Scaffolds in Regenerative Endodontics: A Review

Malli Sureshbabu Nivedhitha¹, Selvam Deepak², Benoy Jacob³, Riluwan Siddique⁴

ABSTRACT

Stem cells, scaffolds, and growth factors, each of which possesses unique biological capabilities, constitute what is known as the tissue engineering triad. 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 in cases with large periapical lesion, and regeneration of periodontal tissue and bone in endo-perio lesions. Scaffolds play a major part in the formation of the extracellular matrix by providing support to cells to adhere, grow, and differentiate. In this review, four major categories of scaffolds (autologous platelet concentrates, nanofibrous scaffolds, injectable scaffolds, and bioactive molecule carrier systems) used in regenerative endodontics have been discussed in detail.

Keywords: Autologous platelet concentrates, Bioactive molecules carrier system, Electrospun nanofibers, Injectable scaffolds, Pulp–dentin regeneration, Regenerative endodontics, Scaffolds.

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INTRODUCTION

Stem cells, scaffolds, and growth factors, each of which possesses unique biological capabilities, constitute what is known as the tissue engineering triad. The differentiation potential of stem cells (undifferentiated cells) to differentiate to a particular tissue in a suitable medium (scaffold) under the influence of growth factors or bioactive molecules, holds significant credibility in regenerative therapeutic procedures.

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 in cases with large periapical lesion, and regeneration of periodontal tissue and bone in endo-perio lesions.

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.

Primarily, scaffolds play a major part in the formation of the extracellular matrix (ECM) by providing support to cells to adhere, grow, and differentiate.

The functions (Fig. 1), ideal requirements (Fig. 2)^{1,2}, and classification of scaffolds (Fig. 3)³ are illustrated in the mind maps.

In this review, four major categories of scaffolds (autologous platelet concentrates [APC], nanofibrous scaffolds, injectable scaffolds, and bioactive molecule carrier systems) used in regenerative endodontics will be discussed in detail.

AUTOLOGOUS PLATELET CONCENTRATES

Platelet-rich Plasma

Platelet-rich plasma (PRP), an autologous first-generation platelet concentrate, has to its credit an abundant reservoir of growth factor content, thereby ideally labelling it as a scaffold.⁴

Platelet-rich Fibrin (PRF)

The second-generation platelet concentrate is composed of an intertwined fibrin mesh of cytokines, glycanic chains, and structural

^{1,2}Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

³Private Practice, Consultant Endodontist, Muthusaram Healthcare Pvt. Ltd., Chennai, Tamil Nadu, India

⁴Private Practitioner, Consultant Endodontist, Karikulam Dental Clinic, Malikampeedika, Aluva, Kerala, India

Corresponding Author: Malli Sureshbabu Nivedhitha, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India, e-mail: nivedhithamallisureshbabu@gmail.com

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glycoproteins. The concoction provides an astounding effect on wound healing and repair.⁵

Dohan enlisted a set of platelet concentrates into four families, according to their leukocyte content and fibrin architecture: pure platelet-rich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and leukocyte- and platelet-rich fibrin (L-PRF).

Platelet concentrates have been widely used in regenerative endodontics as a scaffold material. Platelets are considered the powerhouse of growth factors, released during wound healing for repair and regeneration. In the endodontic literature, a wide array of cases concerning the application of APCs for the management of necrotic immature permanent teeth have been reported, highlighting promising outcomes for the same. There are nine clinical trials^{6–14} and four systematic reviews^{15–18} on this topic. According to the Oxford CEBM, both have a higher level of evidence.

The different outcomes that were evaluated for the regenerative endodontic procedures were healing or reduction

Fig. 1: Functions of scaffold

in size of periapical lesion, apical closure, improvement in the root length and thickness, and pulp vitality. Clinical trials have shown conflicting results in the measured outcomes. The results of systematic reviews have demonstrated that the included clinical trials have a moderate to high risk of bias.

In the systematic review reported by Murray,¹⁶ 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 (Fischer test, $p < 0.0011$) 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 (Fischer tests, $p > 0.05$). The mean success rate seen with PRP and PRF are shown in Figure 4.

Fig. 2: Ideal requirements of scaffold

Fig. 3: Classification of scaffold

In another systematic review done by Metlerska et al.,¹⁷ among the 26 studies included in the systematic review, five were randomized trials. In randomized trials, only three cases were unsuccessful, and those salvaged with APCs exhibited a higher response to pulp vitality tests. In most included subjects, thickening of dentinal wall layers, increase in the length of the root, and even closure of the apical portion of the root were noted.

The concluding remark derived from the meta-analysis was that there was a marked improvement in apical closure and subsequent responses to pulp vitality tests revealed an overall successful treatment outcome.¹⁷ On the other hand, there was no significant increase in root length or dentinal wall thickness. In the subgroup analysis, there was a better effect in apical closure in teeth treated with PRP when compared to PRF, also the pulp vitality tests were adequate for PRP (Fig. 5).

With mounting evidence, a conclusion can be drawn in favor of regenerative endodontics, to serve as a prudent therapeutic option in dealing with immature necrotic teeth, with an overall success rate of 96.5% recorded for the same. However, a longer follow-up period of 5 years or more is essential to gain complete acceptance of the treatment regimen, which is again currently absent in the literature.

Plasma Rich in Growth Factors

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

Fig. 4: Bar chart for the mean success rate of PRP vs PRF

Fig. 5: Bar chart comparing the effectiveness of PRP vs PRF in terms of risk ratio

growth factor-beta (TGF- β), fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor (IGF-I), and hepatocyte growth factor (HGF).¹⁹

PRGF Preparation

A sterile environment is maintained throughout the blood collection procedure, wherein blood is drawn in 3.8% sodium citrate tubes, following which tubes are centrifuged at 460 $\times g$ for 8 min (PRGF System, BTI Biotechnology Institute, Álava, Spain). Blood dissociates into three fractions: the topmost layer of plasma containing platelets, the middle layer enriched with white blood cells also forming a “buffy coat” and finally, the bottom layer comprising of red blood cells. The portion above the first layer, i.e., plasma poor in growth factors (PPGF), and the portion right above the “buffy coat” (PRGF) are dispensed into separate tubes, keeping in mind no additives are to be added. Calcium chloride, the compound used to activate the PRGF concentrate is added to the preparation (50 μ L CaCl₂/mL of preparation), subsequently achieving platelet degranulation and the release of growth factors as an outcome.

In a case series reported by Hengameh Bakhtiar et al., PRGF has demonstrated encouraging outcomes in the management of necrotic immature teeth. After two years of follow-up, 2 teeth showed complete apical closure and in another 2 teeth, apical closure and thickening of the dentinal wall were observed.²⁰ PRGF has also been applied for large through-and-through bone lesions and perforation of sinus membrane with or without bone grafts. Patients’ quality of life was improved during the initial postoperative healing period after the use of PRGF. PRGF has also shown accelerated bone healing in periradicular sur23gery.^{21–23}

Concentrated Growth Factor

The stem cells of the apical papilla (SCAP) tend to exert a “self-renewing” property, which is believed to be the biological mechanism promoting adequate regeneration to acceptable levels highlighting the capability of differentiation into odontoblast-like

cells, ultimately producing tissue, very much similar to the dentin microstructure.^{24,25} The preservation of SCAP proves to be a forerunner in regenerative endodontics promoting the adequate formation of the root from development to its completion.²⁶ A direct infusion of substantial amount of mesenchymal stem cells (MSCs) into the root canal space on induction of bleeding from the apical papilla cannot be expected and whatever quantity begot will be insufficient to reinvigorate the pulp–dentin complex.²⁷ Thus, concentrated growth factor (CGF) succors to stem the regenerative process by serving as a prudent cenote of growth factor content.²⁸ Comparison of all the four platelet concentrates is given in Table 1.

CGF, introduced by Sacco et al., is a second-generation APC capable of bringing about a revolutionary change in regenerative endodontic procedures. To put it in perspective, CGF in comparison with older platelet concentrates exhibits differing characteristics by the manner in which it is concocted. The distinctive centrifugation technique that is subjected to much more improved, distinct, and healthier fibrin matrix, accredited to diverse time intervals and controlled speeds, channeled during the centrifugation process.²⁹ 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.³⁰

Preparation of CGF

A centrifuge machine (MEDIFUGE, Silfradentsrl, S. Sofia, Italy) and a couple of disposable 10 mL non-anticoagulant centrifuge tubes are the equipments to be kept ready at first. Thereafter, after seating the patient, 10 mL of intravenous blood is drawn from the patient, chairside, and transferred to centrifuge tubes. It should be kept in mind that no anticoagulants are used during the preparation process. The tubes are then churned inside, accelerated for 30s, centrifuged at 2700 rpm for 2 min, 2400 rpm for 4 min, 2700 rpm for 4 min, and 3000 rpm for 3 min, decelerated

Table 1: Autologous platelet concentrates

	PRP	PRGF	PRF	CGF
Pioneers	Whitman et al. ⁴	Anitua ¹⁹	Choukroun et al. ⁵	Sacco et al. ²⁹
Generation	1st generation	1st generation	2nd generation	2nd generation
Growth factors	PDGF, VEGF, TGF- β	PDGF, TGF- β , IGF	PDGF, TGF- β , cytokines (sustained release)	BMPs, osteo-conductive fibrin matrix
Anticoagulants	Used	Used	Not used	Not used
Bovine thrombin	Used	Not used	Not used	Not used
Preparation	Blood centrifuged in 2 spins: Soft spin: 1300 rpm—10 min Hard spin: 2000 rpm—10 min	Blood centrifuged at 460 $\times g$ for 8 min CaCl ₂ is added as PRGF activator	Blood centrifuged at 3000 rpm/400 $\times g$ for 10 min	Blood centrifuged at different speeds: 2700 rpm for 2 min, 2400 rpm for 4 min, 2700 rpm for 4 min, and 3000 rpm for 3 min, decelerated for 36 s to stop
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

BMP, bone morphogenetic protein; CGF, concentrated growth factor; PDGF, platelet-derived growth factor; PRF, platelet-rich fibrin; PRF, platelet-rich fibrin; PRP, platelet-rich plasma; VEGF, vascular endothelial growth factor

for 36 s to stop. At the end of the automated pre-programmed cycle, the centrifuge tube encompass a mixture of four separate portions one above the other: the last layer (4th) comprises the red blood cells (RBCs), the one above it (3rd) constitutes the growth factor content, the secondary layer (2nd) is composed of a "buffy coat", and the primary portion (1st) contains serum. The 3rd portion, i.e., the fibrin gel containing the much need concentrated growth factor content (CGF) is separated from the 4th portion of RBCs.

In yesteryears, primary emphasis has been laid upon the incorporation of the most sought-after APCs namely PRP and PRF in a process to regenerate the lost pulp. The main listed disadvantages of the older generation platelet concentrate include high cost, difficulty in centrifugation, handling, and purification procedures. CGF scores over all the aforementioned drawbacks especially in terms of low cost and easy handling, the use of anticoagulants and bovine thrombin are also avoided.³¹ Additional benefit of CGF, when compared to conventional platelet concentrates is that CGF acts as a promising biomaterial releasing growth factors that induce cell migration and proliferation, chemotactic activity on inflammatory cells, stimulates angiogenesis and tissue remodeling.³² CGF plays an important role in implant surgeries and sinus ridge augmentation procedures.³³

CGF in combination with osseograft has several advantages in the formation of sticky bone. The formed product can be molded, prevent both macro and micro-movements, also the mesh network contains growth factors and platelets thereby contributing to accelerated bone regeneration and soft tissue healing. The fibrin interconnection network also helps to prevent the ingrowth of soft tissue into the bone graft.³⁴

CGF accounts for a diverse array of growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF- β 1 and TGF- β 2), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF), of which TGF- β 1 and VEGF predominate. Rodella et al., demonstrated that growth factors TGF- β 1 and VEGF were found in abundance which according to the literature are highly essential for stimulating cell proliferation, matrix rehabilitation, and angiogenesis. The aforesaid study also confirmed the existence of circulating CD34+ cells in CGF and RBC layers which again is an important requisite for the revascularization process.³⁵ While TGF- β 1 stimulates expression of bone morphogenetic proteins (BMPs), curtails matrix metalloproteinases (MMPs) and other enzymes to further promote differentiation of osteoblastic cells,³⁶ VEGF at the same time induces endothelial cell migration and proliferation eventually leading to hyperpermeability of blood vessels.³⁷ In a study done by Park et al., showed a higher level of growth factors, particularly VEGF in CGF as opposed to PRF.³⁸

The results of initial *in-vitro* studies on the usage of CGF in regenerative endodontics are successful. The biological effects of CGF on both SCAP and dental pulp stem cells (DPSC) have been studied extensively. Hong et al.³⁹ evaluated the effects of CGF on SCAP and reported that CGF promoted the proliferation, migration, and differentiation of SCAPs and recommended it as a successful biomaterial for usage in regenerative endodontics. In another comparative study by Hong et al.,⁴⁰ CGF and PRF were compared on the effects of SCAP. The results of the study concluded that both test groups demonstrated significantly greater mineralized areas than the control groups. The expression levels of

alkaline phosphatase (ALP), bone sialoprotein (BSP), dentin matrix protein 1 (DMP-1), and dentin sialophosphoprotein (DSPP) were down-regulated after incubation in CGF and PRF for 7 days, whereas they were significantly enhanced after incubation for 14 days. Also, the expression levels of related genes in the PRF group were statistically higher than those in the CGF group.

Jin et al.,⁴¹ assessed the effects of various concentrations of CGF on DPSC and reported an interesting finding. The low doses (<50%) of CGF increased cell migration, ALP activity, and mineralized tissue deposition, while the cells treated in high doses (50% or 80%) showed no significant difference. Jun et al.,⁴² demonstrated that extracts of CGF upregulated the expression of angiogenesis-related genes in human dental pulp cells and human umbilical vein endothelial cells.

In our previously published case report presentation of two cases, we had used CGF as a scaffold in the endo surgical treatment procedures and had showed promising healing outcomes.⁴³ Also, use of CGF has shown good result in the treatment of NIPT.⁴⁴ In another case report, we had implemented a coalescence of CGF and bone graft (sticky bone) to intercept a large periapical bony lesion and had showcased exceptional results in terms of periapical healing which is probably ascribable to the inclusion of a dynamic combination of CGF and bone grafts to form sticky bone as a means to induce a rapid repair and regenerative process.⁴⁵ In the latest clinical trial, the combination of bone graft along with CGF had been proven to be significantly better than the use of bone graft alone in treating jawbone defects due to various etiologies.⁴⁶

NANOFIBROUS SCAFFOLD

Ideally, a scaffold should accurately reproduce the features of the native ECM at the nanoscale to regulate cellular responses, encourage and regulate specific events at the cellular and tissue levels.⁴⁷ Nanofibrous polymer scaffolds are tailored to show high processing ability, mechanical competence, characteristically biodegradable and biocompatible as well. Certain features, such as interconnected porosity, high surface area and nano fiber dimension propel nano fibrous scaffolds as such much more superior competitor to microfibers or any other morphological arrangements. Most significantly, nanofibrous scaffolds are best-known to stimulate positive cell-ECM interactions, maintain cell phenotype, support differentiation of stem cells, increase cell proliferation, and activate cell signaling pathways by imparting physical and chemical stimuli. The advantages, techniques of fabrication, classification, and recent advances are given in the mind map (Fig. 6).

Electrospinning or electrostatic spinning—by adjustment of electrospinning parameters, fiber morphology is controlled, along with fiber diameter, fiber, and pore size, among different factors identified to influence cell behavior and overall tissue regeneration.⁴⁸ 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.

Intracanal drug delivery of antibiotics using electrospun nanofibers has been extensively studied in *in-vitro* studies.⁴⁹ The antibiotics that are commonly used are ciprofloxacin, metronidazole, and minocycline. They have been tested individually or in different combinations for antimicrobial

Fig. 6: Nanofibrous scaffolds

properties and cytotoxicity against stem cells. These antibiotics have proved to be effective against *Enterococcus faecalis*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*.^{50–52} Replacing minocycline with clindamycin (Clindamycin modified TAP) in the nanofibers showed no visible dentin discoloration. This modified antibiotic paste can be considered as an alternative to the minocycline containing triple antibiotic paste (TAP) which causes staining of tooth structure.⁵³

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). However, TAP concentrations starting from 0.01 to 0.1 mg/mL were not cytotoxic, and also the concentration of 1 mg/mL was solely moderately harmful once applied directly onto SCAP and had no result on viability.⁵⁴ 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.⁵⁵ Antibiotic-containing scaffolds minimize harmful effects of highly concentrated antibiotic pastes on stem cell of apical papilla survival and nonetheless still promote bacterial elimination based on high-performance liquid chromatography (HPLC) analysis data,

in addition to cell toxicity experiments, has supported the claim of an additional biologically friendly strategy compared with the use of antibiotic pastes, since the amount of drugs released occurs more gradually in a lower concentration than in those used in pastes.⁴⁹

Various studies have shown encouraging results for odontogenic differentiation of human DPSC on different nanofibrous scaffolds (poly(lactic acid) (PLA), polycaprolactone (PCL), nano-hydroxyapatite (nHA) incorporated, collagen, gelatin). Future studies will be conducted for carrying dental derived em cells in electrospun nanofibers for optimum dentin–pulp regeneration.⁴⁷

INJECTABLE SCAFFOLD

The root canal system has a complicated morphological structure with a long, narrow channel of the root canal with an average total volume of 20 μ L. It is always a challenge to place the preformed scaffolds (sheets, blocks) into the root canal space. In this regard, an injectable scaffold has several advantages. These include (a) it can be easily implanted into the root canal space by injecting it as it is available in the liquid form, (b) it can easily fill any irregularly shaped

defects, (c) stem cells and bioactive molecules can be easily mixed with the solution before injecting *in situ*, and (d) the placement of scaffold is done in a minimally invasive manner which reduces the risk of infection and improves the comfort.⁵⁶

The requirements, classification, fabrication methods, materials used for injectable scaffolds are shown in the mind map (Fig. 7).⁵⁷ Injectable hydrogels are composed of three-dimensional hydrophilic polymers that absorb water or tissue fluids up to several times their weight (Fig. 8).⁵⁸ These are biocompatible, tunable, water-swollen materials that can be easily injected in their colloidal form, undergoing gelation by chemical (e.g., changes in pH and osmolarity) or physical stimuli (e.g., temperature change). 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.⁵⁸ The properties of hydrogels are given in the mind map (Fig. 9).

Hydrogels made of self-assembly peptides (e.g., PuraMatrix™) show great potential to be used in endodontic tissue engineering because their sequence includes short peptide sequences, like those naturally occurring in tissues, enhancing cell attachment and proliferation. PuraMatrix™ is a successful hydrogel-based nanofibrous scaffold made of 16-mer peptide in an aqueous solution. On interaction with physiological conditions, it polymerizes and forms a biodegradable nanofiber hydrogel scaffold. The self-assembling design presents limitations relative to mechanical properties and structure—examples, the influence of viscosity in

cell proliferation, irregular pore size, and difficulty maintaining the hydrogel throughout the full-length of root canal extension.⁵⁶

Many injectable hydrogels have been evaluated against DPSC, SHED, and SCAP for their survival and differentiation. PuraMatrix promoted the survival and proliferation of DPSC.⁵⁹ SHED encapsulated in PuraMatrix and delivered into whole root canals gave rise to a pulp-like tissue and odontoblasts after transplantation into immunodeficient mice.⁶⁰ In another study, the scaffold was used with DPSC and human umbilical vein endothelial cells (HUVEC) and demonstrated that DPSCs showed higher early vascular network formation by facilitating the migration of HUVECs and by increasing vascular endothelial growth factor (VEGF) expression.⁶¹ Application of scaffold mixed with SCAP without the use of exogenous growth factors and transplanted into molar crowns of mice, formed a viable tissue with odontoblast-like cells.⁶²

A hydrogel composed of PEGDA, HN, and Gn (HyStem C) was assessed against DPSC and it was shown that it maintains viability and facilitates cell spreading in the presence of extracellular matrix proteins.⁶³ Another FDA authorized hyaluronic acid-based hydrogel (Restylane) which has been widely used as facial dermal filler promoted stem cells of apical papilla survival, mineralization, and differentiation into an odontoblastic phenotype.⁶⁴ Shiehzhadeh et al. reported in their case report about the use of injectable scaffold to deliver dental stem cells for the management of large periapical lesions.⁶⁵

Recently, Fukushima et al. have done a systematic review after screening articles based on various hydrogel-based scaffolds used

Fig. 7: Injectable scaffolds

Fig. 8: Hydrogels: Response to stimuli

for regeneration of dental pulp.⁶⁶ 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. PuraMatrix by itself was unable to form dental pulp when DPSC were not present. Synthetic and hybrid hydrogels were unable to attract stem cells from the host. The presence of growth factors in these constructs seems to be of relevance since dental pulp tissue formation was achieved only when the hybrid scaffold was applied with growth factors. All types of hydrogel-based scaffolds, when containing mesenchymal stem cells, can form connective tissue with different degrees of similarity to the dental pulp.⁶

BIOACTIVE MOLECULE DELIVERY SYSTEM

Bioactive molecules are signaling molecules, such as growth factors or chemical cues that control a variety of cellular responses through specific binding of transmembrane or intracellular receptors in a target cell.⁶⁷

Dentin and pulp are two ultrastructurally different tissues. However, they are considered as a single unit (dentin–pulp complex). During physiologic tooth development, bioactive molecules are released in a controlled manner to the desired

location (spatiotemporal release—both location and time-specific). In the regenerative endodontic procedure, there is a great challenge for the accurate release of bioactive molecules in a spatiotemporal manner.⁶⁸

Bioactive molecule carrier system (BACS) aids in mimicking some of the complex physiological processes, overcoming some of the challenges faced in the clinical translation of regenerative endodontic procedures. Also, the biomaterial-based BACS, with the aid of encapsulated bioactive molecules plays an important role in activating critical cellular signals, microenvironmental cues capable of modulating and tailoring cellular responses. The ideal requirements, release mechanisms, and release kinetics of BACS are given in the mind map (Fig. 10).

When the bioactive molecules are administered in a bulk or bolus form the release pattern follows the first-order kinetics. This strategy has the increased concentration initially followed by a drastic decrease in concentration. To overcome this challenge, different strategies have been employed to maintain the release of bioactive molecules. Various carrier materials have been developed to tailor the release of bioactive molecules. In the sustained release strategy, the molecules are released above the therapeutic level for a longer period of time. The more sophisticated controlled-release strategy releases the molecules at a specific rate for a much longer time which follows the zero-order principle.

The most commonly used carrier material is collagen which is a natural polymer. Other natural polymer materials include hyaluronic acid, alginate, and chitosan. These are employed in different forms, such as three-dimensional scaffolds, hydrogels, and micro/nanoparticles for the controlled release. In recent years, chitosan has been used in a nanoparticulate form as a delivery system for bioactive molecules. The molecules tried are the bovine serum albumin (BSA), dexamethasone (DEX), TGF- β 1 and have been tested against the SCAP. The temporally controlled release of BSA and DEX from chitosan nanoparticle carrier systems have promoted the proliferation, ALP activity, and odontogenic differentiation of SCAP.^{69,70} The sustained release of TGF- β 1 enhanced the migration and differentiation of SCAP cells.⁷¹

CONCLUSION

This literature review focuses on the advantages and limitations of various scaffolds used in regenerative endodontics. Autologous

Fig. 9: Hydrogels

Fig.10: Bioactive molecule carrier system

platelet concentrates are the commonly employed scaffold because of their ease of use and biocompatibility. To overcome its drawbacks, other scaffolds are developed. However, many of them are still in laboratory research with promising results. More animal and clinical studies should be done to translate the application of newer scaffolds in regenerative endodontics.

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