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REVIEW ARTICLE **OPEN ACCESS**

Scaffolds in Regenerative Endodontics: A Narrative Review

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ABSTRACT
Regenerative dentistry has been popularized due to advancements in biologic therapies that apply growth and differentiation factors which hasten or induce natural biologic regeneration. 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. 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 to the new developmental approaches. In this review, various categories of scaffolds used in regenerative endodontics will be discussed in detail.
Keywords: Regeneration, scaffold, biologic therapy

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INTRODUCTION

Recently, regenerative endodontics has emerged as a new field that deals with the rejuvenation of the pulp–dentin complex in necrotic immature permanent teeth, regeneration of bone, periodontal ligament and cementum. Scaffolds play a major part in the formation of the extracellular matrix by providing support to cells to adhere, grow, and differentiate. 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 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.

The three key ingredients for regeneration are morphogens, progenitor/stem cells, and the extracellular matrix (ECM) scaffold which are also termed as the triad of tissue regeneration.

STEM CELLS:-

Stem cells are primitive cells found in all multi-cellular organisms that are characterized by self-renewal and the capacity to differentiate into any mature cell type. These stem cells have a great potential for regeneration and may be used to replace or repair damaged cells. There are 2 main types of stem cells – embryonic stem cells and adult stem cells. Embryonic stem cells are totipotent have the capacity to self-renew whereas the adult stem cells have restricted option and have ability to differentiate only via a few possible pathways. Common dental stem cells are DFPCs (Dental follicle precursor cell), DPSCs (Dental pulp stem cell), PDLSCs (Periodontal ligament stem cell) etc.

GROWTH FACTOR :-

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 that affect a

broad range of cellular activities including migration, proliferation, differentiation, and apoptosis of all dental pulp cells, including stem/progenitor cells. 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).

SCAFFOLDS :-

The scaffold can be defined as a three-dimensional microstructural network of biologically active compounds, all working in tandem to ensure the safe delivery of bioactive cells, which are highly essential for facilitating tissue repair and regeneration.

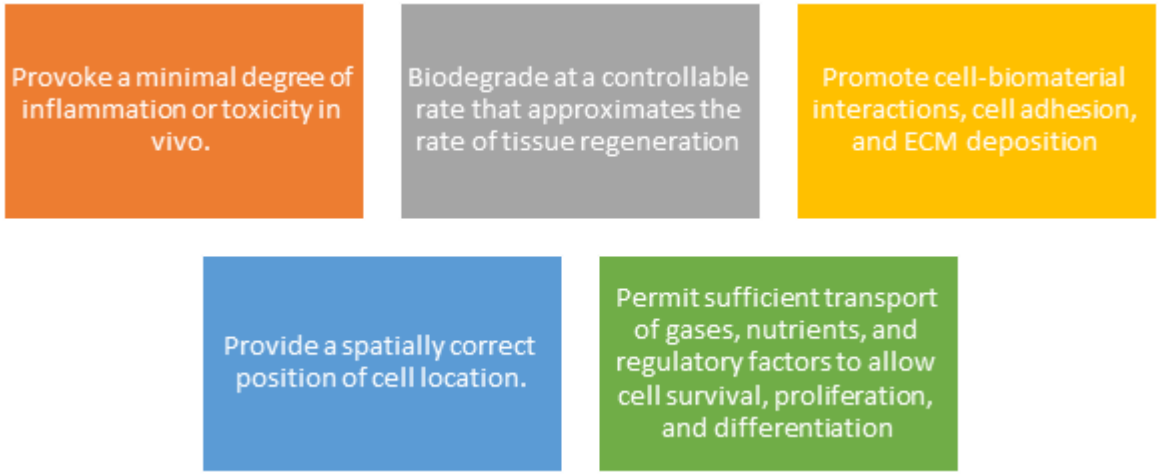

Figure1: Functions of a scaffold

Ideal requirements of a scaffold :-

1) A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout whole structure of both cells and nutrients.
2) Should allow effective transport of nutrients, oxygen, and waste.
3) Biodegradability is essential, since scaffolds need to be absorbed by the surrounding tissues without the necessity of surgical removal.
4) The rate at which degradation occurs has to coincide with the rate of tissue formation.
5) Should be biocompatible.
6) Should have adequate physical and mechanical strength.

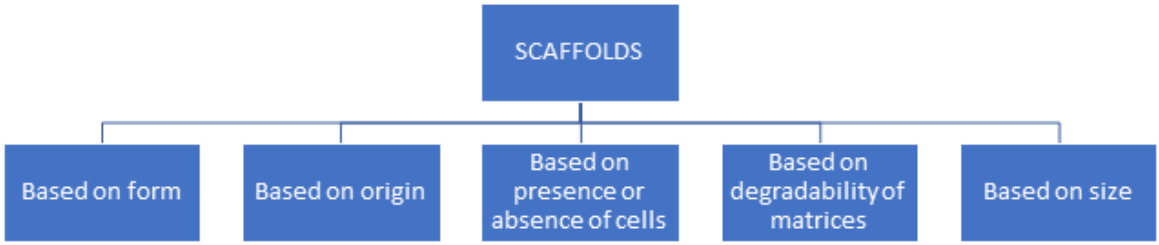

Figure 2: Broad classification of scaffolds

BASED ON FORM	BASED ON ORIGIN			
	Natural/Biological		Synthetic	
	Autologous	Polymer	Bioceramics	Polymer
-Hydrogels -Porous sponges -Sheets -Solid block	-Blood clot -Platelet concentrates	-Collagen -Chitosan -HA dentin matrix	-Calcium/phosphate materials -Bioactive glass	-PLA -PGA -PLGA -PCL

BASED ON PRESENCE OR ABSENCE OF CELLS	BASED ON DEGRADABILITY OF MATRICES	BASED ON SIZE
-Cell free scaffolds -Scaffolds seeded with stem cells	-Biodegradable matrices -Biostable/Permanent matrices (Polytetraflouroethylene, Calciumphosphate)	-Nano (>1µm) -Micro(1-1000µm) -Macro (>1000µm)

Figure 3: Different form of scaffolds classification

AUTOLOGOUS PLATELET CONCENTRATES :-

A) PLATELET RICH PLASMA – PRP is an autologous first generation platelet concentrate that has an ample repository of growth factors thereby categorising it as a scaffold (1) . The platelet concentration exceeds 1 million/ mL which is 5 times more than that of the normal platelet count. The more amount of platelet ensures more amount of growth factors which helps in proliferation of stem cells that induce healing and regeneration. The growth factors involved are PDGFM, TGF-b, VEGF, IGF, epidermal growth factor and epithelial cell growth factor.

B) PLATELET RICH FIBRIN – PRF is a second generation platelet concentrate, also called as Choukroun's PRF after its inventor (2). It is composed of an intertwined fibrin mesh of cytokines, glycanic chains, structural glycoproteins and multitude of growth factors such as PDGF, TGF-β1 and IGF.

In the systematic review reported by Murray, the clinical effectiveness of PRP, PRF, and blood clots to regenerate 222 immature permanent teeth after 1 year of follow-up from 12 articles were compared. The patient's age varied from 6 to 28 years. The included teeth were fractured or decayed (both vital and nonvital teeth) with restorable crowns. The results concluded that for apical closure, the 1st (PRP) and 2nd generation (PRF) platelet concentrates performed significantly better than a blood clot. However, for other parameters like root lengthening, dentinal wall thickness, and periapical healing, PRP, PRF, and blood clots showed no significant difference (3). (Graph1)

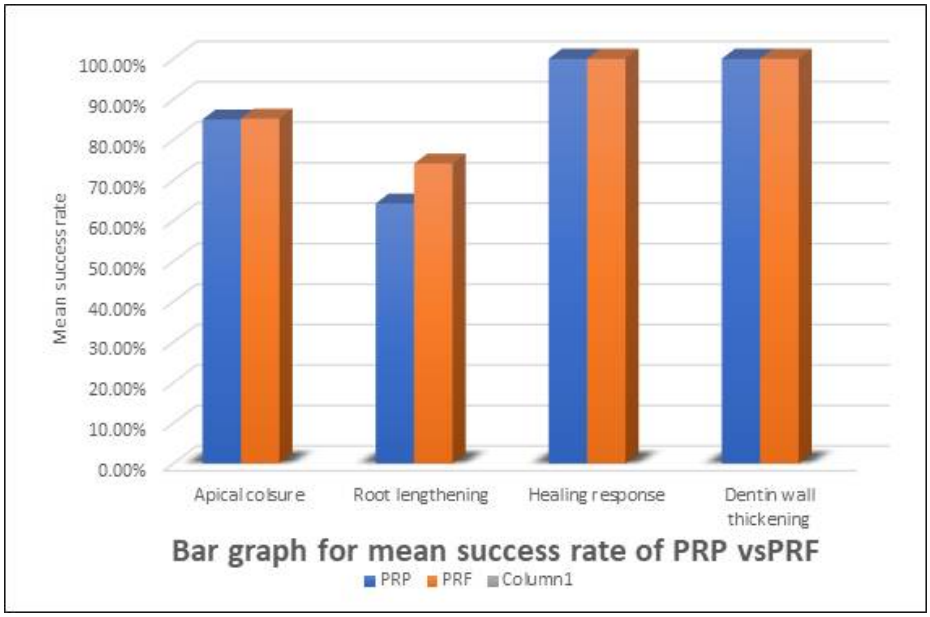

Figure 4: Bar graph for men success rate of PRP vs PRF

C) PLASMA RICH IN GROWTH FACTORS- PRGF technology developed by Anitua, is a first-generation platelet concentrate consisting of a plasma infused autologous platelet concentrate devoid of leucocytes.

PRGF is an autologous platelet concentrate with growth factors in abundance, such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor (IGF-1), and hepatocyte growth factor (HGF). (4)

D) CONCETRATED GROWTH FACTOR – CGF introduced by Sacco et al., is a second generation autologous platelet concentrates. In a comparative study, it was demonstrated that CGF undoubtedly held an edge over PRP and PRF in terms of not only cell proliferation and osteoblastic differentiation but also possessed a richer growth factor content. (5)

Table 1: Autologous Platelet Concentrates

	PRP	PRGF	PRF	CGF
PIONEER	Whitman et al.(1)	Anitua(4)	Choukroun et al.(2)	Sacco et al. (6)
GENERATION	1st generation	1st generation	2nd generation	2nd generation
GROWTH FACTORS	PDGF, VEGF, TGF-β	PDGF, IGF, TGF-β	PDGF, TGF-β, cytokines (sustained release)	BMPs, osteo-conductive fibrin matrix
ADVANTAGES	Easy to prepare, rich in GF, form 3D fibrin that entraps GF	Shortest centrifuge cycle, easy preparation.	Easy to prepare, shorter centrifuge cycles, no coagulant required, long-acting, more effective	Does not require additives, better results compared to PRP, PRF
DISADVANTAGES	Lesser cytokines, short-acting, bovine thrombin—life-threatening coagulopathies, long preparation time.	Less active than PRF, CGF. Low levels of growth factor release.	Used in limited volumes, tissue banks for PRF are unfeasible, shrinks on storage.	Requires special centrifuge machine

Table 2: Biological polymer based scaffolds

	DESCRIPTION	ADVANTAGES	DISADVANTAGES
COLLAGEN	It is a major component of extracellular matrices.	Biocompatible, biodegradable, good tensile strength, shows high alkaline phosphatase activity, forms a trap for osteoinductive activity allows soft tissue and hard tissue formation. Available in porous sponges, gel and sheet form.(7)	Mechanically weak and undergoes rapid degradation and shows contraction.
CHITOSAN	It is produced commercially by the deacetylation of chitin, which is present in the exoskeleton of crustaceans.	Non-toxic, Bioabsorbable, shows fibroblast and odontoblastic proliferation, Available as a porous scaffold, can be made into gel form. Its hydrophilic form enhances cell adhesion and proliferation.	Has low strength, difficult to control the size of the hydrogel, chemical modification can induce toxic behaviour. (8)
GLYCOSAMINOGLYCANS	Hyaluronic acid is one of glycosaminoglycans found in ECM. It supports osteogenesis and provides a milieu for chondrogenesis when exposed to initiating factors.	Biocompatible, biodegradable, non-immunogenic, bioactive, Helps in differentiation of dental mesenchymal cells to odontoblast, in formation of dentin matrix and dental pulp	Highly water soluble, rapidly degrades by enzymes, lacks mechanical integrity. (9)
DEMINERALISED/NATIVE DENTIN MATRIX	It contains 233 total and 68 common proteins, which includes various collagenous and non-collagenous proteins.	Biocompatible, osteoinductive, osteoconductive, Non-immunogenic.(10)	Demineralization is time consuming, it may negatively affect non-collagenous proteins involved in bone formation.(11)

SILK	It has been extensively used for both soft and hard tissue engineering.	Biocompatible, good mechanical strength, elasticity, slow degradation rate which enables proper replacement of fibroin with newly formed tissue.(12)	Compltely degrades within 2 years.(13)

Table 3: Artificial/synthetic scaffolds

	POLYMER	BIOCERAMICS
DESCRIPTION	Synthetic polymer scaffolds include polylactic acid (PLA), poly-L-lactic acid (PLLA), polyglycolic acid (PGA), poly-l-glycolic acid (PLGA), polyepsiloncaprolactone (PCL).	It includes calcium/phosphate materials, bioactive glasses and glass ceramics. Modifications- Addition of various elements help in manipulating the properties of ceramics. Magnesium-based ceramics have helped in improving the mechanical integrity and high rate of bioactivity. Niobium doped fluorapatite bioceramics show increased cell adhesion and permit differentiation of hDPSCs on surface.(17)
ADVANTAGES	Nontoxic, biodegradable, can be manipulated to change various physicochemical properties such as porosity, mechanical stiffness, microstructure etc. Sakai et al. conducted a study showing that PLLA scaffolds promoted dental pulp cell differentiation into endothelial cells and odontoblasts.(14) PLGA used as a scaffold can form dentine like tissue and pulp like tissue in 3-4 months period.(15)	β-Tricalcium phosphate have been used in bone regeneration due to its biocompatibility, osteoconductivity, low immunogenicity and similarity to mineralised tissues. Addition of SiO2 or ZnO helps in improving the mechanical strength Release of dissolution products Ca-P increases the osteoblastic activity of the material.(18)
DISADVANTAGES	Can cause chronic or acute inflammatory host response, and localized pH decrease due to relative acidity cause by byproducts.(16)	Time-consuming fabrications, difficulty of shaping and brittleness. When used alone they have low mechanical properties. They can be combined with polymer scaffolds to overcome this problem.(19)

NANOFIBROUS SCAFFOLDS :-

Preferably, a scaffold should accurately simulate the features of the native extracellular matrix (ECM) at the nanoscale to regulate specific events at cellular and tissue level. Nanofibrous scaffolds are adapted in such a way that they fulfil the above criteria. Nanofibrous scaffolds are best known to reproduce positive cell-ECM interactions, permit stem cell differentiation, maintain cell phenotype, and activate cell signalling pathways by imparting stimuli. (20) Various techniques of fabrication of nanofibrous scaffold are as follows:-

Electrospinning or electrostatic spinning is the most commonly used technique. It consists of application of high electric field to polymer solution that flows through the needle orifice to produce continuous polymer fibres with diameter in range of nanometers or micrometers. Nanofibrous scaffolds have two different applications in regenerative endodontics. The first application is for intracanal drug delivery of antibiotics. The second application is for promoting dentin–pulp regeneration. (21)

A study done by Ruparel et al. has shown that the widely used creamy paste (1 g/mL) of the triple antibiotic mixture is harmful to stem cells from the apical papilla (SCAPs). Nanofiber-based scaffold designed for intracanal drug delivery release antibiotics at lower concentrations, nevertheless antimicrobially effective. Because of the lower concentration of antibiotics released, they are less cytotoxic to the stem cells. (22)

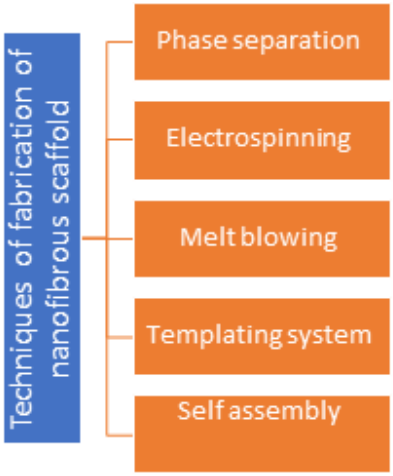

Figure 5: Techniques of fabrication of nanofibrous scaffold

INJECTABLE SCAFFOLDS :-

The root canal system has a complex morphology, which makes it a challenge for the placement of preformed (block, sheets) scaffolds into the root canal space. Hence an injectable scaffold has a lead over the conventional form. These include- It can be easily injected in the root canal space due to its liquid nature, stem cells and bioactive molecules can be first mixed in the liquid media before placement in situ and the placement of the scaffold is done in a minimally invasive manner which reduces the risk of infection. (23)

Injectable hydrogels are composed of three-dimensional hydrophilic polymers that absorb water or tissue fluids up to several times their weight. 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. (24)

Recently, Fukushima et al. have done a systematic review after screening articles based on various hydrogel-based scaffolds used for regeneration of dental pulp. (25) Their conclusion has shown that the appropriate hydrogel scaffold type to be used along with stem cells for regeneration of dental pulp was the natural scaffold. Collagen was the most successful natural scaffold. Even without the use of growth factors, the scaffolds having stem cells were able to support the dentin formation whereas synthetic scaffolds were least preferred.

CONCLUSION

Regenerative endodontics procedures have emerged as a viable alternative for the treatment of tooth with pulpal necrosis. This review article gives an insight on the various scaffolds that are used in regenerative endodontics. It also focuses on advantages and limitations of various scaffolds. Among the various scaffolds, the autologous platelet concentrate is the most widely used one due to their ease of use and biocompatible nature. The clinicians should be aware of the attributes of various scaffolds so that they can select most suitable one for successful results.

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