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Scaffolds Combined with Stem Cells have Synergistic Effect in Regenerative Dentistry

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Abstract

Tissue engineering generates alternatives for tissues regeneration and/or reconstructive surgery. Materials and fabrication technologies are important in designing temporary extracellular matrices (scaffolds), implicated in three-dimensional tissue formation. In this review, we reference the relationship between tissue engineering and materials science. Materials for porous solid-state scaffolds include linear aliphatic polyesters Poly Glycolic Acid (PGA) and their co-polymers Poly Lactic-Co-Glycolic Acid (PLGA), poly (ε-caprolactone) (PCL) and Poly Hydroxyl Butyrate (PHB)]. Biodegradable polymers include Poly Propylene Fumarate (PPF) and phosphoesters. Natural macromolecules such as proteins and polysaccharides including collagen, either denatured as gelatin, or combined with glycosaminoglycan(s), silk, alginate, chitosan, and hyaluronate. Inorganic materials are categorized as porous bioactive glasses or calcium phosphate, β-tricalcium phosphate, hydroxyapatite and their derivatives. Research's on implantable materials for tissue regeneration shed lights on biodegradable polymers and specifically, on synthetic proteins promoting regeneration of the dental pulp despite infectious degradation and necrosis of the pulp tissue.

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Keywords

Tissue Engineering; Scaffolds; Inorganic Porous Materials; Natural Macromolecules; Tissue Regeneration

Introduction

A scaffold may be defined as “the support, delivery vehicle, or matrix for facilitating the migration, binding, or transport of cells or bioactive molecules used to replace, repair, or regenerate tissues”. It should replicate the Extracellular Matrix (ECM) at the nanoscale regulating cell function, and specific events at the cellular and tissue levels. Moreover, scaffolds should be synthesized in order to avoid immune responses [1].

Many polymers, both synthetic (e.g., Poly Lactic Acid (PLA) and natural (e.g. collagen), were used in order to obtain macroporous scaffolds, one of the three crucial elements contributing to tissue engineering strategy. The two others partners are stem cells combined with growth and transcription factors. The polymeric biomaterials used for pulp regeneration are classified into natural or synthetic polymers: natural polymers are considered to provide better biocompatibility, while synthetic polymers allow more control of physicochemical properties, such as the degradation rate, microstructure, and mechanical strength [2].

Apart from demonstrating multilineage differentiation, dental stem cells display positive expression of specific surface antigen markers (e.g., CD44, CD73, CD105). Stem cells have been identified in the dental pulp of permanent or/and deciduous teeth. They are issued from the Stem Cells Apical Papilla (SCAP), gingiva, periodontal ligament, and dental follicle. SCAPs are identified as a soft tissue located at the apex of growing tooth roots. Apical papilla is a source of “primary odontoblasts” that synthesize tubular dentin, as opposed to the “replacement odontoblasts” (including Höhl’cells) implicated in the formation of reparative dentin.

A key benefit related to SCAPs pertains to their apical location that supports tissue survival after pulp degradation. Specifically, SCAP co-expresses STRO-1+ with a range of osteo / dentinogenic markers. From a proliferation standpoint, SCAP demonstrates a higher rate of dentinogenic markers compared to DPSC. Human or animal Stem Cells (SC) include Dental Pulp Stem Cells (DCSC), SC from Exfoliated Deciduous teeth (SHED), SC taking origin from the Apical Papilla (SCAP), from the Periodontal Ligament SC (PDLSC) and Dental Follicle Progenitor Stem Cells (DFPSC). All these SC were identified as components of dental tissues. Periodontitis affects the integrity of oral mucosa and hard tissue (alveolar bone), which may result in tooth loss [3].

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Vascularized soft connective tissue was visualized in the pulp and the cells located at the interface between pulp and dentin appeared to be intimately associated with the dentin wall. Current therapies implies root canal disinfection and regeneration of the pulp-dentin complex despite pulp necrosis.

- Antibiotic pastes used to eradicate canal infection have been demonstrated to impact stem cell survival
- Three-dimensional nanofibers, combined with injectable scaffolds, enriched or not with stem cells and/or Growth Factors (GFs), lead to an increased dental pulp regeneration

Alone or associated with triple antibiotic incorporation, the target was the pulp disinfection (Metronidazole, Ciprofloxacin and Minocycline), prior to electrospinning. Polymer nanofibers were obtained by the creation and elongation of an electrified jet. As an alternative method commonly referred to as thermally-induced phase separation, polymer nanofibers were incorporated in the fabrication of macro/micro pore networks within 3D nanofibrous scaffolds. Recent advances allow to obtain scaffolds that can be easily injected in the desired site. They contribute and serve as delivery vehicles for bioactive factors. Some of the latest developments include the testing of innovative scaffolds/stem cells constructs in conjunction with therapeutic agents, and they are presented as evidence of the translational potential of tissue engineering in regenerative endodontic therapies (Fig. 1).

In a very few cases, a limited number stem cells can be obtained from a dental tissue. Induced Pluripotent Stem Cells (iPSC) were induced by viral introduction of four transcription-factors mediated reprogramming (Oct4, Sox2, Klf4 and c-Myc). Incorporated into a scaffold and in close association with bioactive molecules, they contribute to the formation of reactionary dentin after implantation of cells within the necrotic pulp. Reparative dentin may be induced by such methods, but no cementum nor bone formation were observed after tissue transplantation.

- i. Biocompatibility: After implantation, the tissue engineered construct elicits a negligible immune reaction in order to prevent an inflammatory response.
- ii. Biodegradability: The by-products of this degradation should be non-toxic. In order to allow degradation occurring together with an inflammatory response. An answer combined with controlled infusion of cells such as macrophages is required.
- iii. Mechanical properties: Developing scaffolds with mechanical properties similar to bone and cartilage. Many materials having demonstrated potential in vitro were seen to fail when implanted in vivo due to insufficient vascularization. A balance between mechanical properties and porous architecture allow vascularization, key to the success of any scaffold.
- iv. Scaffold architecture: Scaffolds should have an interconnected pore structure and high porosity to ensure cellular penetration and adequate diffusion of nutrients to cells within

the construct. A porous interconnected structure is required to allow diffusion of waste products out of the scaffold.

- v. Manufacturing technologies: Methods of scaffold fabrication, such as electrospinning, supercritical fluid-gassing, three-dimensional bioprint, and self-assembling cells are used successfully. Nanofibrous scaffolds have been processed via electrospinning, self-assembly, and phase-separation [4].

Figure 1: Perivascular niche and multipotency of mesenchymal stem cells (Oh & Nör, 2015).

Naturally-Derived Polymeric Scaffolds

Attempts to produce scaffolds including polystyrene, Poly-L-Lactic Acid (PLLA), Poly Glycolic Acid (PGA) and Poly-DL-Lactic-Co-Glycolic Acid (PLGA) result from numerous synthetic polymers. While these materials can be fabricated with a tailored architecture, and their degradation characteristics controlled by varying the composition of the individual polymer, polymeric scaffolds have drawbacks including the risk of rejection due to a reduced bioactivity. In addition, concerns exist about the degradation process of PLLA and PGA as they degrade by hydrolysis. Naturally-derived biological materials such as collagen, various proteoglycans, alginate-based substrates and chitosan have been used in the production of

scaffolds. Unlike synthetic polymer-based scaffolds, natural polymers are biologically active and promote excellent cell adhesion and growth. They allow the host cells to produce their own extracellular matrix and replace the degraded scaffold.

In addition to biomechanical signals, cellular behavior is strongly influenced by biological and biochemical signals from the extracellular matrix. Therefore, the use of scaffolds as delivery systems for growth factors, adhesion peptides and cytokines has received considerable attention [5].

Collagen (Type I) combined with glycosaminoglycans is the most important extracellular matrix component. Present in the pulp (sharing this distribution with type III collagen) and dentin, other non-collagenous matrix proteins are implicated in the formation of the tooth ECM. Collagens are the most abundant ECM component of dental pulp. The molecules are advantageous, implicated in the proliferation of pulp SC located in Human Exfoliated teeth (SHED) and their potential differentiation into odontoblasts.

DPSC combined with human endothelial cells exhibited vascularized pulp-like tissue. Cell-cell interactions and migration contribute to successful dental pulp regeneration [6].

Poly Glycolic Acid (PGA) should be oriented to form 13 μ m diameter fibers [7]. Six properties of the scaffold are listed here [8]:

1. The surface should permit cell adhesion and growth
2. Neither the polymer or its degradation products should provoke toxicity when implanted *in-vivo*
3. 3D structures should be process able
4. The porosity should be at least 90% to provide a template for the regenerative tissue
5. The scaffold degradation should match to the tissue regeneration

Utilizing Poly Lactic-co-Glycolic Acid and multistage vector composite microspheres (PLGA-MSV), it was shown that:

1. BMP/ PLGA-MSV microspheres had the ability to release small but effective doses of BMP-2 for 40 days in a controlled, linear fashion
2. The release profile of the BMP/ PLGA-MSV microspheres was dependent on the PLGA coating
3. The PLGA-MSV system did not impact cell metabolic activity
4. BMP-2 released from PLGA-MSV microspheres was capable of osteoinduction of BM-MSCs

This biocompatible, biodegradable, and osteogenic PLGA-MSV microsphere system holds promise as a candidate for the delivery of effective doses of bioactive proteins for pharmaceutical induction of osteoregeneration [9].

Biologically-active growth factors are trapped in the matrix during dentinogenesis, namely Transforming Growth Factor-beta 1 (TGF- β 1) and Bone Morphogenetic Protein 2 (BMP-2), which are keys in driving the odontogenic differentiation of SCAP, as well as VEGF, PDGF and other angiogenic factors. In addition, IGF (insulin-like growth factor) and EGF (Epidermal Growth Factor) were used for vascularization and regeneration.

Different Type of Naturally Devoided Polymeric Scaffolds

The most common polysaccharides used are alginate, hyaluronic acid, chitosan, and starch, but agarose, glucans, and dextran find also their application. The unmodified polymer is used for hydrogel or capsule formation [10].

Early stages of Mesenchymal Stem Cells (MSCs) differentiation towards osteoblasts are characterized by RUNX2 expression, which induces the expression of bone matrix protein genes such as Alpha-1 Type I Collagen (COL1A1), Alkaline Phosphatase (ALP), Bone Sialoprotein (BSP) and Osteocalcin (OCN) in the differentiating cells through the Wnt and BMPs signalling. Mature osteoblasts express osterix and actively secrete bone matrix proteins such as OCN, BSP I/ II, and COL1A1.

Fibrin: Fibrin-based scaffolds have been used for soft tissue engineering and the revascularization of dental pulp as a result of odontoblastic differentiation. Fibrinogen and thrombin are combined to form a fibrin hydrogel. Fibrin can be prepared as a glue or as engineered microbeads. Fibrin is a versatile biopolymer, showing a great potential in tissue regeneration and wound healing [11].

Alginate scaffolds in tissue engineering have intrinsic properties attributed to its biocompatibility, favorable immunogenicity, low cost, and mild gelation requirements. Alginate is one of the most commonly used polysaccharide, as it is easily cross-linked with multivalent cations such as Ca^{2+} .

Hyaluronic acid and derivatives, is a linear glycosaminoglycan (comprised of d-glucuronic acid and N -acetyl-d-glucosamine). With its biocompatibility, enzymatic degradability *in-vivo* and mechanical properties, it is a good candidate for the use in regenerative medicine. HA based scaffolds combined with stem cells have shown to have a synergistic effect in the regeneration capacity [12]. Hydrogels made by cross-linking maleimide-functionalized hyaluronic acid with a cell-adhesive and a degradable peptide, showed a release of these growth factors correlated with the degradation of the scaffold.

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Chitosan derivatives are biocompatible, biodegradable, displaying low cytotoxicity, and immunogenicity. It has a broad-spectrum of antibacterial properties, one of the most frequent composant is a chitosan. It is a glucosamine obtained through deacetylation of chitin. It can act as a polycation due to its deacetylated amino groups and supporting cell attachment, differentiation, and migration, as well as osteoconduction and the promotion of osteoblast growing.

A Carboxymethyl Chitosan-Based Scaffold (CMCS) with TGF- β 1-releasing chitosan nanoparticles (TGF- β 1-CSnp) has been investigated. TGF- β 1 induces the cytological and functional differentiation of odontoblasts which plays an important role in the secretion of the dentin matrix.

Chitosan-alginate is a hybrid biodegradable scaffold. It may serve as temporary skeleton to stimulate new tissue growth. This scaffold provides a favorable environment with high porosity, mechanical and biological properties that can be used for clinical trials.

Cellulose, a polymer of (1-4)-linked- β -d -glucose units, and its derivatives, cellulose may be derived from either plants or bacteria.

Peptide-based scaffolds bind growth factors and display a slow release profile for TGF2 and VEGF. SHED demonstrated to be a population of highly proliferative, clonogenic cells, differentiating into a diversity of cell type expressing STRO-1 and CD146, two MSC markers also present in DPSC, however SHED exhibited higher proliferation rates than DPSC (Demarco et al., 2011).

As major components of the extracellular matrix, Glycosaminoglycans (GAGs) like hyaluronic acid and chondroitin sulfate are a bioinspired class of scaffold materials providing biocompatibility and inherent bioactive properties (like enhanced cell viability) that are beneficial for bone regeneration.

SCAP scaffolds require intracanal blood clot, platelet-rich plasma, alginate, hyaluronic acid, chitosan, PLLA NF-with BMP-2, PLGA-PEG nanoparticles, VitroGel 3D with SDF-1x and BMP-2 constitute scaffolds that can be used for pulp regeneration. Finally, polymeric scaffolds may be used efficiently for dental pulp tissue engineering [2].

Synthetic Scaffolds

Synthetic biomaterials have been used as bone graft substitutes. These biomaterials were initially selected for structural restoration based on their biomechanical properties. Scaffolds were engineered to be bioactive or bioresorbable and to enhance tissue growth. They are

designed to induce bone formation and vascularization. These scaffolds are often porous, made of biodegradable materials that harbor different growth factors, drugs, genes, or stem cells [13].

Poly L-Lactic Acid (PLLA) nanofibrous microspheres was evaluated as a SCAP carrier. PGLA is a copolymer formed by the union of Polylactic Acid (PLA) and Polyglycolic Acid (PGA). Advantages of PLLA NF-MS with controlled BMP-2 release include their inject ability and ability to adapt to the root canal morphology. With similar architecture to collagen, high porosity and a large surface area, NF-MS facilitate cell adhesion, growth, as well as nutrient and waste exchange [14].

PLGA-PEG Nanoparticles The use of the PLGA-PEG nanoparticles scaffold is limited by the clinically-prohibitive costs of production and standardization, as well as the necessity to bank and pre-mix autologous SCAP with scaffolds prior to injection. Furthermore, there was minimal radiographic evidence of continued root formation in length and canal wall thickness suggesting that the injectable PLGA-PEG scaffold may induce apexification while facilitating periapical healing (stem cell-based dental tissue regeneration).

Scaffold properties for SCAP delivery and in other tissue regeneration procedures. Collagen is a natural biomaterial that is widely-used in tissue regeneration applications as a result of its architectural structure to mimic many tissues extracellular matrix and its ability to adapt to the morphology of the target.

Type I collagen-based scaffolds together with dental stem cell-containing blood clots promote intracanal hard tissue formation compared to blood clots alone.

Self-Assembling Peptide Hydrogel (Puramatrix™): It is a synthetic, self-assembling peptide hydrogel that creates a 3D biocompatible, biodegradable, and non-toxic environment to cells. It can be speculated that Puramatrix™ may also prove to be a suitable scaffold for SCAP in regenerative endodontic applications.

Calcium Polyphosphate/calcium phosphate cement: The scaffold also has a chain-like structure with oxygen atoms connecting monomeric subunits that provide easily-accessible sites for hydrolysis to naturally-occurring, readily - metabolized calcium orthophosphate products. As the CPP scaffold degrades, release calcium and phosphorous components, they contribute to the formation of calcified tissues, such as dentin. Calcium Phosphate Cement (CPC) has also been investigated as a convenient scaffold for human DPSC (Fig. 2).

Hydrogel scaffold, maintains viability and support differentiation of dental pulp cells [15]. Puramatrix™ is a self-assembling peptide hydrogel. DPSC cultured in Puramatrix™ survive, proliferate and differentiate into odontoblasts expressing odontoblastic markers: DSPP and DMP-1. This class of materials represents a promising new alternative for tissue engineering [14].

Figure 2: Human dental mesenchymal stem cells (Sharpe 2016).

Conclusion

- First, scaffolds provide architectures for reliable and predictable pulp-dentin complex regeneration. Accordingly, many biomaterials are currently available as scaffolds for regenerative endodontics, including both synthetic nanofibrous microspheres and hydrogels formed from biomimetic (eg, multidomain peptides) or naturally occurring (eg, collagen) molecules. Taking into consideration the deleterious effect of higher concentrations of antibiotics and patient-specific pulp chambers, scaffolds which are endowed with both antimicrobial and injectable capabilities to engineer biocompatible scaffolds that may bring forward amplified of achieving predictable pulp-dentin complex regeneration.
- Second, vascularization possesses superior regeneration feasibility relative to innervation and dentinogenesis.
- Third, it points out that regenerating tubular dentin remains fairly challenging. Organized dentinal tubules in newly formed dentin are less predictable. Accordingly, it is beneficial and significant to provide optimal signals for odontoblastic differentiation of recruited cells.
- Fourth, the efficacy of pulp-dentin complex regeneration is predominantly validated by using histological approaches, in the absence of functional neural and vascular testing. Combination with innervation and vascularization appears to be crucial for the pulp located in the root canal and promotes its regeneration.

- There are two possible scenarios:
 1. The pulp is reversibly inflamed and healthy root canal pulp tissue can remain after a pulpotomy
 2. The pulp, irreversibly inflamed or necrotic, has to be completely removed, and following chemo-mechanical cleaning methods no vital tissue remains inside the root. SCAP may proliferate and recolonize the root

In the first situation, the remnant tissue serves as a source of resident stem cells. The treatment aims to sustain pulp vitality.

Applying the cell homing approach may be appropriate to this less invasive situation as the cell-free scaffold delivers bioactive cues to recruit remaining resident stem cells and induce their differentiation.

However, the characteristics of stem cells might not be reliable to predict a successful outcome due to patient-related variability.

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