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Proteomic analysis of human dental cementum and alveolar bone

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ABSTRACT

Dental cementum (DC) is a bone-like tissue covering the tooth root and responsible for attaching the tooth to the alveolar bone (AB) via the periodontal ligament (PDL). Studies have unsuccessfully tried to identify factors specific to DC versus AB, in an effort to better understand DC development and regeneration. The present study aimed to use matched human DC and AB samples (n = 7) to generate their proteomes for comparative analysis. Bone samples were harvested from tooth extraction sites, whereas DC samples were obtained from the apical root portion of extracted third molars. Samples were denatured, followed by protein extraction reduction, alkylation and digestion for analysis by nanoAcquity HPLC system and LTQ-FT Ultra. Data analysis demonstrated that a total of 318 proteins were identified in AB and DC. In addition to shared proteins between these tissues, 105 and 83 proteins exclusive to AB or DC were identified, respectively. This is the first report analyzing the proteomic composition of human DC matrix and identifying putative unique and enriched proteins in comparison to alveolar bone. These findings may provide novel insights into developmental differences between DC and AB, and identify candidate biomarkers that may lead to more efficient and predictable therapies for periodontal regeneration.

Biological significance

Periodontal disease is a highly prevalent disease affecting the world population, which involves breakdown of the tooth supporting tissues, the periodontal ligament, alveolar bone, and dental cementum. The lack of knowledge on specific factors that differentiate alveolar bone and dental cementum limits the development of more efficient and predictable reconstructive therapies. In order to better understand cementum development and potentially identify factors to improve therapeutic outcomes, we took the unique approach of using matched patient samples of dental cementum and alveolar bone to generate and compare a proteome list for each tissue. A potential biomarker for dental cementum was identified, superoxide dismutase 3 (SOD3), which is found in cementum and cementum-associated cells in mouse, pig, and human tissues. These findings may provide novel insights into developmental differences between alveolar bone and dental cementum, and represent the basis for improved and more predictable therapies.

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1. Introduction

Dental cementum (DC) is a mineralized tissue covering the tooth root, critical for anchoring the tooth to the surrounding alveolar bone (AB) via the periodontal ligament (PDL) (Fig. 1A) [1–3]. DC and AB share a common progenitor cell population in the ectomesenchymal dental follicle, and DC is often described as bone-like, though questions remain whether cementoblasts are merely positional osteoblasts [4,5]. Despite many similarities in morphology and matrix composition, these two mineralized tissues differ in several important respects. Unlike bone, DC is avascular, non-innervated, and grows by apposition with no significant role for turnover or remodeling [1,2]. The process of cementogenesis remains poorly understood at present, though key developmental differences in DC versus AB have been identified through knock-out mouse approaches [2,6–8]. Additionally, AB and DC may respond quite differently to therapeutic interventions in cases where periodontal tissues are lost as a consequence of disease; AB repair occurs more rapidly and predictably, while cementum regeneration is often difficult and unpredictable [9].

Although it is well established that there is overlap between extracellular matrices (ECM) of DC and AB, it has been hypothesized that each matrix contains unique proteins that may provide insight as to their physiologic differences. For both tissues, type I collagen is the primary ECM component, with the remaining organic matrix being composed of varying amounts of noncollagenous proteins (NCPs). These include proteoglycans (e.g., versican, decorin, and biglycan), glycoproteins that are often phosphorylated and sulfated (e.g., osteonectin and arginine–glycine–aspartic acid (RGD) integrin-binding proteins), and gamma-carboxyglutamic acid (gla)-containing proteins (e.g., matrix gla protein, protein S, and osteocalcin). Together, these proteins most likely participate in regulation of cell metabolism, matrix deposition and mineralization, and may contribute to determining the structure and biomechanical properties of the tissue [10,11]. However, the relationship of NCPs to the collagenous framework, the significance of their patterns of distribution, and, particularly, the function of the individual proteins in the presence of various other matrix constituents remain to be determined, and therefore, it is critical to further understand matrix composition of these two mineralized tissues.

The goal of this study was to identify putative unique candidate markers in each tissue using a comparative proteomic analysis of human DC and AB. We hypothesized that physiologic differences and unique development regulation of DC versus AB would be reflected by selectively expressed and unique proteomic profiles for these two tissues. A more comprehensive understanding of the physiology and ECM composition of these two tissues is expected to contribute to more predictable and reliable regenerative approaches.

2. Materials and methods

2.1. Human subjects and sample collection

Dental cementum (DC) and alveolar bone (AB) were harvested from seven clinically healthy human subjects (5 females and 2 males) ranging from 20 to 30 years old. Additional inclusion criteria were a minimum of three semi-included or erupted functional third molars presenting a completely formed root, where extraction was clinically indicated. Human subject studies were approved by the UNICAMP School of Dentistry IRB (008/2011), and all subjects provided a signed written consent, in compliance with the World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects. Following tooth extraction, soft connective tissues adhering to the tooth surfaces were carefully scraped off using a sterile curette, and discarded. Teeth were rinsed in sterile phosphate buffered saline (PBS) several times and DC samples were collected from the apical region of the root using a curette under stereomicroscope. AB fragments were collected from the tooth extraction sites when osteotomy was indicated. After several rinses in PBS to remove potential contaminants, DC and AB samples were ground using a chisel and stored in sterile PBS at −80 °C.

2.2. Sample preparation

Thawed DC and AB samples were denatured by incubation in 100 μL of 0.2% of *Rapi*Gest™ (Waters Corporation, Mildford, MA, USA) and vortexed for 5 min, followed by agitation with 0.5 mm zircon/silica beads in a Mini BeadBeaterTM (Marconi, Piracicaba, SP, Brazil) for 1 min. Homogenized samples were then incubated at 99 °C for 5 min, cooled to room temperature, centrifuged for 1 min at 13,000 rpm to pellet undigested solids, and supernatants were transferred to clean tubes for total protein concentration determination by Bradford's method, using bovine serum albumin (BSA) as a standard. Extracted proteins were reduced by incubation in 5 mM dithiothreitol at 60 °C for 30 min, alkylated by incubation in 15 mM iodoacetamide at room temperature for 30 min, and finally digested with sequencing grade-modified trypsin (Promega, Madison, WI, USA) at 37 °C for 3 h, followed by centrifugation at 14,000 rpm for 10 min. Supernatants were lyophilized and stored at −80 °C prior to analysis.

2.3. Liquid chromatography-high resolution mass spectrometry (LC-MS/MS) analysis

Tryptic peptide mixture samples were reconstituted in a 2% acetonitrile/0.1% formic acid solution, and subsequently applied in cartridges of solid phase extraction by ion exchange (MCX, Waters — Milford, MA, USA) for exclusion of non-ionized small molecules and impurities, following the manufacturer's instructions. Eluents were dried and reconstituted in a 2% acetonitrile/0.1% formic acid solution to a final concentration of 0.4 μg/μL. For nano-LC-MS/MS, 5 μL of each resulting peptide mixture was analyzed in triplicate on a high resolution and high accuracy LTQ-FT Ultra (Thermo Scientific, San Jose, CA, USA) coupled to a nanoAcquity HPLC system (Waters, Milford, MA, USA). Peptides were separated by a 2–90% acetonitrile gradient in 0.1% formic acid using a capillary column prepared and packaged in-house. A laser-puller was used to obtain the tip of the column from a silica capillary of 75 μm of internal diameter and 30 cm in length. The capillary column was packed using reverse phase Jupiter C-12 particles (Phenomenex, Torrance, CA, USA). The nanoelectrospray voltage was set to 2.2 kV and