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Complex cellular responses to tooth wear in rodent molar

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ABSTRACT

The arrangement and roles of the odontoblast and its process in sensing and responding to injuries such as tooth wear are incompletely understood. Evidence is presented that dentine exposure by tooth wear triggers structural and functional changes that aim to maintain tooth integrity.

Mandibular first molars from freshly culled 8 week Wistar rats were prepared for light microscopy ground-sections ($n = 6$), or fixed in 4% paraformaldehyde, decalcified in 17% EDTA, sectioned and stained with antibodies to cyto-skeletal proteins (vimentin (vim), α-tubulin (tub) and α-actin), cellular homeostatic elements (sodium potassium ATPase (NaK-ATPase) and sodium hydrogen exchanger (NHE-1)), and sensory nerve fibres (CGRP) ($n = 10$) for fluorescence microscopy of worn and unworn regions of the mesial cusp.

Immunoreactivity (IR) to vim, actin, NaK-ATPase and CGRP was confined to the pulpal third of odontoblast processes (OPs). IR to tub and nhe-1 was expressed by OPs in full dentine thickness. In areas associated with dentine exposure, the tubules contained no OPs. In regions with intact dentine, odontoblasts were arranged in a single cell layer and easily distinguished from the sub-odontoblast cells. In regions with open tubules, the odontoblasts were in stratified or pseudo-stratified in arrangement.

Differences in structural antibody expression suggest a previously unreported heterogeneity of the odontoblast population and variations in different regions of the OP. This combined with differences in OPs extension and pulp cellular arrangement in worn and unworn regions suggests active and dynamic cellular responses to the opening of dentinal tubules by tooth wear.

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1. Introduction

The dental pulp share intimate spatial and functional relationships with dentine, and is commonly described within a pulp-dentine complex (Yu & Abbott, 2007). Odontoblasts (Ods) perform a range of formative, supportive, sensory and defensive functions throughout life (Ricucci, Loghin, Lin, Spångberg, & Tay, 2014), and the comparison of regions within the same tooth that have been subjected to or spared from injury provides one means of understanding important cellular and hard-tissue responses.

Recognised age-related changes in the pulp-dentine complex including reduced cellularity, particularly within the odontoblast (Od) and subodontoblast (Sod) layers (Morse, 1991; Murray, Stanley, Matthews, Sloan, & Smith, 2002), and changing in volume, structure and permeability of dentine (Moses, Butler, & Qin, 2006; Tjäderhane, Carrilho, Breschi, Tay, & Pashley, 2009). Much understanding has derived from rodent molar studies, which have shown a reduction in odontoblast size and cytoplasmic volume with age (Lovschall, Fejerskov, & Josephsen, 2002), along with physiological occlusal tooth wear, which is apparent within 4 weeks (Schour & Massler, 1949) and consequent hard and soft tissue changes (Kawashima, Wongyaofa, Suzuki, Kawanishi, & Suda, 2006; Lovschall et al., 2002), including reactionary dentine deposition beneath worn cusp tips (Moses et al., 2006).

Dentine exposure by cavity preparation has revealed a reduction of Od numbers, probably resulting from apoptosis (Kitamura, Kimura, Nakayama, Toyoshima, & Terashita, 2001; Murray et al., 2000), but with newly differentiated Od-like cells appearing within 7 days (Ohshima, 1990). Cellular responses may be age-related, since the presence of dendritic like cells in the sub-odontoblast cell layer, with processes extending into the predentine is more prominent after cavity preparation in aged than young rats (Kawagishi, Nakakura-Ohshima, Nomura, & Ohshima, 2006).

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The degree of odontoblast process (OP) extension within dentine remain contentious (Luukko, Kettunen, Fristad, & Berggreen, 2011), with suggestion their limitation to the inner third of dentine (Byers & Sugaya, 1995; Carda & Peydro, 2006; Goracci, Mori, & Baldi, 1999; Yoshiba, Yoshiba, Ejiri, Iwaku, & Ozawa, 2002), whilst others have demonstrated OPs reaching the dentino-enamel junction (DEJ) (Gunji & Kobayashi, 1983; Kagayama et al., 1999; Sigal, Aubin, & Ten Cate, 1985; Sigal, Aubin, Ten Cate, & Pitaru, 1984; Tsuchlya, Sasano, Kagayama, & Watanabe, 2002). In the rat molar, it has been reported that the OPs fully traverse the dentine throughout life in cusp areas, while in the cervical regions, they retract to the inner third as teeth mature (Tsuchlya et al., 2002). In previous work, we have demonstrated the extension of OPs to the rodent incisor DEJ with regional variations and complex branching along their path, especially near the DEJ (Mahdee, Alhelal, Eastham, Whitworth, & Gillespie, 2015).

The arrangement and roles of the Ob and its process in sensing and responding to injuries such as tooth wear are incompletely understood. This report presents evidence from the rat molar that dentine-exposing tooth wear triggers a discrete and thitherto undescribed series of structural and functional changes in that aim to protect deep connective tissues from the oral environment.

2. Methods

In order to investigate tooth changes associated with dentine exposure due to the wear process, this study used ground sections to characterise hard tissue changes and decalcified sections with immunohistochemistry to identify pulp cellular differences.

Ground sections: six lower first molars were extracted from freshly culled male Wistar rats (age 8 weeks; weight 240–300 g). Ground sections (100–105 μm thickness) of the largest, mesial cusp, were examined by light microscopy using objectives ×20 and ×40 to identify variations in tubular arrangement associated with wear process.

Immuno-fluorescence technique: ten lower first molars were extracted as previously, with immediate fixation of the crowns in 4% paraformaldehyde in PBS for 24 h at 4 °C, demineralisation in 17% EDTA (pH 7.4) for 4–6 weeks at 37 °C, washing and incubation in graded sucrose solutions (10%, 20%, 30%) for 24 h each at 4 °C for cryoprotection. Specimens were snap frozen in liquid nitrogen, and stored at −80 °C. During sectioning, sagittal sections were taken parallel to the long axis of the tooth until the three buccal cusps were visible. From this point 20–30 slides with 10 μm thickness were obtained from each tooth in order to standardize the sections to be within the orientation of the odontoblast processes.

The immunofluorescence staining procedure (Eastham, Stephenson, Korstanje, & Gillespie, 2015) was performed as follows: sections were divided randomly into 4 staining groups (approximately 50 slides for each group) and stained by the following combination: monoclonal anti-vimentin structural protein (vim) (mouse 1:5000, BioGenex cat# MU074-UC, UK), accompanied with one of the following:: monoclonal anti-α smooth muscle actin (actin) (rabbit 1:200, Abcam cat# ab32575, UK) detecting microfilament structural protein, monoclonal anti-NaK-ATPase enzyme (rabbit 1:500, Abcam cat# ab76020, UK), polyclonal anti-α tubulin (tub) (rabbit, 1:1000, Gene Tex, cat# GTX102078, UK) to detect the tubulin structural protein, and polyclonal sodium hydrogen exchanger-1 (NHE-1) (rabbit, 1:200, Santa Cruz Biotechnology,cat# sc-28758, UK). Both NaK-ATPase and NHE-1 are enzymes present in the cell membrane and they were used in the present study to detect the cell membrane of the odontoblast processes. One extra staining group used the following antibody combination: monoclonal anti-calcitonin gene-related peptide (CGRP) (mouse 1:500, Santa Cruz Biotechnology, cat# sc-57053, UK) for afferent sensory nerve fibre detection combined with the polyclonal anti-α tubulin. These antibodies have been characterised by the manufacturer and have been used in published studies (see respective manufacturer's data sheets). The primary antibody combination was applied to each section before incubating slides in a humid atmosphere at 4 °C for 24 h. The following day, slides were washed in a three stage cycle (TBS, TBS-T, and TBS) for 20 min each. The secondary antibodies; donkey anti-mouse Alexa Fluor 488 (Molecular Probes®, Invitrogen) and donkey anti-rabbit IgG, Alexa Fluor 594 (Molecular Probes®, Invitrogen), were applied in accordance to the species of primary antibody that had been used and incubated in humidifier for one hour at room temperature. The slides were then washed again in a three stage cycle (TBS, TBS-T, and TBS) for 20 min each, before applying Vectashield hard set mounting medium with DAPI (nucleic acid molecular probe stain) (Vector Laboratories Inc, Burlingame) and placing cover slips.

Control samples were included. The slides were either incubated with PBS instead of the primary antibodies, before staining with the secondary antibodies, or incubated with PBS only.

In order to standardize the region of interest for all the teeth, this study examined only the mesial cusp of the first mandibular molar.The stained sections were examined through ×10, ×20, and ×60 magnification with an Olympus BX61 microscope (Olympus Corporation, Tokyo Japan), using Alexa Fluor 488 and 594 fluorochromes detected via the microscope light source and dichroic mirror, to split excitation and emission light wavelengths. Representative images were captured with a microscope-mounted Olympus XM10 monochrome camera and examined using ImageJ software (Java-based image processing program—National Institute of Health (USA)). For each of the objectives the absolute magnification was determined using a stage graticule. The calibrations using this method were shown in each image. Approximately 50 slides were examined for each group to confirm the accuracy and consistency of the staining technique and to reveal consistent staining phenomena (Gillespie, Markerink-van Ittersum, & De Vente, 2006).

3. Results

In order to identify time dependent changes in the mesial cusp as a model for tooth wear the following descriptions of tooth wear were used (Fig. 1):

Stage 0 (S_0) tooth regions under unworn dentine surface (lateral and central walls of the mesial cusp).

Stage 1 (S_1) tooth regions close to worn dentine surface (at the angle of the cusp from the unworn cusp side).

Stage 2 (S_2) tooth regions of minor worn dentine surface (near the angle of the cusp from the worn cusp side).

Stage 3 (S_3) tooth regions of severe worn dentine surface.

All the ground sections observed in this study showed worn occlusal dentine associated with an underlying area of tertiary dentine close to the pulp space (Fig. 1 big panel). In S_0 regions, the complexity of the dentinal tubules could easily be recognized in the outer dentine with abundant lateral and terminal branching (Fig. 1S_0). The terminal branching seemed to terminate within the DEJ. A similar pattern of the dentinal tubules is also illustrated in S1 region which is located close to the dentine worn surface at the cusp angle (Fig. 1S_1–S_2). In the same image it is apparent that in S_2 region, the dentinal tubules were worn and had lost their outer branching part. In the S_3 region (Fig. 1S_3) the dentinal tubules appeared shorter with the proximity of the worn dentine surface to the pulp space becoming apparent. Considering the region of the tertiary dentine near the pulp (Fig. 1R), there was no continuity of the dentinal tubules between the primary dentine and the newly tissue, which appeared to present tubules with meandering pattern.

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