

## Review Article

### Scaffolds in regenerative endodontics: A review

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#### ABSTRACT

Root canal therapy has enabled us to save numerous teeth over the years. The most desired outcome of endodontic treatment would be when diseased or nonvital pulp is replaced with healthy pulp tissue that would revitalize the teeth through regenerative endodontics. A search was conducted using the Pubmed and MEDLINE databases for articles with the criteria 'Platelet rich plasma', 'Platelet rich fibrin', 'Stem cells', 'Natural and artificial scaffolds' from 1982-2015. 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, and an appropriate scaffold might selectively bind and localize cells, contain growth factors, and undergo biodegradation over time. Different scaffolds facilitate the regeneration of different tissues. To ensure a successful regenerative procedure, it is essential to have a thorough and precise knowledge about the suitable scaffold for the required tissue. This article gives a review on the different scaffolds providing an insight into the new developmental approaches on the horizon.

**Key Words:** Extracellular matrix, pulp, regeneration, scaffolds

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## INTRODUCTION

Regenerative dentistry has been popularized due to advancements in biologic therapies that apply growth and differentiation factors which hasten or induce natural biologic regeneration. Hermann in 1920 described the application of calcium hydroxide for vital pulp therapy which laid the foundation for regeneration of dental tissues. Nygaard Ostby in 1961 evaluated a revascularization method for re-establishing a pulp-dentin complex in permanent teeth with pulpal necrosis.<sup>[1]</sup>

Regenerative endodontics is based on the concept 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.<sup>[2]</sup> 'An exhaustive search was conducted using the Pubmed and MEDLINE databases for articles with the criteria 'Platelet rich plasma', 'Platelet rich fibrin', 'Stem cells', 'Natural and artificial scaffolds' from 1982-2015. All articles were selected, with no inclusion or exclusion criteria'.

Pulp revascularization is defined as re-introduction of vascularity in the root canal system. Although blood vessels are indispensable constituents of dental pulp, pulp regeneration is considered incomplete without an odontoblastic layer lining the dentin surface, nociceptive

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as well as sympathetic and parasympathetic nerve fibers, in addition to interstitial fibroblasts and most importantly, stem/progenitor cells that serve to replenish all pulp cells in the regenerated pulp when they undergo apoptosis and turnover. Thus, a clear distinction between regeneration and revascularization can be made as follows:

- Pulp revascularization = induction of angiogenesis in endodontically-treated root canal
- Pulp regeneration = pulp revascularization + restoration of functional odontoblasts and/or nerve fibers.<sup>[3]</sup>

The three key ingredients for regeneration are morphogens, progenitor/stem cells, and the extracellular matrix (ECM) scaffold.<sup>[1]</sup>

## STEM CELLS

Stem cells are undifferentiated embryonic or adult cells that continuously divide. They can divide and create additional stem cells and differentiate along a specified molecular pathway. Embryonic stem cells are totipotent and have the capacity to self-renew. In contrast, stem cells that reside within an adult organ or tissue have more restricted options, with ability to select a differentiation program from only a few possible pathways<sup>[4,5]</sup> [Table 1].

## 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) that affect a broad range of cellular activities including migration, proliferation, differentiation, and apoptosis of all dental pulp cells, including stem/progenitor cells.<sup>[4]</sup> Bioactive cues that recruit the proper cells are critical in pulp regeneration (transforming growth factors [TGFs]  $\beta$ 1,  $\beta$ 3 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).<sup>[6]</sup>

## SCAFFOLDS

Scaffolds are three-dimensional (3D) porous solid biomaterials designed which

1. Provide a spatially correct position of cell location<sup>[1]</sup>
2. Promote cell-biomaterial interactions, cell adhesion, and ECM deposition
3. Permit 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. Provoke a minimal degree of inflammation or toxicity *in vivo*.<sup>[6]</sup>

Apart from blood cells, most of the normal cells in human tissues are anchorage-dependent residing in a solid matrix called ECM. The best scaffold for an engineered tissue should be the ECM of the target tissue in its native state.<sup>[7]</sup>

### Ideal requirements of a scaffold

- a. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout whole structure of both cells and nutrients<sup>[8]</sup>
- b. Should allow effective transport of nutrients, oxygen, and waste<sup>[9]</sup>
- c. Biodegradability is essential, since scaffolds need to be absorbed by the surrounding tissues without the necessity of surgical removal<sup>[8]</sup>
- d. The rate at which degradation occurs has to coincide with the rate of tissue formation<sup>[18]</sup>
- e. Should be biocompatible<sup>[9]</sup>
- f. Should have adequate physical and mechanical strength.<sup>[9]</sup>

### Classification of scaffolds

- Based on degradability of matrices<sup>[10,11]</sup>
- Based on form<sup>[12]</sup>
- Based on presence or absence of cells<sup>[13]</sup>
- Based on origin<sup>[1]</sup> [Table 2].

### Biological or natural scaffolds

See Table 3.

#### *Platelet rich plasma*

Platelet rich plasma (PRP), an autologous first generation platelet concentrate with a rich source

**Table 1: The most common dental stem cells**

Stem cells	Source
DFPCs	Dental follicle precursor cells
DPSCs	Dental pulp stem cells
PDLSCs	Periodontal ligament stem cells
SCAP cells	Stem cells from apical papilla
SHED cells	Stem cells from human exfoliated deciduous teeth

of growth factors, has been proposed as a potential addendum/substitute scaffold.<sup>[18]</sup> It is easy to prepare, rich in growth factors, and forms a 3D fibrin matrix that helps entrap the growth factors. Platelet concentration in PRP exceeds 1 million/mL, which is 5 times more than that of the normal platelet count.<sup>[19]</sup> More number of platelets increases the number of growth factors secreted by them which helps in the proliferation of stem cells to induce healing and regeneration of tissues.<sup>[20]</sup> It is a concentrated suspension of different growth factors like PDGF, TGF- $\beta$ , IGF, VEGF, epidermal growth factor, and epithelial cell growth factor. These are released via degranulation of alpha granules and stimulate bone and soft-tissue healing.<sup>[21]</sup> The disadvantages of this procedure include drawing blood in young patients, the need of special equipment

and reagents to prepare PRP, and the increased cost of treatment.<sup>[20]</sup>

#### Platelet rich fibrin

Platelet rich fibrin (PRF) is second-generation platelet concentrate named as Choukroun's PRF after its inventor.<sup>[22]</sup> The procedure consists of drawing blood which is collected into test tubes without an anticoagulant and is centrifuged instantaneously. A tabletop centrifuge can be used for 10 min at 3000 rpm or for 12 min at 2700 rpm.<sup>[23]</sup>

The resultant product consists of three layers:

- Acellular platelet poor plasma at peak level
- PRF clot in intermediate level
- Red fraction of red blood cells at the base level.

The blood coagulation starts instantaneously as it comes in contact with the glass surface due to the lack of anticoagulant.<sup>[24]</sup>

#### Biological properties of platelet rich fibrin

PRF can be considered as an immune concentrate with specific composition and a 3D architecture. It contains multitude of growth factors such as PDGF, TGF  $\beta$ 1, and IGF.<sup>[22]</sup>

#### Attributes of platelet-rich fibrin

- Ideal biomaterial for pulp-dentin complex regeneration
- Prevents the early encroachment of undesired cells, thereby acts as a viable barrier between desired and undesired cells
- Healing and inter positional biomaterial
- Accelerates wound closure and mucosal healing due to fibrin bandage and growth factor release.<sup>[25]</sup>

#### Biochemical analysis of platelet-rich fibrin

PRF consists of an intimate assembly of cytokines, glycan chains, structural glycoproteins enmeshed

**Table 2: Classification of scaffolds**

Based on degradability of matrices <sup>[11]</sup>	Based on form <sup>[12]</sup>	Based on presence or absence of cells <sup>[13]</sup>
Biodegradable scaffolds	Solid blocks	Cell free scaffolds
Permanent or biostable scaffolds	Sheets	Scaffolds seeded with stem cells
	Porous sponges	
	Hydrogels (injectable scaffolds)	
Based on origin <sup>[1]</sup>		
Biological or natural scaffolds		Artificial or Synthetic scaffolds
Platelet rich plasma <sup>[14]</sup>		Polymers <sup>[1]</sup>
Platelet rich fibrin <sup>[15]</sup>		PLA
Collagen		PGA
Chitosan		PLGA
Glycosaminoglycans/hyaluronic acid		PCL
Deminerlized or native dentin matrix <sup>[1]</sup>		Bioceramics <sup>[1]</sup>
Blood clot <sup>[16]</sup>		Calcium/ phosphate materials
Silk <sup>[17]</sup>		Bioactive glasses
		Glass ceramics

PLA: Polylactic acid; PGA: Polyglycolic acid; PLGA: Poly(lactide-co-glycolide) acid; PCL: Polycaprolactone

**Table 3: Attributes of commonly used natural scaffolds<sup>[26]</sup>**

Blood clot	PRP	PRF
Lesser cytokines compared to PRP and PRF	Lesser cytokines than PRF	Maximum concentration of the cytokines
Rate of clot formation corresponds to inherent body clotting time	Addition of thrombin for conversion of fibrinogen to fibrin in PRP leads to drastic activation and rapid polymerization leading to dense network of monofibers poor in cytokine concentration	Slow physiological polymerization allows the formation of flexible three-dimensional fibrin network that supports cytokine enmeshment and cellular migration
Slower healing compared to PRP and PRF	Slower healing compared to PRP. Limited bone and dentine regeneration	Faster and stronger healing kinetics than PRP
Not rich in growth factors	Maximum release of morphogens occurs before the actual cell ingrowth, fewer signaling molecules are left for osteoblasts and odontoblasts from the surrounding tissues Inhibits differentiation of BMSC Fibrin matrix susceptible to washout in surgical field	Releases its growth factors steadily with the peak level reaching at 14 days corresponding to the growth pattern of periapical tissues Shows proliferation and differentiation of BMSCs Stronger stable fibrin matrix

PRP: Platelet-rich plasma; PRF: Platelet-rich fibrin; BMSCs: Bone mesenchymal stem cells

within a slowly polymerized fibrin network. These biochemical components have well known synergistic effects on healing processes. Fibrin is the natural guide of angiogenesis. Fibrin constitutes a natural support to immunity.<sup>[22]</sup>

#### *Collagen*

Collagen is the major component in extracellular matrices, and provides great tensile strength in tissues. As a scaffold, collagen allows for easy placement of cells and growth factors and allows for replacement with natural tissues after undergoing degradation.<sup>[27-29]</sup>

#### *Advantages*

It is biocompatible, biodegradable, has a good tensile strength, simulates natural ECM of dentin, demonstrates high alkaline phosphatase activity, allows soft tissue and hard tissue formation, forms a trap for osteoinductive factors.<sup>[29,30]</sup> Collagen may also be processed into a variety of formats, including porous sponges, gels, and sheets, and can be crosslinked with chemicals to make it stronger or to alter its degradation rate.<sup>[30]</sup>

#### *Disadvantages*

It is mechanically weak and undergoes rapid degradation, undergoes contraction (shrinkage).<sup>[31,32]</sup>

#### *Chitosan*

Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi. The properties of chitosan affect the formation of pores in the scaffolds, thereby influencing the mechanical and biological properties.<sup>[33,34]</sup>

#### *Advantages*

Chitosan is nontoxic, easily bioabsorbable, shows antibacterial activity, has gel forming ability, increases alkaline phosphatase activity, shows fibroblast and odontoblastic proliferation.<sup>[35,36]</sup> It is a porous scaffold that can be molded into any shape and its hydrophilic property enhances cell attachment and proliferation.<sup>[35,37]</sup>

#### *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.<sup>[35]</sup>

#### *Glycosaminoglycans*

Hyaluronic acid (HA) is one of the glycosaminoglycans in ECM and plays important roles in maintaining morphologic organization by preserving extracellular spaces, and it has been reported to have excellent potential for tissue engineering. This supports osteogenesis and can provide an environment facilitating chondrogenesis when exposed to its initiating factors.<sup>[38,39]</sup>

#### *Advantages*

It helps in differentiation of dental mesenchymal cells to odontoblasts, contributes to formation of dentin matrix and dental pulp, is biocompatible, biodegradable, bioactive, non immunogenic, and nonthrombogenic, plays a beneficial role in wound healing, can be used as an injectable scaffold and also as HA sponge.<sup>[39-41]</sup>

#### *Disadvantages*

HA is highly water soluble, it degrades rapidly by enzymes such as hyaluronidase<sup>[42]</sup>, especially when not in the form of hydrogel and lacks mechanical integrity in an aqueous environment. However, these drawbacks can be overcome by cross linking and modification of HA.<sup>[42]</sup>

#### *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.<sup>[43]</sup>

#### *Advantages*

Demineralized dentin matrix (DDM) is nonimmunogenic and mechanically superior.<sup>[44]</sup> There is a release of bioactive molecules with DDM that signal associated dentinogenic events.<sup>[45]</sup> It shows direct induction of differentiating odontoblast-like cells and indirect matrix synthesis leading to odontoblast differentiation.<sup>[46]</sup> It has proved to be biocompatible, osteoinductive, and osteoconductive.<sup>[47]</sup>

#### *Disadvantages*

Tooth demineralization is time consuming (usually 2–6 days). Drawback of demineralization is that prolonged acid exposure may negatively affect noncollagenous proteins involved in new bone formation.<sup>[48,49]</sup>

#### *Silk*

Silk-based biomaterial scaffolds have been extensively used for both soft and hard tissue engineering.<sup>[50]</sup>

### Advantages

They are biocompatible and have the ability to support the attachment, proliferation, and differentiation of many different cell types. Silk fibroin (SF) is an enzymatically degradable material, which can be processed into water insoluble implants, injectable hydro gels, and porous sponges.<sup>[50]</sup> The ability of SF to support vascularization with good anticoagulant activity and platelet response is encouraging for tissue engineering research and clinical therapy in dentistry.<sup>[51]</sup> It has good mechanical strength, elasticity, biodegradability, morphologic flexibility, oxygen and water permeability, and a slow degradation rate that enables gradual replacement of fibroin with newly formed tissue.<sup>[17,52]</sup> SF is less immunogenic and inflammatory, compared with either poly(lactic-co-glycolic acid) (PLGA) or collagens.<sup>[53]</sup>

### Disadvantages

Hard tissue formation consists of osteodentin.<sup>[54]</sup> Complete degradation of silk scaffold occurs after 2 years.<sup>[55]</sup>

## Artificial or synthetic scaffolds

### Polymers

A number of synthetic polymers such as polylactic acid (PLA), poly-L-lactic acid (PLLA), polyglycolic acid (PGA), PLGA, and poly(ε-caprolactone) (PCL) have been used as scaffolds for pulp regeneration.<sup>[1]</sup>

### Advantages

The synthetic polymers are nontoxic, biodegradable, and allow precise manipulation of the physicochemical properties such as mechanical stiffness, degradation rate, porosity, and microstructure.<sup>[3]</sup> Synthetic polymers are generally degraded by simple hydrolysis, whereas natural polymers are mainly degraded enzymatically.<sup>[56]</sup>

PLLA is a very strong polymer and has found many applications where structural strength is important. Experiments were carried out by Sakai *et al.* and Cordeiro *et al.* showing PLLA scaffolds promoted dental pulp cell differentiation into endothelial cells and odontoblasts.<sup>[57,58]</sup>

PGA has been used as an artificial scaffold for cell transplantation, and degrades as the cells excrete ECM.<sup>[31]</sup>

PLA is an aliphatic polyester, more hydrophobic than PGA.<sup>[59]</sup>

PLGA was used as a scaffold to demonstrate that dentin-like tissue formed and pulp-like tissue could

be regenerated after 3–4 months.<sup>[60]</sup> PLGA in a 50:50 mixture has a degradation time of about 8 weeks.<sup>[61]</sup>

PCL is a slowly degrading polymer that have been used toward tissue engineering efforts in bone, either alone or combined with hydroxyapatite.<sup>[62]</sup>

### Disadvantages

Synthetic polymers can cause a chronic or acute inflammatory host response, and localized pH decrease due to relative acidity of hydrolytically degraded byproducts.<sup>[63]</sup>

### Bioceramics

This group of scaffolds refers to calcium/phosphate materials, bioactive glasses and glass ceramics.<sup>[64]</sup> Most common biomaterials in use are calcium phosphate-based (Ca-P) bioceramics.<sup>[64]</sup> Ca-P scaffolds include β-TCP or HA and have been widely tested for bone regeneration owing to their properties of resorption, biocompatibility, low immunogenicity, osteoconductivity, bone bonding, and similarity to mineralized tissues. 3D Ca-P porous granules have proved useful in dental tissue engineering by providing favorable 3D substrate conditions for human dental pulp stem cell (hDPSC) growth and odontogenic differentiation. Addition of SiO<sub>2</sub> and ZnO dopants to pure TCP scaffolds increases its mechanical strength as well as cellular proliferation properties. Glass ceramics based on SiO<sub>2</sub>-Na<sub>2</sub>O-CaO-P<sub>2</sub>O<sub>5</sub> are bioactive and offer good crystallization conditions. Release of dissolution products such as Ca-P enhances the osteoblastic activity of the material.<sup>[65]</sup>

## MODIFICATIONS

Scaffolds made of ceramic can be modified to obtain desired permeability, controlled dissolution rate, and specific surface characteristics to enhance cellular activity. Change in pore size and volume affects the mechanical stiffness of the scaffold. Magnesium-based glass ceramics have improved mechanical integrity and high rate of bioactivity. Niobium doped fluorapatite glass ceramic displays excellent attachment, proliferation, and differentiation of hDPSCs on its surface.<sup>[66]</sup>

### Disadvantages

Bioceramics have a time-consuming fabrication, lack of organic phase, nonhomogeneous particle size and shape, large grain size, difficult porosity control, difficulty of shaping, brittleness, slow degradation rate, and high density.<sup>[66]</sup> When used alone, the bioceramics have low mechanical strength and are

brittle. To overcome this disadvantage, they can be combined with polymer scaffolds.<sup>[67]</sup>

## CONCLUSION

REPs have emerged as viable alternatives for the treatment of immature teeth with pulpal necrosis. The clinicians should be aware of the attributes of various scaffolds so that they can select most suitable one for successful results. Combinations of various scaffoldssuch ashydroxyapatite-polymer gels can be used to compensate for their individual shortcomings, which is a significant advantage. Through the use of computer-aided design and 3D printing technologies, scaffolds like polymers can be fabricated into precise geometries with a wide range of bioactive surfaces. Such scaffolds have the potential to provide environments conducive to the growth of specific cell types such as pulpal cells. Future in regenerative endodontics is very promising owing to the discoveries and advancements in scaffold technology.

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