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Review Article

Scaffold based Regenerative Endodontics: Present & future

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Abstract: Most common treatment modality for a missing teeth these days is either a denture or implant based prosthesis. Great level of patient satisfaction has also been achieved with these two treatment options but scientifically speaking, both of these aren't biologically totally in coherence with the host system as the chances of foreign body rejection is always with them. Hence as the research continues in this modern era of dentistry, it is inevitable to open the roadway for an exact biologically duplicate model for what is to be replaced. Talking about the regenerative endodontics, various techniques have been employed for the same. But the most promising and encouraging technique has been the scaffold based regeneration. Three dimension scaffolds which mimic the cells natural extracellular matrix as close as possible are being extensively studied these days and have emerged as a promising option for the replacement of missing tooth with an entirely biological prosthesis, which can probably be called as a "Bio-Tooth".
Keywords: endodontics, Bio-Tooth, scaffold, foreign body.

Introduction:

What seem to be a fiction 20-25years ago is now within the realms of possibility. A complete tooth regeneration is now being seen as an approachable possibility with the introduction of scaffold based tooth regeneration techniques and a much bigger role of teeth tissue engineering in achieving the same. Scaffold is a surface on which cell can adhere, grow, migrate, attach and proliferate for the structural and functional replacement of target tissue.it is very important that the scaffold and extra cellular matrix resemble each other as closely as possible. The type and selection of scaffold along with its various properties which includes physical and chemical as well as surface topology plays an important role in the complete tooth morphogenesis [1-3]. Talking about the scaffold cells, it is very important that we consider their selection on a very precise nano and micro scale level because of the increase sensitivity of cells to any micro or nano level changes. Hence it's very important that scaffold cells are in coherence with the ECM at nano and micro levels. The chemical and physical properties provided by the scaffold should be same as natural surrounding tissue as far as compatibility, adhesion, migration, proliferation, degradation and mechanical strength is concerned. Apart from the above mentioned features one of the most important feature that has to be simulated in a scaffold is interconnected porosities as seen in ECM to allow for the placement of cell and growth factors to take place and also to support the vascular ingrowth for oxygen and biochemical transport [4,5,6]. In this article we are going to review various scaffold designs with their advantages and limitations along with recent developments in this interesting area of dentistry which promises to revolutionize the field of regenerative endodontics.

Biological scaffolds

Collagen

Collagen is one of the most abundant natural polymers found in the human body ranging from cartilage, bone, skin, sinews and etc. It is constantly being investigated for tooth regeneration because of its excellent biocompatibility, bioactivity and an ideal scaffold for cell proliferation, migration and differentiation [7,8,9]. Apart from above mentioned qualities, collagen closely resembles the ECM, has got low immunogenicity and cytotoxicity and excellent efficiency and can closely adapt to various forms and shapes. Prescott et al. seeded dental pulp stem cells (DPSC) on collagen scaffold for 6 weeks and they reported a pulp like tissue regeneration [10]. But a study by Zhang et al showed that the newly generated DPSCs, by growing them on a collagen were similar to connective tissue rather than dentin like tissue [11].

Chitosan

If we deacetylate chitin to 50%, chitosan is achieved which is soluble in aqueous soluble media. Its use in tissue engineering is increasing day by day because of high biocompatibility and biodegradability [12,13]. When DPSCs and HAT-7 dental epithelial cells were seeded on a 3D multi-layered co culture scaffold then after 24 days, Ca deposits were seen, indicating its potential in promoting mineralisation of tooth. Macro scale bio-mimetic structure with tunable mechanical properties and good DNA carrier are some of its unique properties. Plasmid encoding platelet-derived growth factor B (PDGFB) gene carrying chitosan was combined with coral by Zhang et al [14] to construct a porous chitosan/coral scaffold, these were then seeded on human periodontal ligament cells (HPLCs) and implanted into athymic mice and named gene-activated scaffold. Natural coral is mainly composed of calcium carbonate. The result clearly showed that HPLCs performed better on seeded scaffold as compare non-seeded scaffold [14].

Silk

Use of silk is increasing in bone tissue engineering [15,16] day by day because of its unique ability to convert into other peptides such as arginine, glycine etc. silk has been approved by food and drug administration (FDA) [15] because of its unique biocompatibility, biodegradability and high host acceptance as it is highly non-immunogenic. Four different types of minute scaffolds with a diameter in the range of 250 – 500nm have been manufactured which greatly coincides with the chemistry of ECM. These scaffolds were subsequently seeded with tooth bud cells and implanted for 4 days. Results showed the formation of bioengineered mineralized tissue that was more active in 550 mm pore RGD-containing scaffolds and least active in 250 mm pore sized scaffolds without RGD [17].

Alginate

It is a natural polysaccharide and has excellent biocompatibility, mildness of gelation and low immunogenicity. Experiments with rat dental pulp derived cells and human dental pulp cells after having been seeded on alginate scaffold have shown promising results in the form odontoblast like cells and calcification similar to tooth calcifications i.e. hydroxyapatite [18,19].

Hyaluronic acid

It can be chemically and structurally modified in various tooth tissue engineering applications because it's excellent biocompatibility and biodegradability. But it cannot be used alone as a scaffold and has to be combined with growth factors for development of pulp with sound dentin.

Peptides

Because of their nano scaled topology, peptides can be readily and easily incorporated into either organic or inorganic constituents and a new type of nano materials can be manufactured. Inter protein interactions and protein folding are the mainstay of assembling peptides. The basic reason for the self-assembling property of peptides is the core ABA motif of which they are made up of. The central B block consisting of both hydrophobic and hydrophilic molecules in alternate fashion provides the main thrust for self-assembly. In water, they assemble on opposing sides of the peptide backbone, and dimers form because of the tendency of the hydrophilic residues to shield from the water. These dimers string together and form sandwich- like β-sheet nanofibers, 6 nm wide and 2 nm high, where hydrogen bonding occurs along the fibre axis[20]. The flanking region is made up of charged residues, which make the molecules water-soluble and offer the possibility of cross-linking via oppositely charged ions. Since the peptide chains are made of naturally occurring amino acids, the resulting materials are non-immunogenic and biodegradable.

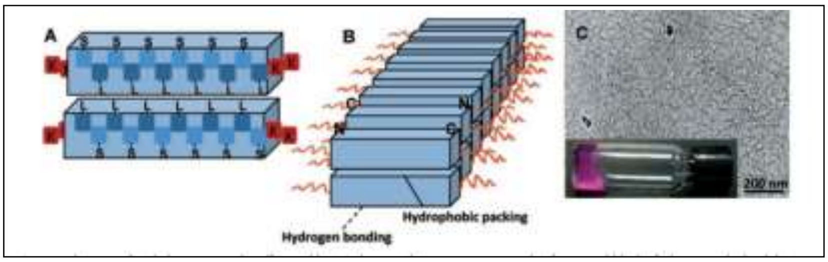

Fig-1: Schematic of multidomain peptide self-assembly. [A] The peptide monomers are made of a central block of alternating hydrophilic (S = serine) and hydrophobic (L = leucine) residues. [B] Dimers form because of the tendency of the hydrophobic amino acids to shield from the water. Positively charged residues (K = lysine) provide water solubility and aid in cross-linking and stabilization of the nanofibers after the addition of negatively charged ions, such as phosphate. The dimers string together, forming a nanofiber, which is stabilized by hydrogen bonding along the fiber axis. N: N-terminus. C: C-terminus. [C] The TEM image shows the nanofibrous network, macroscopically, and gelation is observed [34].

Ceramic scaffolds

Dentin has mineralised phase and a soft hydrogel phase made up of type I collagen. Because of the same composition of calcium sulfate salts and inorganic components of dentin, these can provide some additional advantages in regenerative endodontic therapy. The literature suggests that the practice of hydroxyapatite (HA) scaffolds are effective for regeneration of dentin or a dentin—pulp complex [21,22]. When hDFSCs were seeded on the Synthetic HA scaffold (ENGIpore©) and incubated for 6 weeks under ideal conditions an intense attachment and colonization of polygonal-shaped cells to the HA scaffold was observed and there was the presence of dense material around the cell cluster, which was similar to dentin.

Synthetic polymers

Higher mechanical strength and flexibility in comparison to biological scaffolds have led to the wide spread use of synthetic scaffolds in regenerative medicine. Experiments by Young et al have shown that tooth tissue similar to the natural tooth can be manufactured red by seeding HDPSCs onto biodegradable polyglycolate/ poly-L-lactate (PGA/ PLLA) [23-26]. Another interesting research reported that when stem cells from human exfoliated deciduous teeth were seeded into a synthetic open cell construct made from D, D-L, and L-polylactic acid led to regeneration of pulp tissue scaffolds.

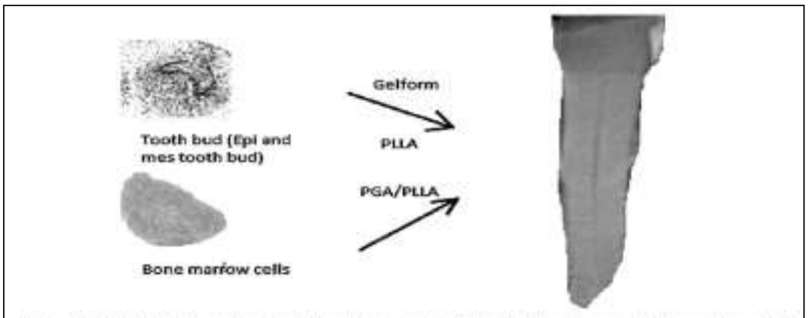

Illustration of Hybrid tooth construction. Abbreviations: epi, dental epithelial cells; mes, dental mesenchymal cells

Fig-2: Hybrid tooth construction Epi: dental epithelial cells, Mes: dental mesenchymal cells.

Hydrogels

Self-assembling peptides or hydrogels have visco elastic properties similar to that connective tissue. They allow fast diffusion of nutrients and metabolites and the possibility of homogenous cell encapsulation. The use of multidomain peptides is furthermore advantageous because of an automated synthesis process, high control over the material properties, a wide range of chemical functionalities, and the possibility for tailoring toward specific applications. Single amino acids serve as building blocks which can be used in a modular fashion, and the peptide sequence determines the properties of the resulting material. Bioactive motifs can already be incorporated into these systems during the synthesis process.

Ideal requirements of scaffold
Biodegradability

When the multiple domain peptides (MDP's) get organised into βsheets, they tend to have poor water solubility and the fibre process reminiscent raises concerns that this material cannot be biodegraded and can lead to amyloid accumulation and probably leading to Alzheimer's disease. Therefore in order to overcome this problem a proteolytic degradation was programmed into the MDP's in the form of hexapeptide, MMP-2 cleavage motif LRG. This intervention allowed the degradability of the MDP's. Therefore Hydrogels offer a unique property of customisation as far as biodegradability is concern.

Cell matrix interactions

Cell adhesion, can be initiated by various short peptide motifs, which closely resemble ligands on molecules abundant in natural ECM. These short peptide motifs can be incorporated into self-assembling peptides during synthesis process. Integrin-binding tripeptide RGD [27], REDV [28], PHSRN [29], or KNEED [30] etc. are the sequences which have been derived from the fibronectin. Similar motifs can be found in laminin [31] and collagen[32]. Because of this indirect method of binding, even growth factors can be incorporated into these self-assembling peptides.

Viscoelastic Properties

Physiologically cells tend to migrate from softer to stiffer environment. They maximum motility in the matrix with intermediate stiffness. Having MDP's with a range of matrix moduli would be of great advantage, since this might provide another means of controlling cell behaviour. Works of Dr. Hartgerink's laboratory demonstrated how the mechanical properties of MDP hydrogels can be varied through the modification of the peptides' chemical functionality [33]. Relatively minor changes in fibre surface chemistry (changing amino acid residues in the central block motif and/or the flanking region) resulted in changes in matrix rigidities.

CONCLUSION

Self-assembling peptides hold a great promising future as far as regenerative endodontics and creation of "Bio Tooth" is concerned. Customisation, such as incorporation of antibacterial substances, interaction and growth factors, all pave a great way for the regenerative endodontics. With the recent results from transplantation experiments, optimized scaffolding systems might contribute toward the development of therapeutic strategies for regenerative endodontic approaches in the near future.

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