Adipose Stem Cells as Alternatives for Bone Marrow Mesenchymal Stem Cells in Oral Ulcer Healing

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Background and Objectives: Adipose tissue is now recognized as an accessible, abundant, and reliable site for the isolation of adult stem cells suitable for tissue engineering and regenerative medicine applications.

Methods and Results: Oral ulcers were induced by topical application of formocresol in the oral cavity of dogs. Transplantation of undifferentiated GFP-labeled Autologous Bone Marrow Stem Cell (BMSCs), Adipose Derived Stem Cell (ADSCs) or vehicle (saline) was injected around the ulcer in each group. The healing process of the ulcer was monitored clinically and histopathologically. Gene expression of vascular endothelial growth factor (VEGF) was detected in MSCs by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Expression of VEGF and collagen genes was detected in biopsies from all ulcers. Results: MSCs expressed mRNA for VEGF MSCs transplantation significantly accelerated oral ulcer healing compared with controls. There was increased expression of both collagen and VEGF genes in MSCs-treated ulcers compared to controls.

Conclusions: MSCs transplantation may help to accelerate oral ulcer healing, possibly through the induction of angiogenesis by VEGF together with increased intracellular matrix formation as detected by increased collagen gene expression. This body of work has provided evidence supporting clinical applications of adipose-derived cells in safety and efficacy trials as an alternative for bone marrow mesenchymal stem cells in oral ulcer healing.

Keywords: Bone marrow, Mesenchymal stem cells, Oral ulcer

Introduction

Wound healing is a process by itself. But a proper, functional and even aesthetic healing of a wound is not only a process but an objective as well. It depends on a great variety of factors for a wound to heal properly, and many of these factors could be managed and modified by our knowledge and skill.

There is an important need to stimulate the healing of acute and chronic wounds to a level that is not presently possible with standard care measures or recently developed innovative approaches. An area of research that holds promise for the treatment of difficult-to-heal wounds is stem cell application. The bone marrow is an important source of hematopoietic stem cells that regularly regenerate components of the blood, and non-hematopoietic stem cells including mesenchymal stem cells (MSC). There are several potential mechanisms by which autologous stem cells could significantly contribute to wound healing. Under appropriate conditions stem cells can rejuvenate or rebuild tissue compartments (1).

The phenotypic definition of MSCs has been hampered by the heterogeneity of this population (2, 3). Heterogeneity

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occurs among cells harvested from a single anatomic site and also occurs between cells harvested from different anatomic sites. Bone marrow and adipose tissues are currently the major sources for MSCs that are being used for pre-clinical and clinical studies. Adipose stromal cells (ADSCs), though exhibiting differences, still share basic characteristics with bone marrow-derived cells (BMSCs) (4, 5).

Recently, increasing focus is being placed on the use of bone marrow-derived MSC. In a first but preliminary report, such autologous cultured cells could bring about closure of long-standing and hard-to-heal wounds. (1) These MSCs-or human bone marrow stromal stem cells-can be cultured, expanded and then transplanted into the injured site or, after seeding in/on shaped polymer scaffolds, placed back in the patient to generate appropriate tissue constructs. It is a rather time consuming procedure for the patients and the laboratory employees. These MSC have a low cell number upon harvesting so they need to be expanded and then transplanted to the injured site or seeded on/in a polymer scaffold. The last option will take more time and besides the time that is necessary in the laboratory, the patients need to be operated twice. First to collect the MSCs and-or stabilize the defect and then again to place the scaffold or transplant the MSCs. That's why researchers are now looking for alternative sources for MSCs (6).

Adipose tissue might be a promising alternative source of stem cells that could have far reaching effects on several fields including bone engineering. We already know that stem cells derived from adipose tissue are capable to differentiate into adipocytes, chondrocytes, osteoblasts and myoblasts, like MSCs. Adipose tissue is easier to harvest and because we have enough of adipose tissue the number of stem cells upon harvesting should be larger (7).

Therefore, we carried out this study to evaluate the efficacy of ADSCs to be an alternative for BMSCs in oral ulcer healing.

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.

I. Bone marrow mesenchymal stem cell 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. (8) and Kadiyala et al. (9). In brief, the bone marrow aspirate was layered onto Histopaque-1077 (Sigma, St. Louis, MO, USA) 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 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 $2.5 \times 10^5/\text{cm}^2$ 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 (10) and neurocytes (11). Differentiation into osteocytes was achieved by adding 1 to 1,000 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 cell homogenate. MSCs were used in this study upon reaching 70 to 80% confluence (12).

II. Mesenchymal stem cell isolation from adipose tissue and their characterization: According to Lobna et al. (13) adipose tissue was excised from both the omentum and the inguinal fat pad of dog under general anesthesia. 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 cul-

ture 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. At 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 resuspended 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. MSCs in culture were characterized by their adhesiveness and fusiform shape, by flow cytometry for MSC surface markers CD45[−] and CD29⁺. MSCs differentiation into chondrocytes and osteocytes was confirmed as in previous published work (14, 15).

III. Labeling of MSCs: Undifferentiated MSCs were harvested and were labeled with green fluorescent protein (GFP) using monster green fluorescent protein vector and lipofectamin 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 μ g GFP plasmid /well of cells. GFP plasmid was incubated with lipofectamin 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 complete medium (2 ml per well). For imaging GFP autofluorescence of MSCs, unstained slides were directly analysed and green autofluorescence detected by inverted fluorescence microscopy (Leica DM IRB, Leica, Wetzlar, Germany) (15, 16).

IV. *In vivo* transplantation of undifferentiated GFP-labeled MSCs in induced oral ulcer: Autologous undifferentiated GFP-labeled MSCs were injected locally in the experimental canines following chemically induced oral ulcer in both treated groups as mentioned in the study design.

V. 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 Corp. Carlsbad, 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 μ g) 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.

VI. 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 μ L of reaction mixture contained 12.5 μ L of SYBR Green Maxima (Fermentas, USA), 1 μ L of each primers (10 μ mol/L), and 1 μ L 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 (15, 17).

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

VIII. Cell transplantation: Dogs were randomly divided into three equal groups each of six dogs. In group 1, six dogs received PBS only (as a control group), while group 2, six dogs were treated by submucosal injection of autologous ADSCs (2×10^7) suspended in 200 μ l phosphate buffered saline (PBS). and finally group 3, six dogs were treated by submucosal injection of autologous BMSCs (2×10^7) suspended in 200 μ l phosphate buffered saline (PBS). Dogs were injected with either autologous MSCs

Table 1. Sequences of primers for conventional PCR

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

F and R indicate forward and reverse primers, respectively.

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) (18). 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 $\pm$ 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 and ADSCs

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 (Fig. 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%) (Fig. 2). 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 (Fig. 3).

MSCs homing & 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 (Fig. 4).

Fig. 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, $\times 10$).

Fig. 2. Flow cytometric characterization analyses of MSCs. Cells were uniformly negative for CD45, and positive for CD29.

Clinical and histopathological assessment

It seems that BMSCs and ADSCs 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 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 no statisti-

cally significant difference among the three groups (Fig. 5 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 viable tissue (Fig. 6). 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. The group treated with ADSCs only epithelial regeneration had already shown and proliferating epithelial cells extended inward over the outer epithelium of the wound and the stroma was lightly infiltrated with polymorphnuclear and macrophages which had aggregated at the wound, while in BMSCs group complete healing of surface epithelium with signs of increased epithelial proliferation was demonstrated with increased thickness of surface epithelium and mild subepithelial inflammatory infiltrate (Fig. 7).

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

Cell therapy has come into attention in all medical fields as a new paradigm for future medicine in which reproductive cells such as stem cells are used to restore organ functions after damage by disease or trauma. The basic mechanisms by which MSCs might improve wounds are (a) paracrine communication with resident wound cells, infiltrating inflammatory cells, and antigen present-

Fig. 3. Green auto fluorescence of GFP-labeled MSCs in vitro before transplantation.

Fig. 4. (A) Arrows directed towards adipose GFP-labeled MSCs after transplantation in canine induced oral ulcer. (B) Arrows directed towards bone marrow GFP-labeled MSCs after transplantation.

Fig. 5. (A, B, 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 (ADSC)		Group 3 (BMSC)		p-value
	Mean	SD	Mean	SD	Mean	SD	
Day 0	32	3.5	32	2.9	32	4.1	1.000
3 days	32 ^a	4.2	26 ^b	3.2	25 ^b	4.1	0.002*
7 days	29 ^a	4.1	21 ^b	2.7	20 ^b	2.6	<0.001*
10 days	17 ^a	3.7	13 ^b	3.1	13 ^b	2.3	<0.001*
15 days	13	2.6	12	3.1	12	2.3	1.000

*Significant at $p < 0.05$. ^{a,b}Means with different letters are statistically significantly different according to Duncan's test.

Fig. 6. Clinical follow up: (I) Control group: (A) oral ulcer at zero time & submucosal injection of saline alone. (B) At 15 days. (II) BMSCs group: (C) oral ulcer at zero time & submucosal injection of MSCs. (D) At 15 days. (III) ADSCs group: (E) Oral ulcer at zero time & submucosal injection of MSCs. (F) At 15 days.

ing cells or (b) their differentiation into resident cells or (c) both (19, 20).

Recently, some studies have reported on the wound-healing effects of adult stem cells by proliferating fibroblasts and secreting cytokines. Re-epithelization and angiogenesis were observed after application of bone-marrow-derived mesenchymal stem cells to wound sites. Additionally, mesenchymal stem cells help in the formation of granulation tissues at wound sites (20). Kim et al. (21) performed an experiment on the wound-healing effects of ADSC and observed that they are mainly mediated by stimulating collagen synthesis of dermal fibroblasts.

There have been reports that collagen synthesis mediated by the activation of fibroblasts plays an important role in skin rejuvenation. In this process, variable cytokines such as IGF, EGF, IL-1, TNF- α , TGF- β and growth factors participate in collagen synthesis (21, 22). At first, these factors are released from platelets, and then variable cytokines and growth factors are secreted from inflammatory cells and fibroblasts, which act as the target tissue (22). In this study, ulcers treated with stem cells showed more expression of collagen gene than ulcers that received saline alone in the control group.

The interrelationship between MSCs and the vascula-

Fig. 7. Ulcers receiving MSCs showed better healing by histopathologic examination of oral tissue biopsies. (I) H&E stain of control group: (A) low power view of ulcerated surface epithelium with multiple degenerative foci (H&E, x100). (B) A higher power of intense chronic inflammatory cell infiltrate with vascular dilatation (H&E, x400). (II) H&E stain of BMSCs group: (C) complete healing of surface epithelium with signs of hyperplasia and hyperparakeratosis (H&E, x40). (D) High magnification of the previous figures showing hyperkeratinization and epithelial hyperplasia. There is mild superepithelial inflammatory infiltrate (H&E, x200). (III) H&E stain of ADSc group: (E) only epithelial regeneration had already shown and proliferating epithelial cells extended inward over the outer epithelium of the wound (H&E, x100). (F) the stroma was lightly infiltrated with polymorphnuclear and macrophages which had aggregated at the wound (H&E, x200).

Table 3. Semiquantitation of PCR products of collagen and VEGF gene expression in oral tissue

	Control (n=6)	ADSC (n=6)	BMSC (n=6)	p-value
Collagen DNA concentration ($\mu\text{g/mL}$)	632.7 $\pm$ 51.5 ^a	1693.4 $\pm$ 101.2 ^b	1763.4 $\pm$ 120.2 ^b	<0.001*
VEGF DNA concentration ($\mu\text{g/mL}$)	775.6 $\pm$ 91.52 ^a	887.3 $\pm$ 153.8 ^a	1595.9 $\pm$ 201.58 ^b	<0.001*

*Significant at $p < 0.05$. ^{a,b}Values with different letters are statistically significantly different.

ture is another area of relevance for wound repair. Granulation tissue formation is a critical early step in the healing process (23). One of the therapeutic functions of MSCs is the early induction of granulation tissue (24, 25). This is followed by the stabilization of the neovascular network as wounds begin to heal. A current theory of BMSCs and ADSCs origin places these cells in perivascular domains in their respective organs (26, 27). In addition, analyses of newly isolated BMSCs and ADSCs have shown that these cells express markers characteristic of pericytes (26). The native pericyte function of these cells may be retained in wound tissues. Pericytes are microvascular support cells that exhibit phenotypic characteristics intermediate between myofibroblasts and smooth muscle cells (28). The neovasculature attracts pericytes through the release of the chemokines platelet-derived growth factor-BB (PDGF-BB). This interaction could explain, at least in part, the motive force behind MSCs migration into wound tissue. Thus, therapeutic functions of MSCs in wounds likely include early induction of granulation tissue and stabilization of neo vasculature.

The discrepancy led researchers to study the paracrine effect of BMSCs in angiogenesis. Indeed, they found that BMSCs conditioned medium promoted endothelial tube formation and that BMSCs expressed high levels of VEGF and Ang-1 but not Ang-2. Notably, BMSCs treatment resulted in significantly increased amounts of Ang-1 and VEGF in the wounds. In our study there was increase expression of VEGF in both MSCs treated groups as VEGF plays a key role in angiogenesis by stimulating endothelial cell proliferation, migration, and organization into tubules (29, 30). Moreover, VEGF increases circulating endothelial progenitor cells (30). Angiogenesis-formation of new microvessels from preexisting vessels-is essential for healing of ulcers. The source of such blood vessels in control group is from uninjured ones adjacent to the injured area. 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 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.

We now know that adipose tissue contains cells with the phenotypic characteristics resembling those of mesenchymal stem cells from the bone marrow. Both of them have a proliferation potential and a similar expression pattern of surface markers. BMSCs and ADSCs also exhibit both the multilineage potential in vitro, differentiating toward the osteogenic lineage when cultured in the presence of established lineage-specific differentiation factors. Lee et al. (31) also demonstrated that there is a similarity between ADSCs and BMSCs supported by gene expression. Gene array analysis revealed that less than 1% of genes were differentially expressed between ADSCs and BMSCs. These data support the notion that ADSCs cells and BMSCs originate from common precursors also proposed by, Bianco et al. (32).

Compared with MSCs harvested from bone marrow, ADSCs cells are (i) easier to obtain, under local anaesthesia with less pain after the procedure (ii) they carry a relative lower donor site morbidity and (iii) are available in large numbers: 4×10^7 cells/100 cm³ fat aspirates versus 1×10^5 cells/30 cm³ bone marrow aspirates. Obtaining large numbers of stem cells at harvest (1) reduces the required amount of harvested fat tissue, which is most beneficial in non-obese individuals, and (2) would reduce or even eliminate the need for costly and lengthy tissue culture expansion that would subject the patient to a second procedure. For example, for the expansion of MSCs an expansive "Good Medical Practice (GMP)-facility is required, which not all hospitals have. Furthermore the special media needed for this expansion, the extra costs and higher risk of infections during expansion makes MSCs from bone marrow aspirates not a favorable cell type to work with. ADSCs cells on the other hand can be harvest, detected and selected from lipoaspirates, stimulated by

growth factors and given back to the patient all in one procedure (33-35). Adipose tissue is therefore considered as one of the favourable alternatives to bone marrow aspirates as a source of MSCs as ADSCs significantly reduced the wound size and accelerated the re-epithelialization from the edge. Collectively, these data suggest that ADSCs are constitutionally well suited for wound healing and secretory factors derived from ADSCs promote wound healing.

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. The various clinical trials demonstrate that fat-derived therapy is not a dream, but is becoming a reality. Surprisingly, results so far suggest that the efficiency of ADSCs in regenerative medicine could be related more to their capacity to modulate immunity and/or inflammation than to their differentiation potentials. The physiological relevance of this phenomenon needs to be better documented, as this could lead to improved efficiency and perhaps new therapeutic possibilities for these cells. However, it is reasonable to speculate that one type of cells will not be able to cover all therapeutic applications and that it will be necessary to fully delineate the respective applications for the various types of cells.

In conclusion, now that we know that there are multiple stem cell reservoirs available for research and clinical applications, we should consider to use that reservoir that is (i) available in a large volume with limited morbidity of the surrounding tissue upon harvest; and (ii) accessible without or with the lowest amount of pain. Furthermore the reservoir should (iii) hold cells which are capable of differentiation into osteoprogenitor cells, produce bone seeded on biomaterial like scaffolds and (iv) can be detected by simple laboratory methods. In my opinion adipose tissue provides us with all the mentioned above. In this context, it is reasonable to suggest that, each time ADSCs display effects more or less similar to other cell types, the inherent advantages of adipose tissue will favour its use over cells from other sources.

Potential conflict of interest

The authors have no conflicting financial interest.

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