Vegueta. Anuario de la Facultad de Geografía e Historia
22 (8), 2022
eISSN: 2341-1112
DOI: 10.5281/zenodo.7024532

3D BIO PRINTED SCAFFOLD WITH BIOMATERIALS AND PERIODONTAL LIGAMENT STEM CELLS FOR ENHANCING PERIODONTAL REGENERATION IN A PORCINE MODEL

B.Dinesh
Department of Dental Surgery, Government Stanley Medical College and Hospital, Chennai 600001, Tamilnadu, India

D.J.Mukesh Kumar
Centre for Oral Cancer Prevention Awareness and Research (Centre of Excellence) Sree Balaji Dental College and Hospital, Chennai, Tamilnadu, India

S. Suresh Kumar
Centre for Materials Engineering and Regenerative Medicine. Bharath Institute of Science and Technology, BIHER, Selaiyur. Chennai 600073, Tamilnadu, India

N. Aravindha Babu
Centre for Oral Cancer Prevention Awareness and Research (Centre of Excellence) Sree Balaji Dental College and Hospital, Chennai, Tamilnadu, India

P. Madhavan
Prof. S A College of Education, SardarPatel Road, Old Mahabalipuram Road (IT Highway), Chemmancherry, Chennai-600 119, Tamilnadu, India, professormadhavan@gmail.com

Abstract
Various methods for creating artificial scaffolds promoting periodontal tissue regeneration, there has been put forward. This paper discusses and analyses advancements in stem cell technologies and scaffold architecture with a primary focus on Periodontal Ligament (PDL) regeneration findings from clinical trials and in vivo pre-clinical research. Almost all of those advancements include the usage of polymeric materials including various surface-based nano-topography, patterns, and printing techniques complexes multiphasic composite scaffolds by many sections to accommodate for the architectural differences between periodontal tissues. Despite the improved effort put into making these scaffolds and their undeniable effectiveness in directing and assisting TR, the right cell source is still required to produce new tissue, in addition to a variety of biological and mechano-chemical prompts from an extracellular matrix (ECM) that act as a biophysical stimulus for cellular development and differentiation. A cutting-edge, promising technology called cell sheet engineering enables the production of cells in sheets while maintaining ECM elements. There is still work to be done to improve regenerative outcomes in the direction of the functional organization of the produced tissues because the ideal

**Autora de correspondencia / Corresponding author.*

combination of those elements has not yet been identified.

Keywords: 3D Bioprint, Periodontal ligament, and regeneration, Porcine models, Scaffolds, Stem Cells, Implants.

Introduction

With a high frequency in adults at 30 as well as older, periodontitis is an infectious oral disorder caused by bacteria that can cause severe periodontal tissue deterioration [1]. When the illness is severe, the bone tissue is destroyed, leaving crater-like holes around the tooth roots. The treatments of these intrabony defects poses a significant challenge because they can be addressed with traditional root exfoliation and healing preparation without the emergence of novel connective tissue [2] and because any periodontal pockets that are still present can be unsightly and serve as catalysts for further degeneration. The primary objective of periodontal therapy at the moment was the regrowth of all periodontal cells simultaneously, including novel alveolar bone, cement, and periodontal ligament (PDL). To do this, surgical therapy depends on the Guided TR (GTR) technique, where the underlying tissues are allowed to repair and regenerate while the proper membranes are used to shield the bone effect from epithelial tissue down growth [3].

Several studies have tried to engineer an appropriate atmosphere aimed at periodontal tissue regeneration (TR) by utilizing suitable monitoring messages, progenitor cells, carrier structures, or extracellular matrix (ECM), along with sufficient blood supply, required for regrowing periodontal tissues, skeletal, composite resin, as well as PDL [4-6]. Even though the majority of research focuses on bone regeneration and also that the majority of surgical problems rely on directed bone tissue regeneration; more recently, steps have concentrated on PDL, bone, as well as cementum regeneration [7-9]. The parallel alignment of fresh, highly ordered collagen fibers that were put into the bone and cement that had been regenerated is the most important component of the entire complex of periodontal tissues, and new initiatives are focused on achieving this ultimate objective.

Periodontal Tissues

Periodontal Ligament

Root cementum and Alveolar bone were separated using a periodontal ligament, a fibrous connective tissue that spans 100-400 m. This derives from neural crest-derived ectomesenchyme, distinguished via significant cell population heterogeneity [10], abundant blood supply [11], as well as a neural network [12]. Before tooth emergence, PDL development begins with root formation [13, 14]. Hertwig's epithelial root sheath (HERS), created via the inner as well as the outer epithelium of the enamel organ, was where root formation begins following the creation of dentin and enamel within the region of cementoenamel junction in near future. Thus, the production of dentin from odontoblasts is induced by HERS, which also determines the size and quantity of tooth roots [14]. HERS disintegrates and loses bond with tooth root once root dentin begins to form. However, a Mallasez epithelial cell still exists today as a reminder of it. The disintegration of dental follicle cells by HERS (DFCs) comes into connection with freshly created dentin, causing cementoblasts to develop cementoid tissue and begins to secrete, which is later calcified to cementum the starting deposition of enamel matrix proteins from HERS cells is a factor in cementogenesis near the apex of the root, as depicted in Figure 1 [15, 16].

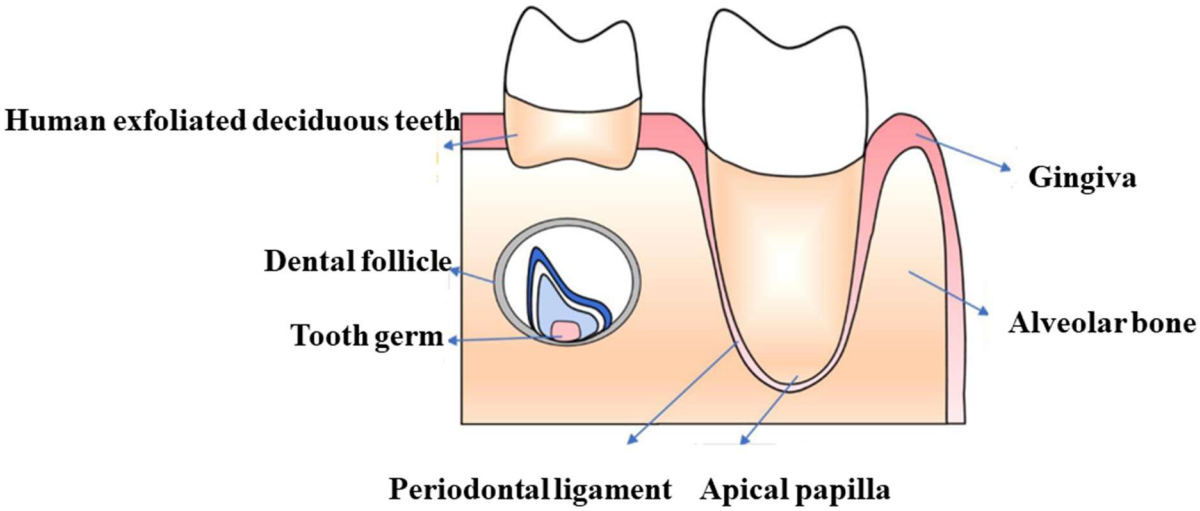

Figure 1. Periodontal Ligament.

The dental follicle's cells undergo differentiation to become fibroblasts, which are in charge of making PDL fibers. PDL fibers gradually elongate during tooth formation and begin to sprout out of both alveolar-based bone and cementum. Collagen fibers are loosely arranged close to the cementum, which turns parallel to the tooth root during the initial stage of PDL development. The position of the nearby teeth appears to have an impact on the direction of the fibers as they alter during tooth eruption. Dento-gingival, transseptal, as well as alveolar crest fiber groups first form when teeth first erupt towards their oral cavity, followed by the appearance of occlusal contact fibers and the roots apical third[17]. Dense In the cervical portion of the root, Sharpey's fibers seem to protrude out of alveolar bone and stretch toward these PDL space's slim cementum-anchored fibers. After the teeth have completed their full occlusal function, they get heavier, were arranged into discrete bundles, and acquire their final size and alignment [18]. These fibers' primary characteristic—which is crucial for PDL regeneration—is that they are encased by cementum and bone. Fibers made of collagen, reticulin, and oxytalan make up the PDL fibrous matrix. Type I collagen makes up the majority of the collagenous 90 percent of PDL fibers. They give the PDL its structural stability, whereas oxytalan fibers that emerge as the root develops and the PDL's vascularization network appear to contribute to vascular support [19]. According to a report, HERS and cementoblasts play a part in the growth of oxytalan fibers during root development, which accounts for their closer proximity to the cementum [20]. PDL fibers are classified as apical, oblique, inter-radicular, horizontal, and fibers that reside among the roots of multirooted teeth depending on where they are located and how they are oriented.

In PDL, many cell populations coexist [21]. They can be broadly split into two lineages, one of which consists primarily of fibroblastic cells, and the other of which contains cells like osteoblasts that are in charge of producing mineralized tissue. Although PDL fibroblasts were the most general cell type [22], other cell types have also been found in PDL, including osteoblasts, osteoclasts, fibroblasts, cementoblasts, Malassezian epithelial rests, macrophages, endothelial-, and so on. The ability of PDL-based cells to develop into an angiogenic, neurogenic, and adipogenic phenotype under the right growth conditions and inductive medium has been demonstrated in numerous investigations [23-25]. Because PDL-derived cells are highly heterogeneous and multipotent PDL cells must be isolated to be used effectively in PDL regeneration, primary

cells are identified by the expression of PDL marker genes like periostin [26], as well as markers resembling those of bone mesenchymal stem cells. Additionally, they discovered as PDLSCs exhibit STRO-1/CD146 markers that are comparable to stem cells of mesenchyma. The ability of PDLSCs to express additional MSC markers, including CD10, 13, 29, and 105, has also been demonstrated in numerous investigations [27, 28]. PDLCSs also fail to exhibit the hematopoietic progenitor cell markers like CD-14, 34, 45, and HLA-DR, confirming their somatic behavior [29]. The extracellular matrix, which is primarily made up of fibers, is produced and maintained by PDL fibroblasts [30]. These cells produce different bioactive chemicals involved in tissue remodeling and wound healing as well as fibrillar collagen, which is synthesized and digested [31]. The PDL fibroblasts function as a device that absorbs mechanical load by having the ability to endure as well as disperse the large occlusal loads put forth during mastication [32]. In addition to offering defense against strong mastication forces, PDL collaborates with gingival tissues to create a potent barrier against oral microorganisms.

Periodontal ligament Stem cells (PDLSCs)

Stem cells have self-renewal, colony formation, as well as cell diversity [33]. Stem cells can either stay in the stem cell niche or respond to signals, or they can leave circumstances for self-renewal or differentiation into particular cell lineages [33]. Several different stem cell types have been isolated at this time and published in human studies. You can classify stem cells into induced pluripotent stem cells (iPSCs), induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), MSCs stem cells, and mesenchymal stem cells [34]. PDLSCs have been identified, in in vivo, and in vitro tests have been performed for assessing their capacity to grow into different types of tissues. PDLSCs exhibit multipotent differentiation abilities, including the capacity to separate as adipogenic, osteogenic, as well as brain cell lines [35]. PDLSCs are employed in regenerative medicine due to their capacity for multiple differentiations, which makes them a resource of cells for both dental TR and the restoration of non-dental tissues like nerves and bones [36, 37].

Description of PDLSCs

A tooth was secured by surrounding alveolar bone having fibrous periodontal ligament. According to reports, this structure is a cause of adult stem cells that may restore alveolar bone tissue and keep the periodontium in a state of homeostasis. Third molar teeth that have been extracted contain human PDLSCs, which possess self-renewal capacity as well as cell distinction to diverse specialized cells [38]. The ability of hPDLSCs to distinguish between osteoblasts, chondrocytes, adipocytes, and other lineages like neurons has been demonstrated in earlier research [39, 40]. For clinical research and dental stem cell therapy, the multipotent properties of hPDLSCs are essential.

CD146/MUC18 and STRO-1, markers of MSCs, were articulated by isolated hPDLSCs, which have a fibroblastic and spiky appearance [35]. Additionally, immune-compromised mice that have received hPDLSC transplants by tricalcium phosphate (HA-TCP) and hydroxyapatite develop PDL-similar tissues/cementum that resembles Sharpey's fibers, which connect that cementum as well as alveolar bone [15, 35].

PDLSCs as well as DPSCs transplanted into swine help to regenerate dental tissue and restore functional tooth-like structures [20]. Additionally, hPDLSCs express scleraxis, similar to cells from tendons and ligaments, supporting high occlusal forces on food mastication, bruxism, as well as compressing [35]. Through the

formula of the Twist genes and S100A4, hPDLSCs prevent osteogenesis by preserving the gap between the cementum and the surrounding alveolar bone [41, 42].

PDLSCs are adult stem cells, although they are more potent than stimulated iPSCs, which were comparable to hESCs. Using particular pluripotent gene markers and the detection of telomerase production, iPSCs from hPDLSCs have been proven [43, 44]. The cells can distinguish a larger range of cell types than other somatic stem cells thanks to PDLSCs' more potent iPSCs.

Cell-Guided PDL Regeneration

Regeneration of the PDL was a difficult and ambitious undertaking because it requires an extremely coordinated spatiotemporal remedial process that comprises the development of bone inside the periodontal defect, cementogenesis, and PDL fibers. Its adhesion to the surface of the root [45]. Additionally, difficulties resulting from the avascular structure of teeth surface, the microbial buildup, and the theoretically difficult working environment brought on by the restricted access are all factors [45]. Using tissue engineering (TE) Lately, aiming to perhaps regenerate several different tissues and organs, including [46] The periodontium. A method of TE includes the usage of the 3D scaffold, coupled with biologically active chemicals and cells, and it may control the healing processing and avoid the aforementioned difficulties [46].

Post-natal progenitor cells have increasingly been used in cell-based PDL regeneration efforts during the past few years, making them a desirable option for TE applications. Due to its capability to differentiate into many cell types, including immunomodulation, anti-apoptosis, angiogenesis, and cell-based conscription, MSCs are currently the cell type that is most frequently used for cell-based regeneration [47]. Preceding efforts at cell-based periodontal regeneration have included diverse types of cells, which includes bone marrow MSCs (BMMSCs) and gingival fibroblasts (GFs).

PDLSCs were cells that were separated from PDL and has a unique capacity to regenerate complex PDL tissues while sharing characteristics with MSCs [48]. The most frequent type of cell seen in gingival tissue, gingival fibroblasts (GF), are known to alter their behavior and relocate into periodontal abnormalities [49]. When used in regenerative applications, it has been discovered that GF can express proteins related to bone and can form mineralized tissue, supporting the theory that they have stem cell properties [49]. A BMMSC has shown evidence of substantial proliferation and multicellular differentiation lines, but their use in periodontal abnormalities has produced some inconsistent results outcomes [50]. The morphology of the defect has a direct bearing on their efficacy where grade III furcation and fenestration show evidence of enhanced bone growth BMSCs used in three-wall intrabony flaws, however, have little success influencing the growth of newer bone [50]. Due to their accessibility, ability to be collected in large quantities without surgery, and capacity for multilineage differentiation, dental pulp stem cells (DPSCs) were viable for cellular sources in TE uses [51]. Administration of DPSCs has been discussed as a potential therapeutic approach, and they are used in vivo to target periodontal regeneration. Their usage might help with bone regeneration, although it's yet unclear how well they work with cementum or PDL regeneration [52].

However, because of the immune response brought on by the breakdown of the scaffolds, TE uses do not always produce the intended consequences [45]. Lower survival of expanded and developed cells in vitro before embedding into the living organism, failure of inoculated cells to connect to the point of embedding, and an absence of vascularization in revascularizing the point of interest are additional issues that can arise

[53, 54]. To address the issues with TE procedures, strategies that use extracellular matrix (ECM) creation in vitro previous in cellular transplantation, like cellular sheets/pellets, have attracted interest.

The preservation of ECM proteins is made possible by the unique technology renowned as cell sheet engineering, which enables some cell acquisition in the sheet structure to lack the use of proteolytic enzyme techniques [55] as shown in Figure 2. Cell sheets have been harvested using a variety of techniques, including the usage utilization of polymerized human fibrin-coated dishes, T-responsive culture dishes, as well as the use of Vitamin C therapy [56] temperature-responsive culture usage. The initial technique adopted for the creation of cell sheets was dishes and has been used most frequently instigated using a material called Poly(N-isopropyl acrylamide) (PIPAAm), a polymer that responds to temperature [57]. PIPAAm created a smart bio-interface that allows temperature adjustment to influence the adhesion of cells [58]. Under typical 37˚C cell culture conditions, hydrophobic surfaces, permit the growth of cells. Under these values of the critical temperature of 32ºC, however, the surface develops hydrophilic, causing detachment of cells on the culture surface and lacking the need for proteolytic enzymes [57]. This method has many merits over traditional ones, including minimal cell loss, preservation of the veracity of protein adhesion like E-cadherin as well as laminin 5, and retention of ECM types of machinery released via cells [57]. Direct application of cell sheets into a problem area is possible in two ways: as a coating (several sheets can overlap and form a 3D structure), or by shrinking and generating a cell-based pellet.

This study focuses to explain 3D Bio printed scaffold with biomaterials and periodontal ligament stem cells for enhancing periodontal regeneration utilizing a porcine model, through spread over cell sheet technology and scaffold concepts.

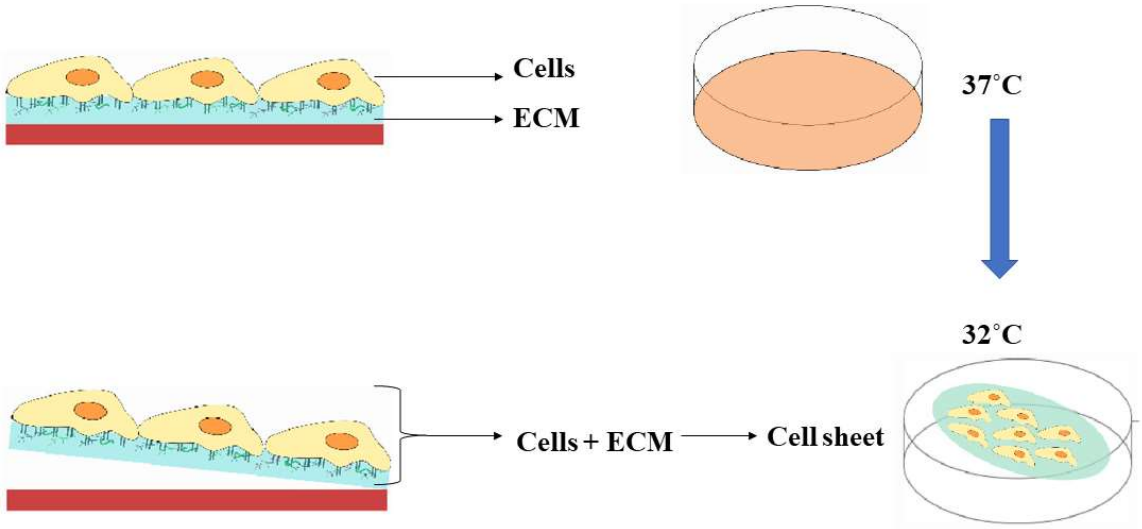

Figure 2. Cell sheet harvesting schematic diagram.

In Vivo Studies

The included throughout Vivo research was using the intrabony/furcation periodontal defect mode or the boundaries periodontal hole mode as some orthotopic model, also several ectopic forms for examining the effectiveness of TE housing projects of scaffolds, matrices, membrane, hydrogels, etc. to regenerate tissue, as depicted in Figure 3.

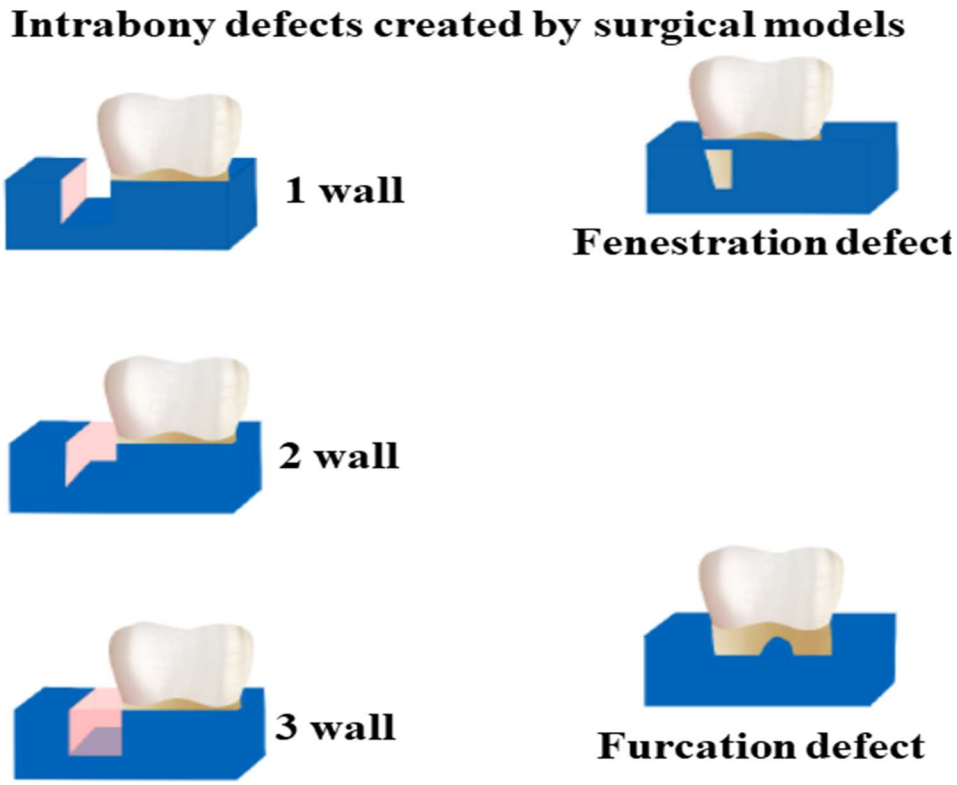

Figure 3. Most general used periodontal defect models for periodontal tissue regeneration

Cell Sheet Engineering

Ectopic Models

26 research evaluated the ability of cell sheet transplantation to regenerate periodontal tissue using ectopic models. In 25 of these experiments, naked mice were utilized; rats were used in one study. To mimic orthotopic conditions and evaluate each cell sheet's potential for periodontal regeneration, studies utilized numerous biomaterials, including chemically conditioned root dentin (CCRD), ceramic bovine bone (CBB), etc. Two investigations used bioengineered tooth roots (bio-roots) made of HA/TCP and covered on those cell sheets to implant the cell sheet/material composite into the implantation of jawbone implantation sockets [59, 60]. Additionally, two investigations [61, 62] evaluated the ability of cell sheets mixed with titanium particles to regenerate tissue. Washio et al. [63], titanium implant complexes with hPDLCs sheets are implanted for defects in mandibular bone, and cell analysis demonstrated that tissues on titanium surfaces are similar to PDL and cementum. These results are encouraging for efforts to develop a constant periodontal complication that surrounds the dental implant in near future.

In the included research, a diversity of pretreatments are used to enhance the ability of cell sheets to regenerate. The Vc pretreatment was chosen as the preferred approach for creating cell sheets in five investigations [64, 65]. The impact of pulse having less intensity ultrasonic (LIPUS) inducement on a sheet of PDLSC development as well as periodontal TR in vivo was examined by Li et al. [66]. Their findings demonstrated that LIPUS-treated PDLSC sheets had an advantage over the untreated PDLSC sheet group in terms of ECM production and PDL-like TR. In comparison to the control group, pre-treated hPDLSC sheets displayed

considerably greater levels of mineralization as well as collagen ligament accumulation, which allowed the development of a PDL cementum-like complex [81]. To create cell pellets for use in periodontal TE, hPDLSC sheets were changed into conditioned media (CM) from growing apical tooth germs by Yang et al. [67]. The CM-treated group was found to have cementum-like mineralized as well as PDL-like fibrous-based tissues, whereas the control group that did not receive CM infrequently produced cementum/PDL-like tissue [67]. Two investigations [68, 69] utilized platelet-rich derivatives; first utilized platelet-rich plasma (PRP) for pre-treatment, and second utilized platelet-rich fibrin (PRF), as biologically absorbable scaffolding. In comparison to the untreated control, PRP pretreatment dramatically improved PDLSC osteogenic differentiation and boosted bone and collagen production in vivo [69]. Additionally, when paired with jaw BMMSC rather than PDLSC sheets, the utilization of PRF, a bioabsorbable-based scaffold is helpful in the case of PDL as well as bone tissue production [68].

Five reports examined the effects of coculture of various cell types on the characteristics and efficiency of cell sheets, and also their capacity for regeneration. The features of the cell sheet produced by the coculture scheme appear to be improved when PDLSCs are cocultured by various cell lines. For more precise, hPDLSCs and hBMMSCs were cocultured, and the resulting mixed cellular sheet was employed for making some cellular pellets that were useful in ectopic relocation and demonstrated improved cementum/PDL-like TR by neovascularization in comparison with some cell pellets which is non-mixed [70]. Additionally, these in vivo cell sheets administration from PDLSCs coculturing by urine-derived stem cells (USCs) led to improved expression stages of ECM/bone associated proteins as well as genes, development of complex tissue comparable to the native periodontal tissue [71-73]. PDLSCs as well as human umbilical vein endothelial cells (HUVECs) are used to develop triple-cell sheets, either by combining the diverse cell sheets/by coculturing the cells together. Panduwawala et al. discovered that both of these circumstances led to the formation of periodontal fibers that were similar to PDL and vascular lumens [73]. When cocultured with DFCs, Liu et al. evaluated some regeneration potential of PDLSCs in certain healthy persons as well as individuals with periodontal disease (PPDLSCs) [74]. The stemness of PPDLSCs and HPDLSCs seemed to be enhanced by DFCs, and the cocultured HPDLSC was able to redevelop the PDL complex while the PPDLSC sheet had poor fiber adhesion and inflammatory cells detected in the regenerated tissue [74].

Orthotopic Models

The healing potential of cellular sheet transplantation in periodontal deficiency studies, with or without biological materials, was evaluated in 20 investigations using orthotopic models. Through a variety of animal models and experimental techniques, the studies looked into the possibility of cell sheets to promote in vivo rejuvenation of periodontal tissue. Diverse periodontal defect models are utilized, including the one-wall bone effect study in two studies [75, 76], the three-wall intrabony defect model in two studies [77, 78], and the horizontal defect model in one study [79], the fenestration defect model in another study [80], the dehiscence defect model in another study [81], the class III furcation defect model in another study [82], the two-wall intrabony defect model in another study [83], and the class III. In one-wall surgically generated deficiencies in dogs, Tsumanuma et al. [76] evaluated the effects of 3-layered cellular sheets from various cell lines. They discovered that the usage of PDLC sheets led to the formation of more recently developed thicker or cellular/acellular cementum, more solid collagen fibers, as well as augmented PDL development in comparison to APC/BMMSC sheets. The efficiency of PDLC/ DFC sheets near regenerative periodontal was evaluated

using the two-wall intrabony effect with dogs in the study by Guo et al. [83]. Whilst novel periodontal accessory was seen in both groups, only the DFC sheet group showed full periodontal regeneration connecting PDL and cementum. This group also showed improved bone production when compared to the PDLC sheets. While Yang et al investigation's revealed comparable periodontal regeneration ability between SHED and DFC sheets [75]. More particular, the regeneration of tissues of experimental groups had collagen fibers as well as fibroblasts that were aligned perpendicularly and in a well-developed manner, much like natural PDL [75]. Another research demonstrated the dominance of applying a complex cell sheet made up of both osteoblastic cells and PDLCs over applying a single cell sheet made up of either cell line [84]. The restoration of the functional joining among the alveolar bone and tooth root was achieved in detail by the application of complex cell sheets, as opposed to control groups, which showed only partial recovery in the development of both soft or mineralized tissue [84]. Vaquette et al. identified PDLC sheets and BMMSC showed identical outcomes for the generation of new bones, cementum, and PDL revival afterward 10 weeks, while these groups showed greater restorative capacity in comparison with GF sheets [81]. This was when associating 3 diverse cell sheets with the needle-inserted dehiscence periodontal effects on sheep.

Different techniques for obtaining cell sheets, usage of cultural dishes which are temperature-responsive, an administration of Vc, along with their impact on the periodontal regenerative capacity of these PDLSC sheets were examined by Wei et al. [85]. When these sheets were applied in a defective location, more bone/cementum-like matrix was formed than when PDLSC sheets with the help of temperature-responsive culturing dishes were used [86]. Two investigations examined the impact of various pretreatments, including inflammatory activation and hypoxia, on the capacity for cell sheets to regenerate [83, 87]. According to Yu et al. work's PDLSCs' ability to regenerate mineralized tissue, cementum, and PDLs in vivo was improved by a 24-hour hypoxic pretreatment [87]. Various nanomaterials, including HA/TCP, Matrigel, CBB, gel/ polycaprolactone scaffold, polyglycolic acid (PGA), TDM, platelet-rich fibers, as well as porous -TCP, was mixed with the cell sheets in the various investigations. Zhao et al study.'s [89] evaluated the impact of platelet-rich fibrin granules on PDLSCs sheet remains to direct regeneration of periodontal. In comparison to the other groups, the combined administration of PDLSC/PRF is even more successful in renewing PDL-like tissues as well as preventing ankylosis and irritation in the tooth reimplantation model employed in this investigation [89].

In a medically produced periodontal deficit in rat maxillary molars, Iwasaki et al. used a decellularized amniotic membrane (amnion) rather than a designed cell sheet, with or without the PDLSCs. Four weeks after this transplant, periodontal TR was improved by the presence of PDLSCs, according to the findings of the histological and radiological investigations [90]. Jiang et al. [91] researched PCL/GE nanofibers, 15-deoxy-D12,14-prostaglandin J2 (15d-PGJ2) nanoparticles, which are decellularized sheets from human PDL fibroblasts were combined as potential choices for periodontal regeneration. Regardless of whether PCL/GE nanofibers were present or not, the use of decellularized hPDLCs sheets led to effective bone tissue ingrowth and PDL and cementum-like tissue development on the mandibular first molar’s root. Some favorable role of the decellularized matrix was established by Farag et al. [92] in their assessment of the impact of PCL incorporated PDLSCs sheets scaffold with periodontal regeneration in a rat. More specifically, compared to the PCL scaffolds alone, the decellularized sheets showed a much higher fresh attachment of periodontal fibers, and the regenerated PDL fibers are arranged and introduced by a perpendicular alignment to the root surface

[92]. A report by Yang et al. [93] demonstrates that TDM/HA/TCP particles shared having DFCs lead to enhanced bone development in comparison with the groups without materials. In addition, in comparison with some control groups devoid of cells, the addition of DFCs possesses some favorable impact on bone growth density as well as the degrees of PDL-like tissue creation [93].

In Vivo Studies with Scaffolds for PDL Regeneration

Regeneration of injured periodontal tissues, a wide range of biological materials in the kind of modest, biphasic, or multiphasic scaffolds is suggested. These 2D and 3D constructs were created using the ideas of total regeneration of the periodontal or partial regeneration of particular sections, like PDL or bone tissues, with the majority of them concentrating on bone regeneration and examining the osteogenic potential of substances. Different degradable or non-degradable membranes are used to avert epithelial down-growth for enabling the repair of injured periodontal ligaments and connective tissues since GTR represents the "gold standard" within periodontal surgical procedures. With the aid of additional connected or loaded substances and growth factors, the usage of scaffolds purposes to create bio-compatible and bio-active platforms that can result in sensible and regulated cell relocation, propagation, and differentiation to support TR. In this way, various animal species and in vivo studies, like surgical scaffold insertion, have been studied. Generated ectopic tissue growth or periodontal abnormalities with subcutaneous implantation in creatures.

Periodontal Defect Model

The fenestration periodontal defect model or furcation or intrabony periodontal defect models were used in in vivo research assessing scaffolds. The main procedure for the furcation model involves surgically creating one/two-wall bone effects to close the premolars and molars roots, removing cementum and PDL, as well as placing a scaffold right next to root dentin; these defects are typically 5 mm in the direction of apical-corona. Beagle dogs were mostly employed in investigations estimating several therapeutic techniques aimed at periodontitis as well as periodontal tissue regeneration in scaffolds [94]. Their usage is justified by the fact that their oral microflora and periodontal tissues resemble those of humans [95]. Additionally, it is possible to perform proper hygiene without sedating the animals, which guarantees their comfort and minimizes the possibility of issues with proper healing and regeneration. Dog studies have important drawbacks in that they do not use lateral movements while chewing and have a higher rate of bone remodeling, which could lead to strong regenerative potential and better in vivo study outcomes [96].

There are two main surgical methods in use; one involves making intrabony defects and the other involves making supra-alveolar critical-size furcation defects [97]. The beagle or other dog models have been used to test hydrogels [98, 99], scaffolds loaded with nanoparticles made of polymeric material or collagen [100], and mixed micropatterned scaffolds made of polymeric material [101], either loaded with cells or not. In the growth of acellular cementum on roots surfaces as well as the production of PDL-like tissue, the FGF-2 usage in conjunction with a nano-Beta-TCP collagen scaffold proved beneficial [102]. Momose et al. [103] made the inline suggestion, using a collagen hydrogel scaffold that had been incorporated using FGF-2. Even though both studies confirmed the existence of fibrous tissue similar to PDL, which failed to detect tissue addon or the development of functional Sharpey's fibers. Associated with beta-TCP, which showed minimal osteoid deposition, bioceramic diopside ceramics were more effective at producing significant amounts of cementum, bone, as well as collagen fibers [104]. Collagen sponges and cell-filled with some organic bovine bone or chitosan composite scaffolds produced larger amounts of new bone or cementum with thick PDL fibers [105],

oriented perpendicularly or obliquely [106]. On the other hand, Liu et al. who have tried BMSCs filled with collagen hydroxyapatite scaffolds packed showed a restricted beneficial effect, which could be accounted for by the restricted cell subsistence inside their scaffold caused by certain low blood flow in labial alveolar bone [107]. The schematic representation of supra-alveolar periodontal defect in the canine is given in Figure 4.

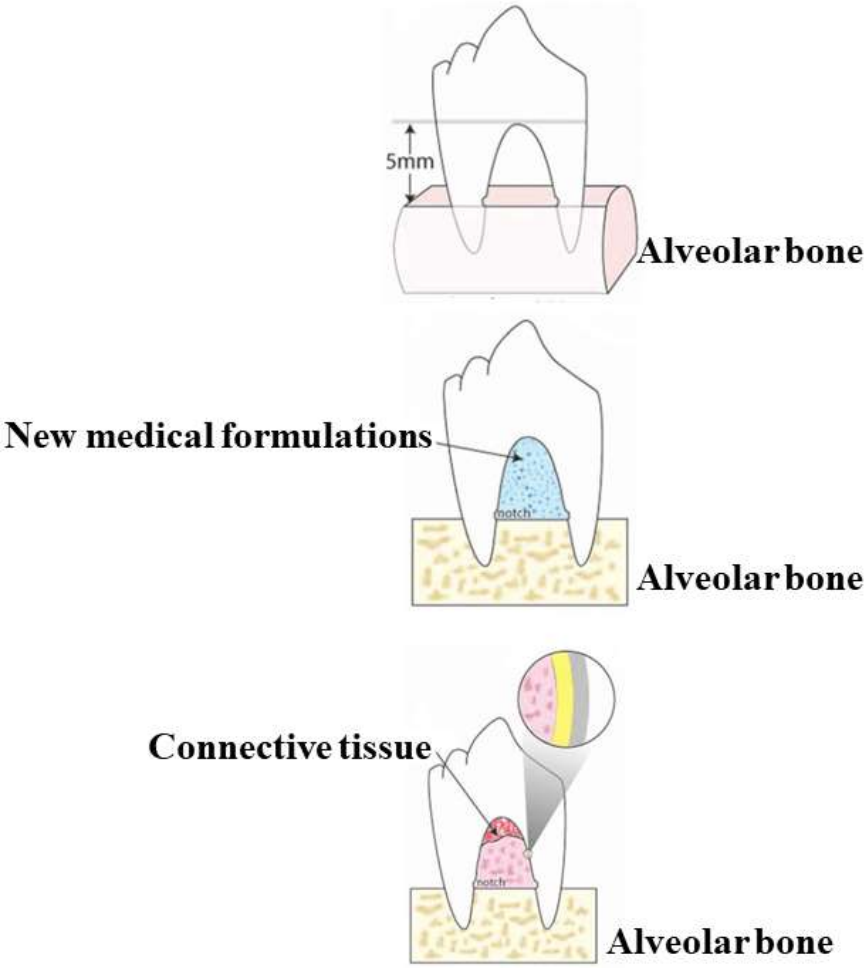

Figure 4. Supra-alveolar periodontal defect in the canine.

There is surprisingly little research investigating PDL regenerative scaffolds in tiny pigs, although these animals have been employed as more practical and trustworthy animal models in numerous dental investigations [108, 109]. Numerous research in recent years has shown that pigs are a suitable animal species because their bone structure, morphology, pace of healing, and remodeling are thought to be similar to those of humans [110]. Pigs also have a temporomandibular joint that is physically and functionally similar to that of humans, and because they are omnivores, which chew using their lateral jaw motions, making them a better prototype for simulating some mastication cycle. Still, these are constrained by their natural features, which include larger teeth surrounded by more bone, a longer junctional epithelium, and several oral microflorae. The periodontal lesion standard in scaffolds having micro pig combined for regenerative PDL has only been employed in a few studies [108, 111]. DSCs were seeded and maintained in hybrid tooth constructions made using PGA/PLLA as well as PLGA scaffolds in bone and tooth sections, correspondingly, for 12 & 20 weeks [108]. Despite these developments, a novel cementum little fibrous tissue in the periodontal ligament

resembled Sharpley's fibers. To maximize regenerations using limited inflammatory stages in periodontal wound healing, a scaffold using hyaluronic hydrogel that releases an IL-1-receptor antagonist was employed [111]. Despite the formation of cementum-like tissue as well as the anchoring of PDL-like fibers to cementum, the IL-1-receptor antagonist group did not show any discernible effects.

The most common animal species utilized to execute the periodontal defect model in recent papers is the rat species. Rats have dominated periodontal TR studies despite having continuous tooth periodontal and eruption remodeling with bone and cementum collocation, which may produce optimal outcomes in regards to possible regenerative ingredients and methods. These factors, along with rats' ease of handling, low maintenance costs, and lack of ethical or social concerns have also contributed to this. Before moving on to larger animal trials, the rat periodontal fenestration defect models having extraoral operating technique was typically employed as well as extensively adopted. This approach has the benefit of preventing gingival tissue ingrowth, but it is technically more difficult [112].

For PDL regeneration, much research has combined Sprague-Dawley, Fischer 344, Wistar, and athymic nude rats with various scaffolding materials. In periodontal deficiencies in rats, different fibrous polymeric structures, primarily electrospun hydrogel scaffolds [113-115], In comparison to uncoated substrates, a biomimetic F/CaP coating technique is used on PCL scaffolds and were successful in generating fresh alveolar bone, PDL, and cementum. On PCL scaffolds, primary HPDLs seeding cells with cell PDLSCs and sheets produced a complex periodontal tissue with PDL fiber angulation that was comparable to native tissue. Contrarily, PDL-like tissue was ranged parallel with root when basic PCL scaffolds were utilized [9, 92], and just a few fibers were needed to introduce the cementum layer. A biphasic scaffold was created, including an amorphous PCL sector for bone development and a micropatterned PLGA/PCL compartment for PDL repair [116]. The PDL segment was seeded with hPDLs, the bone segment with hGFs, so each compartment was altered to include vectors expressing BMP-7 (for the bone section) and PFGD (PDL compartment). Different combinations of PDL segment micropatterning and gene transfer were assessed, as well as the micropatterned PDL consistently produced the best results for obliquely oriented PDL development regardless of single/dual gene transfer.

Rabbits were employed for studies to evaluate therapeutic variables for the management of periodontitis [117, 118], but their usage in studies to assess the regeneration of periodontal/peri-implant tissues is relatively restricted [119]. This is a result of their distinct from human bone structure and remodeling rate. To implant scaffolds of tri-layered nanocomposite hydrogels using FGF 2 (PDL compartment) with platelet-rich plasma (PRP)-derivative anabolic hormones, Sowmya et al. [7] first induced lesions with white rabbits maxilla in New Zealand.

They concluded that while PDL, bone, and cementum, were all produced within some tri-layered scaffolds, the introduction of growth factors led to the development of more organized bone tissue. Sheep and mice are further options for PDL regeneration, however with very little data. Research done by, Vaquette et al. [81] was using some ovine periodontal defect prototypes to examine whether varying types of cells for seeding biphasic electrospun scaffolds of PCL/Beta-TCP could affect PDL rejuvenation in various ways. They found that while robust cementogenesis and PDL renewal are demonstrated in cases in which PDLCs and Bm-MSCs are propagated, devoid of cementum creation is seen in the usage of GCs. Beta-TCP scaffolds are grown on gene-transfected BMSCs as well as transplanted in nude BALB/c mice periodontal abnormalities in the work of

Zheng et al. [120]. Scaffolds of Beta-TCP are required for gene-transfected BMSCs as well as nude BALB/c mice periodontal abnormalities are implanted in the study of Zheng et al. [120]. PDL and cementum could be rebuilt using cell-seeded scaffolds, whereas imperfections of scaffolds of tidy beta-TCP produced fibrous tissues, neither cementum/oriented fibers. These results show that, regardless of their content or form, scaffolds cannot, by themselves, induce regeneration of PDL and attachment towards bone/cementum. In many investigations, loading scaffolds using cell seeding or growth aspects are considered successful for supplying larger quantity bone. The topographical signals required in cells for encouraging PDL regeneration as well as the entire periodontal tissue complexes were delivered by multiphasic/patterned scaffolds which resemble the operational divisions in the periodontium. The calcium-based element was another feature of scaffolds that contributes to the development of functional PDL, albeit it is unclear whether this component's chemical affinity for bones and cementum or its topographical orientation is what drives PDL regeneration.

Clinical Studies Involving Caffolds Combined with Cells

Stem cell-based therapies are another promising method for treating periodontal TR. Several reservoirs of stem cells, including PDL, bone marrow, and dental pulp, gently exfoliate gingival, deciduous teeth, as well as umbilical cords of humans, have been listed in the literature. Allogenic MSC isolation out of the anthropological umbilical cord was less expensive as well as less intrusive when compared to conventional isolation of cells techniques. Additionally, cells can develop into cementoblasts, osteoblasts, as well as PL fibroblasts, were having the capacity for self-renewal, making them pluripotent. As a part of this, Dhote et al. [121] used umbilical cord MSCs having scaffolds of TCP and platelet-derived growth factor-BB (PDGF-BB), as well as they, discovered a substantial increase in radiographic hole and diagnostic reference level fillings compared with open flap debridement (OFD). During chronic advanced periodontitis treatments, Ferrarotti et al. [122] employed micrografts of autologous DPSC which are directly applied with collagen sponges. The clinical and radiological characteristics were reported to be better by the authors when compared to control sites that only received collagen sponge treatment. Baba et al. [123], repaired inrtabony imperfections in 10 individuals using stem cells of autologous bone marrow combination using PRP with the aid of woven-fabric composites made out from scaffolds of poly-L-lactic acid, likewise documented safety and positive clinical outcomes. For subsequent comparisons, there was no control group, though. In contrast, Chen et al. [124] found no appreciable variations among demineralized scaffolds of bovine bone incorporated with some PDL-derived MSCs as well as the scaffold itself in terms of clinical or radiographic observations. Following 12 months of observation, the physicians concluded that the implantation of MSCs and scaffolds was safe because they did not notice any adverse reactions or alterations in blood composition. In a pilot clinical investigation on 20 patients, Sanchez et al. [125] did not discover any additional therapeutic advantages of PDL MSC-based stem cell transplantation relative to xenogenic substitute material alone for 12 months.

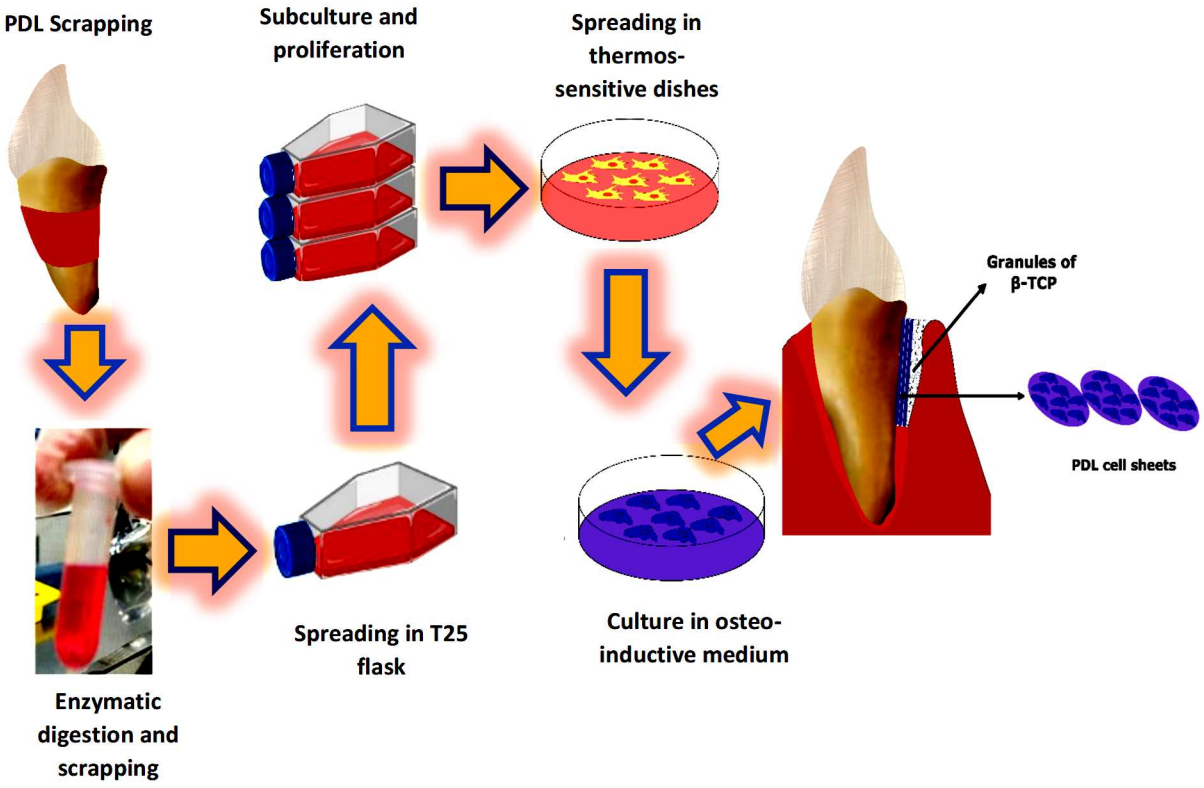

Figure 5. Clinical study for cell sheet transplantation

Three different approaches to treating periodontal bone abnormalities were evaluated survey by Apatzidou et al. [126]: a combination with alveolar bone marrow MSCs which is collagen frameworks incorporated as well as autologous fibrin/platelet lysate (aFPL), a scaffold of collagen made of aFPL, and devoid off scaffolding control. Follow-up in subsequent years, no intergroup alterations are found, although all treatment modalities significantly improved clinical outcomes as radiographical bones filled with the healing of soft tissue. These studies hypothesized a long-term MSCs-based treatment usage may be beneficial for abnormalities with complex, non-contained morphologies. Iwata et al. [127] revealed a quite different method for treating severe bone defects, using cell sheets derived from PDL in conjunction with TCP, as shown in Figure 5.

Summary:

MSC-based therapies for periodontal regeneration are further assessed in histologic and long-term randomized clinical trials. This conclusion can be drawn as a result of clinical research utilizing scaffolds as well as cells in periodontal TR. Aside from that, several significant factors, including safety, the MSCs immunogenicity, latent threats, as well as cost-effectiveness, were taken into account on cell-based gingival therapies are implemented for therapy selection in clinical practices. Table 1 shows the different clinical research cells and scaffolds for periodontal tissue regeneration.

Table 1. Clinical research includes cells and scaffolds for periodontal tissue regeneration.

Type of scaffold	**Cell types**	**Study type**	**Experimental group**	**Number of subjects used**	**Results**
Collagen gel	hGF	Clinical	hGF from attached gingiva was added to collagen gel. Each patient: 1 tooth treated with a periosteal fenestration technique (control group) or a tissue engineered mucosal graft (test group).	9 patients (18 sites), 3 months	The mean amount of linked gingiva was greater at test sites than at control sites.
Bio-Oss	Autologous PDLSCs	Single center, randomized clinical trial	Group 1: GTR and PDLSC + Bio-oss® and Group 2: GTR + Bio-oss® (control group).	30 patients, 12 months	No statistically significant variations were identified among groups.
Xenogeneic bone substitute (XBS)	PDL-MSCs	quasirandomized controlled pilot clinical trial	Patients with moderate and severe chronic periodontitis with one or two wall defects: (1) XBS + PDLMSCs and (2) XBS (control)	20 patients, 12 months	No significant differences among groups, lower morbidity, and safety of cell-based therapy
Collagen sponge	Autologous DPSCs	Randomized controlled trial	Patients with severe periodontitis: (1) DPSC micrografts seeded onto collagen sponge (n = 15), (2)	29 patients, 12 months	Significantly greater clinical link level gain and bone defect fill in test groups

			collagen sponge alone (n = 14, control).		
Collagen scaffolds with autologous fibrin	Autologous alveolar bone marrow MSCs	A proof-of-principle randomized clinical study	Group-1 (n = 9) BMMSCs seeded into collagen scaffolds, and aFPL. Group-2 (n = 10), the collagen scaffold/aFPL seeded with a BMMSCs Group-3 (n = 8) no scaffold, minimal access flap surgery	27 subjects with advanced periodontitis, 12 months	Significant clinical enhancements with no inter-group variations Better clinical outcomes in Groups 1 and 3, over 2nd.

Discussion and Concluding Remarks

The expansion of scaffolds using the regeneration of PDL has been tested in vivo using a variety of materials. Aliphatic polyesters like PGA, PLA, its copolymers, PCL, and PLGA have all been the subject of in-depth research [128]. The predominant polymer is PCL, which is employed as a membrane or in composite scaffolds with other polymers like PLGA or inorganic minerals like HA/TCP. These preferred PCL usage results in accessibility, affordability, and a greater standard of modifiability [129]. It has a high degree of crystallinity (50–60%) and slower in vivo hydrolysis, yet its physicochemical and mechanical characteristics can be easily modified to suit various requirements. A variety of electrospun PCL scaffolds have been created, including electrospun or multiphasic scaffolds [130] for good simulating periodontal tissue architecture as well as cementum formation. Electrospun PCL membranes were also established for common drug delivery having obstacles for encompassing periodontal deficiencies [92]. Periodontal regeneration has been aided in various ways by calcium phosphate minerals and biomimetic calcium phosphate layers. The most widely used of these are HA followed by bioactive glasses [7, 131] and various ceramics.

In two in vivo experiments [9, 132], calcium phosphate coatings were used, and the results showed increased bone or cementum prepositional phrases. Even though it does not appear to be able to efficiently regenerate periodontal tissues whether adopted with other scaffolding materials or growth factors, _-TCP is the substance that is employed almost entirely in clinical applications. Biphasic calcium phosphate (BCP) is employed in a randomized clinical study [133], and then scaffolds made of calcium phosphate were created using 3D printing in BCP as well as utilized in a case study [134]. To replicate bone resorption rate, these composites, which consist of HA and TCP with a 60:40 ratio of HA to TCP, serve as a buffer over the rapid dissolution rate of _-TCP as well as the lower dissolution of HA [135].

Table 2. Merits and demerits of the various substances employed for periodontal regeneration.

Materials	Merits	Demerits
Natural	Better biocompatibility as well as cellular affine	Important deterioration rates Weaker machine-driven features
Synthetic	Better Physico-chemical/mechanical features Higher inconsistency in deterioration rate as well as resorption kinetics	Lower biological activity
Ceramic	Composition identical to bone tissue Osteoconductivity induces bone healing	Non-compatible cellular encapsulation Fragility Numerous cellular reactions as per its surface features

For periodontal regeneration, sponges and hydrogels have also been employed as scaffolds [136]. Collagen is the main substance employed in this regard, along with HA, FGF2, and BCP. Collagen hydrogels may be employed successfully in the form of drug carriers and offer valuable qualities like easy use, minimal toxicity, reasonable cost, and viable synthesis. Each of the materials utilized in periodontal regeneration scaffolding has a distinct advantage compared to the others, and in most cases, multiple materials employed in diverse combinations lead to superior outcomes. The best method for manufacturing scaffold sections appears to be the creation of multiphasic scaffolds having various biomimetic microtopography as well as patterning, as this effectively directs cell growth to produce PDL-like tissues having proper position. This morphology of sophisticated scaffold structures is still very different from the typical architecture of periodontal tissues, despite advances in manufacturing technology. The foundation for individualized treatment of devolved periodontal tissues can be laid by utilizing novel scanning technologies which may transmit through greater precision with periodontal deficiency towards cutting-edge milling machines which is employed cutting-edge technology for creating suitable scaffolds, surface micro/nano-patterning, as well as topography. To accommodates the intricate nano topography of the periodontal architecture, this technique has intrinsic limitations in terms of cost and resolution. When comparing various materials and methods for regenerating periodontal tissues, animal models have proven to be quite useful. As most imperfections are surgically formed and do not resemble the actual destabilization sites even-after the establishment of periodontitis, which may not be able to mimic medical symptoms. Additionally, they are unable to account for aggravating factors like the aggressive existence of bacterial types, general illnesses, mass immune system, or occlusal parafunction. Next is the lack of clinically significant stress which might influence some regeneration's reaction in altering the necessary molecular as well as cellular pathways when assessing PDL scaffolds for model's regenerations that do not incorporate periodontal tissue environments, such as placement of subcutaneous layers/calvaria models. Even while defined protocols are more or less consistently followed in the majority of investigations, there are still faces some differences in the study methodology and materials used, which makes it nearly hard to directly compare data and come to reliable conclusions. The simultaneous regeneration of bone and

cementum is necessary for tissue formation regeneration.

Because although new bone production has been seen in the majority of investigations, in vivo cementum regeneration remained difficult due to the lack of particular expression markers and differentiation mechanisms. The cementum attachment proteins (CAP), as well as some cementum protein-1 (CEMP-1), are two indicators that were identified in cementoblast's findings [137, 138]. Cementoblasts and their progenitor cells have been found to express CEMP-1, whose increased expression in PDLCs supports cementoblast's development while its decreased expression suggests osteoblastic or periodontal differentiation [139]. SPON1, MiR-628-5p, as well as PTPLA were found to have enhanced expression during the early phases of cementogenesis, but miR-628-5p as well as miR-383 limit CEMP-1 countenance [140]. Cementogenesis's last stages suggest, that CEMP-1 and PDPLA expression are boosted [140]. The periodontal complex restoration process, which consists of cementum, alveolar bone, as well as PDL tissues, has been successfully treated by cell sheet engineering, primarily in preclinical studies but also in one clinical research. The periodontal cell sheet engineering uses PDLSCs as cell sources. Applications of cell sheet engineering encounter difficulties that are inherent to the technology along with some therapies of cell-based in general.

The availability of such medicines to the general public is constrained by the expensive and legally restricted processes of cell separation as well as in vitro growth. Cell sheet engineering, a technologically complex process that can be difficult to handle and stabilize, and appropriate addon in situ were essential in regeneration results [81]. and Clinical, as well as preclinical investigations, have confirmed the efficacy and security of autologous transplantation of PDLSC sheets. Nevertheless, many restrictions make this therapeutic approach difficult, including the patient's advanced age, periodontitis, and a dearth of cell supply [140]. Cell isolation and growth as a methodology is a less time and costly method, making it less frequently an appealing or favorable treatment option for the patient [141]. Cell exclusion and expansion are used to fabricate a reliable and constant invention resulting in quality standards with Good Manufacturing Practices (GMP). To produce cell banks and widely available products, shifting focus to an allogeneic cell source could be more time- and cost-effective while simultaneously offering a potential cure for individuals with the aforementioned restrictions. Allogeneic cell sheets are a secure and efficient replacement [77, 78]. By preventing bacterial invasion, safeguarding against inflammatory peri-implantitis, dispersing enhanced occlusal load, and protecting against peri-implantitis, PDL renewal on surfaces of Ti using cell sheets engineering was demonstrated to be viable. However, clinical data have not yet corroborated those hypotheses, and more research is required to determine the long-term durability of the Ti surface as well as this PDL-like regenerative tissue. In clinical practice, this TR method based on a mix of scaffolds, bioactive compounds, and/or stem cells (MSCs) may be considered a substitute for previous surgical periodontal procedures. The advantages of scaffold-based therapy are unclear because these results of treatments are already suggested in clinical studies as well as series of clinical cases varied widely. Clinical factors like diagnostic reference level and radiographic bone fill were assessed in the majority of alveolar bone rejuvenation clinical studies. Due to ethical concerns, there is a lack of information about histologic examination to support periodontal regeneration in humans. Large-cohort studies must conduct additional risk-benefit and unfavorable effect assessments.

1. Eke, P.I.; Thornton-Evans, G.O.; Wei, L.; Borgnakke, W.S.; Dye, B.A.; Genco, R.J. Periodontitis in US Adults: National Health and Nutrition Examination Survey 2009–2014. J. Am. Dent. Assoc. **2018**, 149, 576–588.e6.
2. Caton, J.; Zander, H.A. Osseous repair of an infrabony pocket without new attachment of connective tissue. J. Clin. Periodontol. **1976**, 3, 54–58.
3. 3. Stavropoulos, A.; Bertl, K.; Sculean, A.; Kantarci, A. Regenerative Periodontal Therapy in Intrabony Defects and Long-Term Tooth Prognosis. Dent. Clin. N. Am. **2022**, 66, 103–109.
4. Li, Y.; Jin, F.; Du, Y.; Ma, Z.; Li, F.; Wu, G.; Shi, J.; Zhu, X.; Yu, J.; Jin, Y. Cementum and periodontal ligament-like tissue formation induced using bioengineered dentin. Tissue Eng. Part A **2008**, 14, 1731–1742.
5. 6. Requicha, J.F.; Viegas, C.A.; Muñoz, F.; Azevedo, J.M.; Leonor, I.B.; Reis, R.L.; Gomes, M.E. A tissue engineering approach for periodontal regeneration based on a biodegradable double-layer scaffold and adipose-derived stem cells. Tissue Eng. Part A **2014**, 20, 2483–2492.
6. Zhang, Y.;Wang, Y.; Shi, B.; Cheng, X. A platelet-derived growth factor releasing chitosan/coral composite scaffold for periodontal tissue engineering. Biomaterials **2007**, 28, 1515–1522.
7. Sowmya, S.; Mony, U.; Jayachandran, P.; Reshma, S.; Kumar, R.A.; Arzate, H.; Nair, S.V.; Jayakumar, R. Tri-Layered Nanocomposite Hydrogel Scaffold for the Concurrent Regeneration of Cementum, Periodontal Ligament, and Alveolar Bone. Adv. Healthc. Mater. 2017, 6, 1601251.
8. Yu, M.; Luo, D.; Qiao, J.; Guo, J.; He, D.; Jin, S.; Tang, L.; Wang, Y.; Shi, X.; Mao, J.; et al. A hierarchical bilayer architecture for complex tissue regeneration. Bioact. Mater. 2022, 10, 93–106.
9. Daghrery, A.; Ferreira, J.A.; de Souza Araújo, I.J.; Clarkson, B.H.; Eckert, G.J.; Bhaduri, S.B.; Malda, J.; Bottino, M.C. A Highly Ordered, Nanostructured Fluorinated CaP-Coated Melt Electrowritten Scaffold for Periodontal Tissue Regeneration. Adv. Healthc. Mater. 2021, 10, 2101152.
10. Lekic, P.; Mcculloch, C.A.G. Periodontal ligament cell populations: The central role of fibroblasts in creating a unique tissue. Anat. Rec. 1996, 245, 327–341.
11. 15. Birn, H. The vascular supply of the periodontal membrane. J. Periodontal Res. 1966, 1, 51–68.
12. Trulsson, M. Sensory-motor function of human periodontal mechanoreceptors. J. Oral Rehabil. 2006, 33, 262–273.
13. Cho, M.I.; Garant, P.R. Development and general structure of the periodontium. Periodontology 2000 2000, 24, 9–27.
14. Xiong, J.; Gronthos, S.; Bartold, P.M. Role of The Epithelial Cell Rests Of Malassez In The Development, Maintenance And Regeneration Of Periodontal Ligament Tissues. Periodontology 2000 2013, 63, 217–233.
15. Sculean, A.; Nikolidakis, D.; Nikou, G.; Ivanovic, A.; Chapple, I.L.C.; Stavropoulos, A. Biomaterials for promoting periodontal regeneration in human intrabony defects: A systematic review. Periodontology 2000 2015, 68, 182–216.
16. Wang, J.; Feng, J.Q. Signaling Pathways Critical for Tooth Root Formation. J. Dent. Res. 2017, 96, 1221–1228.
17. Dean, R. The Periodontal Ligament: Development, Anatomy and Function. J. Oral Health Dent. Manag. 2017, 16, 1–7.

18. de Jong, T.; Bakker, A.D.; Everts, V.; Smit, T.H. The intricate anatomy of the periodontal ligament and its development: Lessons for periodontal regeneration. J. Periodontal Res. 2017, 52, 965–974.
19. Strydom, H.; Maltha, J.C.; Kuijpers-Jagtman, A.M.; Von Den Hoff, J.W. The oxytalan fibre network in the periodontium and its possible mechanical function. Arch. Oral Biol. 2012, 57, 1003–1011.
20. Kira-Tatsuoka, M.; Oka, K.; Tsuruga, E.; Ozaki, M.; Sawa, Y. Immunohistochemical expression of fibrillin-1 and fibrillin-2 during tooth development. J. Periodontal Res. 2015, 50, 714–720.
21. Shinagawa-Ohama, R.; Mochizuki, M.; Tamaki, Y.; Suda, N.; Nakahara, T. Heterogeneous Human Periodontal Ligament-Committed Progenitor and Stem Cell Populations Exhibit a Unique Cementogenic Property under in Vitro and in Vivo Conditions. Stem Cells Dev. 2017, 26, 632–645.
22. McCulloch, C.A.G.; Bordin, S. Role of fibroblast subpopulations in periodontal physiology and pathology. J. Periodontal Res. 1991, 26, 144–154.
23. Li, J.; Wang, Z.; Huang, X.; Wang, Z.; Chen, Z.; Wang, R.; Chen, Z.; Liu, W.; Wu, B.; Fang, F.; et al. Dynamic proteomic profiling of human periodontal ligament stem cells during osteogenic differentiation. Stem Cell Res. Ther. 2021, 12, 98.
24. 35. Deng, C.; Sun, Y.; Liu, H.; Wang, W.; Wang, J.; Zhang, F. Selective adipogenic differentiation of human periodontal ligament stem cells stimulated with high doses of glucose. PLoS ONE 2018, 13, e0199603.
25. 36. Choi, S.; Cho, T.J.; Kwon, S.K.; Lee, G.; Cho, J. Chondrogenesis of periodontal ligament stem cells by transforming growth factor-3 and bone morphogenetic protein-6 in a normal healthy impacted third molar. Int. J. Oral Sci. 2013, 5, 7–13.
26. Tomokiyo, A.; Maeda, H.; Fujii, S.;Wada, N.; Shima, K.; Akamine, A. Development of a multipotent clonal human periodontal ligament cell line. Differentiation 2008, 76, 337–347.
27. Song, J.S.; Kim, S.O.; Kim, S.H.; Choi, H.J.; Son, H.K.; Jung, H.S.; Kim, C.S.; Lee, J.H. In vitro and in vivo characteristics of stem cells derived from the periodontal ligament of human deciduous and permanent teeth. Tissue Eng. Part A 2012, 18, 2040–2051.
28. Wang, L.; Shen, H.; Zheng, W.; Tang, L.; Yang, Z.; Gao, Y.; Yang, Q.; Wang, C.; Duan, Y.; Jin, Y. Characterization of stem cells from alveolar periodontal ligament. Tissue Eng. Part A 2011, 17, 1015–1026.
29. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.C.; Krause, D.S.; Deans, R.J.; Keating, A.; Prockop, D.J.; Horwitz, E.M. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006, 8, 315–317.
30. Jiang, N.; Guo,W.; Chen, M.; Zheng, Y.; Zhou, J.; Kim, S.G.; Embree, M.C.; Songhee Song, K.; Marao, H.F.; Mao, J.J. Periodontal Ligament and Alveolar Bone in Health and Adaptation: Tooth Movement. Front. Oral Biol. 2015, 18, 1–8.
31. Naveh, G.R.S.; Lev-Tov Chattah, N.; Zaslansky, P.; Shahar, R.; Weiner, S. Tooth-PDL-bone complex: Response to compressive loads encountered during mastication—A review. Arch. Oral Biol. 2012, 57, 1575–1584.

32. Naveh, G.R.S.; Lev-Tov Chattah, N.; Zaslansky, P.; Shahar, R.; Weiner, S. Tooth-PDL-bone complex: Response to compressive loads encountered during mastication—A review. Arch. Oral Biol. 2012, 57, 1575–1584.
33. Roobrouck VD, Ulloa-Montoya F, Verfaillie CM. Self-renewal and differentiation capacity of young and aged stem cells. Exp Cell Res. 2008;314:1937–44.
34. Beyer Nardi N, da Silva Meirelles L. Mesenchymal stem cells: isolation, in vitro expansion and characterization. Handb Exp Pharmacol 2006;(174):249–282.
35. Seo BM, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004;364:149–55.
36. Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res. 2005;8:191–9.
37. Liu J, Yu F, Sun Y, et al. Concise reviews: characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells. 2015;33:627–38.
38. Zhu W, Liang M. Periodontal ligament stem cells: current status, concerns, and future prospects. Stem Cells Int. 2015;2015:972313.
39. Lee JH, Um S, Song IS, Kim HY, Seo BM. Neurogenic differentiation of human dental stem cells in vitro. J Korean Assoc Oral Maxillofac Surg. 2014;40:173–80.
40. Osathanon T, Sawangmake C, Nowwarote N, Pavasant P. Neurogenic differentiation of human dental pulp stem cells using different induction protocols. Oral Dis. 2014;20:352–8.
41. Komaki M, Karakida T, Abe M, et al. Twist negatively regulates osteoblastic differentiation in human periodontal ligament cells. J Cell Biochem. 2007;100:303–14.
42. Kato C, Kojima T, Komaki M, et al. S100A4 inhibition by RNAi up-regulates osteoblast related genes in periodontal ligament cells. Biochem Biophys Res Commun. 2005;326:147–53.
43. Wada N, Wang B, Lin NH, Laslett AL, Gronthos S, Bartold PM. Induced pluripotent stem cell lines derived from human gingival fibroblasts and periodontal ligament fibroblasts. J Periodontal Res. 2011;46:438–47.
44. Nomura Y, Ishikawa M, Yashiro Y, et al. Human periodontal ligament fibroblasts are the optimal cell source for induced pluripotent stem cells. Histochem Cell Biol. 2012;137:719–32.
45. FakhryA, Ratisoontorn C,VedhachalamC, et al. Effects of FGF-2/-9 in calvarial bone cell cultures: differentiation stage-dependent mitogenic effect, inverse regulation of BMP-2 and noggin, and enhancement of osteogenic potential. Bone. 2005;36:254–66.
46. Maegawa N, Kawamura K, Hirose M, Yajima H, Takakura Y, Ohgushi H. Enhancement of osteoblastic differentiation of mesenchymal stromal cells cultured by selective combination of bone morphogenetic protein-2 (BMP-2) and fibroblast growth factor-2 (FGF-2). J Tissue Eng Regen Med. 2007;1:306–13.
47. Lee JH, Um S, Jang JH, Seo BM. Effects of VEGF and FGF-2 on proliferation and differentiation of human periodontal ligament stem cells. Cell Tissue Res. 2012;348:475–84.
48. Hidaka T, Nagasawa T, Shirai K, Kado T, Furuichi Y. FGF-2 induces proliferation of human periodontal ligament cells and maintains differentiation potentials of STRO-1(+)/CD146(+) periodontal ligament cells. Arch Oral Biol. 2012;57:830–40.

49. Osathanon T,Nowwarote N,Manokawinchoke J, Pavasant P. bFGF and JAGGED1 regulate alkaline phosphatase expression and mineralization in dental tissue-derived mesenchymal stem cells. J Cell Biochem. 2013;114:2551–61.
50. Lee TH, KimWT, Ryu CJ, Jang YJ. Optimization of treatment with recombinant FGF-2 for proliferation and differentiation of human dental stem cells, mesenchymal stem cells, and osteoblasts. Biochem Cell Biol. 2015;93:298–305.
51. Hammarstrom L. Enamel matrix, cementum development and regeneration. J Clin Periodontol. 1997;24:658–68.
52. Beertsen W, McCulloch CA, Sodek J. The periodontal ligament: a unique, multifunctional connective tissue. Periodontol 2000. 1997;13:20–40.
53. Brookes SJ, Robinson C, Kirkham J, Bonass WA. Biochemistry and molecular biology of amelogenin proteins of developing dental enamel. Arch Oral Biol. 1995;40:1–14.
54. Sculean A, Schwarz F, Becker J, Brecx M. The application of an enamel matrix protein derivative (Emdogain) in regenerative periodontal therapy: a review. Med Princ Pract. 2007;16:167–80.
55. He J, Jiang J, Safavi KE, Spangberg LS, Zhu Q. Emdogain promotes osteoblast proliferation and differentiation and stimulates osteoprotegerin expression. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97:239–45.
56. Kemoun P, Gronthos S, Snead ML, et al. The role of cell surface markers and enamel matrix derivatives on human periodontal ligament mesenchymal progenitor responses in vitro. Biomaterials. 2011;32:7375–88.
57. Kato H, Katayama N, Taguchi Y, Tominaga K, Umeda M, Tanaka A. A synthetic oligopeptide derived from enamel matrix derivative promotes the differentiation of human periodontal ligament stem cells into osteoblast-like cells with increased mineralization. J Periodontol. 2013;84:1476–83.
58. Borrione P,Di Gianfrancesco A, PereiraMT, Pigozzi F. Platelet-rich plasma inmuscle healing.AmJ Phys Med Rehab. 2010;89:854–61.
59. Wei, F.; Song, T.; Ding, G.; Xu, J.; Liu, Y.; Liu, D.; Fan, Z.; Zhang, C.; Shi, S.;Wang, S. Functional tooth restoration by allogeneic mesenchymal stem cell-based bio-root regeneration in swine. Stem Cells Dev. 2013, 22, 1752–1762.
60. Gao, Z.H.; Hu, L.; Liu, G.L.; Wei, F.L.; Liu, Y.; Liu, Z.H.; Fan, Z.P.; Zhang, C.M.; Wang, J.S.; Wang, S.L. Bio-root and implant-based restoration as a tooth replacement alternative. J. Dent. Res. 2016, 95, 642–649.
61. Washio, K.; Tsutsumi, Y.; Tsumanuma, Y.; Yano, K.; Srithanyarat, S.S.; Takagi, R.; Ichinose, S.; Meinzer, W.; Yamato, M.; Okano, T.; et al. In Vivo Periodontium Formation Around Titanium Implants Using Periodontal Ligament Cell Sheet. Tissue Eng. Part A 2018, 24, 1273–1282.
62. Gao, H.; Li, B.; Zhao, L.; Jin, Y. Influence of nanotopography on periodontal ligament stem cell functions and cell sheet based periodontal regeneration. Int. J. Nanomed. 2015, 10, 4009–4027.
63. Washio, K.; Tsutsumi, Y.; Tsumanuma, Y.; Yano, K.; Srithanyarat, S.S.; Takagi, R.; Ichinose, S.; Meinzer, W.; Yamato, M.; Okano, T.; et al. In Vivo Periodontium Formation Around Titanium Implants Using Periodontal Ligament Cell Sheet. Tissue Eng. Part A 2018, 24, 1273–1282.

64. Wei, F.; Song, T.; Ding, G.; Xu, J.; Liu, Y.; Liu, D.; Fan, Z.; Zhang, C.; Shi, S.;Wang, S. Functional tooth restoration by allogeneic mesenchymal stem cell-based bio-root regeneration in swine. Stem Cells Dev. 2013, 22, 1752–1762.
65. Gao, Z.H.; Hu, L.; Liu, G.L.; Wei, F.L.; Liu, Y.; Liu, Z.H.; Fan, Z.P.; Zhang, C.M.; Wang, J.S.; Wang, S.L. Bio-root and implant-based restoration as a tooth replacement alternative. J. Dent. Res. 2016, 95, 642–649.
66. Li, H.; Zhou, J.; Zhu, M.; Ying, S.; Li, L.; Chen, D.; Li, J.; Song, J. Low-intensity pulsed ultrasound promotes the formation of periodontal ligament stem cell sheets and ectopic periodontal tissue regeneration. J. Biomed. Mater. Res. Part A 2021, 109, 1101–1112.
67. Yang, Z.; Jin, F.; Zhang, X.; Ma, D.; Han, C.; Huo, N.; Wang, Y.; Zhang, Y.; Lin, Z.; Jin, Y. Tissue engineering of cementum/periodontal-ligament complex using a novel three-dimensional pellet cultivation system for Human periodontal ligament stem cells. Tissue Eng. Part C Methods 2009, 15, 571–581.
68. Wang, Z.S.; Feng, Z.H.; Wu, G.F.; Bai, S.Z.; Dong, Y.; Chen, F.M.; Zhao, Y.M. The use of platelet-rich fibrin combined with periodontal ligament and jaw bone mesenchymal stem cell sheets for periodontal tissue engineering. Sci. Rep. 2016, 6, 28126.
69. Xu, Q.; Li, B.; Yuan, L.; Dong, Z.; Zhang, H.; Wang, H.; Sun, J.; Ge, A.; Jin, Y. Combination of platelet-rich plasma within periodontal ligament stem cell sheets enhances cell differentiation and matrix production. J. Tissue Eng. Regen. Med. 2017, 11, 627–636.
70. Xie, H.; Liu, H. A Novel Mixed-Type Stem Cell Pellet for Cementum/Periodontal Ligament–Like Complex. J. Periodontol. 2012, 83, 805–815.
71. Zhang, H.; Liu, S.; Zhu, B.; Xu, Q.; Ding, Y.; Jin, Y. Composite cell sheet for periodontal regeneration: Crosstalk between different types of MSCs in cell sheet facilitates complex periodontal-like tissue regeneration. Stem Cell Res. Ther. 2016, 7, 168.
72. Yang, X.; Xiong, X.; Zhou,W.; Feng, G.; Zhang, Y.; Dai, H.; Zhou, J. Effects of human urine-derived stem cells on the cementogenic differentiation of indirectly-cocultured periodontal ligament stem cells. Am. J. Transl. Res. 2020, 12, 361–378.
73. Panduwawala, C.P.; Zhan, X.; Dissanayaka,W.L.; Samaranayake, L.P.; Jin, L.; Zhang, C. In vivo periodontal tissue regeneration by periodontal ligament stem cells and endothelial cells in three-dimensional cell sheet constructs. J. Periodontal Res. 2017, 52, 408–418.
74. Liu, J.;Wang, L.; Liu,W.; Li, Q.; Jin, Z.; Jin, Y. Dental follicle cells rescue the regenerative capacity of periodontal ligament stem cells in an inflammatory microenvironment. PLoS ONE 2014, 9, e108752.
75. Yang, X.; Ma, Y.; Guo,W.; Yang, B.; Tian,W. Stem cells from human exfoliated deciduous teeth as an alternative cell source in bio-root regeneration. Theranostics 2019, 9, 2694–2711.
76. Tsumanuma, Y.; Iwata, T.; Washio, K.; Yoshida, T.; Yamada, A.; Takagi, R.; Ohno, T.; Lin, K.; Yamato, M.; Ishikawa, I.; et al. Comparison of different tissue-derived stem cell sheets for periodontal regeneration in a canine 1-wall defect model. Biomaterials 2011, 32, 5819–5825.
77. Cao, Y.; Liu, Z.; Xie, Y.; Hu, J.; Wang, H.; Fan, Z.; Zhang, C.; Wang, J.; Wu, C.T.; Wang, S. Adenovirus-mediated transfer of hepatocyte growth factor gene to human dental pulp stem cells under

good manufacturing practice improves their potential for periodontal regeneration in swine. Stem Cell Res. Ther. 2015, 6, 249.
78. Hu, J.; Cao, Y.; Xie, Y.; Wang, H.; Fan, Z.; Wang, J.; Zhang, C.; Wang, J.; Wu, C.T.; Wang, S. Periodontal regeneration in swine after cell injection and cell sheet transplantation of human dental pulp stem cells following good manufacturing practice. Stem Cell Res. Ther. 2016, 7, 130.
79. Tsumanuma, Y.; Iwata, T.; Kinoshita, A.; Washio, K.; Yoshida, T.; Yamada, A.; Takagi, R.; Yamato, M.; Okano, T.; Izumi, Y. Allogeneic Transplantation of Periodontal Ligament-Derived Multipotent Mesenchymal Stromal Cell Sheets in Canine Critical-Size Supra-Alveolar Periodontal Defect Model. Biores. Open Access 2016, 5, 22–36.
80. Nakajima, K.; Abe, T.; Tanaka, M.; Hara, Y. Periodontal tissue engineering by transplantation of multilayered sheets of phenotypically modified gingival fibroblasts. J. Periodontal Res. 2008, 43, 681–688.
81. Vaquette, C.; Saifzadeh, S.; Farag, A.; Hutmacher, D.W.; Ivanovski, S. Periodontal Tissue Engineering with a Multiphasic Construct and Cell Sheets. J. Dent. Res. 2019, 98, 673–681.
82. Yang, B.; Chen, G.; Li, J.; Zou, Q.; Xie, D.; Chen, Y.; Wang, H.; Zheng, X.; Long, J.; Tang, W.; et al. Tooth root regeneration using dental follicle cell sheets in combination with a dentin matrix-based scaffold. Biomaterials 2012, 33, 2449–2461.
83. Guo, S.; Kang, J.; Ji, B.; Guo,W.; Ding, Y.;Wu, Y.; Tian,W. Periodontal-Derived Mesenchymal Cell Sheets Promote Periodontal Regeneration in Inflammatory Microenvironment. Tissue Eng. Part A 2017, 23, 585–596.
84. Raju, R.; Oshima, M.; Inoue, M.; Morita, T.; Huijiao, Y.; Waskitho, A.; Baba, O.; Inoue, M.; Matsuka, Y. Three-dimensional periodontal tissue regeneration using a bone-ligament complex cell sheet. Sci. Rep. 2020, 10, 1656.
85. Wei, F.; Qu, C.; Song, T.; Ding, G.; Fan, Z.; Liu, D.; Liu, Y.; Zhang, C.; Shi, S.;Wang, S. Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity. J. Cell. Physiol. 2012, 227, 3216–3224.
86. Wang, Y.; Pi, C.; Feng, X.; Hou, Y.; Zhao, L.; Wei, Y. The influence of nanoparticle properties on oral bioavailability of drugs. Int. J. Nanomed. 2020, 15, 6295–6310.
87. Yu, Y.; Bi, C.S.; Wu, R.X.; Yin, Y.; Zhang, X.Y.; Lan, P.H.; Chen, F.M. Effects of short-term inflammatory and/or hypoxic pretreatments on periodontal ligament stem cells: In vitro and in vivo studies. Cell Tissue Res. 2016, 366, 311–328.
88. Guo, S.; Kang, J.; Ji, B.; Guo,W.; Ding, Y.;Wu, Y.; Tian,W. Periodontal-Derived Mesenchymal Cell Sheets Promote Periodontal Regeneration in Inflammatory Microenvironment. Tissue Eng. Part A 2017, 23, 585–596.
89. Zhao, Y.H.; Zhang, M.; Liu, N.X.; Lv, X.; Zhang, J.; Chen, F.M.; Chen, Y.J. The combined use of cell sheet fragments of periodontal ligament stem cells and platelet-rich fibrin granules for avulsed tooth reimplantation. Biomaterials 2013, 34, 5506–5520.
90. Iwasaki, K.; Komaki, M.; Yokoyama, N.; Tanaka, Y.; Taki, A.; Honda, I.; Kimura, Y.; Takeda, M.; Akazawa, K.; Oda, S.; et al. Periodontal regeneration using periodontal ligament stem cell-transferred amnion. Tissue Eng. Part A 2014, 20, 693–704.

91. Jiang, Y.; Liu, J.M.; Huang, J.P.; Lu, K.X.; Sun,W.L.; Tan, J.Y.; Li, B.X.; Chen, L.L.;Wu, Y.M. Regeneration potential of decellularized periodontal ligament cell sheets combined with 15-deoxy-D12,14-prostaglandin J2 nanoparticles in a rat periodontal defect. Biomed. Mater. 2021, 16, 045008.
92. Farag, A.; Hashimi, S.M.; Vaquette, C.; Bartold, P.M.; Hutmacher, D.W.; Ivanovski, S. The effect of decellularized tissue engineered constructs on periodontal regeneration. J. Clin. Periodontol. 2018, 45, 586–596.
93. Yang, H.; Li, J.; Hu, Y.; Sun, J.; Guo,W.; Li, H.; Chen, J.; Huo, F.; Tian,W.; Li, S. Treated dentin matrix particles combined with dental follicle cell sheet stimulate periodontal regeneration. Dent. Mater. 2019, 35, 1238–1253.
94. Kantarci, A.; Hasturk, H.; Van Dyke, T.E. Animal models for periodontal regeneration and peri-implant responses. Periodontology 2000 2015, 68, 66–82.
95. 138. Wancket, L.M. Animal Models for Evaluation of Bone Implants and Devices: Comparative Bone Structure and Common Model Uses. Vet. Pathol. 2015, 52, 842–850.
96. Yang, H.M.; Hu, K.S.; Song, W.C.; Park, J.T.; Kim, H.J.; Koh, K.S.; Kim, H.J. Innervation patterns of the canine masticatory muscles in comparison to human. Anat. Rec. 2010, 293, 117–125.
97. Rios, H.F.; Giannobile, W.V. Preclinical Protocols for Periodontal Regeneration. In Osteology Guidelines for Oral & Maxillofacial Regeneration; Giannobile, W.V., Nevins, M., Eds.; Quintessence Publishing Co., Ltd.: New Malden, UK, 2011; pp. 77–102.
98. Huang, R.Y.; Tai, W.C.; Ho, M.H.; Chang, P.C. Combination of a biomolecule-aided biphasic cryogel scaffold with a barrier membrane adhering PDGF-encapsulated nanofibers to promote periodontal regeneration. J. Periodontal Res. 2020, 55, 529–538.
99. Momose, T.; Miyaji, H.; Kato, A.; Ogawa, K.; Yoshida, T.; Nishida, E.; Murakami, S.; Kosen, Y.; Sugaya, T.; Kawanami, M. Collagen Hydrogel Scaffold and Fibroblast Growth Factor-2 Accelerate Periodontal Healing of Class II Furcation Defects in Dog. Open Dent. J. 2016, 10, 347–359.
100. Ogawa, K.; Miyaji, H.; Kato, A.; Kosen, Y.; Momose, T.; Yoshida, T.; Nishida, E.; Miyata, S.; Murakami, S.; Takita, H.; et al. Periodontal tissue engineering by nano beta-tricalcium phosphate scaffold and fibroblast growth factor-2 in one-wall infrabony defects of dogs. J. Periodontal Res. 2016, 51, 758–767.
101. Pilipchuk, S.P.; Fretwurst, T.; Yu, N.; Larsson, L.; Kavanagh, N.M.; Asa'ad, F.; Cheng, K.C.K.; Lahann, J.; Giannobile, W.V. Micropatterned Scaffolds with Immobilized Growth Factor Genes Regenerate Bone and Periodontal Ligament-Like Tissues. Adv. Healthc. Mater. 2018, 7, e1800750.
102. Ogawa, K.; Miyaji, H.; Kato, A.; Kosen, Y.; Momose, T.; Yoshida, T.; Nishida, E.; Miyata, S.; Murakami, S.; Takita, H.; et al. Periodontal tissue engineering by nano beta-tricalcium phosphate scaffold and fibroblast growth factor-2 in one-wall infrabony defects of dogs. J. Periodontal Res. 2016, 51, 758–767.
103. Momose, T.; Miyaji, H.; Kato, A.; Ogawa, K.; Yoshida, T.; Nishida, E.; Murakami, S.; Kosen, Y.; Sugaya, T.; Kawanami, M. Collagen Hydrogel Scaffold and Fibroblast Growth Factor-2 Accelerate Periodontal Healing of Class II Furcation Defects in Dog. Open Dent. J. 2016, 10, 347–359.
104. Zhang, Y.; Li, S.; Wu, C. The in vitro and in vivo cementogenesis of CaMgSi2O6 bioceramic scaffolds. J. Biomed. Mater. Res. Part A 2014, 102, 105–116.

105. Inukai, T.; Katagiri,W.; Yoshimi, R.; Osugi, M.; Kawai, T.; Hibi, H.; Ueda, M. Novel application of stem cell-derived factors for periodontal regeneration. Biochem. Biophys. Res. Commun. 2013, 430, 763–768.
106. Zang, S.; Jin, L.; Kang, S.; Hu, X.;Wang, M.;Wang, J.; Chen, B.; Peng, B.;Wang, Q. PeriodontalWound Healing by Transplantation of Jaw Bone Marrow-Derived Mesenchymal Stem Cells in Chitosan/Anorganic Bovine Bone Carrier into One-Wall Infrabony Defects in Beagles. J. Periodontol. 2016, 87, 971–981.
107. Liu, Z.; Yin, X.; Ye, Q.; He, W.; Ge, M.; Zhou, X.; Hu, J.; Zou, S. Periodontal regeneration with stem cells-seeded collagenhydroxyapatite scaffold. J. Biomater. Appl. 2016, 31, 121–131.
108. Zhang, W.; Abukawa, H.; Troulis, M.J.; Kaban, L.B.; Vacanti, J.P.; Yelick, P.C. Tissue engineered hybrid tooth-bone constructs. Methods 2009, 47, 122–128.
109. Fawzy El-Sayed, K.M.; Mekhemar, M.K.; Beck-Broichsitter, B.E.; Bähr, T.; Hegab, M.; Receveur, J.; Heneweer, C.; Becker, S.T.; Wiltfang, J.; Dörfer, C.E. Periodontal regeneration employing gingival margin-derived stem/progenitor cells in conjunction with IL-1ra-hydrogel synthetic extracellular matrix. J. Clin. Periodontol. 2015, 42, 448–457.
110. Kantarci, A.; Hasturk, H.; Van Dyke, T.E. Animal models for periodontal regeneration and peri-implant responses. Periodontology 2000 2015, 68, 66–82.
111. Fawzy El-Sayed, K.M.; Mekhemar, M.K.; Beck-Broichsitter, B.E.; Bähr, T.; Hegab, M.; Receveur, J.; Heneweer, C.; Becker, S.T.; Wiltfang, J.; Dörfer, C.E. Periodontal regeneration employing gingival margin-derived stem/progenitor cells in conjunction with IL-1ra-hydrogel synthetic extracellular matrix. J. Clin. Periodontol. 2015, 42, 448–457.
112. Rios, H.F.; Giannobile, W.V. Preclinical Protocols for Periodontal Regeneration. In Osteology Guidelines for Oral & Maxillofacial Regeneration; Giannobile, W.V., Nevins, M., Eds.; Quintessence Publishing Co., Ltd.: New Malden, UK, 2011; pp. 77–102.
113. Takeuchi, T.; Bizenjima, T.; Ishii, Y.; Imamura, K.; Suzuki, E.; Seshima, F.; Saito, A. Enhanced healing of surgical periodontal defects in rats following application of a self-assembling peptide nanofibre hydrogel. J. Clin. Periodontol. 2016, 43, 279–288.
114. Chien, K.H.; Chang, Y.L.;Wang, M.L.; Chuang, J.H.; Yang, Y.C.; Tai, M.C.;Wang, C.Y.; Liu, Y.Y.; Li, H.Y.; Chen, J.T.; et al. Promoting Induced Pluripotent Stem Cell-driven Biomineralization and Periodontal Regeneration in Rats with Maxillary-Molar Defects using Injectable BMP-6 Hydrogel. Sci. Rep. 2018, 8, 114.
115. He, X.T.; Li, X.; Xia, Y.; Yin, Y.; Wu, R.X.; Sun, H.H.; Chen, F.M. Building capacity for macrophage modulation and stem cell recruitment in high-stiffness hydrogels for complex periodontal regeneration: Experimental studies in vitro and in rats. Acta Biomater. 2019, 88, 162–180.
116. Pilipchuk, S.P.; Fretwurst, T.; Yu, N.; Larsson, L.; Kavanagh, N.M.; Asa'ad, F.; Cheng, K.C.K.; Lahann, J.; Giannobile, W.V. Micropatterned Scaffolds with Immobilized Growth Factor Genes Regenerate Bone and Periodontal Ligament-Like Tissues. Adv. Healthc. Mater. 2018, 7, e1800750.
117. Hasturk, H.; Jones, V.L.; Andry, C.; Kantarci, A. 1-Tetradecanol Complex Reduces Progression of Porphyromonas gingivalis–Induced Experimental Periodontitis in Rabbits. J. Periodontol. 2007, 78, 924–932.

118. Hasturk, H.; Kantarci, A.; Ebrahimi, N.; Andry, C.; Holick, M.; Jones, V.L.; Van Dyke, T.E. Topical H2 antagonist prevents periodontitis in a rabbit model. Infect. Immun. 2006, 74, 2402–2414.
119. Slotte, C.; Lundgren, D.; Sennerby, L.; Lundgren, A.K. Influence of preimplant surgical intervention and implant placement on bone wound healing. Clin. Oral Implants Res. 2003, 14, 528–534.
120. Zheng, B.; Jiang, J.; Chen, Y.; Lin, M.; Du, Z.; Xiao, Y.; Luo, K.; Yan, F. Leptin Overexpression in Bone Marrow Stromal Cells Promotes Periodontal Regeneration in a Rat Model of Osteoporosis. J. Periodontol. 2017, 88, 808–818.
121. Dhote, R.; Charde, P.; Bhongade, M.; Rao, J. Stem cells cultured on beta tricalcium phosphate (-TCP) in combination with recombinant human platelet-derived growth factor—BB (RH-PDGF-BB) for the treatment of human infrabony defects. Stem Cells Mediat. Regen. 2016, 10, 17–32.
122. Ferrarotti, F.; Romano, F.; Gamba, M.N.; Quirico, A.; Giraudi, M.; Audagna, M.; Aimetti, M. Human intrabony defect regeneration with micrografts containing dental pulp stem cells: A randomized controlled clinical trial. J. Clin. Periodontol. 2018, 45, 841–850.
123. Baba, S.; Yamada, Y.; Komuro, A.; Yotsui, Y.; Umeda, M.; Shimuzutani, K.; Nakamura, S. Phase I/II Trial of Autologous Bone Marrow Stem Cell Transplantation with a Three-Dimensional Woven-Fabric Scaffold for Periodontitis. Stem Cells Int. 2016, 2016, 6205910.
124. Chen, F.M.; Gao, L.N.; Tian, B.M.; Zhang, X.Y.; Zhang, Y.J.; Dong, G.Y.; Lu, H.; Chu, Q.; Xu, J.; Yu, Y.; et al. Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: A randomized clinical trial. Stem Cell Res. Ther. 2016, 7, 33.
125. Sánchez, N.; Fierravanti, L.; Núñez, J.; Vignoletti, F.; González-Zamora, M.; Santamaría, S.; Suárez-Sancho, S.; Fernández-Santos, M.E.; Figuero, E.; Herrera, D.; et al. Periodontal regeneration using a xenogeneic bone substitute seeded with autologous periodontal ligament-derived mesenchymal stem cells: A 12-month quasi-randomized controlled pilot clinical trial. J. Clin. Periodontol. 2020, 47, 1391–1402.
126. Apatzidou, D.A.; Bakopoulou, A.A.; Kouzi-Koliakou, K.; Karagiannis, V.; Konstantinidis, A. A tissue-engineered biocomplex for periodontal reconstruction. A proof-of-principle randomized clinical study. J. Clin. Periodontol. 2021, 48, 1111–1125.
127. Iwata, T.; Yamato, M.; Washio, K.; Yoshida, T.; Tsumanuma, Y.; Yamada, A.; Onizuka, S.; Izumi, Y.; Ando, T.; Okano, T.; et al. Periodontal regeneration with autologous periodontal ligament-derived cell sheets—A safety and efficacy study in ten patients. Regen. Ther. 2018, 9, 38–44.
128. Park, C.H.; Rios, H.F.; Jin, Q.; Bland, M.E.; Flanagan, C.L.; Hollister, S.J.; Giannobile, W.V. Biomimetic hybrid scaffolds for engineering human tooth-ligament interfaces. Biomaterials 2010, 31, 5945–5952.
129. Varoni, E.M.; Vijayakumar, S.; Canciani, E.; Cochis, A.; De Nardo, L.; Lodi, G.; Rimondini, L.; Cerruti, M. Chitosan-Based Trilayer Scaffold for Multitissue Periodontal Regeneration. J. Dent. Res. 2018, 97, 303–311.
130. Chen, X.; Liu, Y.; Miao, L.;Wang, Y.; Ren, S.; Yang, X.; Hu, Y.; Sun,W. Controlled release of recombinant human cementum protein 1 from electrospun multiphasic scaffold for cementum regeneration. Int. J. Nanomed. 2016, 11, 3145–3158.

131. Zhang, Y.; Miron, R.J.; Li, S.; Shi, B.; Sculean, A.; Cheng, X. Novel MesoPorous BioGlass/silk scaffold containing adPDGF-B and adBMP7 for the repair of periodontal defects in beagle dogs. J. Clin. Periodontol. 2015, 42, 262–271.
132. Costa, P.F.; Vaquette, C.; Zhang, Q.; Reis, R.L.; Ivanovski, S.; Hutmacher, D.W. Advanced tissue engineering scaffold design for regeneration of the complex hierarchical periodontal structure. J. Clin. Periodontol. 2014, 41, 283–294.
133. Venkatesan, N.; Lavu, V.; Balaji, S.K. Clinical efficacy of amniotic membrane with biphasic calcium phosphate in guided tissue regeneration of intrabony defects- a randomized controlled clinical trial. Biomater. Res. 2021, 25, 15.
134. Mangano, C.; Giuliani, A.; De Tullio, I.; Raspanti, M.; Piattelli, A.; Iezzi, G. Case Report: Histological and Histomorphometrical Results of a 3-D Printed Biphasic Calcium Phosphate Ceramic 7 Years After Insertion in a Human Maxillary Alveolar Ridge. Front. Bioeng. Biotechnol. 2021, 9, 614325.
135. Liu, B.; Lun, D. xing Current application of -tricalcium phosphate composites in orthopaedics. Orthop. Surg. 2012, 4, 139–144.
136. Hernández-Monjaraz, B.; Santiago-Osorio, E.; Ledesma-Martínez, E.; Alcauter-Zavala, A.; Mendoza-Núñez, V.M. Retrieval of a periodontally compromised tooth by allogeneic grafting of mesenchymal stem cells from dental pulp: A case report. J. Int. Med. Res. 2018, 46, 2983–2993.
137. Alvarez-Pérez, M.A.; Narayanan, S.; Zeichner-David, M.; Rodríguez Carmona, B.; Arzate, H. Molecular cloning, expression and immunolocalization of a novel human cementum-derived protein (CP-23). Bone 2006, 38, 409–419. [CrossRef] [PubMed]
138. Liu, H.W.; Yacobi, R.; Savion, N.; Narayanan, A.S.; Pitaru, S. A collagenous cementum-derived attachment protein is a marker for progenitors of the mineralized tissue-forming cell lineage of the periodontal ligament. J. Bone Miner. Res. 1997, 12, 1691–1699.
139. Komaki, M.; Iwasaki, K.; Arzate, H.; Narayanan, A.S.; Izumi, Y.; Morita, I. Cementum protein 1 (CEMP1) induces a cementoblastic phenotype and reduces osteoblastic differentiation in periodontal ligament cells. J. Cell. Physiol. 2012, 227, 649–657.
140. Iwata, T.; Mizuno, N.; Nagahara, T.; Kaneda-Ikeda, E.; Kajiya, M.; Kitagawa, M.; Takeda, K.; Yoshioka, M.; Yagi, R.; Takata, T.; et al. Identification of regulatory mRNA and microRNA for differentiation into cementoblasts and periodontal ligament cells. J. Periodontal Res. 2021, 56, 69–82.
141. Onizuka, S.; Iwata, T. Application of periodontal ligament-derived multipotent mesenchymal Stromal cell sheets for periodontal regeneration. Int. J. Mol. Sci. 2019, 20, 2796.
142. Iwata, T.; Washio, K.; Yoshida, T.; Ishikawa, I.; Ando, T.; Yamato, M.; Okano, T. Cell sheet engineering and its application for periodontal regeneration. J. Tissue Eng. Regen. Med. 2015, 9, 343–356.