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Progress in the use of dental pulp stem cells in regenerative medicine

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Abstract

The field of tissue engineering is emerging as a multidisciplinary area with promising potential for regenerating new tissues and organs. This approach requires the involvement of three essential components: stem cells, scaffolds and growth factors. To date, dental pulp stem cells have received special attention because they represent a readily accessible source of stem cells. Their high plasticity and multipotential capacity to differentiate into a large array of tissues can be explained by its neural crest origin, which supports applications beyond the scope of oral tissues. Many isolation, culture and cryopreservation protocols have been proposed that are known to affect cell phenotype, proliferation rate and differentiation capacity. The clinical applications of therapies based on dental pulp stem cells demand the development of new biomaterials suitable for regenerative purposes that can act as scaffolds to handle, carry and implant stem cells into patients. Currently, the development of xeno-free culture media is emerging as a means of standardization to improve safe and reproducibility. The present review aims to describe the current knowledge of dental pulp stem cells, considering in depth the key aspects related to the characterization, establishment, maintenance and cryopreservation of primary cultures and their involvement in the multilineage differentiation potential. The main clinical applications for these stem cells and their combination with several biomaterials is also covered.

Key Words: *cell culture, clinical applications, dental pulp stem cells, DPSCs, scaffold, tissue engineering*

Introduction

The general principles of tissue engineering involve three essential components: identification of appropriate cells, development of three-dimensional (3D) scaffolds and inductive morphogenic signals to regenerate tissues and restore normal organ function [1,2]. In this framework, research is focusing on the use of stem cells (SCs) as the starting point in tissue engineering.

Various SC types have been described in the literature, including embryonic SCs, adult somatic SCs (mesenchymal, hematopoietic and endothelial SCs) and induced pluripotent SCs, artificially derived from adult differentiated somatic cells [3,4]. There is little controversy surrounding human adult SCs because they are not associated with the ethical concerns that embryonic SCs present. SC therapy represents a promising tool, with a focus on the therapeutic potential for regenerative medicine and other biomedical applications [2]. Bone marrow and adipose tissue are conventional sources of mesenchymal stromal cells (MSCs), but the highly invasive cell collection protocols, together with the considerable risk of donor site morbidity, have led to the search for alternative tissues [5,6]. Dental pulp SCs (DPSCs), with their easier surgical access, are a promising SC alternative. The noninvasive nature of DPSC isolation methods compared with other adult tissue sources makes those cells a valuable source of MSCs for tissue repair and regeneration.

Dental pulp is located in the central pulp cavity of each tooth, called the "pulp chamber," and contains a heterogeneous population represented by fibroblasts, endothelial cells, neurons, odonto-osteoprogenitors, inflammatory and immune cells [7,8]. DPSCs were first isolated in 2000 by Gronthos *et al.* [9] from the pulp tissue of third molars. Human DPSCs (hDPSCs) are ectodermal-derived SCs that originate during tooth development from ectodermal cells that migrate from the neural tube to the oral region and finally they differentiate into mesenchymal cells [7,8,10]. This feature confers them special biological properties of MSCs and neural crest SCs. Dental pulp is enclosed into the dental cavity surrounded by mineralized dentin, generating a kind of sealed niche that preserves it from environmental

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differentiation stimuli and maintains SCs in the adult tissue [11]. These cells are responsible for the maintenance and repair of periodontal tissue, have a high proliferation rate and low immunogenicity and exhibit plasticity for multilineage differentiation [7–9]. In fact, they are known to differentiate into various cell lineages such as osteoblasts, chondrocytes, adipocytes, odontoblasts, neural cells and myocytes, among others [8,12,13].

In tissue engineering, the selection of a suitable scaffold and its interaction with SCs is also critical. Biomaterials that act as a scaffold for tissue repair and reconstruction should be able to support cell adhesion and ingrowths while mimicking target tissue and supporting angiogenesis. These biodegradable 3D scaffolds incorporate biological signals that allow them to act as a bioactive platform to precisely control SC behavior [14–17].

As the tissue-engineering industry enters the clinical setting, new and encouraging questions arise. Animal-derived components have traditionally been widely used in SC cultures for more than a century. However, these harbor concerns for human cell therapy, such as the risk of viral, bacterial, fungal and prion contamination and the possible induction of immune rejection of the transplanted cells into the host [3,18]. For these reasons, the development of xeno-free cell culture protocols capable of expanding SCs without affecting their differentiation represents a real challenge to generating cell-based products that meet Good Manufacturing Practice (GMP) standards [5,19].

The aim of this review is to summarize the current knowledge of dental pulp SCs with a special focus on the characterization, establishment, maintenance and cryopreservation of primary cultures and their involvement in the multilineage differentiation potential. The main clinical applications using these SCs, together with the various scaffold biomaterials needed to achieve regeneration of damaged tissue, are also covered.

Dental pulp SCs versus other SCs

SCs are the good cell candidates for use in regenerative medicine because of their biological characteristics. SCs are undifferentiated cells that have the capacity of self-renewal in addition to potential for differentiation into mature specialized cells [20]. With regard to their differentiation potential, SCs can be classified into totipotent pluripotent, multipotent and unipotent cells.

Multipotent adult SCs (ASCs), also referred to as somatic stem cells, are found in quiescent and undifferentiated states in a numerous tissues. ASCs begin to self-renew and differentiate into specialized cells after tissular damage or in homeostasis maintenance. ASC utilization in tissue-engineering techniques could optimize response after transplantation because their autologous origin would avoid possible rejection.

The best known example of multipotent ASCs is the hematopoietic SC (HSC), which can be readily harvested from bone marrow and umbilical cord blood (UCB) [21,22]. Moreover, MSCs can be isolated from a large variety of fetal and extraembryonic tissues and numerous tissues from children and adults [23]. MSCs are capable of differentiating into various mesodermal cell lineages, and their hypo-immunogenic and immunosuppressive characteristics confer excellent suitability for even allogenic cell transplantation [24–26]. MSCs obtained from bone marrow have been widely used in experimental studies, but because of the accompanying pain and morbidity in obtaining them, alternative sources are being sought, including the placenta, human umbilical cord and amniotic fluid. In this sense, DPSCs are a promising source of stem cells for tissue-engineering therapies because of their low cost and greater accessibility versus the costly and invasive techniques required for other ASC isolation. Thus, DPSCs can be obtained without adverse effects on the health of pulp tissue of permanent teeth, habitually from third molars, supernumerary or orthodontically unnecessary teeth [7,27,28].

Dental pulp is derived from ectodermal cells that grow at the periphery of the neural tube and after migrating to the oral region differentiate into cells of the mesenchymal phenotype, therefore some authors have described them as “ectomesenchyma” [11]. Because of their origin, stem cells derived from dental pulp can differentiate into mesodermal and non-mesodermal tissue cells, including osteoblasts, adipocytes, chondrocytes and myocytes, as well as neuronal and endothelial cells, hepatocytes and melanocytes [29–33]. Furthermore, DPSCs do not express the major histocompatibility complex class II antigen on their surface and hold immunoregulatory properties that are able to induce activated T-cell apoptosis [25,34]. Several soluble factors and cytokines secreted by DPSCs could be immunomodulator candidates, among which prostaglandin E2, transforming growth factor beta (TGF-β), hepatocyte growth factor (HGF), interleukin (IL)-6 (IL-6), IL-10, nitric oxide and Fas ligand may be included [35,36]. These immunomodulatory factors may have a profound effect on clinical cell therapy by T-lymphocyte function inhibition and up-regulation of T-cell regulatory stimulating immune tolerance.

Isolation, culture and cryopreservation of DPSCs

In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy