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Scaffolds in Regenerative Endodontics- A Review

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Abstract

Although conventional methods such dental root canal therapy and apexification treatments have been effective in treating sick or infected root canals, these techniques fall short in their ability to restore healthy pulp tissue in teeth that have undergone treatment. The goal of regeneration-based treatments is to rejuvenate teeth by swapping out unhealthy pulp tissue for damaged or necrotic pulp tissue. The use of regenerative techniques in dental offices has the potential to significantly raise the standard of living for patients. This review paper provides an in-depth description of the scaffolds utilized in regenerative endodontic techniques while also offering a glimpse into the upcoming novel developing strategies.

***Keywords**: Scaffold, Autologous Scaffold, Synthetic Scaffold*

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Introduction

Historically, materials and treatment options had limited the ability of dentists to treat diseased tissues. Traditional dentistry had no adequate therapeutic strategies for immature teeth with pulp necrosis. These were subjected to apexification procedures leaving them fragile and prone to fracture, or they were extracted. However, because of our growing grasp of biological ideas pertaining to the regeneration of dental tissues, conventional dentistry has recently experienced a quick revolution.[1] Hermann first documented the use of calcium hydroxide for vital pulp treatment in 1920, which lay the groundwork for dental tissue regeneration. In 1961, Nygaard Ostby examined the effectiveness of revascularization in restoring the pulp-dentin complex in permanent teeth with pulpal necrosis.[2] Beyond the standard strategies that are based primarily on infection management, regenerative techniques aim to manage the damaged tissue. The promise of reviving the non-vital tooth is offered by regenerative endodontics. It focuses on replacing diseased and traumatized pulp tissue with healthy pulp tissue.[3] Regenerative endodontics is based on tissue engineering, which employs stem cells, scaffolds, and growth

factors as three essential components to carry out regenerative treatment.[4] Because tissues are organized as three-dimensional structures, the proper scaffolding is required to place stem cells in the proper location and control their differentiation, proliferation, and metabolism. Extracellular matrix molecules regulate stem cell development, and a suitable scaffold may selectively bind and localise cells, hold growth hormones, and degrade over time due to biodegradation. Different scaffolds make it easier for various tissues to regenerate. To ensure a successful regenerative procedure, it is essential to have a thorough and precise knowledge about the suitable scaffold for the required tissue.[5]

Scaffolds

Scaffolds are three-dimensional (3D) porous solid biomaterials created to:

1. Ensure a spatially correct position of cells [2]
2. Encourage cell adhesion, cell-biomaterial interactions, and ECM deposition
3. Enable the proper movement of nutrients to promote the survival, proliferation, and differentiation of cells.
4. Decompose at a controlled rate that is roughly equivalent to the rate of tissue renewal.
5. Induce a minimal amount of toxicity or inflammation in vivo. [6]

Most of the normal cells in human tissues, with the exception of blood cells, are anchorage dependent and are found inside the ECM, a dense matrix. The ECM of the target tissue in its natural condition makes for the ideal framework for a target tissue.[7]

Ideal requirements of a scaffold

a. To promote cell seeding and diffusion of both cells and nutrients throughout the whole structure, a high porosity and a sufficient pore size are required [8].

b. Should enable efficient waste, oxygen, and nutrition transport [9]

c. Because scaffolds must be absorbed by the surrounding tissues without requiring surgical removal, biodegradability is crucial [8].

d. Degradation must proceed at a pace that is equal to or faster than the rate of tissue formation [10].

e. should be biocompatible [9]

f. Should have adequate physical and mechanical strength.[9]

Classification Of Scaffolds (Table 1)[5]

Based on degradability of matrices [11,12]

Based on form [13]

Based on presence or absence of cells [14]

Based on origin [1]

Table 1: Classification of Scaffolds [5]

Based on degradability of matrices	Based on form	Based on presence or absence of cells
• Biodegradable scaffolds • Permanent or biostable scaffolds	• Solid blocks • Sheets • Porous sponges • Hydrogels/ Injectables	• Cell free scaffolds • Scaffolds seeded with stem cells
Based on Origin		
Biological or natural scaffolds	**Artificial or synthetic scaffolds**	
• Platelet rich plasma (PRP) • Platelet rich fibrin (PRF) • Collagen • Chitosan • Glycosaminoglycans/ hyaluronic acid • Demineralized or native dentin matrix • Blood clot • Silk	**A) Polymers** • Polylactic acid (PLA) • Polyglycolic acid (PGA) • Polylactic-coglycolic acid (PLGA) • Polyepsiloncaprolactone (PCL) **B) Bioceramics** • Calcium/ Phosphate materials • Bioactive glasses • Glass ceramics	

Biological or Natural Scaffolds

Autologous Scaffolds

The synthetic scaffolds are limited in their biological inertness whereas natural scaffolds although provide abundant biological signals and degrade into physiologically smaller compounds that are xenogeneic or allogeneic and increase the risk of pathogen transmission and undesirable inflammatory and immunological reaction resulting from regenerated tissues and organs.[15]

To overcome such limitations, the autologous extracellular matrix scaffolds are used. These scaffolds are safe, eliminate unfavorable host responses, and promote optimal tissue regeneration.[15]

Blood Clot

Inducing bleeding and generating an intracanal blood clot to provide a scaffold for pulp-dentin regeneration is the current approach employed in regenerative endodontics. The purpose to provide a blood clot as a scaffold is to introduce platelet -derived growth factors and mesenchymal stem cells into the canal space for possible pulp tissue regeneration.[16] Induction of bleeding helps transfer SCAP from the periradicular tissues of the tooth into the root canal space through the apical foramen to eradicate foreign stem cells in an immature tooth with open apices. While inducing bleeding, endogenous hemostatic factor enters the canal space and creates a fibrin clot, which helps SCAP survive and helps in proliferation. [16]

Advantages: [16]

a. Low cost
b. Simple procedure
c. Short setting time
d. Host compatible autologous growth factors

Disadvantages: [16]

a. Unstable
b. Inconsistent outcomes
c. Excessive bleeding and hemostasis in some patients
d. Inadequate mechanical strength

Platelet Rich Plasma (PRP)

Marx et al. introduced platelet rich plasma (PRP) in 1988.[17] It is an autologous first-generation platelet concentrate that contains a high concentration of growth factors and can be employed as a blood clot substitute scaffold. It is easy to prepare, has fibrin matrix that helps to entrap growth factors. Platelet concentration is five times higher than normal platelet count (1 million/ml).[18]

Vascular endothelial growth factor (VEGF), transforming growth factor-b (TGF-b), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin like growth factors 1 and 2 are the most important growth factors released by platelets in PRP (IGF-1 and IGF-2). [19]

Method of preparation [20]

- 8 to 10 ml of autologous whole blood is drawn into a citrated tube.
- Sample tube is spun in a standard centrifuge for 10 minutes at 2400 rpm to produce PPP (platelet poor plasma)
- PPP is taken in a syringe with long cannula and a second centrifugation of 15 minutes at 3600 rpm is performed.
- Second supernatant is also taken in a long cannula.
- For each 8 ml of blood, the supernatant produce is 0.6-0.7 ml which is platelet rich plasma (PRP) that can be used for surgical procedures.

Classification of PRP [20]

1. Platelet concentrates can be classified into four types, according to Ehrenfest et al. and is based on the separation of products using two important parameters: cellular content (mainly leukocytes) and fibrin architecture. They are:
2. P-PRP,
3. L-PRP,
4. Pure platelet-rich fibrin (P-PRF) and
5. Leukocyte-and-platelet-rich fibrin (L-PRF).

According to DeLong et al. in 2012, the 'PAW' classification system was published. It specifies that PRP should be used based on three factors: the total quantity of platelets (P), how platelet activation happens (A), and whether or not white cells are present (W).

P1 (baseline [i.e. concentration in whole blood]) to P4 (>1.2 9 106 platelets/mL), activation as exogenous (X) or not, and white blood cells and neutrophils as above or below baseline.

Advantages: [20]

(i) Host compatibility
(ii) Abundance of autologous growth factor
(iii) Revascularization rates are increased
(iv) Cost friendly

Disadvantages: [20]

(i) Difficulties in blood collection
(ii) Variability in composition
(iii) Reduction in rapid growth factor
(iv) Weak mechanical strength
(v) Complexity in clinical formation

Platelet Rich Fibrin (PRF)

Platelet rich fibrin (PRF) is a second-generation platelet concentrate made from autologous platelets and leukocytes that produces a complex fibrin matrix. Choukroun et al. were the first to create PRF in 2001 in France. [21] PRF promotes healing of soft and hard tissues and can be used as scaffolds in tissue engineering.

Method of preparation [21]

The equipment which is necessary for PRF preparation include a PC-02 table centrifuge and blood collection kit that contains a 24-gauge butterfly needle and 9 ml blood collection tubes.

- Around 5ml of whole venous blood sample is taken without anticoagulant in 10 ml tubes and centrifuged immediately at 3000rpm for 10 minutes.
- This process results in blood to contact with the test tube wall that activates platelets and initiate coagulation cascade.
- Centrifugation process is done at 3000rpm for 10 minutes immediately.
- After few minutes, most of the platelet that adhere with the tube wall gets activated due to absence of any anticoagulants which results in initiation of coagulation cascade.

The final product is made up of three layers:

a. Peak level of the straw colored fraction of acellular platelet deficient plasma (PPP).
b. Intermediate-level PRF clot
c. Red fraction of red blood cell (RBCs) at the base level

The circulating thrombin then transforms the fibrinogen that is concentrated in the higher part of the tube into fibrin. Between the red blood cells at the bottom and the acellular plasma at the top, a fibrin clot forms in the middle of the tube. The platelets become trapped in the fibrin clot, which is then taken from the test tube with surgical tweezers and separated from the other layers with sterile scissor. After that, the PRF is compressed between sterile gauze pads to create a membranous film that can be easily packed into the root canals, allowing us to get PRF for clinical usage.

Platelet-derived growth factor (PDGF), transforming growth factor 1 (TGF1), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), fibroblast growth factor, and other growth factors are all found in PRF.

Advanced PRF [21]

An improved PRF form which contains a greater number of white blood cells known as advanced platelet-rich fibrin (A-PRF) was produced by Choukroun *et al* . Leukocytes are very important immunocytes that are capable of directing various cell types in the healing process of the wound. Cell populations are pushed to the bottom of the collection tube by high centrifugal forces. Reduced centrifugal g-force allows for a rise in leukocyte counts in the PRF matrix. Since then, decreasing centrifugal g-force has resulted in an increase in total leukocyte count in PRF matrix constructs (now known as advanced PRF or A-PRF). In comparison to L-PRF and PRP, the release of numerous growth factors was much higher in A-PRF.

Advantages: [21]

a) Ideal biomaterial used for pulp-dentin complex regeneration procedure.
b) Acts as a barrier between desired and undesired cells by preventing early intrusion of undesired cells.
c) Can be used as a healing and inter positional biomaterial.
d) Release of growth factors and fibrin bandage can result in wound closure and mucosal healing.

Disadvantages: [21]

a) Tissue banks for PRF are impractical. They are highly donor-specific and cannot be used to create allogenic transplant tissue.
b) Due to its properties of shrinkage and alternation of structural integrity as well as decreased growth factors content, it cannot be stored.

Concentrated Growth Factors (CGF)

Sacco developed CGF, an upgraded second-generation platelet concentrate, in 2006.[22] It contains abundant cytokines and can be used in the study of bone regeneration but has limited use as a pulp capping agent.[22] CGF promotes proliferation and osteo induction of PDL stem cells in vitro and bone tissue engineering in vivo.[22]

Fibroblast growth factors (FGF), transforming growth factor (TGF), platelet deficient growth factor (PDGF), insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF) are growth factors present in CGF.[23]

The growth factors regulate cell proliferation, migration, matrix remodelling, differentiation and angiogenesis.[23]

Method of preparation [22]

- 9ml of intravenous blood was drawn from healthy volunteer into sterile vacuette tubes without anti-coagulant solutions.
- The tubes were immediately kept into a Medifuge MF200 performing CGF centrifugal program as: : 30 seconds acceleration, 2700 r.p.m. for 2 minutes, 2400 r.p.m. for 4 minutes, 2700 r.p.m. for 4 minutes, 3000 r.p.m. for 3 minutes, and 36 seconds deceleration and stop.
- Three layers were formed after centrifugation:

a) Upper layer- serum
b) Interim phase – Fibrin buffy coat
c) Liquid phase – Growth factor
d) Lower layer- red blood cells

- CGF gel layer were taken out and pressed it onto the membrane.
- CGF membrane was cut into small pieces of 3 x 3 mm^2 and immersed into saline.

Functions: [24]

a) CGF is a hemostatic and tissue-sealing fibrin tissue glue.
b) It stimulates osteogenesis and enhances wound healing.
c) CGF enhances the wound's stability, which is necessary for the attachment of new connective tissue to the root surface.
d) It reduces scarring and enhances epithelial, endothelial, and epidermal regeneration.
e) Because of the large concentration of leukocytes, it possesses antibacterial capabilities.
f) On chronic non-healing wounds, it functions as an anti-antigenic agent.
g) It also acts as a scaffold for the attachment of cytokines and cellular migration.

Collagen

Extracellular matrices mostly consist of collagen, which gives tissues their high tensile strength. Collagen functions as a scaffold that facilitates the insertion of cells and growth factors and permits the replacement of degraded tissues with natural tissues.[25]

Advantages

It permits the creation of both soft tissue and hard tissue, is biocompatible and biodegradable, has a strong tensile strength, has high alkaline phosphatase activity, and acts as a trap for osteoinductive agents.[25,26]

Disadvantages

It is mechanically weak and undergoes rapid degradation, undergoes contraction (shrinkage).[27,28]

Chitosan

Chitin, the structural component of crustacean exoskeletons (such those of crabs and prawns), and the cell walls of fungus are used to make chitosan for commercial use. Chitosan's characteristics have an impact on the scaffolds' pore development, which has an impact on their mechanical and biological properties.[29,30]

Advantages

Chitosan is non-toxic, readily bio-absorbable, has antibacterial action, boosts alkaline phosphatase activity, and promotes the growth of fibroblasts and odontoblasts.[31,32] It is a porous scaffold that is flexible enough to be shaped as desired, and its hydrophilic quality promotes cell adhesion for growth.[33,34]

Disadvantages

It has low strength and inconsistent behavior with seeded cells, difficult to accurately control the size of the hydrogel pores, chemical modifications of chitosan structure could induce toxicity.[31]

Glycosoaminoglycans

Hyaluronic acid (HA), one of the glycosaminoglycans present in the ECM, has demonstrated to provide immense potential for tissue engineering. HA promotes osteogenesis and can create an environment that facilitates chondrogenesis.[34,35]

Advantages

It promotes the conversion of dental mesenchymal cells to odontoblasts, aids in the formation of the dentin matrix and dental pulp, is non-immunogenic, non-thrombogenic, biocompatible, and biodegradable, and aids in wound healing. It can also be used as an injectable scaffold and as a HA sponge.[35-37]

Disadvantages

In an aquatic environment, HA loses mechanical integrity and is extremely water soluble. It also degrades quickly by enzymes like hyaluronidase [42], especially when it is not in the form of a hydrogel. Cross-linking and HA modification, however, can get around these restrictions.[38]

Demineralized or native dentin matrix

The organic matrix of dentin is known to contain 233 total and 68 common proteins, including a variety of collagenous and non-collagenous proteins. Dentin is dominated by a rich ECM and not cells.[39]

Advantages

The mechanically superior demineralized dentin matrix (DDM) is nonimmunogenic. With DDM, bioactive molecules are released to stimulate related dentinogenic processes. It demonstrates the direct stimulation of odontoblast-like cells that are differentiating as well as indirect matrix synthesis that results in odontoblast differentiation. It has been demonstrated to be osteoconductive, osteoinductive, and biocompatible.[40-43]

Disadvantages

Tooth demineralization is time consuming (usually 2–6 days). Drawback of demineralization is that prolonged acid exposure may negatively affect non-collagenous proteins involved in new bone formation.[44,45]

Silk

Silk-based biomaterial scaffolds have been extensively used for both soft and hard tissue engineering.[46]

Advantages

They can support many distinct cell types' of adhesion, proliferation, and differentiation and are biocompatible. Enzymatically biodegradable silk fibroin (SF) may be converted into porous sponges, injectable hydrogels, and implants that are insoluble in water. For tissue engineering research and therapeutic therapy in dentistry, the capacity of SF to enhance vascularization with strong anticoagulant action and platelet reactivity is attractive. It possesses excellent mechanical strength, elasticity, biodegradability, morphologic flexibility, oxygen and water permeability, and a moderate breakdown rate that allows fibroin to be gradually replaced by newly created tissue. SF is less immunogenic and inflammatory, compared with either polylactic-co-glycolic acid (PLGA) or collagens.[46-49]

Disadvantages

Hard tissue formation consists of osteodentin.[50] Complete degradation of silk scaffold occurs after 2 years.[51]

Artificial or Synthetic Scaffolds

A) Polymers

A number of synthetic polymers such as polylactic acid (PLA), poly-l-lactic acid (PLLA), polyglycolic acid (PGA), PLGA, and polyepsiloncaprolactone (PCL) have been used as scaffolds for pulp regeneration.[5]

Advantages

The physicochemical features, such as mechanical stiffness, degradation rate, porosity, and microstructure, of the synthetic polymers may be precisely controlled and they are non-toxic and biodegradable. Natural polymers are mostly broken down by enzymes, but synthetic polymers are often broken down by simple hydrolysis.

Since structural strength is vital in many applications, PLLA, an extremely strong polymer, has been used in several studies. Sakai et al. and Cordeiro et al. conducted experiments that demonstrated how PLLA scaffolds

stimulated the development of dental pulp cells into odontoblasts and endothelial cells.[52-54]

PGA is a synthetic scaffold that has been utilized for cell transplantation; it degrades when the cells secrete ECM. It is a more hydrophobic aliphatic polyester than PGA. After 3–4 months, dentin-like tissue developed, and pulp-like tissue could regenerate using PLGA as a scaffold. A 50:50 blend of PLGA degrades after around 8 weeks. PCL is a polymer that slowly breaks down and has been utilised in bone tissue engineering either alone or in conjunction with hydroxyapatite.[55-58]

Disadvantages

Synthetic polymers can cause a chronic or acute inflammatory host response, and localized pH decrease due to relative acidity of hydrolytically degraded by products.[59]

B) Bioceramics

This category of scaffolds includes glass ceramics, bioactive glasses, and calcium/phosphate materials. The most frequently employed biomaterials are calcium phosphate-based (CaP) ceramics. Due to their characteristics of resorption, biocompatibility, low immunogenicity, osteoconductivity and resemblance to mineralized tissues, CaP scaffolds, such as -TCP or HA, have been extensively investigated for bone regeneration. By offering ideal 3D substrate conditions for hDPSC development and odontogenic differentiation, 3D CaP porous granules have shown potential for dental tissue engineering.[60]

Nanofibrous Scaffold

Nanofibrous polymer scaffolds were developed with specific objectives in mind, including high processing efficiency, mechanical proficiency, and characteristic biodegradability and biocompatibility. Most importantly, nanofibrous scaffolds are best-known for their ability to transmit physical and chemical stimuli that induce favourable cell-ECM interactions, preserve cell phenotype, facilitate stem cell development, boost cell proliferation, and activate cell signalling pathways. There are two applications of nanofibrous scaffolds in regenerative endodontics. The first is for the intracanal administration of antibiotics. The second is to encourage the regeneration of the dentin-pulp complex.

Intracanal drug delivery of antibiotics using electrospun nanofibers has been extensively studied in *in-vitro* studies.[61] The most often employed antibiotics are ciprofloxacin, metronidazole, and minocycline. They have been examined for their capacity to kill microorganisms and their cytotoxicity towards stem cells individually or in various combinations. These antibiotics have been shown to be effective against *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Enterococcus faecalis*.[62-64]

The odontogenic development of human DPSC on several nanofibrous scaffolds, including poly(lactic acid) (PLA), polycaprolactone (PCL), nano-hydroxyapatite (nHA), collagen, and gelatin, has demonstrated positive results in a number of investigations. Future research will be done to determine the best way to regenerate the dentin-pulp by encapsulating dental-derived stem cells in electrospun nanofibers.[65]

Injectable Scaffold

Placing the prepared scaffolds (sheets, blocks) within the root canal is always challenging. In this regard, an injectable scaffold has a number of benefits, such as (a) its ease of implantation into the root canal space by injection due to its liquid form, (b) its ease of filling any irregularly shaped defects, (c) its ease of mixing stem cells and bioactive molecules with the solution before injecting in situ, and (d) its minimally invasive placement, which lowers the risk of infection and increases patient comfort.[66]

Injectable hydrogels are biocompatible, hydrophilic substances that are simple to inject in colloidal form and proceed through either chemical or physical (such as temperature, pH and osmolarity) gelation processes. It can be easily injected into narrow root canal spaces and can be modified to deliver chemotactic and angiogenic agents to drive stem cell homing and supportive angiogenesis.[67]

Given that they include short peptide sequences similar to those found naturally in tissues, self-assembling peptide hydrogels (like Pura MatrixTM) have a lot of potential for tissue engineering. These sequences promote cell adhesion and proliferation. Successful hydrogel-

based nanofibrous scaffold called Pura Matrix TM is built of 16-mer peptide in an aqueous solution. It polymerizes in response to physiological circumstances and creates a biodegradable nanofiber hydrogel scaffold. The self-assembling design has drawbacks in terms of mechanical qualities and structure, such as difficulties in maintaining the hydrogel over the whole length of root canal extension and the effect of viscosity on cell proliferation.[66]

SHED encapsulated in Pura Matrix and delivered into whole root canals gave rise to a pulp-like tissue and odontoblasts after transplantation into immunodeficient mice.[68] In another research, the scaffold was used in conjunction with DPSC and human umbilical vein endothelial cells (HUVEC) and showed that DPSCs formed more early vascular networks by promoting HUVEC migration and upregulating VEGF expression.[69] When a scaffold combined with SCAP was applied without the use of exogenous growth factors and implanted into the mouse molar crowns, a viable tissue including odontoblast-like cells was created.[70]

Conclusion

Regenerative medicine provides many advantages for restorative dentistry in terms of restoration survival rates and longterm treatment prognosis. Patient demand is staggering both in scope and cost, because tissue engineering therapy offers the possibility of restoring natural function instead of surgical placement of an artificial prosthesis. Such translation will require the partnership of researchers and skilled clinicians who can effectively apply advances in knowledge to appropriate cases and develop novel therapies which can be realistically introduced into the clinic. As regenerative endodontics has clinical orientation, the success of the field relies on the final introduction of such therapies into clinical practice at large. The future will show which of the multiple approaches in regenerative endodontics will withstand the test of clinical usage.

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