further confirmed. Also, further research is required to determine strategies to remove harmful factors and improve homing of MSCs to the area of injury. New strategies could mean smaller quantities of MSCs necessary for infusion, thereby attaining the intended therapeutic goal with greatest efficiency and efficacy. To achieve this goal, cell migration and tracking studies must be conducted in various *in vivo* environments along with *in vitro* laboratory studies. Through these studies, optimized culture conditions can be established to cultivate MSCs with enhanced homing ability and expressing the appropriate homing receptor. This is also essential to improve the vascular conditions, so that introduced cells can easily migrate to damaged sites.

CONCLUSION

In conclusion, results from this animal model show that BMSCs may be labeled with green fluorescent protein (GFP), in order to know the distribution of these cells after local or systemic administration, and in fact there is a growing body of evidence that the wound healing effects were partially achieved with systemic route and point to an additional direction of the intralesional route of stem cell administration to achieve acceptable oral mucosal regeneration after induction of oral ulcer.

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