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Structure, chemical composition and mechanical properties of human and rat cementum and its interface with root dentin

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Abstract

This work seeks to establish comparisons of the physical properties of rat and human cementum, root dentin and their interface, including the cementum–dentin junction (CDJ), as a basis for future studies of the entire periodontal complex using rats as animal models. In this study the structure, site-specific chemical composition and mechanical properties of cementum and its interface with root dentin taken from 9- to 12-month-old rats were compared to the physiologically equivalent 40- to 55-year-old human age group using qualitative and quantitative characterization techniques, including histology, atomic force microscopy (AFM), micro-X-ray computed tomography, Raman microspectroscopy and AFM-based nanoindentation. Based on results from this study, cementum taken from the apical third of the respective species can be represented as a woven fabric with radially and circumferentially oriented collagen fibers. In both species the attachment of cementum to root dentin is defined by a stiffness-graded interface (CDJ/cementum–dentin interface). However, it was concluded that cementum and the cementum–dentin interface from a 9- to 12-month-old rat could be more mineralized, resulting in noticeably decreased collagen fiber hydration and significantly higher modulus values under wet conditions for cementum and CDJ ($E_{\text{rat-cementum}} = 12.7 \pm 2.6$ GPa; $E_{\text{rat-CDJ}} = 11.6 \pm 3.2$ GPa) compared to a 40- to 55-year-old human ($E_{\text{human-cementum}} = 3.73 \pm 1.8$ GPa; $E_{\text{human-CDJ}} = 1.5 \pm 0.7$ GPa). The resulting data illustrated that the extensions of observations made from animal models to humans should be justified with substantial and equivalent comparison of data across age ranges (life spans) of mammalian species.
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Keywords: Cementum; Interfaces; Structure; Chemical composition; Animal model

1. Introduction

Destruction of tissues, including the periodontal ligament (PDL), cementum and bone, can cause loss of teeth as a result of periodontitis [1]. Key challenges in regeneration of attachment include (i) understanding degradation of the tissues associated with disease progression and (ii) regeneration of the interfaces that bind the oral tissues together. Due to the difficulty in obtaining well-preserved block sections of human periodontal tissues, including alveolar bone, PDL and cementum, this work addresses the first challenge by defining an animal model and establishing comparisons between the physical properties of rat and human cementum, root dentin and the cementum–dentin interface. Subsequently, periodontitis can be induced and the sequential degeneration of structure, chemical composition and mechanical properties of periodontal tissues in the animal model can be studied. For the second challenge, tissue engineering can be used to create novel scaffolds.

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Rats are considered to be good experimental models because the periodontal anatomy of a rat molar is very similar to that of a human [2]. Additionally the genetic, clinical radiographic and histological aspects of the rat periodontium are similar to the human periodontium [2,3]. The similarities published to date include the cementum structure [4–7], accumulation of significant amounts of cementum at the apical end with age, and localization of proteoglycans within cementum, dentin [5,6,8–12] and the cementum–dentin interface [13]. Although there is some information about cementum structure and its attachment to root dentin [5,6], little is known about the correlation between structure, chemical composition and mechanical properties of cementum and the cementum–dentin interface with root dentin in rat molars.

The objective of this study was to compare the structure, defined by collagen fiber orientation using histology and atomic force microscopy (AFM); chemical composition, defined by spatial distribution of organic (C–H stretch at 2940 cm^{-1}) and inorganic (PO_4^{3-} ν_1 mode at 960 cm^{-1}) contents; and the elastic modulus values of human and rat cementums and the respective cementum–dentin interfaces taken from physiologically equivalent age groups. The structure was studied using histology, atomic force microscopy (AFM), and micro-X-ray computed tomography (MicroXCT™). The site-specific elastic modulus values were determined using AFM-based nanoindentation technique (Triboscope, Hysitron, Minneapolis, MN). Some comparisons were made with previously published results using human specimens especially with regards to the human cementum–dentin junction (CDJ) [14].

2. Materials and methods

2.1. Specimen preparation for AFM, AFM-based nanoindentation and Raman microspectroscopy

2.1.1. Human specimens

Mandibular molars from 40- to 55-year-old males ($n = 6$) requiring extractions as a part of dental treatment were collected following a protocol approved by the UCSF Committee on Human Research. The teeth were sterilized using 0.31 Mrad of γ-radiation [15]. Three-millimeter-thick transversely cut blocks were taken from the apical third of the root (Fig. 1a). The blocks were mounted on AFM steel stubs (Ted Pella Inc., Redding, CA) using cyanoacrylate adhesive (MDS Adhesive QX-4, MDS Products Inc., Anaheim, CA) and ultrasectioned as described below.

2.1.2. Rat specimens

Male RA3 Sprague–Dawley rats, 9–12 months old ($n = 5$), physiologically equivalent to 40- to 55-year-old human males, were obtained using animal tissue transfer according to guidelines by Committee on Animal Research, UCSF. Due to size limitations of rat teeth, the rat mandibles were hemisected, and the half-mandibles with teeth intact (Fig. 1b) were used. Three-millimeter-thick transverse sectioned blocks were prepared from half-mandibles using a low-speed saw (Isomet, Buehler, Lake Bluff, IL). The specimen blocks were mounted on AFM steel stubs (Fig. 1c) using cyanoacrylate adhesive and were ultrasectioned.

All specimens were ultrasectioned with an ultramicrotome (Ultracut E, Reichert-Jung, Vienna, Austria) using a diamond knife (Micro Star Technologies, Huntsville, TX), as previously described [16]. The specimens were ultrasectioned until the cementum in the apical third (Fig. 1a) of the rat molar (Fig. 1c) was exposed. Ultrasectioning was used because it has been shown to be an effective method [16] for creating the relatively flat surface necessary for determining structure, chemical composition and mechanical properties using an AFM, Raman microspectroscopy and AFM-based nanoindentation.

2.2. Specimen preparation for histology

The transversely cut human mandibular molars and the other halves of the rat mandibles were stored in 10% neutral buffered formalin for 2 weeks followed by end-stage decalcification [17] using Cal-EX II decalcifying solution (Fisher Scientific, Fair Lafwn, NJ). The specimens were dehydrated with 80%, 95% and 100% Flex alcohol (Richard-Allan Scientific, Kalamazoo, MI) before being embedded in paraffin (Tissue Prep-II, Fisher Scientific, Fair Lawn, NJ) and sectioned on a rotary microtome (Reichert-Jung Biocut, Vienna, Austria) using disposable steel blades (TBF™ Inc., Shur/Sharp™, Fisher Scientific, Fair Lawn, NJ). The paraffin serial sections were mounted on Superfrost Plus microscope slides (Fisher Scientific, Fair Lawn, NJ) deparaffinized with xylene followed by staining with Sirius red F3B (C.I. 35782) and picric acid (American MasterTech Scientific Co., Lodi, CA). 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 software (Media Cybernetics Inc., Silver Spring, MD). Polarized light was used to enhance birefringence of collagen stained with picrosirius red [18], thus illustrating collagen fiber orientation. A similar staining procedure was implemented for sections taken from human molars.

2.3. Specimen preparation for micro-X-ray tomography

Ground sections, 200 μm thick, were cut longitudinally from hemisected rat mandibles using a low-speed saw (Isomet, Buehler, Lake Bluff, IL). The sections were thinned to ~150 μm by sequential polishing using silicon carbide grit of sizes 1200 and 2400 (Buehler) followed by fine polishing using a diamond suspension slurry of grades 6, 3, 1 and 0.25 μm (Buehler). The specimens were ultrasonicated for 10 s between polishing steps to remove any abrasives. An identical procedure was used to make 150-μm-thick longitudinal specimens from human teeth.