Chapter 14
Tissue Engineering in Periodontal Regeneration

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1 Introduction

Millions of people around the world suffer from tooth loss caused by irreversible periodontium destruction due to acute trauma, extensive caries, or severe periodontal disease. Periodontal disease is a serious health issue that can affect quality of life. One of the main therapeutic goals of today's medicine is to develop novel regenerative treatments for periodontal tissues [1, 2].

The periodontium is a complex organ consisting of both mineralized and soft connective tissues. It includes the periodontal ligament (PDL), gingiva, cementum, and alveolar bone, generally named as the "attachment apparatus" (Fig. 14.1) [3]. The attachment apparatus fastens the tooth to surrounding bone and acts as a bumper to absorb the energy and forces from mastication. Periodontitis can endanger the integrity, health, and function of periodontal tissues [4].

The periodontal ligament (PDL) is an active connective tissue that is capable of continual adaptation to preserve tissue size and width. PDL acts like an anchor that connects the tooth to the alveolar socket and as a cushion to absorb the mechanical forces and loads resulting from mastication. Hence, PDL establishes the substratum of the periodontium and determines the tooth life span [5].

Accumulation of bacteria and other pathogenic microorganisms in the subgingival biofilm can lead to tissue inflammation called periodontitis. Some risk factors that increase the probability of having periodontitis include aging, smoking [6], and

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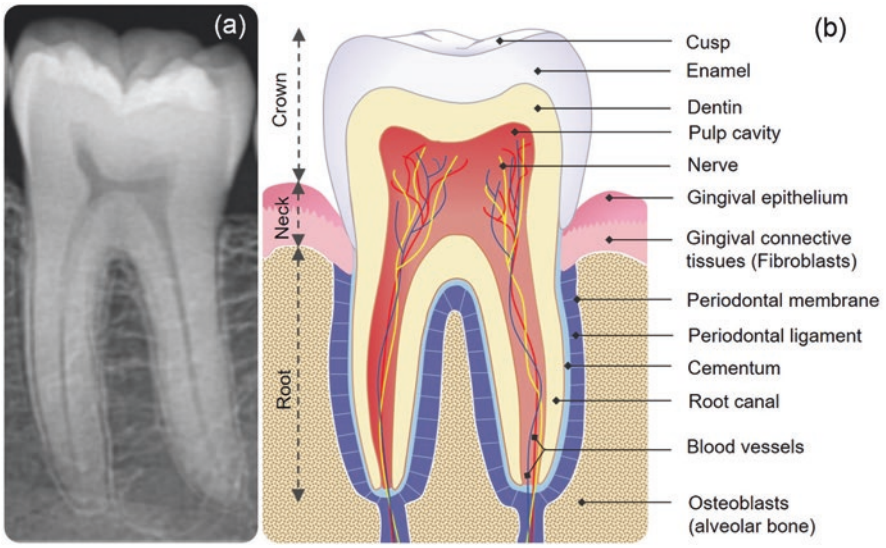

Fig. 14.1 (**a**) Radiographic and (**b**) schematic structure of tooth and attachment apparatus

systemic disorders, such as diabetes, cardiovascular disease, rheumatoid arthritis, and adverse pregnancy outcomes [7]. Untreated periodontitis can lead to early tooth loss due to the inevitable destruction of the periodontium [6, 8].

There is an increasing demand for alternatives to replace and treat the diseased tissues and organs, such as destructed periodontal tissues. One of the major challenges in clinical periodontics has always been treating and managing periodontal defects, including intrabony defects and destructed cementum and PDL [9, 10]. Periodontal regeneration intends to repair the damaged periodontal tissues, both soft tissues (i.e., PDL) and hard tissues (i.e., alveolar bone and cementum) [2, 9].

Six tissues are typically involved to reconstruct a periodontal lesion, including the PDL, cementum, alveolar bone, gingival connective tissue, gingival epithelium, and all related vasculature [3]. Periodontal regeneration is one of the most complicated procedures to occur in the body [2].

In periodontal tissue engineering (TE), regeneration of a periodontal defect is achieved by stimulating the self-recovery capability of the periodontium. Therefore, the appropriate balance of cells and stimulating molecules, along with a durable matrix to control the regrowth of periodontal tissue, is necessary. It is important to prevent soft gingival tissue from growing into the defect so as to preserve the space for new bone formation and achieve functional regenerated tissue [4].

The regeneration ability varies for each of the mentioned tissues [11]. For instance, alveolar bone can regenerate bone that is similar to the original tissue, while the regenerative ability for the cementum and PDL is very limited and slow [12, 13]. For the first time, TE was suggested by Langer and Vacanti in 1993 as a possible technique for periodontal tissue regeneration [14].

Many regenerative treatment techniques have been established so far, such as the use of bone grafts and guided tissue/bone regeneration (GTR/GBR) [8, 15]. However, their success rates are poor and limited. Therefore, researches turned to stem cell-based techniques for periodontal regeneration. Nevertheless, these techniques also have limitations. For example, scaffold degeneration can be caused by inflammation, necrosis, unstable transplanted cells, etc. [16, 17].

In this chapter, a perspective into the fundamental principles of TE and its application in periodontal disease treatments is discussed based on the recent studies in TE and regenerative medicine.

2 Conventional Approaches for Periodontal Regeneration

Since the 1980s, several approaches have been established to enhance regeneration of periodontal tissues. The results of these methods have had limited success and poor clinical predictability [17, 18]. The main conventional methods are listed as follows.

2.1 Bone Grafts

Using grafts/biomaterials containing bone-inducing substances and bone-forming cells can result in bone formation. The biological function of bone grafts can be divided into three categories: osteogenesis (formation of new bone from living stem cells in graft materials), osteoinduction (bone formation by recruitment of immature cells by graft materials to become active osteoblasts), and osteoconduction (known as bone growth on the surface of the graft material) [17, 19]. There are several types of grafts used for bone regeneration.

2.1.1 Autologous Bone Grafts

Autologous bone grafts, also known as autografts, are harvested from one site on the patient's body and transplanted to another site [20, 21]. Autologous bone grafts have been regarded as the gold standard in bone defect treatment because they only contain self-bone-forming cells. These cells can induce osteogenesis and are therefore able to integrate into the host bone more quickly and completely [21–23].

2.1.2 Allogeneic Bone Grafts

Allogeneic bone grafts, also known as allografts, are bone tissues harvested from a genetically distinct source of the same species [24, 25]. Considering the limitation of autologous bone grafts, such as limited amount of obtained graft and surgical

processes, allografts are considered the best substitute to autografts. However, it should be noted that allografts are more immunogenic in comparison to autografts and have a higher risk of failure [23, 26].

2.1.3 Alloplastic Grafts

Alloplastic grafts are usually made from hydroxyapatite (prepared from bioactive glass) or calcium carbonate. Hydroxyapatite (HA) graft is the most used graft today due to its biocompatibility and osteoconduction potential. Calcium carbonate grafts are used less due to rapid resorption of the material and subsequent risk of fragile bone [27]. The major disadvantages of using bone grafts in periodontal regeneration include the risk of infection, surgical challenges, donor site morbidity, limited amount of graft in autologous and allogeneic grafts [28–30], and the risk of fibrous encapsulation associated with alloplastic materials [31, 32]. In addition, they generally result in tissue repair rather than true regeneration and cannot be used in all clinical situations [33, 34].

2.2 Guided Tissue Regeneration (GTR)

The GTR technique aims to enhance the natural healing potential of the PDL and alveolar bone [4, 35–37]. If a periodontal defect is left empty after flap debridement, oral epithelium cells and fibroblasts grow down into the site of the defect, forming an unwanted fibroepithelial tissue that prevents the formation of a functional periodontal tissue [38–42].

It was considered that if the PDL and alveolar bone cells initially colonized the root surface and adjacent alveolar bone instead of gingival cells, the formation of a long junctional epithelium would be prevented, and a functional periodontium would be formed [4, 43].

In this method, a membrane with variable porosity is employed to cover the root surface, acting as a barrier to oral epithelium cells and fibroblasts, and promote the natural growth of bone and PDL cells (Fig. 14.2) [22]. GTR has been the gold standard approach for regeneration of intrabony and interradicular defects for more than a decade [40, 44]. However, several studies demonstrate that the outcomes of GTR therapies have been limited and unpredictable [1, 45–47].

3 Cell-Based Approaches for Periodontal Regeneration

Figure 14.3 shows three indispensable elements in cell-based regeneration in periodontal tissue engineering, including progenitor cells, signaling molecules, and scaffolds. They will be discussed in detail as follows.

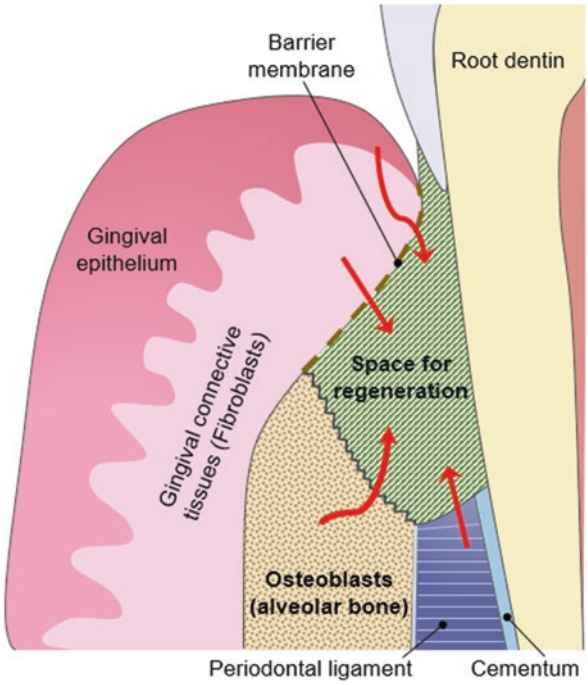

Fig. 14.2 Schematic representation of guided tissue engineering (GTR), involving a barrier membrane to prevent the growth of oral epithelium cells and fibroblasts into the bone defect

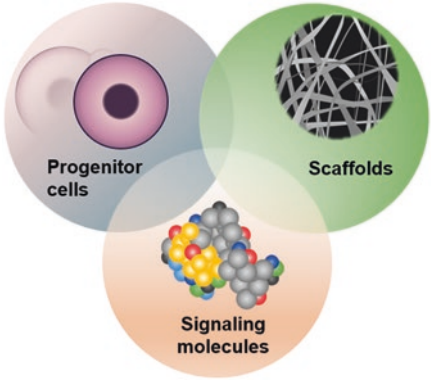

Fig. 14.3 The key components for periodontal tissue engineering

3.1 Progenitor Cells

Stem cells are undifferentiated cells that have the potential for self-renewal, giving rise to more stem cells, and differentiation into various cell types in reaction to external signals [48].

To date, several types of stem cells have been introduced for periodontal regeneration studies, including mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs). Considering the ethical issues of ESC usage, MSCs are more accepted for regeneration purposes [49]. MSCs were initially isolated from bone marrow-derived mesenchymal stem cells (BMMSCs) and were found to promote periodontal bone defects [50, 51].

Obtaining BMMSCs requires a bone marrow aspiration process, which is painful and traumatic. Additionally, the number of cells harvested is limited [51]. Therefore, subsequent research has aimed to harvest MSCs from oral-derived tissues such as the periodontal ligament, gingiva, dental pulp, dental follicles, apical papilla, and human exfoliated deciduous teeth [50, 52–56]. The most critical step in TE is selecting the stem cell population [32, 57]. Several types of mesenchyme-derived cells have been studied for periodontal regeneration.

3.1.1 Intraoral Mesenchymal Stem Cells and Periodontal Regeneration

Intraoral tissues can be used as a source of stem cells for periodontal regeneration. Some of the main intraoral-derived mesenchymal stem cells used in periodontal regeneration are listed below.

3.1.2 Periodontal Ligament Stem Cells (PDLSCs)

The periodontal ligament (PDL) is a specialized, dynamic connective tissue derived from the dental follicle and originating from neural crest cells [5, 58]. PDLSCs can be a good source of autologous stem cells for bone tissue engineering. They possess classic characteristics of stem cells, such as small size, slow cellular cycle, and expression of several stem cell markers [53, 59, 60]. They are also capable of differentiating into cells, including osteoblast-like cells, adipocytes, chondrocytes, and neurocytes, which can colonize on scaffolds [61–63]. PDLSCs can express all surface markers and immunomodulatory ability like BMMSCs [5, 50, 64]. They are able to grow faster than BMMSCs, although their osteogenic potential has been found to be lower than BMMSCs and dental pulp stem cells (DPSCs) [53, 59, 65].

For the first time in 1998, transplanted autologous PDL cells were used to promote periodontal regeneration in an animal study. The results suggested that autologous PDL cells can promote regeneration in vivo [3, 66]. Isaka et al. [67] placed PDL cells in a surgically created defect in an autologous dog model. Their results showed the formation of new cementum, while formation of alveolar bone was limited. Dogan et al. [68, 69] showed that seeding PDL cells in an autologous blood clot under a Teflon membrane supported regeneration of surgically created furcation and fenestrations. Seo et al. [70] harvested PDLSCs from human impacted third molar and found that these cells successfully could differentiate into PDL, cementum, alveolar bone, nerves, and blood vessels [50, 71–73]. A more recent study by Dan et al. [74] showed that PDL cells have more periodontal regenerative capacity

compared to other periodontal-derived counterparts such as gingival fibroblasts and alveolar bone osteoblasts. All these studies confirm that PDL cells do have a regenerative capacity.

Dental Follicle Stem Cells (DFSCs)

The dental follicle is a mesenchymal tissue. During the tooth development process, the dental follicle encircles the tooth germ. During root formation, dental follicle progenitors create the periodontal components, such as the PDL, alveolar bone, and cementum [75, 76]. DFSCs, which were first isolated from the dental follicle of human third molar, are the progenitor cells of osteoblast, cementoblasts, and fibroblasts. They can differentiate into osteoblasts, cementoblasts, adipocytes, and neurons [77] and produce cementum and bone [78, 79]. Bay et al. found that co-culturing of DFSCs with Hertwig epithelial root sheath (HERS) cells enhances the ability of DFSCs to regenerate cementum and PDL after transplantation [80]. Yokoi et al. [79] transplanted DFSCs subcutaneously to immunocompromised mice. They found that PDL-like structures with type I collagen began forming, indicating the potential of DFSCs in regenerating PDL.

Gingival Epithelial Cells and Fibroblasts

Okuda et al. [81] cultured gingival epithelial cell sheets that were harvested from human gingival tissue for chronic desquamative gingivitis treatment. The results showed that autologous gingival epithelial sheets enhanced gingival regeneration. In another study, autologous gingival fibroblasts were used for patients with deficient attached gingiva, and this resulted in successfully increasing the keratinized tissue width [82].

GINTUITTM is an allogeneic cellular product, which is comprised of allogeneic cultured keratinocytes and fibroblasts in bovine collagen. McGuire et al. [83] indicated that the product is an effective therapy approach for repairing the keratinized gingiva.

Periosteal Cells

The periosteum is a structure consisting of two layers. The outer layer is a fibrous layer containing fibroblasts, collagen, elastin, nerves, and a vascular network. The inner layer is a highly cellular layer comprised of osteoblast-like cells that support bone generation and bone reformation. The periosteum is considered a structure with regenerative capacity [84], as it has been found that cultured periosteum is capable of differentiation into an osteoblastic lineage. Autologous cultured periosteum sheet samples combined with HA and coagulated platelet-rich plasma (PRP) showed significant improvements in human infrabony defects and clinical attachment gain [85].

Dental Pulp Stem Cells (DPSCs)

Dental pulp stem cells (DPSCs) were the first recognized human dental stem cells [86]. They have been harvested from human third molars [86, 87]. The dental pulp contains a variety of cells, including fibroblasts, neural cells, vascular cells, and undifferentiated stem cells. DPSCs are anatomically located on the most vascularized areas of the pulp. They possess multipotent differentiating and self-renewal ability. They can differentiate into osteoblasts, odontoblasts, neural cells, and chondrocytes in vitro [59, 88–90]. DPSCs can successfully be isolated and characterized from human extracted teeth, inflamed pulp tissue [91], supernumerary teeth [92], and natal teeth [93] by a variety of approaches. For example, D'Aquino et al. [94] showed that DPSCs isolated from human teeth, along with collagen sponge implants, have improved mandibular bone tissue regeneration in patients.

DPSCs have also been reported to have immunomodulatory properties on mice [95]. They have several similar features to BMMSCs; however, their osteogenic potentials are limited in comparison [86]. Human autologous DPSCs, along with HA or beta-tricalcium phosphate (TCP), have shown capability of forming bone and cementum [32, 86, 89, 96]. However, the effect of these stem cells on periodontal regeneration has been inconsistent to date [32].

Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

Miura et al. [55] described SHED as clonogenic cells with high proliferation capacity that can differentiate into several cell types. They transplanted SHED in vivo and found that they were able to promote bone generation. These stem cells are mainly obtained from children's tooth pulp tissue around ages 6 to 12 [97]. Obtaining SHED is simple and beneficial because of six reasons: (1) They are less mature compared to permanent teeth, so they possess a higher proliferation ratio. (2) They have the flexibility of differentiating into a variety of cells, including osteoblasts, adipocytes, odontoblasts, and neural cells. (3) They are easily achievable. (4) They are convenient for use in young patients with mixed dentition. (5) There is no need to sacrifice a tooth. (6) Obtaining process is atraumatic [76].

According to previous studies, SHED are not able to differentiate directly into osteoblasts, but they can induce in vivo bone generation by forming osteoinductive patterns to employ osteogenic cells in rats. Therefore, it has been concluded that deciduous teeth, in addition to providing a guidance for permanent tooth eruption, are associated with inducing bone generation during permanent tooth eruption [55]. Although both DPSCs and SHED are obtained from pulp tissue, they show noticeable differences in proliferative potentials [98]. SHED possess a higher proliferation ratio compared to DPSCs and can differentiate into a variety of mesenchymal lineages [55, 99].

Stem Cells from Apical Papilla (SCAP)

SCAPs were isolated and introduced by Sonoyama et al. from premature roots of human third molars [54]. They can differentiate into several mesenchymal lineages such as osteoblasts, odontoblasts, chondrocytes, adipocytes, smooth muscle cells, and neurons in vitro [100–102]. In comparison to DPSCs, SCAPs possess a higher proliferation rate and mineralization capacity. However, compared to BMMSCs, they have lower adipogenic capacity [54, 101]. It has been reported that SCAPs possess immunomodulatory properties [103].

In one study, SCAPs were transplanted into immunocompromised rats using HA and tricalcium phosphate particles as dentin-like carriers. Human SCAPs and PDLSCs were also transplanted into mini pigs. The results showed successful root and periodontal regeneration. In addition, an in vivo study on human SCAPs, along with porous ceramic discs that were transplanted into immunosuppressed rats, showed that hard tissues can be formed [104].

3.1.3 Extraoral Mesenchymal Stem Cells and Periodontal Regeneration

Extraoral tissues can be used as a source of stem cells for periodontal regeneration. The main extraoral-derived mesenchymal stem cells used in periodontal regeneration are listed below.

Bone Marrow-Derived Mesenchymal Stem Cells (BMMSCs)

Bone marrow-derived mesenchymal stem cells (BMMSCs) have been the most studied among mesenchymal stem cells. Human BMMSCs are pluripotent stem cells which originate from the bone marrow and can differentiate into multiple mesenchymal linages such as osteoblasts, chondrocytes, and adipocytes [105–107]. BMMSCs have been found to generate the cementum, periodontal ligament, and alveolar bone, indicating that the bone marrow can be a convenient source for periodontal regeneration [105].

Pittenger et al. [107] aspirated bone marrows from 350 donors and found differentiation of MSCs into bone, cartilage, and fat. Kuo et al. [108] reported that BMMSCs can induce the generation of PDL, odontoblasts, and cementum from DPSCs. Kawaguchi et al. [109] showed that autologous bone marrow-derived mesenchymal cells promote periodontal regeneration in surgically induced class III furcation defects in dogs. Other studies also showed that BMMSCs were able to control diabetes in animal models [110] and stimulate wound healing [111].

Adipose Tissue-Derived Stromal Cells (ATSCs)

Adipose tissue is another extraoral source of mesenchymal stem cells. Lately, adipose tissue-derived stem cells have been extensively studied as an applicable source of cells for regenerative medicine [112, 113]. They have been introduced as a convenient source of stem cells because they are abundant and easy to obtain [17]. Various studies have confirmed the capability of ATSCs' differentiation into osteogenic, neurogenic, adipogenic, myogenic, and chondrogenic cells [114–116]. ATSCs, in combination with platelet-rich plasma (PRP), have been shown to induce alveolar bone and PDL-like structures fabrication in mice [117].

3.2 Signaling Molecules

Another major TE approach for periodontal regeneration is to stimulate cells near the defect area using biological signals. Table 14.1 summarizes different types of signaling molecules in addition to their effects and applications. The main signaling molecules are listed as follows.

3.2.1 Insulin-Like Growth Factors (IGFs)

Insulin-like growth factor (IGF) is a hormone with a similar molecular structure to insulin, which has different forms. IGF-1 is an effective chemotactic agent that enhances the formation of new blood vessels and promotes the formation of bone and cementum. It causes in vitro protein synthesis and periodontal ligament fibroblasts mitogenesis [118]. Studies on non-human primate models showed that IGF-1 cannot modify periodontal wound healing by itself [126]. Lynch et al. [127] proposed using IGF-1 along with platelet-derived growth factor-B (PDGF-B) to increase periodontal regeneration. IGF-2 is an active factor in bone formation which abounds in bone as a growth factor. Although it helps in bone formation, it is not as effective as IGF-1 [128].

3.2.2 Platelet-Derived Growth Factor (PDGF)

PDGF is a growth factor that controls cell differentiation and growth. It consists of two polypeptide chains encoded by two dissimilar genes, PDGF-A and PDGF-B [5]. PDGF can enhance periodontal tissue regeneration by stimulating mitosis of PDL cells and synthesis of gingival fibroblast hyaluronate [2]. Clinical studies have shown that using PDGF-B improves the treatment of periodontal bone defects. It enhances the rate of filling bone defects and improvement of attachment level, along with reduction of gingival recession [129].

Table 14.1 Signaling molecules, theirs effects, and applications

Signaling molecules	Effects	Applications	Ref.
Insulin-like growth factor (IGF)	Enhances the formation of new blood vessels and promotes the formation of bone and cementum	Protein synthesis, periodontal ligament fibroblasts mitogenesis	[118]
Platelet-derived growth factor (PDGF)	Controls cell differentiation and growth	Periodontal tissue regeneration	[2, 5]
Bone morphogenetic protein (BMP)	Interacts with specific receptors on the cell surface	Development of new blood vessels, bone fabrication	[2, 119]
Fibroblast growth factor (FGF)	Triggers development of new blood vessels and stimulates differentiation and proliferation of mesenchymal cells	Tissue regeneration, wound healing, and angiogenesis	[120]
Transforming growth factor-beta (TGF-β)	Adjusts and stimulates several biological processes and components such as embryonic growth and immune regulation	Induces cells to grow in soft agar	[121, 122]
Periodontal ligament-derived growth factor (PDL-CTX)	Periodontal regeneration without chemotactic effect on epithelial cells or gingival fibroblasts	Autocrine chemotactic agent for periodontal ligament cells	[2, 123]
Enamel matrix derivative (EMD)	Stimulates differentiation of mesenchymal cells including osteoblasts	Enamel formation, root and attachment apparatus development	[44, 124]
Platelet-rich plasma (PRP)	Developing grafting procedures, decreasing periodontal healing time, and improving bone quality	Source of growth factors such as PDGF and TGF-β	[1, 125]

As mentioned earlier, the combination of PDGF-B and IGF-1 can improve periodontal regeneration; at initial stages of wound healing after a surgery, it increases the formation of the periodontal attachment apparatus [130].

3.2.3 Bone Morphogenetic Proteins (BMPs)

BMPs are growth factors that interact with specific receptors on the cell surface. There are several BMPs that originate from the human body. Three of these are bone morphogenetic protein-2, bone morphogenetic protein-3 (known as osteogenin), and bone morphogenetic protein-7. All are highly involved in periodontal regeneration [119]. BMP stimulates development of new blood vessels and bone fabrication [2]. BMPs have morphogenetic potential and are important in conducting migration and attachment of stem cells onto scaffolds to increase the response of stem cells to BMPs [131].

3.2.4 Fibroblast Growth Factor (FGF)

The FGF is one of the heparin-binding growth factors involved in tissue regeneration, wound healing, and angiogenesis [120]. FGF triggers development of new blood vessels and can stimulate differentiation and proliferation of mesenchymal cells [120]. It is a signaling protein that is isolated from regular tissue in two forms: acidic FGF (a-FGF) and basic FGF (b-FGF) [2].

There are several different forms of FGF. FGF2 is the most studied FGF and can attach to heparin to develop new blood vessels and induce mitosis [132]. In the wound healing process, it can adjust different cellular functions, including proliferation and migration, among others [133]. In vivo studies of FGF2 in non-human primates show that it can trigger regeneration of periodontal tissue with new cementum and alveolar bone creation [134]. Furthermore, it can improve bone formation by enhancing the rate of differentiation of osteoprogenitor cells. FGF2 is a capable candidate for regenerating soft and hard periodontal tissues because it can trigger the migration and proliferation of ligament cells [55, 70, 90, 95]. An in vivo study on skin defect of mice indicated that regeneration of soft tissue can be accelerated by b-FGF [135].

3.2.5 Transforming Growth Factor-Beta (TGF-β)

TGF-β is highly concentrated in human bone and platelets, and it can induce cells to grow in soft agar. TGF-β adjusts different types of biological processes such as differentiation of adult stem cells, embryonic growth, immune regulation, etc. [121]. It stimulates several biological processes and components, including fibronectin and osteocalcin biosynthesis, chemotaxis of osteoblasts, bone matrix deposition, type I collagen, and periodontal ligament fibroblast proliferative activity, as well as rising ECM production. Moreover, it decreases the connective tissue destruction due to the reduction of metalloproteinases and plasminogen activator inhibitor (PAI) synthesis [2, 122].

TGF-β is composed of three 25 kDa homodimeric mammalian isoforms, including β1, β2, and β3. TGF-β1 can result in proliferation of MSCs, wound healing, enhanced ECM formation, inhibition of inflammation, and production of preosteoblasts, chondrocytes, osteoblasts, and collagen [136–138]. In addition, it has been reported that TGF-β1 raises cell surface proteoglycan genes in PDL cells [139, 140], assists in DNA and fibronectin synthesis, and produces protein acids [141, 142].

3.2.6 Periodontal Ligament-Derived Growth Factor (PDL-CTX)

Periodontal ligament-derived growth factor (PDL-CTX) is a novel polypeptide growth factor from human periodontal cells [143]. It is a specific autocrine chemotactic agent for periodontal ligament cells, which is one thousand times more

effective than other growth factors, including IGF, PDGF, and TGF [2]. Furthermore, PDL-CTX can have a favorable effect on periodontal regeneration because it does not have a chemotactic effect on epithelial cells or gingival fibroblasts [123].

3.2.7 Enamel Matrix Derivative (EMD)

Enamel matrix proteins are produced by ameloblasts and are responsible for growth of HA crystals and enamel mineralization [144]. In addition to their role in enamel formation, they are also involved in root and attachment apparatus development [44]. EMD is available commercially in an injectable gel solution form known as Emdogain, which consists of enamel proteins amelogenin, ameloblastin, amelotin, tuftelin, and enamelin [3]. Emdogain was the first signaling product that could successfully regenerate periodontal tissue [22]. It has been reported that EMD can stimulate differentiation of mesenchymal cells including osteoblasts. Hejil et al. [124] applied EMD in intrabony defects; the results showed 66% bone fill in defected areas.

3.2.8 Platelet-Rich Plasma (PRP)

PRP is a concentration of autologous plasma isolated by centrifugation of patient's blood. PRP acts as a source of growth factors and contains growth factors such as PDGF and TGF-β [125]. Various commercial PRP kits are available to facilitate chair-side PRP isolation for clinicians. Although there is not enough convincing information about PRP benefits for periodontal regeneration, it seems that PRP can be advantageous for developing grafting procedures, decreasing periodontal healing time, and improving bone quality [1].

3.3 Scaffolds

To utilize the maximum potential of stem cells, an isolated three-dimensional (3D) environment should be provided to allow the cells to proliferate in three dimensions and be transferred into the defected area [145]. Scaffolds can be in the form of a sponge, gel, or complex network of pores and channels. All the scaffolds used in TE are designed to degrade gradually after implantation in the targeted site, being replaced by new tissue [1, 146]. The major challenge in TE is to develop regeneration in three dimensions and promote angiogenesis over the entirety of the scaffold.

The main roles of scaffold include as follows [132]: (1) It serves as a framework to supply physical support for the regenerating area, to preserve the shape of the defect and to prevent surrounding tissue from collapsing into the defect. (2) It provides a 3D substratum for ECM production, cell adhesion, and migration. (3) It

serves as a barrier with selective permeability to confine the migration of cells. (4) It serves as a growth factor delivery vehicle for cells [2].

Additionally, the key features of an ideal scaffold are (1) biocompatibility, biodegradability, and nontoxicity; (2) being able to preserve migration, attachment, proliferation of the cells, and production of new ECM; (3) having enough mechanical strength to endure physical stress and being able to preserve the surrounding bone from stress; (4) having an intrinsic network of interconnected pores; and (5) providing appropriate conditions for neovascularization [147].

3.3.1 Biomaterials Used as Scaffolds

To date, a variety of biocompatible materials have been used to fabricate scaffolds, including polymers, metals, ceramics, and proteins [148]. The following is the list of main biomaterials used as scaffolds.

Ceramics

Bioceramics have a long history of use for joint and tooth implants [149]. Ceramics used in bone TE are natural and synthetic hydroxyapatite (HA) and beta-tricalcium phosphate (TCP). They are biocompatible and osteoinductive and, due to being protein free, are not able to induce immunological reactions [2]. HA is a natural bioceramic found in hard tissues such as dentine and enamel [149] and was one of the first materials used as scaffolds. It can be synthetic or derived from natural sources like bovine bone or coraline [2].

Some studies suggest nanostructured HA as a potent material due to its good biocompatibility and bone integration ability [150, 151]. TCP is a natural material consisting of calcium and phosphorous and is used as a ceramic bone substitute [2].

Metals and Alloys

Titanium and its alloys, cobalt-chromium alloys, and stainless steel are used in fabrication of scaffolds [149]. Titanium is the most common material used for implants due to its decent biocompatibility, osteointegration potential, and ability to be laminated with various polymers [152]. However, metals and ceramics have very limited potential to be used as effective scaffolds because they are not biodegradable and cannot be processed easily [148].

Polymers

Polymers have been widely used for TE due to their biodegradability and capability of being processed [153–156]. There are two types of polymer materials: synthetic polymers and natural polymers [148]. The most biodegradable polymers are

polyesters, polycaprolactone, polyanhydride, polycarbonate, polyfumarate, and polyorthoester [157–162]. The most widely used polymers in TE include polyesters like polyglycolic acid (PGA), polylactic acid (PLA), and their copolymer poly(lactic-co-glycolic acid) (PLGA) [163–166].

Synthetic Polymers

Polyglycolic acid (PGA), a simple and linear polymer of glycolic acid, is the first polymer scaffold used in TE. For the first time in the 1980s, PGA was used alone as a scaffold in the form of mesh for renal injury treatment. It is also used as a bone fracture fixation implant and suture material [2, 149].

Polylactic acid (PLA) is a polymer of lactic acid and is more hydrolysis resistant and hydrophobic than PGA. The copolymer of PLA with PGA is PLGA. It was first used as a suture material (Vicryl) in 1974 and degrades in 8 weeks [167]. It was the first FDA-approved copolymer and has been the first candidate for use in dental tissue regenerations due to its biocompatibility, structural strength, controllable degradation, and ability to deliver growth factors. PLGA can also be used in combination with other polymers like gelatin [155].

Polymethylmethacrylate (PMMA) is a highly biocompatible, but nondegradable, polymer. Due to its excellent biocompatibility, it has been widely used for mandibular reconstruction and repairing skull defects and bone cements in clinical procedures [168, 169]. Nevertheless, PMMA promotes fibrotic tissue formation [169].

Naturally Derived Polymers

Naturally derived polymers include proteins derived from natural ECM or polysaccharides. They have been widely used in TE [170–172].

Chitosan is a biocompatible, nontoxic, and non-immunogenic carbohydrate polymer derived from chitin, which is found in crustacean shells [173] and has shown improvement in bone regeneration and wound healing, as well as antibacterial activity [174, 175] and bioadhesive character [176]. Chitosan is capable of being made into different shapes and structures such as membranes [177], fibers [178], sponges [179], paste [180], microspheres [181], and porous scaffolds [182]. These characteristics make it suitable to be used as a scaffold for tissue regeneration. Chitosan can also be used as a copolymer with other materials [165, 183].

Collagens are made by several cell types [184]. Collagens can be formed into various forms and structures such as sheets, gels, sponges, fibers, and films [1, 185, 186]. However, collagen scaffolds have not shown enough mechanical strength, and their degradation rate is not convincing. Therefore, crosslinking agents such as formaldehyde, polyepoxy, and glutaraldehyde compounds have been used to enhance the thermal, mechanical, and biological properties of collagen [1, 187].

Fibrin is a blood component critical for hemostasis. It is produced from fibrinogen and thrombin during hemostasis and enhances wound healing [1, 149]. After

tooth extractions, blood clots have been used as natural scaffolds for promoting bone healing process [149]. Fibrin is widely used as a biopolymer scaffold in periodontal TE due to its biocompatibility, biodegradability, and simple preparation and handling process [149]. Fibrin scaffolds are also available in combination with other polymers, such as fibrin-PEG blend [172, 188]. Fibrin hydrogels are used as a heparin-binding delivery system and cell seeding during inkjet printing process [172].

Hydrogels are a new type of biomaterials that are injectable into the periodontium. They are made of viscous polymers, which are composed of synthetic or natural macromolecules [189–191].

Alginate is a hydrogel isolated from brown seaweed and bacteria [192]. It is biocompatible and nontoxic and can have a slow gelling time depending on temperature and concentration [193]. Its limitations include uncontrollable degradation and low viscoelasticity. These can be improved by using alginate incorporation with HA [194].

4 Cell Sheet Technique

Cell sheet technique is a novel approach for harvesting and delivering cells in TE [195]. Conventionally, in TE proteolytic enzymes are utilized to fragment the ECM and release the cells, which could impair cell functions and damage the cell membrane because the proteolytic enzymes hydrolyze cell membrane proteins [32]. This technique prevents the enzymatic digestion of proteins, keeping the normal cell and ECM interactions [196]. In other words, it is possible to harvest a complete cellular sheet with intact ECM and cell-cell junctions [3]. This technique has been used recently to regenerate periodontal defects [197, 198].

Cell sheet engineering involves thermo-responsive systems including poly(N-isopropylacrylamide) (PIPAAm) polymer for cell culturing [3]. This polymer is hydrophilic at temperatures greater than 32 °C and hydrophobic when temperatures are lower than 32 °C. In addition, cells are prone to attach to hydrophobic surfaces. These properties are beneficial for detaching the cell sheets from cultures [32].

Harvested cell sheets are delicate and fragile. Thus, manipulation and implantation of them can be challenging. Therefore, 3D biocompatible scaffolds such as hyaluronic acid, fibrin gel, and ceramic bovine bone have been developed to increase stabilization and strength, also facilitating the manipulation and results [32].

Cell sheet technology has been utilized for various TE applications, such as cornea transplantation using corneal epithelial cell sheets and myocardial tissue regeneration using cardiomyocyte cell sheets [199, 200]. They have been extensively applied to improve periodontal regeneration in animal studies using dogs and rats [201].

5 Conclusions

During the last few decades, a rapid development has been reported in periodontal regeneration methods. Recent studies have emphasized the importance of wound stability and space preservation for a predictable and optimal tissue regeneration, but they are not achievable by current clinically applied techniques. Successful results can be feasible by utilizing a combination of cells, signaling molecules, and scaffolds to construct the anatomy based on the complex structure of periodontal tissues and defects. Three-dimensional scaffolds are the key for complete periodontal regeneration. Application of tissue engineering on periodontal regeneration is still in its initial stages and requires more investigation. Recent advances in material science, tissue engineering, and microscopy techniques provide a brighter perspective for more predictable regenerative therapies for periodontal defects in the near future.

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