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The tooth attachment mechanism defined by structure, chemical composition and mechanical properties of collagen fibers in the periodontium

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Abstract

In this study, a comparison between structure, chemical composition and mechanical properties of collagen fibers at three regions within a human periodontium, has enabled us to define a novel tooth attachment mechanism. The three regions include, (1) the enthesis region: insertion site of periodontal ligament (PDL) fibers (collagen fibers) into cementum at the root surface, (2) bulk cementum, and (3) the cementum–dentin junction (CDJ). Structurally, continuity in collagen fibers was observed from the enthesis, through bulk cementum and CDJ. At the CDJ the collagen fibers split into individual collagen fibrils and intermingled with the extracellular matrix of mantle dentin. Under wet conditions, the collagen fibers at the three regions exhibited significant swelling suggesting a composition rich in polyanionic molecules such as glycosaminoglycans. Additionally, site-specific indentation illustrated a comparable elastic modulus between collagen fibers at the enthesis (1–3 GPa) and the CDJ (2–4 GPa). However, the elastic modulus of collagen fibers within bulk cementum was higher (4–7 GPa) suggesting presence of extrafibrillar mineral.

It is known that the tooth forms a fibrous joint with the alveolar bone, which is termed a gomphosis. Although narrower in width than the PDL space, the hygroscopic CDJ can also be termed as a gomphosis; a fibrous joint between cementum and root dentin capable of accommodating functional loads similar to that between cementum and alveolar bone. From an engineering perspective, it is proposed that a tooth contains two fibrous joints that accommodate the masticatory cyclic loads. These joints are defined by the attachment of dissimilar materials via graded stiffness interfaces, such as: (1) alveolar bone attached to cementum with the PDL; and (2) cementum to root dentin with the CDJ. Thus, through variations in concentrations of basic constituents, distinct regions with characteristic structures and graded properties allow for attachment and the load bearing characteristics of a tooth.
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Keywords: Atomic force microscopy (AFM); Interface; Collagen fibers; Periodontium; Nanoindentation

1. Introduction

Biomechanical function of a load bearing system is defined by the physical properties of the tissues and their interfaces (interphases). However, inflammation due to disease progression leads to degraded physical properties resulting in loss of attachment and function. This study was performed to provide a foundation for tissue engineering formulations for tooth attachment by defining physical characteristics of tissues and interfaces within the human periodontium.

The periodontium of a human tooth includes three mineralized tissues: alveolar bone, cementum and root dentin, of which cementum and alveolar bone interface with a soft fibrous tissue; the periodontal ligament (PDL), forming a fibrous joint or gomphosis [1,2]. Periodontal fibers and Sharpey's fibers in acellular cementum seemingly attach to root dentin [3–5]. Additionally, they were reported to be calcified in acellular cementum [3,4].

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In addition, the acellular cementum fibers intermingle with the dentin matrix [6]. More recently, similar observations on acellular cementum were reported [7]. Although not clear in studies by Dewey [5], results presented to date were only on acellular cementum.

Cementum is attached to root dentin via a hydrophilic fibrous cementum–dentin junction (CDJ) [8]. The CDJ is also known as the innermost cementum layer, intermediate cementum or collagen hiatus and consists of collagen fibrils and remnants of epithelial cells of Hertwig's epithelial root sheath (HERS) [9]. Additionally, it was suggested not to be a distinct matrix layer but a composite of non-collagenous proteins and collagen fibrils [10]. Despite the existing theories and these observations, no physical property associations between the fibrous CDJ and the PDL insertions into cementum, also known as Sharpey's fibers (SFs) have been made.

In this study the attachment of bulk cementum to root mantle dentin was investigated with respect to the PDL and previously reported hydrophilic CDJ [8]. This study sought to demonstrate that the collagen fibers within the CDJ are continuous with the Sharpey's fibers in human cellular cementum, using various high-resolution imaging techniques and histology. The structure at micro- and nanoscales was investigated using light and atomic force microscopy (AFM) techniques and the site-specific mechanical properties were studied using AFM-based nanoindentation.

2. Materials and methods

2.1. Specimen preparation for light microscopy

Mandibular molars from varying ages (19–81 yr) were sterilized using 0.26 Mrad of gamma-radiation [11]. Several 2–3 mm thick transverse sections perpendicular to the longitudinal axis of the tooth root were made using a diamond wafering blade and a low speed saw (ISOMET, Buehler, Lake Bluff, IL) under wet conditions. The specimens were ultrasonicated in deionized water for 10 s (L&R Ultrasonic Cleaning System, Kearny, NJ) after preparation to remove any abrasives from coarse sectioning. The transverse sections were fixed in 2.5% glutaraldehyde for 8 weeks followed by end-stage decalcification [12] using Cal-EX II decalcifying solution (Fisher Scientific, Fair Lawn, NJ). The specimens were embedded in paraffin and sectioned on a rotary microtome (Reichert-Jung Biocut, Vienna, Austria) using disposable steel blades (TBF^{TM} Inc., Shur/Sharp^{TM}, Fisher Scientific, Fair Lawn, NJ). The paraffin sections were mounted on Superfrost Plus microscope slides (Fisher Scientific, Fair Lawn, NJ) deparaffinized with xylene followed by staining with either Safranin O' or Masson's trichrome. The stained tissues were characterized using a light microscope (BX 51, Olympus America Inc., San Diego, CA) and analyzed using Image Pro Plus v6.0 (Media Cybernetics, Inc., Silver Spring, MD). Polarized light was used to generate a strong contrast, thus enhancing the features of interest, including orientation of collagen fibers.

2.2. Specimen preparation for AFM and AFM-based nanoindentation

The specimens for AFM and AFM-based nanoindentation were prepared using an ultrasectioning method, which was described previously [13]. Ultrasectioning was used to generate a relatively flat surface to maintain orthogonality between the nanoprobes of the AFM and AFM-based nanoindenter and the specimen. In brief, unfixed transverse sections were glued to AFM steel stubs (Ted Pella, Inc., Redding, CA) using a cyanoacrylate adhesive (MDS Adhesive QX-4, MDS Products Inc., Anaheim, CA). The specimens were ultrasectioned using an ultramicrotome (Ultracut E, Reichert-Jung, Vienna, Austria) and trimmed with a glass knife (Electron Microscopy Laboratory—UCSF, San Francisco, CA). Final trimming of the specimens was performed using a diamond knife (Micro Star Technologies, Huntsville, TX) by removing 300 nm thick ultrasections at a speed of 0.75 mm/s, thus creating a sectioned block surface for AFM and AFM-based nanoindentation under dry and wet conditions.

2.3. AFM and AFM-based nanoindentation of ultrasectioned cementum

The structure from the root surface of cementum to mantle dentin including the CDJ was characterized under dry and wet conditions using an AFM (Nanoscope III, Multimode, DI-Veeco Instruments Inc., Santa Barbara, CA). A Si_3N_4 tip attached to a 'V-shaped' type 'D' microlever with a nominal spring constant of 0.06 N/m (DNP, Veeco Probes, Camarillo, CA) at a scanning frequency of 1.5 Hz was used to scan the surfaces of the ultrasectioned block specimens. The nominal radius of curvature of the tip was less than 50 nm [13]. Areas as large as $100 \times 100\,\mu m^2$ were evaluated using a 'J' type piezo scanner. The structural changes of the Sharpey's fibers (PDL inserts), along with the CDJ within each specimen block under dry and wet conditions were determined using the scanned areas and Nanoscope III version 5.2 software (Nanoscope III, Multimode, Veeco Instruments Inc., Santa Barbara, CA).

Nanomechanical tests on dry and wet specimens were performed using an AFM, to which a load–displacement transducer (Triboscope Micromechanical Test Instrument, Hysitron Incorporated, MN) was attached [8]. A sharp diamond Berkovich indenter with a radius of curvature less than 100 nm (Triboscope Micromechanical Test Instrument, Hysitron Incorporated, Minneapolis, MN) was fitted to the transducer. The scan speed, scan area and load displacement of the indenter on the specimen were controlled by a computer. After indentation, the AFM piezo was used to scan the indented area. Immersion of the specimen and indenter in deionized water allowed measuring the mechanical properties closer to *in vivo* conditions. The maximum load used was 500 μN, with load, hold, and unload for 3 s each when measuring the reduced elastic modulus 'E_r'. Fused silica was used as a calibration standard for the AFM-nanoindenter [14]. The E_r of regions of interest was determined using the following equation:

$$S = \left[\frac{2}{\sqrt{\pi}}\sqrt{AE_r}\right],$$

where 'S' is the stiffness obtained from the load-displacement curve and 'A' is the contact area [15].

3. Results

3.1. Structure analysis using light and atomic force microscopes

These results are divided into sections demonstrating that: (1) collagen fibers are continuous from root surface through bulk cementum, pass through the CDJ and enter the root dentin; (2) the collagen fiber-rich region at the root surface and CDJ have similar compositions; and (3) nanomechanical properties of the collagen-rich region at the root surface and CDJ have similar values.