NEW PERSPECTIVE ARTICLE

Dental Pulp Regeneration: Insights from Biological Processes

Regeneración pulpar: Perspectivas desde los procesos biológicos

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ABSTRACT

One of the major approaches on dental research in this century is the development of biological strategies (tissue engineering) to regenerate/biomineralize lost dental tissues. During dentin-pulp regeneration, the interaction between stem cells, signaling molecules, biomaterials and the microenvironment in the periapical area drives the process for pulp tissue engineering. Understanding the signaling mechanisms and interactions involved with the biological process for the formation of a new tissue is essential. The knowledge of the micro-environment is the key for the application of tissue engineering. The present article is a short review of the current state of this topic, with the purpose of showing insights of pulp regeneration.

KEYWORDS

Pulp regeneration; Molecular signals; Scaffolds; Stem cells.

RESUMEN

Actualmente la investigación en odontología se orienta al desarrollo de estrategias basadas en principios biológicos (ingeniería de tejidos) para la regeneración/biomineralización de estructuras dentales perdidas. El proceso de regeneración del complejo dentino-pulpar está guiado por la compleja interacción entre las células indiferenciadas de origen dental (DTSC), moléculas de señalización y biomateriales con el microambiente donde se va a restablecer. Es esencial comprender detalladamente, los mecanismos de señalización e interacciones involucradas en los procesos biológicos para la formación de un nuevo tejido, además de la identificación de los componentes presentes en los tejidos dentales implicados en este proceso (características del microambiente), ya que representan la base sobre la cual se debe emplear la ingeniería de tejidos. El presente articulo es una breve revisión del estado actual del tema, con el fin de entender el proceso de regeneración pulpar, basado en la comprensión de los fundamentos biológicos.

PALABRAS CLAVE

Regeneración pulpar; Señalización molecular; Andamios; Células madre.

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The recovery of a lost, damaged organ or tissue is one of the most frequent, devastating and costly issues in human health care (1). Tissue engineering is an emerging interdisciplinary science that applies the principles of biology and engineering in regenerative medicine to improve or replace the biological function of cells, tissues and organs damaged by intrinsic or extrinsic factors (1-5).

These procedures are based on the interactions of these essential components: stem cells, morphogens and scaffolds. Two primary approaches have been developed: 1) *cell-based approaches*, which involve the seeding of undifferentiated cells and suitable biochemical inductors in designed scaffolds to be implanted *in vivo*; and 2) *cell-free approaches*, which propose that stem cell populations that are already present on the host can be recruited into a bioactive scaffold by chemotaxis and growth factors to induce cell migration, proliferation and differentiation (3,4,6).

Currently, dental research applies tissue engineering protocols and explores the potential of cell-based products as alternative therapies to replace tissues such as pulp, dentin and bone (7-9). Regenerative endodontic procedures attempt to apply biologic procedures to substitute the lost/damaged dentin-pulp complex tissue to restore biological function (10).

The biologic rationale for endodontic treatment is the prevention or treatment of apical periodontitis through the disinfection of the root canal space, the conformation of the canal and the filling with an inert material (10,11). However, in spite of efforts to preserve as much dental structure as possible, evidence suggests that endodontic treatment causes loss of a significant amount of dentin, resulting in a non-vital and weakened tooth, especially when clinicians are dealing with dental trauma on an open apex tooth (4). In a retrospective study, Caplan *et al.* (12) found that the removal of pulp in a compromised tooth may lead to tooth loss in comparison with teeth with normal developed tissues (12). The ideal treatment to solve this problem would involve maintaining the vital pulp to allow complete development and to prevent apical periodontitis through regenerative procedures (13).

Under these criteria, Dr. Jacques Nör (9) stated that when there is a need to endodontically treat a tooth with incomplete root development, the principles of regenerative medicine may be applied. The key elements for dental pulp engineering are a) molecular signals that induce the differentiation of cells that constitute dental pulp, b) cells that will respond to these signals and c) scaffolds that will either carry or attract these cells and will provide an environment to proliferate, differentiate and develop a tissue with the characteristics and functions of normal pulp (4,5,9). However, it is necessary to study and understand the principles of tooth development and the various pathways involved in cell differentiation to produce a functional replacement tooth or any other organ using tissue engineering technologies (5,14).

MOLECULAR SIGNALS

The process of tooth formation implies different and multiple interactions between the developmental tissues mediated by regulatory signals (15). These signals are required to stimulate the differentiation of dental pulp stem cells and are necessary for the synthesis and secretion of mineralized matrix in hard tissues (4,5,9).

Morphogens are proteins that bind to specific cell membrane receptors and induce a cascade of processes that coordinate all cellular functions, influencing vital processes such as cell division, cell signaling, matrix synthesis and proliferation (9,16). Though they are quite versatile, these proteins are classified according to their activity(4). For example, cytokines are related with immune and inflammatory reactions and growth factor functions

as stimulators or inhibitors of growth by controlling the rates of cell proliferation, differentiation and stem cell activity (4,5).

Grontos *et al.* (17) suggested that biochemical pathways are involved in the differentiation of dental pulp stem cells (DPSCs) into functional odontoblasts. Indeed, several regulators of bone formation (e.g., bone morphogenetic proteins - BMPs) are related to odontoblast development. It is well known that fibroblast growth factor, epidermal growth factor and tumor necrosis factor-α, among others, regulate the proliferation and differentiation of odontoblast precursors. Proteins such as dentin matrix protein 1 (DMP-1), fibronectin, collagen type 1, alkaline phosphatase, osteonectin, osteopontin, bone sialoprotein, osteocalcin, dentin sialoprotein (DSP) and dentin phosphoprotein (DPP) are involved in the formation of the mineralized matrix of either bone or dentin (17). However, it is still necessary to identify a more global morphogenetic signaling pathway and their functions and sequences to propose a signaling strategy.

Collagen fibers and non-collagenous proteins (NCPs) constitute the organic dentin matrix. The NCPs of dentin play an important role in the biomineralization of the extracellular matrix, mainly DMP1, DPP and DSP, which function as regulatory factors (15). Goldberg *et al.* found that, in dental therapy, some molecules within the extracellular matrix may have the potential as bioactive agents for pulp repair or tissue engineering (18). In accordance, Tomson *et al.* (19) demonstrated that after complete root development, dentin retains "fossilized" growth factors that can be released when the dentin is solubilized. These dentin matrix components potentially influence cellular events for dentine repair and regeneration (4,19). Nevertheless, Nör suggests that the ideal combination of signals required to engineer all of the cellular components of a fully functional dental pulp is still unclear (9).

STEM CELLS

Stem cells are the second key element for dental pulp engineering. These nonspecialized cells have the capacity for self-renewal and continuously divide and differentiate into specialized cells. Embryonic stem cells found in early stages of development are capable of unrestrictedly forming new tissues and organs (totipotent). Nevertheless, due to ethical and legal concerns, their use is controversial. Postnatal stem cells also share some characteristics with embryonic stem cells, the main difference being their limited capacity for specializing into diverse cells types (multipotent). As an advantage, they can be found in some tissues at any stage of life; therefore, their use is more accessible (20).

Subpopulations of postnatal stem cells have been found in adult tissues, such as bone marrow, adipose tissue, brain, skin, muscle and dental tissues(16). In 2000, Grontos *et al* (17) discovered stem cells obtained from the pulp of third molars (DPSCs). Songtao Shi *et al.* (21) identified other types of human dental pulp stem cells obtained from exfoliated deciduous teeth (SHEDs) and stem cells from the apical papilla (SCAPs)(22) and periodontal ligament (PDLSCs)(16,23).

The discovery of DPSCs evokes an important and accessible source of undifferentiated cells, with the potential for use by either seeding or recruitment in many potential therapies. DPSCs are highly proliferative and can specialize themselves into a number of cell types, such as odontoblasts, neural progenitors, osteoblasts, chondrocytes and adipocytes (3,16). In this scenario, the possibility to develop strategies for dental tissue engineering could begin successfully with endodontic regeneration.

Furthermore, dental pulp tissue has an inherent potential associated with the healing process. As a defense response, odontoblasts can

be replaced via the migration and differentiation of resident DPSCs. These cells produce a collagenous matrix and then mineralize to maintain the balance and vitality of the dental pulp. Understanding the intrinsic mechanisms associated with the protective capacity of the dental pulp is the fundamental basis for applying similar approaches to induce pulp regeneration therapeutically (6,10,24).

Isolated DPSCs may differentiate into odontoblast-like cells in vitro, positive to mineralizing factors and, therefore, suitable for novo dental-pulp complex formation (17, 25-27). The research group led by Nör showed that SHEDs and DPSCs are capable of regenerating well-vascularized tissue with morphological and functional characteristics that closely resemble those of human dental pulp (3,28,29). Among these studies, Cordeiro et al. reported the engineering of dental pulp tissue using a biodegradable scaffold with SHEDs. The engineered tissue exhibited a similar architecture and histologic structure when compared to human dental pulp (3) and demonstrated the feasibility of engineering a well-vascularized dental tissue.

SCAFFOLDS

The association of previously described key elements must develop into a helpful microenvironment, called bioactive scaffolds. These microenvironments should provide a framework for stem cell attachment, differentiation and proliferation guided by morphogenic molecules. In addition, these scaffolds should be suitable for nutrient and oxygen diffusion to develop a fully functional tissue as naturally as possible (9,24).

Scaffolds can be molded (rigid and made for specific purposes) or injectable (delivered gels adaptable to the site for tissue regeneration). Both can be functionalized by enhancing the conditions for cell attachment and improved with morphogenic signals that supplement the host conditions and guide stem cells differentiation (4,24).

However, based on the finding that dentin-derived proteins are capable of inducing the full differentiation of DPSCs into odontoblasts, it has been suggested that the necessary morphogenic signals are already present in the root canal. As Piva et al. suggested, it is critical to protect dentin-derived factors from degradation and to enhance their accessibility and mobilization by treating the dentin surface. Taking advantage of these "well-prepared" host molecules, is essential to provide an adequate attachment for the stem cells to provide efficient vascularization for oxygen and nutrient perfusion and the chemotaxis of circulating progenitor cells by the application of functionalized scaffolds (24,29,30).

The accomplishment of an ideal scaffold to regenerate dental pulp tissue is a critical procedure. The translation of laboratory findings into clinical use represents a significant challenge. The approach should allow the formation of connective tissue within the confines of the dental root and must simultaneously be suitable for the recruitment or formation of blood vessels and neuronal structures through the apical foramen (4).

Several materials and models have been developed, e.g., polymers with suitable flexibility and degradability, synthetic polymers (poly-L-lactic acid) as a model for mechanic studies, co-polymers (poly-lactic-co-glycolic acid), gelatin and others. However, it has been demonstrated that hard polymer scaffolds are not practical for clinical applications. Therefore, injectable scaffolds seem to be the best option (4,24,28).

Injectable hydrogels penetrate throughout the root canal system and are conducive to stem cell survival and proliferation. Available options such as alginate hydrogels or self-assembling peptide hydrogels have been used. For example, Puramatrix® is a commercially available peptide matrix that instantly polymerizes, forming a biodegradable scaffold. Cavalcanti *et al.* showed that

these scaffolds allowed odontoblastic differentiation of dental pulp stem cells in vitro, demonstrating that this protocol was a promising alternative for dental pulp tissue engineering (4,24,28,31). The goal for future studies must be focused on achieving an optimum scaffold that resembles the microenvironment observed during tooth development.

VASCULAR AND NEURAL SUPPLY

To develop a novel strategy for dental pulp engineering, studies cannot focus merely on DPSC differentiation into odontoblasts. Indeed, to maintain and preserve the tissue, their specialization into supporting cells or the chemotaxis of vascular endothelial and neural cells is required. Vascular and neural supplies represent challenges, as they must be maintained and supplied through the foramen. Some strategies have been proposed to address this problem, such as embedding angiogenic and neurogenic factors into the scaffolds, the application of a prevascularized matrix, utilization of polymers containing vessel-like networks, and, more recently, taking advantage of the inherent vasculogenic potential of endothelial cells (9,24).

Successful tissue regeneration depends directly on the formation of an effective vascular network to provide oxygen, nutrients, immune cells and the recruitment of circulating cells. Moreover, substrates required for mineralization, such as calcium or phosphate, become available for odontoblasts through the blood vessels. It might be necessary to provide (or attract) endothelial cells to enhance the process of tissue vascularization to sustain viability and tissue vitality over time. Furthermore, innervation is essential for the regulation of the cells involved in the regeneration process, providing a protective effect with essential roles in inflammation and tissue repair (9,16,24).

The comprehension of strategies to improve these processes becomes one of the most critical challenges to dental pulp engineering. Despite all of the progress in the field, several processes remain unclear in pursuing the formation of a functional vascular network and efficient innervation for the clinical engineering of dental tissue.

Regenerated dental pulp must be both structurally and functionally as similar as possible to the natural tissue (32). The complexity of this tissue still challenges clinicians and investigators. Remarkable progress has been achieved over a short amount of time. Evidence suggests that the engineering of new dental pulp may be feasible. However, attaining full comprehension of the biological events underlying pulp-dentin complex formation is essential to regenerate this tissue and is key to achieving its application from the clinical perspective.

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