Regenerative Approaches in Periodontics

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1 Introduction

Periodontal diseases are some of the most common diseases of humanity. According to the Global Burden of Disease Study in 2010, severe periodontal disease was the sixth most common health condition in the world. This disease had a worldwide prevalence of 11.2%, and affected some 743 million people, with that number increasing every year [1, 2].

Damage to the periodontium can occur due to trauma, gingivitis, periodontitis, or age-related loss of tissue. Destruction of the periodontium eventually may result in the loss of teeth and surrounding tissues. The ultimate goal of regenerative periodontal treatment is to prevent tooth loss by achieving complete regeneration of the periodontium [3].

Conservative periodontal therapy, which continues to be used effectively today, encompasses measures to control biofilm. It focusses on the removal of both supragingival and subgingival dental plaque biofilm, the removal of infected cementum, and the correction of problematic aspects of restorations such as overhangs [4–6]. Conservative periodontal treatment and supportive periodontal therapy (SPT) is essential as a foundation for any surgical interventions, and good control of biofilm must ideally be achieved prior to all attempts at periodontal reconstructive and regenerative surgery.

Periodontal regeneration can only be achieved by creating the appropriate microenvironment for the differentiation of specific cell types that constitute the periodontium. Epithelial cells (keratinocytes) and fibroblasts can proliferate faster than other cells in the periodontium, and controlling the proliferation of fibroblasts and epithelial cells poses a major challenge after surgical periodontal treatment [7]. All regenerative treatment approaches are based on maintaining a separation that controls the tendency of epithelial cells and fibroblasts to overgrow during the healing period and to give an opportunity for others cells from the periodontium to proliferate and differentiate [4].

In recent decades, deficiencies in the methods and outcomes of traditional surgical treat-

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ment approaches for periodontal disease have led researchers to investigate new regenerative treatment methods involving tissue engineering technologies [8]. Tissue engineering is defined by Groll et al. as "an interdisciplinary field that provides the restoration and function of biological units using the principles of engineering and life sciences" [9].

A combination of conservative periodontal therapy and new techniques in tissue engineering has created a new regenerative direction that is the focus of much current periodontal research. Amongst the disciplines within dentistry, periodontology has arguably gained the most benefited from advances in tissue engineering. Ongoing developments in this field have been very promising, and they underpin the success of periodontal regeneration methods used in clinical treatments [4, 8].

2 Periodontium

The periodontium is a specialized multi-tissue structure in the oral cavity. It contains both mineralized and soft tissues, and it comprises the cementum, gingiva, periodontal ligament and alveolar bone. The periodontal apparatus is responsible for providing the maxillary and mandibular teeth with the necessary support and protection for their normal functions [10]. Each of the four components of the periodontium has its own architecture, composition, and differentiation, yet they all work in function together as one entity in the oral cavity [11].

Gingiva
This is the most superficial part of the periodontium, and it includes the free gingiva, the attached gingiva and the interdental gingiva. This soft tissue overlies the alveolar bone. Its epithelial layer lines the sulcus of teeth and is the first barrier that prevents microbial invasion of the underlying structures.

Periodontal Ligament
This is a vascular fibrous structure positioned as the interface between two hard tissue structures, the cementum and alveolar bone. It supports the tooth in its socket. The stem cells that are present in the periodontal ligament have an astonishing ability to differentiate into other cell types, especially in cases of injury.

Cementum
This is an avascular calcified tissue that covers the dentine of the tooth root. It consists of a primary acellular type and a secondary cellular type. The cementum is attached to the alveolar bone through periodontal ligament fibers (Sharpey's fibers). Cementoblasts produce the intrinsic collagenous matrix of cementum [10].

Alveolar Bone
Alveolar bone or the alveolar process is the mineralized part of the periodontium, that is attached to the cementum of the root through the Sharpey's fibers of the periodontal ligament [12].

Many different factors that can cause destruction and loss of the periodontium. These include both systemic, developmental, and acquired diseases (genetic, endocrine, connective tissue diseases, acquired immunodeficiency, neoplasms) as well as oral diseases (periodontal diseases and oral malignancies). Periodontal destruction can also be caused by non-disease factors such as traumatic occlusal forces. Since these various conditions and diseases can cause the overall oral health of patients to deteriorate, a corrective approach or therapeutic intervention is necessary to stop the process of damage, limit its extent, and maximize the health status of the affected area [13].

3 Nature of Periodontal Healing and Regeneration

"*Periodontal regeneration*" is the concept where materials and procedures are used to induce reconstruction of a part or the whole of the periodontal tissues [14]. It involves complex biological cooperation, where different cells and bioactive proteins are responsible for interacting, with the goal being to reproduce the previous normal function and architecture of the tissues. In

an ideal scenario, coordination of the four compartments would give rise to a connection between the new cementum and new alveolar bone through Sharpey's fibers, giving a functional periodontal ligament [15].

A true visualization of the repaired tissue can only be achieved at the histological level, where all the periodontal structures can be viewed. At the clinical level, comparing probing and attachment levels before and after treatment can provide an indication of treatment outcomes. Conventional intraoral radiography for evaluating bone fill in the defect sites is not considered reliable, since a certain amount of mineralization needs to occur before it can be visible on a radiograph. Methods such as digital subtraction radiology, cone beam volumetric tomography, and computer-assisted densitometric image analysis can provide more information on changes to bone. In some cases, re-entry surgery to the treated site is used to visualize the healing, however even this method cannot show fully what type of outcome has occurred [12].

Periodontal regeneration is the ultimate outcome of any periodontal therapy. Conventional surgical and non-surgical periodontal therapy debrides the root surface to prepare it for the healing process. There are usually two main paths for this healing—either by regeneration, where complete renewal of the tissue function and structure takes place or by repair, which results in compromised clinical outcomes [10]. At the microscopic level, the repair is the most common and default outcome with conventional surgical and non-surgical periodontal therapy.

Successful reconstruction of the lost tissue requires collaborative efforts from progenitor cells in order to deposit new tissue and allow this to mature. Some of these events require careful coordination, for example, growth of alveolar bone should occur in a coronal direction towards the soft tissue, with no adjacent bony part at the other end. This is a unique situation for bone that is not found elsewhere in the body [11].

The healing of periodontal wounds is a complicated process, for many reasons, including the proximity to an avascular tooth surface, and the potential ingress of pathogenic microorganisms into the surgical site [11]. It is also challenging because the goal is to form new attachment, but there is a loss in the regulatory messages needed to direct this process [16].

During the normal healing process, as can be shown in surgically induced bone defect models in rats, bone formation starts at the bony part of the wound, and after that gradual thickening and mineralization of cementum can be observed around the apex of the tooth. Periodontal ligament fibers are the last to integrate into both cementum and bone [17].

Like any other wound in the body, in a periodontal wound a fibrin clot forms between the wound edges. In a periodontal wound, this clot forms between the wound margin and the root surface. It is replaced later by granulation tissue, and hopefully by a new connective tissue attachment. However, as the clot sits between a hard tissue (the root) and a soft tissue, microscopic movements of these create tensile forces, which can readily displace the clot. Rapid migration of epithelium causes space to becomes epithelialized. The net result is that the root surface has a long junctional epithelium (LJE) on its surface, rather than periodontal ligament fibers. The outcome of an LJE is the most common form of repair in periodontal defects after open or closed periodontal debridement. The epithelium has the highest migration rate, so will always dominate in the wound healing sequence if it is not excluded. This is why in periodontal regeneration, the epithelium must be excluded. If the clot can be maintained in a stable position against the root, it is more likely that a new connective tissue attachment will emerge on the root surface [18].

Ideally, the outcome of periodontal healing is to reconstruct the periodontium, and eliminate periodontal pockets (Fig. 1). Reconstruction involves restoring the proper anatomical and functional relationships between the junctional epithelium, connective tissue, the periodontal ligament, the cementum, and the bone. Any lost tissue should be replaced. This is especially challenging in terms of the alveolar bone [12].

In 1976, Melcher et al. proposed the tissue compartment hypothesis [19]. According to this, there is a "first mover" advantage, so the type of cell which is the pioneer at the defect area will determine the outcome. Therefore, there is usu-

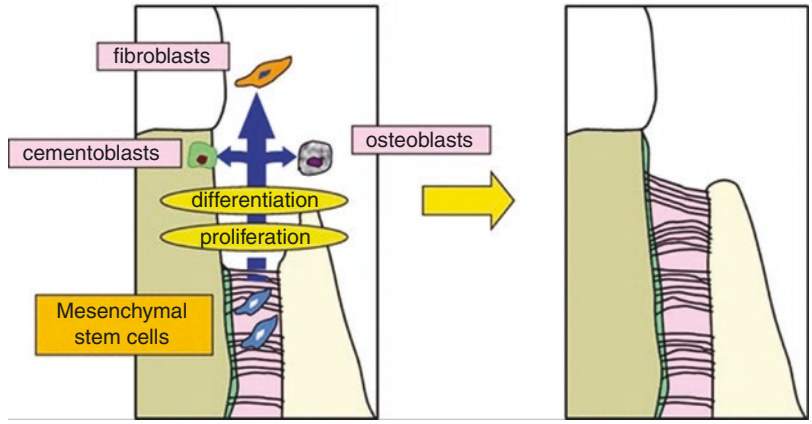

Fig. 1 Periodontal ligament cells differentiation and regenerative capacity [23]

ally one or a combination of four possible healing outcomes that can occur after conventional periodontal treatments:

- *Long Junctional Epithelium*, where epithelial cells are the first dominators.
- *Recession*, where new connective tissue attachment is formed apically to the cemento-enamel junction (CEJ) but without regeneration of periodontal ligament.
- *Ankylosis*, where the ligament is lost and there is the union of bone and tooth, with resultant tooth resorption.
- *Recurrence of the pocket* is also a possibility in cases of repair [20].

4 Regenerative Capacity of Periodontal Ligament Cells

Various experiments have revealed that neither osteoblasts nor gingival connective tissue cells have the ability to produce new connective tissue attachment and ankylosis will result in the areas where the periodontal ligament does not exist. As the periodontal ligament contains mesenchymal stem cells and periodontal stem cells, it should have the ability to produce other cell lineages. This regenerative capacity is regulated by several growth factors, including fibroblast growth factor, transforming growth factor-β, insulin growth factors 1 and 2, platelet-derived growth factor, and bone morphogenic proteins. These can activate cells to divide. Signals from growth factors and cytokines drive the differentiation of stem cells [21].

Mesenchymal cells of the periodontal ligament are of particular interest as they express high levels of markers for bone, cementum, and fibroblasts. When grown in culture in various conditioned media, periodontal ligament mesenchymal cells show elevated levels of markers for an osteoblastic lineage (RUNX2, ALP, OPN and COL1), a cementoblastic lineage (CEMP1, CAP), and increased content of both fibronectin and collagen. These extracellular matrix components increase the ability of the cells to attach. They also regulate how the cells interact with other cells and tissues in the periodontium [22].

5 Types of Periodontal Defects

Achieving periodontal regeneration for bone defects of various shapes is one of the most important challenges of surgical periodontal therapy. The approach used for reconstructive periodontal management varies according to the type of tissue that has been lost. Periodontal defects can be classified as soft tissue defects, hard tissue defects, or a combination of both [24].

Osseous (hard tissue) defects are classified according to the level of the periodontal pocket base in reference to the alveolar crest, into either suprabony defects (supracrestal) or infrabony defects (subcrestal).

The latter includes;

(A) Intrabony defects are classified according to the number of remaining osseous walls (one, two, or three-wall defects). The lesser the number of residual walls, the more difficult it is to get bone fill to restore the defect. Some defects have less walls remaining coronally than at the basal part. These are termed "combination intrabony defects."
(B) Craters. These occur frequently in the mandibular posterior segment. The buccolingual concave shape of bone resorption involves two neighboring teeth, or the septum between the buccal and lingual walls is resorbed. When the osseous defect is between the roots of multirooted teeth, these are known as inter-radicular defects.

Glickman et al. classified osseous defects according to their vertical and horizontal dimensions. Later, a classification of the vertical dimension of furcation defects was introduced [25]. Hamp et al. classified horizontal bone loss numerically, where Class I has less than 3 mm loss of periodontal tissue; Class II has more than 3 mm loss of periodontal tissue, but the probe cannot go through the defect, and Class III is a through-and-through horizontal loss of tissue [26]. This classification is still one of the most commonly used in clinical practice [25].

Soft tissue defects can present as gingival recession, where the gingival margins have become apical to the CEJ. It can affect the total width of the attached gingiva, on one tooth or many teeth. Classically, marginal soft tissue recession has been classified descriptively as shallow or deep, or wide or narrow. In 1985, Miller et al. proposed four categories for gingival recession, in relation to the mucogingival relationship and the alveolar bone status. Class I refers to recessions in which the gingival margin is coronal to the mucogingival margin. In Class II, the gingival margin reaches the mucogingival margin or beyond, with no bony defect. Class III is when the gingival margin is at the mucogingival margin, and is accompanied by interproximal alveolar bone loss and/or tooth malposition, while Class IV describes severe alveolar bone loss and/or considerable tooth malposition [27]. This classification, although very popular, is currently seen as inadequate, as some clinical cases do not belong to either Class I nor II (e.g., when interproximal bone loss occurs while the gingival margin is still coronal to the mucogingival margin). Another drawback of this classification is that it ignores the issue of the recession on the palate, which can be of significance to clinical management [27].

A newer classification of the gingival recession that was adopted by the World Workshop of Periodontology, classifies the gingival recession with respect to clinical attachment loss at the interproximal area. In Type 1, there is no attachment loss interproximal, and the interproximal CEJ cannot be seen. In both Type 2 and Type 3, there is the loss of interproximal attachment (from the CEJ to the most apical point of the pocket at both the mesial and distal sides) and is compared to the buccal attachment loss (from the CEJ to the most apical point of the pocket at the buccal side). If it is less than or equal to the buccal attachment loss, then it is classified as Type 2, and if it is higher it comes under Type 3 gingival recession. This classification is seen as treatment directed and can be combined with assessment of other gingival parameters, such as the width of keratinized gingiva and the gingival phenotype or biotype [28].

6 Current Surgical Treatment Protocols in Regenerative Periodontology

Periodontitis results from the host response to the accumulation of dental plaque on the roots of the teeth. In a susceptible patient, long-standing inflammation of the supporting structures of the teeth results in loss of alveolar bone. Subsequently, apical migration of the soft tissue can manifest as gingival recession, with loss of interdental papilla.

Comprehensive treatment of advanced periodontitis involves the regeneration of both hard

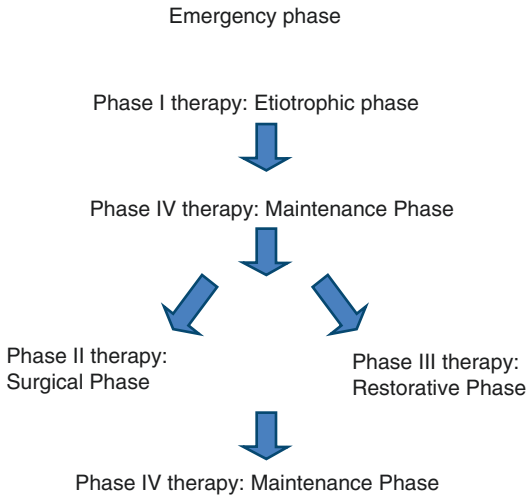

Fig. 2 Flow chart of comprehensive periodontal treatment divided into four phases. Phase I therapy involves the removal of all aetiologic factors. Following re-evaluation, patients with a healthy periodontium are placed in the maintenance phase. Patients not responding adequately to phase I therapy are treated with surgical periodontal therapy

and soft tissue components of the periodontium. As discussed earlier, non-surgical periodontal therapies (NSPT) remain the cornerstone of periodontal treatment, and the majority of cases are treated with NSPT. Periodontitis is initially treated with NSPT (phase I) either as a preparatory phase for further treatment or as a definitive treatment. The decision to move the patient towards either surgical periodontal therapy (phase II) or maintenance therapy (also known as supportive periodontal treatment (SPT)) (phase IV) is made following re-evaluation of the clinical situation (Fig. 2). However, a portion of cases in advanced stages do not respond to NSPT and will require surgical periodontal therapy [29].

7 Team of the Periodontist and the General Dental Practitioner

Around the world, in many countries, there is an aging population. Many older patients have retained a significant portion of their natural teeth, and this has resulted in an increased demand for comprehensive periodontal treatment. Advanced periodontitis is best treated by a shared approach, wherein both the general dentist and the periodontist work together towards improving and maintaining the periodontal health of the patient [30]. The general dentist screens and identifies cases of advanced periodontitis, and refers them to a periodontist. They also coordinate the patient's care and provide their routine restorative and preventive oral health care. By the general dentist explaining the various available treatment options, pointing out the advantages and disadvantages, the patient is able to make their own choices based on evidence [29, 30]. The periodontist carries out active and comprehensive periodontal treatment, whereas the general dentist maintains the treated periodontium by providing supporting periodontal treatment.

7.1 Indications for Surgical Periodontal Therapies

1. The presence of advanced stages of periodontitis. In the current classification of periodontitis, this would translate into stage III and stage IV, grade C periodontitis [31].
2. Patients not responding to NSPT, as evidenced by the presence of multiple residual periodontal pockets ≥6 mm, would be ideal candidates for surgical periodontal therapy [32]. The decision to use surgical techniques for a patient is dependent on the response of the patient towards NSPT at the re-evaluation visit.
3. The presence of bone defects, including intrabony defects (three-wall defects) and craters that would benefit from the use of regenerative bone grafts or membranes (regenerative periodontal surgery) [33].
4. The presence of bony contours or abnormalities that need re-contouring to establish an ideal periodontal architecture (resective periodontal surgery) [34].

7.2 Contraindications for Surgical Periodontal Therapies

1. Poor oral hygiene or patients in whom maintenance of optimal oral hygiene is difficult.
2. The presence of risk factors including uncontrolled diabetes mellitus (≥7% HbA1C), and continued use of tobacco (smoking ≥20 cigarettes/day), since these are known to impair healing and to result in poor surgical outcomes.

The main goals of surgical therapy are to: enhance accessibility to root deposits for root debridement and scaling, reduce or eliminate pockets by resection of the pocket wall, expose the diseased zone to perform regenerative techniques, and gain access for resection of periodontal defects [29–32].

8 Periodontal Flaps

As discussed above, periodontal surgical treatment is applied where preventive periodontal treatment is not sufficient. It aims to completely eliminate the cause of the disease and to ensure as much regeneration of the destroyed tissue as possible. Periodontal flaps can be designed in different ways to provide access to the periodontal defect region.

"A periodontal flap is a section of mucosa and/or gingiva surgically separated from the underlying tissues to provide access and visibility to the bone and root surface" [35]. Periodontal flaps are classified according to the exposure of bone, after flap reflection, into either partial thickness flaps or full-thickness flaps. Flaps can also be classified based on their placement after the operation, whether displaced (coronally, apically or laterally) or undisplaced. Also, according to the management of the interdental papilla, flaps can be classified as conventional flaps or papilla preservation flaps [32]. Table 1 lists the major features of the main types of periodontal flaps.

Various techniques and methods have been utilized in surgical periodontal procedures, including the modified Widman flap, the papilla preservation flap, and various modifications of these [32].

9 Classification of Periodontal Surgical Procedures

Periodontal surgerical procedures are broadly classified into resective and regenerative periodontal surgical procedures.

9.1 Resective Periodontal Surgical Procedures

Resective periodontal surgery involves eliminating bony irregularities, to create a stable peri-

Table 1 Various classifications of periodontal flaps and their main features [32]

	Based on	Flap type	
1	Bone exposure	**Full-thickness flaps/Mucoperiosteal flaps** all the soft tissue, including the periosteum, is reflected to expose the underlying bone	**Partial thickness flaps/Mucosal flaps/Split thickness flap** includes only the epithelium and a layer of the underlying connective tissue. The bone remains covered by a layer of connective tissue, including the periosteum
2	Placement of the flap after surgery	**Displaced flaps**: after the completion of the surgery, flaps are moved either coronally or apically	**Undisplaced flaps**: after completion of the surgery, flaps are placed back in the same position and sutured
3	Management of the papilla	**Conventional flaps** are used when the interdental spaces are too narrow. In this procedure, the papilla is split into two (labial and lingual) portions	**Papilla preservation flaps** are indicated when there are wide interdental spaces. One papilla is incorporated into either the buccal and lingual flaps. This flap is commonly used whenever regenerative periodontal products are used

odontal architecture that will assist the patient in maintaining their oral hygiene. Essentially, this approach sacrifices some bone for the creation of a stable periodontal architecture. In recent decades, the scientific community has realized the importance of regenerating and conserving bone. Therefore, resective periodontal surgical approaches have become less popular, and today increased emphasis is directed towards regenerating bone [33–35].

9.2 Regenerative Periodontal Surgerical Procedures

Regenerative periodontal therapy aims to reconstruct and reconstitute the tooth-supporting structures that have been lost through periodontitis or because of other causes. Regenerative periodontal procedures involve the use of various regenerative materials and techniques for the regeneration of the lost portions of the periodontium. The most commonly used products in periodontal regeneration are bone grafts and barrier membranes. Each of these materials has certain distinct advantages and disadvantages [33–35].

10 Regeneration of Bone and Bone Grafts

After the placement of bone grafts into osseous defects, several steps of healing follow. The process finally culminates in the complete integration of the bone graft into the surrounding native bone, with the goal being that the new bone displays similar structural and biochemical properties to the native bone. Bone regeneration requires three main components including cells (osteoblasts), a scaffold (which provides the structural framework for the development of clotting, maturation, remodelling, and for the recreation of the Haversian canal systems), and signals (such as from bone morphogenic proteins and other signals that can induce the formation of bone) [36].

The regenerative potential of a bone graft is based on the properties of osteogenesis, osteoinduction, and osteoconduction.

- *Osteogenesis* is the formation of bone by osteoblasts.
- *Osteoinduction* is the process by which proteins and cellular signalling molecules present in the graft will induce neighbouring mesenchymal stem cells to differentiate into osteoblasts and thereby produce bone.
- *Osteoconduction* is the presence of a matrix/scaffold that helps with the organization of the population of cells in the scaffold and the creation of a system similar to native bone.

Apart from these three main criteria for ideal bone grafts, other relevant criteria include: the ease with which the graft can be procured, its cost, its biocompatibility (e.g., being free from antigenic properties that would evoke an inflammatory response), and the provision for an adequate amount of the graft. Table 2 lists the various criteria for an ideal bone graft. However, an ideal bone graft will likely never exist, and any bone graft will be a compromise in terms of its various properties. The selection of the bone graft to be used by the clinician will be based on the circumstances and needs of the individual case [37].

11 Classification of Bone Grafts

Bone grafts are classified based on their source, as follows: autografts, allografts, xenografts and alloplastic materials [38].

Table 2 Features of an ideal bone graft [36, 37]

An ideal bone graft should	
1	Have osteogenic potential, meaning it should contain some osteoblasts that can directly produce bone.
2	Have a scaffold framework (structural framework for the development, maturation, and remodelling of tissue).
3	Have signals for the induction of mesenchymal stem cells to differentiate into osteoblasts.
4	Be biocompatible, and unable to elicit an inflammatory immunological response.
5	Be free from any virus or prion-related infection.
6	Be economical.
7	Be available in sufficient quantity to fill or replace the lost portion of the bone.

11.1 Autografts

Autogenous bone grafting involves harvesting bone grafts from a donor site, and then using this bone fill the bone defect within the same individual from whom the graft was taken, thereby reducing the concerns of allergenicity. Autogenous bone grafting is predictable and is considered to be the gold standard because it has the three essential criteria: osteogenic cells, osteoinductive growth factors, and a scaffold for the growth of cells. Autogenous grafting materials include cortical bone, cancellous bone, and bone marrow transpirates [37]. Among these, cancellous and bone marrow transpirates are considered to be more osteogenic due to their higher osteogenic potential and vascularity. Iliac crest, tibia, fibula, and ribs are the most favoured extraoral sites, whereas the symphysis of the mandible, tori/exostoses, the retromolar pad, healing wounds, extraction sites, and the region of the maxillary tuberosity are the most common intraoral sites [38].

With autogenous bone grafts, the main disadvantages are the necessity of a second surgical site and the limited amount of bone that can be harvested. In patients with immunocompromised conditions or elderly patients, the creation of a second surgical site may result in considerable donor site morbidity and further complications, including postoperative infections. Autografts also need to be done by an experienced clinician who is capable of harvesting from the donor tissue while causing minimal damage to that site. Although autogenous bone is inexpensive as it is retrieved from the same patient, there are additional expenses because of the additional effort required by the surgeon. Such factors make autografts less appealing than other options [38, 39].

11.2 Allografts

Allografts are grafts taken from the same species, i.e., from humans, however, the donor is genetically different from the recipient [39]. All allografts carry a risk of blood-borne virus transmission, which is eliminated by rigorous screening and testing of potential cadavers for such transmissible diseases. There are three main types of allografts: (1) frozen, (2) freeze-dried bone allograft (FDBA) and (3) demineralized freeze-dried bone allograft (DFDBA). Fresh unprocessed bone and frozen bone from cadavers are not used due to the chance of spreading an infection.

FDBAs contain the mineralized skeleton for the population of native cells. FDBAs require a long period for neovascularization of the mineralized skeleton and conversion into new bone. This mineralized framework, without any growth factors, makes FDBAs osteoconductive, but not osteoinductive. The majority of periodontal defects treated with FDBA have shown complete bone fill or more than 50% bone fill [40]. A mixture of autogenous grafts and recombinant human bone morphogenetic protein 2 (rhBMP2) with FDBA provides improved results with complete regeneration of bone defects [40, 41].

DFDBAs or demineralized bone matrix (DBM) has the inorganic content removed, retaining the organic portion and its osteoinductive properties. The DBM or a DFDBA contains bone morphogenic proteins (BMPs). These are acidic polypeptides from the transforming growth factor-β family, and they initiate the differentiation of mesenchymal stem cells into osteoblasts. Higher levels of BMPs in the graft favours increased oxygen tension, thereby helping in the production of bone [41]. To date, DFDBAs are the only bone graft material that has been shown to provide complete regeneration using histological methods [42]. The American Association of Tissue Banks (AATB) is a scientific organization that provides guidelines for standard setting and the use of donated human tissues. The majority of nations have developed their own tissue bank associations following the guidelines of the AATB. The British Association for Tissue Banking for the United Kingdom and Australian Tissue Banking Association for Australia are examples of some of the tissue banks that are now involved in regulating harvesting and processing of allografts [42–44].

11.3 Xenografts

Bone grafts transferred from other species, including animals, to humans are known as xenografts. Commonly used products come from cows, pigs, or from natural marine coral [44]. Tissue banks procure bone grafts from animals, and process them using a battery of intense physical and chemical purification methods to remove components that may be antigenic, and also components that may spread infection, such as prions [43]. Testing of donor animals for bovine spongiform encephalopathy reduces the risk of disease transmission from bone grafts. Additionally, removal of the entire organic portion virtually eliminates the chances of prion-related infections, since prions are proteinaceous in nature. The remaining inorganic portion of the bone serves as a scaffold, within which neovascularization takes place [39].

Bio-Oss®, manufactured by Geistlich, is one of the most commonly used xenograft materials of bovine origin. Bovine bone has an inorganic structure that is similar to that of human bone, thus favouring its osteoconductive activity. Additionally, inorganic bovine bone has a high degree of porosity, thus increasing the chances of osteoconductivity and angiogenesis. Other similar products are available from various manufacturers.

The combination of P-15, a synthetic cell-binding peptide, with a bovine-derived bone graft, has been shown to provide improved clinical results when compared to DFDBA alone in the treatment of human periodontal intrabony defects [45]. Biocoral, on the other hand, contains calcium carbonate derived from natural coral and is this material is resorbable. Biocoral has a large amount of porosity, and it does not cause fibrous encapsulation, thus giving it high osteoconductivity [46].

11.4 Alloplasts

These are synthetic or semi-synthetic inorganic bone graft materials. Hydroxyapatite, calcium phosphate, β-tricalcium phosphate, and bioactive glasses are the most commonly used alloplastic materials [43, 44]. Alloplasts are often combined with growth factors or antimicrobial agents to improve their efficacy. The addition of autografts in small quantities to alloplasts provides a mixture with osteoinductive potential. However, this approach involves the harvesting of autografts, with the possibility of complications as discussed earlier.

Alloplasts with active additive agents have two major functions: the alloplast itself acts as a replacement material, while the added active agent helps induce bone formation, or prevents secondary infection [47, 48].

Bone grafts are also classified based on their regenerative potential, as outlined in Table 3.

Table 3 Classification of bone grafts based on their regenerative potential [43, 44]

1. Osteogenic/ osteoproliferative bone grafts	Possess osteoblasts, thus having the highest amount of regenerative potential. These would be considered as the gold standard among bone grafts
2. Osteoinductive bone grafts	Possess certain molecules, which could induce neighbouring mesenchymal stem cells to convert into osteoblasts and thereby lay down bone. For example, bone morphogenic proteins have the ability to stimulate mesenchymal stem cells to convert them to osteoblasts and produce bone
3. Osteoconductive bone grafts	Provide a meshwork for the formation of bone. These grafts are dense and non-resorbable. They act as a scaffold wherein bone from the adjacent area can form new bone. However, the majority of osteoconductive grafts are non-resorbable, so the use of these grafts for implant site development is not recommended

12 Guided Tissue Regeneration

Periodontitis causes the loss of both the hard and the soft tissue of the periodontium (Fig. 3a). Any periodontal surgical procedure involves the removal of the diseased pocket epithelium, resulting in a surgical wound. The

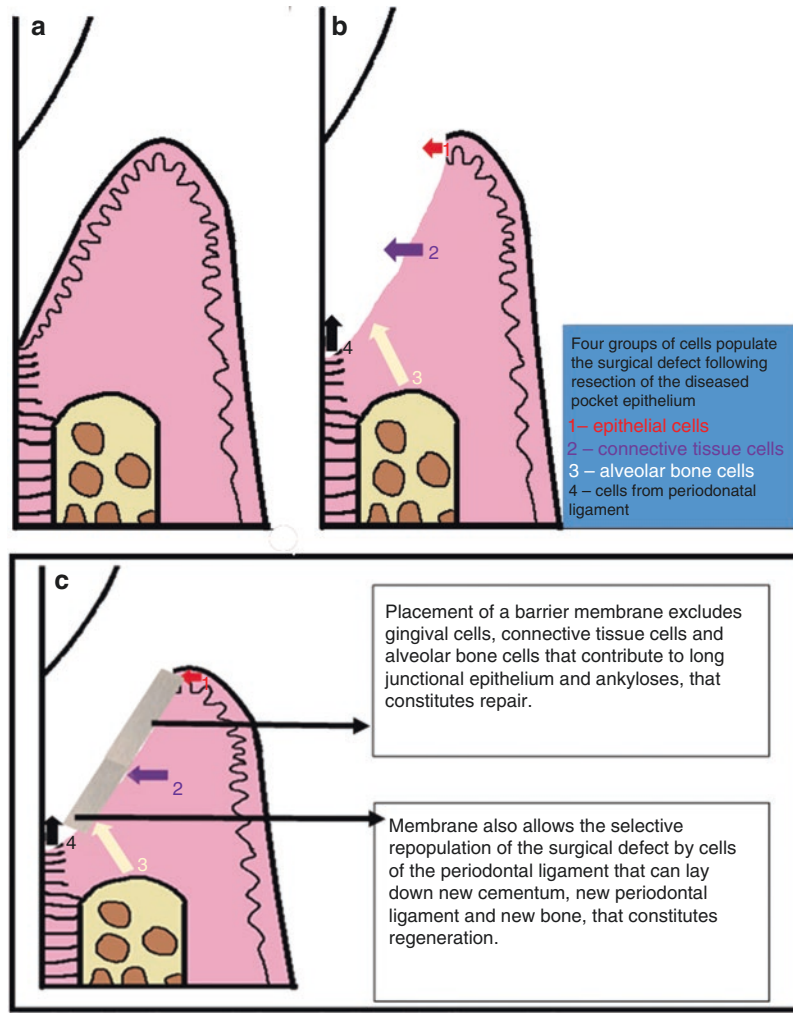

Fig. 3 (**a**) Periodontitis results in loss of supporting tissues of the periodontium. (**b**) Surgical excision or removal of the diseased pocket epithelium results in the repopulation of the surgical area by four groups of cells: (1) epithelial cells, (2) connective tissue cells, (3) alveolar bone cells, and (4) cells from periodontal ligament. (**c**) Placement of the barrier membrane, leading to cell exclusion and selective repopulation of the surgical defect

surgical wound can contribute four types of cells for possible repair/regeneration: epithelial cells, connective tissue cells, alveolar bone cells, and cells of the periodontal ligament (Fig. 3b) [49].

As discussed earlier, the repopulation of the defect by epithelial cells and fibroblasts results in the formation of an LJE, which constitutes repair rather than regeneration. The LJE situation remains susceptible to the recurrence of periodontitis. The population of the surgical site by alveolar bone cells results in ankylosis, which subsequently leads to replacement resorption, tooth mobility, and finally tooth loss. On the other hand, repopulation of the defect by cells of the periodontal ligament can result in the formation of new periodontal ligament, new bone, and new cementum.

Since the migratory rate of epithelial and connective tissue cells is faster than that of cells of the periodontal ligament, periodontal regeneration requires specific methods to prevent the migration of the epithelial and connective tissue cells into the periodontal defect, otherwise, it will heal by repair and not by regeneration as desired [49, 50].

The concept of isolating/preventing epithelial, connective tissue cells and osteoblasts (all of which are non-desirable cells for regeneration) by means of a barrier membrane, and at the same time guiding the periodontal ligament cells into the defect, results in the formation of new peri-

odontal ligament, new cementum and new bone (Fig. 3c). This concept is known as "*guided tissue regeneration*" or *GTR* [50, 51].

12.1 Barrier Membranes

Various regenerative barrier membranes are used for the purposes of periodontal regeneration. The ideal barrier membrane should possess various properties including being nontoxic, non-carcinogenic, non-antigenic, and sterile. The membrane should be able to maintain space, so that it can withstand the forces of suturing, and the weight of the overlying flap, so that under normal function including masticatory forces it does not collapse into the periodontal defect. At the same time, the membrane should be sufficiently flexible and easy to use, so that the clinician can mould and shape the membrane to adapted to the particular architecture of the periodontal defect that is being treated. Systematic reviews have shown that the use of barrier membranes and bone grafts results in improved results compared to open flap debridement alone [51].

Membranes should be bioresorbable, as this eliminates the need for a second stage surgical procedure to remove the membrane. In cases where they are non-resorbable, they should be easily retrievable. Additionally, membranes should have a long shelf life, and they should be inexpensive. Currently, the ideal barrier membrane does not exist, and the existing membranes that are available on the market represent a compromise of these various properties.

Barrier membranes are classified into either non-resorbable and resorbable types [52]. Expanded polytetrafluoroethylene (ePTFE) was used for the earliest work in guided tissue regeneration. Reinforcement of these membranes with a mesh or exoskeleton of very thin titanium, greatly improved their mechanical strength, and their ability to preserve the space at the surgical site. However, such non-resorbable membranes need to be removed after the initial healing period, necessitating additional surgical procedure, trauma to the tissues, and additional expenses to the patient [52].

Among the resorbable membranes, collagen membranes have been studied extensively. These are biocompatible, and also somewhat hemostatic in nature. They possess chemostatic properties, allowing them to induce the migration of host cells and to facilitate primary wound closure. This reduces the chances of membrane exposure and the associated secondary infection of the surgical site. Their hemostatic properties promote the establishment of a clot and improve the overall stability of the wound site. However, collagen membranes are delicate, and they lack mechanical strength and therefore can collapse into the defect. Hence, placement of bone grafts beneath a collagen membrane provides improved clinical outcomes [53].

There are many disadvantages of using membranes for periodontal regeneration. Exposure of the membranes leads to localized infection which causes failure of the procedure. Exposed membranes have been shown to harbour significantly higher levels of *Aggregatibacter actinomycetemcomitans,* a bacterial species that produces potent leukotoxins, resulting in worse clinical outcomes. Membranes must be placed in areas with sufficient soft tissue, to ensure complete coverage of the membrane. In larger defects, placement of membranes without underlying bone grafts may result in the collapse of the membrane [54].

As well as these available materials for periodontal regeneration, enamel matrix proteins have become an important addition to the armamentarium for guided tissue regeneration in recent years. Before the formation of cementum, a thin layer of enamel matrix proteins, known as amelogenins, is deposited onto the root surface. This layer of enamel matrix proteins plays a vital role in the formation of cementum, as they initiate differentiation of periodontal ligament cells into cementoblasts. Following the isolation of enamel matrix proteins from various animal and human sources, they have been investigated extensively. The use of enamel matrix proteins or amelogenins enhances the formation of periodontal tissues in osseous defects. Enamel matrix proteins have been widely used alone and combination with other materials [55].

13 Periodontal Plastic Surgery

"Periodontal (and peri-implant) plastic surgery encompasses the surgical procedures performed to prevent or correct anatomical, developmental, traumatic or disease-induced defects of the masticatory mucosa (gingiva), lining mucosa (alveolar mucosa) or bone" [56]. Clinicians need to consider the thickness of the gingival tissue during the planning of periodontal plastic surgeries, taking into account a phenomenon termed as "periodontal phenotype," previously known as the periodontal biotype [57].

Patients with thin friable gingival tissue, a minimal amount of attached gingiva, thin underlying alveolar bone and long, narrow, conical crowns are known as having "thin periodontal phenotype." This tissue is more prone to gingival recession and interdental papilla loss, which results in poorer clinical outcomes after periodontal operations.

Patients with thick fibrotic gingival tissue, wide zones of attached gingiva, thick underlying alveolar bone and short-wide crowns are considered to have a "thick periodontal phenotype." This gingival tissue type is resistant to gingival recession and provides better clinical outcomes following periodontal plastic surgical procedures [58]. Placement of soft tissue grafts can convert a thin periodontal phenotype to a thick periodontal phenotype [59]. Figure 4 lists the major differences between thin and thick periodontal phenotype.

Soft tissue defects include gingival recession, interdental papilla loss, and mucogingival deformities. These defects can be treated, with several options available including autografts, allografts, and xenografts.

Autografts include a lateral pedicle flap, free gingival grafts, and sub-epithelial connective tissue grafts. Free gingival grafts are easier to harvest, however, they result in a colour mismatch between the grafted site and the adjacent tissues. Sub-epithelial connective tissue grafts are considered as the gold standard among soft tissue grafts [60].

For the treatment of periodontal soft tissue defects, gingival tissue can be harvested from the hard palate [61]. However, these procedures are technique sensitive and can result in a number of postoperative complications including pain, bleeding from the palate, exposure of the underlying bone and its necrosis, paresthesia, and permanent anesthesia due to damage to the greater palatine nerve [62]. Periodontal dressings are placed in the palate to protect the donor site and held in place by an acrylic stent that must be worn by the patient during the immediate postoperative period to retain the dressing. Additionally, in some individuals, autografts harvested from

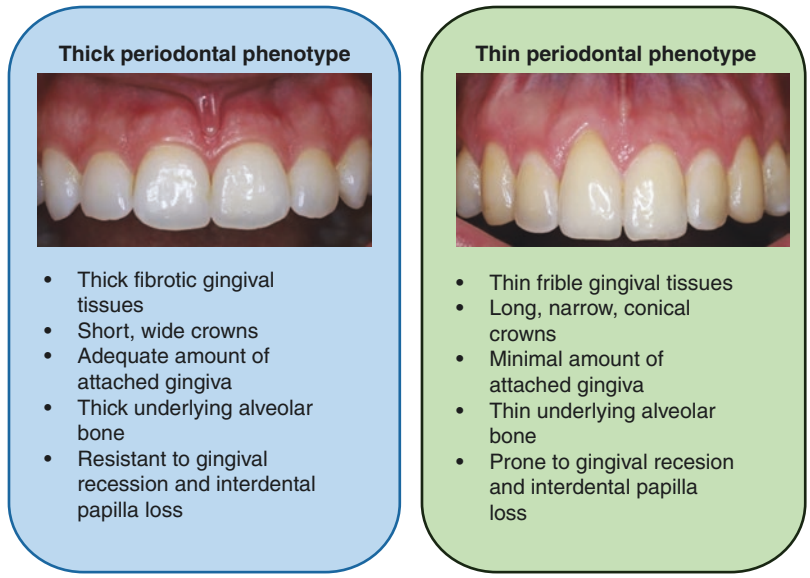

Fig. 4 Differences between thick and thin periodontal phenotypes [61] (Courtesy of Dr. Aya Alali and Dr. Salah Mortaja from the University of Queensland School of Dentistry, Australia)

the palate do not have an adequate thickness of tissue.

Because the majority of these complications are related to the creation of a second surgical site while harvesting the graft, various techniques have been developed to avoid the need for a second surgical site. Such options include allografts and xenografts. Table 4 lists the various soft tis-

Table 4 Various soft tissue grafts, their sources, advantages, and disadvantages [63]

Name of the graft	Type of graft	Source	Advantages	Disadvantages
Lateral pedicle flap	Autograft	Gingiva from adjacent teeth	Technique sensitive	Adjacent area may have an inadequate amount of tissue, so the technique can be used in very limited instances
Sub-epithelial connective tissue grafts	Autograft	Palatal mucosa, rarely from retromolar pad and adjacent edentulous site	Considered as the gold standard among soft tissue grafts. Can be harvested from the palate, so is economical	Creates a second surgical site with related possible complications including: impaired wound healing, severe pain, necrosis of the bone, profuse bleeding from the palate, paresthesia, and permanent anesthesia of the palate
Free gingival/ epithelial grafts	Autograft	Palatal mucosa, rarely from retromolar pad and adjacent edentulous site	Can be harvested from the palate, so is economical	Similar disadvantages associated with harvesting donor graft from the second surgical site, as mentioned above. Mismatch in the color between the grafted area and normal gingival tissue. Donor site heals by secondary intention, so donor site is painful for long period
Alloderm® regenerative tissue matrix	Allograft	Tissue banks obtain tissue from the skin of donated human cadavers. Following thorough processing, antigenicity is removed to prevent transmission of virus and infections	Will be useful in multiple recession defects wherein more quantity is essential. Correction of cancer surgeries. Correction and repair of burn injuries. Used instead of autografts	Expensive
MucomatrixX®	Xenograft	Consists of collagen and elastin derived from animal tissue, to remove all antigenic properties	Provides a stable three-dimensional matrix consisting of collagen and elastin	Expensive
Mucograft® collagen matrix	Xenograft Porcine origin	Consists of pure porcine collagen, following sterilization and processing, to remove prion-related infections	The product contains pure collagen	Expensive
Platelet-rich fibrin (PRF)	Autologous	PRF is obtained by autologous means and is compressed as a membrane	Preparation is easy with a simple centrifuge machine and is inexpensive. Contains growth factors that assist in the regeneration	PRF membranes are thin, so suturing may lead to tear of the membrane

sue graft options, with their relevant advantages and disadvantages [63].

14 Concept of Periodontal Tissue Engineering

As discussed in the introduction, the periodontium and the tooth form a highly developed functional structure [64, 65]. Periodontal diseases (such as gingivitis and periodontitis) are one of the most common inflammatory diseases seen in adults, and they cause high financial costs for their direct treatment [2].

Advance periodontitis can cause numerous problems such as increased mobility and loss of teeth, aesthetic problems, halitosis, and loss of masticatory efficiency, leading to changes in the diet and in food selection. When one takes into consideration the cumulative effect of untreated periodontitis on systemic health conditions, such as diabetes, periodontal disease is the oral disease that has potentially the most negative impact on the quality of life of an individual [66].

In line with developments in technology and science, periodontal treatment has also changed over time. In the early stages of periodontology, non-surgical and surgical treatment was directed towards removing the biofilm and the tissue that was thought to be infected. Subsequent histological examinations of these interventions led to a better understanding of periodontal regeneration. It soon became apparent that traditional closed debridement allowed epithelial cells to migrate to the root surface, reaching this before other cell types, and thus providing by creating a long junctional epithelium. Using this traditional approach meant that full regeneration of periodontal tissues could not be achieved [67].

Once the concept of guided tissue regeneration (GTR) had been developed, and begin to be used more widely as a surgical procedure for regeneration of the periodontium, it was realized that preventing epithelial cell migration using barrier membranes was only one of the first parts of the overall solution. Later work showed the importance of bone substitutes, root surface demineralization procedures, and the need to combine tissue regeneration protocols with periodontal treatment [68]. The sequential development of the concepts is shown in Fig. 5.

The term periodontology and tissue engineering were used together for the first time about 20 years ago [68, 69]. Modern periodontal tissue engineering targets the treatment of damaged periodontium using cells, bioactive molecules, and scaffolds together, in various combinations to achieve periodontal regeneration [65].

14.1 Stem Cells and Cell Sheet Technology

The concept of tissue engineering for periodontal regeneration can be classified into scaffold-free or scaffold-based approaches, depending on whether biomaterials are used or not. In the scaffold-free concept, cells can be placed directly into a periodontal defect without a cell carrier. This regenerative approach, which includes a single stem cell type or a combination of stem cell types, is called "cell sheet" technology [8, 70–72].

Iwaka et al. stated that according to the cell sheet engineering principle, at 37 °C cells adhere to and proliferate on the surface of a temperature-sensitive polymer comfortably, because the polymer is anhydrous and compact at this temperature. At temperatures below 32 °C, layers of cells with extracellular matrix proteins are spontaneously separated from the temperature-sensitive polymer [73]. This approach to release the cell sheets from the culture flask without the need to use any enzymes or other chemicals opened up a broader research field.

Periodontal ligament stem cells (PDLSCs), bone marrow-derived mesenchymal stem cells (MSCs), dental pulp stem cells (DPSCs) and fat-derived stem cells (ADSCs) have been investigated to determine their ability to induce periodontal regeneration [70–72]. There are now several studies showing promising results for the application of cell sheets in various in vivo models. Raju et al. recently reported successful in vivo periodontal regeneration, in a large peri-

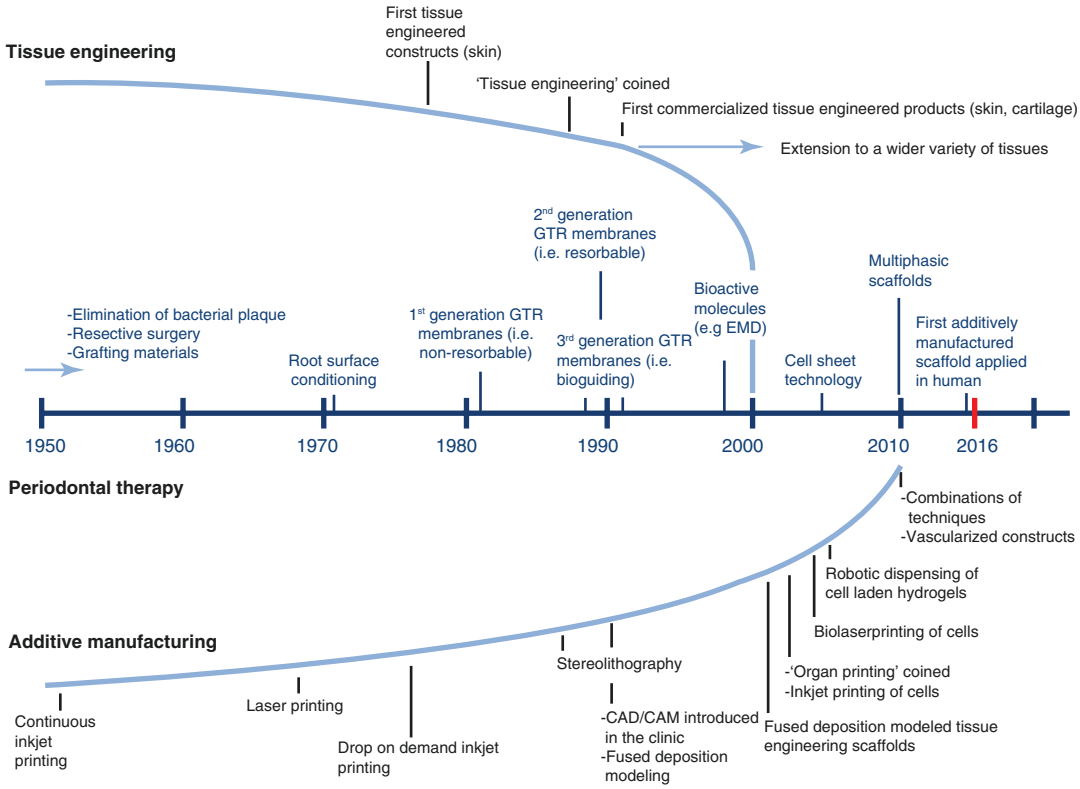

Fig. 5 Reflection on historical developments in tissue engineering and additive manufacturing to periodontal regenerative therapy. In the past 20 years, applications in the periodontology field of tissue engineering have focused on enabling regeneration [69]

odontal defect, fabricating a three-dimensional cell sheet, including a bone-ligament component, by layering together periodontal ligament cells and osteoblast-like cells [74].

Despite the enormous promise of the cell sheet approach, the use of robotic systems for sterile cell culture in the transplantation of allogeneic frozen cells significantly increases the cost of cell therapies, limiting the widespread use of this technology [73, 75].

14.2 Biomaterials for Periodontal Tissue Engineering

In periodontal tissue engineering, various biomaterials with different targets can be used alone or in combinations. As discussed earlier, while those materials with relatively high mechanical strength, such as hydroxyapatite (HA), are used for filling defects in cases involving the regeneration of alveolar bone and cementum, softer polymeric materials, such as collagen, which have relatively low mechanical strength, are used for periodontal ligament and gingiva regeneration [8].

Ceramic biomaterials are used particularly in hard tissue engineering, due to their similar structures and chemical composition. HA, calcium phosphate (CaP), calcium sulfate [9], and bioactive glass (BG) are the most extensively researched bioceramics in periodontal regeneration [69].

HA was one of the first biomaterials used in periodontal tissue engineering. It can be derived from natural bovine bone or used as a pure synthetic material [76]. Besides their advantages such as slow degradation, and their ability to stimulate and enhance the proliferation of osteoblasts, bioceramics also have disadvantages, such as fragility and low bioactivity [69, 76].

Polymers used in periodontal tissue engineering are divided into two groups: synthetic polymers and natural polymers. Synthetic polymers such as polylactic acid (PLA), polycaprolactone (PCL), copolymer poly lactic-co-glycolic acid (PLGA) and polyglycolic acid (PGA) have properties that be adjusted readily, and they can be manufactured with impeccable repeatability. Nevertheless, during the printing process of synthetic polymers, they pass through stages that are inherently harmful to the viability of cells, such as high temperature. These procedures complicate attempts to combine cells and polymers [69]. Natural polymers include biomaterials such as albumin, hyaluronic acid, cellulose, chitosan, and collagen [76].

These various biomaterials can be altered or unified into composite materials to create a suitable microenvironment and used in scaffolding systems to induce periodontal regeneration. However, complete imitation of the unique architecture of the periodontium remains challenging, even with currently available biomaterials, and this area still requires much further research [8].

14.3 Scaffolds

The ideal scaffold used in tissue engineering should prevent the collapse of the site during wound healing; it should promote the ingrowth of cells and blood vessels, and be easy to manage. The main aspects of scaffolds include their morphology (including porosity), mechanical strength, and chemical composition. The latter affects their rate of degradation [4, 77–79]. Manufacturing technologies, such as three-dimensional (3D) printing, provide precise control over the architectural and dimensional aspects of scaffolds, to ensure that they are conducive for reproducing the unique structure of periodontium [80].

Scaffolds for use in periodontal regeneration are inspired by guided tissue regeneration. Specially designed biomaterial scaffolds protect the surgical site and promote the formation of periodontal tissues during healing [3].

Scaffolds for periodontal tissue engineering can be monophasic or multiphasic. In monophasic scaffolds, the cells are encapsulated in various hydrogels, or they are planted on carrier scaffolds and then placed in periodontal defects. During wound healing, monophasic scaffolds play a protective role in maintaining the shape of the periodontal defect, and transporting the necessary cells as a carrier. Another role of monophasic scaffolds in periodontal regeneration is to protect the wound area against the challenge of bacterial infection from the oral environment, for example, by releasing antibacterial agents. Furthermore, monophasic scaffolds can be used to deliver growth factors to the defect region [3, 77]. Although monophasic scaffold technology is theoretically a simple concept, such scaffolds can be utilized for many purposes, by using different combinations of biomaterials and cell types [14].

The periodontium has a unique layout and contains many different structures, cells, and tissues. In order to perform periodontal regeneration successfully, each sub-tissue group that constitutes the periodontium must create coordinated sub-regenerations within a certain sequence (Fig. 6). One of the most important developments in periodontal tissue engineering is to emulate this complex regeneration system, by designing multiphasic scaffolds, each representing a different periodontium tissue [14].

In periodontal regeneration, multiphasic scaffolds mainly target the regeneration of soft-hard tissue interfaces, that is, between the alveolar bone and periodontal ligament, and between the periodontal ligament and the cementum. Thus far, biphasic and triphasic scaffolds have been introduced to periodontal tissue engineering [77].

Park et al. reported the usage of a biphasic scaffold, produced with polyglycolic acid for the bone phase and polycaprolactone for the periodontal ligament phase [81]. Following this study, the same technique was tested in vivo in a periodontal defect model. Perpendicularly oriented microchannels guided the periodontal fibers [82].

Lee et al. fabricated a scaffold from a mixture of PCL and hydroxyapatite (90%/10%), and used this to create a triphasic scaffold. These three

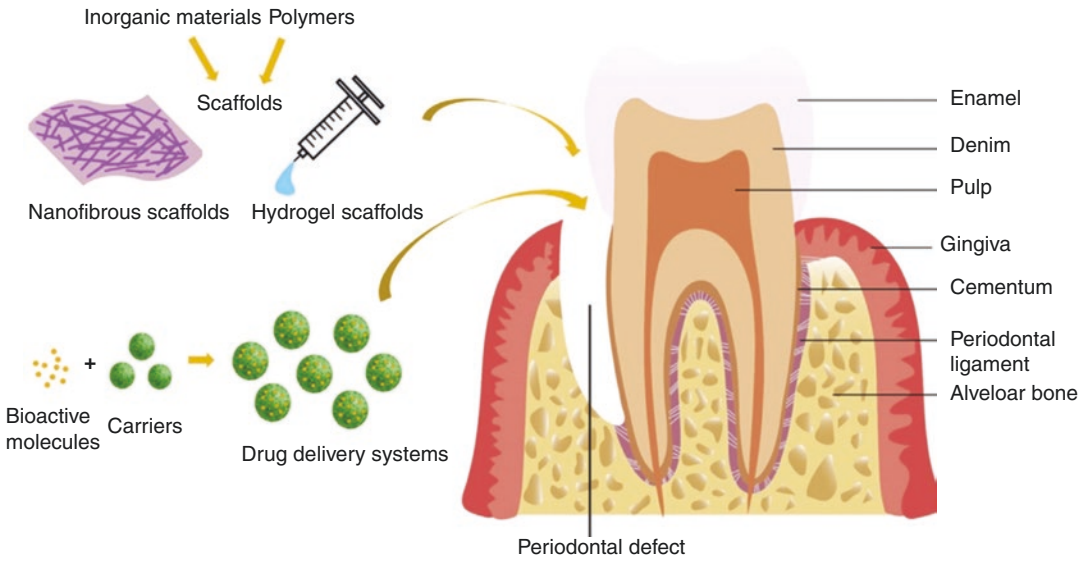

Fig. 6 Different approaches used in modern periodontal tissue engineering. Various combinations of cells, biomaterials and bioactive molecules can be the key to achieve complex periodontal regeneration in the future [8]

compartments consisted of 100 μm, 600 μm and 300 μm microchannel architecture, representing the cementum-dentine interface, the periodontal ligament, and the alveolar bone respectively. In this study, distinctive tissue phenotypes were formed after 4 weeks of separate in vitro incubation with periodontal ligament stem cells (PDLSCs), alveolar bone stem cells (ABSCs) or dental pulp stem cells (DPSCs). PDLSCs made up tissues that were rich in collagen I, while mineralized tissues were produced by DPSCs, PDLSCs, and ABSCs. This approach is a promising strategy for complete periodontal regeneration involving different tissue types [83].

14.4 Growth Factors

Growth factors are proteins that direct cellular behaviour in the relevant tissue, during physiological remodelling. In order to fulfill their duties, they must be released to the region at specific times and in sufficient doses. They can be delivered to the site directly, from cells, or transported there by a carrier. To date, growth factors that have been used for periodontal regeneration include bone morphogenic proteins, enamel matrix derivatives, vascular endothelial growth factor, platelet-derived growth factor, transforming growth factor and fibroblast growth factor [65].

Enamal Matrix Derivatives

The main component of Enamal Matrix Derivative (EMD) is amelogenin. Amelogenin is a unique extracellular matrix protein that directs mineralization of the enamel. It stimulates new bone formation and wound healing, when used under appropriate physiological conditions. There are studies showing that EMD can mimic odontogenesis and promote the stimulation of cementoblasts on the root surface [4, 55, 84].

The largest controversy around EMD is regarding the gel structure of the material, and whether or not it will remain in the wound environment after surgery. However, immunohistochemical evaluations have shown that EMD does continue to stay on the root surface for some 4 weeks after application. EMD is commercially available for clinical usage (Emdogain®) in more than 100 countries [84, 85].

Bone Morphogenic Proteins

Bone Morphogenic Proteins (BMPs) cover more than 20 growth and differentiation factors. These proteins are also members of the TGF-β superfamily [11]. BMPs, along with their receptors, have been proven to exist in periodontal tissues. Due to the potential use of BMPs to improve periodontal wound healing and regeneration, most research has been done on BMP-2, BMP-7, and BMP-3. The United States, Food and Drug Administration (FDA) approved the use of BMP-2 for maxillary sinus augmentation and localized ridge augmentation. Animals studies using BMP-2 in alveolar ridge augmentation have provided promising results [65].

Fibroblast Growth Factor-2

Fibroblast Growth Factor-2 (FGF-2) has been investigated for periodontal treatment due to its angiogenic and mitogenic effects in wound healing. FGF-2 increases the proliferation of fibroblasts. Additionally, it increases the release of osteoblast differentiation factors, thus accelerating bone formation. This protein has been approved for use in Asia and in the USA for periodontal clinical research [86].

Platelet-Derived Growth Factor

Platelet-Derived Growth Factor (PDGF) is arguably the most well-researched growth factor in dentistry and it plays an important role in wound healing. PDGF is chemotactic for periodontal ligament cells, causing them to migrate into the defect area. Additionally, PDGF enhances fibroblast proliferation and collagen synthesis. PDGF has several isotypes (AA, AB, BB). Recombinant human PDGF isotype BB (rh PDGF-BB) is available commercially in the United States for use in periodontal therapy [4, 87].

15 Soft Tissue Engineering

The simultaneous exposure of the root surface in the oral cavity and the apical relocation of the gingival margin from the cemento-enamel junction constitutes gingival recession. In most cases, there is resorption of buccal alveolar bone. While GTR techniques were used initially to treat gingival recession, it became clear that this approach had numerous limitations in thin phenotype cases and in Miller type 1 situations [88, 89].

As discussed earlier, the total renewal of the periodontium involves the regeneration of both soft and hard tissues. Surgical applications that aim to achieve only the regeneration of soft tissues can be performed for cases of gingival recession and mucogingival defects where there are keratinized tissue deficiencies [88, 90], to address problems with aesthetics and cervical dentinal hypersensitivity due to the exposure of the root surface in the oral cavity [89]. For mucogingival surgical procedures, autogenous grafts (free gingival grafts and connective tissue grafts) remain the "gold standard," even though both have drawbacks including the necessity for a secondary surgical procedure at the donor site a risk of postoperative complications such as paraesthesia and hemorrhage, and the limited amount of material that can be harvested.

There is a wide range of biomaterials on the market, designed for use with different technologies for mucogingival applications. These biomaterials may be allogenic (e.g., AlloDerm®), xenogeneic (e.g., Mucograft®), or tissue engineering materials (e.g., living cell constructs). Only Alloderm and Mucograft have been examined extensively. Both have been relatively successful as alternatives to autogenous grafts for mucogingival surgery [90].

In past years, various biomaterials have been used in periodontal regenerative medicine as a connective tissue scaffold, involving various tissue culturing techniques and tissue engineering approaches. Recent tissue engineering research has investigated the possibility of using synthetic biomaterials with live-cell constructs as an alternative to autografts for mucogingival surgery [91]. However, the performance of live cell constructs has not yet been fully explored in human trials. Live cell constructs have been assumed to improve the wound environment through wound coverage, growth factor interactions, and enhanced matrix accumulation [92], however such mechanism remains largely speculative at present.

16 Challenges in Periodontal Regenerative Therapies

One of the most thought-provoking points regarding clinical applications of regenerative periodontal medicine is the various limitations. To achieve optimal outcomes, every clinician should appreciate the challenges, contraindications, and limitations related to the technique being used, and the patient and site that the technique is being used on [11].

16.1 Variables Influencing Periodontal Regeneration

Predictable regeneration of periodontal tissues remains as a major challenge. Anatomically, the periodontium is a combination of hard and soft tissues. The hard tissue compartment consists of alveolar bone and root cementum, whereas the soft tissue compartment consists of the periodontal ligament and the gingiva [14]. A range of regenerative approaches have been developed to regenerate the periodontium, including GTR, bioactive molecules, bone grafts, soft tissue grafts, and tissue engineering. The results of these diverse applications are still not predictable [93–97].

On the other hand, the progress of periodontal regenerative medicine has been increasing year by year, with many recently invented materials and techniques. All bioactive materials also need progenitor cells to fulfill their purpose. The various progenitor cells (pre-fibroblasts, pre-cementoblasts, and pre-osteoblasts) must first migrate to the defect site, proliferate, and then mature into functional periodontium tissues, in the correct sequence. The success of this depends on the presence of suitable growth factors and the control of the progenitor cell gene expression [98, 99].

From this standpoint, it can be concluded that periodontal regeneration depends on the following variables:

(a) A sufficient supply of suitable progenitor cells, with the ability for polarization into the required mature tissue-forming phenotypes (fibroblasts, cementoblasts, and osteoblasts).
(b) An adequate cavity or space for regeneration of the tissues to take place, which is maintained by a physiological or therapeutically placed biomaterial.
(c) The proper signals to coordinate cellular polarization and tissue maturation [11].

In addition to these variables, patient factors (systemic factors, personal habits), selecting the appropriate surgical technique, local factors (tooth type and defect type) and the surgeon's experience also influence the outcomes. All these variables must be considered together during the planning of periodontal regeneration therapy [100].

16.2 Patient-Related Factors

Patient-related variables that affect the outcome of periodontal regenerative approaches include oral hygiene habits, systemic conditions, and smoking. As well, variables such as age, genetics, and stress have been suggested to have negative effects on periodontal regenerative therapy, but there is not sufficient scientific data at present to support this.

During the first few weeks after initial periodontal therapy, observing a patient's compliance and motivation in regards to changing personal habits is highly informative [100]. If the patient manages to perform satisfactory oral hygiene, this provides some optimism, since poor plaque control will hinder all regenerative periodontal processes. Many studies have demonstrated the value of professional maintenance and high standards of plaque control, both for non-surgical conservative periodontal approaches, as well as periodontal surgery, to maintain gains in clinical attachment and to reduce probing depths over the long term [100, 101].

Smoking is a substantial modifying factor for periodontal disease [102]. It impairs wound healing after surgical therapy and after non-surgical treatment [103, 104]. During wound healing, smoking reduces fibroblast cell attachment and blood flow through the tissues [105]. Smokers have worse periodontal inflammatory

conditions compared to non-smokers [106–108]. Furthermore, smoking causes worse clinical outcomes from regenerative periodontal treatment [102, 109], with less gain of alveolar bone in periodontal defects [110–114].

Other patient factors include systemic health. It is now recognized that there is a complex interplay between oral health, periodontal health, and systemic health. The concept of "periodontal medicine" is well researched, with various associations (mostly due to common risk factors) between periodontal health and 57 different systemic conditions (including in descending order of importance diabetes mellitus, pregnancy, rheumatoid arthritis, respiratory diseases, cardiovascular diseases, psoriasis, and chronic kidney diseases). The periodontium can be an important aspect of the total body burden of infection [115], however, effects of periodontal treatment on the progression of these systemic conditions has yet to be clearly documented. One notable exception is diabetes mellitus, which has a bidirectional relationship with uncontrolled periodontitis. There is a higher prevalence and greater severity of periodontal disease in poorly controlled diabetic patients than in individuals without diabetes [116–118].

16.3 Surgical Technique

Periodontal intrabony defects vary in topography from wide to narrow and very deep to shallow. Most surgical periodontal protocols are effective in the treatment of intrabony 1, 2 and 3 wall defects or a combination of them [33]. Appropriate surgical techniques and materials selection are important. Surgical approaches that use a papilla preservation flap design, and suture techniques that ensure tissue integrity, stability, and primary closure of tissues, are associated with better regenerative results [119–121]. In 2019, a meta-analysis reported that papilla preservation flaps improve the clinical outcome of regeneration procedures and should be considered a surgical pre-requisite [122].

The selection of the correct material in periodontal regenerative therapy is equally as important as choosing the correct surgical technique. However, even with the proper material, complications can still occur, such as membrane exposure after surgical intervention. Exposure of the membrane negatively affects the outcome of regenerative surgical procedures, especially when it is related to a non-resorbable membrane.

This has led to the design of modified surgical approaches to maintain interdental periodontal tissues and reduce the chances of membrane exposure [123, 124]. Cortellini et al. demonstrated that the prevalence of membrane exposure could be decreased with the utilization of specifically designed flaps that protect the interdental tissues by using the modified papilla preservation technique [119, 121, 125]. Numerous studies have demonstrated that membranes that become exposed to the oral area are readily infected with bacteria, and this bacterial contamination (whether of non-resorbable or resorbable membranes) lowers attachment gains in intrabony defects [126–130].

Better periodontal regenerative results can be achieved if the patient is worked up with a meticulous pre-surgical examination, to explore the anatomy of the defect, the interdental space, and determine which type of material is going to be used [131]. A multicenter randomized study has shown that shallow and deep bone defects are likely to give similar regenerative outcomes [132]. However, each patient has their unique characteristics, and each defect is unique. No one periodontal regenerative protocol can address all possible defects by itself. Consequently, there must be a clinical decision pathway that leads the surgeon to select the best surgical approach for periodontal regeneration of each individual defect [33, 34].

16.4 Local Factors

The defect and the tooth condition have an important impact on the success of periodontal regeneration locally. These aspects should be evaluated clinically and radiographically.

Defect Factors

The predictability of periodontal regenerative therapy is influenced by the local anatomy and

the nature of the periodontal defect. Tonetti et al. showed that intrabony periodontal defects deeper than 3 mm show superior probing attachment and alveolar bone gain, than shallow defects after GTR [133]. Tonetti et al. also reported that the morphology of the defect can influence the results, with greater regeneration of deep, narrow defects with more remaining bone walls [133, 134]. In a different study, Tonetti et al. showed that better regeneration was achieved in non-smokers and for intrabony defects with three walls [135].

Tooth Factors

Endodontic status and tooth mobility are potential factors of interest. Ehnevid et al. demonstrated that inadequately treated and endodontically compromised teeth respond less favorably to periodontal regenerative approaches [136, 137]. On the other hand, when performed precisely, endodontic therapy does not impact the healing outcome and the long-term results when treating deep intrabony periodontal defects with regenerative protocols [138].

According to a multicenter clinical trial, greater tooth mobility is related to worse clinical outcomes for periodontal regenerative protocols [120]. In particularly, Miller grade III mobility adversely affects the outcomes. However, teeth with mobility lower than 1 mm horizontally can successfully be treated with periodontal regenerative protocols [139].

Aside from these various factors, there is one further very important factor that has a strong impact on the outcomes of regenerative therapy: *operator experience*. Predictable clinical outcomes require meticulous diagnosis and treatment, hence the clinical skills of the operator influence the success of periodontal regenerative treatments [131, 140].

17 Future of Regenerative Periodontics: What Is Next?

Current developments in the field of regenerative periodontology are exciting. While many techniques in tissue engineering have produced promising results from in vitro and in vivo studies, few methods have been applied to humans. In 2015, Rasperini et al. reported the first human case of surgical treatment of a periodontal defect with a 3D printed scaffold. A customized 3D laser sintered approach was used in the production of this scaffold, which incorporated PCL and a combination of HA powders. The scaffold was used to treat a very large periodontal defect. It did not cause any inflammatory reaction in the first 6 months. However, due to exposure of the scaffold after 12 months, it was found to be clinically inadequate [141].

One of the most important developments in biological fabrication is the production of a whole tissue or organ by utilizing bioprinting techniques. The goal of biological printing is to build a particular tissue or a whole organ by using the living cells of the individual, placed in an extracellular matrix environment. Improvements in biological printing have led to progress in some branches of medicine, whereas the biological production of oral tissues using the same method is still in its early stages.

Raveendran et al. reported an optimization study involving the 3D bioprinting of periodontal ligament cells, where a gelatin methacryloyl hydrogel (GelMA) was used as the carrier. Various parameters such as the concentration of the hydrogel, the printing pressure, and the aperture of the needle used for printing influenced the resolution and dimensional stability of the bioprinting process [80].

Gene therapy, on the other hand, had led to new approaches to treat hereditary dental diseases [4]. Gene therapy usually involves placing relevant genes in an individual's cells to achieve an increase in the release of a specific growth factor. Gene therapy can be performed by two methods. In the first method, the gene vector is placed directly into the target region (in vivo). Alternatively, in a second approach, the selected cells are harvested, expanded, and then genetically transduced, for example, using an adenovirus vector or another vector (ex vivo) [142].

Although gene therapy is a promising method, it is debatable whether the use of adenovirus for this purpose is safe [143]. Variations in immune

responses to gene therapy may also expose problems. However, the approach of stimulating the genes of the cells towards targeted tissue regeneration may emerge as a very important treatment alternative in the future, not only in periodontology, but in other disciplines [4, 143].

As discussed earlier, the combined use of growth factors and 3D scaffolds with biological materials for periodontal regeneration is one of the most important developments in tissue engineering. However, the biomolecules used in the production of growth factors are expensive, and the production of 3D scaffolds is a highly demanding process. Furthermore, even the use of a low dose of biomolecules can cause side effects in some individuals. These reasons make it difficult for innovative regenerative treatment protocols to be accessible for everyday clinical usage [75]. However, various research groups from all around the world are working to make these protocols more user-friendly for clinicians.

18 Conclusion

The ultimate goal of periodontal regenerative medicine is to prevent tooth loss by reproducing the supporting tissue of the tooth. Regenerative treatment with the combination of available surgical techniques and materials is generally limited to defined types of periodontal defects. Periodontium regeneration must rest on a solid biological rationale, histological evidence, as well as evidence of achieving the desired clinical outcomes [144].

Recent developments have combined cells, appropriate biomaterials, and growth factors for periodontal regeneration. There have been many other positive developments in the regeneration of complex alveolar bone defects. Techniques for the production and design of scaffolds are becoming more predictable [3].

It is an extremely difficult task to completely renew periodontal tissues, both functionally and morphologically. Current scientific studies state that any single regenerative approach is unlikely to be successful for every purpose. A key point is that migration of soft tissue into the periodontal defect should be prevented, and appropriate pioneering signals should be released. Therefore, the use of multiple layer manufacturing systems for tissue engineering seems to hold the greatest promise [145].

Any regenerative approaches must ensure effective control of local infections caused by microbial pathogens during healing. Therefore, a suitable strategy must consider how best to limit bacterial growth, [4] and ensure that patient's maintain good levels of plaque control. Clinical improvements after regenerative therapy can be maintained for a long period of time, in most treated areas, if patients do not smoke and continue to maintain high standards of oral hygiene [89]. A further factor that influences outcomes is the surgeon's ability to choose and then apply the best surgical technique, based on patient-specific and region-specific criteria.

New periodontal regenerative approaches seem promising for the transition to clinical practice in the not too distant future. Each method that has been successful in in vitro approaches, however, needs to be tested extensively in vivo in animal models and then in human clinical trials. Any biological products that are to be used in daily treatments must be free of pathogens and of high quality and meet the required regulatory approvals.

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