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Elastogenesis in the Periodontal Tissue Evaluated at Electron-Microscopic Levels

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Abstract

In the elastogenesis in the periodontal tissue, microfibrils in the bundle are linked to one another through delicate filaments found as the deposition of high electron-density. The elastic fiber system in the periodontal ligament is characterized by the presence of only oxytalan fibers composed of numerous microfibrils without the central core. In contrast, elaunin fibers are distinguished from oxytalan fibers by the high electron-dense core labeled with tannic acid-uranyl surrounded by numerous micro fibrilsin the putative gingival lamina propria, in the connective tissue surrounding the alveolar bone, and in the periodontal space of the enamel side of incisors. Their cross section represents a tube-like structure of the microfibrils showing peripheral electron-dense mantle on its outskirts intensely stained with the tannic acid-uranyl acetate solution at a higher magnification. These electron-dense mantles on the outskirt of microfibrils are aggregated like a patchwork composed of middle grade of dense cores surrounded by many microfibrils. The patchwork-like electron-dense cores seem to be gradually aggregated into a larger central core of elaunin or elastic fibers with higher electron-density. The present findings on the morphogenesis of elastic fiber system are strongly supported by biochemical results clarified so far.

Keywords: Elastogenesis; Oxytalan fibers; Elaunin fibers; Elastic fibers; Periodontal tissue

Introduction

The biomechanical character of the periodontal tissue is important in its reaction upon functional forces. Several previous researches suggest that the elastic fiber family contributes to the biomechanical character and behavior of the periodontal tissue [1-3] although collagen has been the primary subject of investigations in this field. A large number of studies have reported that collagen fibers play a valuable role in the biomechanical character and behavior of the periodontal tissue [1], and several results claim that oxytalan fibers could also contribute to it [2,3]. The elastic fiber system is a major insoluble part of extracellular matrix/matricellular proteins that constitutes the connective tissues with elasticity: The elastic system fibers can be distinguished into three groups, oxytalan, elaunin and elastic fibers [4]. Oxytalan fiber system comes to a more expanded stage with the growth of root of the tooth. A close association between oxytalan fibers and the vascular system also exists later in life, suggesting their role in vascular support [2,3]. Oxytalan fibers occur organized into bundles consisting of only microfibrils, while elaunin and elastic fibers have a central cross-linked core of elastin surrounded by a sheath of microfibrils. Elaunin fibers contain a smaller proportion of elastin than elastic fibers [5]. In our previous investigations [2,3], the growth and distributional patterns of the oxytalan fiber system were examined in the developing periodontal ligament of rats using the specific staining for oxytalan, elastic and collagen fibers, and electron-microscopic analyses. Oxytalan staining clearly confirmed the earliest oxytalan fibers in a well-staged tooth germ of embryonic day 18 (E18). At adult stage, we identified oxytalan fibersin the periodontal membrane but not any elaunin and elastic fibers, whereas three different elastic system fibers were detected in the gingiva at the same developmental stage. On the contrary, recent studies have reported that elastin-immuno reactive fibers were localized mainly in the close vicinity of blood vessels in the apical region of the periodontal ligament and could be identified as elaunin fibers [6-8]. After the oxytalan fiber system appeared in the tooth germ tissue of E18, much remains to be deciphered about the time-lapse changes of the elastic fiber system in the periodontal tissue. Thus, the purpose of the present study is to reveal the detailed morphogenesis of elastic fiber system in the periodontal tissue.

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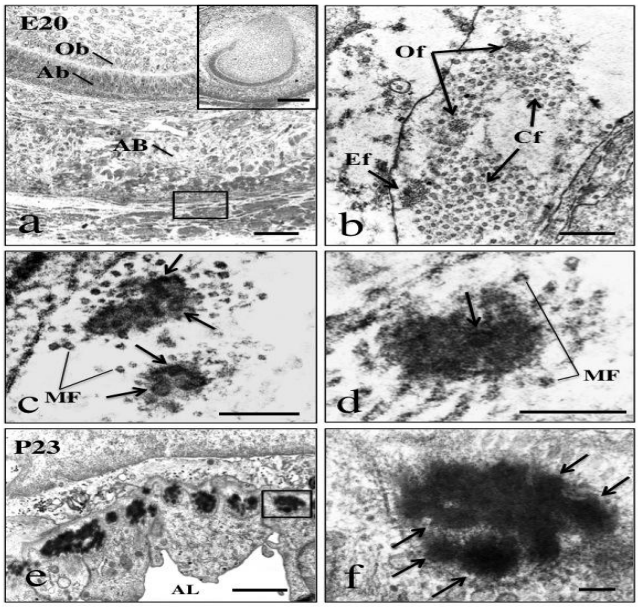

Figure 1: Tooth germ of a bell-stage in a lower incisor at embryonic day 20 (E20) of rats (**A-D**) and arteriole distributed in the mucosa of hard palate at postnatal day 23 (P23) of them (**E,F**). Figures **B-F** stained with the tannic acid-uranyl acetate solution. Inset in figure A (toluidine blue-stained semi-thin section) represents lower magnification of the transverse section of tooth germ and figures **B-D** represent higher magnification of the region adjacent to alveolar bone (AB), marked by a black rectangle in figure **A**. Oxytalan (Of), elaunin (Ef), and collagen fibers (Cf) are observed (**B**). A large number of tube-like microfibrils (Mf) are seen organized into loose small bundles. Microfibrils in the bundle appear to be cross-linked to one another through the deposition of high electron-density (arrows in **C**). The intense deposition of higher electron-density (arrow in **D**) is apparently expanded into a central cross-linked core surrounded by numbers of microfibrils (Mf). Numerous sub-endothelial elastic fibers are observed as the depositions of higher electron-density (**E**). Figure F represents higher magnification of a black rectangle in figure **E**. A central core of elastic fibers is composed of cross-linking assemblies with higher electron-density (arrows in **F**) deposited onto several microfibrils. Ab ameloblast; Ob odonto blast; AL arteriole lumen. Scale bar = 0.5 mm for **A**; scale bar = 0.2 mm for inset in figure **A**; scale bar = 400 nm for **B**; scale bars = 100 nm for **C** and **D**; scale bar = 1 μm for **E**; scale bar = 100 nm for **F**.

Morphogenesis of Elastic Fiber System in the Periodontal Tissue

A large number of tube-like micro fibrils of 10-12 nm in width were seen organized into loose small bundles in the region adjacent to alveolar bone of a well-staged lower incisor germ at E20 of rats (Figure 1A, and 1B). Microfibrils in the bundle appeared to be linked to one another through delicate, approximately 3-nm-diameter filaments possibly of fibro nectin, found as the deposition of high electron-density (Figure 1B and 1C) [2]. Elaunin and collagen fibers were also observed in the same area (Figure 1B). Elaunin fibers were detected as the ultra-structure like a patchwork composed of the middle grade of central cross-linked cores surrounded by many micro fibrils (Figure 1C and 1D). In contrast, oxytalan fibers were composed of only numerous microfibrils without the central core (Figure 1B). At the stage of E20, both oxytalan and elaunin fibers were already confirmed in the putative gingival lamina propria, in the connective tissue surrounding the alveolar bone, and in the periodontal space of the enamel side of incisors. A small number of elaunin fibers were distinguished from oxytalan fibers by the presence of the high electron-dense core labeled with tannic acid-uranyl, surrounded by numerous microfibrils. The cross section of micro fibrils revealed a tube-like structure showing gradual development of peripheral electron-dense mantle on its outskirts intensely stained with the tannic acid-uranyl acetate solution at a higher magnification (Figure 1C and 1D). These microfibrils were distributed to surround a central core-like structure. These electron-microscopic results are in accord with those obtained from in situ hybridization method for tropoelastin (in submission). In an arteriole distributed within the mucosa of hard palate at postnatal day 23 (P23) of rats, numerous sub-endothelial elastic fibers were observed as the depositions of higher electron-density (Figure 1E). A central core of elastic fibers was composed of cross-linking assemblies with higher electron-density deposited onto several microfibrils (Figure 1F). Oxytalan fibers composing of numerous microfibrils, elaunin fibers composed by patch-like amorphous cores and microfibrils, and elastic fibers with intensely-labeled core and a few microfibrils are observed in the sub epithelial layer of gingival lamina propriain adult rats. Elaunin and elastic fibers are distributed also in the deep supra-alveolar layer of gingival lamina propria. Oxytalan fibers appear to be more densely distributed in the sub-epithelial layer of the lamina propria, elastic fibers in the supra-alveolar layer, and elaunin fibers between both layers. Furthermore, we have conducted our investigation with the greatest care using the cross section of gingiva in the adult rats of P60 in order to reveal the characteristic structures of the elastic fiber system. The central core of elastic fibers is labeled as dark homogenous staining using tannic acid-uranyl acetate and lead citrate, such as those distributed in the arteriolar wall (Figure 1F).

Morphological Mechanism in Elastogenesis in the Periodontal Tissue

The morphological mechanism of elastic fiber assembly that leads to deposition and cross-linking of elastin along microfibrils remains largely unknown. Elastic fibers are characterized by the presence of two morphologically distinct components: microfibrils and polymerized elastin [9]. Microfibrils, which are 10-12 nm filaments in the extracellular matrices [2], are mainly composed of large extended glycoproteins, fibrillin-1 and -2 [10,11]. In addition, they serve as scaffolds for deposition of tropoelastin [12], together with several kinds of proteins that are associated with them, such as latent transforming growth factor (TGF) β-binding proteins (LTBPs) [13,14] and microfibrils-associated glycoproteins (MAGPs) [15,16]. Latent TGF β binding protein-2 (LTBP-2), which belongs to the fibrillin/LTBP family but lacks binding to the latency-associated propeptide-TGFβ complex (small latency complex or SLC), interacts with DANCE/fibulin-5 and facilitates fibrillin-1 microfibril-dependent elastin deposition [17]. DANCE/Fibulin-5 was shown to bind N-terminal fibrillin-1 fragments and co-localize with microfibrils [18,19]. It was further demonstrated that fibulin-5 binding to fibrillin-1 markedly potentiates binding between fibrillin-1 fragments and tropoelastin, acting as an adaptor to form a ternary complex [20]. In another experiment, fibulin-5 was shown to bind tropoelastin or fibrillin-1 without influencing the overall complex formation and it was suggested that fibulin-5 facilitates targeting and deposition of tropoelastin onto microfibrils [21]. Although the precise sequence of events during tropoelastin deposition onto microfibrils is not completely determined, these data collectively suggest that DANCE/fibulin-5 binds tropoelastin to navigate onto a fibrillin-1 dominant microfibrillar scaffold while simultaneously mediating proper coacervation prior to cross-linking (Figure 2). When LTBT-2 is knocked down, elastin preferentially deposits onto fibrillin-2 microfibrils. LTBP-2 can regulate DANCE deposition

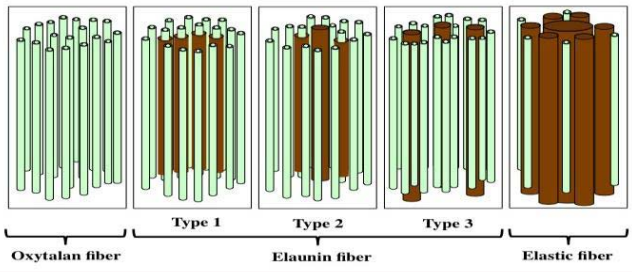

Figure 2: Schematic diagram showing elastic system fibers consisting of three groups of oxytalan, elaunin and elastic fibers. Oxytalan fibers are organized into bundles consisting of only microfibrils (green-colored tubules), while elaunin and elastic fibers have a central cross-linked core of elastin (brown-colored substances) surrounded by a sheath of microfibrils. Elaunin fibers contain a smaller proportion of elastin than elastic fibers. The growth patterns of the elaunin fiber system are designated as types 1, 2 and 3.

on microfibrils [17]. The DANCE and elastin deposition on micro fibrills is dependent on fibrillin-1 in the presence of LTBP-2, and down regulation of LTBP-2 causes fibrillin-1-independent depositon of DANCE and elastin on fibrillin-2 or other potential microfibrils. LTBP-2 might fuction as a molecular switch that determines which microfibrils DANCE/fibulin-5 should be deposited on, thereby regulating subsequent assembly of elastic fiber components [17]. The expression of fibrillin-1 and -2 is differentially controlled, but overlaps in the development of elastic tissues [22]. Although fibrillin-1-mutated mice develop normal elastic matrices [23] and fibrillin-2-mutated mice show syndactyly but normal elastogenesis [24,25], more deficient in both fibrillin-1 and -2 were recently reported to exhibit impaired elastogenesis [26]. However, why only a part of microfibrils deposit elastin to form elastic fibers and fibrillin-1 and -2 microfibrils are differentially utilized in elastogenesis remains unclear. Fibrillin-1 microfibrils are dependent on the fibro nectin matrix for deposition, and knockdown of fibro nectin completely abolishes the formation of fibrillin-1 microfibrils [27,28].

Concluding Remarks

Considerable progress has been made in the last decade in determining the biochemical properties of fibulins and their biological functions during elastogenesis in the periodontal tissue. The present findings on the morphogenesis of elastic fiber system in the periodontal tissue are strongly supported by the biochemical results. However, further studies on the localization of microfibrils of fibrillin-1 or -2 and several kinds of proteins such as fibulinsor LTBPs will be required to elucidate the morphological mechanism in elastic fiber assembly of the periodontal tissue.

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