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Global proteome profiling of dental cementum under experimentally-induced apposition

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ABSTRACT

Dental cementum (DC) covers the tooth root and has important functions in tooth attachment and position. DC can be lost to disease, and regeneration is currently unpredictable due to limited understanding of DC formation. This study used a model of experimentally-induced apposition (EIA) in mice to identify proteins associated with new DC formation. Mandibular first molars were induced to super-erupt for 6 and 21 days after extracting opposing maxillary molars. Decalcified and formalin-fixed paraffin-embedded mandible sections were prepared for laser capture microdissection. Microdissected protein extracts were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), and the data submitted to repeated measure ANOVA test (RM-ANOVA, alpha = 5%). A total of 519 proteins were identified, with 97 (18.6%) proteins found exclusively in EIA sites and 50 (9.6%) proteins exclusively expressed in control sites. Fifty six (10.7%) proteins were differentially regulated by RM-ANOVA ($p < 0.05$), with 24 regulated by the exclusive effect of EIA (12 proteins) or the interaction between EIA and time (12 proteins), including serpin 1a, procollagen C-endopeptidase enhancer, tenascin X (TNX), and asporin (ASPN). In conclusion, proteomic analysis demonstrated significantly altered protein profile in DC under EIA, providing new insights on DC biology and potential candidates for tissue engineering applications.
Significance: Dental cementum (DC) is a mineralized tissue that covers the tooth root surface and has important functions in tooth attachment and position. DC and other periodontal tissues can be lost to disease, and regeneration is currently unpredictable due to lack of understanding of DC formation. This study used a model of experimentally-induced apposition (EIA) in mice to promote new cementum formation, followed by laser capture microdissection (LCM) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) proteomic analysis. This approach identified proteins associated with new cementum formation that may be targets for promoting cementum regeneration.

1. Introduction

Dental cementum (DC) is a mineralized tissue that covers the tooth root surface and is part of the periodontal attachment complex. DC is present in human teeth in two primary types, acellular and cellular cementum. Acellular cementum anchors collagen fibers from the periodontal ligament (PDL), promoting attachment to the surrounding alveolar bone (AB). The apical cellular cementum adjusts the post-eruptive tooth position. DC is produced by cementoblasts, derived from the differentiation of ectomesenchymal cells from the dental follicle [1,2]. During cementogenesis of the cellular type of DC, some cementoblasts are entrapped within the cementoid and become cementocytes, residents of the DC that dwell within lacunae and feature dendritic processes within a lacunocanalicular system, similar to osteocytes of the bone [3]. While it is now understood that osteocytes modulate bone homeostasis and remodeling, act as mechanical sensors, and play a role in endocrine regulation of mineral metabolism [4], it remains unclear whether cementocytes play an active role in either cementum formation or repair.

Like bone, the DC extracellular matrix (ECM) is primarily composed of collagens (predominantly type I and smaller amounts of types III, IV, V, XI, and XII), several non-collagenous proteins thought to regulate mineralization, such as bone sialoprotein (BSP) and osteopontin (OPN), and proteoglycans, including biglycan (BGN) and decorin (DCN) [1,5]. Unlike bone, DC is avascular, non-innervated, and grows by apposition with no physiological role for remodeling or turnover. While it is known that DC regeneration is possible, current clinical strategies to regenerate periodontal tissues often lack a biologic basis for

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treatment and have unpredictable outcomes with limited regeneration, especially for DC [6]. Studies focusing on transgenic animals have identified novel regulators of cementogenesis [7–9]. Comparative proteomic analysis of human DC vs. alveolar bone (AB) identified differentially expressed proteins that may be associated with physiological differences between the two tissues [10].

In order to identify factors involved in neocementogenesis, or new cementum formation, that may be novel targets for cementum regeneration, we used a previously established model of mouse molar supereruption [11,12]. Experimentally-induced apposition (EIA) in mandibular molars of mice, by extraction of maxillary molars, was shown to generate new DC formation. By using laser capture microdissection (LCM) on EIA and control tissues, we harvested DC and performed proteomic profiling by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The resulting analysis identified significantly differentially expressed proteins resulting from EIA, as well as evidence for cementocyte cell activity.

2. Materials and methods

2.1. Animal model and histological processing

Animals were housed within the University of Campinas animal facility at the Piracicaba Dental School, and all experimental procedures were approved by the University Committee for Ethics in Animal Research (Protocol # 2160-1). Male Swiss Webster mice (35 days of age) were kept in plastic cages on a 12-hour light/dark cycle at 25 to 30 °C, with rodent chow and tap water ad libitum. To promote experimentally induced apposition (EIA) of DC, we used a previously verified mouse model wherein extraction of maxillary molars removed mandibular molars from occlusion (i.e. they become unloaded), promoting increased appositional growth of cellular DC on the tooth apex [11,12]. The experimental side (right or left) was chosen randomly, and first and second maxillary molars were extracted while the mouse was under anesthesia (Fig. 1A). The contralateral side was used as the untreated control. Experimental groups were divided as follows: 1) control at 6 days after surgery (control-6D) (n = 7); 2) control at 21 days after surgery (control-21D) (n = 7); 3) EIA at 6 days after tooth extraction (EIA-6D) (n = 7); and 4) EIA at 21 days after tooth extraction (EIA-21D) (n = 7). Following euthanasia, mandibles were dissected, fixed in 10% Protocol® buffered formalin (Fisher Diagnostics, USA), and processed for histology according to a protocol previously described for laser capture microdissection (LCM) of decalcified and formalin-fixed paraffin-embedded (FFPE) samples [13]. Longitudinal 5 μm thick sections of the mandibular first molars were obtained and mounted onto PEN membrane glass slides (Applied Biosystems, USA). Sections were deparaffinized in two changes of xylene for 2 and 5 min respectively, air dried for 5 min, and then immediately microdissected.

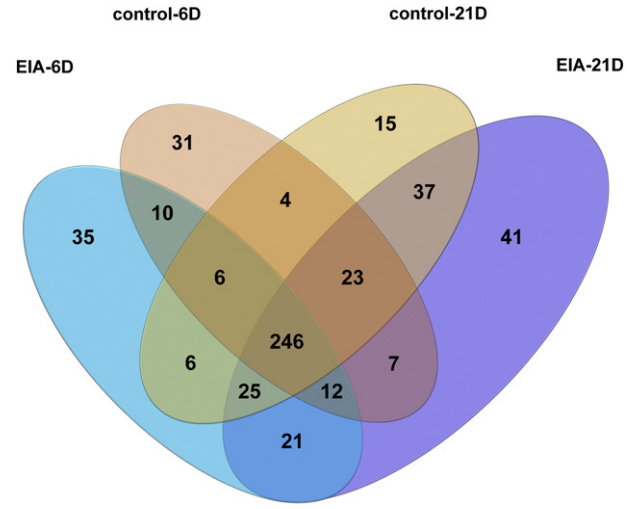

Fig. 2. Proteomic profile of dental cementum. Area-proportional Venn diagram showing the distribution of the total 519 proteins identified in dental cementum (DC) under experimentally-induced apposition for 6 and 21 days (EIA groups), as well as respective control groups at the same time points. Proteins were considered as an identified protein when detected in, at least, one of the seven individual samples. The number of exclusive proteins to each group and shared proteins in every intersection of the diagram are shown.

2.2. Fluorescence microscopy

To verify DC apposition in EIA experimental groups, animals were administered fluorochromes to label mineralizing surfaces. Five animals were given two intraperitoneal injections of calcein (20 mg/kg) at 24 h and 17 days, and one injection of tetracycline (20 mg/kg) at 9 days after tooth extraction. At day 21 post-surgery, mandibles were harvested, fixed in 10% formalin solution for 48 h, rinsed in water for 72 h, and

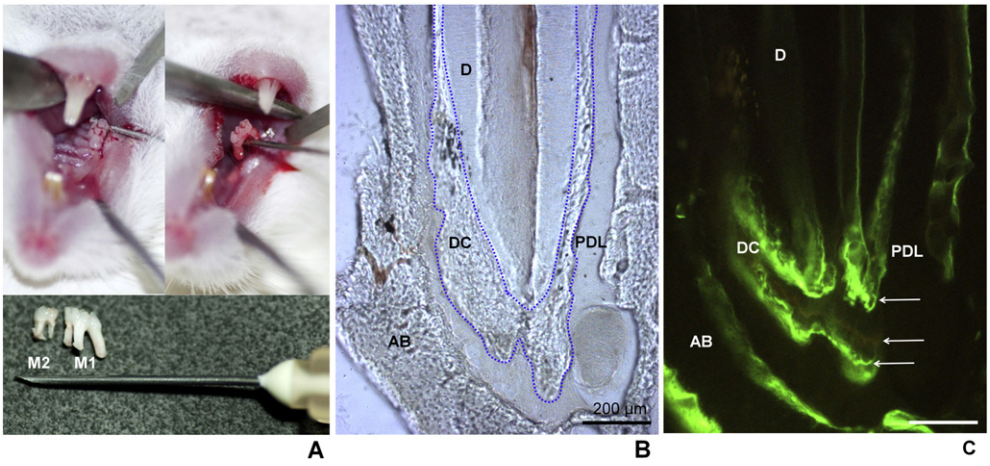

Fig. 1. Experimentally-induced dental cementum apposition model in mouse. (A) Extraction of first (M1) and second (M2) maxillary molars. The antagonist lower molars were kept out of occlusion, inducing super-eruption and apposition of dental cementum (DC) and alveolar bone (AB). (B) Light and (C) fluorescence microscopy images of a longitudinal section of the mesial root of the first mandibular molar. Blue dotted lines in B indicate the DC-dentin (D) border. The white arrows in panel C point to the fluorescent labels formed by the incorporation of fluorochrome markers during DC apposition. Calcein (administered at 24 h and 17 days after surgery) is observed as two intense green lines, while tetracycline (administered 9 days after surgery) is observed as the faint yellow line between the tetracycline labels.