Clinical evidence indicates that in physiological and therapeutic conditions a continuous remodeling of the tooth root cementum and the periodontal apparatus is required to maintain tissue strength, to prevent damage, and to secure teeth anchorage. Within the tooth’s surrounding tissues, tooth root cementum and the periodontal ligament are the key regulators of a functional tissue homeostasis. While the root cementum anchors the periodontal fibers to the tooth root, the periodontal ligament itself is the key regulator of tissue resorption, the remodeling process, and mechanical signal transduction. Thus, a balanced crosstalk of both tissues is mandatory for maintaining the homeostasis of this complex system. However, the mechanobiological mechanisms that shape the remodeling process and the interaction between the tissues are largely unknown. In recent years, numerous 2D and 3D in vitro models have sought to mimic the physiological and pathophysiological conditions of periodontal tissue. They have been proposed to unravel the underlying nature of the cell–cell and the cell–extracellular matrix interactions. The present review provides an overview of recent in vitro models and relevant biomaterials used to enhance the understanding of periodontal crosstalk and aims to provide a scientific basis for advanced regenerative strategies.

1. Introduction

1.1. Periodontal Tissue Crosstalk

The periodontal tissue is a complex connective structure composed of diverse cell types that continually interact to maintain alveolar bone, gingiva, cementum, and periodontal ligament.[1] Of these four tissues that establish the periodontium, the periodontal ligament (PDL) is closely attached to the alveolar bone and cementum, which in turn connects tooth to the bone socket (Figure 1A,B). PDL is a fibrous tissue composed of heterogeneous cell populations (fibroblasts, cementoblasts, osteoclasts, osteoblasts, osteocytes, endothelial cells), and non-cellular components (made mostly of collagen fibers and non-collagenous extracellular matrix [ECM] proteins and polysaccharides) that are all intertwined with the nerve fibers and blood vessels.[2] It serves as a cushion for the mechanical stimuli created during mastication and provides a protective barrier to the microorganisms in the oral cavity.[3,4]

The periodontal apparatus is a unique structure. Nowhere else in the body does a soft tissue have a comparable ability to regulate hard tissue remodeling due to mechanical stress (i.e., shear, tension, and compression). During local inflammation pro-inflammatory enzymes, cytokines and chemokines are released and immune cells are recruited, inducing local remodeling.[3,7,8] This periodontal tissue crosstalk is essential in order to regulate local tissue reshaping and is central to maintaining the physiological function of the tissue (Figure 1C). However, when this system is disturbed the mentioned interactions do not work properly. This can provoke a breakdown of soft and hard tissue accompanied by cytokine and prostanoid cascades, leading to tissue destruction. Even if the inflammation is resolved by treatment and some of the bone and connective tissue is regenerated, restoration of degenerated tooth supporting tissues remains a challenge.[9]

1.2. Periodontal Remodeling

In reaction to mechanical stimulation like orthodontic forces, other dental interventions or trauma the local tissue produces inflammatory responses (i.e., a synthesis of pro-inflammatory mediators including enzymes, cytokines, and chemokines) in order to induce local tissue remodeling. This naturally occurring process is regulated mainly by biomolecules that are released by the connecting periodontal tissue, but also by cells from the innate immune system that migrate to the PDL.[10] The remodeling in the PDL is controlled by two different regulative processes, both of which have inflammatory reactions in the background, but are
induced either by bacteria or non-bacterial stimuli. It is essential to differentiate the origins of these inflammatory reactions, although they can occur simultaneously in some cases. Various mechanical trauma or injuries caused by chemical agents can cause “sterile inflammation,” a type of inflammation unrelated to the presence of specific microorganisms. Nevertheless, the reaction of the local tissue is similar to microbially induced inflammation. This means that even in the case of “sterile inflammation” signs typical of microorganism presence are observed, such as the recruitment of neutrophils and macrophages that accompanies the intensified release of pro-inflammatory cytokines and chemokines.[11] A strong correlation between these two common types of inflammations of the periodontium also share biochemical actors that can regulate the processes (e.g., pattern recognition receptors [PRRs][12]). A finely tuned immune response is therefore a critical factor for reaching homeostasis while allowing healing process of the periodontium to conclude properly. There is a differentiation between the endogenous molecules termed damage-associated molecular patterns (DAMPs) and the exogenous molecules, conserved structural moieties that are found in microorganisms, termed pathogen-associated molecular patterns (PAMPs).[11] Thus, the periodontal remodeling caused by inflammatory reactions can be classified as follows:

A) Periodontal remodeling regulated by sterile (non-bacterial) conditions: an endogenous process that takes place physically under usual daily masticatory stress on the tissue. The subsequent ability of the tissue to renew itself is a special feature of the periodontium.[13] As such, it provides the basis for the controlled tissue forming process induced by environmental stimulation including orthodontic force and tooth movement. To understand this remodeling process it is crucial to explore the mechanisms of the cellular crosstalk that occurs in the periodontium.

B) Periodontal remodeling caused by the second type of inflammation, known as infectious periodontal disease or periodontitis: an exogenous process induced by bacteria.[13–15] This is frequently mentioned as inflammatory periodontal disease observed in patients. In clinical practice, bacterial induced periodontal inflammation (often triggered by insufficient dental hygiene) results in degradation and loss of the periodontal tissue.

Hence, the current view is that periodontal tissue homeostasis is regulated by the same tissue crosstalk that is induced when the sterile type of remodeling takes place.[16] The mechanobiological mechanisms shaping this remodeling process are largely unknown as it is the molecular backgrounds of different cell types that actively interact during periodontal regeneration.

In this review, we will focus on recently proposed 3D in vitro models and relevant biomaterials selected to mimic the native alveolar bone and periodontal tissue. We will discuss the importance of existing models for providing a scientific basis for advanced regenerative strategies, while unravelling multifaceted (non)cellular interactions during endogenous periodontal tissue reshaping. The recent literature will be reviewed to evaluate current approaches that may be applied for more extensive study of actual (non)cellular crosstalks taking place in the periodontium during remodeling regulated by sterile (non-bacterial) inflammation.

1.3. Periodontal Tissue Remodeling

To avoid pathologies and tissue degeneration during the PDL remodeling process, a finely tuned balance between bone material resorption and formation is of outmost importance (Figure 2A,B). A controlled hard tissue resorption is performed by osteoclasts that reach the local area from periodontal blood vessels.[7,8] New bone material is provided by osteoblasts and functional osteoprogenitor cells (PDL and bone stem cells), while formation of cementum formation is a result of cementoblasts.
activity.\textsuperscript{[17–19]} Cementoblasts located proximate to fibroblasts on the tooth root cementum have recently been shown to have central importance for the PDL-root connection and have therefore become the focus of interest in the scientific community.\textsuperscript{[20]} The functional network of PDL and tooth root cementum is crucial to ensuring tooth stability and health.\textsuperscript{[21]} Imbalanced remodeling may induce an immunologic overreaction resulting in enhanced periodontal tissue resorption. This can reduce the anchorage of teeth accompanied by tooth root resorption or loss of tooth periodontal and cementum connection.\textsuperscript{[22,23]} As a result, undesirable side effects in the orthodontic therapy may occur, leading to decreased stability and in some cases tooth loss.\textsuperscript{[24]}

1.4. Molecular Mechanisms of Periodontal Remodeling and Orthodontic Tooth Movement

The unique character of alveolar bone crosstalk in periodontal tissue opens a variety of research possibilities on complex systemic levels. For example, in this tissue we find variable aspects of periodontal remodeling that can be studied in the context of osteoimmunology, activation of specific internal molecular mechanisms (e.g., cell signaling, mechanotransduction, autophagy), or alteration of the ECM features.\textsuperscript{[25,26]} Each of these processes is controlled by a dynamic interaction between principal cellular elements of the PDL that includes osteoblasts, fibroblasts, cementoblasts, and fiber-like ECM. All together, these components are crucial for adjacent alveolar bone formation and regeneration into functional moieties. In addition, the skeletal and the immune systems share a multitude of regulatory molecules, including cytokines, receptors, signaling molecules, and signaling transducers, thereby mutually influencing each other.\textsuperscript{[27]} Recently, the scope of osteoimmunology has been extended to evolving concepts in bone-immune interactions in health and disease, and a broad range of molecular and cellular interactions have been introduced in the field.\textsuperscript{[20]}

Besides numerous cellular and molecular events, mechanical forces are also indispensable actors in the periodontal homeostasis maintenance. This homeostasis is impaired during orthodontic tooth movement (OTM),\textsuperscript{[28]} which triggers a coordinated response of all cellular components tending to reestablish the balance of the system.\textsuperscript{[29]} During this movement, different mechanical stresses (i.e., shear, compression, and tension) are applied to the teeth and transferred to the alveolar bone through PDL activating a series of transcriptional, post-transcriptional, and translational modifications.\textsuperscript{[30]} Mechanosensory cells of the PDL tissue perceive mechanical stimuli from the surrounding microenvironment and transduce them into cellular signaling cascades. These changes comprise the activation of specific genes and proteins resulting in the release of soluble factors that together modulate the local microenvironment in reaction to the perceived mechanical forces.\textsuperscript{[31]} The mechanical forces contribute substantially to stem cell differentiation towards osteogenic-derived lineages. Osteoblasts then cover the periodontal space while inducing new alveolar bone generation. Osteoblasts can be obtained from different cell sources, including periodontal ligament stem cells (PDLSCs). In fact, upon osteoblast-specific stimulation, these cells differentiate toward functional osteoblasts.\textsuperscript{[32]} This is possible due to PDLSC’s self-renewal, multilineage differentiation, and immunomodulatory properties because of which PDLSCs are very important for periodontal homeostasis maintenance. The mechanobiology signaling that sustains the functionality of PDLSCs is still under investigation. Nevertheless, some key molecules regulating the effects of internal and external physiological forces that control cell proliferation and ECM synthesis throughout the entire PDL apparatus have been identified.\textsuperscript{[4,31]} PDL cells are also known to have a strong impact on local osteoclastogenesis, since they upregulate Connexin43 following the application of mechanical forces. This results in an enhanced expression of osteogenic markers such as Runt-related transcription factor 2 (RUNX2), Osterix (Osx), and Osteoprotegerin (OPG), and in a...
corresponding downregulation of the osteoclastogenic marker Receptor activator of nuclear factor kappa-B ligand (RANKL).\cite{34}

The RANKL/RANK/OPG as well as high-mobility-group box protein 1 (HMGB1) are important regulatory pathways of periodontal immunity and tissue homeostasis.\cite{15,16} When mechanical loading is absent, PDL cells attract osteoclast precursors via Intercellular Adhesion Molecule 1 (ICAM1) presentation and support the expression of osteoclastogenesis-beneficial molecules such as RANKL, macrophage colony-stimulating factor (MCSF), HMGB1, and tumor necrosis factor-α (TNF-α).\cite{37,38} During mechanical loading and OTM, alteration of the ECM features occurs due to a release of soluble factors, that may exhibit either positive or negative effects on PDL remodeling and eventually trigger a local inflammatory response.\cite{39,40} Periodontal ligament fibroblasts (PDLFs), PDLSCs, and cementoblasts have been investigated in vitro and in vivo models focusing on the secretion of cytokines and growth factors turnover during remodeling and regeneration.\cite{10,41} All of these modifications inevitably activate mechanotransduction signals that advance the internal signal propagation cascades and gene transcription. In fact, the periodontium is a highly mechanoresponsive tissue, with canonical Wnt pathway as a main actor of the process.\cite{44,45}

In addition to activation of the Wnt pathway, mechanical loading results in cytoskeletal rearrangements when the mechanosensory protein complex is linked to a kinase cascade system.\cite{39} The mechanosensory protein complex contains Talin, Vinculin, Tensin, Paxillin, Src, and focal adhesion kinase (FAK). Activated extracellular signal-regulated protein kinase (ERK) then enters the nucleus triggering the transcription of several additional regulatory genes.\cite{46} Also, the level of Chemokine (C-C Motif) Receptor 5 (CCR5) increases in the PDL in a time-dependent manner when exposed to tension and compression forces.\cite{47,48}

As our awareness about the molecular background of alveolar bone remodeling, PDL regeneration, and OTM is increasing, the chances for more successful therapeutic methods expand.\cite{49} The periodontal-cementum interface and the functional base of the existing crosstalk also need more in depth investigation at the molecular and cell levels. Meeting this goal will require the development of adequate in vitro reconstructions of native-like PDL systems that consider their multi-functionality. Therefore, besides conventional 2D cell systems used for approaching PDL-related cell processes,\cite{10} the 3D bioengineered PDL models are promising means for a better approximation of the periodontal-cementum interface.\cite{50}

2. Bioengineering

Periodontal tissue engineering aims to enhance local tissue regeneration capacities in order to allow the restoration of proper tissue structure and its functionality. This is possible thanks to the development of numerous biomimetic ECM supports that sustain cell growth and cell functionalization while guaranteeing the restoration of physiologically necessary biological structures.\cite{51} One bioengineering approach considers the introduction of 3D scaffolds to favor the growth of embedded or adjacent cells during neo-tissue generation. Moreover, these structures can be used as carriers of specific growth or pro-differentiation factors that can finely tune the biological processes required in periodontium during its repair.\cite{52}

2.1. Cell Sources for Periodontium Bioengineering

Several types of stem cells can be used for periodontal tissue regeneration and tooth repair. They include bone marrow mesenchymal stem cells (BMSCs), dental pulp stem cells (DPSCs), PDLSCs, stem cells from human exfoliated temporary teeth (or stem cells from human exfoliated deciduous teeth—SHEDs), dental follicle stem cells (DFSCs), and dental epithelial stem cells (DESCs) (Figure 3). The most explored cell type in PDL regenerative studies is PDLSC.

Human PDLSCs were first isolated from extracted wisdom teeth by Seo and coworkers.\cite{51} PDLSCs express a range of cementoblastic and osteoblastic/odontoblastic markers, including alkaline phosphatase (ALP) and ECM phosphoglycoproteins. After implantation of PDLSCs in vivo, the cells form a periodontal-like tissue with root cementum and collagenous ligament fibers. They generate a thin layer of cement-like tissue on the wearer’s surface made up of compacted collagen fibers resembling the PDL. The collagen fibers then crosslink with the newly formed cement-like structure, mimicking the physiological attachment of Sharpey’s fibers in root cementum.\cite{51} These results underline the central role of cementum tissue in controlling periodontal homeostasis and suggest that the cells are able to differentiate into osteoblasts, cementoblasts and fibroblasts, and to build up an ECM similar to periodontal tissues. Using the cloning technique, Seo and colleagues were able to demonstrate that only a small percentage of the cells of the periodontal ligament have stem cell character, sharing instead morphological, phenotypical, proliferative, and functional features specific for multipotent adult mesenchymal stem cells (MSCs) (Figure 4A–J). These cells alone promote cell turnover and tissue homeostasis and serve as a resource for renewable progenitor cells, including cementoblasts, osteoblasts, and fibroblasts, which can mature throughout their lifetimes or migrate to the periodontium. As PDLSCs are of mesodermal origin, they are compared in many studies with MSCs, the natural precursor cells of bone, cartilage, and adipose tissue in terms of their surface marker expression. Indeed, PDLSC/PDLFs and MSCs share a common phenotype, presenting, for example, CD73, CD90, and CD105 molecules on their surface while lacking hematopoietic markers.\cite{22,23,54} PDLSCs possess additional characteristics similar to MSCs, in particular their trophic phenotype, immunomodulatory features, and the fact that they are non-immunogenic since they do not present MHC complex molecules on their surface (HLA-DR negative and HLA-ABC positive).\cite{55} In addition, PDLSCs demonstrate a migratory behavior similar to MSCs since they respond to the hepatocyte growth factor, a factor identified as chemoattractant for MSCs to guide them toward wound regions.\cite{36,37} Compared to other stem cells in the alveolar bone, for example, dental pulp derived stem cells, PDLSCs express more Scleraxis, a transcription factor regulating tendon and ligament development. These cells have the ability to form Sharpey’s fiber-like collagen bundles that are connected to tooth root cementum.\cite{58} Together with the fact that PDL cells can be easily isolated from fresh and cryopreserved periodontal ligaments, they are also important for remodeling and tissue regeneration of the periodontal system.\cite{59} Recently, induced pluripotent stem cells (iPSCs) have gained attention as a new cell source that can provide applicable solutions in the field of periodontal regeneration.\cite{60} Due to their main
Figure 3. The source of stem cells for the regeneration of periodontium. SGSCs—salivary gland-derived stem cells, DFSCs—dental follicle stem cells, TGPCs—tooth germ progenitor cells, DPSCs—dental pulp stem cells, PDLSCs—periodontal ligament stem cells, SHEDs—stem cells from human exfoliated deciduous teeth, SCAP—stem cells from the apical papilla, BMSCs—bone marrow-derived MSCs, OESCs—oral epithelial progenitor/stem cells, GMSCs—gingiva-derived MSCs, PSCs—periosteum-derived stem cells. Reproduced with permission. [53] Copyright 2012, Elsevier.

Figure 4. Isolation of adult human PDLSC. A) Extracted human third molar showing PDL attached to the surface of the roots (arrow). B) Single colonies formed after PDLSCs were plated at low density and colony-forming efficiency was evaluated after staining with toluidine blue. C) Magnification of the single colony from panel (B) is presented. D) Cell proliferation was measured by bromodeoxyuridine (BrdU) labelling for 24 h. The number of BrdU-positive cells was expressed as a percentage of total number of cells. The results are presented as median ± SD. E,F) Immunocytochemical staining showed that cultured PDLSCs were expressing STRO-1 (red; E) and CD146/MUC18 (green; F), two early mesenchymal progenitor markers. PDL tissue was also positive for STRO-1 antibody with G) immunohistochemical and H) fluorescence staining. I) Clonogenic assays was done with unfractionated (bulk), STRO-1 negative (STRO-1−) and STRO-1 positive (STRO-1+) cell fractions shows number of colonies as median ± SD. J) RT-PCR (left) and northern blot analysis (right) showed that cultured PDLSCs (P) expressed higher levels of Scleraxis, a transcription factor specifically expressed in tendon cells, compared with DPSCs (D) and BMSCs (B). GAPDH was used as internal control. Reproduced with permission. [5] Copyright 2004, Elsevier.
feature of pluripotency, iPSCs from the same donor can be used as the precursor for the generation of additional cell types that are essential for the reconstruction of periodontium. The expectations for this direction of research are high and the potential for its applications to expand in the years to come is great.

2.2. Bioengineering Approaches for PDL Tissue Generation

Implementation of multipotent stem cells in combination with biomimetic ECM (non-cellular component) introduces substantial advances in the field of periodontium engineering. The non-cellular component of PDL is principally type I collagen, which creates bundle-like structures. It is abundantly produced by PDLFs and is organized into collagen fibers. Collagen turnover rate is high and the orientation of collagen fibers is defined by the type of the applied mechanical forces. Collagen I is a preferential type of collagen found in bones and for this reason it is largely used for the creation of hydrogel-like structures seeking to simulate the functionalization of stem cells toward bone production. The specific physicochemical characteristics of collagen make it highly suitable for tissue engineering. In fact, the in vitro 3D collagen hydrogels have been developed to more closely approach the in vivo anatomy of the PDL and, by simulation of the bone matrix formation, alveolar bone as well. Although the strength of these hydrogels is not sufficient to completely support tooth growth, their mechanical and chemical features can be easily modified and tuned to reach this goal. These modifications can also be applied to mold their interactions with adjacent cells. However, apart from collagen, numerous other hydrogels (natural or synthetic) have been proposed for the study of periodontium regeneration (Figure 5) and will be mentioned in the following sections.

2.3. 3D In Vitro Models for PDL to Study Effect of OTM

The material used for bioengineering a 3D PDL system must be biocompatible with all the types of previously described cells and allow their proper interaction (pro-adherent feature), their expected self-organization, and hence their functionalization without causing an inflammatory response. Although 3D models have been proposed mainly as tissue-engineered structures for in vivo implantation, they could also be considered as an optimal in vitro approach for unraveling the underlying nature of the cell–cell and the cell–extracellular matrix interactions. In addition, 3D models are important segments in the in vitro experimental setup of periodontium since they allow a more accurate characterization of the force magnitude to be applied in order to achieve clinically relevant osteogenic and osteoclastic responses. Their characteristics depend on the purpose to be achieved and the type of tissue that needs to be reproduced (Table 1). The features of the 2D models have been thoroughly discussed elsewhere.

One of the 3D models that can be useful for the mechanotransduction studies was achieved by cultivating PDL cells in a thin sheet composed of poly(lactic-co-glycolic acid) (PLGA) scaffolds with high porosity. This system has been proposed as an optimal synthetic polymer that successfully replaces...
Table 1. General characteristics of 3D models for in vitro periodontium fabrication.

| 3D in vitro approach | Specifications | Cell support material | Cell types explored | Potential | Limitations | Ref. |
|----------------------|----------------|-----------------------|---------------------|-----------|-------------|------|
| Engineered biomimetic ECM (hydrogel) substrate used as a support for the growth of one or more cell types | Collagen*, HA*, Alginate*, Gelatin*, Fibrin*, PLGA**, PEG**, Pluronic F127**, PCL**, GelMA*** | PDLSC, PDLF, MSC, Endothelial cells, Osteoblasts, Osteoclasts | - Suitable for the in vitro and/or in vivo studies for OTM, wound healing, as growth factor carrier - Easy manipulation and in situ imaging of multicellular structures - Possible chemical modifications for improving mechanical and adhesive properties - Suitable for static and dynamic in vitro analyses - Suitable for non-invasive in vitro studies - Less time-consuming than in vivo studies - Possibility of high-throughput in vitro analyses | - In some cases chemical modification is required to improve cell adhesion - Natural hydrogels share mechanical properties closer to ligament features - No consensus in data analyses - Finely tuned biochemical properties for each PDL structure to have it functional | [66,69–73] |
| Porous scaffolding structures for sustained or guided cell growth | Ceramic biomaterials# biodegradable glass, PLA, PGA | Cementoblasts, PDLSC, MSC | - Implants or drug delivery for tailored scaffold production - Easier multicomponent system fabrication - Fabrication of more complex geometrical structures - Controlled strength properties and degradation rate | - Slow degradation rate, bone-oriented applications - Limited real-time visualization - More laborious - Need to be combined with other material to reproduce different PDL structures | [74–78] |
| Bioprinting with natural, synthetic, and semi-synthetic bioinks | PLGA, PLA, PCA | PDLSC, MSC | - Tailored scaffold production - Easier multicomponent system fabrication - Fabrication of more complex geometrical structures - Controlled strength properties and degradation rate | - Limited number of easily printable bioinks - Protocol optimization can be time consuming - Need to have programming knowledge - Resolution limitations (directly dependent on the nozzle characteristics) | [79,80] |

Collagen, hyaluronic acid (HA), alginate, gelatin, fibrin (*—natural); Poly(lactic-co-glycolide) (PLGA), polyethylene glycol (PEG), Poloxamer 407 (Pluronic F127), and poly-caprolactone (PCL) (**—synthetic); Methacrylated gelatin (GelMA) (**—semi-synthetic), # Ceramic biomaterials (calcium sulfate and calcium phosphates). Cells: Periodontal ligament stem cells (PDLSCs), periodontal ligament fibroblasts (PDLFs), mesenchymal stem cells (MSCs), dental pulp stem cells (DPSCs).
collagen-based scaffolds since it has physical characteristics more similar to PDL. In fact, PLGA is a material prevalent in biomedical engineering as it can provide diverse grafts, prosthetics, implants, and micro- or nanoparticles. Its stability can be easily tuned, thus affecting the biodegradation rate itself. By studying the range of compression forces required to induce specific cell response within a defined time-frame, their value in 3D conditions has been shown to greatly exceed those employed in 2D cell systems (mean values 15 and 2 g cm\(^{-2}\), respectively). This characteristic is essentially dependent on the stiffness of the model used in the study and must be precisely determined for each in vitro application in order to allow cell differentiation towards the desired cell lineage. A reduced time-dependent proliferation rate of the cells is a function of the cells’ differentiation and hence these two processes coincide in vivo. Similar to the in vivo situations, a 3D model’s pro-osteoclastogenic ability is highly dependent on the applied compressive forces, which shape the level of previously described osteoclastogenesis inducers such as RANKL, cyclooxygenase-2, parathyroid hormone-related protein, and interleukin (IL)-11. The application of mechanical forces leads not only to modifications of ECM composition in PLGA scaffolds, but inevitably reflects on the behavior of the adjacent cells as well. These forces strongly influence the proliferation rate of PDLCs and their overall functioning. In fact, the cells activate the release of transforming growth factor-beta (TGF-\(\beta\)) and the secretion of proteolytic enzyme matrix metalloproteinases (MMPs) in response to these mechanical forces. While the production of MMPs is triggered by pro-inflammatory factors (such as TNF-\(\alpha\) and IL-1\(\beta\), -6, -12), TGF-\(\beta\) impedes their enzymatic activity by promoting synthesis of TIMPs, the specific endogenous inhibitors of MMPs. The role of each of these molecules may therefore be crucial in regulating the balance between PDLC proliferation on the one hand and degradation of collagen fibers in the surrounding ECM on the other. This implies the existence of a highly accurate sensing system that is active throughout orthodontic treatments and is able to finely tune these two substantially diverse processes of production and elimination, both of which are required for controlled ECM homeostasis. At the same time, growth differentiation factor 15 (GDF15) plays an important role in the regulation of PDL homeostasis during OTM. This secreted ligand binds to the TGF-\(\beta\) family receptors after stressful stimuli and cellular injury is detected due to OTM. The main impact of GDF15 as a soluble factor is in the regulation of bone turnover, which is dependent on the activity of MMPs released in the ECM. In this way, GDF15 likely balances the alveolar bone remodeling during OTM. This notion strengthens the necessity of close interaction among the different cell types of the periodontium for an orchestrated homeostasis regulation.

2.4. 3D In Vitro Models to Study Regenerative Potential of Bioengineered Constructs

PDMS (polymethylsiloxane) has also been exploited for in vitro modeling of PDL tissue. In order to allow adherence of PDL cells to its otherwise hydrophobic surface, it is pre-treated with vacuum plasma and then coated with fibronectin. The resulting in vitro model allows studies of PDL tissue stretching and sustains PDLF differentiation towards bone-like cells. One of most intriguing features of PDL tissue is its highly organized alignment of connective fibers, which is not easily reproducible in vitro. On the other hand, this structure is required in order to guarantee complete functionality of periodontium. Therefore, besides providing structural support against internal pressure, scaffold matrices help in achieving a desirable orientation of the fibers inside the tissue. An approach that leads toward acquiring scaffolds with the topographic attributes of native tissue involved a combination of biodegradable poly(e-caprolactone)-poly(ethylene glycol) (PCE) nanofibers obtained by electrospinning and immersed into chitosan (CHI). In this manner more ECM proteins, not only collagen I but also collagen III, are produced while sustaining the expression of Periostin in vivo. The organization and localization of each marker is driven by nanofiber PCE scaffolds embedded in the CHI, opening a new frontier in PDL regeneration. Adequate cell alignment is also important for assuring their directed movement within collagen I based scaffolds. Together with porosity, it is becoming evident that reproduction of functional PDL in 3D conditions also requires accurate orientation of the microstructures. Moreover, the addition of minerals into specific positions within such structures establishes a possible triggering event for the selective and hierarchal mineralization of collagen-based ECM. This process is dependent on the dentin matrix protein-1 analogues. As reported by Zuo and colleagues, both the type of ECM and also the spatial control of biomineralization need to be finely tuned in the proposed 3D structures for functional PDL tissue generation. Calcium ions are especially important in this scenario since they bind easily to the phosphate groups positively influencing the mineralization within the tissue. It is therefore essential to achieve the concentration gradient of organic phosphate within the 3D structures of PDL, with higher concentration rates in the parts that are closer to alveolar bone and lower on the side approaching PDL. On the other hand, these findings reinforce the relevance of multiphasic scaffolding systems, which show promise in overcoming limited PDL regeneration. More complex structures have been proposed by Sowmya and colleagues who adopted biofabricated scaffolds that fulfill the biophysical properties required at a microscale level for the growth of different types of PDL-deriving cells. The nano-composite hydrogel scaffolds made of chitin–PLGA and nano-bioactive glass ceramic were loaded with growth factors or re-combinant proteins to achieve a calibrated tri-layered structure. The system allowed local cell differentiation and spatiotemporal organization toward three distinct compartments of PDL tissue, namely cementum, PDL, and alveolar bone (Figure 5). Similarly, tissue specific growth factors can be distributed within the channels of the structures with distinct microarchitecture allowing intersection into the multifaceted periodontium. Adoption of the encapsulation of the PLGA micro-sphere carrying molecular drivers (amelogenin, connective tissue growth factor, and bone morphogenetic protein (BMP)-2) into three distinct zones (Figure 6) led here to a desired triggering of the stem cell differentiation process during periodontal management.

Less examined components of the PDL tissue during fabrication of optimal 3D replacements are blood vessels and innervation. The inclusion of endothelial cells is therefore a required step in the preparation of an artificial multicellular
tissue with dimensions greater than 100–200 microns in order to allow optimal oxygen and nutrient supply. Their reorganization within the 3D systems should prime for the formation of neo-vessels. As described previously, mechanoreceptors are also the integrative component of PDL anatomy and are crucial in transforming mechanical information into biochemical signals during OTM. The proper modification of hydrogels embedded with stem cells can generate the microenvironmental conditions that support growth of nerve cells in PDL. In particular, the alginate and hyaluronic acid (HA) hydrogels loaded with nerve growth factor (NGF) can direct the differentiation of stem cells toward neuronal-like phenotypes. A combination of more cell types may offer additional advantages to the alginate/HA hydrogel 3D model with local NGF loading in the regeneration of PDL where, in addition to stromal components, nerves can also be achieved. Of course, providing all of the cellular components together with adequate ECM is challenging. Implementation of tissue specific growth factors along with the iPSCs embedded in the hydrogels can provide a path to the improvement of current in vitro periodontium models. In fact, the initial application of BMP-6-releasing hydrogels made of CHI/gelatin/glycerol phosphate appears to provide a suitable 3D environment for sustained growth and differentiation of iPSC toward bone and connective tissues. Nevertheless, the behavior of iPSCs within hydrogels should be thoroughly tested to provide more data that will endorse their future use. One of the approaches that can be adopted for a rapid 3D in vitro evaluation of the regenerative potential of a specific biomaterial embedded with different types of cell, including iPSCs, has been proposed by Koch and colleagues. This strategy could be useful in the first round evaluation of the pro-regenerative features of the newly proposed biomaterials (Figure 7). In addition, the proposed model allows the integration of biochemical components within hydrogels, broadening its applicability in periodontal-oriented studies. In this manner,
predictions of the success of novel polymeric biomaterials to trigger regenerative processes in periodontium can be made before moving to in vivo validations.

2.5. Scaffold-Free Engineering Approaches to Study Local Tissue Crosstalk

The scaffold-free approach in the context of the use of connecting cell-sheets is largely accepted in PDL tissue regeneration.[102] The development of a temperature-responsive culture dish allows for the fabrication of a confluent layer of cells that is easily detached without using standard chemical approaches. Application of a temperature gradient to release a cell layer thus develops intact membrane with pro-adherent receptors while conserving the ECM components favoring cell attachment.[103] In fact, this scaffold-free tissue engineering method favors PDL-derived cells self-assembling in a structure that mirrors the complexity of periodontium.[104] More importantly, organized cell structures are maintained in vivo where they express key molecules required for PDL remodeling. These structures are also suitable for a co-culture of additional cell types. Besides PDL-derived cells, endothelial cells are also easily manageable in these scaffold-free conditions.[105] The possibility of having active neo-vascularization processes within the proposed 3D structures is extremely advantageous since it increases the possibility of building thicker tissue-like constructs avoiding necrotic areas. The complexity of cell-sheets increases the possibility of reaching native anatomy of periodontium. Moreover, it enhances the success of approaching the development of a functional bone-ligament connection as found in PDL.[106] All of these characteristics make cell-sheet technology a useful approach for future translation into the clinic, although it is more likely they will need to be combined with other bioengineering methods in order to assure a large-scale tissue injury regeneration in clinical trials.[107] In fact, the initial tests for improved interaction between alveolar bone and PDL when applying cell-sheets in vivo are encouraging. The combined use of cell-sheets with biphasic scaffolds speaks in favor of this direction the opportunity of using different primary cells for generation of multicellular moieties that successfully integrate and crosstalk in periodontium.[108] In this manner, well-defined region-specific tissue phenotypes (dental pulp, periodontal ligament, and alveolar bone) can be assured, enabling their suitable functionalization in vivo while strengthening further implications in the clinical modalities used for treating problems related to proper regeneration of periodontal tissue.

2.6. Bioengineered 3D Constructs for In Vivo Implantation

Recently, a variety of animal models have been explored in the periodontal tissue engineering field.[109] They have been used for the subcutaneous transplantation of scaffolds loaded with human PDL or stem cells[110] and for the evaluation of the regenerative capacities of bioactive and biomimetic scaffold types for both hard and soft parts of periodontal tissue.[111] In addition, the effectiveness of autologous periodontal cell types for periodontal tissue complex regeneration have been extensively studied in vivo.[112] In fact, animal experiments are still the gold standard approach used to unravel fundamental biochemical mechanisms on the cellular and on the tissue level.[113] However, due to ethical reasons and interest in improved animal welfare, the implementation of small and large animals for research purposes must be limited only to cases where no other options are available. Therefore, an important current research task is the development of new strategies suitable for the application of the “3Rs principle” (reduction, refinement, and replacement)[114] in the context of animal experimental design. Sophisticated 3D in vitro models offer powerful tools in this context as they can provide new insights into cell biological mechanisms and help to refine animal experiments. This brings us to the question of whether all previously discussed 3D models are already at the stage for their functional validation in vivo or whether they might require additional in vitro assessments before moving to the implantation step. Following the principle of 3Rs, we may need to enhance the current in vitro platforms for sustained growth of 3D in vitro models of periodontal tissue. For example, a long term growth of described 3D models could be sustained by introducing specifically designed bioreactors[115] that would enable the optimization of more consistent in vitro protocols for assessment of the underlying nature of the cell–cell and the cell–ECM interactions. In this way, 3D models can also be more critically evaluated in the pre-implantation step assuring the refinement of animal experimentation.

3. Bioprinting Technology for Advanced 3D In Vitro Models

The technology of bioprinting has significantly improved the functionality of tissue engineered 3D in vitro models in recent years. This is because bioprinting enables the manufacture of cell-laden hydrogel constructs in an anatomically spatial manner.[116] Thus, bioprinting is of interest for 3D in vitro models mimicking periodontal tissue and to investigate its remodeling capacity.[117] The number of papers using bioprinting for PDL in vitro modeling is very limited. However, we can learn how to design suitable in vitro models for investigation of the alveolar bone crosstalk in periodontal tissue from published studies that deal with other bioprinted tissue replicas.

Most of the published bioprinted models were manufactured using the microextrusion method, where rods of cell-laden hydrogel are deposited in a computer-controlled manner.[118] The reasons for the frequent use of extrusion-based bioprinting include its simplicity, the ease of operation of the printers, and finally the availability of a great number of different extrusion-based bioprinters on the market (Figure 8A–E). So far, there has been one study available in which the microextrusion bioprinting technique was applied to investigate the basic cellular response of printed periodontal ligament cells.[79] The bioprinted PDL cells embedded in GelMA and PDLC showed negligible cytotoxicity and began elongating and proliferating with time. In principle, the drop-on-demand (DoD) technique, that is, the deposition of single small droplets, is preferred compared to microextrusion bioprinting because of the higher resolution that can be achieved.[119,120] Moreover, the shear stress that is applied on the cells during printing, a potential source of cell damage, is lower using the DoD technique compared to the microextrusion method.[121–124] However, if highly viscous hydrogels are to
Figure 8. Bioprinting process. A) Bioprinting technology uses patients’ own cells proliferated in vitro and subsequently 3D printed. Hydrogels are carrier fluids for the cells, which can be gelled post-printing to enable the 3D build-up of cell-laden structures. The bioprinted cell-laden structures can then be developed toward functional tissues intended as replacement for deceased or lost native tissues in vivo. Due to the high regulatory standards for tissue engineered medical products, until now bioprinted constructs are more frequently used as in vitro testing platforms for novel drugs or as advanced in vitro 3D models to investigate fundamental scientific questions. Bioprinted in vitro models could be used in future to mimic alveolar bone crosstalk in periodontal tissue. B–E) Different bioprinting methods have been developed in recent years. Among these, inkjet (B) and extrusion bioprinting (D) are of special interest for the manufacturing of periodontal tissue in vitro, as these methods are capable of manufacturing cell-laden structures even at the millimeter scale, which is only barely possible with laser-assisted methods (C). Stereolithography (E) can build 3D components on the milli- and centimeter length scale, but requires cytotoxic photoinitiators and UV light for the gelling process, which results in low cell viability post-printing. With the inkjet method (B), a comparable high resolution can be achieved, especially if microvalves are used as nozzles. Reproduced with permission.[125] Copyright 2016, Elsevier.

be processed, only the microextrusion bioprinting method can be used.

This discussion underlines the fact that the bioprinting technique itself is only one part of the process to realize suitable 3D in vitro models. A key component is the hydrogel.[64] The hydrogel must fulfill technical (rheology, stiffness, relaxation) as well as biological (cytocompatibility, gas permeability, active ligands) requirements. 3D bioprinting techniques can arrange different cell types in a mimetic 3D configuration. However, the self-organization of the embedded cells during a subsequent maturation process in a bioreactor is required to remodel the cell-laden-hydrogel construct into a fully functional tissue substitute.[126] A principle challenge of 3D bioprinting is the kinetics of the gelation mechanism of the processed hydrogels. The hydrogel should have a low viscosity in the printing nozzle but should gel very quickly at the moment when the cell-laden droplet (or cell-laden extruded strand) hits the building platform. Most gels need some time before they are completely gelled, making the use of the bioprinting technique for the build-up of 3D hydrogel components with defined geometry a challenge. Different methods have been developed to address this issue. One expedient strategy is to print the cell-laden hydrogels submerged beneath a support fluid that preserves the defined 3D shape of the hydrogel construct until the gelation process has been completed. Among the support fluids proposed for this purpose are perfluorocarbon,[127] due to its cytocompatibility, very high density, and hydrophobic qualities, and gelatin,[128,129] because it works as a sacrificial material that can be released after printing by slightly increasing the temperature. The diffusion range within a native tissue is limited to \( \approx 150-200 \, \mu \text{m} \).[130] Thus, if a 3D in vitro artificial tissue construct can be achieved at the millimeter length scale it is essential to enable the process of vascularization if longer cultivation periods are required. In vitro vascularization in bioprinted structures has been successfully achieved using co-cultures of endothelial cells and stem cells in GelMA,[131] and in tailored blends made of collagen type I and GelMA[132] as well as collagen type I and agarose.[133] Microvascular formation can additionally be influenced by cell-mediated traction forces.[134] Such forces occur due to the actin network of the cell and can result in contraction of the bioprinted cell-laden hydrogel construct. The effect of forces on in vitro vascularization are relevant for potential periodontal tissue models that are intended to investigate the mechanisms of OTM and local tissue regeneration. Thus, the bioprinting method holds great potential for the manufacturing of models that aim to mimic the alveolar bone crosstalk in periodontium.

4. Conclusions
Numerous 2D in vitro models in which periodontal ligament cells were challenged to mimic physiological and pathophysiological conditions have been proposed during the past decade. They have allowed the underlying nature of cell–cell and cell–ECM interactions to be unraveled and have contributed substantially to current understandings of many biological aspects related to a unique tissue such as periodontium. However, the very same complexity of PDL is missing in currently available models, limiting detailed investigation of this tissue. To reach a more profound understanding of the cellular, molecular, and biophysical background of periodontal crosstalk and to provide a scientific basis for advanced regenerative strategies of PDL tissue, various innovative 3D in vitro models are currently being explored. Toward this goal, the development of suitable hydrogels and the
implementation of bioprinting technology offer great potential to achieve a spatiotemporal organization of several cell types that would together generate functional native periodontium. If combined with patient-derived cells, these structures could then permit not only better comprehension of the biological cues relevant for regeneration processes, but also create a basis for more reliable in vitro testing of the tailored treatment options that are emerging in periodontal apparatus related pathologies.

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Conflict of Interest
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[1] K. G. Silvério, T. L. Rodrigues, R. Dela Coletta, L. Benevides, J. S. Da Silva, M. Z. Casati, E. A. Sallum, F. H. Nociti, J. Periodontol. 2010, 81, 1207.
[2] H. Maeda, N. Wada, A. Tomokiy0, S. Monnouchi, A. Akamine, Prospective Potency of TGF-B1 on Maintenance and Regeneration of Periodontal Tissue, Elsevier Inc., Amsterdam 2013.
[3] M. C. Meikle, Eur. J. Orthod. 2005, 28, 221.
[4] V. Krishnan, Z. Davidovitch, Am. J. Orthod. Dentofacial Orthop. 2006, 129, 469.e1.
[5] B.-M. Seo, M. Miura, S. Gronthos, P. M. Bartold, S. Batoul, J. Brahimi, M. Young, P. G. Robey, C. Y. Wang, S. Shi, Lancet 2004, 364, 149.
[6] M. Pereira, E. Petretto, S. Gordon, J. H. D. Bassett, G. R. Williams, J. Behmoraas, J. Cell Sci. 2018, 131, jcs216267.
[7] F. d’Apuzzo, S. Cappabianca, D. Ciavarella, A. Monsurrò, A. Silvestrini-Biavati, L. Perillo, Sci. World J. 2013, 1, 1.
[8] Y. Li, L. A. Jacox, S. H. Little, C.-C. Ko, Kaohsiung J. Med. Sci. 2018, 34, 207.
[9] H. Kanzaki, M. Chiba, Y. Shimizu, H. Mitani, J. Bone Miner. Res. 2002, 17, 210.
[10] M. Li, C. Zhang, Y. Yang, Bone Jt. Res. 2019, 8, 19.
[11] C. Y. Chen, G. Nufiez, Nat. Rev. Immunol. 2010, 10, 826.
[12] O. Takeuchi, S. Akira, Cell 2010, 140, 805.
[13] G. Hajishengallis, R. P. Darveau, M. A. Curtis, Nat. Rev. Microbiol. 2012, 10, 717.
[14] A. V. B. Nogueira, M. Nokhbehaim, S. Eick, C. Bouraual, A. Jäger, S. Jepsen, C. Rossa, J. Deschner, J. A. Cirelli, Mediators Inflammation 2014, 2014, 425421.
[15] R. Cortés-Veyra, C. Rosales, E. Uribe-Querol, J. Immunol. Res. 2016, 2016, 1.
[16] C. Kirschneck, J. Fanghänel, U. Wahlmann, M. Wolf, J. C. Roldán, P. Proff, Ann. Anat. 2017, 210, 32.
[17] D. D. Bosshardt, J. Dent. Res. 2005, 84, 390.
[18] Y. Bai, Y. Bai, K. Matsuza3ka, S. Hashimoto, T. Fukuyama, L. Wu, T. Miwa, X. Liu, X. Wang, T. Inoue, Bone 2011, 48, 1417.
[19] B. L. Foster, F. H. Nociti, E. C. Swanson, D. Matsa-Dunn, J. L. Berry, C. J. Cupp, P. Zhang, M. J. Somerman, Calcif. Tissue Int. 2006, 78, 103.
[20] M. Tsukasaki, H. Takayangi, Nat. Rev. Immunol. 2019, 19, 626.
[21] Z. Davidovitch, V. Krishnan, Am. J. Orthod. Dentofacial Orthop. 2009, 135, 222.
[22] S. Fujii, H. Maeda, N. Wada, A. Tomokiy0, M. Sait0, A. Akamine, J. Cell. Physiol. 2008, 215, 743.
[23] G. T.-J. Huang, S. Gr0nthos, S. Shi, J. Dent. Res. 2009, 88, 792.
[24] J. Lindhe, E. Bressan, D. Cecchinato, E. Corrâ0, M. Toia, B. Liljenberg, Clin. Oral Implants Res. 2013, 24, 172.
[25] S. Memmert, A. V. B. Nogueira, A. Damana0, M. Nokhbehaim, S. Eick, T. Divnic-Resnik, A. Spahr, B. Rath-Deschner, A. T0ll, W. Götz, J. A. Cirelli, A. Jäger, J. Deschner, Clin. Oral Invest. 2018, 22, 2933.
[26] L. Chen, S. Mo, Y. Hua, J. Periodontal 2019, 90, 1170.
[27] H. Takayangi, J. Periodontal Res. 2005, 40, 287.
[28] M. Shimono, T. Ishikawa, H. Ishikawa, H. Matsuza3ka, S. Hashimoto, T. Muramatsu, K. Shim0, K.-I. Matsuza3ka, T. Inoue, Microsc. Res. Tech. 2003, 60, 491.
[29] J.-C. Park, J.-M. Kim, I.-H. Jung, J. C. Kim, S.-H. Cho, K.-S. Cho, C.-S. Kim, J. Clin. Periodontal. 2011, 38, 721.
[30] Q. Ma, Z. Ma, M. Liang, F. Luo, J. Xu, C. Dou, S. Dong, J. Cell. Physiol. 2019, 234, 12498.
[31] T. P. Garlet, U. Coelho, J. S. Silva, G. P. Garlet, Eur. J. Oral Sci. 2007, 115, 355.
[32] C. Zhang, J. Li, L. Zhang, Y. Zhou, W. Hou, H. Quan, X. Li, Y. Chen, H. Yu, Arch. Oral Biol. 2012, 57, 1395.
[33] X. Xing, Y. Zhang, X. Wu, B. Zhao, J. Ji, X. Xu, J. Periodontal Res. 2009, 44, 286.
[34] S. Li, H. Zhang, S. Li, Y. Yang, B. Huo, D. Zhang, J. Orthop. Res. 2015, 33, 1003.
[35] M. Yamaguchi, Orthod. Craniofacial Res. 2009, 12, 113.
[36] M. Wolf, S. Lossdö0rfer, R. Craveiro, A. Jäger, Innate Immun. 2014, 20, 688.
[37] S. Ogawa, H. Kitaura, A. Kishikawa, M. Noguchi, A. Marahleh, Y. Nara, Y. Ochi, I. Mizoguchi, PLoS One 2019, 14, e0223989.
[38] D. Sokos, V. Everts, T. J. de Vries, J. Periodontal Res. 2015, 50, 152.
[39] M. Janic, D. Docheva, O. Trickovic Janic, A. Wichelhaus, U. Baumlert, Stem Cells Int. 2018, 2018, 1.
[40] E. B. Rego, T. Inubushi, A. Kawazoe, M. Miyauchi, E. Tanaka, T. Takata, K. Tanne, Arch. Oral Biol. 2011, 56, 1238.
[41] K. Diercke, A. König, C. J. Lux, R. Erber, Eur. J. Cell Biol. 2012, 91, 402.
[42] Niederau Christian, Craveiro Rogerio B., Azraa Irma, Brockhaus Julia, Bastan Assia, Kirschneck Christian, Wolf Michael, Selection and validation of reference genes by RT-qPCR for murine cementoblasts in mechanical loading experiments simulating orthodontic forces in vitro Scientific Reports 2020, 10, (1), https://doi.org/10.1038/s41598-020-67449-w.
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