Expert Opinion

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Extracellular matrix administration as a potential therapeutic strategy for periodontal ligament regeneration

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Introduction: The current strategies employed for the treatment of connective tissue disease include the application of stem cells, the use of functional molecules that can reorganize tissue integrity and cellular activities to recover connective tissue function. Approaches to the regeneration of periodontal tissue, which is the tooth-supporting connective tissue, have made some progress recently and provide a useful experimental model for the evaluation of future strategies to treat connective tissue diseases such as periodontal disease.

Areas covered: The ultimate goal of periodontal tissue regeneration is to reconstruct the ligament structure that will sustain the required mechanical force to connect with mineralized tissues such as cementum and alveolar bone. In this review, we discuss the proposed use of extracellular matrix (ECM) administration therapy as an additional therapeutic strategy to stem cell transplantation and cytokine administration in the current field of periodontal tissue regeneration therapy.

Expert opinion: Although various available tissue engineering technologies can now achieve periodontal tissue regeneration, ECM administration therapy is likely to play an essential future role in the development and regeneration of periodontal tissue and attenuate the signaling events that mediate tissue degradation. Hence, ECM administration could serve as a novel technology in periodontal tissue regeneration and also as a viable approach to alleviating connective tissue disorders such as Marfan’s syndrome.

Keywords: connective tissue, microfibril, PDL, regenerative therapy


1. Introduction

Periodontal tissue is a tooth-supporting tissue comprising periodontal ligament (PDL), cementum and alveolar bone. This tissue thereby plays an important role in the maintenance of the occlusion system. Among the components of periodontal tissue, the PDL consists mainly of an extracellular matrix (ECM) that provides the physical properties to withstand mechanical stress in cooperation with the cementum and alveolar bone. Dysfunction of the PDL occurs as a result of periodontal disease, an inflammatory disorder involving the irreversible destruction of periodontal tissues and requiring the regeneration of PDL as a treatment for recovering occlusion function [1,2]. Periodontal disease is caused by pathogenic microflora including Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola, and the resulting inflammation extends deep into the periodontal tissue and causes the loss of PDL, cementum and alveolar bone [3]. Chronic periodontal disease is the most common form of this disorder, showing a prevalence rate of > 90% in adults.
over 60 years of age [4]. Furthermore, this disorder is the major cause of tooth loss in adults of over 40 years and its more severe forms has a worldwide prevalence of up to 20% according to the World Health Organization [5]. Blocking the progression of periodontal disease has been achieved by mechanically removing bacterial biofilm with conventional periodontal and/or surgical treatments. These treatments can reduce the destruction of periodontal tissue and diminish inflammation in the affected region. However, achieving adequate periodontal tissue regeneration remains a problem, particularly in cases where the disease has caused large defects in the periodontal tissue.

The current advances in future regenerative therapies have been influenced by many previous studies of embryonic development, stem cell biology and tissue engineering technologies [6-9]. To restore the partial loss of organ functions and to repair damaged tissues, attractive concepts that have emerged in regenerative therapy is stem cell transplantation into various tissues and organs [10] and cytokine therapy, which has the potential to induce the activation and differentiation of tissue stem/progenitor cells [11]. Tooth tissue stem cells and the cytokine network that regulates tooth development, and dental tissue cell growth and differentiation, have been well characterized at the molecular level [12,13]. The regeneration of periodontal tissues is being made clinically possible by the transplantation of mesenchymal stem cells which can differentiate into PDL cells, cementoblasts and osteoblasts, or through the local application of cytokines to stimulate the proliferation and differentiation of these stem cells [14-17]. Although these therapies are effective and contribute to periodontal tissue repair, these interventions will likely be improved by an enhanced understanding of the development of periodontal tissues, particularly those involved in the formation of PDL, cementum and alveolar bone.

The ECM is a biologically active molecule composed of a complex mixture of macromolecules that, in addition to serving a structural function, profoundly affect the cellular physiology of an organism [18]. Previous findings have revealed that ECM components including type I collagen, type III collagen, laminin, decorin, peristin, f-spondin, tenasin-N and PLAP/Aspirin are highly expressed during PDL formation [19,20]. Since the ECM is regulated in a tissue-specific manner, these structures could enhance periodontal regeneration by promoting the differentiation of cells required for the synthesis of PDL, bone and cementum [21,22]. Among the ECM formations in the PDL, fibrillin-1, a major component of the microfibrils that regulate tissue integrity and elasticity, has been shown to contribute to the formation and maintenance of this ligament. An abnormal PDL structure in association with the progressive destruction of microfibrils has been observed in a Marfan’s syndrome (MFS) mouse model and has characteristics that are similar to those of fibrillin-1 dysfunction [23]. These findings have strongly suggested that microfibril formation through fibrillin-1 assembly plays an important role in PDL formation and function. However, the molecular mechanisms of fibrillin-1 microfibril assembly remain unclear as the microfibril-associated molecule that regulates or stabilizes fibrillin-1 microfibril formation has not yet been identified. Recent findings have revealed that ADAMSL6 is essential for the development and regeneration of the PDL through the direct interaction of fibrillin-1 to promote microfibril assembly [23,24]. These findings have also suggested that the administration of fibrillin-1 microfibrils provides a novel therapeutic strategy for the treatment of periodontal disease.

We here review the present status of the periodontal tissue regeneration technologies that focus on the molecular mechanisms underlying development, regeneration and tissue engineering of periodontal tissue, and also discuss the potential of ECM administration therapy through the promotion of microfibril assembly as a novel therapeutic strategy for the essential functional recovery of periodontal tissue.

2. Development processes in periodontal tissue

The PDL has essential roles in tooth support, homeostasis and repair, and is involved in the regulation of periodontal cellular activities such as cell proliferation, apoptosis, the secretion of extracellular matrices, resorption and repair of the root cementum and remodeling of the alveolar bone [25-27]. To develop future methods to regenerate damaged PDLs, it will be important to understand the molecular basis of PDL development.

2.1 Molecular mechanisms underlying periodontal tissue development

The PDL is derived from the dental follicle (DF), which is located within the outer mesenchymal cells of the tooth germ and can generate a range of periodontal tissues including
the PDL, cementum and alveolar bone [21]. The DF is formed during the cap stage of tooth germ development by an ectomesenchymal progenitor cell population originating from the cranial neural crest cells [28]. Given the critical role that the progenitor cell population in the DF appears to play in the development of periodontal tissue, the developmental processes in this tissue are of considerable interest in terms of further understanding the biology of these cells (Figure 1). The differentiation of the DF proceeds as follows: i) during the tooth root-forming stage, the Hertwig’s epithelial root sheath (HERS) comprising the inner- and outer-dental epithelia that initiate tooth root dentin formation is fragmented into the Malassez epithelium resting on the tooth root surface; ii) the DF migrates to the surface of the tooth root and differentiates into cementoblasts to form the cementum matrix [29,30]; iii) at almost the same time, the DF differentiates into the PDL on the cementoblasts in order to insert collagen fibers, known as Sharpey’s fibers, into the cementum matrix. Fiber insertion also takes place along the alveolar bone and iv) both bone- and PDL-derived fibers finally coalesce in the PDL to form the intermediate plexus, which resembles periodontal tissue [31-33].

The DF has long been considered to be a source of multipotent stem cells (DFSCs), since these cells have the ability to migrate onto the tooth root surface to form periodontal tissue including cementum, PDL and alveolar bone during the tooth root-forming stage [32,34-37]. Previous studies have indicated that DF cells can form PDL-like tissues and cementum/bone-like structures after implantation into immunodeficient mice [38,39], supporting the notion that stem cells that can differentiate into PDL, cementoblast, osteoblast lineages are present in the DF [34,35]. To regenerate periodontal tissue, functional molecules which promote the differentiation of DFSCs into PDL need to be elucidated to enable a proper understanding of the mechanisms underlying periodontal tissue formation, including the pathways pertaining to PDL cell, cementum and alveolar bone differentiation.

2.2 Functional molecules involved in DF differentiation

Although the molecular mechanisms of DF development and differentiation remain to be determined, previous gene expression studies of mouse molar root development have suggested that some growth factors, including bone morphogenetic protein (BMP) 4, growth and differentiation factors (GDFs) 5, 6 and 7 [40-43], epidermal growth factors [44], Shh [45-47] and insulin-like growth factor (IGF)-1 [48], are involved in the growth or differentiation of the DF. Transcriptional factors such as Scleraxis, Gli, Msi1, Mix2 and Runx2 have also been shown to be involved in the differentiation of the DF into cementoblasts and in the mineralization of cementum [39,43,46,49]. Among these factors, GDFs and scleraxis are the most well characterized that are involved in tendon/ligament morphogenesis, suggesting that PDL development shares similar molecular mechanisms to those of tendon/ligament morphogenesis. With regard to cemenogenesis/osteogenesis of the DF, treatment of this tissue with BMP-2 and BMP-7 has been found to induce mineralization ability. In addition, previous findings suggest that PDL cells harbor mineralization inhibitory mechanisms that enable them to maintain a ligament structure across the mineralized tissue, including the alveolar bone and cementum, during PDL development [50-52]. These observations strongly suggest that the tendon/ligament-related cytokines, the BMPs, and inhibitors of mineralization are linked to the restoration of the tendinous structure of the PDL. The mechanisms involving these factors may also have a role in preventing ankylosis of the PDL.

3. Regeneration therapies for PDL defects

A partial restoration of periodontal tissue has been achieved previously using a guided tissue regeneration (GTR) technique which provides an adequate space and favorable niche for the repair of periodontal defects using barrier membrane [53]. From the results of these GTR therapies, regeneration of the PDL has been shown to be critical for recovering the connection between the cementum on the root surface and the alveolar bone.

To regenerate periodontal tissue that has been destroyed by periodontal disease requires the recruitment of PDL stem cells (PDLSCs) to properly reconstitute the PDL structure including its extracellular components such as the collagen and elastic fibril systems [32,33]. Recent studies of stem/progenitor cells have provided considerable new insights that have furthered our understanding of PDLSCs, which can differentiate into periodontal tissue cell lineages such as PDL, cementum and alveolar bone [14,54]. PDLSCs will have utility for the future development of stem cell transplantation therapies and tissue engineering applications to restore periodontal organ function as they replace damaged areas with enriched and purified stem cells and thereby achieve PDL repair (Figure 2) [14]. The biological potential of PDLSCs to stimulate the regeneration of periodontal tissue can now be realized by the local application of human recombinant cytokines.

3.1 Stem cell therapies

PDLSCs have been isolated from human PDL tissue by single-colony selection and magnetic activated cell sorting. PDLSCs express the mesenchymal stem cell markers STRO-1 and CD146/MUC18, and can differentiate into cementoblast-like cells, adipocytes and fibroblasts [14]. In addition, PDLSCs show the capacity to generate a cementum/PDL-like structure and contribute to periodontal tissue repair on transplantation into immunocompromised rodents. Clonal PDLSC analysis has further revealed that these cells show a similar phenotype to DFSCs since they also express RUNX-2, Col I, ALP, OPN, OCN, RANKL, OPG, scleraxis, peristin, Col XII and alpha-SMA mRNAs [54]. Importantly, PDL tissue collected from one tooth can give rise to many stem cells because of their high proliferation capacity ex vivo. Recently also, it has been shown that the transplantation of autologous PDLSCs obtained from the
extracted teeth of miniature pigs can regenerate and repair a surgically created periodontal defect [55]. This finding suggests that PDLSCs obtained from an easily accessible tissue resource and expanded \textit{ex vivo} using wisdom teeth might represent a feasible therapeutic approach to the reconstruction of tissues destroyed by periodontal disease.

In addition to the clinical application of stem cell transplantation, cell sheet engineering therapies for periodontal tissue regeneration are now being developed for clinical application [56,57]. In this technology, temperature-responsive dishes are used to harvest the cell sheets through a simple decrease in the temperature, thus avoiding the use of proteolytic enzymes [58]. The use of this method allows PDL cell sheets to be easily harvested and transplanted into periodontal defects \textit{in vivo} [56,57,59,60]. PDL cell sheets have the potential to induce periodontal regeneration, including the reformation of the PDL and cementum. The available data also suggest that this technique has the appropriate efficacy for periodontal regeneration in patients with periodontal disease.

### 3.2 Cytokine therapies

Some new treatments that accelerate the regeneration of periodontal tissue by local application of human recombinant cytokines have now been established. This approach stimulates the proliferation and differentiation of stem cells/progenitors from the PDL into hard tissue-forming cells. The local application of human recombinant cytokines such as platelet-derived growth factor (PDGF) and IGF-1 [16,61], BMP-2 [62,63], TGF-\(\beta\) [64], osteogenic protein (OP)-1 [65] and brain-derived neurotrophic factor (BDNF) [66] stimulates and promotes the regeneration of regional periodontal tissue in animal models. The potency of PDGF-BB plus \(\beta\)-tricalcium phosphate (\(\beta\)-TCP, an osteoconductive scaffold) in periodontal tissue regeneration in human has also been recently reported [67]. In addition, a clinical Phase I study of fibroblast growth factor (FGF)-2 has shown that it stimulates the regeneration of periodontal tissue lost due to periodontal disease and demonstrated the safety of this treatment [15]. The results of this trial were clinically interpreted as a demonstration of...
the efficacy of FGF-2 in stimulating the regeneration of periodontal tissue. These findings collectively suggest that cytokine therapy has great clinical potential for achieving the partial regeneration of periodontal tissue.

4. Novel approaches to periodontal tissue regeneration using ECM administration therapy

ECM components organized in the PDL not only reflect the functional requirements of this matrix such as mechanical stress and storage of signaling molecules, but also regulate the tissue framework during development and regeneration [21]. Diseases affecting ECM function such as MFS have been shown to increase the susceptibility to severe periodontal disease due to a dysfunction of the PDL through a microfibril insufficiency, suggesting that fibrillin-1 microfibril formation plays a central role in PDL formation [68-74]. In addition, a new therapeutic concept has proposed that a fibrillin-1 microfibril insufficiency can be corrected by the administration of ECM components [23].

4.1 Periodontal disease and MFS

MFS is a severe, systemic disorder of connective tissue formation and can lead to aortic aneurysms, ocular lens dislocation, emphysema, bone overgrowth and severe periodontal disease [68,75,76]. MFS has an estimated prevalence of 1 in 5000 – 10,000 individuals [77]. Fibrillin-1 comprises one of the major insoluble ECM components in connective tissue microfibrils which provides limited elasticity to tissues and stores cytokines such as TGF-β [78,79] (Figure 3A). Various mouse models of MFS have now been established via gene targeting or missense mutations in which germline mutations in fibrillin-1 lead to progressive connective tissue destruction due to fibrillin-1 fragmentation in association with an insufficiency of fibrillin-1 microfibril formation [72,74,75]. Hence, it is largely accepted that MFS is caused by insufficient fibrillin-1 microfibril formation in various connective tissues [76]. The study of PDL provides a useful experimental model not only for investigating the molecular pathogenesis of MFS, but also for evaluating novel therapeutic strategies for the improvement of microfibril disorders. This is because the principal elastic fiber system of the PDL, the oxytalan fiber, is composed of fibrillin-1 microfibrils and does not contain significant amounts of elastin [80-82]. Indeed, an abnormal PDL in association with progressive destruction of microfibrils is an obvious phenotype in the MFS mouse model [23]. Hence, PDLs will likely be more susceptible to breakdown in MFS compared with other elastic tissues composed of both elastin and fibrillin-1 (Figure 3B).

A structural insufficiency of fibrillin-1 microfibrils arises in MFS and leads to activation of TGF-β and its regulatory targets.
protein domains with the ADAMTS protease, including thrombospondin type I repeats, a cysteine-rich domain and an ADAMTS spacer, but lack the catalytic and disintegrin-like domains. Among the novel ADAMTSL family molecules, ADAMSL6β is essential for the development and regeneration of the PDL [23]. ADAMTSL6β was recently found to associate with fibrillin-1 microfibrils through its direct interaction with the N-terminal region of fibrillin-1, and thereby promote fibrillin-1 matrix assembly both in vitro and in vivo [24]. Another study has indicated that fibronectin is an essential component during the assembly of fibrillin-1 through its interaction with the C-terminal region of fibrillin-1, thus suggesting the potential for improved microfibril assembly through the regulation of fibrillin-1-associated proteins including ADAMTSL6β [83,84]. In an animal model of MFS microfibril disorder [85], ADAMSL6β expression can rescue fibrillin-1 microfibril formation through the promotion of fibrillin-1 microfibril assembly (Figure 4A). More importantly, the local administration of ADAMTSL6β was found to be highly effective in accelerating the wound healing of periodontal tissues through the restoration of microfibrils (Figure 4B). Further evidence for the impact of ADAMTSL6β on microfibril assembly is its suppression of TGF-β signaling, a pathway which is known to contribute to elastolysis in MFS.

These findings have demonstrated that microfibril assembly induced by ADAMTSL6β is essential not only for fibrillin-1 microfibril restoration but also for the inhibition of the pathological activation of TGF-β. Thus, ECM administration therapy such as microfibril assembly could form the basis of a novel therapeutic approach to PDL regeneration and the treatment of periodontal disease in MFS patients.

5. Conclusions

Regenerative therapies for periodontal disease that use the cells of the patient to repair the periodontal defect have been proposed in a number of studies [86-88]. PDL-derived stem cells such as PDLSCs can differentiate into all of the periodontal lineages that contribute to cell turnover in the steady-state and would thus be useful cell sources for regenerative therapies to treat periodontal disease following tissue injury [89-91]. Treatments that partially regenerate damaged PDLS through local application of cytokines have now been established, and such regenerative therapies have provided a very useful and feasible clinical study model for the future design of stem cell and cytokine therapies [15,61,92]. Although partial regeneration of the periodontal tissue has been established, methods to achieve the functional regeneration of large defects caused by severe periodontal disease are still lacking. To address this, it is essential to better understand the molecular mechanisms underlying PDL development and to thereby identify the appropriate functional molecules that induce the differentiation of stem cells into periodontal lineage cells for the successful reconstruction of periodontal tissue [31,38]. Investigations of the molecular mechanisms of fibrillin-1 microfibril assembly via ADAMTSL6β during
PDL formation will make substantial contributions to this endeavor [23]. In addition, since microfibrils play an important role in maintaining connective tissue integrity, including the aorta, lung and skin, we are hopeful the ECM administration therapy will in the future encourage the development of PDL regeneration for the treatment of periodontal disease as well as connective tissue disorders such as MFS [75,77].

6. Expert opinion

As described above, the partial regeneration of connective tissue damaged by pathological microflora has been achieved by regeneration therapy using stem cell transplantation and the local application of cytokines. Identification of the stem cells in the PDL or DF has enabled the development of protocols to regenerate the PDL and these have proved to be useful model systems for the development of connective tissue regeneration therapies [15,17]. One of the major research obstacles in PDL regeneration studies is the identification of all of the key functional molecules that drive PDL development. The establishment of ECM administration therapy such as fibrillin-1 microfibril assembly is ultimately critical for the development of new therapeutic approaches for periodontal disease and MFS [76]. MFS fibrillionopathies have been explained by the structural insufficiency of fibrillin-1 microfibrils leading to the activation of TGF-β and its regulatory targets [9].

A variety of MFS therapies have been developed to date, including surgical therapy for aortic root aneurysms that are life-threatening [78], traditional medical therapies such as β-adrenergic receptor blockade for slow aortic growth and to decrease the risk of aortic dissection, and novel approaches based on new insights such as the pathogenesis of insufficient fibrillin-1 microfibril formation and the deregulation of TGF-β activation [79]. In the case of periodontal disease in MFS, surgical therapy or regeneration therapy is performed using stem cells or cytokines to recover damaged periodontal tissue (Figure 5, left panel).

In contrast to these approaches, the administration of ADAMTS6β to fibrillin-1 microfibrils may represent a new ECM administration therapy which is viable for the treatment of the periodontal disease of MFS [28]. The evidence indicates that ADAMTS6β is capable of enhancing microfibrils even in the case of a fibrillin-1 haploinsufficiency. Hence, ECM
administration therapy through the promotion of microfibril assembly by ADAMTS6β may have potentially novel therapeutic benefits for the treatment of periodontal disease and disorders associated with MFS (Figure 5, right panel).

In conclusion, we here introduce the concept that a fibrillin-1-associated protein such as ADAMTS6β, which induces microfibril assembly, should be considered as an ECM administration agent for the treatment of periodontal disease and improvement of connective tissue disorders such as MFS. The exogenous application of recombinant ADAMTS6β improves fibrillin-1 microfibril assembly, indicating that the reinforcement of fibrillin-1 microfibrils by ADAMTS6β may represent a new treatment for periodontal disease which is accessible from oral cavity in MFS patients. Since elastolysis occurs continuously in aortic aneurysms arising in MFS cases, the chronic administration of ADAMTS6β may be required for the stabilization of microfibrils to prevent progressive tissue destruction. It will also be necessary to develop methodologies for the systemic administration of ADAMTS6β to induce fibrillin-1 microfibril assembly in connective tissue for the treatment of life-threatening conditions such as an aortic aneurysm (Figure 5, right panel). Hence, an ECM administration therapy involving ADAMTS6β has the capacity to facilitate drug discovery for treating periodontal diseases, and MFS-associated disorders.

Declaration of interest

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Extracellular matrix administration as a potential therapeutic strategy


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