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Dental-derived stem cells and biowaste biomaterials: What's next in bone regenerative medicine applications

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Abstract: The human teeth and oral cavity harbor various populations of mesenchymal stem cells (MSCs), so called dental-derived stem cells (D-dSCs) with self-renewing and multilineage differentiation capabilities. D-dSCs properties involves a strong paracrine component resulting from the high levels of bioactive molecules they secrete in response to the local microenvironment. Altogether, this viewpoint develops a general picture of current innovative strategies to employ D-dSCs combined with biomaterials and bioactive factors for regenerative medicine purposes, and offers information regarding the available scientific data and possible applications.

Introduction

Mesenchymal stem cells (MSCs) are undifferentiated high proliferative cells, well known for their self-renewal and differentiation properties in multiple cell lines, in response to the microenvironmental conditions in which they grown (Charitos *et al.*, 2021).

The International Society for Cellular Therapy (ISCT) in 2005 had established benchmarks for defining MSCs with the following minimal criteria: (a) must be plastic-adherent and fibroblastoid under standard culture conditions (b) must display immunophenotypic expression of CD73, CD90, CD105 and a lack of expression of CD34, CD45, CD14, CD19, CD79a, CD31 and HLA-DR surface markers (c) and lastly, (d) must have the capacity to minimally differentiate into adipocytes, osteocytes, and chondroblasts *in vitro* (Horwitz *et al.*, 2005; Praveen Kumar *et al.*, 2019).

MSCs are also able to produce immunomodulatory factors, leading to the creation of a regenerative microenvironment. Thanks to these properties, MSCs may play a main role in regenerative medicine, and some clinical studies have demonstrated that MSCs from different sources may have the ability to repair injured tissues, such as bone (Salgado *et al.*, 2020; Gugliandolo *et al.*, 2021) and corneal tissue engineering (Nosrati *et al.*, 2021a), wound healing and muscle (Martínez-Sarrà *et al.*, 2017), cartilage regeneration (Mata *et al.*, 2017; Dang *et al.*, 2021), as well spinal cord injuries (Asadi-Golshan *et al.*, 2021).

To gather the rising demand for human tissues and organs since of diseases or accidents, scientific research is progressively more focusing on tissue engineering and regenerative medicine. The core common methods of set up

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tissues and organs are (I) the addition of bioscaffolds, growth factors, and MSCs into a culture medium for *in vitro* organization and (II) the implantation of bioscaffolds and growth factors for *in vivo* organization (Wang *et al.*, 2020). Comparable to the natural extracellular matrix (ECM), topography, morphology and composition of scaffold manage cell behavior, showing influence on cell adhesion, proliferation, differentiation and migration.

Consequently materials structure, architecture and composition should reproduce the native extracellular matrix (ECM) as more strict as it is possible to entirely mimic target tissue environment. Besides, scaffolds should be immunologically inert or influence minimal immunological reaction (Litowczenko *et al.*, 2021; Boccaccio *et al.*, 2018).

In general, the present viewpoint develops a general overview of current innovative strategies to (I) employ D-dSCs combined with biological waste biomaterials, from renewable and biodegradable bioactive compounds, to obtain new materials having lower cost and comparable properties, for bone regenerative medicine purposes, and (II) offers information regarding the available scientific data and possible applications of D-dSCs use.

Following this approach, biowaste could be considered as a high value resource which could be converted into sustainable materials and new active factors fully complying with the circular economy concept.

Dental-derived stem cells (D-dSCs)

In the early begin of the current millennium, moved by the isolation of MSCs from bone marrow (BMSCs), stem cells form oral tissues were firstly isolated from dental pulp (DPSCs).

During the last 20 years, the concept of Dental-derived stem cells (D-dSCs), was associated to a specific kind of MSCs, obtained by specific methods, i.e., separating tissues around human teeth (Figs. 1A and 1B).

So far, nine types of dental stem cells were successfully isolated, including DPSCs, stem cells from human exfoliated deciduous teeth (SHEDs), apical papilla stem cells (SCAPs), periodontal ligament stem cells (PDLSCs), dental follicle stem cells (DFSCs), gingival mesenchymal stem cells (GMSCs), human tooth germ stem cells (TGPCs), alveolar bone mesenchymal stem cells (ABMSCs) and human periapical cyst mesenchymal stem cells (hPCy-MSCs), as well reported in scientific literature (Lei *et al.*, 2021; Paduano *et al.*, 2016, Ballini *et al.*, 2007a; Cantore *et al.*, 2018; Ballini *et al.*, 2015; Mason *et al.*, 2014).

During development of the maxilla and mandible, neural crest cells (NCCs) delaminate from the neural tube and migrate towards the maxillary and mandibular primordia (Blentic *et al.*, 2008) where interact with local tissue and initiate a differentiation process that gives rise to ectomesenchymal derivatives express several factors which play an important role during the differentiation of bone, cartilage, and teeth (da Silva Sasso *et al.*, 2021).

Moreover, some explorative studies, showed the differential expression of pro-angiogenic factors for D-dSCs by suggesting this MSCs populations could act as scaffolds, potentially promote the vascularization of hard (Hilkens *et al.*, 2014) and soft (Nosrati *et al.*, 2021b) tissues. Since oral tissues develop from migrating cranial cells, dental-derived MSCs display ready availability and high proliferation abilities (Chen *et al.*, 2021; Wang and Cao, 2019; Zhang *et al.*, 2019; Matsui *et al.*, 2018; Carnevale *et al.*, 2018; Inada *et al.*, 2017; Huang *et al.*, 2017; Aguilar and Lertchirakarn, 2016; Pan *et al.*, 2016).

Apart from their direct cellular activity following stem/progenitor cells engraftment, D-dSCs/MSCs mark on regenerative medicine is indirectly mediated through paracrine effects (El Moshy *et al.*, 2020). In fact, throughout the release of trophic and modulatory bioactive factors (secretome) into the surrounding environment, they can influence tissue homeostasis, can stimulate cellular migration, proliferation, immunomodulation and promote tissue regeneration (El Moshy *et al.*, 2020).

In addition D-dSCs exhibit peculiar advantages first of all, for the easy accessible localization, compared to other sites (i.e., iliac crest), and secondly for using post-natal tissues (by-passing ethic concerns), of samples that most of the time are extracted for clinical issues (i.e., orthodontics reasons, impacted teeth, etc), becoming in this way a biowaste specimen ready to use in wider circular economy concept.

The DPSCs, SHEDs, SCAPs and ABMSCs, posses the advantages of potential clinical applications in the field of angiogenesis and/or dental/pulp complex formation, as well PDLSCs and DFSCs show features for periodontal

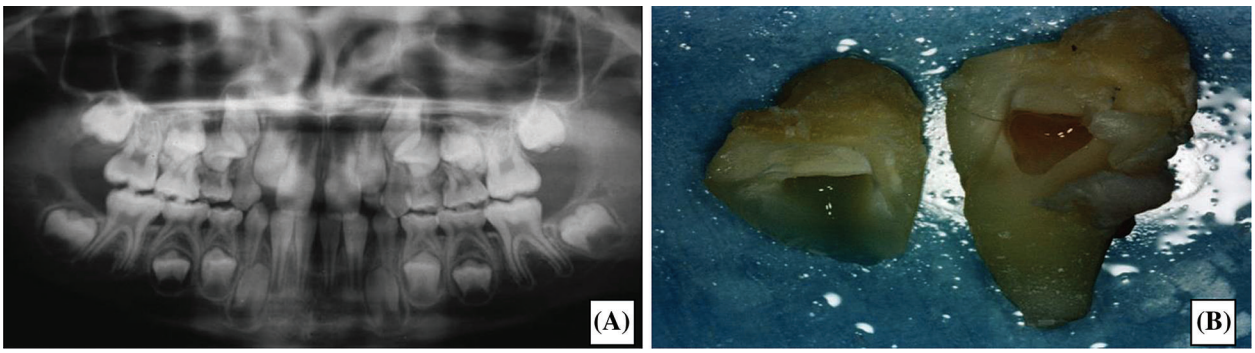

FIGURE 1. Clinical Images. (A) Orthopantomogram of different MSCs sources: immature dental tissues follicle/germ (DFSCs/SCAPs/TGPCs), Dental Molars (DPSCs), Deciduous Teeth (SHEDs) and alveolar bone (ABMSCs). (B) After the cut, the pulp chamber and its content were revealed. The tooth pulp provided DPSCs. For clinical images, written and verbal information was given to the patient before the procedure, and written informed consent was obtained, in full accordance with the World Medical Association Declaration of Helsinki on experimentation involving human subjects, as revised in 2008.

tissue regeneration (Chen *et al.*, 2021; Mason *et al.*, 2014; Ballini *et al.*, 2007b).

On the other hand, GMSCs, TGPCs and hPCy-MSCs seems to demonstrate potential applications in nerve regeneration (Chen *et al.*, 2021; Paduano *et al.*, 2016) (Table 1).

The limitation reported in literature, in general for all type of MSCs, included the D-dSCs family, are most of the time limited to the specimen collection contamination, culture conditions/passages, and dependent for the specific clinical purpose as well the safety issues (Lee, 2018; Ballini *et al.*, 2017).

Current perspectives in regenerative medicine applications

The similarity of gene expression profiles between D-dSCs, osteoblast precursors and BMSCs has recently been reported, as well their stemness properties (Posa *et al.*, 2021; Cantore et al. 2017; Ballini *et al.*, 2017; Mori *et al.*, 2010). In fact D-dSCs have revealed the expression of transcription factors like kruppel-like factor 4 (Klf4), octamer-binding transcription factor 4 (OCT4), homeobox transcription factor Nanog (Nanog), v-myc avian myelocytomatosis viral oncogene homolog (c-Myc), and SRY (sex determining region Y)-box 2 (Sox2), that plays a key regulatory activity in the stem cell self-renewal process in replenishing mature cells that constantly die due to normal and constant tissue turnover reprogramming (Ballini *et al.*, 2019). In this light, Takahashi and Yamanaka showed in 2006 (Charitos *et al.*, 2021) that the introduction of specific genes encoding transcription factors such as OCT4, Nanog, Klf4, c-Myc and Sox2 in keeping the pluripotency status of stem cells has been well confirmed either in embryonic stem cells (ESCs) or in induced pluripotent stem cells (iPS), keeping them in a stemness state preventing their differentiation, and sustaining their self-renewal.

The term "tissue engineering" identifies tissue regeneration procedures of the human organism through the seeding of cells on structures (scaffolds) made up of specific materials and with peculiar characteristics, their cultivation in special reactors (bioreactors) until the colonization of the same scaffold, and to the neo-formation of a bioengineered tissue (Bettini *et al.*, 2020; Cantore *et al.*, 2018; Nosrati *et al.*, 2020). The recent trend is the scaffolds creation for the *in vitro* genesis of bio-tissues (Wang *et al.*, 2020); in this case, bioabsorbable polymeric materials used in 3D printers are employed, generally with fused deposition modeling (FDM) techniques (filament extrusion) or with bioprinting (nozzles that spray small amounts of liquid material). In studies on animal models, some authors have tested the isolation and use of D-dSCs and bio-scaffolds for bone regeneration, obtaining neo-formation of bone tissue in the grafted sites (Shoushrah *et al.*, 2021).

Bio-scaffolds material properties in bone regeneration are a key factor for maximizing cell recruitment and differentiation and to minimize local induced inflammatory response after *in situ* implantation. Such materials are selected according to the following characteristics: (i) ability to provide appropriate mechanical support to the tissue, (ii) ability to determine the digestibility of the scaffold, and (iii) proficiency to trigger the appropriate immune response to promote tissue regeneration and healing (Li *et al.*, 2019). In this light, is possible that natural biomaterials can control macrophage activation and lower the foreign body reaction mediating constant cross-talk with inflammatory cells (Li *et al.*, 2019; Swartzlander *et al.*, 2015).

Currently, still if in the pioneering phase, there is the use of polyphenols in the regeneration of bone tissue. In particular, has been investigated that, the oligostylbenes (natural compounds) deriving from the recovery of waste of agri-food processes (waste compounds), in association with D-dSCs/MSCs, can promote the early consolidation and mineralization of bioengineered tissue with a significant decrease in the healing time (Di Benedetto *et al.*, 2018; Murgia *et al.*, 2019; Posa *et al.*, 2020). Preliminary experiments have shown an increased deposition of mineralized matrix in osteoblastic differentiated MSCs in

TABLE 1

Main D-dSCs markers and related functions

Marker	Function	References
CD146	Cell adhesion molecule and an integral membrane glycoprotein at the intercellular junction	Matsui *et al.* (2018)
CD271 or p75 neurotrophin receptor (NTR)	Well-conserved transmembrane pro-neurotrophin/neurotrophin receptor that plays critical roles in the maintenance of nerve cell viability and in tooth development and epithelial-mesenchymal transition (EMT).	Pan *et al.* (2016)
STRO-1	Marker that recognizes a trypsin-insensitive epitope on perivascular cells, and has shown enhanced proliferation potential	Carnevale *et al.* (2018)
Alkaline phosphatase (ALP)	Expressed in undifferentiated cells. ALP is also a marker of neuronal progenitor cells, human myogenic progenitor cells (also called "pericytes"), and BMMSCs	Inada *et al.* (2017)
Nuclear factor I-C (NFIC)	Key regulator of tooth development	Zhang *et al.* (2019)
Wnt inhibitory factor 1 (WIF1)	Enhance the dentinogenic differentiation potential in SCAPs via its regulation of OSX	Wang and Cao (2019)
CD39 and CD73	CD39 and CD73 are coexpressed on GMSCs and regulate T cells, which play a catalytic role to promote immune regulation and reduce inflammation	Huang *et al.* (2017)
CD24	Specific surface marker, which can distinguish SCAP from DPSCs and BMSCs	Aguilar and Lertchirakarn (2016)

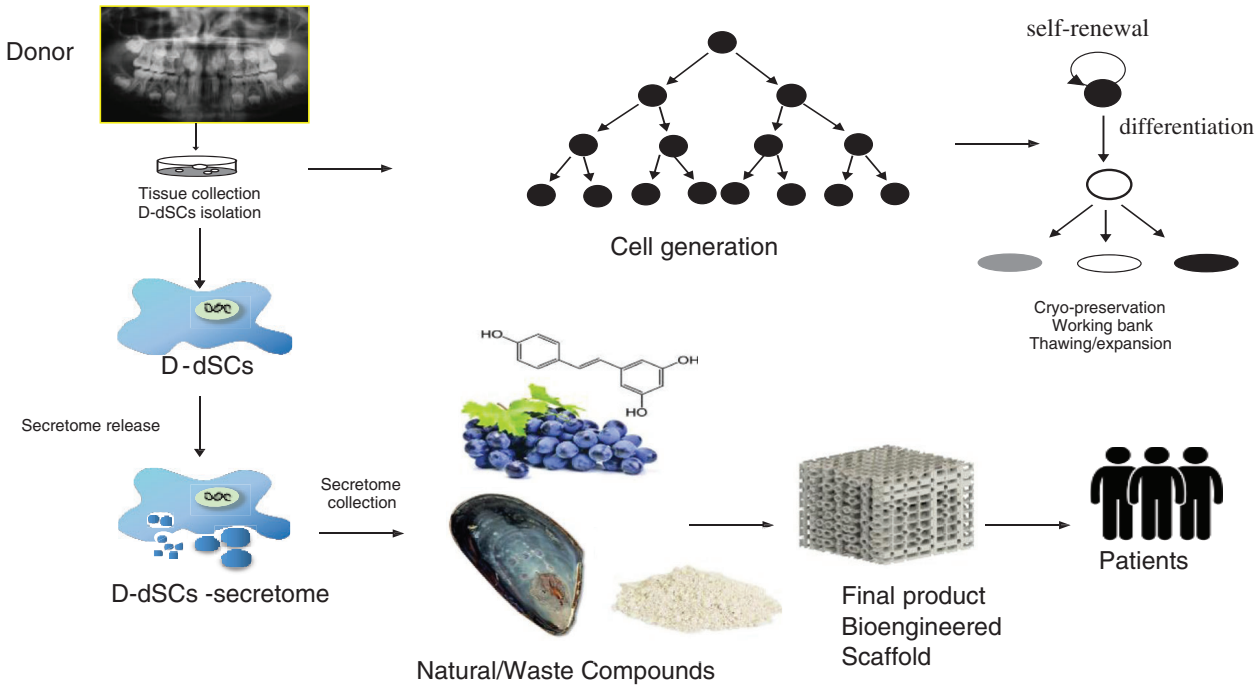

FIGURE 2. From waste to regenerative medicine: biotechnological applications of D-dSCs in the context of innovative sustainability and circular economy.

presence of natural compounds/waste compounds of agri-food origin, as well as potential antioxidant, anti-aging and anti-tumor capacities have been documented (di Benedetto *et al.*, 2018; Di Domenico *et al.*, 2020). These biological characteristics could have an impact on the immunomodulatory and therapeutic potentials of D-dSCs *in vivo* since, in the implantation phase, the contact of the stem cells with the bacteria through the Toll-like receptors (TLRs), triggers the activation of an inflammatory response inducing vascularization and osteogenesis (Fehrmann *et al.*, 2020). To date, it has been shown that the TLRs expression pattern could influence the therapeutic potential of adult MSCs during *in vivo* transplantation (Fehrmann *et al.*, 2020; Fawzy El-Sayed *et al.*, 2017).

Consequently, it is possible to speculate that the intrinsic antibacterial, anti-inflammatory and antioxidant activity of natural compounds/waste compounds could be inhibitory on the production of pro-inflammatory molecules, in the maintenance phase of a bio-scaffold loaded with D-dSCs *in vivo* interacting, in a positive way, with the oral microbiota (Vázquez *et al.*, 2020). Furthermore, in the literature are reported *in vitro* studies about the realization of scaffolds deriving from a mixture of organic compounds included in some easily available waste compounds, such as egg shells or mussels (*Mytilus edulis*), useful for the recovery of calcium phosphates in the form of hydroxyapatite (HA), hair keratin (improvement of scaffold properties by cell adhesion), and jellyfish, rich in bioactive compounds (collagen) already used in nutraceuticals and cosmeceuticals (Scialla *et al.*, 2020; Arslan *et al.*, 2017). The bio-scaffold obtained according to the principles of the circular economy, can be additionally soaked with polyphenols (strengthening osteogenic and osteoinductive effects, anti-inflammatory, antibacterial and antioxidant properties) thanks to diatomaceous flour, which also act to consolidates the scaffold's mechanical properties (Bonifacio *et al.*, 2020) (Fig. 2).

Current limitations

While the preclinical evidence showing the regenerative and immunomodulatory potential of the D-dSCs/MSCs secretome continues to expand rapidly, the clinical studies revolving around this hypothesis are still scarce (Ferreira *et al.*, 2018). Even if D-dSCs/MSCs have an innate potential to induce and/or contribute to regeneration, this potential is now known to be greatly influenced by diverse extrinsic factors such as the tissue source of the D-dSCs/MSCs, the health status and age of the MSCs donor, the batch/lot of serum used for the *in vitro* culture of the D-dSCs/MSCs, passage number, oxygen concentration (Ferreira *et al.*, 2018), and in general for cell culture, the presence/absence of a pro-inflammatory, apoptotic or genotoxic environment when the cells are infused (Mori *et al.*, 2009; Ferreira *et al.*, 2018; Mori *et al.*, 2007; Lovreglio *et al.*, 2006). The success of D-dSCs/MSC transplantation relates to large scale *in vitro* expansion of therapeutically qualified cells under Good Manufacturing Practice (GMP) conditions, although a standard therapeutic cell dose of D-dSCs/MSCs, the route of administration and the number of doses are still being optimized. Although culture expansion of D-dSCs/MSCs has been widely used, a few issues need to be highlighted (Praveen Kumar et al., 2019; Ballini *et al.*, 2018).

Conclusions

Dental-derived stem cells are today considered as an intriguing milestone of the regenerative medicine. In summary, an advanced understanding of the interactions among D-dSCs/MSCs, immune cells, and biomaterials and natural compounds that modulate immunoregulation and response to inflammation could be translated into the development of advanced biomaterials and change the way that experts approach tissue regeneration. Such advancements can open

new horizons toward more efficient personalized therapies and optimized clinical outcomes. Starting from the contents of the present viewpoint, the scientific community will be stimulated to experiment new ideas, to improve the knowledge of D-dSCs, and to speed up their clinical application, so to improve regenerative medicine approaches.

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