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Three-Dimensional Printing and Biomaterials for Periodontal Regeneration

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Abstract: *Periodontal regenerative therapy results in the augmentation of the attachment and bone level in addition to reduction in pocket depth with minimal soft tissue recession. Various regenerative approaches such as GTR, bioresorbable grafts, 3-D printing scaffolds were developed, of which 3-D printing is the most innovative technology. In 3D printing, an ideal structure has been built layer by layer from CAD/digital 3D model. 3D scaffolds in the form of bone graft substitutes are found to be advantageous over commonly used grafting materials. Various biomaterials such as natural, synthetic, bioceramics and metals are used for preparing these scaffolds. Biomaterials can be combined to regenerate the complex structure of the bone-PDL-cementum apparatus by designing layered materials and cells to biomimetically regenerate periodontal structures completely and synchronously. New biomaterials are dramatically broadening the options for advanced periodontal regeneration therapeutics and various old biomaterials are being modified to elicit better biological and systemic responses.*

Key Word*: Biomaterials, Natural and synthetic biomaterials, 3D printing, Periodontics, Regeneration, Scaffolds*

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I. Introduction

Periodontal disease is a chronic inflammatory disease of periodontium and is characterized by progressive destruction of the periodontal ligament and alveolar bone, eventually leading to tooth loss. In support of the treatment for periodontally diseased tooth, along with the periodontal various regenerative approaches such as GTR, bone grafts, 3-D scaffolds were developed to achieve periodontal tissue formation.[1] However, the clinical outcomes of these approaches are variable and unpredictable. Therefore, challenges remain in the field of regeneration of periodontal apparatus with the formation of alveolar bone-periodontal ligament-cementum complex simultaneously.

The objectives of periodontal regenerative therapy is to augment the attachment and bone level in addition to reduction in pocket depth with minimal soft tissue recession.[2] 3D scaffolds in the form of bone graft substitutes are found to be advantageous over commonly used grafting materials.[3]They are biocompatible materials which can mimic the extracellular matrix of native bone and provide a 3-dimensional environment.[4,5] Various biomaterials are used for preparing these scaffolds. Each biomaterial has its specific chemical, physical and mechanical properties and provide a template for the reconstruction of the defects by effectively promoting stem cell proliferation and differentiation and guide new tissue formation.[6] New biomaterials are dramatically broadening the options for advanced periodontal regeneration therapeutics and various old biomaterials are being modified to elicit better biological and systemic responses.

II. Clinical application of 3-D printing in periodontics

The 3-D printing of scaffolds is a fascinating alternative to the traditional periodontal regenerative techniques. These scaffolds tend to create the architectural structure of the periodontal tissues and is made up of various materials such as natural or synthetic polymers, bioceramics or metals. 3-D printing is used in various aspects of periodontology such as periodontal repair and regeneration, socket preservation, sinus or bone augmentation, guided implant placement and in periimplant maintenance.

1. 3-D printed bioresorbable scaffold for GBR/GTR

3-D printed scaffold is a recent development in the field of tissue engineering. And these 3-D printed multiphasic scaffolds consist of both hard (i.e., bone and cementum) and soft tissues (such as PDL, gingiva) components of periodontium. Scaffolds will therefore promote the formation of cementum, PDL, bone and they also re-establish connection between them.

Injectable and absorbable scaffolds were developed for bone regeneration purposes of which calcium phosphate cement is most commonly used. And to establish vascularization, a tri-culture system was introduced by Zhang et al in 2017 that includes hiPSCs-MSCs, Human umbilical vein endothelial cells and pericytes. Xia Chen et al in 2018 used gold nanoparticles to be incorporated into the CPCs and found out that it improved cell adhesion, proliferation and osteogenic induction on CPC. Kämmerer et al in 2017 used Collagen Scaffolds, Growth Factors, and Periodontal Ligament Stem Cells for treatment of peri-implant bone defects in vivo and found that it will lead to an enhancement of peri-implant bone growth.[7] Ossix® Volumax, a new collagen scaffold for GBR and GTR with improved volume maintaining features has the potential to augment thin tissue around implants, esthetic deficiencies and correct residual dehiscence after regenerative procedures.

2. Socket preservation

Following tooth loss, there is an anticipatory resorption of bone that leads to loss of height and width of the alveolar ridge. Socket preservation will help to compensate for resorption by reducing bone resorption and accelerating bone formation.

Araujo-Pires et al in 2015 investigated the novel PLGA/CaP scaffold (OsteoScaf™)in the healing of tooth extraction sockets in humans and the quantitative analysis of CBCT showed less bone resorption and also new bone tissue in direct apposition to the scaffold demonstrating its osteoconductive nature.[8] Wang et al in 2013 used adrenomedullin delivery in microsphere-scaffold composite for remodelling of the alveolar bone following tooth extraction and this study suggest that local application of ADM has the potential to preserve the residual alveolar ridge and accelerate the alveolar bone remodeling.[9] Nie et al in 2019 used nano-hydroxyapatite mineralized silk fibroin porous scaffold for tooth extraction site preservation. The study demonstrated the effect of nHA inorganic crystals on osteogenic differentiation of MC3T3-E1 cells which indicates that the MSF scaffolds improved osteogenesis and reduced the height of alveolar bone resorption after tooth extraction.[10] Park et al in 2017 evaluated 3D-printed polycaprolactone scaffold combined with β-TCP powder for alveolar bone augmentation in beagles defect model and the results indicated the potential of 3D-printed porous PCL scaffolds to promote alveolar bone regeneration for healing of defects.[11]

3. Sinus and bone augmentation

The main advantage of 3-D printing is sinus and bone augmentation as their ability to replicate the bony architecture and will form microporous internal structure of graft.

Giardino in 2006 used a non-porous poly-DL-lactide tubular chamber filled by demineralised bone matrix (DBM) and bone marrow stromal cells (BMSC) in combination, as a scaffold for guided bone regeneration (GBR) in an experimental model using the rabbit and acquired favourable results.[12] Shin et al in 2004 conducted a study to assess bone formation from mesenchymal stem cells (MSCs) on a novel nanofibrous scaffold in a rat model in which a highly porous, degradable poly(*e*-caprolactone) (PCL)scaffold with an extracellular matrix-like topography was used and determined adequate mineralization and type I collagen deposition.[13] Xu and co-workers used polylactic acid nanofibers scaffold with chitosan for bone tissue engineering and found out that it enhanced the mineralization ability, and made them more beneficial for the attachment and growth of cells.[14]

D'Alessandro et al in 2016 histologically studied bovine bone matrix/poly(L-lactic-*co*-ε caprolactone)/gelatin hybrid scaffold (SmartBone®) for maxillary sinus augmentation and the data indicates that SmartBone® is biocompatibility, non-immunogenic, osteoconductive, promotes fast bone regeneration, leading to mature bone formation in about 7 months.[15] Shayesteh et al in 2008 used human mesenchymal stem cells loaded into a β-tricalcium phosphate/hydroxyapatite scaffold in sinus augmentation procedures and the clinical and histological findings suggest that it may enhance bone formation in the maxillary sinus area.[16]

4. 3-D printing for implant placement

With increase in implant placement, in account with various studies, there is also an increase in the complications related to that. 3-D printed surgical guides will enhance the guided implant placement thereby preventing many complications.

Bae et al in 2017 developed and assessed 3D-printed scaffold with rhBMP-2 for an implant surgical guide stent and bone graft material simultaneously.[17] Falisi et al in 2013 used 3D cartilage scaffolds for the stabilization of implants and bone regeneration with the fit-lock technique and the values of implant stability increase progressively over time.[18]

Almansoori and co-workers in 2021 used mesenchymal stem cells and platelet-rich plasma-impregnated polycaprolactone-β tricalcium phosphate bio-scaffold around dental implants and found out that PCL-TCP scaffold is compatible for regeneration of bone in defects around dental implants and also it improved implant stability.[19]

III. Biomaterials used for 3-D scaffolds

3-D scaffold properties are influenced by various factors including the constituent biomaterials. Further, the choice of biomaterials will affect the adhesion, cell proliferation and regeneration outcome. The first biomaterial used for clinical applications are natural polymers such as proteins and polysaccharides. Recently many research studies have come forward to develop a variety of novel biomaterials and composites with enhanced properties. The scaffold materials can be made up of natural or synthetic materials, bioceramics and metals.

1.Natural polymers

Natural polymers consist of collagen, glycosaminoglycans and proteoglycans. They have a very good biocompatibility, hydrophilic properties and good biodegradation and also possess good cell recognition and improves cell interaction with surrounding tissues whereas it lacks bioactivity, which is a desirable factor in hard tissue formation[4]. So to overcome these limitations, natural polymers are combined with more bioactive materials (such as bioceramics) or synthetic polymers/ metals which are mechanically strong.[20] Collagen and chitosan are the most commonly studied natural biomaterials for periodontal regeneration.

a) Collagen

It is the most commonly expressed protein and in tissue engineering, it serves as an important biomaterial. It has good biocompatibility with no antigenic potential and promotes cell proliferation and wound healing. Perhaps because of its poor mechanical strength and fast degradation rate , various studies on collagen modification has been carried out.[21] Collagen scaffolds with greater mechanical strength can be formed by cross-linking with certain molecules such as formaldehyde, glutaraldehyde, polyepoxy compounds or carbodiimides[22,23] or with inorganic molecules.[24] Lee et al., in 2008 studied biodegradable polymer coated collagen. He also developed nano-HA collagen/poly(L-lactide) scaffold and found out that it enhances cell proliferative viability and bone regeneration. Due to its size effects and surface phenomena at the nanoscale, nanoHA possessed unique properties such as high surface-to-volume ratios, biomimetic morphologies and reactivities, which make it more favourable in bone tissue engineering.[25,26] Kato et al., in 2015 modified collagen by adding BMP-2 and noticed an improvement in periodontal attachment without ankylosis.[27] Nakamura in 2019 introduced collagen sheet with FGF-2 which inhibited epithelial downgrowth into the periodontal defect and also enhanced regeneration.[28] Shimauchi et al in 2013 investigated the effect of nano-HA on BMP-2 expression in human PDL cells and found out that Nano-HA selectively increased the expression of BMP-2 in dose- and time-dependent manner. [29]

Scaffolds can also be designed to release growth factors that induce cellular differentiation and tissue growth in vitro or cell migration into the wound site in vivo. For example, by using resorbable collagen sponge surgical implanted with rhBMP-2, there is an enhancement of bone regeneration in intrabony periodontal defects and collagen scaffolds loaded with BMP-2 and=or 7 have also been successfully used in applications to induce bone formation, which has led to the development of Food and Drug Administration–approved collagen-BMP products for the treatment of such bone defects. The combination of rhBMP-2 delivered in an absorbable type I collagen sponge was approved by the Food and Drug Administration in 2004 as INFUSE Bone by the European Union in 2002 as InductOs .At present, in a particulate bone-derived type I collagen matrix osteogenic protein-1 (OP-1) delivered and is available in the United States and the European Union as OP-1 Implant.[30]

b) Chitosan

Chitosan because of its superior properties such as good biocompatibility, biodegradability, non-immunogenicity and anti-microbial properties it has been widely used in periodontal regeneration procedures. It also minimizes scaffold contamination thereby preventing postoperative infections, and failure of scaffold.[20] Shen et al in 2018 combined chitosan nanoparticles with PLA nanofibers and noticed improved hydrophilicity and mechanical properties over PLA alone.[31] Another study by Liao et al in 2020 combined chitosan with hydroxyapatite and amelogenin scaffolds and observed an enhanced antibacterial activity.[32]

Porous chitosan scaffolds were prepared through a freeze-drying process and loaded with an adenoviral (Ad) vector encoding BMP-7 and the results showed that the scaffold containing Ad-BMP-7 exhibited higher alkaline phosphatase activity and that expression of osteopontin and bone sialoprotein were up-regulated. After implantation into the periodontal or peri-implant defects, bone formation in Ad-BMP-7 scaffolds was greater than that in other scaffolds at 4 or 8 weeks, demonstrating the potential of chitosan scaffolds combined with adbmp-7 in bone tissue engineering.

2) Bioceramics

Bioceramics due to its excellent biocompatibility, hydrophilic properties and their bioactivity, is considered as the material of choice for bone reconstruction. The main advantage of bioceramics scaffolds is their osteoconductive and osteoinductive properties. But the slow degradation rate of ceramic is disadvantageous for periodontal regeneration.[33]

Hydroxyapatite and β-TCP are the two main biodegradable bioceramics that have been widely used as scaffold for bone regeneration.[33] HA shares the same biochemical composition as bone tissue, and allows adhesion and proliferation of osteoblasts, but it is resorbed very slowly in vivo. Tricalcium phosphate β (β-TCP) induces the formation of strong bond between bone and calcium phosphate, with higher rate of resorption. The two-phase ceramic (BCP) is produced by the combination of HA and β -TCP. Its properties include control of its bioactivity, a very good stability and allows the induction of bone growth especially in very large defects. And in addition, its degradation rate can also be controlled.[4] In an *in vitro* study by Casthilho et al in 2014, he reported that BCP showed good cytocompatibility with significantly higher osteoblastic cell viability and cell proliferation levels than in pure TCP scaffolds. Zhong et al in 2014 prepared TCP/CS scaffolds and noticed that the prepared TCP/CS scaffolds promotes significantly higher cell proliferation of human periodontal ligament (PDL) than pure CS scaffold.[34] According to a 6-month study by. Abdal-Wahab, et al. in 2020 β-TCP combined with BMP-2 and PDGF applied with collagen membrane had high clinical attachment level and radiographic bone gain.[35] Scaffolds containing nano-HA offers a new approach for inducing periodontal cell differentiation. Nano-HA increases the protein synthesis of PDL cells and improves the activity of alkaline phosphatase, induces cell differentiation, effectively promotes periodontal tissue regeneration and formation of new teeth attachments. Whereas Ogawa et al in 2016 introduced scaffolds composed of nano β-TCP combined with FGF-2 which showed enhanced cell infiltration and periodontal hard tissue regeneration as compared to collagen scaffold.[36]

Another most studied bioceramics are bioactive glasses (BG), made of silicone oxide and substituted calcium which in contact with biological fluids, a layer of calcium phosphate is formed on the surface of the bioglass which allows chemical bonding with the surrounding bone. It can be combined with both hard and soft tissue scaffolds and it also has a slow rate of degradation as it sets to a converted HAP like material.[20] Inspite of all the qualities of bioceramics, they have a low mechanical strength and fracture resistance so they are often too brittle to form reliable 3D structure with desired shape and dimension. But a recent study by Tarafder et al and S. Bose et al in 2012 enabled a 3D printing of bioceramics into patient-specific anatomic shape and dimension of scaffolds.[37,38]However, at the sites that require high mechanical stability for longer duration, the application of bioceramics are limited because high temperature/pressure-based fabrication process often shrink the 3D structure.

3) Synthetic polymers

Synthetic polymers have been predominantly used for scaffolds materials to replace the non-resorbable membrane-PTFE. It can be of two types-Degradable and Non-degradable.[6] **Degradable** polymers include polyesters, polyorthoesters, polylactones, polycarbonates, polyanhydrides, polyphosphazenes, etc., whereas **Non-degradable** polymers being used are PE, PTFE, PMA, PAA, PU, polyether, polysiloxanes, etc. The most used materials are aliphatic polyethers such as polycaprolactone, polylactic acid, polyglycolic acid and their co-polymer poly(lactic-co-glycolic) (PLGA). [4]

Polycaprolactone

It is the most widely used aliphatic polyether in the medical field and in especially in craniofacial repair over the past thirty years. Because of its interesting properties, it is usable for many 3D printing techniques. It is biocompatible with a very long resorption time and a high mechanical resistance. It has low melting temperature of ~60°C and rapid solidification due to its semi-crystallinity so that it can be used for temperature based-printing technique. But polycaprolactone is hydrophobic, with lower cellular affinity, a decrease in cellular responses and surface interactions. Although they lack bioactivity, aliphatic ethers are more advantageous because of its moldable characteristic during manufacture and have good mechanical properties.[39,40]

As most of the synthetic polymers are hydrophilic, various specific modification have been performed to improve cell-scaffold interaction. Polymers can be biofunctionalized in two different ways- pre-polymerization functionalization and post-polymerization functionalization.[41,42]

Various synthetic polymeric scaffolds have been investigated for periodontal regeneration. PLGA/PCL composite scaffolds with FGF-2 and bone marrow mesenchymal stem cells (MSCs) resulted in improved periodontal tissue healing by 6 weeks *in vivo* in a rat model.[43] A multi-phase composite scaffolds consisted of micro-patterned PCL/PLGA for PDL and amorphous PCL for bone with PDGF-BB and BMP-7 for delivery from the scaffold showed enhanced regeneration of bone-periodontal ligament interface in a rat fenestration defect model.[44]

4) Metals

As first-generation materials for bone substitutes, metallic 3D scaffolds are commonly used for load-bearing areas compared with ceramics or polymers because of their high mechanical strength, fatigue resistance, and printing processability. Commonly used metallic biomaterials in 3-D printed scaffolds include titanium, stainless steels, cobalt-chromium (Co–Cr) based alloys, and magnesium (Mg). The size of scaffold can also be enhanced by metals to improve the mechanical properties. But metallic biomaterials possess certain disadvantages. The first main one is their lack of biological recognition on the material surface and to overcome this surface modification processes can be carried out.[45] Another main limitation is the possible release of toxic metallic corrosion by-products that can cause inflammatory and allergic reactions which can eventually lead to biocompatibility loss and cause tissue loss. To avoid this a proper treatment of the material surface should be performed which also helps to create a direct bonding with the tissue.[46]

a) Titanium and its alloys

These are commonly used metal alloy on the basis of good biocompatibility, mechanical properties and elasticity. In an optimal situation, it can osseointegrate with bone.[47] In addition, it forms a very stable passive layer of TiO_2 on its surface which is responsible for its superior biocompatibility. But it has a major disadvantage of nondegradability which requires to be removed. Porous Ti scaffolds with 3D architecture has its benefits such as vascularization, nutrient and gas transport, and cell seeding.[48]

Titanium-aluminium-vanadium alloys have better mechanical properties than commercially pure titanium (cp Ti) and is most commonly used. However, because of toxic elements such as vanadium concerns arises and these have led to the development of new beta titanium alloys which contains nontoxic alloying elements like Ta, Nb, Zr.[49]

Further by the introduction of second-generation titanium alloys including Ti-15Mo-5Zr-3Al, Ti-15Zr-4Nb-2Ta-0.2Pd, Ti-12Mo-6Zr-2Fe, Ti-15Mo-3Nb-3O and Ti-29Nb-13Ta-4.6Zr there is enhancement of biocompatibility.

In order to determine the optimum pore size, Yang et al. in 2016 fabricated screw shaped Ti6Al4V dental implant prototypes by laser beam melting (LBM) with three controlled pore sizes (200, 350 and 500 μm) and showed improved attachment, proliferation and differentiation on both 350 and 500 μm pore size implants. When EBM processing was used, Ti6Al4V scaffolds have better anti-corrosion ability with reduced precipitates of harmful Al and V ions compared to wrought scaffolds.[50]

Several studies have indicated that this material is suitable for supporting the growth and osteogenic expression of bone marrow cells. Various surface treatments can be done such as addition of fibronectin, collagen or calcium phosphate, to regulate the rate and amount of bone formation by implanted cells into titanium mesh scaffolds .[51,52]

b) Tantalum

Porous tantalum is a biomaterial with its specific physical and mechanical properties such as high-volume porosity (>80%) with fully interconnected pores (which allows secure and rapid bone in growth) and modulus of elasticity similar to native bone (which minimizes stress shielding).[53] This trabecular metal has been shown to be highly biocompatible in several animal models with studies supporting substantial cortical bone ingrowth between the trabecular network as well as high levels of bone growth onto the scaffold itself. Further, it offers better osteoconduction than other technologies used for biological fixation. Durham S.R. *et al.* in 2003 done a study by implanting tantalum mesh for the repair of large cranial defects into 8 patients and found out that tantalum mesh used with HA cement and fixed with Ti plates provided internal structural support and also increased the stability of the construct.[54]

c) Magnesium

In the past decade, magnesium and its alloys have been researched and found to be extremely appealing materials for orthopaedic applications with a great potential in bone tissue engineering because of their appropriate mechanical properties which is close to native bone and are also completely biodegradable which eliminates the need for a second surgery to retrieve the scaffold. [55] The corrosion by-products of Mg and its alloys are also biocompatible and do not elicit adverse reactions. They are osteoconductive, have a role in cell attachment, and tend to increase the expression of osteogenic markers in vitro.[56]

d) Nickel-titanium alloy (NITINOL)

Nitinol ,one of the most promising titanium implants possesses a mixture of novel properties such as shape memory effect (SME), enhanced biocompatibility, superplasticity, and high damping properties.[57] With the elastic modulus of the Nitinol and the compressive strength close to that of the bone and due its good biocompatibility porous NiTi have been used in the treatment of scoliosis.[58] Nitinol is highly biocompatible, more than stainless steels. However, due to the toxicity by the release of Ni ions, certain surface modifications such as oxidation treatment of NiTi can be performed to obtain a Ni-free surface and several alternative Ni-free shape memory alloys, mainly Nb-based.[59]

5) Synthetic hydrogels

Hydrogels are a new class of biomaterials that can be potentially injected into the periodontium. These biomaterials are composed of a viscous polymer made of synthetic or natural hydrophilic macromolecules that are able to form a hydrogel after physical, ionic, or covalent cross-linking.

Hydrogels are an appealing scaffold material because of their structural similarity to the extracellular matrix of many tissues. These hydrogels have been utilized as scaffold materials for drug and growth factor delivery, engineering tissue replacements, and a variety of other applications.[60] A variety of synthetic materials may be used to form hydrogels for tissue engineering scaffolds. These materials include poly(ethylene oxide) (PEO), poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), poly(propylene furmarate-co-ethylene glycol) (P(PF-co-EG)), and polypeptides.[61] It can be reproducibly produced with specific molecular weights, block structures, degradable properties, and crosslinking modes which in turn determine gel formation dynamics, crosslinking density, and material mechanical and degradation properties.

PEO is currently FDA approved for several applications in medicine and is one of the most commonly applied synthetic hydrogel polymers for tissue engineering. PEO and poly(ethylene glycol) (PEG) are hydrophilic polymers that can be photocrosslinked by modifying each end of the polymer with either acrylates or methacrylates. This modified PEO or PEG is mixed with the appropriate photoinitiator and crosslinked via UV exposure to form the hydrogel.[62]

Another synthetic hydrophilic polymer widely used in space filling and drug delivery applications is poly(vinyl alcohol) (PVA). It can be physically crosslinked with aqueous polymer solutions or chemically crosslinked with glutaraldehyde, succinyl chloride, adipoyl chloride, and sebacoyl chloride to form hydrogels. A newer synthetic hydrogel copolymer, P(PFco-EG) has been created for use as an injectable carrier for bone and blood vessel engineering.[63]

In addition, it is possible to construct thermally reversible hydrogels from block copolymers of PEO and poly(l-lactic acid) (PLLA) and PEG and PLLA. And also, degradable PEO and PEG hydrogels have been formed by synthesizing copolymers containing hydrolytically degradable poly(lactic acid) (PLA) and enzyme specific cleavage sequences of oligopeptides.[64]

6) Hybrid materials

With respect to all the previously described biomaterials used for scaffolds, each one has its own advantages and individual limitations. So, in order to produce a “synergistic effect” in the overall resulting properties and improve the mechanical, biological, and degradation properties of a scaffold, two or more different biomaterials can be combined. These scaffolds are referred to as “composite” or “hybrid” scaffolds. Many combinations of materials and surface modifications are investigated to stimulate desirable specific responses at the molecular level. It was shown that the synergistic combination of two types of materials can produce new structures that possess novel properties.[46] Composite scaffolds used for tissue engineering applications can be “polymer/ceramic,” “ceramic/metal,” or “polymer/metal.” However, various literature data confirms that composite scaffolds support attachment, proliferation, and differentiation of osteoblasts while maintaining the final shape of newly formed bone.

Recently, metal-ceramic-polymer hybrid materials have also been used for the fabrication of load-bearing scaffolds. Composite scaffolds have been used in many clinical cases and proved necessary results in the reconstruction of structural diseases and bone defects. But the porous polymer/ceramic composites cannot satisfy the requirements for hard tissue repair.[65] For example, scaffolds made with HA or tricalcium phosphates (TCP) are very stiff and brittle and they have different mechanical and viscoelastic properties from bone.[66] Hybrid constructs of porous Ti/TCP ceramic and cells have been investigated and have demonstrated better osteogenic properties compared with Ti scaffold alone.[67]

Polymer/Ceramics

Polymer/ceramic based scaffolds have been studied by many researchers in the past years. Kaplan and co-workers in 2008 investigated the combinations of calcium phosphate/polyesters for scaffold production and developed a scaffold from silk fibroin-polyaspartic acid coated with calcium phosphate.[68] This study showed cell-viability, proliferation and osteogenic differentiation. Li et al. in 2009 used two polymers, PLGA and PCL, with a layer of calcium phosphate and a gelatin coating and found that MC3T3 cells got adhered to the regions with higher calcium phosphate content along the scaffold, indicating that the mineralization gradient affects the adhesion/proliferation of cells and physical properties of the scaffold.[69] Osyczka and co-worker in 2011 developed a 3D scaffold of PLGA along with bioactive glass from Silica and Calcium.[70]The results showed good mechanical properties for both bioglass types. Other studies have investigated the use of α and β-Chitin hydrogel/nanobioactive glass ceramic/nanosilver composite scaffold for periodontal regeneration and antibacterial activity and this composite scaffold showed characteristics such as antibacterial activity, bioactivity and controlled degradation.

Metal/Ceramic

Various studies have reported the development of metal/ceramic scaffolds, which have been shown to possess favourable characteristics, regarding mechanical properties and bioactivity including cell attachment, proliferation, and differentiation). Yang and co-workers in 2009 developed a biodegradable and bioactive scaffold composed of magnesium with a coating of β-TCP.[71] *In vitro* results of this study showed good cell adhesion and bioactivity. In similar studies, when tantalum and titanium were used, osteoconductivity and ostoeinductivity was improved.[72] Lee et al in 2012 fabricated a scaffold composed of (biphasic calcium phosphate) ZrO2 and PCL layers and it was also shown that PCL incorporated into BCP gave scaffolds high biodegradability, cell attachment and proliferation.[73] Boccaccini and co-workers in 2015 synthesized and characterized a new boron-containing bioactive glass-based scaffold coated with alginate cross-linked with copper ions and found out that the coated scaffolds exhibited bioactivity comparable to the uncoated one.[74]

Recent studies by Dimitriesvska et al in 2011 using porous ceramic coated TiO2 as scaffolds demonstrated adhesion, growth and osteoblastic differentiation of hMSCs in the TiO2-HA nanocomposite; however, applications in bone implants are limited because of their bioinertness.[75]

Metal/Polymers

Chemical and physical properties may influence the osteointegration of implant surface, allowing protein adsorption between implanted biomaterials and the biological environment. Thus, various strategies are put forward to create a bond between the implant and the living host tissue. Recently, metallic implants with polymer coating have been used for many for these purposes.

Lagoa et al. developed a partially biodegradable implant from titanium, polylactide, HA, and calcium carbonate and concluded that the implant presented mechanical stability, biocompatibility, and partial biodegradability. [76] Helary and co-workers in 2009 have used poly(sodium styrene sulfonate) polyNaSS on oxidized and grafted Ti samples and poly- NaSS/(Methylacrylic acid) MA grafted onto Ti6Al4V alloy surfaces.[77] They reported that cell adhesion and differentiation on grafted Ti was higher than on oxidized titanium and titanium due to the presence of active sites that interact with extracellular proteins. Oughlis et al. in 2011 developed a scaffold from polyNaSS polymer and titanium. It showed cell viability, proliferation and osteoblastic differentiation of hMSCs on this scaffold.

IV. Conclusion

Periodontal regeneration has evolved through various different concepts, philosophies, and biomaterials. To repair the periodontal defects, advanced methodologies such as GTR/GBR, tissue engineering, 3-D printing have been evolved which helps to replicate the multi-layer architecture of the native tissue.[78] 3D printing technology has been used in various fields including 3D‑ printed scaffold for socket preservation, periodontal repair and regeneration, sinus and bone augmentation, peri‑ implant maintenance, and implant education. 3D‑ printed scaffolds show predictable outcome in periodontal regeneration and also in implant placement using 3D printing surgical template, which increases the accuracy and reduces the incidences of complication.[4]

Various novel biomaterials are used to prepare these 3-D scaffolds.[68] New researches on the novel scaffold biomaterials have the potential to greatly enhance the periodontal bone-ligament-cementum regeneration effectively, as well as it also have an impact on other areas of tissue engineering and regenerative medicine.[80]To promote further development of tissue engineering and clinical applicability and availability of the novel biomaterials, it is important to optimize fabrication and application techniques that provides satisfactory interaction and limitation of biologic functions. So, in the future it will become increasingly important to consider the concepts of scaffolds and the various biomaterials used to prepare these scaffolds which not only regenerate the complex structure of the bone-PDL-cementum apparatus but also will be biocompatible and elicits appropriate tissue reactions.[52]

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