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Implications of cultured periodontal ligament cells for the clinical and experimental setting: a review

Julie Teresa Marchesan¹, Christina Springstead Scanlon¹, Stephen Soehren¹, Masato Matsuo², and Yvonne L. Kapila¹

¹ Department of Periodontics and Oral Medicine, University of Michigan, School of Dentistry, Ann Arbor, MI 48109

² Department of Oral Anatomy, Kanagawa Dental College, Yokosuka, Kanagawa, 238-8580, Japan

Abstract

The periodontal ligament (PDL) is a key contributor to the process of regeneration of the periodontium. The heterogeneous nature of the PDL tissue, its development during early adulthood, and the different conditions to which the PDL tissue is exposed to *in vivo* impart on the PDL unique characteristics that may be of consequence during its cultivation *in vitro*. Several factors affecting the *in vivo* setting influence the behavior of PDL fibroblasts in culture. The purpose of this review is to address distinct factors that influence the behavior of PDL fibroblasts in culture — *in vivo-in vitro* transitions, cell identification/isolation markers, primary PDL cultures and cell lines, tooth-specific factors, and donor-specific factors. Based on the reviewed studies, the authors recommendations include the use of several identification markers to confirm cell identity, use of primary cultures at early passage to maintain unique PDL heterogeneic characteristics, and noting donor conditions such as age, systemic health status, and tooth health status. Continued efforts will expand our understanding of the *in vitro* and *in vivo* behavior of cells, with the goal of orchestrating optimal periodontal regeneration. This understanding will lead to improved evidence-based rationales for more individualized and predictable periodontal regenerative therapies.

Keywords

periodontal; human; fibroblast; cell culture; regeneration

INTRODUCTION

Regeneration of periodontal tissues is an important endpoint of therapy for periodontal disease. Significant progress has been made in understanding the different cells and factors involved in healing, leading to numerous regenerative materials for managing periodontal

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Address for correspondence: Yvonne L. Kapila, D.D.S., Ph.D., Department of Periodontics and Oral Medicine, University of Michigan, School of Dentistry, 1011 N. University Ave., Ann Arbor, MI 48109-1078, Tel. 734-615-2295; Fax 734-763-5503, ykapila@umich.edu.

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defects. However, current clinical techniques are still unpredictable, and complete regeneration of the periodontium remains unattainable (1, 2).

Periodontal disease is complex and multifactorial, and the combination of factors leading to disease is distinct among patients. More individualized therapies directed toward providing the ideal environment for regeneration may be important for a predictable and complete outcome. Hence, a further understanding and continued investigation of the distinctive factors that influence the heterogeneous cellular behavior of periodontal ligament (PDL) cells are still needed.

The PDL tissue is a key contributor to the process of regeneration of the periodontium (3). Although the PDL tissue is 0.15 to 0.38 mm wide (4), it is quite complex and composed of several different cell populations (Fig 1). Osteoblasts and osteoclasts are found on the alveolar bone side of the PDL tissue. Cells in the Epithelial rests of Malassez and cementoblasts are located near the root surface of the tooth (4–9). Endothelial cells create a rich vascular network and are found throughout the PDL tissue (Fig 2) (10), along with fibroblasts, macrophages, undifferentiated adult/mesenchymal stem cells, and neural elements. Taking the tissue complexity into account, regeneration of the periodontal apparatus may be more challenging when compared to tissues with fewer cell populations, and when further considering the constant bacterial challenge in the oral environment.

In addition, the different conditions to which the PDL tissue is exposed to in the oral cavity may impart this tissue with unique characteristics, such as epigenetic alterations mediated by systemic disease, environmental factors, bacterial challenge, and stage of development, that may carry forward into the culture process. The PDL tissue most commonly used as a donor for cell cultivation fully develops and remodels during early adulthood. Premolars erupt at 10–12 years of age, and root formation is complete at 12–13 years of age when occlusion is achieved. Third molars generally erupt at 17–21 years of age, and the roots completely form by 18–25 years of age. In the permanent dentition, tooth formation, eruption, and occlusion are related to synchronized changes in PDL metabolism (11). Cells within the PDL tissue have different morphology and protein expression depending on their stage of development and on tooth eruption. Thus, since the PDL is harvested for cell cultivation at different phases of development and under different conditions, this may influence cell behavior in culture.

The purpose of this review is to examine the different factors shown to influence PDL fibroblast behavior in culture — *in vivo-in vitro* transitions, cell biomarker identification, primary PDL cultures and cell lines, tooth-specific factors, and donor-specific factors. The distinctive nature of the PDL cells and the factors that influence their behavior should be considered when interpreting results and in designing therapeutics for regenerative purposes.

FACTORS INFLUENCING PDL BEHAVIOR IN VITRO

In vivo-in vitro transitions

There are gaps in knowledge in PDL biology that arise from discrepancies between *in vitro* cellular behavior and *in vivo* cellular performance. For studies evaluating the regenerative potential of PDL cells, two transitions are important: 1) the transition from the tooth-PDL tissue to the *in vitro* setting and 2) the transition from the *in vitro* setting back to the tooth (Fig 3). Upon transfer from the tooth to the cell culture environment, PDL cells undergo alterations in their biochemical behavior (12). mRNA transcript expression analysis of fresh PDL tissue compared to PDL cell isolates in culture revealed that 6.0% of 54,000 probes (47,000 genes) examined were expressed at higher levels in fresh tissues, and 4.1% were expressed at higher levels in fibroblast cultures. A preferential mRNA expression, associated

with mineralized tissues, was observed in fresh PDL, including osteopontin (50-fold), pleiotrophin (14-fold), alkaline phosphatase (8-fold), osteomodulin (8-fold), periostin (6.5-fold), fibromodulin (5-fold), and BSP-2 (7-fold). However, PDL cells in culture preferentially expressed several factors associated with the regulation of differentiation, including gremlin (a soluble BMP antagonist), stanniocalcin-2 (a novel, putative inhibitor of mineralized tissue formation), and follistatin (a soluble TGF- β antagonist). In general, protein expression levels reflected the differences in RNA expression (12). Interestingly, follicular dendritic cell-secreted protein and tenascin-N were uniformly observed throughout fresh PDL tissue but were absent in PDL cell cultures (13, 14). Although these differences cannot be overcome, they should be noted. The regenerative impact of the differences observed between the *in vitro* and *in vivo* environments remains to be determined. Animal knockout models may provide a useful setting to analyze the importance of proteins that have been altered in culture and that may have a bearing on periodontal regeneration.

Cell identification

PDL fibroblasts are the main cellular component of the periodontium and they represent a heterogeneous population of cells that express a rich repertoire of proteins. PDL fibroblasts have cementoblast-like and/or osteoblast-like properties, including the capacity to form mineralized nodules *in vitro*, express bone-associated markers, modulate osteoclastogenesis, and form cementum-like tissues *in vivo* (3, 15–17). When compared to gingival fibroblasts, genes encoding transmembrane proteins and cytoskeleton-related proteins tend to be up-regulated in PDL fibroblasts (18).

The *in vitro* isolation and characterization of primary PDL fibroblasts is usually based on i) the anatomical site selected for harvesting (middle third of the root), ii) the harvesting method chosen (explant or digestion) (19), and iii) the cell culture medium chosen to promote fibroblast growth. Although there is currently no defined marker that uniquely represents this cell population, various secreted matrix proteins and cytoskeletal proteins have been used to characterize PDL fibroblasts. Protein and mRNA markers previously utilized for identifying PDL fibroblasts *in vitro* include: periostin (20–22), runt-related transcription factor-2 (Runx2) (20), N-cadherin (23), twist (24), cementum attachment protein (25, 26), neural-cell adhesion molecule-1 (NCAM-1) (27), cementum protein-23 (16), sclerostin (28), periodontal-ligament associated protein (PLAP)-1/aspurin (29, 30), fibromodulin (31), periodontal ligament specific (PDLs)-17 (32, 33), S100A4 calcium binding protein (27, 34), tissue factor pathway inhibitor-2 (35), major histocompatibility complex-D- β (35, 36), uncoordinated-like protein (37), and laminin- 2/4 and -8/9 (38, 39). Nine genes whose expression is likely to be unique in the PDL tissue have been identified, but their presence *in vitro* is yet to be confirmed (29). Due to the non-specificity of a single marker, the use of more than one marker is ideal for PDL fibroblast identification *in vitro*. The choice of the markers could be based on the known function of the protein (Table 1) and the design and purpose of the study. For example, if the purpose of the study is to examine cementum formation, primary PDL cell cultures could be initially characterized by both a non-specific marker for PDL cells in addition to a cementum-associated marker. Depending on the treatment and culture conditions, some PDL fibroblasts cultures are shown to express proteins such as cementum-attachment protein (40). In addition, 15% of PDL fibroblasts were shown to express cementum-derived protein-23, compared to 98% expression in cementoblasts (16). Because some PDL cells are more prone to a cementoblast phenotype, this may help determine if the response is dependent on the agent being tested or on the cells being evaluated.

Primary PDL cultures and cell lines

Although immortalized cell lines may help establish more uniform results among different studies, there are unique benefits to using primary cultures for regenerative studies. Primary PDL cell cultures used at early passages have the advantage of maintaining the rich phenotypic and functional heterogeneity of fibroblasts characteristic of the original tissue. PDL cells used at late passages (passage 20) exhibited different morphology and biological activity when compared to those at early passages (passage 7) (41, 42). In addition, PDL fibroblasts derived from 5 donors were evaluated for changes during in vitro cell passage. A decrease in the number of STRO-1 positive cells was observed, along with a decrease in expression of tenomodulin, collagen type XII, and alkaline phosphatase (43). Therefore, to preserve that original heterogeneity as much as possible and minimize clonal cell selection, PDL fibroblasts should not be used at late passages (no more than 7 passages). Using more than one PDL donor tissue and consideration of the factors mentioned in this review will yield more physiologically relevant and therefore more clinically meaningful results.

A critical issue that can occur when using immortalized cell lines is the inadvertent incorporation of intraspecies and interspecies cross-contamination. In addition, 18% to 36% of cell lines recently evaluated had the tissue of origin misidentified. Such cultures have been used at high-passage numbers, donated by other research laboratories, and generated misleading results. Authentication tests to confirm the identity of immortalized cell lines is now a requirement from funding agencies (44). General pros and cons of utilizing cell lines or primary cultures can be found in Table 2.

Immortalized PDL cell lines have been established from mouse molars (20, 45, 46), human impacted third molars (47, 48), human premolars extracted for orthodontic reasons (48–51), human molars and premolars (52), a mandibular premolar from a miniature swine (22), and the teeth of a patient with Marfan's syndrome type I (53).

Tooth-specific factors

Few studies have evaluated PDL cells derived from human primary teeth. Since the root and the PDL tissue are generally resorbed when primary teeth exfoliate and the goal of regenerative approaches is not designed for primary teeth, permanent teeth are preferable for PDL tissue collection and cell culturing. Differences between primary and permanent teeth have been observed in mechanical stretching studies, expression of TGF- β , and proliferation (54, 55).

The teeth most commonly used as a source for PDL fibroblast cultures are premolars (35) and third molars (43), usually extracted for orthodontic reasons and because they are generally disease-free. Culturing PDL cells from teeth compromised by periodontal disease is controversial; it seems that sections of the ligament from these teeth may be useful sources of healthy tissue as further discussed below.

Tooth eruption—Tooth eruption is classically divided into three phases: pre-eruptive, eruptive pre-functional, and eruptive functional (when occlusion is achieved) (56). Studies show different complex signaling and gene expression profiles during each phase, with over 300 genes involved in tooth development (57). In addition to metabolic changes, the size, number, and morphology of the cells are altered during eruption and PDL development. The size of cells increases continuously during PDL development, starting with an average cytoplasmic size of 300 μ m in the dental follicle stage and reaching 1,006 μ m in the newly developed PDL (58). In the pre-eruptive phase, the tooth remains cradled intra-osseously within a mesenchymal tissue, the dental follicle, which gives rise to the PDL tissue. F-spondin and Golgi protein 49kDa are expressed specifically in the dental follicle and in early

PDL tissue *in vivo* (13, 59). The eruptive pre-functional phase starts when teeth begin to move toward the surface of the jaws. As root formation begins and progresses, the amorphous character of the dental follicle is gradually replaced by the structured morphology of the PDL (60). During tooth eruption, type XII collagen expression is first detected in the dental follicle/PDL region and increases in the PDL as the tooth erupts and achieves occlusion (61). In developing molars, gene expression of bone morphogenetic protein family members and growth and differentiation factors -5, -6, and -7 is at high levels during fiber bundle formation in the PDL tissue, but is undetectable after root formation is complete (62). Expression of calcium-binding protein S100A4 is correlated with tooth eruption status. S100A4 protein expression is low in the PDL of teeth undergoing eruption, but high in the PDL of erupted teeth. Proteins from the S100 family are known to be involved in the regulation of a number of cellular processes, such as cell cycle progression and differentiation (63). Other proteins, such as tenascin-N, are strongly expressed in terminally differentiated PDL tissue (13).

Eruptive Functional Phase –Tooth Movement, Mechanical Forces, and Occlusion—The PDL anchors the tooth to the alveolar bone and is usually exposed to mechanical forces (including occlusal forces during mastication) that result in active tissue remodeling. Mechanical forces are shown to alter PDL fibroblast morphology and orientation, and affect the synthesis of proteins. Uncoordinated-like protein is expressed *in vitro* and *in vivo* under tissue remodeling conditions, including mechanical stress, and on the tension side of the PDL during tooth movement (37). Other proteins affected by mechanical forces in PDL fibroblasts include collagen type I, type XII, and fibronectin (64, 65). A recent study identified 17 genes up-regulated under mechanical stress in human PDL cells *in vitro*, including homer homolog 1 and glutamate receptor ionotropic N-methyl-d-aspartate 3A, which are related to glutamate signaling. Up-regulation of glutamate signaling-associated gene expression was also observed at tension sites in the PDL tissue under orthodontic tooth movement *in vivo* (66). Studies applying *in vitro* forces show that human PDL fibroblasts have increased synthesis of type I collagen and fibronectin, and decreased tropoelastin production compared to unstretched cells (65, 67). The amount of oxytalan fibers also increases under stretched conditions *in vitro*, whereas the gene expression of fibrillins seems unaffected (68).

Loss of normal occlusal forces leads to atrophic changes in the PDL, such as narrowing of the PDL space, disorientation and reduction in size and number of collagen fibers, and vascular constriction (69, 70). High expression levels of the calcium binding protein, S100A4, have been proposed as a marker of maturation of the PDL tissue in erupted teeth. In addition, inhibition of S100A4 expression in human PDL cells *in vitro* was related to a reduction in osteoblast-related gene expression (63), suggesting that S100A4 may be involved in osteogenic processes within the PDL.

These studies provide evidence that the developmental stage, mechanical forces, and occlusion of the tooth have a significant impact on PDL cell properties. Thus, PDL fibroblasts may behave differently depending on the developmental phase from which they were harvested, which could skew the interpretation of *in vitro* results.

Tooth health status—Some studies have harvested and cultivated PDL fibroblasts derived from patients with periodontal disease (71, 72). To date, only two studies have directly evaluated whether periodontal disease affects the cell's behavior *in vitro* (73, 74) and they report differing results. In one study, PDL cells harvested from diseased and healthy conditions were not different regarding morphology, integrin profile, motility, and bone nodule formation. Cells also showed no difference when assayed for adhesion, proliferation, and chemotaxis in response to fibronectin (73). The concept that cells

harvested from teeth with periodontal disease would have a similar response as those derived from healthy patients is in agreement with results of a classic histological study from 1976. Waerhaug showed that subgingival plaque leads to cellular infiltration, collagenolysis, and severe chronic inflammation of the soft tissue, which is limited to a 1 to 2 mm zone in the immediate vicinity of the plaque (Fig. 4) (75). This would mean that cells close to the plaque would be affected by the chronic inflammation, and PDL cells at a 3 mm distance and beyond would be unaffected. However, a recent study showed that PDL fibroblasts derived from periodontally diseased teeth had different morphology and gene expression in vitro when compared to cells derived from healthy teeth, even after multiple cell passages. The cytoplasm of PDL fibroblasts from diseased teeth contained large amounts of free ribosomes and glycogen deposits, with a dilated and vesiculated rough endoplasmic reticulum, while PDL cells derived from healthy controls did not show such morphology (74). In addition, cells from healthy PDL tissues produced more alkaline phosphatase in cell culture, and were capable of significantly increasing that activity under mineralizing conditions when compared to PDL fibroblasts derived from diseased sites. Significant up-regulation of 46 cytokines, chemokines and their receptor genes was observed in fibroblasts derived from diseased PDL tissues in vitro, which corresponded to 54% of the total genes. The greatest degree of up-regulation detected was in IL-6, IL-8, IL-1 β , TNF- α , and chemokines CCL1, CCL21, and CCL17. The authors suggest that the necessary responses for maintaining a healthy periodontium are suppressed in PDL tissue compromised by periodontal disease. This concept is in agreement with the evolving field of epigenetics, where the environment leads to changes in DNA methylation that influence gene expression (76). The environmental factors range from stress to infection and accumulate in cells throughout the lifespan of an organism, permanently altering their gene expression. We speculate that the differences found in these studies could be explained by the amount of plaque accumulated on the teeth and the amount of inflammation of the adjacent PDL tissue compromised by periodontal disease. Although both studies used PDL tissue derived from the middle third of the root, the extent of the chronic infection may have been different. More studies are needed to explore whether the behavior and regenerative potential of cells is altered when a tooth is periodontally compromised.

Donor-specific factors

Some studies have evaluated the effect of the donor's systemic condition on PDL fibroblast behavior in culture. We will review the conditions that have shown such effect.

Age—As PDL tissue ages, it becomes progressively thinner, cellular mitotic activity decreases, inorganic matrix production slows down, and soluble collagen becomes less abundant (77). Cell density is reported to vary depending on age, function, and animal species evaluated (78). Murine studies show that PDL tissues from aged and young animals differ in the area occupied by endoplasmic reticulum, number and size of intercellular contacts, intracellular collagen profile, and osteogenic potential — all of which are reduced in vitro when isolated from older animals (79, 80). Changes in the PDL rate of proliferation and cytoskeleton were also observed, including reduced expression of vimentin, cytokeratin 19, and vascular endothelial growth factor (81, 82). The in vitro life span of human PDL fibroblasts is longer when isolated from adolescents (15–19 years old) than when isolated from adults (20–80 years old), as demonstrated by senescence-associated β -galactosidase staining and population doubling. No differences were found in cells derived from the 20 to 59 year old group, as well as from the 30 to 80 year old group. Statistical differences were found between the 20–29 year old donor group and the 60–80 year old group. (72). In addition, the telomere length of osteogenic PDL cells did not show correlation with donor age, but did correlate with a reduction in alkaline phosphatase activity (83). Therefore,

donor age seems to affect the *in vitro* life span of PDL fibroblasts. Furthermore, *in vitro* biological activity seems to diminish with increasing donor age.

Smoking—Smoking is the primary behavioral risk factor for periodontitis, resulting in loss of the periodontal apparatus (84–87). Among the more than 4,000 chemicals present in tobacco smoke, nicotine is a key modulator of cellular processes, including cell growth, motility, and differentiation. Nicotine has a negative biological effect on PDL cells *in vitro* by decreasing their proliferation, migration, attachment, alkaline phosphatase activity, and chemotaxis in a concentration-dependent manner (88, 89). Cigarette smoke inhalation and nicotine administration are also known to enhance murine bone loss in ligature-induced periodontitis models. Furthermore, up-regulation of cytokines and proteases, such as MMP-2, TNF- α , and IL-1 β , are associated with smoking-mediated periodontal breakdown (90–92). Recently, functional nicotinic acetylcholine receptors were identified in healthy human PDL cells, and murine experiments supported an increase in their expression and in IL-1 β expression in experimental periodontitis (93). However, differences in nicotine metabolism between humans and rodents, and the fact that high concentrations of compounds are used in experimental settings, should be noted. To our knowledge, no study has addressed the effects of *in vivo* nicotine administration and smoke inhalation on human PDL cellular behavior *in vitro*. From a regenerative perspective, it may be important to understand which biological functions are altered in PDL cells as a result of smoking. These alterations may be overcome by using regenerative materials that address these biological changes.

Marfan Syndrome—Marfan syndrome type I is caused by mutations in the gene encoding fibrillin-1, a protein present in the elastic fiber system. Immortalized cells derived from a patient with Marfan syndrome were compared to healthy PDL cells *in vitro*. Lower expression of periostin, osteocalcin, and collagen type I and XII was found in PDL cells derived from the patient with Marfan syndrome. Activated TGF- β was more abundant and osteopontin was hardly expressed when compared to healthy controls (53).

Cleidocranial dysostosis—Cleidocranial dysostosis is caused by mutations in the RUNX2 gene and characterized by anomalies in bone and disturbances in tooth development. PDL cells from two patients diagnosed with cleidocranial dysostosis were evaluated for gene expression of osteoblastic markers. Among the factors evaluated, RANKL expression was reduced compared to that of healthy donors (94). Thus, general systemic genetic alterations of proteins that are also present in the periodontal apparatus may impact PDL gene expression and function.

Diabetes—PDL fibroblasts derived from patients with diabetes type I show the same growth response to high glucose *in vitro* when compared to those derived from healthy patients (95). Some PDL cells that were growth-inhibited by high glucose showed reduced expression of basic fibroblast-growth factor, while platelet-derived growth factor –AA, –BB, insulin-growth factor-1, and TGF- β remained unchanged. Cell growth was more affected when cells were exposed to long-term glucose treatment (> 1 week). These data suggest that glucose levels affect the behavior of cells derived from both diabetic and healthy patients. Based on this study, diabetic patients with controlled glucose levels have PDL cells that behave similarly *in vitro* compared to those from healthy patients. However, more studies are needed to address changes that may influence the regenerative potential of PDL cells from diabetic patients.

Thus, to better understand the outcome of *in vitro* results and their potential applications to the regeneration process, an evaluation of the donor health status should be noted prior to the cell culture isolation process.

Recommendations

This review discusses some of the challenges faced when developing methods for periodontal regeneration. The heterogeneous nature of the PDL tissue, its development during early adulthood, and the different conditions to which the PDL tissue is exposed to *in vivo* impart it with unique characteristics that should be considered. Several factors affecting the *in vivo* setting are retained and influence the behavior of PDL fibroblasts in culture. Based on a review of several studies, two distinct transitions impact cell behavior and should be noted in experimental protocols for the cultivation of PDL fibroblasts. To date, there is not a singular specific marker to identify PDL fibroblasts from other cells. Ideally, cell identification should be confirmed by the use of several markers monitored during cell passage, and based on the needs of the study design. The use of primary cultures at early passages is ideal for maintaining the unique and rich PDL cellular heterogeneity (96–101). Tooth developmental stage may have effects on protein expression after PDL cell harvest and during the culture phase. Studies are still inconclusive in determining whether periodontal disease alters the PDL fibroblast profile in culture. This discrepancy could be related to the degree of disease and inflammation that the donor tooth and PDL tissue were exposed to. The donor condition should be noted including the patient's age, since this may change cell behavior. Few studies have evaluated systemic conditions of the patient and the impact that those may have on PDL fibroblast behavior. PDL cells from diabetic donors behaved similarly to cells derived from healthy patients, however, elevated glucose levels altered PDL cell behavior *in vitro*. Although smoking clearly shows a negative effect on cell behavior, it is still unknown to what degree it affects human PDL tissue. PDL cells derived from patients with Marfan syndrome and cleidocranial dysostosis showed different expression profiles compared to healthy controls. Consideration of these factors is important in the study design and interpretation of results.

It is important to have a large donor base to normalize for cellular heterogeneity and various factors that can influence cells in culture. The studies reviewed here show that PDL tissues from different patients may have different repertoires of proteins or genes and this may be of consequence for achieving complete periodontal regeneration. This implies that patients not responding to treatment with the use of one type of regenerative therapy may benefit from another. Having information regarding the patient's condition and tooth condition could aid therapy prediction. Continued efforts will expand our understanding of the *in vitro* and *in vivo* behavior of cells, with the goal of orchestrating optimal periodontal regeneration. This understanding will lead to improved evidence-based rationales for more individualized and predictable periodontal regenerative therapies.

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Figure 1.

Schematic view of the rich and complex nature of the periodontal tissue, including the cellular, vascular and extracellular matrix elements. The different components are depicted with different colors: osteoblasts in orange and osteoclasts in pink in proximity to the bone; fibroblasts in green following collagen fiber orientation; stem cells in purple, differentiating into a fibroblast-like or osteoblast-like cell; cementoblasts in blue and epithelial cells rest of Malassez (ERM) in aqua in proximity to the cementum; blood vessels emerge from the alveolar bone, along with nerve fibers.

Figure 2.

Microvasculature of the periodontium in a premolar tooth of a beagle dog prepared using the resin cast model (10). A and B) Vascular network under light microscope of the gingival (G) and periodontal ligament (PDL) tissues; alveolar bone (AB) is also depicted; C) Rich vascular network of the PDL tissue emerging from the bone observed by scanning electron microscopy under 12X; inferior alveolar nerve (IAA) is also depicted; D) Volkmann's canals (VC) observed at 50X, and E) AB and PDL observed at 150X, with a double arrow depicting the thickness of the PDL vascularization.

Figure 3.

Transitions that can occur when periodontal ligament fibroblasts are used for regenerative purposes. Transition 1 happens when the healthy donor tooth is taken to the cell culture environment. Transition 2 happens when the cells are taken from the culture environment to the *in vivo* setting, the latter compromised by periodontal disease.

Figure 4. Schematic figure showing the zone of destruction created by the periodontal biofilm. The safe zone for tissue collection of periodontal ligament is represented as a distance of 1 to 2mm in the immediate vicinity of the infectious biofilm.

Table 1

Markers used for periodontal ligament fibroblast identification.

Factor	Functions	References
Periostin	Bone formation, mechanical loading	Saito et al. 2002; Ibi et al 2007; Rios et al. 2008; Iwata et al 2010
Runx2	Essential for osteoblast differentiation	Saito et al 2002;
N-cadherin	Mineral nodule formation	Lin et al. 1999
NCAM-1	Unknown	Iwata et al 2010
Twist	Negative regulator of osteoblast differentiation	Komaki et al. 2007
Cementum attachment protein	Mineralization; collagen-like protein; partial homology to collagen I and XII	Pitaru et al. 1995; Bar-Kana et al. 1998; Pitaru et al. 2002
Cementum protein-23	Unknown function; potential role in cementum formation; related to collagen type X	Alvarez-Perez et al. 2006
Sclerostin	Mineralization	Jager et al. 2010
Asporin/PLAP-1	Positive/Negative regulator of mineralization	Yamada et al. 2001; Yamada et al. 2007
SI00A4 calcium binding protein	Inhibitor of mineralization	Duarte et al. 1998; Park et al. 2001; Iwata et al 2010.
Fibronectin	Fibril assembly	Lallier et al.2005
PDLs-17	Unknown	Park et al. 2001
Major histocompatibility complex-D-β	Receptor molecules	Ohyama et al. 2002; Fujita et al. 2007
Tissue factor pathway inhibitor-2	Extracellular matrix regulation	Fujita et al. 2007
Uncoordinated-like protein	PDL fibroblast differentiation, mechanical stress	Kim et al. 2007
Laminin- 2/4 and 8/9	Chemotaxis of gingival epithelial cells	Ohshime et al. 2006

Table 2
Advantages and disadvantages of using cell lines or primary cultures for studying periodontal ligament fibroblasts.

Cell Lines	Primary Cell Culture
Readily obtained from established labs	Obtaining cells requires institutional human research committee approval, patient availability and consent, and the process of cell isolation
Cells can be used at higher passage numbers (average of 50 passages)	PDL fibroblasts should be used in lower passage numbers (7–10 passages)
Possibility of cells being evaluated beyond safe passage numbers can influence cell metabolism and results	Cells exhibit diminished proliferation and reach senescence as passage number increases, providing control of safe passage numbers
Lower resemblance to the original tissue	Higher resemblance to the original tissue
A fewer number of different cell lines is sufficient to normalize for heterogeneity	A higher number of donors is necessary to normalize for heterogeneity between patients; registration of factors that might influence cell behavior is suggested
Possibility of cross-contamination is higher (without authentication tests) due to higher passage number and lack of documentation when obtained from colleagues	Possibility of cross-contamination is lower