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Plasticity of human dental pulp stromal cells with bioengineering platforms: A versatile tool for regenerative medicine

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ABSTRACT

In recent years, human dental pulp stromal cells (DPSCs) have received growing attention due to their characteristics in common with other mesenchymal stem cells, in addition to the ease with which they can be harvested. In this study, we demonstrated that the isolation of DPSCs from third molar teeth of healthy individuals allowed the recovery of dental mesenchymal stem cells that showed self-renewal and multipotent differentiation capability. DPSCs resulted positive for CD73, CD90, CD105, STRO-1, negative for CD34, CD45, CD14 and were able to differentiate into osteogenic and chondrogenic cells. We also assayed the angiogenic potential of DPSCs, their capillary tube-like formation was assessed using an *in vitro* angiogenesis assay and the uptake of acetylated low-density lipoprotein was measured as a marker of endothelial function. Based on these results, DPSCs were capable of differentiating into cells with phenotypic and functional features of endothelial cells. Furthermore, this study investigated the growth and differentiation of human DPSCs under a variety of bioengineering platforms, such as low frequency ultrasounds, tissue engineering and nanomaterials. DPSCs showed an enhanced chondrogenic differentiation under ultrasound application. Moreover, DPSCs were tested on different scaffolds, poly(vinyl alcohol)/gelatin (PVA/G) sponges and human plasma clots. We showed that both PVA/G and human plasma clot are suitable scaffolds for adhesion, growth and differentiation of DPSCs toward osteoblastic lineages. Finally, we evaluated the interactions of DPSCs with a novel class of nanomaterials, namely boron nitride nanotubes (BNNTs). From our investigation, DPSCs have appeared as a highly versatile cellular tool to be employed in regenerative medicine.

1. Introduction

Among mesenchymal stromal cells (MSCs) of autologous origin, human dental pulp stromal cells (DPSCs) have received growing attention in recent years firstly due to the easy accessibility of the tissue in any adult individual.

Dental pulp tissue is thought to be derived from migratory neural crest during development (Peters and Balling, 1999). Dental pulp is a soft connective tissue and its main functions are to produce dentin, and to maintain the biological and physiological vitality of the dentin. DPSCs are multipotent stromal cells derived from neural crest and mesenchyme and have the capacity to differentiate into multiple cell lineages. Firstly, Gronthos et al. (2000, 2002) have isolated post-natal stromal cells from the human dental pulp of permanent teeth. Other stromal cell populations from surrounding tissues of the tooth have been isolated from periodontal ligament, human exfoliated deciduous teeth, apical papilla and

Abbreviations: DPSCs, dental pulp stromal cells; MSC, mesenchymal stromal cells; SHEDs, stromal cells from human exfoliated deciduous; BM-MSCs, bone marrow-derived mesenchymal stromal cells; NTFs, neurotrophic factors; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; PPP, platelet poor plasma; US, ultrasounds; LIUS, low intensity US; LFUS, low frequency US; PVA, poly(vinyl alcohol); G, gelatin; BNNTs, boron nitride nanotubes; α-MEM, minimum essential medium alpha modification; FBS, fetal bovine serum; PDT, population doubling time; CPs, chondrogenic pellets; HUVECs, human umbilical vein endothelial cells; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

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dental follicle precursor cells (Miura et al., 2003; Seo et al., 2004; Sonoyama et al., 2006). These post-natal populations have MSC-like features, namely the capacity for self-renewal and the potential to differentiate into multiple lineages including osteoblasts and chondroblasts (Huang et al., 2009). However, DPSCs show higher self-renewal ability, immunomodulatory capacity and proliferation *in vitro* than bone marrow-mesenchymal stromal cells (BM-MSCs); furthermore, they preferentially differentiate to osteoblasts rather than into adipocytes (Gronthos et al., 2000; Pierdomenico et al., 2005). Although the majority of studies have focused their attention on the ability of DPSCs to differentiate into odontoblast-like cells (Almushayt et al., 2006; Cordeiro et al., 2008; Paula-Silva et al., 2009) or osteoblasts (Laino et al., 2005, 2006; D'Aquino et al., 2007), it has also been discovered that they are capable of differentiating into other cell types, including smooth muscle cells (Kerkis et al., 2006; Gandia et al., 2008) and neurons (Arthur et al., 2008; Kadar et al., 2009). DPSCs express nestin and glial fibrillary acidic protein and, under appropriate stimuli, are capable of differentiating into functionally active neurons (Arthur et al., 2008), influencing endogenous recruitment of neural stem cells and generating neurospheres (Sasaki et al., 2008). Neural stem cell markers, such as nestin, expressed in DPSCs reflect the neural origin of dental pulp (Kerkis et al., 2006; Estrela et al., 2011).

Recently, we have investigated specific molecular profiles of human stromal stem cell populations derivated from different tissues, particularly with regard to the global HOX gene family expression profile (HOX code) and their three amino acid loop extension co-factor subfamilies (Picchi et al., 2013). The different levels of HOX expression detected in stromal cells with different potency strongly suggest that HOX genes may not only reflect positional and embryological cell identity, but also indicate the cellular position within the stem cell hierarchy. Such considerations support the growing evidence that HOX code provides a "biological fingerprint" to distinguish stem cell populations (Chang et al., 2002; Moens and Selleri, 2006). Our previous study shows that DPSCs exhibit extremely low levels of expression of a few HOX genes, confirming previous findings, and in line with the neuroectodermal origin of DPSCs (Couly et al., 2002; D'Antò et al., 2006). It is known that neural crests are HOX negative and indeed the few active HOX genes in DPSCs are expressed at barely detectable levels.

MSCs appear to exert paracrine trophic effects through the secretion of bioactive molecules (Caplan and Dennis, 2006; Caplan, 2007) as the neurotrophic factors (NTFs). In particular, brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) produced by DPSCs, have been shown to have a crucial influence over neurons in the central nervous system such as motor neurons and dopaminergic neurons of the substantia nigra (Nosrat et al., 2001, 2004). In our previous study, we found that high levels of BDNF, NGF transcripts were constitutively expressed by DPSCs and that the neuroprotective effect of DPSCs against two neurotoxins on an *in vitro* model of Parkinson's could be due to soluble factors, such as BDNF and NGF, released by DPSCs (Nesti et al., 2010). DPSCs may thus be an alternative source in cell therapy for neurological diseases.

Recent highlights have started considering stem cells not only as the final therapeutic product, but rather as part of complex bioengineered therapeutic strategies. In this view, MSCs can be fully appreciated as a versatile tool in combination with biomaterials and biomedical devices to guide their commitment in various human body tissues (Kshitiz et al., 2012; Kinney and McDevitt, 2013). Therefore, stem cell plasticity thus represents a master feature. The potential use of stem cells in bioengineering-based therapies includes tissue engineering and nanotechnology. As a proof of concept, we tested the biological response of DPSCs in three different bioengineering platforms. As an example of biomedical device-assisted platforms for tissue regeneration, ultrasounds (US) represent a non-invasive and versatile tool that has been shown to promote tissue repair, such as cartilage healing in animal models of articular cartilaginous defects (Cook et al., 2001). Studies on the underlying mechanism leading to cell response to US stimuli are ongoing, however preliminary evidence in rat chondrocyte cultures have highlighted that US can enhance the synthesis of proteoglycans depending on their application cycles and intensity (Parvizi et al., 1999). In line with these observations, recent reports have pointed out that low intensity US (LIUS) can exert a favorable effect on the proliferation, extracellular matrix synthesis and chondrogenic differentiation of MSCs *in vitro* (Lee et al., 2006; Shah et al., 2013). Differently from LIUS, used for diagnostic purposes, low frequency US (LFUS) (range 20–100 kHz) is used in sonophoresis and dentistry since they transfer higher mechanical energy. In our study we tested the efficacy of LFUS on DPSC pellets under chondrogenic differentiation with respect to traditional pellet culture.

Moreover, we investigated DPSCs for tissue engineering applications, using biocompatible three-dimensional spongy materials to assess their differentiation capability. Poly(vinyl alcohol) (PVA) has been among one of first synthetic macromolecules employed in both implantable and non-implantable medical devices (*e.g.*, contact lenses, artificial meniscus) due to its characteristics including low protein adsorption, biocompatibility, high hydrophilicity, easy processability and chemical inertia (Alves et al., 2011). Additionally, PVA can be easily added to biologic molecules to obtain bioartificial matrices with specific architectural features (Cascone et al., 2004). Among which, gelatin (G) is a natural protein derived from collagen able to promote cell adhesion (Dubruel et al., 2007). Spongy scaffolds based on PVA and G have been successfully tested with gingival fibroblasts, being reported as very promising substrates for tissue engineering (Moscato et al., 2008). In the present study, PVA/G sponges were prepared and investigated *in vitro* for three-dimensional growth and osteogenic differentiation of DPSCs.

Finally, nanomedicine is emerging as a powerful source of innovative cell-targeted-therapies based on nanoengineered materials, whose toxicological risks have yet to be disclosed. The balance of these features has thus become an intriguing challenge for successful development of therapeutically effective nanosystems. In particular, due to their small size, nanoparticles can enter the cell membrane and be used as non-viral vectors. Boron nitride nanotubes (BNNTs) are a class of ceramic nanoparticles with a tubular shape that have attracted recent attention due to their excellent physical properties and putative biocompatibility (Ciofani and Danti, 2012). In this study, we report our preliminary findings on the interactions of BNNTs with DPSCs, aimed at supporting the future disclosement of novel nanomedicine-based therapies.

In summary, the specific aims of the current study are to improve understanding of the biological properties of human DPSCs. Moreover, we have investigated DPSC growth and differentiation in different bioengineering applications, evaluating their multipotent potential in regenerative medicine.

2. Materials and methods

2.1. Extraction and isolation of human DPSCs

Human dental pulps were obtained from molars of healthy subjects ($n = 10$) 18–35 years of age, after informed consent (Oral Surgery Department, Santa Chiara Hospital, Pisa, Italy) according to a protocol approved by the local University committee on Ethics in Medicine. Each subject, before extraction, was checked for systemic and oral diseases and pre-treated a week before with professional dental hygiene. Before extraction, the dental crown was rinsed with a 0.2% chlorexidin gel (Dentosan, Johnson & Johnson Medical S.p.A., Rome, Italy) for 2 min. Radicular dental pulps were obtained, using