

Human exfoliated deciduous teeth and oral mucosa: promising applications in tissue regeneration

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ABSTRACT

In the last three decades, the constantly increasing need for therapies, efficiently preventing and/or treating human diseases, has raised the interest in Regenerative Medicine (RM). RM is based on employing mesenchymal stem cells (MSCs), that showed to have great proliferation, self-renewal and multi-lineage differentiation potential, *in vitro* as well as *in vivo*. The opportunity of an accessible, painless and low-cost reservoir of MSCs constitutes the first important step of a successful regenerative therapy to include in the current clinical practice. Oral cavity has recently demonstrated to contain different MSCs niches: dental pulp from permanent and deciduous teeth, periodontal ligament, dental follicle, apical papilla and mucosa. MSCs from dental pulp of deciduous teeth, naturally lost in pediatric age, and the oral mucosa have shown to be easily harvested and to have a promising regenerative potential. Thus, the aim of the paper is to review the potentialities of human exfoliated deciduous teeth stem cells (SHEDs) and oral mucosa stem cells (OMSCs) in RM, with the purpose of their use as accessible source of MSCs for the future of pediatric patient.

Introduction

Regenerative Medicine (RM) and Tissue Engineering (TE) are currently among the most discussed topics in medicine. Since their origin as medical branches, their goals have been to provide new strategies not only to treat diseases but also to regenerate injured tissues and organs supporting healthcare. RM and TE are based on the application of mesenchymal stem cells (MSCs), growth factors and scaffolds, artificial or natural biomaterials used as cellular support^{1,2}. The key of the regenerative process are the properties of MSCs, multipotent cells characterized by clonogenicity, self-renewal and ability to differentiate towards different cellular lineages and regenerate various types of tissues³.

Several MSC sources have been identified as valuable sources for cellular therapy in tissue regeneration (TR). Bone marrow (BM) represents the first identified source of adult MSCs, even if the collection of BM-MSCs presents limitations due to the invasive and painful methods to harvest the samples. Moreover, even if very promising for regenerative purposes, the number, the differentiation potential and the maximal life cycle of BM-MSCs, decrease with the age of the subject⁴⁻⁶. Thus, other MSC sources have been investigated: the umbilical cord blood and the adipose tissue can more easily

provide a greater number of MSCs with respect to BM by a less invasive method^{7,8}.

Oro-facial mesenchymal stem cells

More recently, the efforts from many researchers were focused on detecting other available sources of stem cells, leading to the characterization of the oro-facial MSCs. Many studies widely demonstrated the promising *in vitro* and *in vivo* TR potential of the oral MSCs. Noteworthy, the oral cavity contains different “niches” of stem cells: dental pulp from permanent and deciduous teeth, periodontal ligament, dental follicle, apical papilla and the three subtypes of mucosa: the masticatory mucosa, namely hard palate and gingiva; the specialized mucosa, represented by the dorsal surface of the tongue, and the lining mucosa, which includes buccal mucosa, the internal surface of the lips, the ventral surface of the tongue, the soft palate, and the alveolar mucosa⁹⁻¹⁶.

Among the oro-facial MSCs, human exfoliated deciduous teeth stem cells (SHEDs) and oral mucosa stem cells (OMSCs) have recently attracted the attention of many studies focused on TR therapies, for several reasons (See Table 1)^{9-13, 17}:

1. the surgical procedures necessary to harvest the MSC are easy and painless;
2. the MSC isolation methods are simple, fast and low-cost;

3. as all recognized SCs, they express the canonical surface markers (e.g. CD29, CD73, CD105, CD44, CD146, CD49c, SSEA-4 and Stro-1) and nuclear markers (e.g. NANOG, Oct-4 and SOX2);
4. they show a great self-renewal ability and multi-lineage differentiation potential.

It has been widely demonstrated that SHEDs and OMSCs, besides the expression of canonical MSC surface and nuclear markers without any expression of hematopoietic stem cell markers, in presence of specific growth factors, differentiate towards different lineages (e.g. osteocytes, chondrocytes and adipocytes), also expressing the related specific lineage markers^{9, 11-13, 17}. Moreover, they are considered a very homogeneous population, with even a greater TR potential with respect to BM-MSCs^{9,18-21}.

The investigations on stem cell properties of oro-facial MSCs were recently extended to inflamed tissues, showing as MSCs deriving from inflamed tissue have similar regenerative properties as the healthy counterpart, in terms of stem cell marker expression, proliferation and multi-lineage differentiation abilities. *Alongi et al* demonstrated that the human dental pulp stem cells (hDPSCs) from inflamed tissue have no difference in the MSC profiles if compared to control tissue-derived hDPSCs. Even if some stem cell properties may be affected and altered under inflammation condition, most of them remained potent in

Table 1: OMSCs and SHEDs *in vitro* and *in vivo* stem cell properties

Ref/Author/Year	MSCs	ESC and MSC markers	<i>In vitro</i> regeneration	<i>In vivo</i> regeneration
Miura et al (2003) [10]	SHEDs	STRO-1, CD146	Neurons, adipocytes and odontoblasts	Bone and dentin
Zhang et al (2009) [13]	OMSCs	Oct-4/SSEA-4, Oct-4/STRO-1, CD29, CD73, CD90/Thy-1 and CD105	Neurons, adipocytes and osteoblasts	Connective tissue
Wang et al (2012) [17]	SHEDs	STRO-1, CD146, CD29 and CD105	Osteocytes and adipocytes	Bone
Tomasello et al (2017) [22]	OMSCs	STRO-1, CD146, Cd29 and SSEA-4	Osteocytes	—
Marynka-Kalmani et al (2010) [25]	OMSCs	Oct-4, Sox-2, SSEA-4, CD29, CD73, CD90, CD105 and CD166	Neurons, adipocytes, chondrocytes and osteocytes	Bone, fat, striated muscle and neural tissue
Zhang et al (2012) [26]	OMSCs	CD73, CD90, CD44, CD105	Adipocytes, chondrocytes and osteocytes	Bone
Machado et al (2012) [30]	SHEDs	CD29, CD73 and CD105	Osteocytes and odontoblasts	Bone/dentin
Nakamura et al (2009) [31]	SHEDs	CD29, CD73, CD105 and CD44	—	—
Nakajima et al (2018) [36]	SHEDs	CD29, CD44, CD73, CD105, CD146	Osteocytes	Bone
Ginani et al (2018) [37]	SHEDs	CD90, CD73, CD105	Osteocytes, adipocytes	—
Huang et al (2018) [39]	SHEDs	CD90, CD73, Oct4	Osteocytes	—
Tang et al (2011) [41]	OMSCs	CD90, CD29, CD44, CD105, CD146, STRO-1	Osteocytes, adipocytes and chondrocytes	Connective tissue
Treves-Manusevitz et al (2013) [43]	OMSCs	CD29, CD73, CD90, CD105, CD166	Osteocytes, adipocytes and chondrocytes	Bone
Mitrano et al (2010) [44]	OMSCs	CD90, CD105, CD73, CD44, CD13	Osteocytes, adipocytes and chondrocytes	—
Rajan et al (2017) [45]	OMSCs	CD29, CD90, CD73, CD105, CD44, Oct4, SOX-2, SSEA-4	Osteocytes, adipocytes and chondrocytes	—

MSCs=mesenchymal stem cells; **ESC**=embryonic stem cell; **SHEDs**= human exfoliated deciduous teeth stem cells; **OMSCs**=oral mucosa stem cells.

self-renewal and multi-lineage differentiation *in vitro* as well as *in vivo*^{22, 23}.

The demonstrated great potential of SHEDs and OMSCs^{16, 17, 24-26} and the relatively easy collecting and harvesting method, led us to suggest the opportunity to use the deciduous teeth, naturally lost in pediatric age, and/or the oral mucosa, for TR purposes of patients.

For this reason, the aim of this paper is to review and clarify the regeneration potential of SHEDs and OMSCs.

Deciduous teeth and oral mucosa: two promising reservoir of mesenchymal stem cells for the presence and the future of pediatric patient

Easily accessible and with a great regenerative ability, SHEDs are valuable candidate progenitors for TR^{10, 27}. The natural intrinsic mechanism involving the transition from deciduous to permanent teeth makes them a reliable source of MSCs, eventually employable in the future of pediatric patient. Various studies showed that, as DPSCs, SHEDs have not only interesting *in vitro* and *in vivo* TR potential, being able to repair bone, cartilage, blood vessels and adipose tissue, but also a higher proliferation rate, increased population doubling and stemness properties for longer periods, compared to DPSCs and BM-MSCs^{28-31 17, 32, 33}. These properties make them a simple, straightforward and convenient source of stem cells for treatment of many current or potential diseases of the pediatric patient, with minimal or no trauma³²⁻³⁴.

Nakajima et al. compared bone tissue regeneration of SHEDs with that of DPSCs and human bone marrow stem cells (hBMSCs) by micro-computed tomography and histological analysis, demonstrating that SHEDs have equal bone formation ability to hBMSCs and DPSCs, and produce larger osteoids and wider distribution of collagen fibers than other sources, resulting in an efficient capability of bone defect regeneration³⁵.

More recent studies also focused on their potential neuroprotector effects, showing that SHEDs, after transplantation in a spinal cord injury murine model, were able to induce anti-apoptotic factor activity, resulting in the neuroprotective effects on moto-neurons³⁶.

Finally, it was also demonstrated that the proliferation and the differentiation ability of SHEDs may be enhanced by chemical and physical treatments. Two recent studies showed that physical treatments, as laser irradiation, are able to enhance the *in vitro* proliferation of SHEDs^{37, 38}. Moreover, in presence of specific factors, SHEDs showed higher proliferation and differentiation ability: chitosan scaffolds, functionalized with divalent metal phosphates, enhance the osteo-differentiation ability of SHEDs³⁹. *Araújo et al.* analysed the effects of mineral compounds on the regenerative properties of SHEDs and demonstrated a

proliferative and differentiation advantage⁴⁰. The studies above described open up to the possibility of a regenerative procedure based on SHEDs, supported or not by specific physical or chemical factors.

If on one side it is worth discussing about SHEDs TR potential, on the other side, it is also important talking about the surprising properties of OMSCs. As described above, the human oral mucosa is divided in the masticatory, the specialized and the lining mucosa¹⁶. Many laboratories have worked up-to-now to analyze and characterize the different stem cell populations, contained in the oral mucosa, by the classical MSCs hallmarks as self-renewal, *in vitro* and *in vivo* multi-lineage differentiation and expression of typical stem cell markers¹⁶.

Izumi et al. successfully isolated and characterized an oral keratinocyte-enriched population of progenitor-stem cells: able to *in vitro* form colonies, they *ex vivo* regenerate an oral mucosa equivalent²⁴.

Also *Marynka-Kalmani et al.* identified in oral connective tissue (i.e. the lamina propria) another promising source of stem cells; the population of SCs was heterogeneous: part of it expressed embryonic stem cell transcription factors as Oct4, SOX2 and SSEA-4 part of it expressed the canonical MSC markers as CD29, CD73, CD90, CD105, CD166 and Stro1. They were able to differentiate towards mesodermal and ectodermal lineages²⁵.

Many recent works also focused on investigating the regenerative properties of cells isolated from gingival tissue. Gingival mesenchymal stem cells (GMSCs) have been demonstrated to express MSCs markers, to have a high clonogenic and proliferation ability and, when in specific growth condition, show multipotency differentiation ability into several cellular lineages. They, indeed, resulted positive for MSCs markers as CD90, CD105, CD73, CD44 and CD13, and negative for hematopoietic markers as CD34, CD38, CD45 and CD54 and in presence of specific growth factors, they differentiated towards osteoblasts, adipocytes and chondroblasts, confirmed by the expression of osteogenic markers (i.e. bone sialoprotein, alkaline phosphatase and osteocalcin), adipogenic markers (i.e. peroxisome proliferator-activated receptor γ) and chondrogenic markers (i.e. collagen 2A1 and aggrecan)⁴¹⁻⁴⁴.

Noteworthy, neuroprotection and immune-modulation of GMSCs were recently investigated. The conditioned medium by human GMSCs is able to increase the levels of neuroprotective factors (e.g. neurotrophins and anti-inflammatory cytokines) resulting in inhibition of apoptosis after injury and neuroprotection⁴⁵. GMSCs are able to inhibit proliferation of peripheral blood lymphocytes, increasing the expression of anti-inflammatory factors; indeed, the infusion of human GMSCs in an experimental model of colitis decreases the resulting effects of inflammation

(e.g. colon inflammation, weight loss, injury on intestinal mucosa)¹³.

In conclusion, even if more studies will be necessary to make SHEDs and OMSCs available for clinical use, the up-to-now knowledge about their TR potential is encouraging and represents a hopeful starting point for future therapeutic treatments.

Concluding remarks

Regenerative medicine is a constantly evolving interdisciplinary field, deriving from the collaboration of different scientific figures as biologists, clinicians, technologists, chemists, nanotechnologists that cooperate together to develop procedures aimed to manipulate and control cells and the relative surrounding environment, for TR purposes². The need of therapies in all different biomedical areas and the promising properties of the adult MSCs led to the real possibility of successful therapies able to treat human diseases, in some cases otherwise not treatable, and this represents an attracting perspective for the future of medicine. After years of studies focused on BM-MSCs and adipose tissue mesenchymal stem cells (AT-MSCs), which have well-known great regeneration potential, but whose harvesting and isolation procedures implicate high costs and painful clinical practices, the aim of many studies is now more and more focused on developing an easier, painless and more economic procedure. Oral cavity has been identified as a rich and more accessible reservoir of MSCs. Noteworthy, oral mucosa and pulp tissue from deciduous teeth, naturally lost in pediatric age, represent a promising and accessible source of MSCs.

Nevertheless, even if promising candidates for regenerative therapies, there are also some limitations of the above described MSCs use in clinical practice. The MSCs *in vitro* cell culture needs fetal bovine serum (FBS) that provides nutrients to cells. However, FBS addition could affect the safeness of MSCs for clinical use, since it could result toxic for patients. A recent study shows a little difference in SC marker expression and differentiation ability of DPSCs and BM-MSCs, grown in serum/xeno-free medium⁴⁶. Thereby, it is necessary to develop a safe and qualified protocol that both allowed MSC growth and expansion enough to have a good amount of cells for therapies, and ensured the safeness of cell application in clinics; it is the base of a safe translational research.

Another limitation of oro-facial MSC clinical use is the availability of cells throughout the whole patient's life. For instance, SHEDs need to be cryo-preserved until the adult age of patient; but a valuable cryo-preservation protocol is still under investigation⁴⁷⁻⁴⁹. Instead, this is not a limitation of OMSCs since the mucosa is always easily accessible and available, and there is no necessity of long-term preservation.

Finally, the lack of a standardized protocol for clinical application and the scarcity of human clinical trials evaluating the pros and cons of the use of oro-facial MSCs still limit their employment in clinical practice.

Many studies are still necessary to develop a safe protocol for oro-facial MSC clinical use; however, the up-to-now results represent the encouraging idea of an ever-available ready-to-use autologous reservoir of MSCs to use for treatment of any disease occurring during the childhood (e.g. burns, orthopaedic disorders, spinal cord dysfunction, hypospadias, physeal injuries and genetic diseases as bullous epidermolysis) or adult age, without need of additional surgical sites or economic cost, once everyone naturally lost milk teeth.

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Abbreviations

AT-MSC	Adipose tissue mesenchymal stem cell
BM	Bone Marrow
BM-MSC	Bone marrow mesenchymal stem cell
CD	Cluster of differentiation
DPSC	Dental pulp mesenchymal stem cell
FBS	Fetal bovine serum
GMSC	Gingival mesenchymal stem cell
hBMSC	Human bone marrow stem cell
hDPSC	Human dental pulp mesenchymal stem cell
MSC	Mesenchymal stem cell
NANOG	Homeobox protein NANOG
OCT4	Octamer-binding transcription factor 4
OMSC	Oral mucosa stem cell
RM	Regenerative medicine
SC	Stem cell
SHED	Human exfoliated deciduous teeth stem cell
SOX-2	SRY (sex determining region Y)-box 2
SSEA-4	Stage-specific embryonic antigen-4
TE	Tissue engineering
TR	Tissue regeneration

Conflicts of interest

The authors declare that there is no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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