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Literature Review Article

Dental pulp stem cells in endodontic research: a promising tool for tooth tissue engineering

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Abstract

Introduction: Millions of teeth are saved each year by root canal therapy. Although current treatment modalities offer high levels of success for many conditions, an ideal form of therapy might consist of regenerative approaches in which diseased or necrotic pulp tissues are removed and replaced with healthy pulp tissue to revitalize teeth. The practice of dentistry is likely to be revolutionized by biological therapies based on growth and differentiation factors that accelerate and/or induce a natural biological regeneration. **Literature review:** This review summarizes current knowledge, barriers, and challenges in the clinical use of adult stem cells, scaffolds, and growth factors for the development and evaluation of regenerative endodontic therapies.

Introduction

There is a high rate of success in retention of teeth by endodontic therapy. However, many teeth are not restorable because of apical resorption and fracture, incompletely formed roots, or carious destruction of coronal structures. One novel approach to restore tooth structure is based on regenerative endodontic procedures by application of tissue engineering. Langer and Vacanti [19] defined tissue engineering as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function. The key elements of tissue engineering are stem cells, morphogen, and a scaffold of extracellular matrix.

Regenerative endodontic procedures can be defined as biologically based procedures designed to replace damaged structures, including dentin

and root structures, as well as cells of the pulp-dentin complex. Regenerative dental procedures have a long history, originating around 1952, when Hermann reported on the application of $Ca(OH)_2$ in a case report of vital pulp amputation [13]. Subsequent regenerative dental procedures include the development of guided tissue or bone regeneration (GTR, GBR) procedures and distraction osteogenesis [2] the application of platelet rich plasma (PRP) for bone augmentation [17]. The purpose of this article is to review the biological principles of tissue engineering and the hurdles that must be overcome to develop regenerative endodontic procedures.

Literature review and discussion

The major areas of research that might have application in the development of regenerative endodontic techniques are (a) postnatal stem cells, (b) scaffold materials, (c) morphogen/growth factors, (d) implantation.

Adult or postnatal stem cells

The most valuable cells for regenerative endodontics are postnatal or adult stem cells. All tissues originate from stem cells. A stem cell is commonly defined as a cell that has the ability to continuously divide and produce progeny cells that differentiate (develop) into various other types of cells or tissues. Based on their origin, there are two main types of stem cells - embryonic stem cells (ES cells) and postnatal or adult stem cells (AS cells).

Embryonic stem cells are stem cells derived from the inner cell mass of an early, preimplantation stage embryo known as a blastocyst. ES cells are pluripotent cells, which mean that they can give rise to all differentiated cell types derived from all three germ layers. There are limited numbers of publications about ES cells in pulp regeneration, due to the restricted policies regarding ES cell research over the past few years. The possible donor-host rejection of human ES cells is another concern [3]. This explains why researchers are now focusing attention on developing stem cell therapies using postnatal stem cells donated by the patients themselves or their close relatives. Postnatal stem cells have been sourced from umbilical cord blood, umbilical cord, bone marrow, peripheral blood, body fat, and almost all body tissues, including the pulp tissue of teeth [8].

Pulp stem cells

To date, four types of human dental stem cells have been isolated and characterized: (i) dental pulp stem cells (DPSCs) [12] (ii) stem cells from exfoliated deciduous teeth (SHED) [21] (iii) stem cells from apical papilla (SCAP) [31] (iv) periodontal ligament stem cells (PDLSCs) [28]. These dental stem cells are considered mesenchymal stem cells (MSCs) and possess different levels of capacities to become specific tissue forming cells. Sometimes pulp stem cells are called odontoblastoid cells, because these cells appear to synthesize and secrete dentin matrix like the odontoblast cells they replace [23]. After severe pulp damage or mechanical or caries exposure, the odontoblasts are often irreversibly injured beneath the wound site. Odontoblasts are postmitotic terminally differentiated cells, and cannot proliferate to replace subjacent irreversibly injured odontoblasts. The source of the odontoblastoid cells that replace the odontoblasts and secrete reparative dentin bridges are resident undifferentiated mesenchymal cells [26].

Dental pulp stem cells or (DPSCs) are multipotent mesenchymal type of stem cells that have the future potential to differentiate into a variety of other cell types including cardiomyocytes to repair damaged cardiac tissue following a heart attack [9], neurons to generate nerve and brain tissue [25], myocytes to repair muscle [18], osteocytes to generate bone [11], chondrocytes to generate cartilage and adipocytes to generate fat.

DPSCs can differentiate to odontoblasts, which makes them the most promising candidate for dentin-pulp complex regeneration. After being transplanted into immunocompromised mice, these cells generated mineralized dentin with highly organized tubular structures. Histological analyses revealed a well-defined layer of odontoblast-like cells, with characteristic processes extending into tubular structures within the regenerated dentin, and a highly vascularized pulp tissue center. The orientation of the collagen fibers within the dentin was perpendicular to the odontoblasts-like cell layer, similar to the naturally formed dentin [12].

Scaffolds

The second component of tissue engineering is a physical scaffold. Tissues are three-dimensional structures, and an appropriate scaffold is needed to promote cell growth and differentiation. It is known that extracellular matrix molecules control the differentiation of stem cells, and an appropriate scaffold might selectively bind and localize cells, contain growth factors, and undergo biodegradation over time [10].

Scaffold requirements

To achieve the goal of pulp tissue reconstruction, scaffolds must meet some specific requirements: (a) A scaffold should contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development (b) Scaffold should be effective for transport of nutrients, oxygen, and waste. It should be gradually degraded and replaced by regenerative tissue, retaining the feature of the final tissue structure (c) A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients [16].

Scaffold materials

(a) Platelet-rich plasma (PRP) is autologous, fairly easy to prepare in a dental setting, rich in growth factors, degrades over time, and forms a 3-dimensional fibrin matrix. (b) Bone sialoprotein acts as a scaffold material. When bone sialoprotein is implanted in the pulp tissue, up-regulation of secretory activity of extracellular matrix of newly generated odontoblasts and a thick reparative dentin formation are observed [4]. (c) Alginate hydrogel facilitates pulpal wound healing with hydration properties and tethering growth factors. (d) Mineral trioxide aggregate (MTA) sets in the presence of moisture, prevents microleakage, is biocompatible, and promotes reparative dentin formation [32]. The synthetic materials include polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL), which are all common polyester materials that degrade within the human body [1].

Most DPSCs studies have focused on the regeneration of the dentin-pulp complex revealing for the most part, poorly organized dentin-pulp complex-like structures with random shapes and orientations. In contrast, for clinical applications, the regenerated tissue needs to be highly organized. A regenerated highly vascularized soft tissue core with surrounding hard tissue seal would result in the best prognosis. Mooney *et al*. [22] reported that human DPSCs seeded onto a 3D PGA matrix and grown in vitro formed new tissue with a cellularity similar to that of native pulp.

Morphogen/growth factors

The third components of tissue engineering are morphogen. Morphogen can be used to control stem cell activity, such as by increasing the rate of proliferation, inducing differentiation of the cells into another tissue type, or stimulating stem cells to synthesize and secrete mineralized matrix. A variety of growth factors have successfully been used for dentin-pulp complex regeneration, including transforming growth factors (TGFs) [6], bone morphogenetic proteins (BMPs) [29], platelet-derived growth factor (PDGF) [35], insulin-like growth factor (IGF) [20].

Bone morphogenetic proteins (BMPs) were originally isolated from demineralized bone matrix. Recombinant human BMP2, BMP4, and BMP7 have been shown to induce reparative/regenerative dentin formation in vivo [24]. Recombinant human insulin-like growth factor-I with collagen membrane induces complete dentin bridging and tubular dentin formation [20]. Other investigators have shown that dentin or application of a dentin extract rich in growth factors will promote formation of an odontoblast phenotype [14]. Extracts of dentin promote growth, because many growth factors are embedded into the dentin matrix during dentinogenesis.

Growth factors, especially those of the transforming growth factor beta (TGFβ) family, are important in cellular signaling for odontoblast differentiation and stimulation of dentin matrix secretion. These growth factors are secreted by odontoblasts and deposited within the dentin matrix where they remain protected in an active form through interaction with other components of the dentin matrix [30]. Thus, the growth factors should be used in conjunction with postnatal stem cells to accomplish the tissue engineering replacement of diseased tooth pulp.

Implantation

In pulp implantation, replacement pulp tissue is transplanted into cleaned and shaped root canal systems. The source of pulp tissue may be a purified pulp stem cell line that is disease or pathogen-free, or is created from cells taken from a biopsy, that has been grown in the laboratory. The pulp stem cells must be organized into a three-dimensional structure that can support cell organization and vascularization. This can be accomplished using a porous polymer scaffold seeded with pulp stem cells.

The technique for creating replacement pulp tissue is using a three-dimensional cell printing technique [7]. In this technique an ink-jet-like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue [27]. Hydrogels are injectable scaffolds that can be delivered by syringe. The three-dimensional cell printing technique can be used to precisely

position cells, and this method has the potential to create tissue constructs that mimic the natural tooth pulp tissue structure. The ideal positioning of cells in a tissue engineering construct would include placing odontoblastoid cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cells. However, early research has yet to show that three-dimensional cell printing can create functional tissue in vivo.

Pulp revascularization

Pulp necrosis of an immature tooth as a result of caries or trauma could arrest further development of the root, leaving the tooth with thin root canal walls and blunderbuss apices. The absence of an apical constriction makes root canal treatment problematic because of the difficulty to obtain a seal with conventional obturation methods. The thin root canal walls render it susceptible to fracture. Regeneration of the pulpal tissue of an infected immature tooth might take place if suitable environment is possible with absence of intrapulpal infection and scaffold conductive to tissue ingrowth. Under these circumstances the pulpal space might become repopulated with mesenchymal cells arising from dental papilla or apical periodontium [5, 33]

Whole tooth regeneration

Tooth-like tissues have been generated by the seeding of different cell types on biodegradable scaffolds. A common methodology is to harvest cells, expand and differentiate cells in vitro, seed cells onto scaffolds, and implant them in vivo, in some cases, the scaffolds are re-implanted into an extracted tooth socket or the jaw.

Ikeda *et al.* (2009) [15] reported a successful fully functioning tooth replacement in an adult mouse achieved through the transplantation of bioengineered tooth germ into the alveolar bone in the lost tooth region. This technology was proposed as a model for future organ replacement therapies. The bioengineered tooth, which was erupted and occluded, had the correct tooth structure, hardness of mineralized tissues for mastication, and response to noxious stimulations such as mechanical stress and pain in cooperation with other oral and maxillofacial tissues. This study represents a substantial advance and emphasizes the potential for bioengineered organ replacement in future regenerative therapies.

Xu *et al.* (2008) [34] seeded tooth bud cells from the rat on scaffolds fabricated from silk fibroin with 2 different pore sizes that were either used as fabricated or treated with the Arg-Gly-Asp attachment site (RGD) binding peptide. These tissue-engineered constructs were placed in the omenta of athymic adult rats for 20 weeks prior to analysis. The larger pore sizes, as well as scaffolds treated with RGD, resulted in more mineralized osteodentin-like tissue.

Although most dental tissues are regenerated, the success rate for achieving the correct arrangement of a natural tooth is only 15-20%. Further studies are, therefore, required to consistently achieve reconstituted and structurally sound teeth.

Future directions

Despite the impressive progress in tissue engineering approaches to regenerative pulp therapy, numerous challenges remain.

1. A major challenge facing regenerative techniques is the ability to obtain a sufficient number of autogenous cells for scaffold seeding. One reason may be because cells isolated from adult tissues are often difficult to expand *in vitro* and generally do not maintain their phenotype. Technologies that facilitate high-throughput approaches are of particular interest for stem cell evaluation for dental tissue regeneration.
2. For regeneration of the dental pulp, fabrication of vascularized scaffolds is likely a key requirement. Technologies to fabricate tissue-engineered scaffolds with micro-engineered capillary beds are a promising advance toward a tissue-engineered tooth.
3. Advances in growth factors or drugs to control the activity of cells must be sought out.

Conclusion

An interesting question, the origin of the new pulp tissue still remains to be answered. One of the most challenging aspects of developing a regenerative endodontic therapy is to understand how the various component procedures can be optimized and integrated to produce the outcome of a regenerated pulp-dentin complex. The future development of regenerative endodontic procedures will require a comprehensive research program directed at each of these components and their application. The regenerative therapy will revolutionize the future endodontics with the synergistic confluence of advances in signaling

pathways underlying morphogenesis and lineage of stem/progenitor cells by morphogen such as BMPs and synthetic scaffolds.

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