Role of Angiogenesis in Endodontics: Contributions of Stem Cells and Proangiogenic and Antiangiogenic Factors to Dental Pulp Regeneration

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Abstract

Introduction: Dental pulp regeneration is a part of regenerative endodontics, which includes isolation, propagation, and re-transplantation of stem cells inside the prepared root canal space. The formation of new blood vessels through angiogenesis is mandatory to increase the survival rate of re-transplanted tissues. Angiogenesis is defined as the formation of new blood vessels from preexisting capillaries, which has great importance in pulp regeneration and homeostasis. Here the contribution of human dental pulp stem cells and proangiogenic and antiangiogenic factors to angiogenesis process and regeneration of dental pulp is reviewed. **Methods:** A search was performed on the role of angiogenesis in dental pulp regeneration from January 2005 through April 2014. The recent aspects of the relationship between angiogenesis, human dental pulp stem cells, and proangiogenic and antiangiogenic factors in regeneration of dental pulp were assessed. **Results:** Many studies have indicated an intimate relationship between angiogenesis and dental pulp regeneration. The contribution of stem cells and mechanical and chemical factors to dental pulp regeneration has been previously discussed. **Conclusions:** Angiogenesis is an indispensable process during dental pulp regeneration. The survival of inflamed vital pulp and engineered transplanted pulp tissue are closely linked to the process of angiogenesis at sites of application. However, the detailed regulatory mechanisms involved in initiation and progression of angiogenesis in pulp tissue require investigation. *(J Endod 2015;41:797–803)*

Key Words

Angiogenesis, proangiogenic and antiangiogenic factors, pulp regeneration, pulp stem cells

In blood vessel formation, the terms *vasculogenesis* and *angiogenesis* have been distinctively discussed. Vasculogenesis is defined as the formation of the primary vascular plexus from preexisting vascular precursor cells in the embryo (1). However, angiogenesis is the formation of new blood vessel from preexisting capillaries (1) and is responsible for the majority of the blood vessels formed during physiological and pathologic conditions (2, 3). Angiogenesis is initiated as a result of insufficient oxygen and nutrient supply and is regulated by tightly balanced production of numerous stimulatory and inhibitory chemobiological molecules such as growth factors, cytokines, matrix metalloproteinases (MMPs), endogenous angiogenesis inhibitors, transcription factors, adhesion molecules, and also components of the extracellular matrix (ECM) (4–8). The therapeutic modulation of angiogenesis process includes antiangiogenic therapies for fighting against malignancies (9–13) and proangiogenic therapies in repairing cardiovascular diseases and wound healing disorders by new blood vessels supplying blood to damaged tissues (9, 10, 14).

Human dental pulp is a highly vascularized tissue, which because of its vascular network and progenitor or postnatal dental pulp stem cells (DPSCs) has an impressive naturally inherent regenerative capacity (15–17). Dental pulp regeneration is part of the regenerative endodontic concept, which provides replacements for damaged tooth structures including pulp-dentin complex (18). It is a field in regenerative medicine and a branch of tissue engineering, which uses stem cells, biochemical factors, and engineering materials to replace lost or impaired biological tissues (19, 20). After isolation, the tissue-engineered stem cells are propagated in special medium and transplanted inside the prepared root canal space to develop into new pulp tissue (18). The success of tissue engineering depends on oxygen and nutrient transport to the implanted cells. If blood supply cannot be established rapidly, necrosis of the transplant will occur (21). This rule is also applicable to dental pulp regeneration, where angiogenesis is a key to both the development and regeneration of the dentin-pulp complex (17, 22). Angiogenesis establishes the blood supply and brings the oxygen, nutrition, and prevascular stem cells for regeneration (23).

Here we discuss an overview of the role of angiogenesis in dental pulp regeneration and the proangiogenic or antiangiogenic factors involved. The main aspects pursued in this review include the following:

1. The evaluation of the current state and the research trend regarding the role of angiogenesis in regenerative endodontics from January 2005 through April 2014.

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2. The determination of the elements or components, such as the stem cells or proangiogenic or antiangiogenic factors, that are involved directly in the angiogenesis process in dental pulp regeneration and the field of regenerative endodontics.
3. The clarification of strong and weak points regarding the angiogenesis events in dental pulp regeneration to introduce the present challenges and complexities in regenerative endodontic procedures that should be taken into consideration in research studies. Regarding the current state, in the conclusion section for each evaluated heading, the current state and research trends have been introduced and also the weak points or challenges are addressed to aid future studies to target these points.

Materials and Methods

Purpose of Review

The present review was conducted to evaluate the role of angiogenesis in dental pulp regeneration. Specifically, the potential effects of cell-related factors such as the contribution of stem cells and the proangiogenic and antiangiogenic factors in dental pulp regeneration were reviewed through the literature.

Inclusion and Exclusion Criteria

The inclusion criteria considered all articles including review studies, *in vitro* or *in vivo* studies, and case reports in peer-reviewed journals published in English from January 2005 through April 2014 that evaluated the cellular and elemental factors that are directly related to angiogenesis process in dental pulp regeneration. The studies that investigated the effects of angiogenic factors in regenerative endodontic procedures were included, whereas other investigations that did not address these criteria were excluded.

Search Methodology

The search methodology used in this review article included electronic searches that were done in the PubMed database by using key words mentioned in the MeSH headings regarding the role of angiogenesis in the dental pulp regeneration and the stimulatory or inhibitory factors in pulpal angiogenesis process.

Search Strategy

In the electronic search of scientific papers in the PubMed database the following key words were used in combination with angiogenesis: dental pulp regeneration, dental pulp stem cells, orthodontic forces, proangiogenic growth factors such as vascular endothelial growth factor, fibroblast growth factor, platelet-derived growth factor, angiopoietins, matrix metalloproteinase, stem cell factor, and antiangiogenic growth factors. A number of full-text articles and the reference lists of the relevant articles were also evaluated to supplement the search. The evaluation of the eligibility and finding of relevant data was done independently by 2 reviewers. A third reviewer was selected for resolving of any disagreements met during evaluation processes.

Dental Pulp Regeneration

Contributions of DPSCs and Angiogenesis. In stem cell biology, which is a part of tissue regenerative procedures, the highly potential stem cells, which are provided from vital tissues, are propagated and used for treatment of different pathologic conditions. Among different sources for stem cells such as umbilical cord blood, bone marrow, peripheral blood, and adipose tissue, the most common sources of stem cells used in this field are the mesenchymal stem cells isolated from bone marrow (BM-MSCs) (24, 25). In addition, DPSCs are introduced as another source for tissue regeneration procedures (18, 24, 25). DPSCs are postnatal, multipotent stem cells with similarities and some limitations to BM-MSCs (18, 26). Several authors have also reported some advantages in clinical usage of DPSCs including lower mortality rate, less legal or ethical issues, easy access from extracted teeth, and cryopreservation without losing their multi-differentiation potential (24–28).

Sieveking and Ng (29) found 2 distinct roles for stem cells such as BM-MSCs. They may act in a paracrine fashion through expression of proangiogenic factors. Alternatively, they may differentiate into endothelial cells and directly participate in neoangiogenesis. The activity of human dental pulp cells in secretion of proangiogenic factors has been well-documented by many authors (30–35). Bronckaers et al (30) demonstrated that human dental pulp stem cells (hDPSCs) are able to induce angiogenesis in a paracrine fashion through expression of a wide range of angiogenic factors including vascular endothelial growth factor (VEGF) and monocyte cheomtactic protein-1 (MCP-1). Furthermore, hDPSCs can stimulate the migration of endothelial cells through activation of the PI3K/AKT and MEK/ERK signaling pathways *in vitro*. Other authors have also shown that DPSCs are able to secrete a variety of proangiogenic factors including VEGF, fibroblast growth factor 2 (FGF-2), platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), MMP-9, and transforming growth factor beta (TGF-β) and promote the migration and tubulogenesis activity of endothelial cells (22, 31–35) (Fig. 1).

Concerning the endothelial differentiation potential of hDPSCs, several investigators have indicated that in the presence of specialized differentiation medium, hDPSCs can express some of the endothelial cell markers including CD31, CD105, CD34, and von Willebrand factor *in vitro* (36–38). Janebodin et al (39) indicated that DPSCs can resemble perivascular supporting cells and induce more mature blood vessels when co-cultured with endothelial cells.

The dental pulp stem cells of exfoliated teeth (SHED) are capable of differentiating into endothelial-like cells (40, 41). Bento et al (41) indicated that VEGF/MEK1/ERK signaling pathway is a key regulator of the endothelial differentiation of DPSCs. Liu et al (42) showed that the inhibition of miR-424 might assist the dental pulp regeneration process. Kim et al (43) reported that a sudden increase in SIRT1 gene expression can up-regulate the angiogenic markers such as VEGF, FGF-2, and endothelial cell adhesion molecules such as vascular endothelial cadherin and platelet endothelial cell adhesion molecule-1. Dissanayaka et al (44) reported that the direct co-culture of DPSCs and endothelial cells can enhance the expression of angiogenic phenotype *in vitro*. Nakashima and Iohara (45) found that CD31(−)/CD146(−) or CD105(+) cell types isolated from dental pulp, in the presence of stromal cell–derived factor-1, can produce pulp tissue including vascular and neuronal structure in 14 days, formation of dentin in 35 days, and impose trophic action on endothelial cells. Other cells of pulp tissue seem to have remarkable effects on angiogenesis process. Tran-Hung et al (22) co-cultured dental pulp fibroblasts with human umbilical vein endothelial cells and indicated that fibroblasts can induce angiogenesis through secretion of FGF-2 and VEGF (Table 1).

Proangiogenic and Antiangiogenic Factors

Mechanical Stimulation. Muscle contractions promote angiogenesis through enhanced production of nitric oxide from vasodilated blood vessels (9). Higher capillary shear stress also increases the expression of VEGF and angiogenesis in skeletal muscles (46). Derringer et al (47,48) detected an increase in the number of microvessels in pulp tissue of orthodontically moved teeth that was due to the elevation of the angiogenic growth factors. They later used