Dental Pulp Stem Cells: Their Potential in Reinnervation and Angiogenesis by Using Scaffolds

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Abstract

Dental pulp is a highly vascularized and innervated tissue containing a heterogeneous stem cell population with multilineage differentiation potential. Current endodontic treatments focus on the preservation of the pulp tissue and the regeneration of dental pulp after pathological insults. Human dental pulp stem cells (hDPSCs) are currently investigated as stem cell–based therapy for pulp regeneration and for peripheral nerve injury in which neurons and Schwann cells display limited regenerative capacity. We have developed a neuronal differentiation protocol for hDPSCs that requires neurosphere formation before neuronal maturation. Moreover, Schwann cell differentiation of hDPSCs in our group revealed that differentiated hDPSCs have acquired the ability to myelinate and guide neurites from dorsal root ganglia. Besides their dynamic differentiation capacity, hDPSCs were shown to exert a paracrine effect on neural and endothelial cells. Analysis of hDPSC conditioned medium revealed the secretion of a broad spectrum of growth factors including brain-derived neurotrophic factor, nerve growth factor, vascular endothelial growth factor, and glial-derived neurotrophic factor. Application of the conditioned medium to endothelial cells promoted cell migration and tubulogenesis, indicating a paracrine proangiogenic effect. This hypothesis was enforced by the enhanced formation of blood vessels in the chorioallantoic membrane assay in the presence of hDPSCs. In addition, transplantation of 3-dimensional–printed hydroxyapatite scaffolds containing peptide hydrogels and hDPSCs into immunocompromised mice revealed blood vessel ingrowth, pulplike tissue formation, and osteodentin deposition suggesting osteogenic/odontogenic differentiation of hDPSCs. Future studies in our research group will focus on the pulp regeneration capacity of hDPSCs and the role of fibroblasts within the pulp extracellular matrix. *(J Endod 2017;■:1–5)*

Key Words

Angiogenesis, biomaterials, dental pulp regeneration, dental stem cells, neurogenesis

Significance

Dental pulp tissue is a strongly vascularized and innervated soft connective tissue. Because the completion of root development is affected by disturbances in normal pulp physiology, the treatment of necrotic immature permanent teeth remains a challenge. The implementation of a dental pulp stem cell–based approach could potentially overcome current treatment-associated concerns. Given their regenerative potential as well as their pronounced neurogenic and angiogenic properties, hDPSCs are a promising tool in regenerative endodontic procedures.

Dental pulp tissue can be characterized as a strongly vascularized and highly innervated soft connective tissue. Although it is considered to have many specialized physiological functions, dental pulp is also very vulnerable to a variety of insults, such as infections, caries, and trauma. Because the completion of root development is easily affected by disturbances in normal pulp physiology, the treatment of necrotic immature permanent teeth remains a challenge. Next to maintaining the inherent strength and structure of the compromised tooth, modern endodontic practice aims to sustain the vitality of pulp tissue by disinfecting the root canal and subsequent induction of a blood clot. This procedure not only causes the release of growth factors but also attracts resident (stem) cells and thus creates an optimal environment for repair and regeneration of the dental pulp (1, 2). However, the exact nature of the regenerated tissue has been difficult to define because different cell populations can invade the pulp cavity with subsequent deposition of bone and cementum as well as ingrowth of periodontal tissue (3–5). Because the vascular supply within teeth is limited by the apical foramen, another recurring issue in cell homing–based approaches is the size of the apex required for proper pulp revascularization (5).

The implementation of a stem cell–based approach could potentially overcome these concerns. *In situ* transplantation of human dental pulp stem cells (hDPSCs), for example, already led to the successful regeneration of vascularized pulp tissue in canine teeth with an apical foramen of 0.7 mm (6, 7). In comparison with a cell homing–based methodology, hDPSC transplantation resulted in a more pronounced volume of dental pulp–like tissue with a higher capillary density (8). Given their

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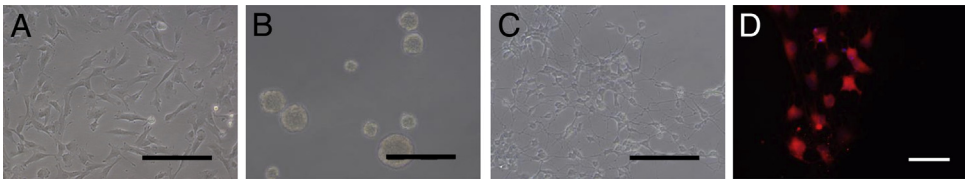

Figure 1. Neurogenic differentiation of neurosphere-derived hDPSCs. (*A*) hDPSCs are able to form neurospheres in the presence of (*B*) basic fibroblast growth factor and epidermal growth factor, which sequentially acquire a (*C*) neuronal morphology with multiple cytoplasmic extensions upon neuronal maturation achieved by cyclic adenosine monophosphate and neurotrophin-3 signaling. (*D*) The neuronally maturated cells were characterized by high NeuN immune reactivity. Scale bars = (*A–C*) 200 μm and (*D*) 50 μm.

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Neurogenic and Angiogenic Properties of Dental Pulp Stem Cells

hDPSCs are a plastic adherent, heterogeneous population of stem cells with multilineage differentiation potential that can be isolated by enzymatic digestion or outgrowth of the pulp tissue (9, 10). hDPSCs express the surface markers CD73, CD90, and CD146 but do not express CD34, CD14, CD45, or human leukocyte antigen-DR (10). These characteristics classify hDPSCs as mesenchymal stem cells (MSCs) according to the guidelines of the International Society for Cellular Therapy (11).

Neuronal and Schwannogenic Differentiation and Their Neuroregenerative Potential

In addition to multilineage differentiation experiments toward classic mesodermal lineages, our group has subjected hDPSCs to neural (12) and Schwann cell differentiation (13) experiments. Differentiating hDPSCs toward neurons and Schwann cells could provide an *ex vivo* expandable alternative to these slowly or not regenerating cells that play an eminent role in host repair. Moreover, our group has also shown the paracrine effect of hDPSCs on various features of neural and endothelial cells.

hDPSCs were first differentiated to functional neuronal cells by Arthur et al (14) and Kiràly et al (15). However, our group was the first to demonstrate single action potential firing by neuronal differentiated hDPSCs (12). Multiple approaches were used to differentiate hDPSCs to neuronal cells, most of which are based on sequential growth factor signaling and/or epigenetic programming to first trigger neuronal induction followed by a maturation step (14, 15). A neuronal induction step based on neurosphere formation (16) before neuronal maturation is essential for neuronal commitment of hDPSCs because subjecting hDPSCs to the maturation medium alone did not lead to differentiated hDPSCs with neuronal features. The step-by-step change in hDPSC morphology during the differentiation protocol is shown in Figure 1*A–D*. Briefly, hDPSCs were stimulated to form neurospheres in low attachment culture plates in the presence of epidermal growth factor and basic fibroblast growth factor for 1 week. Afterward, these neurospheres were collected and replated in laminin/poly-L-ornithine–coated culture dishes, and neurogenic maturation was achieved by subjecting the cells to neurotrophin-3 and cyclic adenosine monophosphate for 4 weeks. In addition to neurogenic differentiation aiming at being used as a potential cell replacement therapy in central nervous system pathologies, hDPSCs have also been subjected to differentiation toward Schwann cells. The goal of Schwannogenic differentiation is to provide a cell-based therapy for peripheral nervous system injuries. In a study performed by Martens et al (13), hDPSCs were successfully differentiated into Schwann cells with the ability to myelinate and guide neurites from dorsal root ganglia *in vitro*. These findings suggest a potential role for hDPSC-derived Schwann cells in promoting peripheral nerve repair.

Another mechanism of action by which hDPSCs are thought to mediate beneficial effects is by the production of various paracrine factors. Our research group showed that hDPSCs produce brain-derived neurotrophic factor, nerve growth factor, vascular endothelial growth factor, and glial-derived neurotrophic factor (12, 13). The production of these factors was confirmed by Mead et al (17), who also showed that hDPSCs secrete higher quantities of these factors compared with bone marrow–derived and adipose-derived MSCs. Supporting these results, this research group showed a paracrine-mediated neuroprotective and neurogenic effect of the hDPSC secretome on axotomized retinal ganglion cells (17).

These encouraging *in vitro* results stimulated the application of hDPSCs in *in vivo* models of neurodegenerative diseases such as spinal cord injury (18) and ischemic stroke (19) as pioneered by Sakai et al and Leong et al, respectively. In a complete hemisection spinal cord injury model, hDPSCs promoted the recovery of hind limb motor function after transplantation in the spinal cord. The mechanisms of action responsible for this recovery were suggested to include the inhibition of apoptosis of the neuronal and glial cells present in the spinal cord, inhibition of axon degeneration, and differentiation toward

Figure 2. Paracrine-mediated proangiogenic effect of hDPSCs. (*A*) hDPSCs significantly increase endothelial migration in a transwell migration assay. (*B*) Endothelial tubulogenesis is notably augmented by hDPSCs in a tube formation assay. (*C*) hDPSCs significantly induce angiogenesis in a chorioallantoic membrane assay.