Periodontology 2000, Vol. 63, 2013, 7–13
Printed in Singapore. All rights reserved

Information generation and processing systems that regulate periodontal structure and function

P. Mark Bartold & Christopher A. McCulloch

The appropriate functions of cells and tissues that ensure the health of multicellular organisms are very dependent on their ability to provide suitable responses to signals that originate in the extracellular environment. These signals include, but are not limited to, chemical and physical cues arising from neighboring cells and tissues. Current thinking in integrative biology suggests that many cell and tissue responses are regulated by cellular networks, which enable adaptation to a wide variety of cues from the environment. In biological systems, signaling and response networks often exhibit operational frameworks that are designated as 'motifs'. For several years it has been thought that an understanding of motifs may provide important insights into response variables that are exhibited by biological networks and that, in turn, regulate cell behavior. However, the complexity of many organs and tissues, such as the periodontium, often precludes the identification of single motifs that can be studied in isolation from other network motifs.

A new approach that has been developed to provide a more detailed understanding of the dynamic nature of motifs is designated as synthetic biology. This new approach enables biologists to test existing theories and to construct and analyze synthetic gene circuits. In turn, this information has enabled the discovery of how motif behavior is regulated in time and space to regulate transcription and the translation of proteins. Because of the complex structure of periodontal tissues and the wide variety of regulatory factors that affect cell function and fate, application of synthetic biology to obtain a better understanding of fundamental regulatory systems in periodontal tissues has been limited. One of the many remarkable features of the periodontium is the preservation of cellular domains in the periodontal ligament, a phenomenon that is consistently observed in numerous mammalian species and is maintained in spite of the application of high-amplitude physical forces and challenges from subgingival biofilms. The precise preservation of spatial dimensions in periodontal tissues, and most notably the periodontal ligament, indicates that signal processing and transduction may be particularly important in the behavior of motifs and in the spatially appropriate expression of matrix molecules that characterize cementum, bone, periodontal ligament, gingival epithelium and connective tissues. Recent work on spatial signal transduction in cellular networks has emphasized the role of various types of feedbacks, switches and oscillators in the signal transduction that characterizes homeostatic mechanisms in complex tissues (1). Cognizant of this background we have assembled a collection of reviews that describe recent progress in the cells, molecules and signaling systems that regulate periodontal structure and the responses of periodontal tissues to microbial and physical challenges. We hope that these reviews will be instructive, not only of current trends in periodontology but also as means of looking ahead to an era when biologically based foundations of stem cell biology can actually make a difference in treatment outcomes.

Molecules expressed by periodontal cells determine not only the structure and function of the periodontium but also influence how these tissues respond to physical forces, infection and inflammation. Currently it is not understood how the biological

activities of molecules expressed in the periodontium are appropriately regulated in time and space to preserve tissue homeostasis, to influence inflammatory responses and to participate in tissue regeneration. As we obtain larger and more comprehensive data sets on the spatio-temporal expression patterns of molecules in the periodontium, and examine the biological significance of these patterns using systems approaches, we will be able to apply fundamental concepts from synthetic biology and motif regulation to develop a fundamental understanding of how the form and the function of the periodontium are generated. Cognizant of the relative dearth of knowledge on fundamental periodontal biology and how this might be applied to a systems approach, in this volume of *Periodontology 2000* we explore new experimental methods and data sets that will help to resolve how periodontal tissues are precisely tuned in health and how these homeostatic mechanisms, when compromised, lead to tissue degradation. We have considered, in some detail, the molecules and cells that regulate tissue form and structure in health, disease and regeneration.

Normal periodontium and homeostasis

In addition to microbial challenge, the periodontium is also subjected to high-amplitude mechanical loading in normal function, which together have complex effects on the structure of periodontal tissues and on the periodontal ligament in particular (18). Masticatory and parafunctional loading of the periodontal ligament have been of considerable long-term clinical interest because of the central role of this tissue in tooth support, proprioception and anchorage of teeth to the alveolar bone. Mechanical forces regulate the coordinated formation and resorption of bone, as well as the organization of periodontal ligament matrix molecules to maintain tooth position over time, which is important for the long-term stability of dental occlusion. Mechanical forces can also contribute to the activation of periodontal ligament fibroblasts, particularly after injury. Currently it is not understood how periodontal ligament cells sense and respond to mechanical forces, but a wide variety of mechanisms has been considered, including the activation of mitogen-activated protein kinases (32). In this issue, Hinz et al. (14) examine the mechanosensing activities of periodontal fibroblasts and their roles in collagen secretion and remodeling, particularly in response to mechanical forces in the periodontal ligament. They describe, in detail, how mechanical forces and mechanoperception are involved in fibroblast activation. Arising from the ideas that are considered in their article is the possibility of developing new treatments that could be used to regulate fibroblast activation and to ameliorate the effects of scar formation. Notably, new pharmacological approaches are being considered that could lead to improved healing of injured periodontal tissues. Much work is needed for these approaches to be of clinical value, including the development and implementation of site-specific delivery systems to overcome systemic effects that may arise with parenteral delivery and a fundamental understanding of how periodontal cells may respond to new drugs and treatments.

The periodontal ligament is a fascinating tissue as a result of its remarkable mechanical (11) and mechanosensory properties and because of its enrichment with stem cells (8). Notably, the apparent enrichment of the periodontal ligament may be important for optimizing clinical procedures to promote periodontal regeneration. While it has been thought, for some time, that the periodontal ligament is involved in periodontal regeneration (9), the role of matrix signaling through adhesion receptors to enable appropriate timing of the proliferation and differentiation of periodontal precursor cells has not been defined. These processes are of considerable importance for wound healing in the periodontium and for establishing functional attachments.

The periodontium exhibits precisely organized cell and tissue arrays, which are important for normal function. These arrays are characterized by precisely organized collagen fiber orientation and by the insertion of mineralized Sharpey's fibers into cementum and bone. In turn, periodontal tissue organization is very much dependent on tight interactions between cells and on the organization of matrix molecules. Integrins are cell-surface receptors that enable attachment of cells to the collagen-rich and mechanically stressed microenvironment of the periodontal ligament. Integrins also provide two-way conduits for signaling between cells and matrix molecules. Barczyk et al. (3) focus on the role of integrins in the periodontal ligament in the context of matrix organization and information processing. The potential roles of integrins in regulating periodontal ligament fibroblast metabolism and stem cell differentiation are considered here, and the contributions of integrins to the organization of the extracellular matrix and to tissue regeneration are described. Evidently, integrins are crucial signaling conduits, but they may

also be important pharmaceutical targets through which periodontal stem cells may be manipulated and recruited to optimize wound healing.

In addition to their roles as matrix adhesion molecules, integrins cluster together upon matrix ligand engagement to participate in the creation of multiprotein adhesion/signaling complexes. These complexes contain hundreds of structural, signaling and adhesion molecules that interact to enable bidirectional cell–matrix signaling. Moreover, adhesion complexes are the sites at which certain inflammatory cytokine receptors (e.g. interleukin-1) are clustered (2, 20). Without receptor clustering, interleukin-1 signal transduction in fibroblasts does not occur. These observations raise interesting possibilities for development of new therapies because if inflammatory cytokine signaling relies on receptor clustering, blockade of receptor clustering could be used to inhibit inflammation. Currently, our understanding of signaling through adhesion receptors and adhesion complexes is at an early stage of development but this restriction suggests new therapeutic possibilities that are independent of conventional approaches in which receptors are blocked (e.g. tumor necrosis factor alpha-blocking antibodies). Wang et al. (29) report on the role of adhesion molecules and their roles in cell migration, matrix remodeling and inflammatory responses to infection. As noted above, cell-adhesion molecules are heterogeneous and dynamic cell-surface structures that are strongly influenced by the type of substrate to which they adhere. New proteomic methods and cell-fractionation procedures are now being used to discover novel components of cell adhesions. Wang et al. (29) consider how cell adhesions in connective tissue cells of the periodontium contribute to information generation and processing, some of which are needed for regulating periodontal structure and function. From the standpoint of drug development for the simultaneous reduction of inflammation and promotion of connective tissue formation, the isolation and study of adhesion complexes could provide new therapeutic targets that may be useful in the clinical management of periodontitis and in periodontal wound healing.

Cell attachment to underlying matrix molecules has been extensively examined in mesenchymal cells but there is less known of the molecules and the regulatory processes that enable epithelial cells to attach to matrix molecules or to tooth surfaces. For many years it has been thought that the epithelial attachment of the gingiva to tooth surfaces is a site of one of the earliest stages of periodontal pocket formation following microbial infection.

Accordingly, an improved understanding of the molecules that mediate epithelial attachment to teeth could provide new insights into pathogenic mechanisms in periodontitis and may yield high-value targets for prevention and therapeutic intervention. Consistent with this notion, Nishio et al. (19) have explored cell-adhesion molecules that mediate attachment of gingival epithelium to the tooth surface, focussing on the expression of odontogenic and ameloblast-associated molecules, such as amelotin, in the junctional epithelium. Here they consider the structure and the function of the junctional epithelium and how the pattern of expression of these molecules impacts the formation and regeneration of the junctional epithelium in wound healing. From the standpoint of preventing periodontitis, the idea of developing topically applied treatments that would preserve the integrity of these adhesion complexes holds considerable promise, but we first need to understand in depth what the key molecules are, how are they assembled and the inflammatory processes that are responsible for their initial degradation.

Antimicrobial peptides play an important role in innate immunity and help to control bacterial colonization and infection. A long-standing observation of human periodontium is that in spite of prolonged microbial biofilm colonization of the dento–gingival junction, most periodontal sites in individuals do not exhibit progressive destruction over time (26) and there appear to be in-borne, but poorly understood, protective mechanisms that prevent apical spread of biofilms. As noted by Nishio et al. (19), the epithelium of the dento–gingival junction probably plays a central role in maintaining periodontal health. Working out the protective role played by specific molecules in junctional and crevicular epithelia would seem to be a productive approach for developing new antidestructive therapeutics. In this context, Greer et al. (13) review the role of defensins and LL-37 in the junctional and oral epithelia. They note that antimicrobial peptides exhibit multiple immune functions, including the ability to regulate the chemotaxis of numerous types of host cells involved in innate and adaptive immunity. Their data point to a central role for these molecules in the earliest phases of gingival inflammation and for host responses in the initiation of periodontal diseases. Arising from their observations are the logical possibilities that the identification and the purification of these molecules could lead to the development of new diagnostics and therapeutics. For example, these approaches could employ defensins as indicators of progression or resistance to disease and as model compounds upon

which to fashion exogenously applied therapies to prevent destruction of the dento–gingival junction by pathogenic biofilms.

Chronic and aggressive periodontal diseases are characterized by a failure to resolve local inflammation in response to disease-associated bacteria in subgingival biofilms (15). Notably, progressive disease seems to involve a relatively small subpopulation of patients who are frequently refractory to current therapies. While current data indicate that the destruction of alveolar bone is associated with modified innate and adaptive immune responses to bacteria, the molecular and cellular natures of the determinants of health and disease have not yet been defined. Recent progress on the identification of subsets of myeloid-lineage cells (particularly neutrophils and monocyte/macrophages) suggests that disease-susceptible patients may exhibit cellular signatures that are associated with periodontal disease or healing. Sima & Glogauer (24) describe how macrophage subsets are involved in the regulation of alveolar bone health. In particular, they consider how macrophage differentiation to pro-inflammatory or anti-inflammatory phenotypes may be useful targets for monitoring disease activity and for modulating immune responses to prevent bone loss. It is possible to envisage that with site-specific and noninvasive sampling of crevicular fluid macrophage subpopulations, better estimates of future disease susceptibility and responses to treatment could be developed. Furthermore, as there are often wide temporal variations of responses to treatments, ongoing and noninvasive monitoring may allow a more precise estimation of when a specific treatment is either 'working' or is not effective.

Defects in mineralization of dentin and cementum lead to a structurally weak dentition; teeth become loose, are prone to infection and are lost prematurely. The mineralization of periodontal ligament fibers, which are manifest microscopically as Sharpey's fibers, at their insertion into tooth cementum and alveolar bone, is a critical process for tooth attachment to the periodontium. Past and current attempts at periodontal regeneration have not been successful in reproducibly enabling the new insertion of Sharpey's fibers into root surfaces that have been previously affected by periodontitis. This failure of predictable reattachment counts as one of the longest standing challenges in dentistry; overcoming this failure would seem to be crucially important for establishing real progress in periodontology. Currently, our understanding of the fundamental mechanisms by which mineralization is regulated at the periodontal ligament–cementum interface is limited. It would be a major advance for dental sciences in general, and for periodontology in particular, if we were to develop a definition of the repertoires of molecules and systems by which Sharpey's fibers are formed and insert into exposed root surfaces in physiological systems. McKee et al. (17) focus on the roles of two novel noncollagenous matrix proteins in mineralization: tissue-nonspecific alkaline phosphatase and phosphate-regulating gene with homologies to endopeptidases on the X-chromosome. Inactivating mutations of these proteins in humans and in mouse models lead to the soft bones and teeth characteristic of hypophosphatasia and X-linked hypophosphatemia, respectively. Insights from these two proteins are likely to provide new glimpses of the regulation of mineralization of the periodontium that is critical for maintenance of oral health and for periodontal regeneration.

Inflammation in periodontal tissues

The resolution of inflammation is critical for preventing chronic inflammatory diseases such as periodontitis. A substantial proportion of periodontal therapies in current use are aimed at suppressing inflammation because it is recognized that uncontrolled inflammation not only is more likely to promote destruction of periodontal tissues but will also restrict periodontal healing following treatment. For many years it has been recognized that the principal structural protein of the periodontium – collagen – is a critical target of zinc-dependent endopeptidases such as collagenases 1 and 2 (4). These enzymes exhibit triple-helicase activity, are abundant in the gingival crevicular fluid of patients with periodontitis and have long been considered as culprits that mediate inflammation-mediated destruction of the periodontium. Collagenases 1 and 2 are members of the matrix metalloproteinase family of proteases that exhibit a remarkably broad range of substrate specificities. These enzymes are now recognized to play central roles in inflammation, tumor formation, tumor development and wound healing. Butler & Overall (6) consider the role of matrix metalloproteinases in biology and describe their use of novel experimental proteomic techniques (degradomics) that were developed in Overall's laboratory. Their research shows that matrix metalloproteinases process a very wide array of substrates. Furthermore, their investigations on the substrates that are

degraded by matrix metalloproteinases clearly demonstrate that these molecules exhibit many functions in health and disease that go well beyond the degradation of matrix molecules. Notably, substrates of matrix metalloproteinases regulate inflammation. Therefore, these molecules may contribute to the regulation of the inflammatory response. These studies hold promise for defining new molecules for the diagnosis of (for example) acute, destructive inflammatory episodes of the periodontium and which could be identified, if sufficiently abundant, in gingival crevicular fluid. These molecules could also serve as new therapeutic targets for the prevention of progressive periodontitis, if delivery methods could be developed to enable their introduction into inflamed periodontium.

It is now recognized that the switch from inflammation to health is not a passive process involving the transition from an environment of predominantly pro-inflammatory mediators to the ultimate disappearance of the inflammatory response and a return to a situation of tissue homeostasis. Recent studies have demonstrated that resolution of inflammation involves a dynamic biologic sequence of events known as 'catabasis'. This process is considered to be just as complicated as the onset of inflammation and includes the orchestrated exit of leukocytes and return of the resident cells to a 'noninflammatory' state (22). This restoration of tissues to a state of homeostasis is tightly regulated and results in the return to health of chronically inflamed tissues affected by robust matrix destruction, fibrosis and frustrated wound healing. Recently, important advances have been made in improving our understanding of the process of inflammation resolution and how it is regulated (30).

In periodontitis, the resolution of inflammation is associated with high levels of cyclooxygenases and associated pro-inflammatory lipid mediators (e.g. prostaglandin E_2). At the initiation of resolution of inflammation, a 'class switch' occurs within neutrophils, and this results in the synthesis of pro-resolving mediators through the activation of pathways that are independent and separate from the pro-inflammatory pathways involving lipid mediators. These mediators are known as lipoxins, resolvins and protectins and exert their pro-resolving effects through a number of complex intracellular processes. The resulting cytokines that are released halt neutrophil migration to the inflammatory site, attract monocytes that do not release pro-inflammatory mediators, enhance phagocytosis of bacteria and apoptotic cells by macrophages, direct the movement of phagocytes away from the site via the lymphatics and stimulate the synthesis of antimicrobial agents (7, 21, 23).

In this volume of *Periodontology 2000*, Freire & Van Dyke (12) focus on these new concepts for the roles of endogenous lipid mediators to regulate cell fate and resolution of inflammation. They point out that resolution of inflammation starts by a class switch of lipid mediators. Classic prostaglandins and leukotrienes change to mediators that promote the resolution of inflammation. Some of these mediators include arachidonic acid-derived lipoxins and aspirin-triggered lipoxins, ω3-eicosapentaenoic acid-derived resolvins of the E series, docosahexaenoic acid-derived resolvins of the D series, protectins and maresins. The authors note that improved recognition of how lipid agonists mediate and target the cellular functions of cells may go a long way toward developing new treatment methods for inflammatory diseases, including periodontitis.

Neutrophils act as primary defence cells in controlling bacterial infection. This function is mediated through binding of their pattern recognition receptors to microbial molecular components. Approximately 10 years ago a new property of neutrophil-mediated microbial killing was noted with the observation of extracellular fiber-like structures, termed 'neutrophil traps' (5). A major role of neutrophil extracellular traps is microbial killing. Interestingly, many bacteria have evolved mechanisms allowing successful evasion and killing by neutrophil extracellular traps. In keeping with the theme of this volume of *Periodontology 2000* we recognize that there are critical processes involved in this part of the innate immune system that involve information generation and processing systems of both the host and bacteria, which lead to the successful control or failure of the infective and inflammatory components of periodontal disease. Indeed, the identification of neutrophil traps and their role in innate immunity has opened a new way of considering the processes by which bacteria are killed but also mechanisms by which collateral damage may occur, ultimately leading to chronic inflammation. In this volume of *Periodontology 2000*, Cooper et al. (10) examine some of the controversial aspects of neutrophil traps and consider how these structures may be involved in the pathogenesis of periodontitis. In particular, they suggest that an exuberant or unregulated release of neutrophil extracellular nets by this innate immune system may contribute to periodontal tissue destruction and are not necessarily protective in susceptible individuals.

Novel cellular approaches for periodontal tissue regeneration

Mesenchymal stem cells from adult tissues exhibit considerable potential utility for use in tissue engineering. Their ability to differentiate into multiple stromal cell lineages is of particular importance for periodontal regeneration. The use of these cells in regenerative therapies relies on their successful re-implantation and integration into host tissues. The risk of immune rejection of these cells would seem to be high. However, recent studies have demonstrated that mesenchymal stem cells exhibit significant immunomodulatory properties that appear to protect them from such rejection (28). These cells can inhibit the proliferation of activated T-cells and exert immunosuppressive effects on B-cells, natural killer cells, dendritic cells and neutrophils. More recently, mesenchymal stem cells derived from periodontal connective tissues have been demonstrated to possess immunosuppressive properties that are mediated, in part, by soluble factors produced by activated peripheral blood mononuclear cells (28). In this volume of *Periodontology 2000*, Wada et al. (27) review the immunosuppressive effects of mesenchymal stem cells on immune cells and the potential mechanisms that are involved in these processes. Further, preclinical animal studies and human clinical trial that utilize mesenchymal stem cells obtained from dental tissues are described here and how they may relate to this novel application of mesenchymal stem cells is discussed. Although it is recognized that considerably more work needs to be undertaken in this field, including further *in-vivo* studies using large-animal models, the immunosuppressive properties of mesenchymal stem cells have 'opened the door' for allografts (humal leucocyte antigen-mismatched cell transplants as an alternative cell source for tissue regeneration).

With regard to the periodontium and its capacity for regeneration, numerous mesenchymal stem cells have been identified in periodontal tissues and have been proposed to have potential use in periodontal-regenerative therapies. Throughout this volume of *Periodontology 2000* we consider the role of molecular and cellular systems in tissue homeostasis, tissue destruction and tissue regeneration. In order to understand how the periodontium behaves in both health and disease, an in-depth understanding of the cellular composition is required, particularly of cells with regenerative capacity. At present it is not known which cell or cells are responsible for successful regenerative outcomes. For some time it has been considered that periodontal regeneration should mimic the processes involved in tooth-root formation and hence the cells and molecules associated with tooth-root development have received considerable attention in recent years. A critical stage in root formation is the disruption of Hertwig's epithelial root sheath and the subsequent deposition of cementum by cementoblasts onto the recently exposed dentin surface, which is probably coated with proteins left behind by Hertwig's epithelial root sheath (16, 25). Hence, Hertwig's epithelial root sheath and its descendants, the epithelial cell rests of Malassez, are thought to play important roles in periodontal homeostasis. In this volume of *Periodontology 2000*, Xiong et al. (31) review the multiple roles of these cells in the prevention of ankylosis and root resorption, and investigate their potential contributions to cementum repair. Recently, ovine epithelial cell rests of Malassez have been demonstrated to contain epithelial stem-cell populations that exhibit functional and phenotypic properties similar to those of mesenchymal stem cells. The ability of the epithelial cells to undergo epithelial/mesenchymal transformation appears to be a unique feature. Hence, Hertwig's epithelial root sheath may produce epithelial cells with stem-cell like properties that have an intriguing capacity to undergo mesenchymal transformation. The epithelial/mesenchymal transformation by the epithelial cell rests of Malassez may provide a hitherto-unrecognized progenitor cell pool for the development of mesenchymal cells, such as periodontal ligament fibroblasts and cementoblasts. Thus, the epithelial cell rests of Malassez may be an important source of stem cells for periodontal regeneration.

Summary

The periodontium comprises complex and highly cellular tissues that are organized into tightly regulated mineralized (cementum and bone) and soft connective tissue (gingiva and periodontal ligament) domains. Collectively, the papers presented in this volume of *Periodontology 2000* highlight the evolving importance of systems biology for obtaining a more global understanding of cell and tissue regulation in the periodontium and the preservation of domain structure. An improved understanding of how the cells and tissues of the periodontium generate and process informational molecules for assembly and maintenance, and respond to various regeneration stimuli, is crucial for the development of new preventive and therapeutic approaches.

References

1. Alam-Nazki A, Krishnan J. An investigation of spatial signal transduction in cellular networks. *BMC Syst Biol* 2012: **6**: 83.
2. Arora PD, Ma J, Min W, Cruz T, McCulloch CA. Interleukin-1-induced calcium flux in human fibroblasts is mediated through focal adhesions. *J Biol Chem* 1995: **270**: 6042–6049.
3. Barczyk M, Bolstad AI, Gullberg D. Role of integrins in the periodontal ligament: organizers and facilitators. *Periodontol 2000* 2013: **63**: 29–47.
4. Birkedal-Hansen H. Role of matrix metalloproteinases in human periodontal diseases. *J Periodontol* 1993: **64**(5 Suppl): 474–484.
5. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science* 2004: **303**: 1532–1535.
6. Butler GS, Overall CM. Matrix metalloproteinase processing of signaling molecules to regulate inflammation. *Periodontol 2000* 2013: **63**: 123–148.
7. Campbell EL, Louis NA, Tomassetti SE, Canny GO, Arita M, Serhan CN, Colgan SP. Resolvin E1 promotes mucosal surface clearance of neutrophils: a new paradigm for inflammatory resolution. *FASEB J* 2007: **21**: 3162–3170.
8. Chen FM, Sun HH, Lu H, Yu Q. Stem cell-delivery therapeutics for periodontal tissue regeneration. *Biomaterials* 2012: **33**: 6320–6344.
9. Chen FM, Zhang J, Zhang M, An Y, Chen F, Wu ZF. A review on endogenous regenerative technology in periodontal regenerative medicine. *Biomaterials* 2010: **31**: 7892–7927.
10. Cooper PR, Palmer LJ, Chapple ILC. Neutrophil extracellular traps as a new paradigm in innate immunity - friend or foe? *Periodontol 2000* 2013: **63**: 165–197.
11. Fill TS, Toogood RW, Major PW, Carey JP. Analytically determined mechanical properties of, and models for the periodontal ligament: critical review of literature. *J Biomech* 2012: **45**: 9–16.
12. Freire MO, Van Dyke TE. Natural resolution of inflammation. *Periodontol 2000* 2013: **63**: 149–164.
13. Greer A, Zenobia C, Darveau RP. Defensins and LL-37: a review of function in the gingival epithelium. *Periodontol 2000* 2013: **63**: 67–79.
14. Hinz B. Matrix mechanics and regulation of the fibroblast phenotype. *Periodontol 2000* 2013: **63**: 14–28.
15. Kinane DF, Preshaw PM, Loos BG; Working Group 2 of Seventh European Workshop on Periodontology. Host-response: understanding the cellular and molecular mechanisms of host-microbial interactions – consensus of the Seventh European Workshop on Periodontology. *J Clin Periodontol* 2011: **38** (Suppl 11): 44–48.
16. Lindskog S. Formation of intermediate cementum I. Early mineralization of aprismatic enamel and intermediate cementum in monkey. *J Craniofac Genet Dev Biol* 1982: **2**: 147–160.
17. McKee MD, Hoac B, Addison WN, Barros NMT, Millán JL, Chaussain C. Extracellular matrix mineralization in periodontal tissues: Noncollagenous matrix proteins, enzymes, and relationship to hypophosphatasia and X-linked hypophosphatemia. *Periodontol 2000* 2013: **63**: 102–122.
18. Naveh GR, Brumfeld V, Shahar R, Weiner S. Tooth periodontal ligament: direct 3D microCT visualization of the collagen network and how the network changes when the tooth is loaded. *J Struct Biol* 2013: **181**: 108–115.
19. Nishio C, Wazen R, Moffatt P, Nanci A. Expression of odontogenic ameloblast-associated and amelotin proteins in the junctional epithelium. *Periodontol 2000* 2013: **63**: 59–66.
20. Qwarnstrom EE, Page RC, Gillis S, Dower SK. Binding, internalization, and intracellular localization of interleukin-1 beta in human diploid fibroblasts. *J Biol Chem* 1988: **263**: 8261–8269.
21. Serhan CN, Brain SD, Buckley CD, Gilroy DW, Haslett C, O'Neill LA, Perretti M, Rossi AG, Wallace JL. Resolution of inflammation: state of the art, definitions and terms. *FASEB J* 2007: **21**: 325–332.
22. Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP, Petasis NA. Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. *J Immunol* 2006: **176**: 1848–1859.
23. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med* 2002: **196**: 1025–1037.
24. Sima C, Glogauer M. Macrophage subsets and osteoimmunology: tuning of the immunological recognition and effector systems that maintain alveolar bone. *Periodontol 2000* 2013: **63**: 80–101.
25. Slavkin HC, Bringas P, Bessem C, Santos V, Nakamura M, Hsu MY, Snead ML, Zeichner-David M, Fincham AG. Hertwig's epithelial root sheath differentiation and initial cementum and bone formation during long-term organ culture of mouse mandibular first molars using serumless, chemically defined medium. *J Periodontal Res* 1989: **24**: 28–40.
26. Socransky SS, Haffajee AD, Goodson JM, Lindhe J. New concepts of destructive periodontal disease. *J Clin Periodontol* 1984: **11**: 21–32.
27. Wada N, Gronthos S, Bartold PM. Immunomodulatory effects of stem cells. *Periodontol 2000* 2013: **63**: 198–216.
28. Wang L, Zhao Y, Shi S. Interplay between mesenchymal stem cells and lymphocytes: implications for immunotherapy and tissue regeneration. *J Dent Res* 2012: **91**: 1003–1010.
29. Wang Y, Wang Q, Arora PD, Rajshankar D, McCulloch CA. Cell adhesion proteins: roles in periodontal physiology and discovery by proteomics. *Periodontol 2000* 2013: **63**: 48–58.
30. White ES, Mantovani AR. Inflammation, wound repair, and fibrosis: reassessing the spectrum of tissue injury and resolution. *J Pathol* 2013: **229**: 141–144.
31. Xiong J, Gronthos S, Bartold PM. Role of epithelial cell rests of Malassez in the development, maintenance and regeneration of periodontal ligament tissues. *Periodontol 2000* 2013: **63**: 217–233.
32. Ziegler N, Alonso A, Steinberg T, Woodnutt D, Kohl A, Müssig E, Schulz S, Tomakidi P. Mechano-transduction in periodontal ligament cells identifies activated states of MAP-kinases p42/44 and p38-stress kinase as a mechanism for MMP-13 expression. *BMC Cell Biol* 2010: **11**: 10.