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RESEARCH ARTICLE

SCAFFOLDS IN REGENERATIVE ENDODONTICS: A REVIEW.

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Abstract

Introduction:-

Pulp tissue regeneration may present an ideal alternative treatment to traditional root canal therapy. The present concept of pulp tissue regeneration includes two possible approaches. [1] The first is revascularization, where a new pulp tissue is expected to grow into the root canals from the remaining tissues exist apically in the root canal.[2] The second includes the replacement of the diseased pulp with a healthy tissue that is able to revitalize the tooth and restore dentin formation process. The stem cell therapy, gene therapy, three-dimensional (3D) cell printing, scaffold implantation, and pulp implantation are suggested for this approach. [3]

In tissue engineering, the selection of a suitable scaffold is critical. Scaffolds can be identified as biocompatible structures, 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 [4-6]

Regenerative endodontics is an emerging field of modern tissue engineering that has demonstrated promising results using stem cells associated with scaffolds and responsive molecules. This article gives a review on the different scaffolds providing an insight into the new developmental approaches on the horizon.

Greenwood and colleagues described regenerative medicine as the “emerging interdisciplinary field of research and clinical applications focused on the repair, replacement, and regeneration of cells, tissues, or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma, and aging.” Regeneration approaches use a combination of scaffolds, stem cells, growth factors, tissue engineering, organ tissue culture, transplantation, and tissue grafting [7]

Scaffold:-

The scaffold provides a physico-chemical and biological three-dimensional micro environment for cell growth and differentiation, promoting cell adhesion, and migration. The scaffold serves as a carrier for morphogen in protein therapy and for cell in cell therapy. Scaffold should be effective for transport of nutrients, oxygen and waste. Scaffolds should be able to support and guide the cell growth and the development of new tissues. The matrix should be able to withstand forces and maintain a potential space for tissue development and should provide a controlled

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vehicle for gene and protein delivery. A large surface area to volume ratio is desirable to allow delivery of high density cells. It should encourage cell migration and differentiation. [8]

Scaffold Implantation to create a more practical endodontic tissue engineering therapy, pulp stem cells must be organized into a three-dimensional structure that can support cell organization and vascularization. This can be accomplished using a porous polymer scaffold seeded with pulp stem cells [9]. A scaffold should contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development [10]. Growth factors were described in the previous section. The scaffold may also contain nutrients promoting cell survival and growth [11], and possibly antibiotics to prevent any bacterial in-growth in the canal systems. The engineering of nanoscaffolds may be useful in the delivery of pharmaceutical drugs to specific tissues [12]. In addition, the scaffold may exert essential mechanical and biological functions needed by replacement tissue [13]. In pulp-exposed teeth, dentin chips have been found to stimulate reparative dentin bridge formation [14]. Dentin chips may provide a matrix for pulp stem cell attachment [15] and also be a reservoir of growth factors [16]. The natural reparative activity of pulp stem cells in response to dentin chips provides some support for the use of scaffolds to regenerate the pulp dentin complex. The other important properties are biocompatibility and biodegradability. Some scaffolds are permanent, while for other scaffolds, they need to be absorbed by the surrounding tissues to avoid interfering with the regenerated tissue. The rate of degradation should coincide with the rate of tissue formation.[17]The latest generations of scaffolds have been engineered to have ideal properties and functional customization: injectability, synthetic manufacture, biocompatibility, nonimmunogenicity, transparency, nano-scale fibers, low concentration, and high resorption rates.[18]

The types of scaffold materials available are natural or synthetic, biodegradable or permanent [19'20]

Biological or natural scaffolds:-

Blood clot:-

The utilization of a blood clot to regenerate dental pulp tissues was first practiced by Ostby and resulted in a growth of granulation tissues, fibrous tissues or cementum-like tissues into the root canals. [21] It is believed that tissues are not able to grow into empty spaces with the absence of suitable scaffolds, [21] it can be suggested that blood clots yield good scaffolds to fill intracanal spaces and aid the growth of new tissues. [22]

The blood clot consists of fibrin matrix that traps cells necessary for tissue regeneration. It also provides a suitable pathway for cells from the periapical area including macrophages and fibroblasts to migrate into the root canal and enhance the new tissue growth.[23,24] The rich content of growth factors allows the blood clot to play an important role in cell differentiation[24,25] and thus, promotion of tissue regeneration.[26] These growth factors include plateletderived growth factor (PDGF), vascular endothelial growth factor (VEGF), and platelet-derived epithelial growth factor, known also as vascular permeability factor.[27,28]

There are several advantages to a revascularization approach. First, this approach is technically simple and can be completed using currently available instruments and medicaments without expensive biotechnology. Second, the regeneration of tissue in root canal systems by a patient's own blood cells avoids the possibility of immune rejection and pathogen transmission from replacing the pulp with a tissue engineered construct. [3]

Generally, tissue engineering does not rely on blood clot formation, because the concentration and composition of cells trapped in the fibrin clot is unpredictable. This is a critical limitation to a blood clot revascularization approach [3]

Platelet rich plasma:-

PRP is referred as a first-generation platelet concentrate. The PRP was introduced to dentistry world in 1997 by Whitman.[29] Platelet-rich plasma the most widely used blood venous derivatives, is a mass of autologous plasma with a high platelet concentration, which is recommended as a scaffold for its abundance of growth factors (GFs) [30,31]. PRP was widely used to promote wound healing after oral maxillofacial, implant, and endodontic surgery and recently for endodontic regenerative procedures.

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. [32]

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. [33] It is a concentrated suspension of different growth factors like PDGF, TGF-b, 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.[34]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. [33]

Platelet rich fibrin:-

PRF is known as a second-generation platelet concentrate. [35] PRF was developed first by Choukroun et al. (2001) [36] this simplified and cost-effective chair side procedure results in a resorbable fibrin matrix enriched with platelets and leukocytes. PRF provides a rich source of growth factors, including platelet derived growth factors (PDGFs), transforming growth factors (TGFs), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF)[36] The growth factors are slowly released during the course of the healing process.[37] Because of the unique character of PRF, it is used as a tissue-engineering material with a wide range of dental applications.[38,39]PRF is currently recommended as a scaffold material for regenerative endodontics.[40].Successful pulp revitalization cases using PRF were reported.[42]

Synthetic polymers:-

Synthetic biodegradable polymers, such as polyglycolic acid (PGA), polylactic acid (PLA) and poly-lacticcoglycolide, have been initially approved by the Food and Drug Administration (FDA) as drug delivery systems.[43,44] The application of these polymers as matrices for cell transplantation was the fi rst suggested by Vacanti et al. [45] Their biocompatibility and a broad range of reproducibility make them attractive for tissue engineering studies.[46-49] Polymers scaffold shape, porosity, mechanical properties, pores diameter, and degradation time can be successfully controlled in the preparation techniques.[50,51] Degradation of synthetic polymers are generally occurred by simple hydrolysis.[52]

PGA:-

The first attempt for pulp tissue engineering in vitro was achieved using PGA with human pulpal fibroblasts. A new tissue-like construct with similar cellularity as in normal pulp tissue could be observed. [53] PGA scaffold enhanced the growth of new blood vessels and the odontogenic differentiation of human fibroblasts when cultured on it [54]

PLLA:-

The poly-L-lactic acid (PLLA) polymer is a widely used FDA-approved biodegradable polymer. [55, 56] PLLA scaffold was able to produce tissue similar in architecture and cellularity to dental pulp tissue when transplanted with human dermal micro vascular endothelial cells [55]

PLA (OPLA):-

PLA is an aliphatic polyester, more hydrophobic than PGA [56] the synthetic open-cell PLA (OPLA) is another promising polymer for dental pulp regeneration. SHED seeded on OPLA and transplanted into cleaned, and shaped canals of human extracted teeth were able to attach to the root canal dentin. [57]

PCL:-

Is a slowly degrading polymer that have been used toward tissue engineering efforts in bone, either aloneor combined with hydroxyapatite. [58]

Poly-lactic-co-glycolic acid:-

Is the first copolymer mixture to gain approval from Food and Drug Administration. PLGA has been used in engineering bone, liver and cartilage.[59] Bertoldi tested a 50:50 PLGA copolymer and stated that it is free of inflammation effects, seems to stimulate bone growth, and decomposes after 6–8 months.[60] They also showed a strong alkaline phosphatase expression, indicating a probable promotion effect on bone cells. El-Backly *et al*.[61] Showed that 50/50 PLGA has good porosity for osteoconduction and that PLG scaffold may act as a suitable matrix to support dental pulp stem cells and their differentiation to form an organized dentine/pulp-like tissue [61] hence we used 50:50 PLGA scaffold. Tooth like structures have been obtained by seeding dental pulp stem cells on PLGA scaffolds. [62]

Bioactive ceramics:-
Calcium phosphate ceramics such as hydroxyapatite (HA), betatricalcium phosphate (β-TCP), and biphasic calcium phosphate (BCP) are totally biocompatible and bioactive crystallized materials. [63, 64]Cells cultured on the porous form of ceramics could attach, proliferate, and expressed dentin sialophosphoprotein, which is a dentin marker. [65]

HA:-
HA [(Ca10 (PO4)6(OH) 2] has been suggested as an effective scaffold for regeneration of dentin and dentin-pulp complex. [66-69]HA is a non-biodegradable ceramic while β-TCP [β-TCP Ca3 (PO4)2] is considered a biodegradable. [70] However, the mechanical properties of TCP are inferior to those of HA. BCP has been developed from HA and TCP to display the advantages of the both ceramics. [71, 72] BCP was widely investigated as a possible scaffold for pulp and dentin tissue regeneration. When pulp-derived cells were mixed with HA or HA/TCP and transplanted subcutaneously in nude mice, bone and dentin-like mineralized tissues were generated.[73,74,75] Moreover, the formation of dentin bridge after pulp capping in pig teeth model was stimulated using pulp cells seeded on HA/ TCP scaffold.[76]

Bioactive glass (BG):-
BG is a group of synthesized surface reactive biomaterials that have an amorphous structure and high mechanical strength. Silicate BG (known by its commercial name: Bioglass) has been traditionally used in BG researches. [77] However, for tissue engineering purposes, new BGs based on borate and borosilicate compositions have been suggested,[78-79] the biocompatibility and controllable degradation rate of these new glass scaffolds have been reported.[80,81] When degrades, BG will be converted into an HA-like substance that is able to bond to soft and hard tissues. The degradation also releases ions that contribute in osteogenesis and angiogenesis. [82, 83] It is well recognized that odontogenesis pathway is very similar to osteogenesis pathway and that odontogenesis and angiogenesis are essential for the successful generation of the dentin-pulp complex. Taken together, all these facts suggest that using BG as a scaffold for dentin and dental pulp engineering might be promising.

Naturally derived scaffold:-
Alginate:-
Alginate is a hydrogel comprising 1,4-linked b-D-mannuronic acid and a-L-guluronic acid, typically derived from brown seaweed and also bacteria.[84]The advantages of alginate are its biocompatibility, low toxicity, and slow gelling time (20–60 minutes), depending on the concentration and temperature.[85] Disadvantages of the material are the inability to control its degradation rate in vivo and its low viscoelasticity, although this can be improved by increased crosslinking or addition of other substances, such as HA.[86] Several studies using alginate and alginate/HA mixtures have been performed in bone and cartilage tissue engineering.[87,88]Alginate has been used in dental engineering to deliver cells and/or growth factors. The alginate hydrogel with either transforming growth factor (TGF)-b1 or acid treatment was applied to slices of human teeth with vital dentin-pulp complex tissues and maintained in culture. Hydrogel with TGF-b1 or acid treatment, but not the untreated control hydrogel, induced dentin matrix secretion and formation of new odontoblast-like cells in the human tooth slices. [87]

Collagen:-
particularly type I collagen, are major constituents of dentin and have been used to provide a 3D culture environment for various types of cells, including stem cells from the dental pulp.[89] Compared with other natural scaffold products including gelatin and chitosan, the dental pulp cells cultured in the type I and III collagen gel exhibited a higher degree of odontoblastic differentiation as shown by alkaline phosphatase activity and expression of osteocalcin, dentin sialophosphoprotein (DSPP), and dentin matrix protein 1 (DMP1).[89 90]Collagen gel can be used alone or in combination with growth factors (eg, TGF-b1, BMP4, FGF2)[91] and other scaffold materials such as chitosan.[92]

Chitosan:-
Chitosan is a polymer derived from the deacetylation of chitin, the major component of crustacean exoskeletons. It can be formulated into an injectable hydrogel. Chitosan/HA blend (polyelectrolyte complex) was used for compatibility studies with mesenchymal stem cells. In a 2:1 blend (HA/chitosan), cells were viable for 72 hours and no cytotoxicity was apparent. [93] The same group used chitosan/pectin scaffolds for bone regeneration with similarly positive results. [94]Chitosan/collagen scaffolds adsorbed with BMP7 were seeded with human adult dental pulp cells and stained positive for dentin matrix proteins DSPP and DMP1, whereas scaffolds without BMP7 were negative.
[92]

Hyaluronic acid:-
Hyaluronic acid sponges were used as 3D scaffolds for the regeneration of dental pulp. In comparison with the collagen sponge, the hyaluronic acid sponge can support cell growth in culture and in vivo from the amputated dental pulp of rat molars, with fewer immunologic reactions as shown by expression of inflammatory cytokines tumor necrosis factor (TNF)-a and interleukin-6, as well as leukocyte infiltration.[95] However, when used as an injectable hyaluronic acid gel for soft-tissue augmentation, adverse hypersensitivity reactions were reported, due to impurities and bacterial contamination.[96]

Fibrin:-
Fibrin consists of the blood proteins fibrinogen and thrombin, which are produced naturally in the body after injury to establish hemostasis and enhance wound healing. Because of their biocompatibility, biodegradability, simple preparation, and manipulation, fibrin scaffolds have been used for multiple purposes (eg, filling in bone cavities, vascular graft, and repairing injuries to urinary tract, liver, and lung) and are also available as mixtures with other polymers such as fibrin-PEG blend. [97], Fibrin glue and platelet-rich fibrin can be prepared fromwhole blood before surgery. The mixture of these 2 components was used as a scaffold for reassembly of porcine tooth bud cells implanted in the extraction socket. After 36 weeks, these implants developed into a complete tooth or an unerupted tooth crown.[98] For reassembly of porcine tooth bud cells implanted in the extraction socket. After 36 weeks, these implants developed into a complete tooth or an unerupted tooth crown. [98]

Injectable Scaffold Delivery:-
Rigid tissue engineered scaffold structures provide excellent support for cells used in bone and other body areas where the engineered tissue is required to provide physical support [99]. However, in root canal systems a tissue engineered pulp is not required to provide structural support of the tooth. This will allow tissue engineered pulp tissue to be administered in a soft three-dimensional scaffold matrix, such as a polymer hydrogel. Hydrogels are injectable scaffolds that can be delivered by syringe [100, 101]. Hydrogels have the potential to be noninvasive and easy to deliver into root canal systems. In theory, the hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure [102]. Past problems with hydrogels included limited control over tissue formation and development, but advances in formulation have dramatically improved their ability to support cell survival [103]. Despite these advances, hydrogels at are at an early stage of research, and this type of delivery system, although promising, has yet to be proven to be functional in vivo. To make hydrogels more practical, research is focusing on making them photopolymerizable to form rigid structures once they are implanted into the tissue site [104].

Three-Dimensional Cell Printing:-
The final approach for creating replacement pulp tissue may be to create it using a three-dimensional cell printing technique [105]. In theory, an ink-jet-like device is used to dispense layers of cells suspended in a hydrogel [106] to recreate the structure of the tooth pulp tissue. The three-dimensional cell printing technique can be used to precisely position cells [107], and this method has the potential to create tissue constructs that mimic the natural tooth pulp tissue structure. The ideal positioning of cells in a tissue engineering construct would include placing odontoblastoid cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cells. Theoretically, the disadvantage of using the three-dimensional cell printing technique is that careful orientation of the pulp tissue construct according to its apical and coronal asymmetry would be required during placement into cleaned and shaped root canal systems. However, early research has yet to show that threedimensional cell printing can create functional tissue in vivo [108].

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 scaffolds such as hydroxyapatite-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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