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Fibrin Scaffold; a Silent Supporter in Dental Pulp Regeneration: A Review

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Abstract
Root canal therapy has enabled us to save numerous teeth over the years. Revitalizing a tooth with diseased pulp or nonvital tooth will be the most desired outcome of any endodontic treatment.Tissues are organized as three-dimensional structures, and appropriate scaffolding is necessary to provide a spatially correct position of cell location and regulate differentiation, proliferation, or metabolism of the stem cells. Extracellular matrix molecules control the differentiation of stem cells. Regeneration of different tissues are facilitated by different scaffolds. An appropriate scaffold selectively localizes, bind cells and contain growth factors. Scaffolds undergo biodegradation over time. Thorough and precise knowledge about the suitable scaffold for the required tissue is needed for successful regeneration. The current review presents how for dentin–pulp complex regeneration, the application of fibrin scaffolds could represent a promising approach.

Key Words: *Regeneration, Stem cells, Growth factors, Fibrin Scaffolds, Hydrogel*

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I. Introduction

Regenerative dentistry has been popularized by recent advances in biologic therapies which hasten or induce natural biologic regeneration. Nygaard Ostby in 1961 evaluated a revascularization method for re-establishing pulp-dentin complex in permanent teeth with pulpal necrosis.

Regenerative endodontics is an offshoot of tissue engineering. "Regenerative endodontic procedures (REPs) have been defined as biologically-based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex with live viable tissues, preferably of the same origin, that restore the normal physiologic functions of the pulp-dentin complex".

The three key ingredients for regeneration are stem cells, growth factors and the extracellular matrix (ECM) scaffold.

STEM CELLS

Stem cells are undifferentiated embryonic or adult cells that retain the capacity to continuously divide, creating additional stem cells. Embryonic stem cells are totipotent and have the capacity to self-renew, whereas stem cells that reside within an adult organ or tissue have the ability to select a differentiation program from only a few possible pathways. [2]

GROWTH FACTORS

Growth factors regulate either transplanted cells or endogenous cells in dental pulp-dentin regeneration. They are polypeptides or proteins that bind to specific receptors on the surface of target cells. e.g., bone morphogenetic protein [BMP] receptors. BMP affect cellular activities like migration, proliferation, differentiation, and apoptosis of all dental pulp cells, including stem/progenitor cells. Bioactive cues that recruit the proper cells are critical in pulp regeneration. eg. transforming growth factors [TGFs] β1, β3are needed for odontoblast differentiation and stimulation of dentin matrix. These events of repair and regeneration can be coordinated and modulated by growth factors such as platelet-derived growth factor (PDGF), TGF, BMPs, vascular endothelial growth factor (VEGF), fibroblast growth factor, and insulin-like growth factor (IGF). [1]

SCAFFOLDS

1.Scaffolds are three-dimensional (3D) porous solid biomaterials which provide a spatially correct position of cell location.
2.Promotion of cell-biomaterial interactions, adhesion of cells, and deposition of extra cellular matrix.

3.Allow sufficient transport of gases, nutrients, and regulatory factors to allow cell survival, proliferation, and differentiation.
4.Biodegrade at a controllable rate that approximates the rate of tissue regeneration.
5.Produce a reduced amount of inflammation or toxicity in vivo. [3]

CLASSIFICATION OF SCAFFOLDS

- Based on degradability of matrices:

Biodegradable scaffolds
Permanent or biostable scaffold

- Based on form:

Solid blocks
Sponges
Porous sheet
Hydrogels (injectable scaffolds)

HYDROGEL
1. Natural: Collagen, Gelatine, Fibrin, Matrigel, Keratin, Alginate , Chitosan, Agarose , Cellulose , Hyaluronic acid, Extracellularised matrix from decellularized tissue.
2. Synthetic: Polylacticacidhydrogel, Polydimethyl siloxane hydrogel, Polyethelene glycol, Poly n propylacrlanamide gel, Vitrogel, Self-assembling peptide hydrogel
3. Hybrid: Alginate/laponite hydrogel, PEG modified natural polymers

- Based on presence or absence of cells:

Cell free scaffolds
Scaffolds seeded with stem cells

- Based on origin:

Biological or Natural Scaffolds:
Platelet rich plasma, Platelet rich fibrin, Collagen, Chitosan, Glycosaminoglycans/hyaluronic acid, Demineralised /Native dentine matrix, Blood clot, Silk.

Artificial or Synthetic scaffolds:
Polymers: Polylactic acid, Polyglycolic acid, Polylactic-co glycolic acid, Polyepsiloncaprolactone
Bioceramic: Calcium/phosphate materials, Bioactive glasses, Glass ceramics [5]

HYDROGELS IN REGENERATION

Hydrogel-based scaffolds are a unique category of three-dimensional polymeric networks with high content of water. They are hydrophilic and biocompatible. They have tuneable degradation patterns and mechanical properties, and can be loaded with various bioactive molecules. Together with this, considerable degree of flexibility and elasticity of hydrogels, make them similar to the cell extracellular matrix (ECM), particularly that of the DP. [4]

Hydrogels are classified into natural, synthetic or hybrid hydrogels that combine natural and synthetic hydrogels. Natural polymers are bioactive, highly biocompatible, and biodegradable by naturally occurring enzymes or via hydrolysis. Synthetic polymers have tuneable mechanical properties, thermostability and durability in comparison to natural hydrogels. Hybrid hydrogels combine the benefits of synthetic and natural polymers. [6]

Natural polymers-based hydrogels mimic natural tissues, they are also liable to be easily permanently damaged due to their poor mechanical properties. On the contrary, the synthetic ones have considerably higher mechanical properties and physicochemical properties but lack natural tissue resemblance.

Role of Hydrogels in Dentin–Pulp Regeneration

The hydrogels act as a space filling material. They also act as carriers for cells and bioactive molecules. For this, hydrogels need to maintain desired volume and structural integrity for the required time.

For dentin–pulp complex regeneration, hydrogels act as carriers of stem/progenitor cells with odontogenic potential, such as dental pulp stem cells, odontoblasts-like cells, Human Umbilical Vein Endothelial Cells, Stem cells from apical papilla (SCAP), SHED: stem cells from human exfoliated deciduous teeth, Bone marrow-derived mesenchymal stem/stromal cells (BMSC), Periodontal ligament stem cells (PDLSCs), endothelial cells and primary dental pulp cells.

They can also act as carriers for the local delivery of antibiotics, such as clindamycin and bioactive molecules, aiming to promote tissue regeneration, such as Vascular endothelial growth factor (VEGF) , Fibroblast growth factors (FGF) , Bone Morphogenetic Proteins (BMPs) , Transforming growth factor beta 1(TGF-1) , stem cell factor , dentonin sequence and RGD cell-binding motifs .After implantation, hydrogels biodegrade allowing release of bioactive molecules that influence the surrounding environment.[7]

Fibrin:

Fibrin is a naturally derived insoluble protein biopolymer, produced through the polymerization of fibrinogen protein (present in blood plasma) under the control of thrombin during blood clotting, which results in the formation of a fibrous polymer network important in haemostasis and wound healing.

It is advantageous compared with most of synthetic polymers and collagen gels when bio-compatibility, cost, cell adhesion, and immune response are concerns. Fibrin has no undesirable immunogenic reactions when obtained from autologous sources. Major advantages of fibrin hydrogels are that they are injectable, reproducible, and mouldable to specific 3D shapes. Mechanical properties of fibrin scaffolds have been notably improved when combined with polyurethane, polyethylene, hyaluronic acid, or calcium phosphate ceramics.

Physical characteristics, cell invasion, and survival characteristics of fibrin scaffolds can be adjusted by manipulating the fibrinogen/thrombin concentration. Increasing fibrinogen content increases stiffness but reduces porosity [8]

Platelet-rich plasma (PRP) therapy is an emerging technology in sports medicine, and it has been used since the mid-1990s in dental and oral surgery. Recently, the patient-derived fibrin (PRF) scaffold (based on PRP technology) operates as an autologous and biodegradable delivery system. PRP/PRF along with collagen membranes are widely used for regeneration of periodontal ligament and alveolar bone for GTR procedures in periodontics and oral surgery.

PRP therapy was shown to be a successful promoter in the healing process. PRP and its associated formulations have yet to show promise in dental pulp regeneration. Fibrin has low mechanical stiffness and undergoes rapid shrinkage and degradation; nonetheless, shrinkage can be reduced by fixing agents, like poly-L-lysine and degradation rate can be controlled by fibril cross-linking or by use of enzyme inhibitors. [9]

Fibrin glue mixed with PRF is used as a scaffold seeded with dental bud cells for regeneration of tooth. PRF is rich in cytokines and GFs, especially platelet-derived GF (PDGF) and tissue GF-β (TGF-β) that have relevant roles in angiogenesis and hard tissue regeneration, respectively. Dental bud cells cultured on the PRF/PRP scaffold regenerated a complete tooth with enamel, dentin, cementum, pulp, blood vessels, and periodontal ligament [6]

Fibrin hydrogel

Fibrin hydrogels are able to function as both two-dimensional (2D) and 3D cell culture scaffold. In the 2D application, the scaffold is prepared and undergoes gelation prior to cell seeding and after the gelation, cells are seeded into the scaffold. In the 3D application, isolated cells are initially suspended in the scaffold precursor solution. Then the cell–fibrin gel precursor solution mixture can be directly injected into the target lesion in which, afterward, the fibrin gel cures. Thus, fibrin hydrogels can also act as a vehicle for cell transplantation.

Advantages:

- Low cost,
- Providing excellent biocompatibility
- Superior cell adhesion properties.
- Easily obtained from autologous sources, thus avoiding undesirable immunogenic reactions.
- Fibrin is biodegradable in a controllable manner
- Non-toxic degradation products and can be readily replaced by cell-derived ECM in a few days.

Disadvantages

- Weak mechanical properties
- the possibility of disease transmission and gel shrinkage.

The shrinkage of the gel can be decreased using fixing agents eg.polyL-lysine .
Mechanical properties of fibrin scaffolds can be easily altered by adjusting the concentration and ionic strength of fibrinogen. The modification of the fibrin polymerization alters mechanical properties through modifying the fibre thickness, degree of porosity and branching of the formed gel. [9,10]

Fibrin and its degradation by-products lack any anti-bacterial activity, which could represent a drawback for the use of fibrin hydrogel in REP, since residual microorganisms in the canal space and dentinal tubules may hinder dentin–pulp complex regeneration.

Fibrin hydrogels are sometimes stabilised by factor XIIIa, which increases crosslinking of fibrinogen molecules, but this strongly slows down hydrogel lysis. Addition of 10 U/mL of factor XIIIa results in decrease of the lysis rate by approximately 45 %.[16]

The persistence of residual bacteria in the root canal after disinfection could be due to bacterial organization into biofilms on the dentin surface, making them more resistant to anti-microbial agents and deep penetration into the dentinal tubules. Residual bacteria affect dentin–pulp complex regeneration through triggering the host immune/inflammatory response. The incorporation of anti-bacterial agents into the fibrin scaffold could represent a viable solution for such a limitation. [11,3]

Fibrin hydrogels have three major disadvantages: shrinkage, low mechanical stiffness, and rapid degradation prior to proper tissue formation. Depending on the application site and whether the disadvantages adversely affect the outcome of regenerative treatments, these disadvantages can be compensated by applying modifications to this scaffold.

STRATEGIES TO IMPROVE FIBRIN-BASED SCAFFOLD PROPERTIES:

1.Pro-regenerative properties
2. Anti-inflammatory properties
3. Antibacterial properties
4. Nanotherapeutics

PRO-REGENERATIVE PROPERTIES

DP regeneration requires the concerted action of growth factors and other bioactive molecules that must be provided to the cells responsible for tissue neoformation in the endodontic space. The scaffold injected into the endodontic space must guide the spatio-temporal cell recruitment and differentiation of various DP cell lineages . Several growth factors have been added to the fibrin scaffold to promote tissue regeneration, including pro-angiogenic factors such as VEGF,PDGF and FGF-2, factors inducing odontoblast differentiation such as BMP-2 and -4, nerve growth promoting factors such as the Nerve growth factor (NGF) and factors promoting Mesenchymal stem cells (MSCs) recruitment Dental Pulp Mesenchymal stem cells (DP-MSC) pre-treatment with FGF-2 promoting angiogenesis within subcutaneously implanted hydrogels.

ANTI-INFLAMMATORY PROPERTIES

DP-MSC expression of the pro-inflammatory cytokines IL-1β, IL-6, and IL-8 upon treatment with lipopolysaccharide (LPS), which is also in favour of a possible role of fibrin in preventing inflammation. MSCs possess immunomodulatory properties through their inhibition of the T-cell response.

ANTIBACTERIAL PROPERTIES

Incorporating drugs or molecules with antibacterial properties at the time of scaffold manufacture such as metronidazole and/or ciprofloxacin into nanofibrous scaffolds, both drugs were shown to inhibit the growth of the endodontic bacteria Enterococcus fæcalis, Porphyromonas spp.and Fusobacterium nucleatum. Recently, chitosan, an antibacterial glycosaminoglycan derived from shrimp shells, was associated with fibrin to provide a fibrin hydrogel with antibacterial properties, the fibrin-chitosan hydrogel strongly inhibited E. faecalis growth [18]

NANOTHERAPEUTICS

The recent development of nanobiotechnologies offers great hope to deliver locally, in a spatially and temporarily controlled manner, antibacterial drugs in small-sized tissues such as the endodontic space and in difficult to reach areas such as dentine tubules. NPs are effective antibiotic carriers because

i. They protect the drug in its native structure
ii.Conserve therapeutic properties
iii. Deliver large amounts of drug at the site of infection and
iv. Limit side effects. [17]

Among the various types of NPs, PLA-NPs received considerable attention over recent decades because of their highly biocompatible and biodegradable nature and their low levels of immunogenicity and toxicity. [11,13]

A nanocomposite fibrin-based hydrogel containing CLIN-loaded PLA-NPs was recently designed to bring the antibiotic into contact with endodontic bacteria. [19]

Addition of CLIN-loaded NPs into a fibrin hydrogel gave it antibacterial and antibiofilm properties against E.fæcalis. Nanotherapeutic strategies thus appear to augment DP regeneration.

Slow release of the molecule increases the exposure time of the bacterial population to the antibiotic. Systemic side effects are minimized by restraining the drug localization within the infected site. Antibiotic-loaded NPs helps in biofilm eradication as they penetrate deep into biofilm matrices, reaching the deepest and most persistent bacteria which are difficult to eliminate. Poly (D,L) Lactic Acid NPs (PLA-NPs) are promising , because of their biodegradable nature, biocompatibility, and low immunogenicity[20]. PLA is a biopolymer with high safety profile which has been approved by the US Food and Drug Administration for various applications. PLA-NPs constitute a versatile carrier of drugs and vaccines and have been used to deliver antifungal and antibacterial drugs such as amphotericin B ,minocycline and pipemidic acid. The PLA-NPs could constitute a suitable antibiotic carrier that, once added to fibrin hydrogel formulation, would provide the hydrogel with antibacterial and antibiofilm properties. Clindamycin (CLIN) was chosen because of its broad spectrum activity against endodontic bacteria. Also, CLIN can be easily incorporated into PLA-NPs owing to its hydrophobicity.[21]

II. Conclusion

Regenerative endodontic procedures have emerged as viable alternatives for the treatment of immature teeth with pulpal necrosis. The clinicians should be aware of the properties of scaffolds for selecting and using the most appropriate scaffolds.

Antibacterial and biocompatible hydrogel may be valuable for DP regeneration in a previously infected endodontic space. Fibrin gel scaffolds have favourable properties that includes adhesion, proliferation and differentiation of stem cells, induction of angiogenesis, along with excellent handling characteristics, especially for RETs. Its properties can be desirably modified according to its application. Platelet concentrates are autologous fibrin-based materials containing growth factors that favour RET. They seem to be promising in this treatment modality. Further clinical studies with higher levels of evidence is required.

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