Chapter 14
Trends in Periodontal Regeneration Therapy: Potential Therapeutic Strategy of Extracellular Matrix Administration for Periodontal Ligament Regeneration

Masahiro Saito

Abstract Current strategy for the treatment of periodontal disease is to application of stem cells or functional molecules that can reorganize tissue integrity, cellular activities and extracellular matrix framework to recover peridontal tissue function. The approach to be regeneration of periodontal ligament (PDL) that is a tooth supporting connective tissue has made a progress for consideration of strategies in regeneration therapy of periodontal tissue damaged by periodontitis. To realize the achieving functional PDL regeneration, the application of stem cells and functional molecules which are essential for PDL regeneration/development must be developed. The identification of stem cells/progenitors and functional molecules that contribute PDL regeneration will substantial contribution for realization of the regeneration therapy as a novel treatment of connective tissue disease. This review describes current strategy of functional PDL regeneration based on development, stem cell biology and tissue engineering after pathological degradation by periodontitis. The present status of the hurdles to this technology are also described and discussed.

Keyword Extracellular matrix • Marfan syndrome • Microfibril • Periodontal ligament • Regeneration therapy

14.1 Introduction

The current advances in future regenerative therapies have been influenced by many previous studies of embryonic development, stem cell biology, and tissue engineering technologies [1, 2]. 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 [3] and cytokine

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therapy, which has the potential to induce the activation and differentiation of tissue stem/progenitor cells [4]. PDL stem cells and the cytokine network that involved PDL formation and dental follicle cell growth and differentiation, have been well characterized at the molecular level [5–7]. Based on these results, 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 [8–10]. 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.

Fibrillin-1 comprises one of the major insoluble extracellular matrix components in connective tissue microfibrils and provides limited elasticity to tissues through microfibril formation [11]. Various mouse models revealed that Marfan syndrome (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 [12]. MFS have been established via gene targeting or missense mutations, with germline mutations in fibrillin-1 leading to progressive connective tissue destruction due to fibrillin-1 fragmentation in association with an insufficiency of fibrillin-1 microfibril formation [13]. Hence, it is largely accepted that MFS is caused by insufficient fibrillin-1 microfibril formation in various connective tissues [14]. Fibrillin-1 has been shown to contribute to the formation and maintenance of periodontal ligament. An abnormal PDL structure in association with the progressive destruction of microfibrils has been observed in a Marfan's syndrome mouse model. These findings have strongly suggested that microfibril formation through fibrillin-1 assembly 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.

14.2 Periodontal Ligament Development

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 [15]. To develop future methods to regenerate damaged PDL, it will be important to understand the molecular basis of PDL development and also how the destruction of the PDL occurs during periodontal disease.

14.2.1 Developmental Process of Dental Follicle

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 [16]. 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 [17]. The differentiation of the DF proceeds as follows: (1) 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 Mallasez epithelium resting on the tooth root surface; (2) the DF migrates to the surface of the tooth root and differentiates into cementoblasts to form the cementum matrix [18, 19]; (3) 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 (4) both bone- and PDL-derived fibers finally coalesce in the PDL to form the intermediate plexus, which resembles tendinous tissue [20].

14.2.2 Tendon/Ligament Related Molecules Involved in DF Development

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 4, growth and differentiation factors (GDFs)-5, 6, and 7 [21, 22], epidermal growth factors [23], Shh [24], and insulin-like growth factor-1 [25], are involved in the growth or differentiation of the DF. Transcriptional factors such as Scleraxis, Gli, Msx1, Msx2 and Runx2 have also been shown to be involved in the differentiation of the DF into cementoblasts and in the mineralization of cementum [20, 26]. 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 [20, 27]. These observations strongly suggest that the tendon/ligament related cytokines regulate induction of extracellular matrix (ECM) component to the formation of the tendinous structure of the PDL. The mechanisms involving fibrous ECM network formation may also have a role in formation of the DF development.

14.3 Microfibril is Essential for PDL Maintenance and Formation

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 tendon/ligament formation [28]. Global gene expression analysis of PDL forming stage have revealed that ECM components including type I collagen, type III collagen, lumican, decorin, periostin, f-spondin, tenascin-N, fibrillin-1 and PLAP1/aspirin are highly expressed during PDL formation [29, 30].

14.3.1 Fibrillin-1 Regulate PDL Formation and Maintenance

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 PDL [31]. Various mouse models of Marfan's syndrome (MFS) have been established via gene targeting or missense mutations, with germline mutations in *fibrillin-1* leading to progressive connective tissue destruction due to fibrillin-1 fragmentation in association with an insufficiency of fibrillin-1 microfibril formation [32–35]. Hence, it is largely accepted that MFS is caused by insufficient fibrillin-1 microfibril formation in various connective tissues. 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 [36]. MFS patient have been shown that periodontal disease is progressed severely compared with non MFS patient [37]. 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.

14.3.2 Strategy of MFS Treatment

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. A variety of MFS therapies have been developed, including surgical therapy for aortic root aneurysms that are life-threatening, traditional medical therapies such as β-adrenergic receptor blockade for slow aortic growth and to decrease the risk of aortic dissection [14]. It has been demonstrated also that

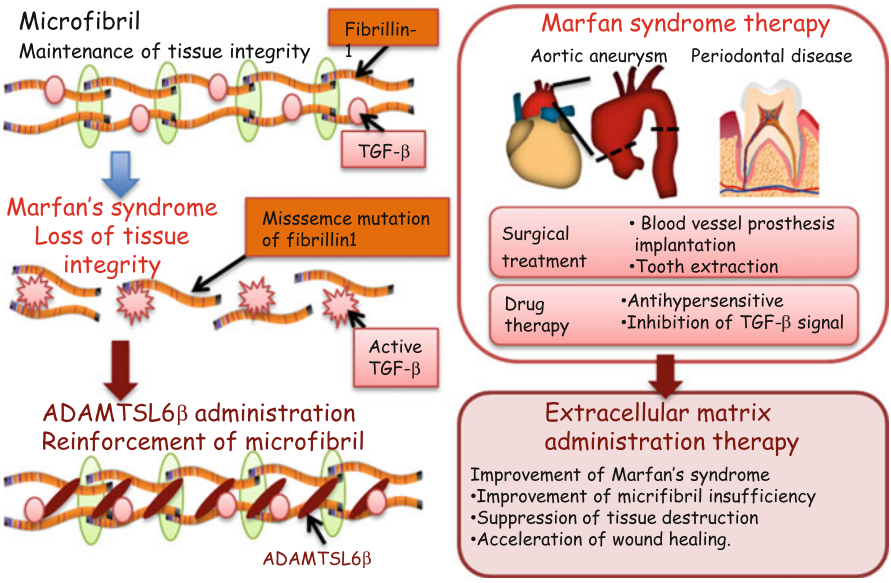

Fig. 14.1 Schematic representation of the MFS and ECM administration therapy as a novel therapeutic strategy for the treatment of MFS. *Left panel*: Fibrillin-1 comprises insoluble extracellular matrix components in connective tissue microfibrils and provides limited elasticity to tissues through fibrillin-1 microfibril formation. Missense mutations of fibrillin-1 leading to progressive connective tissue destruction due to fibrillin-1 fragmentation in association with an insufficiency of fibrillin-1 microfibril formation. ADAMTSL6β is essential for fibrillin-1 microfibril formation and suggest a novel therapeutic approach to the treatment of MFS through the promotion of ADAMTSL6β-mediated fibrillin-1 microfibril assembly. *Right Panel*: A variety of MFS therapies have been developed, including surgical therapy for aortic root aneurysms that are life-threatening, 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 deregulation of TGF-β activation. ECM reinforcement therapy which induces restoration of properly formed microfibrils by ADAMTSL6β is essential not only for improvement of the predominant symptoms of MFS, but also for the suppression of excessive TGF-β signaling induced by microfibril disassembly. Image from published paper [39]

systemic antagonism of Transforming Growth Factor-type beta (TGF-β) signaling through the administration of a TGF-β neutralizing antibody or losartan, an angiotensin II type 1 receptor blocker, has been shown to have a beneficial effect on alveolar septation and muscle hypoplasia in MFS [33, 38] However, another potential therapeutic strategy which remains to be investigated is the reconstruction of the microfibril in connective tissues through the expression or administration of a microfibril-associated molecule that regulates or stabilizes fibrillin-1 microfibril formation. To investigate this concept, it will be necessary to identify molecular mechanisms of microfibril formation and an appropriate fibrillin-1 microfibril associated molecule (Fig. 14.1).

14.4 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 [30]. In addition, a new therapeutic concept has proposed that a fibrillin-1 microfibril insufficiency can be corrected by the administration of ECM components.

14.4.1 ADAMTSL6β Serves as a Novel Molecules that Regulate Microfibril Assembly

A disintegrin-like metalloprotease domain with thrombospondin type I motifs (ADAMTS)-like, ADAMTSL, is a subgroup of the ADAMTS superfamily that shares particular protein domains with the ADAMTS protease, including thrombospondin type I repeats, a cysteine-rich domain, and an ADAMTS spacer, but lacks the catalytic and disintegrin-like domains [40]. A recent study has demonstrated that ADAMTSL2 mutations cause geleophysic dysplasia, an autosomal recessive disorder similar to MFS, through the dysregulation of TGF-β signaling [41]. A homozygous mutation in ADAMTSL4 also causes autosomal-recessive isolated ectopia lentis, another disease similar to MFS which is characterized by the subluxation of the lens as a result of disruption of the zonular fibers [42]. The novel ADAMTSL family molecules ADAMTSL6α and 6β were recently identified by in silico screening for novel ECM proteins produced from a mouse full-length cDNA database (FANTOM). These proteins are localized in connective tissues, including the skin, aorta and perichondrocytes. Among ADAMTSL6, ADAMTSL6β has shown to associated with fibrillin-1 microfibrils through its direct interaction with the N-terminal region of fibrillin-1 and promotes fibrillin-1 matrix assembly in vitro and in vivo [43]. These findings suggest a potential clinical application of ADAMTSL6β as a novel MFS therapy by promoting fibrillin-1 microfibril assembly and regulating TGF-β activation.

It is also suggested that the administration of fibrillin-1 microfibrils provides a novel therapeutic strategy for the treatment of periodontal disease.

14.4.2 ADAMTSL6β Regulates Microfibril Assembly

To investigate whether ADAMTSL6β plays a critical role in microfibril assembly in connective tissues, we generated ADAMTSL6β transgenic mice (TSL6β-TG mice) in which the transgene is expressed in the whole body. Since ADAMTSL6β has shown to be expressed in the aorta and skin, we investigated microfibril assembly of these tissues in the *TSL6β*-TG mice. Immunohistochemical analysis revealed that

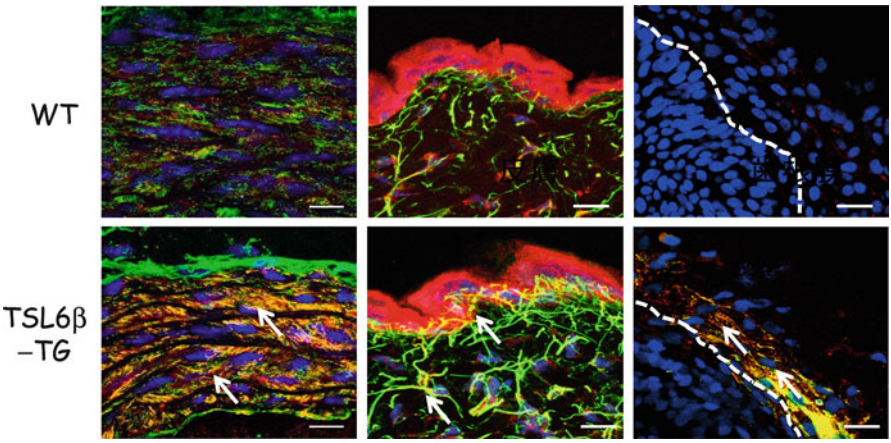

Fig. 14.2 Immunohistochemical analysis of TSL6β-TG mice. Cryosections were prepared from the aortas (*left*), skin (*middle*) or PDL (*right*) of wild type (*upper panel*) or TSL6β TG (*lower panel*) littermates and subjected to double immunostaining with antibodies against ADAMTSL6β (*red*) and fibrillin-1 (*green*). ADAMTSL6β and fibrillin-1-positive microfibrils (*green yellow*) was markedly increased in the aorta and skin of TSL6β TG mice compared with WT mice. Bar = 50 μm Image from published paper [39]

ADAMTSL6β positive microfibril assembly was barely detectable in WT mice but strongly induced in the aorta of TSL6β-TG mice (Fig. 14.2). Histological analysis revealed that microfibrils are clearly increased in the aorta and that microfibril assembly is also induced in the skin and PDL of TSL6β – TG mice. This confirmed that ADAMTSL6β induces fibrillin-1 microfibril assembly in connective tissue such as the aorta, skin and PDL.

14.4.3 ADAMTSL6β Involved in PDL Formation and Repair

To investigate whether ADAMSL6β contributes to PDL formation, we first examined its expression patterns during PDL forming stage of DF in the developing tooth germ. In situ hybridization analysis revealed that ADAMSL6β was strongly expressed in the PDL forming stage of the DF however ADAMSL6β expression was significantly downregulated in the adult PDL. Immunohistochemical analysis further revealed that ADAMSL6β is detectable in assembled microfibril-like structures during the PDL forming stage of the DF, and in organized microfibrils in the adult PDL. Because developmental processes involve similar mechanisms to wound healing, we next determined whether ADAMSL6β is involved in PDL microfibril assembly during wound healing using a tooth replantation model. Histochemical analysis revealed that both fibrillin-1 and ADAMSL6β expressions were found to be clearly induced during wound healing of PDL, but to decrease again after healing. These findings suggested that ADAMSL6β was involved in microfibril formation during PDL formation/regeneration.

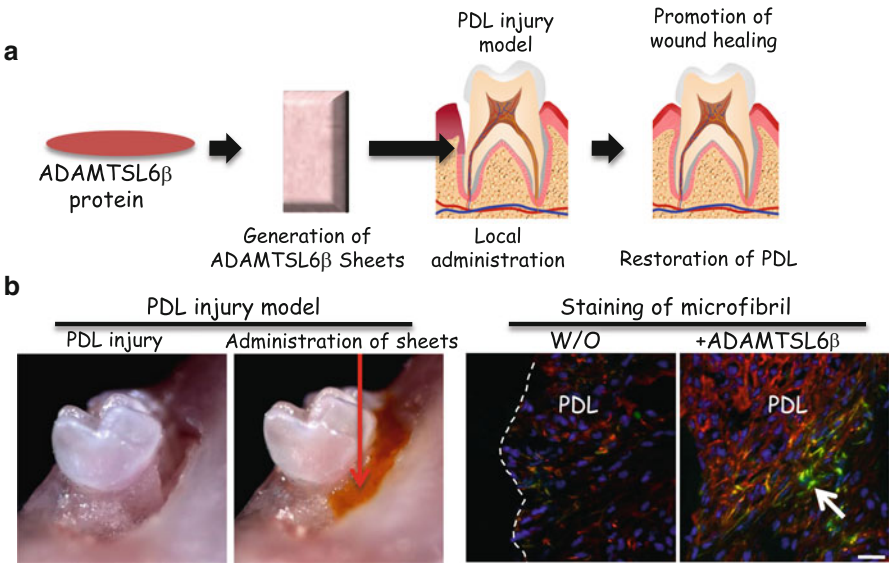

Fig. 14.3 ADAMSL6β improves microfibril disorder in PDL from an MFS model. (**a**) Schematic representation of the local administration of recombinant ADAMSL6β into a PDL injury model (**b**) After injury of PDL by dislocation, collagen gel-containing recombinant ADAMSL6β was then injected into the injured PDL (*left*). Immunohistochemical analysis showed an improvement in fibrillin-1 microfibril assembly (*arrowheads*) induced by the injection of recombinant ADAMSL6β. *WO*: Without treatment of ADAMSL6β. Image from published paper [17]

Since oxytalan fiber, a principal elastic fiber system of PDL is composed of fibrillin-1 microfibrils and does not contain significant amounts of elastin [44, 45], this composition suggests that PDL will have an increased susceptibility to breakdown in MFS compared with other elastic tissues composed of both elastin and fibrillin-1 [46]. We demonstrated that ADAMSL6β is highly expressed in DF during PDL forming stage. In addition, intense expression of ADAMTSL6β can be seen in wound healing process of PDL, indicating that this protein involved in recovery of damaged PDL. Using an animal model of MFS, we demonstrate that local administration of ADAMSL6β can rescue fibrillin-1 microfibril formation through the promotion of fibrillin-1 microfibril assembly in PDL (Fig. 14.3). These results strongly indicate that ADAMTSL6β is essential for fibrillin-1 microfibril formation and suggest a novel therapeutic approach to the treatment of periodontal disease with MFS.

14.5 Conclusion

Regenerative therapy for the periodontal disease has been attempted to use of patient's own cells to recover periodontal defect. Predictable treatment for partial regeneration of PDL damaged by local application of cytokines or stem cell trans

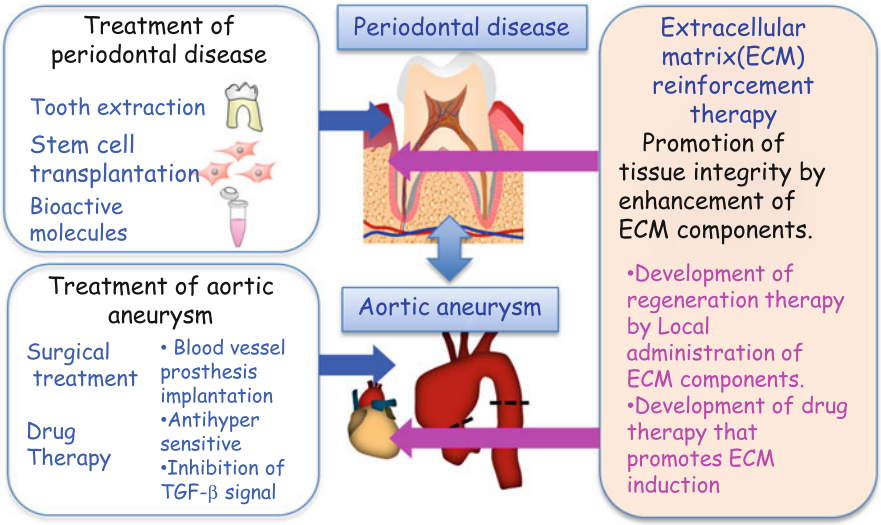

Fig. 14.4 ECM administration therapy as a novel therapeutic strategy of MFS syndrome. ECM administration therapy using ADAMTSL6β which induces microfibril assembly, should be considered in the development of future mechanism-based therapeutics for the improvement of connective tissue disorders such as MFS. Image from published paper [17]

plantation has been established, thus regenerative medicine for PDL has made the most useful study model and is feasible clinical study for the planning of stem cell- and cytokine- therapies [47]. Although partial regeneration of the periodontal tissue has been established, novel treatment must be developed corresponding to regenerate large defect destroyed by severe periodontal disease. To approach this criticism, it is essential to understand the molecular mechanisms of PDL development to identify the appropriate functional molecules of inducing differentiation of stem cells into periodontal lineage cells for successful reconstruction of periodontal tissue [17, 48, 49].

In this review, we proposed that fibrillin-1 associated protein such as ADAMTSL6β, 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 ADAMTSL6β improves fibrillin-1 microfibril assembly, indicating the reinforcement of fibrillin-1 microfibrils by ADAMTSL6β 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 ADAMTSL6β 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 ADAMTSL6β to induce fibrillin-1 microfibril assembly in connective tissue for the treatment of life-threatening conditions such as an aortic aneurysm (Fig. 14.4).

Hence, an ECM administration therapy involving ADAMTSL6β has the capacity to facilitate drug discovery for treating periodontal diseases, and MFS-associated disorders.

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