A First Step in *De Novo* Synthesis of a Living Pulp Tissue Replacement Using Dental Pulp MSCs and Tissue Growth Factors, Encapsulated within a Bioinspired Alginate Hydrogel

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Abstract

Introduction: A living, self-supporting pulp tissue replacement *in vitro* and for transplantation is an attractive yet unmet bioengineering challenge. Our aim is to create 3-dimensional alginate-based microenvironments that replicate the shape of gutta-percha and comprise key elements for the proliferation of progenitor cells and the release of growth factors. **Methods:** An RGD-bearing alginate framework was used to encapsulate dental pulp stem cells and human umbilical vein endothelial cells in a ratio of 1:1. The alginate hydrogel also retained and delivered 2 key growth factors, vascular endothelial growth factor-121 and fibroblast growth factor, in a sufficient amount to induce proliferation. A method was then devised to replicate the shape of gutta-percha using RGD alginate within a custom-made mold of thermoresponsive N-isopropylacrylamide. Plugs of alginate containing different permutations of growth factor–based encapsulates were tested and evaluated for viability, proliferation, and release kinetics between 1 and 14 days. **Results:** According to scanning electron microscopic and confocal microscopic observations, the encapsulated human endothelial cells and dental pulp stem cell distribution were frequent and extensive throughout the length of the construct. There were also high levels of viability in all test environments. Furthermore, cell proliferation was higher in the growth factor–based groups. Growth factor release kinetics also showed significant differences between them. Interestingly, the combination of vascular endothelial growth factor and fibroblast growth factor synergize to significantly up-regulate cell proliferation. **Conclusions:** RGD-alginate scaffolds can be fabricated into shapes to fill the pulp space by simple templating. The addition of dual growth factors to cocultures of stem cells within RGD-alginate scaffolds led to the creation of microenvironments that significantly enhance the proliferation of dental pulp stem cell/human umbilical vein endothelial cell combinations. *(J Endod 2015;41:1100–1107)*

Key Words

Dental pulp stem cells, fibroblastic growth factor, human umbilical vein endothelial cells, RGD alginate, vascular endothelial growth factor

Root canal therapy is currently the preferred treatment for irreversible pulpitis wherein pulp is completely excavated and the root canal is prepared, debrided, and later obturated with gutta-percha (1, 2). What makes the prognosis for root canal treatment questionable are complications such as bacterial metabolite leakage (3), failure to achieve a proper seal (4), fracture upon application of force, and loss of vitality (5). Efforts toward endodontic regeneration have involved implementation of the latest tissue engineering strategies, including root revascularization by forced induction of blood clots, use of postnatal stem cell therapy (6), pulp implantation, injectable biochemically active cell-free scaffolds, 3-dimensional (3D) cell printing, and gene therapy (7). The greatest challenge of tissue engineering the "pulp" is to achieve *in vivo* revascularization from the host blood supply (8). The other barriers to progress in regenerative endodontics are dentinogenesis and maintaining the afferent nerve supply. Emphasis must be placed on replicating the pulp's intricate zonal structure with their functions, namely mesenchymal stem cell self-renewal, odontogenic repair (formation of secondary dentin), and sensory and blood vessel apparatus (9). Bioengineering of the pulp requires a judicious selection and combination of stem cells and morphogens delivered over time in convoluted spatial patterns within a supporting framework. So far, the optimal permutation of factors has not been determined (10).

A variety of dental stem cells have been implemented in pulp bioengineering strategies such as dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth, stem cells from apical papilla, and periodontal ligament stem cells. DPSCs exhibit strong, stable proliferation and self-renewal properties, directly differentiate along the odontoblast lineage, and are the most common dental stem cell used in pulp tissue engineering (11). A key process in early tissue morphogenesis is the interplay between stem cells and vascular endothelium. In a dental context, it has been shown that the coculture of postnatal DPSCs with endothelial cells (ECs) offer superior regenerative potential than when used individually (12). Other than the cellular and

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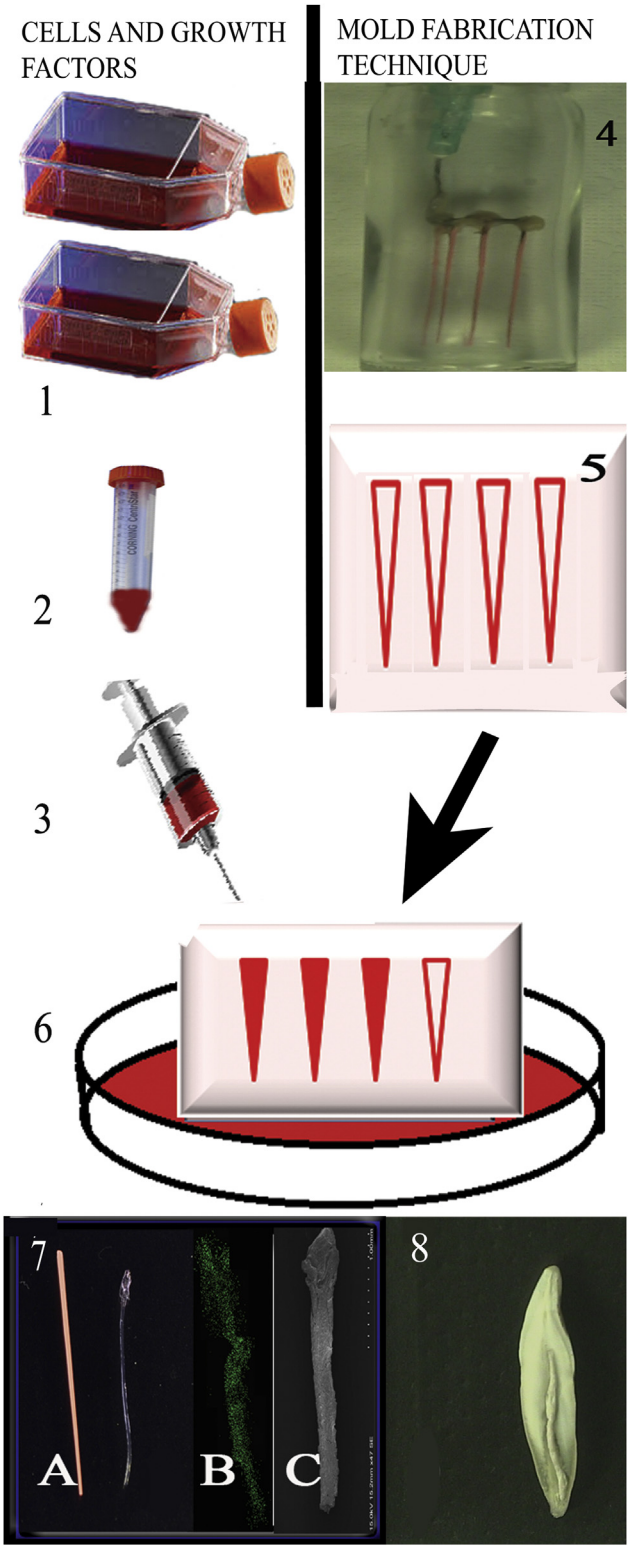

Figure 1. A schematic of the procedure used to fabricate cell-seeded alginate pulp replacement frameworks. Step 1: cultivation and expansion of human cells. Steps 2 and 3: mixing of cells and growth factors in RGD-alginate solution. Steps 4, 5, and 6: fabrication of temperature-responsive sockets and molds. Step 7: (*A*) extruded pulp constructs, (*B*) confocal image, and (*C*) SEM. Step 8: prospective clinical end point filling of pulp cavity.

TABLE 1. Various Test Groups of Pulp Construct

Encapsulate	Quantity (mL)
DPSCs	10^6 cells
DPSCs:HUVECs	10^6 cells in ratio of 1:1
DPSCs:HUVECs+VEGF	10^6 cells in 1:1 ratio of 50 ng VEGF
DPSCs:HUVECs+VEGF: FGF (1:3)	10^6 cells 1:1 ratio in 50 ng VEGF + 150 ng FGF in 1:3 ratio

DPSCs, dental pulp stem cells; FGF, fibroblast growth factor; HUVECs, human umbilical vein endothelial cells; VEGF, vascular endothelial growth factor.

structural environments, the biochemical composition is important in driving tissue production by cells.

Tissue morphogens are small proteins inducing an array of cellular activities even at low concentrations in picogram quantities (10). Bone morphogenetic protein, vascular endothelial growth factor (VEGF), fibroblastic growth factor (FGF-2), and transforming growth factor are the principle growth factors that are being used frequently in conjunction with dental stem cells to induce various tissue structures (9). Cumulative evidence showed that VEGF and FGF in a pulp context specifically lead to angiogenesis, whereas bone morphogenetic protein is proposed to induce dentinogenesis at the pulp periphery (13–15).

All dental cells require a 3D framework to provide safe anchorage and spatial organization and to be used as modules for transplantation to the desired site of reconstruction. Various synthetic and natural scaffold materials are available to build structural environments that mimic natural tissue frameworks, but accurate matching of the biology and biodegradation characteristics to a tissue exists as the major area of difficulty in optimizing scaffold material. Recent documented attempts indicated the formation of dentinlike precursor tissue, with the use of gelatin hydrogels, collagen, peptide-based gels, and polyglycolic acid (PGA) at the boundary within existing dentin (16, 17). Other tissue engineering biomaterials such as alginate have been virtually absent from pulp bioengineering research even though it is suitable for this context. Alginate, which is derived from marine seaweed, is widely available at relatively low costs and is used broadly in experimental tissue engineering as well as by food and drug manufacturers and has Food and Drug Administration approval (18). Continuous efforts are needed to engineer a scaffold that can properly self-organize and support the expansion and differentiation of stem cells into naturally simulated pulp, especially once it is implanted in the root canal.

To start tackling these design flaws, we have engineered a morphologically accurate pulp replacement from stiffened alginate, which express internal cellular and molecular environments necessary for beginning pulp tissue reconstruction. The ultimate purpose of such biomimetic designs for pulp microenvironments is to eliminate the shortcomings of current pulp regeneration strategies by building an endothelial tubule network (protovasculature) that is the foundation for tissues. The presence of these structures guarantees a connection with the host blood supply after implantation. Such a strategy is critical to revitalizing and strengthening the existing diseased tooth structures.

Materials and Methods

Cell Sources and Extraction Procedure

Human umbilical vascular endothelial cells (HUVECs) were obtained commercially (ScienCell Research Laboratories, San Diego, CA) and cultured in endothelial cell medium (ECM) (ScienCell Research Laboratories Cat. No. 1001) at a seeding density of 5000