The Role of Vasculature Engineering in Dental Pulp Regeneration

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Abstract

Creating an optimal microenvironment that supports angiogenesis, cell-cell cross talk, cell migration, and differentiation is crucial for pulp/dentin regeneration. It was shown that dental stem cells being seeded onto a scaffold and transplanted *in vivo* could give rise to a new tissue similar to that of the native pulp. However, the unique structure of the tooth with a pulp space encased within hard dentin allows only a single blood supply from a small apical opening located at the apex of the root canals. Therefore, a further strategy that can address this limitation such as the incorporation of endothelial/endothelial progenitor cells or cells with high angiogenic potential into the transplant is required so that the added cells can contribute to the vascularization within the implant. However, the placement of 2 or more different cell types inside 3-dimensional porous scaffolds is technologically challenging. In contrast to the conventional scaffolding approach, self-assembly of monodispersed cells into 3-dimensional tissue mimics permits true physiological interactions between and among different types of cells without any influence from a secondary material. In this review, we discuss potential strategies that can be used in vasculature engineering in dental pulp regeneration with a specific emphasis on combining prevascularization and scaffold-based or scaffold-free approaches. *(J Endod 2017;43:S102–S106)*

Key Words

Angiogenic factors, dental pulp stem cells, microfabrication, prevascularization, vasculogenesis

Significance

Pulp tissue housed within a rigid space of dentin receives its blood supply only from a small apical opening. Therefore, engineering the vasculature during pulp regeneration is a crucial task. Incorporation of endothelial/endothelial progenitor cells into the transplant, use of angiogenic factor–incorporated scaffolds that allow sustained release, mobilization of angiogenic factors fossilized in dentin, fabrication of endothelialized microfluidic channels, and proangiogenic decellularized matrices are several potential strategies that could overcome this challenge.

Dental pulp is a metabolically active tissue that shows a high capacity for regeneration after injury. However, dental pulp is surrounded by hard tissue, and there are only a few main vessels entering via the apical foramen to supply the pulp tissue. The microvascular arrangement in the dental pulp not only maintains the vitality of pulp tissue but also plays a major role in dentin physiology (1, 2). Therefore, the regenerated pulp/dentin complex should be well vascularized. Securing a good blood supply during dental pulp tissue engineering is a critical and challenging task because of the unique anatomy of pulp space encased by the thick, continuous layer of dentin allowing only a single blood supply from its small (<1 mm) apical canal opening. It is inevitable that the size of the canal opening affects the growth of blood vessels in the regenerating tissue. This was further expressed by the fact that immature teeth with open apices are the best candidates for apexogenesis and revascularization (3).

Vascularization of Engineered Tissues

Irrespective of the type of tissue/organ of interest, after implantation, an engineered 3-dimensional tissue construct requires an adequate vasculature in order to get sufficient amount of nutrients including oxygen and to remove the waste products immediately. However, when transplanted *in vivo*, tissue constructs have to depend solely on the oxygen supply via diffusion from the nearest capillary (4). It has been shown that the diffusion only serves up to a distance of 200 μm from a blood vessel (5) because the majority of the cells undergo apoptosis when the distance from a blood vessel exceeds the latter distance (6). The survival of implanted tissue constructs of greater size requires the formation of a capillary network of its own that can deliver necessary nutrients for the cells. Although host blood vessels start to invade the implanted tissue construct, partly in response to the angiogenic factors secreted by the cells undergoing hypoxia, this process happens very slowly, growing only a few tenths of micrometers per day (4). Therefore, the development of methods to promote rapid vascularization of the tissue construct after *in vivo* implantation is critical for better engraftment and, later, for its functional integration.

During the last decade, there have been major advances in the tissue engineering field in understanding the process of neoangiogenesis and the application of this

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knowledge to enhance vascularization in bioengineered tissues. These can be categorized into 3 main approaches:

(1) incorporation of growth factors,
(2) coculture of progenitor/target cells with endothelial cells, and
(3) microfabrication of vasculature or decellularized matrices (Fig. 1).

Incorporation of Angiogenic Growth Factors

Engineered tissue constructs gain their vascularization mainly via sprouting angiogenesis in which budding of capillaries from preexisting blood vessels into the tissue construct will occur (6). This process is driven by multiple growth factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor, and angiopoietins (7). Among these various angiogenic factors, VEGF is the most investigated and is known for its role in endothelial migration, tube network formation, and maturation of vascular structures (8, 9).

The incorporation of growth factors into biodegradable porous scaffolds is a common approach that has been investigated. VEGF, encapsulated in various scaffolds, has been used in ischemic animal models to investigate its ability to enhance vasculo/angiogenesis. The results showed that VEGF enhances angiogenesis *in vivo* as manifested in higher density and total number of blood vessels (10–12).

Use of Angiogenic Growth Factors in Pulp Regeneration

VEGF, which was initially described as an inducer of vascular permeability (13), is now known for its key role in the promotion of the formation of new capillaries leading to angiogenesis (14). Particularly, VEGF acts on proliferation, migration, and survival of endothelial cells (15). The potential use of VEGF in revasularization and angiogenesis has been assessed in relation to several tissues (11, 16, 17) including dental pulp (18). Several studies have shown that the application of VEGF as an angiogenic factor can enhance the microvessel density of severed human dental pulps (18, 19). In addition, VEGF can also induce endothelial cell survival by inhibiting apoptotic events in dental pulps (19). It was also shown that VEGF can promote chemotaxis, proliferation, and/or differentiation of dental pulp cells (20). Fibroblast growth factor-2 (FGF-2), another potent angiogenic factor, was also examined for its potential use in neovasculogenesis. However, it was found that the inductive effect of FGF-2 on dental pulp angiogenesis was less pronounced than that of VEGF (18).

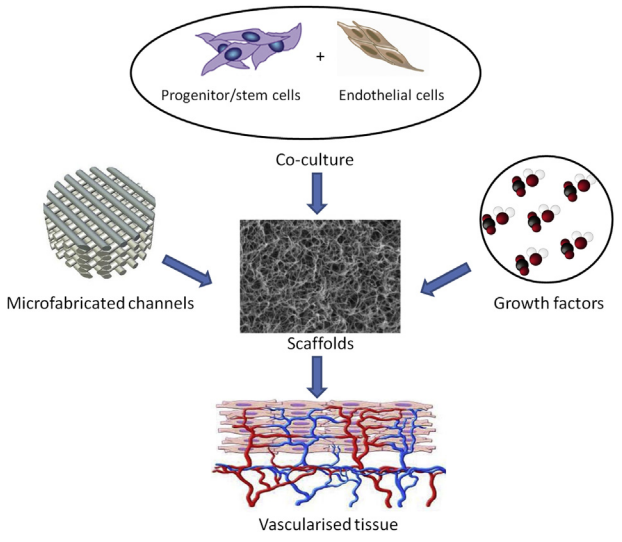

Figure 1. Potential approaches of enhancing vasculature in tissue engineering.

Dentin Matrix as a Source of Angiogenic Factors

During dentinogenesis, odontoblasts receive abundant nutrition via a rich vascular supply fenestrated into the dentin-pulp complex zone. After completion of dentin formation, these blood vessels appear to retract from the odontoblast region and would be later confined to the subodontoblastic area. The angiogenic growth factors secreted during dentinogenesis are sequestered in dentin matrix through their interactions with extracellular matrix proteins and act as a pool of fossilized bioactive molecules (21). It was shown that these growth factors are released in a sustained manner during matrix breakdown in which pulp-dentin repair is needed (22, 23).

These findings have led to the hypothesis that dentin matrix components can be used to increase the vascular supply during reparative dentinogenesis. Smith et al (24) have implanted isolated dentin matrix components in injured sites of dentin and showed that it causes an increase in local vasculature. In an attempt to identify the angiogenic growth factors sequestered in dentin, Roberts-Clark and Smith (21) showed that the dentin matrix contains VEGF, PDGF, FGF-2, and very low concentrations of epidermal growth factor. In a subsequent study, it was shown that dentin matrix components stimulate endothelial cell proliferation and *in vitro* endothelial tube formation in a positive dose-dependent manner (25).

Angiogenic Factor–incorporated Scaffolds with Sustained Release

The encapsulation of growth factors in biodegradable polymer scaffolds that facilitate sustained release was introduced by investigators to overcome some inherent problems associated with cytokine function (26). The incorporation of a single growth factor is not sufficient to induce formation of mature blood vessels (7). Therefore, the combination of 2 or more growth factors has been investigated. Several studies have shown that the delivery of VEGF and PDGF-BB is effective in increasing the density of capillaries. A unique system that can deliver VEGF in rapid release and PDGF-BB in delayed release was described (26, 27). Because PDGF mainly acts on the maturation of vessels after initial vascular structures are formed, this is considered a useful approach in engineering long-lasting vasculature. The combination of VEGF and FGF is another promising blend of growth factors that was shown to be effective in mature vessel formation (28). However, this approach is yet to be investigated in relation to dental pulp regeneration.

Cell-based Approach

The major limitation of the transplantation of angiogenic growth factor–incorporated scaffolds is that the growth factors tend to diffuse out of the scaffold rapidly before exerting their intended effect of attracting endothelial cells to vascularize the implant. Furthermore, continuous release at the implant site could lead to adverse effects such as the induction of tumorigenesis and the development of leaky and abnormal blood vessels.

A further strategy that can address this limitation is to incorporate endothelial/endothelial progenitor cells or cells with high angiogenic potential into the transplant so that the added cells can contribute to the vascularization within the implant.

Prevascularization

Another promising approach that is being researched is to use stem/progenitor cells and endothelial cells to create an early