Functionalized Scaffolds to Control Dental Pulp Stem Cell Fate

Evandro Piva, DDS, MS, PhD,[*†] *Adriana F. Silva, DDS, MS, PhD,*[*†] *and Jacques E. Nör, DDS, MS, PhD*[†‡§]

Abstract

Emerging understanding about interactions between stem cells, scaffolds, and morphogenic factors has accelerated translational research in the field of dental pulp tissue engineering. Dental pulp stem cells constitute a subpopulation of cells endowed with self-renewal and multipotency. Dental pulp stem cells seeded in biodegradable scaffolds and exposed to dentin-derived morphogenic factors give rise to a pulplike tissue capable of generating new dentin. Notably, dentin-derived proteins are sufficient to induce dental pulp stem cell differentiation into odontoblasts. Ongoing work is focused on developing ways of mobilizing dentin-derived proteins and disinfecting the root canal of necrotic teeth without compromising the morphogenic potential of these signaling molecules. On the other hand, dentin by itself does not appear to be capable of inducing endothelial differentiation of dental pulp stem cells despite the well-known presence of angiogenic factors in dentin. This is particularly relevant in the context of dental pulp tissue engineering in full root canals in which access to blood supply is limited to the apical foramina. To address this challenge, scientists are looking at ways to use the scaffold as a controlled-release device for angiogenic factors. The aim of this article was to present and discuss current strategies to functionalize injectable scaffolds and customize them for dental pulp tissue engineering. The long-term goal of this work is to develop stem cell–based therapies that enable the engineering of functional dental pulps capable of generating new tubular dentin in humans. *(J Endod 2014;40:S33–S40)*

Key Words

Angiogenesis, dental pulp stem cells, dentin, morphogenic signals, pulp biology, regenerative endodontics, tissue engineering

A major goal of the health sciences in the 21st century is to develop clinically relevant strategies for tissue regeneration. The reasoning for this goal comes from the realization that the best substitute of an organ/tissue lost because of disease or trauma is the actual organ/tissue. Broadly speaking, this can be achieved either by transplantation or regeneration. Transplantation-based strategies have been successfully used for decades. However, organ/tissue rejection is a major threat that has been addressed with the prolonged use of immunosuppressive drugs, which carry intrinsic risks for the patient. On the other hand, tissue regeneration mediated by targeted activation of host stem cells or the delivery of autologous stem cells may allow for similar results as transplantation-based strategies without the need for chronic immunosuppressive therapies. However, tissue regeneration is certainly not devoid of significant challenges. These challenges include the development of strategies for the recruitment or isolation of appropriate stem cells and the generation of a suitable microenvironment that enables the stem cells to differentiate, proliferate, and give rise to a fully functional organ/tissue in the correct shape and size. Although these are rather substantial challenges, it is becoming increasingly evident that the successful development of tissue regeneration strategies using autologous cells might have long-lasting benefits that surpass potential risks. This review focuses on 1 aspect of tissue regeneration (ie, the development of functionalized scaffolds that provide a conducive microenvironment for controlled differentiation of stem cells and the generation of a new dental pulp for the treatment of necrotic immature permanent teeth).

Tissue Engineering

Tissue engineering is a multidisciplinary science that aims at the development of clinically relevant strategies for the regeneration of a tissue or organ (1). It involves the identification of progenitor cells capable of tissue regeneration when seeded in biodegradable scaffolds and exposed to morphogenic signals (1–3). Scaffolds must be uniquely developed for the regeneration of each specific tissue or organ. Nevertheless, they share common features such as allowing cell attachment, diffusion of nutrients and oxygen, being biodegradable, and having physical properties aligned with those of the tissue/organ to be regenerated (3). In addition, the scaffolds can be functionalized by enhancing conditions for cell attachment and survival and providing morphogenic signals that supplement those coming from the host and enable guidance of stem cell differentiation (3).

Broadly speaking, scaffolds can be divided into (1) casted (ie, fairly rigid and custom-made for specific purposes) and (2) injectable (ie, low viscosity gels that can be delivered and "molded" at the site that requires tissue regeneration). Both types

From the *Department of Operative Dentistry, School of Dentistry, Federal University of Pelotas, Pelotas, RS, Brazil; †Department of Cariology, Restorative Sciences, Endodontics, University of Michigan School of Dentistry, Ann Arbor, Michigan; ‡Department of Biomedical Engineering, University of Michigan College of Engineering, Ann Arbor, Michigan; and §Department of Otolaryngology, University of Michigan School of Medicine, Ann Arbor, Michigan.

This paper is based on a presentation from the International Association for Dental Research (IADR) Pulp Biology and Regeneration Group Satellite Meeting, which was held March 24–26, 2013 in San Francisco, California.

Address requests for reprints to Dr Jacques E. Nör, Department of Cariology, Restorative Sciences, Endodontics, University of Michigan School of Dentistry, 1011 North University Room 2309, Ann Arbor, MI 48109-1078. E-mail address: jenor@umich.edu

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.01.013

of scaffolds can be functionalized with morphogenic signals. Notably, these signals are typically proteins with a short half-life. Therefore, the development of a strategy for controlled release of these proteins is critical to maximize their effects for predetermined time periods. Morphogenetic proteins can be incorporated both into casted scaffolds using copolymers such as poly(lactic-co-glycolic acid) and gas-foaming approaches (4, 5). They can also be mixed with injectable scaffolds such collagen or the self-assembling hydrogel Puramatrix (BD Bioscience, Franklin Lakes, NJ), but in this case it is very difficult to slow down the degradation rate of the proteins. To address this issue, it has been suggested that natural polymers derived from brown algae (eg, alginates), which are biocompatible and present low immunogenicity, can be used in combination with injectable scaffolds to serve as a "slow-release device" for morphogenic signals (6, 7). Notably, the gelation process in the presence of divalent ions at physiological levels is a very simple way to incorporate, protect, and release morphogenic factors from alginate microspheres in a controllable rate (8, 9).

The biological standard for the controlled release of morphogenic factors in dental tissue engineering is the microenvironment observed during tooth development (10, 11). Investigators have attempted to understand this environment as a means to create ideal conditions for guided determination of stem cell fate and dental tissue regeneration (11, 12). The work of many investigators throughout the world identified morphogenic signals that play major roles during tooth development and that can potentially be used therapeutically in tooth regeneration (13–15). Indeed, gene knockouts such as dentin sialophosphoprotein (DSPP) (16), dentin matrix protein 1 (DMP1) (17), Msx homeobox family (18, 19), and amelogenin (20, 21) revealed major tooth developmental defects, indicating that these morphogenic signals are critically involved in these processes. Such findings suggest candidate morphogenic signals that can be either recruited from the surrounding environment or delivered locally with the use of the scaffold to direct stem cell fate and optimize guided pulp tissue regeneration.

In addition to the need for guided differentiation of stem cells into odontoblasts, there is also an important need for their differentiation into supporting cells (eg, vascular endothelial cells and neural cells). Tissue innervation is critical for the functional regulation of the cells involved in pulp regeneration. In addition to the protective effect of the pulp innervation, it also plays important roles in inflammation and tissue repair (22). The rapid induction of a proangiogenic response is crucial not only as a means to provide necessary influx of the oxygen and nutrients required by the high metabolic demands of cells engaged in tissue regeneration but also to enable immunologic responses necessary to protect the emerging tissues from bacterial contamination typically associated with the clinical handling of necrotic teeth. Immune cells such as tissue-infiltrating macrophages require the presence of a functional vascular network to access the regenerated pulp tissue and protect it against bacteria that could possibly remain viable after the treatment of necrotic teeth. Indeed, it is plausible to speculate that access of immune cells to the pulp might play a major role in the successful outcome of necrotic teeth treated with regenerative endodontics–based approaches.

In addition, it is through the blood vessels that substrates required for dentin mineralization (eg, calcium and phosphate) are made available to odontoblasts, which perhaps explains the frequent presence of blood vessels in close proximity to the odontoblastic layer, particularly in pulp actively engaged in regenerative processes. Furthermore, vascularization is a key determinant of mesenchymal cell heterogeneity in dental tissue engineering, presumably by enabling the recruitment of circulating cells to the developing tooth (11). Later in this review, we discuss potential strategies proposed for the rapid vascularization of engineered dental pulps.

Tooth-related Stem Cells

The cells that define the pulp tissue are the odontoblasts, terminally differentiated cells that do not proliferate and that are endowed with the capacity of generating new tubular dentin. Lost odontoblasts can be replaced in normal pulps by resident multipotent stem cells (23, 24) found in permanent teeth (25) or primary teeth (26). They can differentiate into odontoblasts and also into other cell lineages such as osteoblasts, chondrocytes, and neuronal progenitor cells (25–29). Stem cells have also been identified in other oral tissues, such as the apical papilla (30), mesenchymal follicle (31), periodontal ligament (32), and gingivae (33). It is speculated that stem cells from each tissue are somewhat "primed" to regenerate that same tissue, and, therefore, it is likely that the best stem cells for dental pulp tissue engineering are pulp stem cells. However, it is rather unclear at this time what the relative potential of each one of these oral stem cells for dental pulp tissue engineering is.

Dental pulp stem cells are relatively easily obtained from exfoliated primary teeth or permanent teeth extracted for orthodontic reasons. Considered a relatively rich source of mesenchymal stem cells, the interest in dental pulp stem cell isolation and banking has increased substantially in recent years. More importantly, exfoliating primary teeth and permanent teeth extracted for orthodontic reasons overlap temporally with immature permanent teeth of adolescents that are relatively prone to trauma-induced pulp necrosis. Therefore, it is suggested that these teeth are an ideal source of stem cells for dental pulp tissue engineering of necrotic immature permanent teeth. In these cases, the goal is to regenerate a functional dental pulp capable of completing vertical and horizontal root formation (Fig. 1).

In proof-of-principle experiments using the permanent tooth slice/scaffold model (34), we observed that stem cells from exfoliated deciduous teeth differentiate into functional odontoblasts and vascular endothelial cells (35–38). Notably, fluorescent lines created by tetracycline staining of newly formed tubular dentin confirmed that the stem cells from exfoliated deciduous teeth differentiated into mature odontoblasts (38). These experiments suggested the possibility of isolating stem cells from exfoliating primary teeth and transplanting them back in the same patient (autologous transplantation) in clinical scenarios involving pulp necrosis of an immature permanent tooth during the mixed dentition phase.

A critical challenge of the clinical scenario described previously is the need for quick vascularization of the engineered tissue to enable the maintenance of the viability of transplanted cells (39). Indeed, the anatomy of the dental root is a major limiting factor regarding access to vascularization, considering that all blood vessels have to come through a system of narrow foramina located exclusively in 1 end of the tooth. In young, immature teeth, the apical opening of the root is relatively wide. However, in people aged 21 years or older, the dimensions of apical foramen are very narrow (40) and tend to decrease progressively over time. It appears that necrotic immature teeth with open apices are the prime candidates for dental pulp tissue engineering at this stage of development of the technique. Even in these cases, we believe that the success rate of such therapy would benefit from the delivery of a proangiogenic stimulus.

The recent discovery that dental pulp stem cells differentiate into vascular endothelial cells in addition to differentiating into functional odontoblasts (36–38) suggests that these cells can serve as a single cellular source for dental pulp tissue engineering. However, a key observation of these studies is that although dentin-derived morphogenic signals are sufficient to induce odontoblastic differentiation,