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REVIEW ARTICLE

Scaffold Selection for Tissue Engineering in Dentistry

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ABSTRAK

Kejuruteraan tisu dijalankan untuk membaiki dan memulihkan tisu yang mengalami kerosakan atau dijangkiti penyakit yang seterusnya menyebabkan kecacatan menggunakan produk yang dicipta secara teknikal. Kemajuan dalam kejuruteraan tisu telah menjanjikan peluang pendekatan klinikal yang lebih baik dalam rawatan pesakit pergigian, terutamanya dalam bidang regenerasi endodontik, tulang dan tisu periodontal serta penjanaan semula keseluruhan gigi. Ulasan ini merumuskan kriteria pemilihan perancah yang ideal serta menpunyai potensi dalam kejuruteraan tisu bagi bidang pergigian. Sifat biokimia dan fizikal serta pendekatan dalam pembuatan perancah yang berkaitan dengan kriteria pemilihan perancah yang ideal untuk kejuruteraan tisu bagi bidang pergigian turut dibincangkan dalam ulasan ini. Ulasan ini juga membincangkan aplikasi utama kejuruteraan tisu dalam bidang pergigian, seterusnya mewujudkan paradigma untuk kajian penjanaan semula tisu tulang pada masa hadapan menggunakan sel dan perancah yang tertentu sebagai rawatan alternatif dalam pergigian.

Kata kunci: bahan bioserasi, kejuruteraan tisu, pergigian, sel stem

ABSTRACT

Tissue engineering aims to restore lost, damaged, diseased or defective tissues in the human body using engineered or regenerated products. The advancement of tissue engineering has given a promising opportunity for better clinical practice in treating dental patients especially in the fields of endodontic, bone and periodontal tissue as well as whole tooth regeneration. In this review, we briefly summarise the

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possible selection criteria of scaffolds for potential tissue engineering applications in dentistry. Biochemical and physical properties, as well as scaffolding approaches involved in the selection of an ideal scaffold for dental tissue engineering, are also discussed in this review. This review also discussed major applications of tissue engineering in the dentistry field, which can create a paradigm for future studies of tissue regeneration by using selected cells and scaffolds as an alternative treatment in dentistry.

Keywords: biocompatible material, dentistry, stem cells, tissue engineering

INTRODUCTION

The field of tissue engineering has dramatically evolved in the past decades, offering a potential paradigm shift in the current management of diseases involving tissues and organs of the human body and ultimately improve the patient's quality of life. In the dental field, a possible area of implementing tissue engineering is coming to the fore: regenerative endodontics, regeneration of bone for a bony defect, periodontium composed of complex cementum, periodontal ligament tissues and alveolar bone and regeneration of the whole tooth.

Generally, the triad of stem cells-scaffolds-growth factor plays an important role in the success of tissue engineering and regenerative medicine. The stem cells can either be of dental or non-dental origin. Stem cells from bone marrow, adipose tissue, and induced pluripotent stem cells are among non-dental origin widely used in tissue engineering (Chieruzzi et al. 2016; Ude et al. 2018). Dental stem cells are more of interest due to their affinity with target tissues. They have been categorized into three main groups based on their embryonic origin (Chieruzzi et al. 2016; Sharpe 2016): pluripotent stem cells, mesenchymal stem cells, and epithelial stem cells. Pluripotent stem cells, for instance, is dental pulp pluripotent stem cells (DPPSC). Mesenchymal stem cells consist of dental pulp stem cells (DPSC), stem cells from human exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP), and stem cells from periodontal ligament (PDLSC). The cells, which reside in the developing tooth germ, oral epithelium, and salivary gland appear to be in the group of epithelial stem cells.

Advances in the field of cellular and molecular biology have allowed the exploration of growth factor functions and their participation in the regenerative approach. Several commonly used biological mediators are intended to induce and accelerate cell growth in dental tissue engineering, such as (Chieruzzi et al. 2016) i.e. (i) Periodontal regeneration: fibroblast growth factor-2, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF); (ii) Pulp-dentin complex: Stromal

cell-derived factor-1, basic fibroblast growth factor, PDGF, stem cells factor

Scaffolds are biomaterials, matrices, or constructs (Chen & Liu 2016) that act as artificial frameworks to guide the growth of intended tissues. In the past few decades, biomaterial for biomedical application has progressed significantly. This review highlights various scaffolds that previous researchers have explored for tissue engineering in dentistry.

SCAFFOLDS IN TISSUE ENGINEERING

A scaffold is a biomaterial that provides an environment that allows implanted cells to proliferate, differentiate, and form the intended tissue or organ (Chen & Liu 2016). It is designed to perform the following functions i.e. (i) promote interaction of cell-biomaterial state, cell adhesion and deposition of extracellular matrix (ECM); (ii) allow transportation of nutrients, gases, and factors for a cell to survive, proliferate and differentiate; (iii) able to biodegrade at a controllable rate with tissue regeneration; and also (iv) exhibit a minimal degree of inflammation. It can be classified according to their structural, chemical and biological characteristics (Chen & Liu 2016). In general, scaffolds are divided into three groups; natural polymers, synthetic polymers, and bioceramics.

Natural polymer scaffolds enhance the performance of cells in the biological environment since they have better interaction due to their bioactive properties (Proksch & Galler 2018). Natural scaffolds can be obtained from natural sources. Natural scaffolds are categorised into proteins, polysaccharides or nucleic acids (DNA and RNA) (Proksch & Galler 2018; Kelly et al. 2019). Moreover, some natural scaffolds that are actively being used in dental tissue regeneration include proteins like collagen, gelatin, fibrin, and silk as well as polysaccharides like chitosan, hyaluronic acid, alginate, and agarose. These scaffolds usually have excellent biocompatibility for cell attachment and proliferation. Nevertheless, the use of this type of material for load-bearing areas is limited by its physical and mechanical instability (Proksch & Galler 2018).

Synthetic polymers have the advantages of being predictable and have reproducible physical and chemical properties (i.e. porosity, tensile strength, elastic modulus, degradation time). These types of scaffolds also exhibit physicochemical and mechanical properties comparable to biological tissues. They can also be manufactured under a controlled condition that allows production in a larger scale of uniform size and design, making them very useful for biomedical applications (Proksch & Galler 2018; Dorati et al. 2017). Examples of synthetic scaffolds are organic polymers like polylactic acid (PLA), polyglycolic acid (PGA), poly lactide-co-glycolic acid (PLGA), and polycaprolactone (PCL). PLGA and PCL are a few polymers that are commonly used for forming scaffolds in dental tissue regeneration.

Bioactive ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) are often associated

with insufficient biocompatibility and biodegradability, limiting the clinical use of this type of scaffold in tissue engineering (Chocholata et al. 2019). Researchers have overcome this issue by combining synthetic and natural polymers to enhance the aforementioned properties. This type of material is generally known as a composite. Composite materials include a polymer phase with toughness and compressive strength, and an inorganic phase with bioactivity, which improves the mechanical properties and degradation rate (Chocholata et al. 2019). Composite scaffolds of PLGA/HA, PLGA/TCP, PCL/PGA, and zirconia/HA are commonly used in dentistry.

Biochemical Property

Immunogenicity and biocompatibility

These issues usually relate to natural scaffolds since they are obtained from allogenic or xenogenic sources, which may be antigenic to the host and could cause the body to exhibit an immunologic reaction to the scaffold, an inflammatory response, and cytotoxicity to native cells, tissues, or organs. Reconstruction of the craniofacial defect using a xenograft scaffold usually results in disease transmission and stimulation of immunogenicity. Therefore, it is necessary that the scaffold used in the reconstruction of dental tissue exhibit minimal or avoid host immune responses. The immune-inert scaffolds concept was just recently implemented. These scaffolds have an immune-modulatory function that regulates the immune system (i.e., decreased natural killer cell activity and T-and-B-cell-mediated immunity) (Roseti et al. 2017).

Since the scaffold is expected to remain in the human body and lasts for some time, bi-products resulting from the degradation process of the scaffold should not produce any harmful material or element in the body. The scaffold should be able to biodegrade *in vivo* at a certain time that matches with the new matrix production of the developing tissue upon implantation (Nelms & Palmer 2019), with a controllable absorption rate that eventually provides space for new tissue generation (Yi et al. 2016). The degradation of the biomaterial should allow the intended tissue to generate, for instance in spinal fusion, with the requirement being after nine months or longer while the skull or maxillofacial bone required three to six months' degradation (Yi et al. 2016). Moreover, for pediatric patients with mandible defects, future growth of the mandible must be considered. In this case, fixation of the mandible using scaffolds without biodegradable properties prevent mandibular growth over time and could result in facial asymmetry and problems with occlusion as the patient grows (Kakarala et al. 2018). Therefore, recent criteria for ideal scaffold design and development require that scaffolds naturally degrade at an appropriate rate so that there is enough time for bone regeneration, and gradual absorbtion by the human body without generating any side effects.

Physical Property

Porosity (pore volume fraction of scaffold)

The scaffold should offer a void volume for neovascularization, new tissue formation, and remodelling to promote integration with the host tissue upon implantation (Iviglia et al. 2019). The scaffold should also have enough porosity for effective nutrient and metabolite transportation and exchange with the surrounding environments. A porous scaffold is essential i.e. i) in the proliferation and migration of new tissue formation and vascularization, ii) to assist mechanical interlocking between the scaffold and environment for stability, as well as iii) to facilitate, guide, and promote the formation of new tissues (Iviglia et al. 2019). However, the mechanical properties of scaffolds are inversely related to porosity. Hence, an adequate balance between porosity and mechanical properties should be considered when designing an ideal scaffold.

Pore size (pore diameter)

Pore size would have a direct impact on the functionality of the scaffold during application. For bone tissue engineering, the ideal pore size is still a subject of debate among researchers due to conflicting reports. For instance, a pore size >200 μm is reportedly required for osteoconduction and up to 500 μm for vascularization (Cheng et al. 2016). This is due to the nature of the cell-forming bone, osteoblast. As osteoblast is approximately 10-50 μm, in size, but scaffolds with larger pores sizes of 100-200 μm are preferred for the osteoblast to regenerate mineralised bone after implantation (Abbasi et al. 2020). This larger pore size permits macrophages to infiltrate, eliminate bacteria, and induce cells colonisation, migration, and vascularisation *in vivo* (Iviglia et al. 2019). Meanwhile, a smaller pore size of <100 μm reportedly promotes the formation of non-mineralised osteoid or fibrous tissue (Iviglia et al. 2019; Liu et al. 2018). Cheng et al. (2016) reported that scaffolds with a larger pore size caused a greater formation of mature bone by promoting vascularisation, these newly formed blood vessels supply sufficient oxygen and nutrients, thus promoting better osteoblastic activity. However, cell-seeded scaffolds with a pore size greater than 500 μm might be washed away during *in vivo* application. Hence, the appropriate pore size should be within the range of 200-500 μm for better differentiation and vascularization.

Interconnectivity

The interconnectivity feature is essential to enable cell migration and perfusion without a severe concentration gradient in the scaffold that may finally end up in cell death and tissue necrosis (Guda et al. 2014). Additionally, interconnectivity also provides space for cell metabolism (i.e. to nourish new bone and remove wastes) via vascular development. A lack of pore interconnection will result in a poor or low efficiency of changes in nutrients, gas, and waste within the

scaffolds. It is preferable that scaffolds for dental tissue engineering have a 100% interconnecting pore volume, thereby also maximizing the diffusion and exchange of nutrients throughout the entire scaffold pore volume.

Mechanical Property

The scaffolds used in dental tissue engineering should have mechanical properties consistent with the anatomical properties of the implanted place and should have a strong and good working ability with hand tools. Since a tooth is routinely subjected to mechanical loads, scaffolds selected for regenerating dental tissues must have adequate strength properties to support the applied loads. Moreover, sufficient mechanical strength is crucial to maintain cell integrity until new tissues form. Aside from affecting the cell behavior and differentiation potential, the porosity and pore sizes of the scaffolds also affect their mechanical properties. Intensive porosity and pore sizes may facilitate nutrient and oxygen delivery or enable more cell ingrowth, which may compromise the mechanical properties of the scaffold due to a large volume size (Farzin et al. 2019). Although the mechanical property of scaffolds is effected by intensive porosity or pore sizes, the use of materials with high inherent mechanical strength might be a solution to this issue. Moreover, it is important that the material property of the scaffolds matches the native tissue *in vivo*, especially for bone regeneration as new bone should withstand loadings to prevent stress shielding, comparable to the surrounding native bones. Thus, the mechanical property of the intended tissue to be grown should be taken into consideration when designing pore size and porosity, both of which should be incorporated into a scaffold.

SCAFFOLD DESIGN

Major scaffolding approaches have evolved these last few decades especially in the area of tissue engineering including pre-made porous scaffold, decellularised extracellular matrix (ECM), cell sheets with secreted ECM, cell encapsulated in self-assembled hydrogels, and rapid prototyping.

Pre-made Porous Scaffold

This type of scaffold is the most well-established and commonly used scaffold in tissue engineering (Mallick et al. 2015). Natural and synthetic scaffolds are used for manufacturing porous scaffolds. Since this approach relatively offers a precise design for tissue architecture and microstructure, the physicochemical properties are easily engineered to mimic the native ECM in the host tissue. This aids load-bearing tissues where the mechanical properties are important (Mallick et al. 2015; Johari et al. 2017). However, various efforts have been made to overcome the limitation of the cell's ability to penetrate the scaffold without increasing production cost or cell viability. Pre-made porous scaffolds have also has been applied in dentistry, mainly for the regeneration of

augmenting atrophic ridges. One of the greatest challenges facing successful ridge augmentation is to maintain the desired shape after soft tissue closure (D'Amato et al. 2015). Several studies have reported a high rate of bone resorption after the insertion of a pre-made porous scaffold in bone augmented ridges (Aboushelib & Shawky 2017; Berberi & Nader 2016).

Decellularised Extracellular Matrix

Acellular ECM is developed after the removal of the cellular component from allogenic or xenogenic tissue using a combination of physical, chemical, and enzymatic approaches while preserving the natural composition of the basic structural and functional ECM proteins. This approach is popularly used for tissue engineering involving heart valves, vessels, nerves, tendons, and ligaments (Parmaksiz et al. 2016). Scaffolds fabricated with this technique are assumed to have better immunologic properties as cellular antigens where the sources for immunogenic reaction have been removed and replaced with more natural mechanical and biological properties. Besides that, preserved growth factors in the decellularised matrix are also an advantage to facilitate further cell growth after implantation. In dentistry, this approach may work through a combination of acellular products with graft material for treatments of maxillofacial defects, the soft connective tissue of the mouth, and intra-oral mucosal damages (Parmaksiz et al. 2016). In a recent study performed with Alloderm®, an acellular collagen matrix derived from a decellularised human dermis, researchers reported significant improvement in gingival repair by the end of the nine-month observation period (Agarwal et al. 2015).

Cell Sheets with Secreted ECM

This scaffolding approach, using confluent cells coated on a culture dish, is harvested from thermos-responsive polymers without the use of scaffolds (Iwata et al. 2015). The advantage of this approach is the ability to keep ECM proteins and cell-cell interaction intact during implantation. Apart from that, this process can be repeated to form a thicker matrix with multiple laminations of single cells. A variety of cell sheets with secreted ECM are applied in tissue engineering, for instance, using the cell sheet as a source of 3 dimensional (3D) pellet, applying a multi-layered cell sheet, and using the cell sheet to wrap a scaffold (Paz et al. 2018). This approach has been applied for the regeneration of soft tissue such as cornea and myocardium in the biomedical area; with several attempts to produce periodontal apparatus to treat periodontitis in dentistry. The tissues rich in ECM for load-bearing purposes (bones and cartilage) are almost impossible to manufacture with this type of engineering, as the amount of secreted ECM is limited (Paz et al. 2018).

Cell Encapsulated in the Self-Assembled Hydrogel

Encapsulation is a process of entrapping

cells within a semi-permeable membrane or a homogenous solid mass (Kim et al. 2019). Hydrogels are the most commonly used materials for encapsulation; derived from either natural (algae, alginate, agarose, and chitosan) or synthetic (polyethylene glycol and polyvinyl alcohol) sources. Cell encapsulated hydrogel allows cells to retain a structurally supported scaffold for cell proliferation and subsequently degrade when the cells secrete ECM (Johari et al. 2017). This scaffolding approach is most commonly applied in engineering heart muscles, neural, and liver for biomedical applications. Meanwhile, the regeneration of new dental pulp to treat necrotic teeth has been actively applied in dentistry. For example, Gelfoam-encapsulated dental stem cells are actively applied for treatment to regenerate new dental pulp tissue (Kaur et al. 2016). The advantage of this approach is simple, yielding homogeneous cell distribution and enormous viability (Kim et al. 2019). Nevertheless, due to the poor mechanical properties of hydrogels, its application in tissues with load-bearing functions is rare.

Rapid Prototyping

This approach involves fabricating a scaffold directly from a scanned image and a computer model of the defect site (Yuan et al. 2017). Rapid prototyping approaches produce scaffolds that are structurally and mechanically precise to defect sites. The rapid prototyping approach has quickly gained popularity in bone tissue engineering for its high precision, reproducibility, and controllable pore structure. The term "3D printing", which is the most popular to the public, refers to rapid prototyping (Yuan et al. 2017). In 3D printing, scaffold materials are manufactured layer by layer to form a 3D model, thus enabling better control of a scaffold's physical properties. Many polymers are printable, for they often have proper melting ranges require to shape scaffolds. PLA, PCL, PLGA, and porous ceramic are types of scaffold materials usually fabricated using 3D printing. In dentistry, fabricating a scaffold for maxillofacial bone, a temporal mandibular joint disc, a tooth, or periodontal tissue are major applications of this approach (Yuan et al. 2017).

SCAFFOLD AND TISSUE ENGINEERING IN DENTISTRY

The emergence of tissue engineering in a multidisciplinary field sheds new light on the treatment of patients. In dentistry, tissue engineering offers the regeneration of non-dental tissues and dental tissues as well as their supporting structures. There are three main areas of tissue engineering that have been extensively studied for dental application: the regeneration of pulp-dentin complex, the regeneration of bone, and the regeneration of periodontal tissue.

Regeneration of Pulp-dentine Complex

Regenerative endodontics refers to "biologically-based procedures

designed to replace damaged structures, including dentin and root structures, as well as the cells of the pulp-dentin complex'' (Murray et al. 2007). This new treatment modality utilizes the concepts of tissue engineering to restore the canal system and surrounding tissue to a healthy state, thus allowing the root to continue to develop. Regeneration implies proper re-vascularisation and re-innervation of the pulp thus permitting the formation of new dentin (Mitsiadis et al. 2015).

It might be possible to treat pulpal necrosis by generating new dental pulp, in particular, the functional dentin-making odontoblast and vascular endothelial cells. One challenge of manufacturing scaffolds for this application is the small and enclosed space of the root canal environment. In the regeneration of a pulp-dentine complex, the scaffold should have a relatively fast setting time (Ajay Sharma et al. 2015). The use of a soft and injectable scaffold to engineer the pulp-dentine complex is an advantage due to its small size and the difficulty of reaching the receiving site (Chieruzzi et al. 2016). The Puramatrix™ hydrogel scaffold has been used successfully in a tooth slice model (Cavalcanti et al. 2013; Dissanayaka et al. 2015) and full-length human root canals (Rosa et al. 2013), as shown in Table 1. Since vascular endothelial cells are equally important as odontoblast in regenerative endodontics, Dissanayaka et al. (2015) utilized prevascularized PuraMatrix™ using human umbilical vein endothelial cells (HUVEC). This *in vivo* study showed promising histological results for both odontogenic and angiogenic processes. Despite the success, the prevascularised PuraMatrix™ showed limited ability to regenerate pulp-like tissue up to the middle (5 mm) to lower third (3.3 mm) of the root canal, as shown in Table 1. Thus, this method can only be applied for pulp regeneration in a tooth with an open apex. However, the biggest problem of utilizing HUVEC in pulp regeneration is the risk of an immunologic reaction to the host.

A recent of regenerative endodontics approach that has been successfully applied in a clinical setting is based on the bleeding technique, whereby the blood clot will act as a scaffold to deliver the stem cells into the root canal system. Platelet concentrates are also a source of growth factors that are essential in tissue engineering (Bakhtiar et al. 2017). The use of the first generation of platelet concentrates, platelet-rich plasma (PRP) as a scaffold in regenerative endodontics has been evaluated clinically and radiographically showing no significant difference in the success rate between PRP and a conventional blood clot (BC) scaffold, as shown in Table 1 (Bezgin et al. 2015). However, this technique has a complex production procedure, activation, and sudden fibrin polymerization as it requires thrombin as coagulation agents. This may predispose the donor to an immunologic reaction. Thus, platelet-rich fibrin, which are second-generation platelets, was introduced to overcome the disadvantages of PRP. As shown in Table 1, the clinical and

Table 1: Type of scaffold and stem cells that are actively being investigated in dentistry for tissue regeneration

Authors, Year	Type of study	Scaffold and dental cells used	Scaffold properties	Sample size, n	Results
Regenerative Endodontic					
Cavalcanti et al. (2013)	In vitro (tooth slice model)	Puramatrix™ and human DPSC	Fast setting time Biodegradable Injectable hydrogel	Not reported	WST-1 assay showed proliferation of human DPSC after 72 hours and survived for at least three weeks. Odontoblastic differentiation after 21 days in tooth slice with Puramatrix™ as measured by expression of DSPP and DMP-1.
Rosa et al. (2013)	In vivo	1. Puramatrix™ and SHED 2. rhCollagen type I and SHED	Fast setting time Biodegradable Encapsulated injectable hydrogel	6	Expression of odontoblastic marker was observed as early after 7 days and complete by day 21 in Puramatrix™ while in rhCollagen markers only observed by day 14 and complete on day 28. Histologically, connective tissue with multiple blood vessels seen close to dentin in both scaffold occupied full extension of root length and positively confirmed with tetracycline staining to mark new dentin formation.
Dissanayaka et al. (2015)	In vivo	PuramatrixTM and human DPSC + HUVEC	Fast setting time Biodegradable Encapsulated injectable hydrogel Prevascularized scaffold	8	Histological analysis showed the formation of pulp-like tissue in all cell-transplantation group after 4 weeks. Osteodentin/predentin also was observed adjacent to the pre-existing dentin in co-cultured groups. Formation of odontoblast-like cells (positive odontoblast marker) and significant neovascularization (positively stained for CD31) were observed in regenerated pulp-like tissue.
Bezgin et al. (2015)	Clinical trial	PRP + SCAP versus BC + SCAP	Not reported	20 (10 teeth per group)	No significant treatment outcome between PRP and BC scaffold after 18-months follow-up period. PRP group showed a mean of 8.1 months for complete apical closure compared to 9 months in BC group. However, in cases of de novo regeneration, all teeth in the PRP group showed resolution of sign and symptom, 90% showed a various degree of root maturogenesis. 5 teeth in the PRP group give positive responses to vitality testing compared to 2 teeth in the BC group.

Authors, Year	Type of study	Scaffold and dental cells used	Scaffold properties	Sample size, n	Results
Bakhtiar et al. (2017)	Case series	Platelet-rich Fibrin	Biodegradable	4	Resolution of apical radiolucency after 12 months and evidence of apical closure in all four cases, while minor discolouration in all cases and inconsistent evidence of root lengthening observed.
Bone Regeneration					
Behnia et al. (2012)	Clinical trial	Human bone marrow-derived mesenchymal stem cells (hMSCs) + biphasic HA/TCP scaffold	Not reported	3	51.3% bone regeneration observed 3-months post-operatively
Guda et al. (2014)	In vivo	1. Cortical-cancellous organization 2. Trabecular-like architecture 3. Untreated defect	Bilayer scaffold 65% porosity Pore size: 1. Cortical-cancellous organization (200 µm outer, 450 µm inner) 2. Trabecular-like (340 µm)	8	Both bilayer and trabecular architecture promote bone growth with more uniform new bone distribution in microCT of trabecular scaffolds. Foe mechanical strength evaluation, trabecular scaffolds showed greater flexural strength and toughness compared to negative control.
Khojasteh et al.	In vitro	Canine MSC and endothelial progenitor cell + PLGA-coated β-TCP scaffolds containing VEGF	Porosity less than 86.87% Pore size 500 µm	Not reported	Penetration of cell to the depth of 5.5 mm with normal cell metabolism VEGF encapsulating the scaffold significantly increase both proliferation and differentiation of MSC and endothelial progenitor cell
Shim et al. (2017)	In vivo	Canine MSC (dog) + Polycaprolactone-tricalcium phosphate (PCL/TCP)	PCL/TCP Block 70% porosity Pore size >300 µm	4	Higher amount of bone formation in area seeded with PCL/TCP scaffold from histomorphometric analysis
Du et al. (2017)	Clinical trial	Human bone marrow-derived mesenchymal stem cells (hMSCs) + biphasic HA/TCP scaffold	Not reported	3	72.8% bone regeneration observed 3-months post-operatively. Bone formation induces by human bone marrow-MSC with biphasic HA/TCP scaffold were radiographically equivalent to iliac crest bone graft in alveolar cleft repair

Authors, Year	Type of study	Scaffold and dental cells used	Scaffold properties	Sample size, n	Results
Huang et al. (2018)	In vitro	1. human adipose-derived stem cells (hADSC) + PCL 2. hADSC + PCL/HA 3. hADSC + PCL/TCP	Pore size 23EeECM87-317 µm	Not reported	PCL/HA scaffold had a smoother surface than PCL/TCP Addition of HA improve biological but lower mechanical property of scaffold
Periodontal Regeneration					
Requicha et al. (2014)	In vitro	Canine adipose stem cells + scaffolds from starch and poly (ε-caprolactone)	Double layer scaffold	Not reported	Double layer scaffold allowed cells adhesion with the preference of growing on the surface of fiber mesh Alkaline phosphatase assay showed a significant increase of osteogenic differentiation in cells cultured with osteogenic medium
Lee et al. (2014)	Animal model	DPSC, PDLSC, or alveolar bone stem cells + Printed multiphase	Printed Multiphasic Compartmentalised scaffold Polycarprolactione-hydroxylapatite (90:10wt%)	10	PDLSCs formed Collagen-I rich fibers while mineralized tissues by DPSC, PDLSC, and alveolar bone stem cells . Following 6-weeks in vivo implantation, aligned PDL-like collagen fibers inserted into bone and dentin/ cementum-like tissues reported
Park et al. (2014)	Preclinical	Human PDL + PCL-polyglycolic acid biphasic scaffold with fiber-guiding properties	Biphasic Compartmentalised scaffold	48	A significant high defect confirmation in fiber-guiding scaffold, formation of Type I Collagen fibrous bundle and orientation, ligament-like tissue formation and alignment. Histologically, newly formed PDL complex mimic original tissue

Abbreviations: DSPP: dentin sialophosphoprotein; BC: blood clot; DPSC: dental pulp stem cells; SHED: stem cells from human exfoliated deciduous teeth; HUVEC: human umbilical vein endothelial cells; HA: Hydroxyapatite; MSC: mesenchymal stem cells; PRP: platelet-rich plasma; PLGA: Poly(lactide-co-glycolide); PCL: Poly-ε-caprolactone; SCAP: stem cells from apical papilla; PDLSC: stem cells from periodontal ligament; PDL: periodontal ligament; VEGF: vascular endothelial growth factor; TCP: tricalcium phosphate

radiographical evaluation of four case series demonstrated a positive outcome for all cases, i.e.: the resolution of periapical lesion, continuous root development, and apical closure after 18 months recall in immature teeth with necrotic pulps (Bakhtiar et al. 2017).

The platelet concentrates technique, particularly the platelet-rich fibrin clots, is one of the possible successful scaffolds for regenerating pulp complex tissue. However, this method requires an additional phlebotomy, a procedure which is uncomfortable for children. In these cases, injectable hydrogel scaffolds are more practical perhaps with further clinical trials to provide concrete evidence for pulp regeneration. The use of soft injectable hydrogels into a narrow tapering canal is an advantage. Nevertheless, the whole process is quite tedious and time-consuming if it is to be translated into clinical application. Thus, the choice of scaffolds will depend on the clinicians' clinical assessment of the patient's age, level of cooperation, and stage of root development.

Regeneration of Bone

The current practice in treating cranial and maxillofacial defects involves the use of autologous bone. However, this treatment modality brings together a few disadvantages; a second surgery in the donor site with limited shape and some bone pain, swelling, infection, and scarring (Shamsuddin et al. 2017; Farré-Guasch et al. 2015). Thus, the concept of bone tissue engineering could bring a paradigm shift to the gold standard of autologous bone in treating a bone defect in this area. Nevertheless, constructing a potential bone replacement that is structurally, functionally and mechanically comparable to the natural bone in treating bone defect has been a challenge thus far.

Bone tissue engineering applies the concept of seeding osteogenic cells into an osteoconductive scaffold together with the induction of angiogenesis to regulate the metabolism of the cells (Shamsuddin et al. 2017; Khojasteh et al. 2016). This scaffold should be biocompatible and porous to allow the migration, adhesion, proliferation, and differentiation of seeded mesenchymal cells into an osteoblast. The biodegradable property of scaffolds allows the deposition of new bone (Huang et al. 2018). A scaffold with a well-interconnected structure enables the acceleration of bone regeneration and vascularization (Sarker et al. 2015). As in other fields of tissue engineering, extensive works have been conducted in search of suitable scaffolds for bone engineering over the past decades. This effort included the use of natural, synthetic, bioceramic, platelet-concentrated materials as well as mixed composites for different scaffold designs.

Apart from application in soft tissue engineering, natural hydrogel such as gelatin is a potential scaffold for non-load-bearing bone regeneration due to their low mechanical modulus (Jaipan et al. 2017). Gelatin, a protein obtained from the hydrolysis of collagen, has been an attractive candidate for fabricating natural hydrogel due to its

large number of functional groups that can be easily crosslinked. Nevertheless, recent studies have introduced crosslinking between genipin and gelatin to increase gelatin stiffness, support differentiation, mineralisation, increase stability during implantation, as well as significantly inhibit bacterial proliferation (Nguyen et al. 2016; Muhammad Mior et al. 2019; Chang et al. 2019; Sun et al. 2016).

Fibrin, the essential element in clotting and wound healing is biodegradable and degrades within two weeks after implantation (Li et al. 2015). The use of fibrin glue in a large animal model showed significant new bone formation in a surgically prepared alveolar cleft (Yuanzheng et al. 2015). A similar promising result was found in a recent study in which relatively higher new bone formation was observed eight weeks after implantation (Wang et al. 2017). Despite this success, the latter study utilized amniotic fluid-derived stem cells-premixed with PRP gel as compared to bone marrow stem cells in the former study. Wang et al. (2017) postulated the positive synergistic effect of PRP, which could be attributed to the angiogenetic, proliferative and differentiating effect of the growth factor contained in PRP.

Several bioceramic scaffolds from both calcium phosphate-based and glass-ceramic groups have been investigated. HA and β-TCP are among the most studied scaffolds in the former group. HA is the commonly investigated material among researchers as this scaffold shares a similar mineral structure with bone. HA scaffolds with pore sizes ranging from 50 μm to 1000 μm have been investigated both *in vivo* and *in vitro* (Guda et al. 2014; Quinlan et al. 2015). The consensus remains that for osteogenesis, a pore size of more than 100 μm is recommended, whereas to support vascularization, a pore size of more than 300 m is required (Guda et al. 2014). Since interconnectivity is also among the important features of a scaffold, Guda et al. (2014) demonstrated that larger interconnecting uniform pores (400±40 μm) had greater bone regeneration, mechanical strength, and toughness in large segmental defect, as shown in Table 1. However, the study was conducted on a long bone defect, which may not be a true reflection of the cranial bones that are flatter and thinner. Despite excellent osteoconductivity, concerns have been raised regarding the limited biodegradation and bioresorbability properties of HA (Huang et al. 2018). This means HA tends to remain in the body for a long time after implantation. HA is also associated with hardness, fragility, and a lack of flexibility. Hence, their application in a situation in which the scaffold is required to be shaped in a specific form is limited (Chang et al. 2013). In addition, HA is also is not an option in large bone regeneration due to brittleness and low mechanical stability (Huang et al. 2018). Alternately, being one of the most extensive forms of TCP used in tissue engineering, β-TCP has been proven to have good biocompatibility and faster degradation than HA but causes brittleness, poor fatigue resistance, and is difficult to shape (Huang et al. 2018, Arahira et al. 2015).

Many studies have reported on composite materials containing a combination of two or more groups of scaffolds to overcome the drawbacks of each component. Shavandi et al. (2016) reported that the biocompatibility and mechanical property of HA was improved after nanohydroxyapatite with a pore size of 10-30 nm was incorporated into chitin hydrogel. Poly-ε-caprolactone (PCL), a synthetic scaffold, presents long degradation times and poor bioactivity (Huang et al. 2018). Huang et al. (2018) suggested the incorporation of a mixture of HA and TCP into a PCL scaffold as an ideal composite scaffold for bone regeneration. However, a mixture of HA showed a better result compared to TCP (Huang et al. 2018). Shim and co-worker (2017) manipulated the PCL structure by adding TCP to increase the mechanical yield strength of the brittle PCL. The PCL scaffold alone is usually fabricated with 80% porosity and a 0.2-1 mm pore size, which is slightly bigger than the commonly used scaffolds in bone engineering. Although the study successfully generated bone, the main concern was the possibility of seeded cells washing out during *in vivo* application (Shim et al. 2017).

As shown in Table 1, Khojasteh et al. (2016) conducted a study by coating PLGA on highly porous β-TCP and encapsulating it with VEGF with an average pore size of about 500 μm. The highly porous β-TCP coated with PLGA resulted in a scaffold with increased compressive and mechanical strength (Khojasteh et al. 2016). Granular β-TCP was used in a chin reconstruction case due to its resorption profile (De Ruiter et al. 2015). β-TCP degraded faster as compared to crystalline hydroxyapatite but had low mechanical properties due to its brittleness; which in turn, may cause a sudden collapse of newly formed bone (Arahira et al. 2015). Bone regeneration in cleft patients utilizing mesenchymal stem cells (MSC) loaded on HA/β-TCP scaffolds with a combination of PDGF has shown successful bone formation 3-months postoperatively (Du et al. 2017), as shown in Table 1. However, the disadvantages of this study are a lower amount of regenerated bone as compared to the autogenous iliac graft and the absence of a control group for comparison due to ethical issues.

A natural hydrogel can be considered an option for bone regeneration in a non-load-bearing area. Platelet-derived concentrates could also serve as an alternative scaffold since the effect of the growth factor in PRP contributes to the angiogenesis, proliferation, and differentiation of cells. For load-bearing areas, the use of bioceramics is preferred since this type of scaffold offers better mechanical properties for the intended tissue growth. The initiative of mixing bioceramic material into a synthetic scaffold improves the biological and physical property of the scaffold which would enhance cell attachment, proliferation, and differentiation. Hence, the most suitable type of scaffold for bone regeneration will depend on the site and the size of the intended tissue to growth.

Regeneration of periodontal tissues

Periodontitis is an inflammatory disease that may lead to the destruction of

the periodontal tissue. The treatment modalities include non-surgical treatment and periodontal surgery. The ultimate goal for successful periodontal regeneration consists of a newly formed functional periodontal ligament between the regenerated cementum on the root surface and the newly formed alveolar bone. To achieve this successful clinical outcome, a coordinated response between the soft tissue (periodontal ligament and gingiva) and hard tissue (alveolar bone and cementum) component during wound healing is required (Ivanovski et al. 2014). The current approach for treating this periodontal disease is by using guided tissue regeneration which utilises a membrane barrier to allow the selective repopulation of the periodontal defect by cells from the remaining periodontal ligament (Chen et al. 2016). This treatment modality is only applicable to a limited range of clinical scenarios such as with infrabony defects and mandibular molar Class II bifurcation involvement (Ivanovski et al. 2014). Generally, scaffolds in tissue engineering should degrade and resorb as the new tissue regenerates. However, periodontal regeneration involves different tissues and it is important to note that ingrowth and maturation differ between tissues (Ivanovski et al. 2014). Chen and Liu (2016) suggested that scaffolds should remain intact to allow newly formed tissues to mature inside the pores while degradation takes place at a later time. In periodontal regeneration, the scaffold design is equally as important as the type of scaffold. Researchers have investigated various techniques and approaches including compartmentalised designs, computer-aided design based on compartmentalised scaffolds and cell sheet technology. The double-layered scaffold approach is a modification of the traditional guided tissue regeneration technique; the outer phase is designed as an occlusive membrane preventing the invasion of surrounding tissue into a periodontal defect while the inner phase is manufactured with macropores to suit bone regeneration (Requicha et al. 2014), as shown in Table 1. Requicha et al. (2014) reported a positive outcome in their bilayered scaffold manufactured via a combination of starch and a slower degrading polymer, PCL. The results demonstrated the osteogenic differentiation of seeded cells, which are part of the essential structure of periodontium, while the membrane layer promoted cell attachment and proliferation. Because the canine adipose stem cells used grow preferentially on the fiber mesh, the distribution of cells into the interior part is limited so their viability is affected (Requicha et al. 2014).

An extensive work by Park and co-workers (2014) on a multi-phasic scaffold demonstrated the role of computational design and 3D printing in periodontal tissue regeneration, as shown in Table 1. The designed fiber-guiding scaffolds promoted the formation and orientation of Periodontal Sharpey's fiber i.e. Type-I collagen bundles embedded within the cementum and alveolar bone that respond to mechanical forces (Park et al. 2014). However, these fiber-guiding

scaffolds may increase the treatment cost because of the use of computer technology. Well-trained personnel are also required to deliver this treatment to the patient.

The promising results from various studies serve as a foundation for a future paradigm shift in treatment modalities for periodontal diseases; from replacement to regeneration. Nevertheless, due to the complexity of the periodontal tissue, the development of suitable scaffolds is also challenging. The recent approach of using compartmentalised scaffolds seems successful but brings together disadvantages of cost and training issues in clinical application.

CONCLUSION

Scaffolds are an important component of tissue engineering. The scaffold used in dental tissue engineering must exhibit minimal immune response. It must also be biocompatible with an appropriate degradation rate, have adequate porosity, and a pore size with a 100% interconnecting pore to maximize the diffusion and exchange of nutrients. Sufficient mechanical strength is also a crucial property of the scaffold. Scaffolding approaches such as the pre-made porous scaffold, the decellularised extracellular matrix, cell sheets with a secreted extracellular matrix, cells encapsulated in a self-assembled hydrogel, and rapid prototyping can be used to produce more reliable and functional scaffolds for dental tissue engineering. With the emergence of tissue engineering, the development of scaffold from various sources, designs, and properties has shed new light on the treatment of patients with dental diseases. Advances in dental tissue engineering may become available for clinical application. However, one of the challenges in realizing this idea is the aggregate cost required to introduce such technology to clinicians and patients. Therefore, the production of affordable, reproducible, and clinically-safe scaffolds should be considered so that this technology could become accessible to all clinician as well as the patient. Moreover, advanced research in scaffolds with tissue-specific considerations in relation to target tissue composition and interfaces, structural, and functional relationships deserves more attention among researchers.

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