Treatment of Oral Ulcers in Dogs Using Adipose Tissue-Derived Mesenchymal Stem Cells

Alamoudi N M* / El Ashiry E A** / Farsi N M*** / El Derwi D A **** / Atta H M*****

Aim: *Adipose tissue Derived Mesenchymal Stem cells (ADMSCs) represent a promising tool for new clinical concepts in supporting cellular therapy. The goal of this study was to investigate the effects of ADMSCs transplantation on oral ulcer healing in dogs.* ***Study design:*** *Mesenchymal stem cells were isolated from adipose tissues of dogs obtained by suction-assisted lipectomy (liposuction), by dish adherence and were expanded in culture. Oral ulcers were induced by topical application of formocresol in the oral cavity of 18 dogs. The dogs were classified into 3 groups. Either autologous ADMSCs, Corticosteriod (Dexamethasone) or vehicle (saline) was injected. The healing process of the ulcer was monitored histopathologically. Gene expression of vascular endothelial growth factor (VEGF), platelets derived growth factor (PDGF), epidermal growth factor (EGF) and collagen was assessed in biopsies obtained from all ulcers ''as healing markers'', by real time polymerase chain reaction (PCR).* ***Results:*** *ADMSCs group showed significantly accelerated oral ulcer healing compared with the Dexamethasone and control groups. There was increased expression of VEGF, PDGF, EGF and collagen genes in ADMSCs-treated ulcers compared with Dexamethasone and controls.* ***Conclusion:*** *ADMSCs transplantation may help accelerate oral ulcer healing, possibly through the induction of angiogenesis by VEGF and PDGF, as well as epithelial and connective tissue proliferation as evidenced by increased EGF and collagen gene expression.*

Keywords: *Adipose tissue- Derived mesenchymal stem cells, corticosteroid, oral ulcer, dogs.*

* Najlaa M. Alamoudi, BDS, MSc, DSc, Professor at King Abdulaziz University, Faculty of Dentistry, Preventive Dental Sciences Department, Pediatric Dentistry Division, Jeddah, Kingdom of Saudi Arabia.

** Eman A. El Ashiry, BDS, PhD, Associate Professor at King Abdulaziz University, Faculty of Dentistry, Preventive Dental Sciences Department, Pediatric Dentistry Division, Jeddah, Kingdom of Saudi Arabia and Lecturer at El Azhar University, Faculty of Dental Medicine for Girls, Pedodontic Department, Cairo, Egypt.

*** Najat M. Farsi, BDS, MSc, Professor at King Abdulaziz University, Faculty of Dentistry, Preventive Dental Sciences Department, Pediatric Dentistry Division, Jeddah, Kingdom of Saudi Arabia.

**** Douaa A. El Derwi, MD, MSc, PhD, Professor of Community Medicine and Public Health, Faculty of Dentistry, Preventive Dental Sciences Department, King Abdulaziz University, Jeddah, KSA, and Professor of Public Health and Community Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt.

***** Hazem M. Atta, MD, MSc, PhD, Professor of Clinical Biochemistry, Faculty of Medicine, Rabigh branch, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia, and Professor of Medical Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt.

Send all correspondence to: Prof. Najlaa M. Alamoudi, King Abdulaziz University, Faculty of Dentistry, Preventive Dental Sciences Department, P.O. Box 80209, Jeddah 21589, K.S.A.

Mobile: 966 - 505622325
Fax: 966 - 02 – 6403316

E-mail: nalamoudi2011@gmail.com
nalamoudi@kau,edu,sa

INTRODUCTION

The oral cavity plays an essential role in many key bodily functions, including mastication, digestion, swallowing, respiration, and communication. Ulceration of the oral mucosa may be due to trauma, recurrent aphthous stomatitis, microbial infections, mucocutaneous disease, systemic disorders, squamous cell carcinoma and drug therapy. Vesicullobullous blistering disorders frequently also present as ulceration due to rupture of initial lesions. Because of the rich innervations of the oral mucosa, most ulcers are invariably painful although an important exception is squamous cell carcinoma, which is often painless, particularly when the tumor is small.[1,2]

In children, trauma is the common cause of ulceration of the oral mucous membranes. Traumatic ulceration may result from physical, chemical, or thermal injury to the tissue. Physical damage to the oral mucosa may be caused by sharp surfaces within the mouth, such as orthodontic appliances, dental restorations, sharp broken teeth, prominent tooth cusps or accidents. In addition, irritation of cheek chewing results in ulceration in some children. Oral ulceration caused during seizures is well-recognized in poorly-controlled epileptics. Chemical or thermal, (burns) injury to the tissue usually occur accidentally and are more painful. Traumatic injuries to the oral mucosa are treated by removing the responsible irritant, after which healing is usually uneventful. Chronic oral ulcers of traumatic origin develop when the low magnitude traumatic stimulus has persisted over time.[3]

Simple mouth ulcers are usually self-limiting and rarely present in general practice. However, severe, recurrent or persistent oral ulceration can be extremely painful as major recurrent aphthous stomatitis (MjRAS) which usually lasts from 4 to 6 weeks. Clinical examination of MjRAS may reveal scarring of the mucosa at sites of previous lesions, due to the severity and prolonged nature of this type of ulcer. Also secondary ulcers as congenital or acquired epidermolysis Bullosa (chronic bullous disease of childhood) are another example. The prolonged and painful ulceration may present significant problems to the patient; difficulty in eating, speaking and swallowing can severely affect a patient's quality of life.[4,5]

In all situations, the aim of the treatment is to provide soft tissue regeneration, hoping for restoring structure, function, and physiology of damaged tissues. In addition to analgesics coupled with supportive therapy during the healing period, a specific therapy may be necessary in many oral ulcer conditions. The mainstay of pharmacologic immunomodulation depends on the proper incorporation of steroid therapy into the management of many intraoral mucosal ulcers such as major recurrent aphthous ulcerations and lichen planus. This class of medications can be used either topically or by injection but can cause some unwanted side effects as the anti-inflammatory effects of corticosteroids cannot be separated from their metabolic effects as all cells use the same glucocorticoid receptor. Steroids block white blood cells from reaching sites of infection, these agents may cause existing infections to get worse or allow new infections to occur. Acne, rashes and sweating is also seen in some patients during steroid therapy. Also steroids may interfere with the way patients metabolize carbohydrates and can cause blood glucose levels to raise. Some weight gain is to be expected during steroid therapy. Because steroids decrease calcium absorption and increase its excretion, they affect bones. Allergic and hypersensitivity reactions to steroids are possible in patients who are susceptible or have had allergic responses to other drugs including difficulty breathing, closing of the throat, swelling of lips and tongue.[6,7]

The repair process of ulcer healing is genetically programmed. It is a series of biologic events that begin as homeostasis but then involve inflammation, cell proliferation, re-epithelialization, formation of granulation tissue, angiogenesis, interactions between various cells and matrix and tissue remodeling, all resulting in scar formation.[8,9] This repair process is controlled and regulated by biologically active substances called growth factors. Growth factors are polypeptides that control the growth, differentiation, and metabolism of cells. These growth factors are hormone-like molecules that interact with specific cell surface receptors to control the process of tissue repair.[10] Although they are present in only nanogram amounts, they exert a powerful influence on wound healing and repair.

Tissue engineering has been defined as "understanding the principles of tissue growth, and applying these to produce functional replacement tissue for clinical use".[11] It refers to the number of ways lost tissue due to trauma or disease might be restored and aims to stimulate the body either to regenerate tissue on its own or to grow tissue outside the body which can then be implanted as natural tissue. Stem cell-based therapy is becoming a promising new approach in almost every medical specialty. We should appreciate that in recent years there have been tremendous scientific activity focused on this area of research (basic, preclinical as well as clinical), and rapidly growing evidence is accumulating to support the therapeutic potential of stem cells. The use of stem cells is a relatively new technological advancement in medicine, and is a heavily debated topic world-wide.[12] Stem cells are often termed the "master" cells of the body because they are unspecialized cells with the ability to renew themselves for many years through cellular division. They can differentiate into different types of cells with specialized functions if proper conditions are available, factors that control cell structure and function can produce these conditions that lead to differentiation.[13] In 2009, Granero-Molto *et al* [14] stated that at the injury site, MSCs could possibly help in repair in two ways: first by differentiating into tissue cells in order to restore lost morphology as well as function and second by secreting a wide spectrum of bioactive factors that help to create a repair environment by possessing anti-apoptotic effects, immunoregulatory function and the stimulation of endothelial progenitor cell proliferation. El-Menoufy *et al* [15] reported that bone marrow–derived MSCs accelerate oral ulcer healing.

Since the discovery and characterization of multipotent MSCs from bone marrow (BM), MSC-like populations from other tissues have now been characterized based on the 'gold standard' criteria established for BM-MSCs.[16-18] Due to certain shortcomings of obtaining the BM-MSCs, including pain, morbidity, and low cell number upon harvest, alternate sources for MSCs have been sought. One of those is MSCs derived from adipose tissues obtained by suction-assisted lipectomy (liposuction).[19-21] MSCs from BM and adipose tissue are morphologically and immunophenotypically similar, but not identical.[22]

Adipose tissue represents a source of cells that may be able to enhance wound healing. ADMSCs are adult stem cells that are easily harvested and of great interest for plastic surgeons. Specifically, ADMSCs secrete angiogenic growth factors that can induce tissue regeneration. ADMSCs could be used in therapies for treatment of chronic, non healing wounds. ADMSCs' studies demonstrate a diverse plasticity, including differentiation into adipo-, osteo-, chondro-, myo-, cardiomyo-, endothelial, hepato-, neuro-, epithelial and hematopoietic lineages, similar to that described for bone marrow derived MSCs.[23,24]

As the quality of mucosal structural restoration may be the most important factor in determining future ulcer recurrences and to avoid the side effects of some medications used for treatment of oral ulcers, so the aim of this study was to investigate the therapeutic potential of adult autologous MSCs derived from Adipose tissues on healing of oral ulcers and to compare the outcome of using ADMCs to that of the conventional use of corticosteroid

MATERIALS AND METHOD

Experimental animals

This is an experimental study performed on dogs. Eighteen dogs with the inclusion criteria of: 1-3 year old male dogs, weighing 8-10.1 kg and had orally and systemically healthy condition were included in the study. These dogs were treated in accordance with the guidelines approved by the Institutional Animal Care and Use Committee of Cairo University. All procedures were carried out under aseptic conditions and general anesthesia. All animals were given antibiotic and analgesic for pain relief after each procedure.

The clinical work was implemented in five phases:

I. Preparation of Adipose- tissue derived Mesenchymal stem cell

Under general anesthesia with isoflurane inhalation, Adipose tissue was excised from both the omentum and the inguinal fat pad of dog according to Tomiyama *et al* [25] The Adipose tissue was resected and placed into a labeled sterile tube containing 15 ml of a phosphate buffered solution (PBS; Gibco/ Invitrogen, Grand Island, New York, USA). Enzymatic digestion was performed using 0.075% collagenase II (Serva Electrophoresis GmbH, Mannheim) in Hank's Balanced Salt Solution for 60 minutes at 37°C with shaking. Digested tissue was filtered and centrifuged, and erythrocytes were removed by treatment with erythrocyte lysis buffer. The cells were transferred to tissue culture flasks with Dulbecco Modified Eagle Medium (DMEM, Gibco/BRL, Grand Island, New York, USA) supplemented with 10% fetal bovine serum (Gibco/BRL) and, after an attachment period of 24 hours, non-adherent cells were removed by a PBS wash. Attached cells were cultured in DMEM media supplemented with 10% fetal bovine serum FBS, 1% penicillin-streptomycin (Gibco/BRL), and 1.25 mg/L amphotericin B (Gibco/BRL), and expanded *in vitro.* When large colonies developed, (80-90% confluence), cultures were washed twice with PBS and the cells were trypsinized with 0.25% trypsin in 1 mM EDTA (Gibco/BRL) for 5 min at 37°C. After centrifugation, cells were re-suspended with serum-supplemented medium and incubated in 50 cm2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures and expanded in vitro until passage three[15]. Cells were identified as being MSCs by their morphology which were fusiform shape, adherence, and their power to differentiate into osteocytes[26] and neurocytes.[27] Differentiation into osteocytes was achieved by adding 1–1000 nM dexamethasone, 0.25 mM ascorbic acid, and 1–10 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, TX, 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 cell homogenate.[28]

II. Induction of oral ulcers and Cell transplantation

The perioral tissues and gingiva were disinfected with povidone iodine (Betadine, Purdue Pharma, Norwalk, CT, USA). Oral ulcers were chemically induced in 18 adult dogs by topical application of a number 4 pellet soaked in a full strength formocresol, and applied to the buccal mucosa in all animals (figures 1 and 2).

After 3 days of ulcer induction dogs were randomly divided into three equal groups, six dogs each according to the type of the injected treatment (figure 3).

Group A (ADMSCs group): six dogs were treated by intralesiosnal submucosal injection of autologous ADMSCs (1×10^7) suspended in 200 µl phosphate-buffered saline (PBS).[29]

Group B (Dexamethasone group**):** six dogs treated by intralesiosnal submucosal injection of corticosteroid therapy injection of Dexamethazone 6mg/1.5ml (Dexamethazone sodium phosphate) (sigma-tec pharmaceutical industries – Egypt – S.A.E).

Group C (control group**):** six dogs treated by intralesiosnal submucosal injection of PBS.

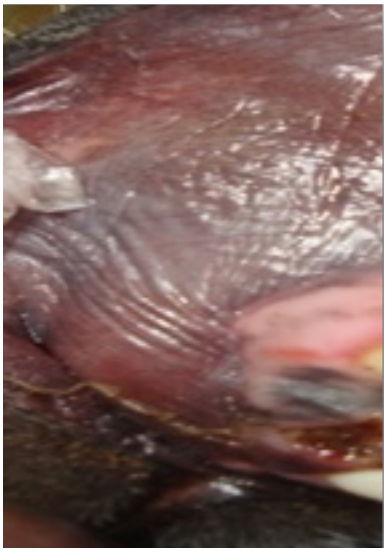


Figure 1. Normal buccal mucosa of dog.

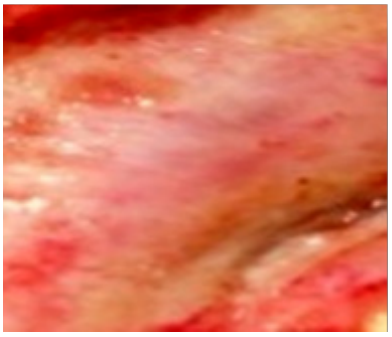


Figure 2. Induced oral ulcer at the buccal mucosa.

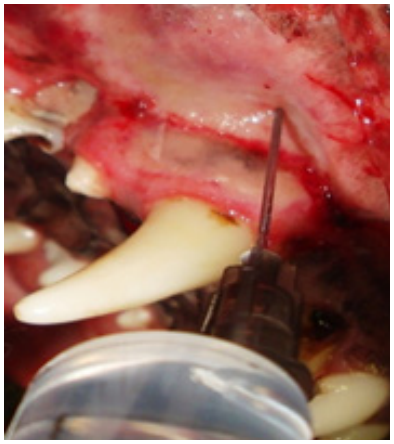


Figure 3. Submucosal injection of the test treatment solution at the oral ulcer At the Buccal Mucosa.

III. Histopathological study

Ulcer tissue biopsy was taken from the three tested groups at the end of the 1st and 2nd week for histopathological study. This examination was carried out after the experimental groups were blinded.

IV. Gene expression growth factors and collagen by real time PCR:

Gene expression of vascular endothelial growth factor (VEGF), platelets derived growth factor (PDGF), epidermal growth factor (EGF) and collagen , as markers of healing were assessed in ulcer tissue biopsies obtained at the first and second weeks .

Table 1. Primer sequences used for RT-PCR

Primer	Sequence
VEGF	F: 5'- ATGAACTTTCTGCTCTCTTGG R: 5'- TCACCGCCTCGGCTTGTC
PDGF	F: 5'- CACTCGGGAGAACAAAGAGA R: 5'- TCTGCACTTCCATCCCAC
EGF	F: 5'- AAT AGT TAT CCA GGA TGC CC 3'- R: 5' ACG CAG CTC CCA CCA TCG TA -3'
Collagen	F: 5'- GAACCTGGCAAACAAGGTC3'- R: 5'- A AGGAGAACCATCTCGTCC -3 '
GAPDH	F: 5' TAT TGT CGC CAT CAA TGA CC3' R: 5' A TAC TCA GCA CCA GCA TCA CC3'

V. Detection of collagen and EGF gene expression using real time PCR (RT–PCR)

Total RNA was isolated from ulcer tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA) according to manufacturers instruction . The RNA sample was dissolved in RNase-free water and quantified spectrophotometrically, concentration of the RNA were assessed using the OD 260/280 ratio. The integrity of the RNA was studied by gel electrophoresis on a 1% agarose gel, containing ethidium bromide .

First-strand cDNA synthesis was performed with the cDNA synthesis kit (Qiagene -USA) by mixing 2 µg total RNA with 0.5 µg of oligo(dT)12-18 primer in a total volume of 12µL. After the mixture was heated at 70°C for 10 min, a solution containing 50 mmol/L Tris•HCl (pH 8.3), 75 mmol/L KCl, 3 mmol/L MgCl2, 10 mmol/L DTT, 0.5 mmol/L dNTPs, 0.5 µL RNase inhibitor, and 200 U Superscript Reverse Transcriptase was added, resulting in a total volume of 20.5 µL. This mixture was incubated at 42°C for 1 h.

VI. Real-time quantitative polymerase chain reaction (PCR)

For real-time quantitative PCR, 5 µLof first-strand cDNA was used in a total volume of 25 µL, containing 12.5 µL 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200 ng of each primer, which is shown in table 1. PCR reactions consisting of 95°C for 10 min (1 cycle), 94°C for 15 s, and 60°C for 1 min (40 cycles), were performed on an ABI Prism 7900 HT Fast Real Time PCR system (Applied Biosystems). Data were analyzed with the ABI Prism 7500 sequence detection system software and quantified using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the GAPDH genes.[29]

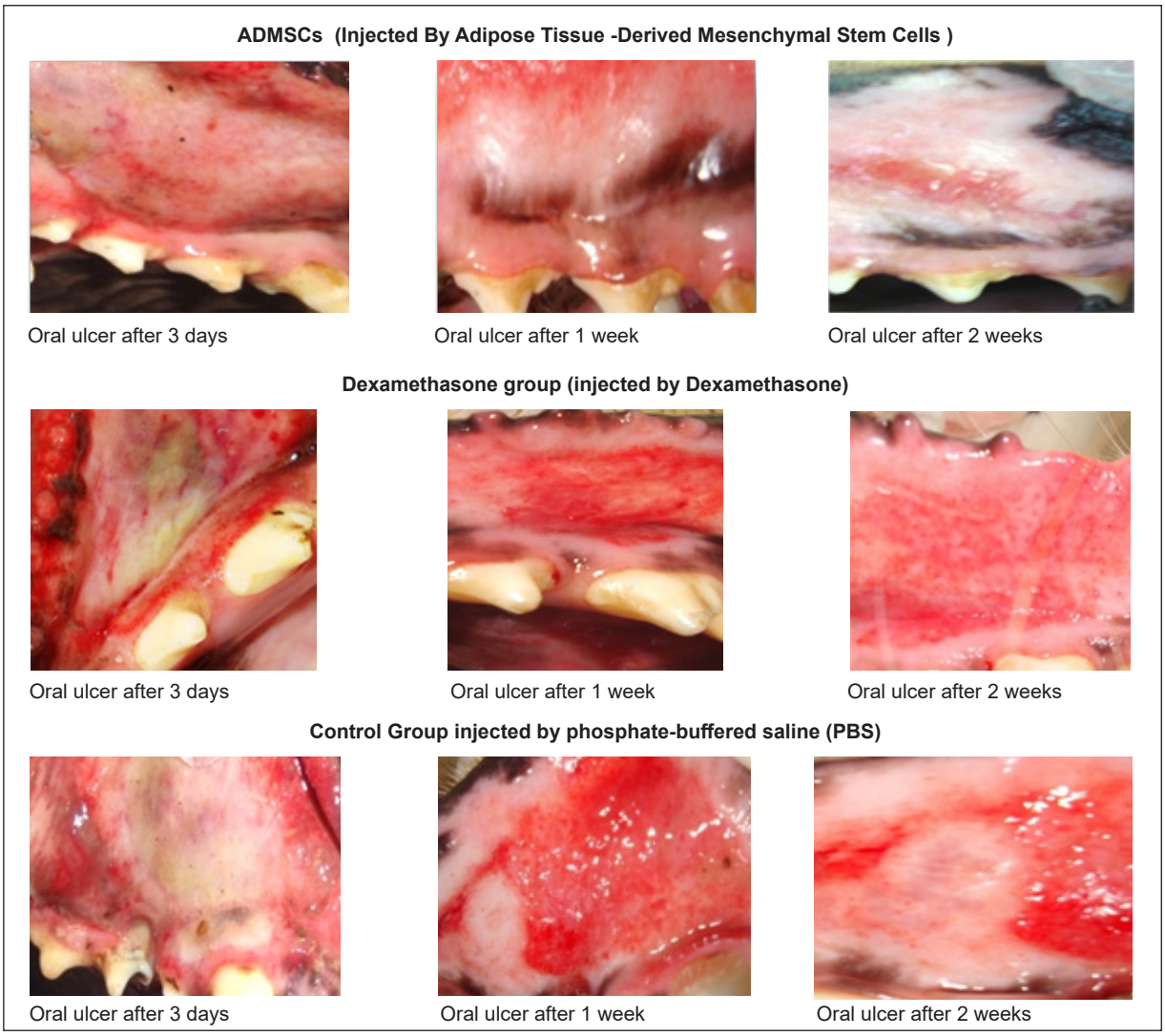


Figure 4. Clinical findings in different experimental groups

Table 2. The Histopathological Findings of Oral Ulcer Tissue Biopsies in the Three Groups at the end of 1st week

ADMSCs group 6 dogs	Dexamethasone group 6 dogs	Control group 6 dogs
Surface epithelium • Complete epithelization of ulcer • Hyperplastic epithelium with hyperkeratinization with regular thickening of the layer. *Subepithelial tissue* • Neovascularization • Inflammation is reduced • Mature fibrous tissue • Architecture appears normal	*Surface epithelium* • Incomplete epithelization of ulcer. • New Epithelium thin. • Inflammatory cells less prominent than in control. *Subepithelial tissue* • Disorganized fibrous tissue with focal dense areas alternating with looser thinner types of collagen (collagen atrophy) • Architecture Is Distorted and Atrophic	*Surface epithelium* • Ulcer filled with exuberant GT & inflammation • Edge: hyperplastic epithelium X keratinization. *Subepithelial tissue* • Full thickness GT with inflammation

Table 3. The histopathological findings of oral ulcer tissue Biopsies in the three groups at the end of 2nd week

ADMSCs group 6 dogs	Dexamethasone group 6 dogs	Control group 6 dogs
Surface epithelium • Complete epithelization of ulcer • Hyperplastic Acathotic epithelium with hyperkeratinization and parakeratosis with irregular thickening of the layer *Subepithelial tissue* • Neovascularization • inflammation is reduced • Mature Fibrous tissue • Architecture appears norm	*Surface Epithelium* • Incomplete epithelization of ulcer • New epithelium thin and is invaded by many inflammatory cells • Keratosis and parakeratosis *Subepithelial Tissue* • GT still present with Telangiectasia in ulcer area • Marked sepsis and scab formation tissue breakdown. inflame in & out of BV • Disorganized mostly loose fibrous tissue (collagen atrophy) with focal dense areas • Architecture is distorted and atrophic	*Surface epithelium* • Ulcer filled with exuberant GT and inflammation • Edge: hyperplastic epithelium X keratinization *Subepithelial tissue* • Full thickness GT with inflammation

Statistical analysis

Statistical analysis was performed using SPSS 18.0 (Statistical Package for Social Studies Inc., Chicago, IL, USA) for Windows. Group Mean ± SD were calculated to quantify the variability in each factor. Comparison between the groups was carried out using Kruskal Wallis test. Statistical significance was set at $P < 0.05$.

RESULTS

Clinical examination of induced oral ulcers tissues after treatment during the 2 weeks evaluation period revealed that, group (A) ulcers that received adipose tissue derived MSCs showed better clinical healing (decrease in bleeding and erythema) than group (B) that received Dexamethasone therapy and group (C) that received phosphate-buffered saline only as control (figure 4).

Histopathological examination of oral tissue biopsy, one and two weeks after treatment of the ulcers revealed that ulcers receiving ADMSCs showed better healing than the Dexamethasone treated group and the control group (tables 2,3 and figure 5).

Gene expression of VEGF: Analysis of the results revealed that expression of VEGF was significantly higher in ADMSCs-treated compared with the Dexamethasone-treated and control groups (table 4).

- **Gene expression of PDGF:** Analysis of the results revealed that expression of PDGF was significantly higher in the ADMSCs-treated group compared with the Dexamethasone-treated and the control groups table (5).
- **Gene expression of EGF**: Analysis of the results revealed that expression of EGF was significantly higher in the ADMSCs-treated group compared with the Dexamethasone-treated and the control groups (table 6).
- **Gene expression of collagen**: Analysis of the results revealed that expression of collagen was significantly higher in ADMSCs-treated group compared with the Dexamethasone-treated and the control groups table (7).

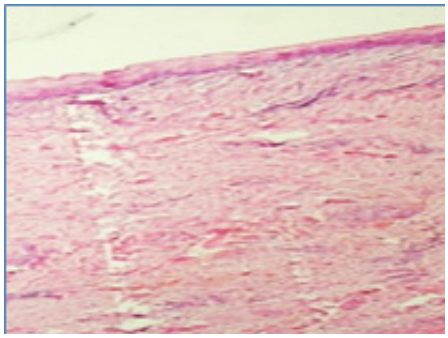
DISCUSSION

Good oral health is important for child's quality of life and well-being for a variety of reasons. Painful, unpleasant oral ulcers present difficulties for maintaining good oral hygiene, eating, drinking, taste, breathing, verbal and non-verbal communication.

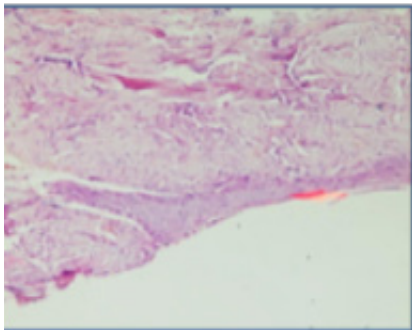
Due to the good vascularity of the oral tissues the majority of oral ulcers heal relatively quickly. In case of severe, recurrent or chronic ulcers, a wide range of treatment therapies were recommended for the symptomatic management to reduce pain and aid healing of lesions. Many patients obtain symptomatic relief from use of topical or systemic corticosteroid preparations; however these medications have many side effects.

Since stem cells possesses the ability to build every tissue in the human body; hence they have great potential for future therapeutic uses in tissue regeneration and repair. Development of effective cellular-based therapies for regenerative medicine may act as a viable alternative treatment to develop a more effective and

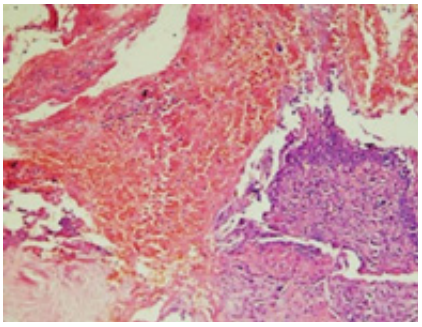
Surface epithelium at the end of 1st Week



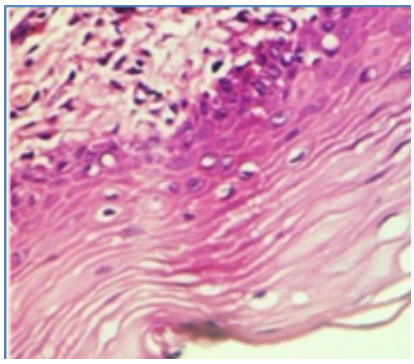
ADMSC Group

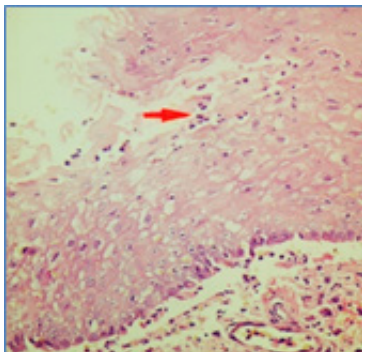


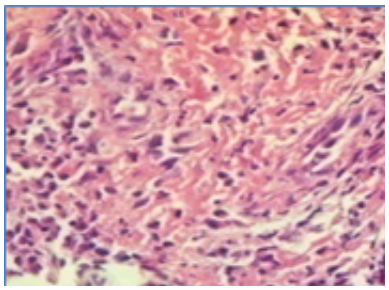
Dexamethasone group

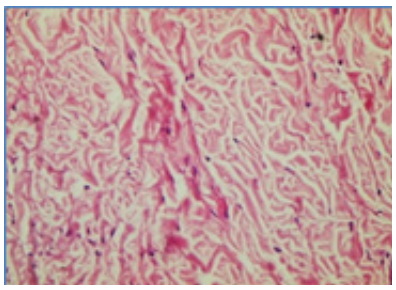


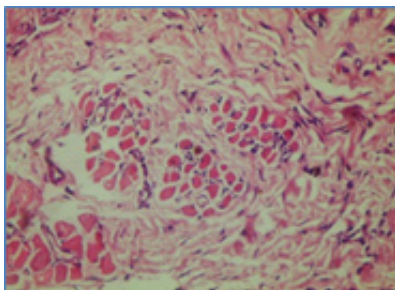
Control Group

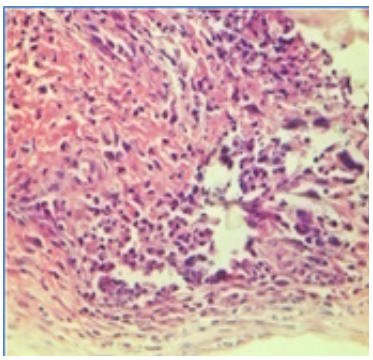


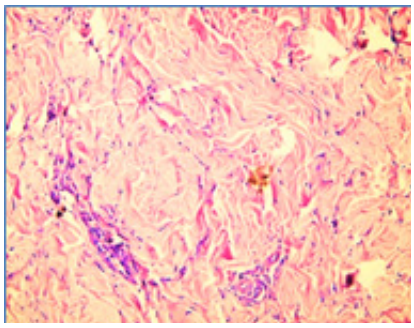


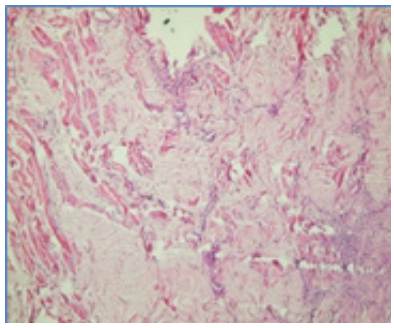


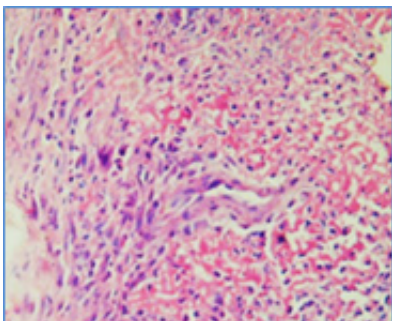












safer therapeutic system for oral mucosal ulceration. Bone marrow contains stem cells that can trans differentiate into osteoblasts, chondrocytes, adipocytes, myocytes, hepatocytes, neural cells, and even epithelial cells of the liver, lung, gastrointestinal (GI) tract, and skin.[30] These findings imply that bone marrow-derived cells in the adult body may be used to recover lost functions of damaged tissues. In 2002, Zuk *et al* [31] showed that human adipose "fat" tissue can be a source of multipotent stem cells .The simple surgical procedure, the easy and repeatable access to the subcutaneous adipose tissue, and the uncomplicated enzyme-based isolation procedures make this adipose tissue source for MSCs most attractive for researchers and clinicians of nearly all medicinal subspecializations. ADMSCs were chosen in this study as they represent an alternative source of autologous adult stem cells that can be obtained repeatedly in large quantities under local anesthesia with a minimum of patient discomfort. A comparative analysis of MSCs obtained from bone marrow, adipose tissue, and umbilical cord clearly showed that ADMSCs were not different regarding morphology, immune phenotype, and success rate of isolating MSCs, colony frequency, and differentiation capacity.[32]

Formocresol is a pulp therapy material in pediatric dentistry. The use of this substance has been taught at dental schools worldwide because of its clinical and radiographic success.[33] Formocresol promotes a coagulation necrosis associated with the inflammatory process with mononuclear cells and dilatation of the vessels with fibril new formation,[34] as it promotes damage and delays the healing process.[35] So, formocresol was chosen to induce oral ulcers in the present study.

This study demonstrated that adipose tissue–derived dish-adherent cells were expandable and implantable to the oral mucosal ulcers and accelerated healing of ulcers induced chemically, which is a model for chronic or recurrent oral ulcers. The cells were able to differentiate into neurocytes and osteocytes and therefore could be defined as adipose tissue –derived MSCs.

In this study histopathological examination of oral ulcer tissue biopsies 1 and 2 weeks after treatment in all tested groups showed those ulcers that received AD MSCs-treated showed better and rapid healing than the Dexamethasone treated and the control groups. These findings are in accordance with some researchers suggested that, the ADMSCs repairs the local tissue either directly or through the promotion of angiogenesis or neovascularization, by the secretion of cytokines, such as vascular endothelial (VEGF), transforming growth factor-β (TGFβ), fibroblast growth factor-2 (FGF-2), and angiopoietin.[36] Others emphasize the relation between pre adipocytes and macrophages, and suggest that the healing effect may be related to an enhanced immune response leading to tissue remodeling and the removal of damaged cells. Lastly, the release of hormones, cytokines, or growth factors by the ADMSCs and local tissue may direct differentiation of the cells.[37] Moreover these results are in agreement with some investigators who stated that patients taking Dexamethasone or other steroids may notice that it takes longer than usual for wounds to heal.[38,39]

Histological investigation of ulcer tissue biopsy at the end of 1st and 2nd week follow up period, showed the presence of marked sepsis, scab formation tissue breakdown and inflammation in and out of BV in the ulcer tissue biopsy of group treated with Dexamethasone. Drugs that suppress normal immune responses can make a person susceptible to infections. They may also decrease a person's ability to fight the start of a new infection. These results are in accordance with researches who concluded that patients on steroids, including dexamethasone, have an increased risk of all types of infections (bacterial, viral, or fungal).[40,41]

The mechanism of healing of oral ulcers, in this study was assessed by Gene expression of VEGF, PDGF, EGF, and collagen, as healing markers. Gene expression of VEGF, PDGF, EGF and collagen was significantly higher in ADMSCs group compared with the Dexamethasone and control groups at the end of both 1st and 2nd week. The role of the studied growth factors was confirmed, where VEGF is considered a potent endothelial mitogen,[42] while PDGF is chemotactic for cells migrating into healing wounds.[43]

EGF enhances cellular proliferation, differentiation and survival.[44] these results are in agreement with El-Menoufy *et al*, who reported that the VEGF and collagen gene expression was significantly higher in oral ulcers treated with BMMSCs compared with saline treated controls. They concluded that MSC treatment improves the quality of mucosal structural restoration which is the most important factor in determining future ulcer recurrence[15]

CONCLUSION

The above results support the reported evidence that adipose tissue might be a promising alternative source of stem cells for therapy. Local injection of autologous ADMSCs appears to be beneficial in regenerating damaged tissues and treatment of resistant oral ulcers, and supersedes corticosteroid treatment. ADMSCs influence the gene expression of local growth factors at the lesion site, facilitating the healing process. Use of this novel modality will surely continue to be explored further in the laboratory and clinically in several oral and dental applications.

ACKNOWLEDGMENT

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah grant No. (106/165/1432).

The authors would like to thank Prof. Dr. Soheir M. Mahfouz, Professor and former Head of Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt, for her outstanding contribution to this work.

REFERENCES

1. Field EA, Longman LP. Tyldesley's *Oral Medicine*, 5th edn. Oxford: Oxford University Press, 2003.
2. Porter SR, Scully C. Adverse drug reactions in the mouth. *Clin Dermatol;18*: 525–532. 2000.
3. Francis B, Quinn F, Matthew W. Ulcerative lesions of the oral cavity. Grand Rounds Presentation, UTMB, Department of Otolaryngology, TX: 2002.
4. Field EA, Allan RB. Review article: Oral ulceration–aetiopathogenesis, clinical diagnosis and management in the gastrointestinal clinic. *Aliment Pharmacol Ther; 18*: 949–962. 2003.
5. Scully C, Gorsky M, Lozada-Nur F. The diagnosis and management of recurrent aphthous stomatitis: a consensus approach. *J Am Dent Assoc;134*:200–207. 2003.
6. Fernandez J, Escorsell A, Zabalza M, Felipe V, Navasa M, Mas A, Lacy A, Ginès P, Arroyo V. Adrenal insufficiency in patients with cirrhosis and septic shock: Effect of treatment with hydrocortisone on survival. *Hepatology;44*:1288–1295. 2006.
7. Porter SR, Scully C, Pedersen A. Recurrent aphthous stomatitis; *Crit Rev Oral Biol Med.; 9*: 306–321. 1998.
8. Tarnawski A. Cellular and molecular mechanisms of ulcer healing lesions from gastrointestinal ulcers. *Dig Dis Sci; 50*(Suppl. 1):S24–33. Review. 2005
9. Tarnawski A S, Ahluwalia A. Molecular mechanisms of epithelial regeneration and neovascularization during healing of gastric and esophageal ulcers. *Curr Med Chem.;19*(1):16-27. 2012.
10. Davidson J. Growth factors in wound healing. *Wounds.;7*(suppl A):53A–64A. 1995.
11. MacArthur BD, Oreffo ROC. Bridging the gap. Nature. 2005; 433(7021):19.
12. Gronthos S, Zannettino ACW. A method to isolate and purify human bone marrow stromal stem cells. In: *Mesenchymal stem cells: methods and protocols*. Prockop DJ, Bunnell BA, Phinney DG, editors. Totowa, NJ: Humana Press, pp. 45-57, 2008.
13. National Institutes of Health Resource for Stem Cell Research. The stem cell in formation Stem Cell Basics page. Http://stemcells.nih.gov/infbasics/defaultpage.asp. Accessed July 21, 2008.
14. Granero-Molto F, Weis K, Miga M, Landis B, Myers T, O'Rear L, Longobardi L, Jansen D, Mortlock M, Spagnoli A. Regenerative effects of transplanted mesenchymal stem cells in fracture healing. *Stem Cells;27*:1887-1898. 2009.
15. El-Menoufy H, Aly LA, Aziz MT, et al. The role of bone marrow-derived mesenchymal stem cells in treating formocresol induced oral ulcers in dogs. *J Oral Pathol Med.;39*(4):281-9. 2010.
16. Prockop DJ. Marrow stromal cells as stem cells for non hematopoietic tissues. *Science.;276:*71-74. 1997.
17. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simoneti DW, Craig S, AMrshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science;284*:143-147. 1999.
18. Stan Gronthos S, Zannettino A, Hay S, Shi S, Graves S, Kortesidis A, Simmons J. Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. *J Cell Sci;116*:1827-1835. 2003.
19. Mareschi K, Biasin E, Piacibello W, Aglietta M, Madon E, Fagioli F. Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood. *Haematologica; 86*:1099-1100. 2001.
20. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell W, Katz AJ, Penhaim P, Lorenz P, Hedrick P. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng;7*:211-228. 2001.
21. Mizuno H, Zuk PA, Zhu M, Lorenz HP, Benhaim P, Hedrick MH. Myogenic differentiation by human processed lipo aspirate cells. *Plast Reconstr Surg;109*:199-209. 2002.
22. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells;24*:1294-1301. 2006.
23. Aust L, Devlin B, Foster SJ, Halvorsen YD, Hicok K, du Laney T, Sen A, Willingmyre GD, Gimble JM. Yield of human adipose-derived adult stem cells from liposuction aspirates. *Cytotherapy;6*:7-14. 2004.
24. Rodriguez AM, Elabd C, Amri EZ, Ailhaud G, Dani C. The human adipose tissue is a source of multipotent stem cells. *Biochimie; 87*:125-128. 2005.
25. Tomiyama K, Murase N, Stolz DB, Toyokawa H, O'Donnell DR, Smith DM, Dudas JR, Rubin JP, Marra KG. Characterization of transplanted green fluorescent protein+ bone marrow cells into adipose tissue. *Stem Cells; 26*:330-338.2008.
26. Yamazoe K, Mishima H, Torigoe K, Iijima H, Watanabe K, Sakai H, Kudo T. Effects of atelocollagen gel containing bone marrow-derived stromal cells on repair of osteochondral defect in a dog. *J Vet Med Sci; 69*:835-839. 2007.
27. Jaiswal N, Haynesworth S, Caplan A, Bruder S. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *J Cell Biochem; 64*: 295–312. 1997.
28. Hou L, Cao H, Wang D, et al. Induction of umbilical cord blood mesenchymal stem cells into neuron-like cells in vitro. *Int J Hematol; 78*: 256–61. 2003.
29. Kenneth J. Livak and Thomas D. Schmittgen: Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 22DDCT. *Methods; 25*:402–408. 2001.
30. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow derived stem cell. *Cell;105*: 369–377. 2001.
31. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell;13*(12): 4279-4295. 2002.
32. Izadpanah R, Trygg C, Patel B, Kriedt C, Dufour J, Gimble JM, Bunnell BA. Biologic properties of mesenchymal stem cells derived from bone marrow and adipose tissue. *J Cell Biochem;99*:1285-1297. 2006.
33. Boeve C, Dermaut L. Formocresol pulpotomy in primary molars: a long-term radiographic evaluation. *ASDC J Dent Child;49*:191–6. 1982.
34. AnaTeresa S, Luis C, Lizeti T, Oliveira R. Histological analysis of the association between formocresol and endotoxin in the subcutaneous tissue of mice. *Braz Dent J;19*: 33–39. 2008.
35. Yamasaki M, Nakamura H, Kameyama Y. Irritating effect of formocresol after pulpectomy in vivo. *Int Endod J*; 27:245–251. 1994.
36. Neels JG, Thinnes T, Loskutoff DJ. Angiogenesis in an in vivo model of adipose tissue development. *Faseb J; 18*:983-985. 2004.
37. Charrière G, Cousin B, Arnaud E, André M, Bacou F, Penicaud L, Casteilla L. Preadipocyte conversion to macrophage. Evidence of plasticity. *J Biol Chem.;278*:9850-9855. 2003.
38. Guidry JA, George JN, Vesely SK, Kennison SM, Terrell DR. Corticosteroid side-effects and risk for bleeding in immune thrombocytopenic purpura: patient and hematologist perspectives 2009. John Wiley & Sons *A/S European Journal of Haematology 83*:175–182.
39. Stanbury RM, Graham EM. Systemic corticosteroid therapy-side effects and their management. *br j ophthalmol;82*:704–708. 1998.
40. Suzuki B, Peterson D, Siegel M. Medications influencing immune response and wound healing. Grand Rounds Oral-Sys Med; 2:159–168. 2007.
41. Moraes LA, Paul-Clark MJ, Rickman A, Flower RJ, Goulding NJ, Perretti M. Ligand-specific glucocorticoid receptor activation in human platelets. *Blood; 106*:4167-4175. 2005.
42. Gerritsen M, Tomlinson J, Zlot C, Ziman M, Hwang S. Using gene expression profiling to identify the molecular basis of the synergisticactions of hepatocyte growth factor and vascular endothelial growth factor in human endothelial cells. *Br J Pharmacol;140*: 595–610. 2003.
43. Sabine W, Richard G. Regulation of Wound Healing by Growth Factors and Cytokines. *Physiol Rev;83*:835-870. 2003.
44. Herbst RS. Review of epidermal growth factor receptor biology. *Int J Radiation Oncology Biol Phys;59*(2):21–26. 2004.