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Qualifying the lapped enamel surface: A profilometric, electron microscopic and microhardness study using human, bovine and ovine enamel

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ABSTRACT

Objective: When enamel specimens are prepared for erosion and abrasion studies, the assumption is often made that specimens prepared in the same way will have the same baseline surface characteristics. This study aimed to test the null hypothesis that there are no significant differences in baseline surface characteristics of human, ovine and bovine enamel specimens prepared using the same method.

Design: Twenty enamel slabs were prepared from bovine, human and ovine incisor crowns and polished with 3 μm aluminium oxide paste. Roughness average (Ra), bearing parameters (MR1, MR2, Rpk, Rk, Rvk), surface microhardness and scanning electron microscopy (SEM) were used to compare the different tissues. One way Analysis of Variance (ANOVA) was used to quantitatively compare surface characteristics between tissue types.

Results: Human, bovine and ovine enamel roughness and microhardness were significantly different to one another at baseline ($P < 0.001$); ovine enamel was the roughest and softest, and bovine enamel was the smoothest and hardest. SEM allowed a visual comparison to be made between tissue types, confirming the quantitative data.

Conclusions: Enamel from human, bovine and ovine specimens showed significantly different surface characteristics after lapping and polishing. The null hypothesis is rejected, recognising that the same preparation techniques will not necessarily result in consistent baseline roughness or surface characteristics between tissue types. Surface studies should lap and polish samples with a standardised approach, whilst ensuring that baseline data are recorded for comparison.

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1. Introduction

Obtaining intact human teeth is becoming increasingly difficult, primarily due to increased oral health and a subsequent increase in retention time. Researchers are turning to more accessible and consistent sources as substitutes such as bovine or ovine incisor teeth.[1]

When enamel specimens are prepared for erosion and abrasion studies, the assumption is often made that

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specimens prepared in the same way will have the same baseline surface characteristics. A literature search revealed that most studies fail to report baseline surface characteristics after preparation. For those that do, specimens are inspected relatively superficially under the light microscope for surface defects, or hardness tested in order to select specimens of similar mechanical properties.[1] More recent work using bovine and human enamel reported significant differences between the roughness averages (Ra) of bovine and human enamel at baseline, despite being prepared in the same way.[2] Further, SEM analysis shows that although bovine enamel has a similar microstructure to human enamel, the bovine enamel shows a greater presence of interprismatic substance and an aggregation of 'fibril-like' structures around the prisms.[3] SEM work has also shown that bovine crystallites are larger than human crystallites by a factor of 1.6 (57 nm c.f. 36 nm respectively).[4] SEM analysis of ovine enamel[5] shows that superficial enamel tends to be more irregular than bovine or human enamel (straight columns are disturbed, and crystallites from both interprismatic and the prism cores tend to coalesce). Similar findings were reported by Grine[6] and most recently by O'Brien[7] who observed through optical microscopy that this enamel decussation was more widespread in ovine enamel than human or bovine; it was also reported that enamel tufts frequently observed in human enamel were absent completely from the ovine specimens. Microhardness testing results show similar properties for human and bovine specimens[8] reporting Knoop hardness values between 244 and 337.[9] Only limited data is currently available to facilitate comparison of the microhardness of ovine enamel, which is reportedly around 90% as hard as human specimens.[7]

2. Aim of the study

There is clear potential for consistently lapped and polished enamel samples to yield inconsistent baseline surfaces between species. This study aimed to test the null hypothesis that there are no significant differences in baseline surface characteristics of human, ovine and bovine enamel specimens prepared using the same method.

3. Materials and methods

3.1. *Specimen collection and preparation*

Extracted human lower permanent incisor teeth were collected from the adult Dental Emergency Clinic at Newcastle Dental Hospital, UK between February 2008 and February 2011. The teeth were stored in a 1% sodium p-toluenesulfonylchloramide (Chloramine-T, Sigma–Aldrich, UK) solution and suitable teeth, showing no signs of coronal caries or tooth surface loss were entered into the Newcastle Tissue Bank (Human Tissue Act license number 12534), stored at 4 °C in a fresh solution of Chloramine-T. Consent from the donors was not required on the condition that the specimens could not be used to identify the donor.

Bovine permanent incisor teeth were harvested on two occasions – March 2010 and December 2010 from the same abattoir – Linden Foods, Burradon, Cramlington (Registered Plant Number 2056, Food Standards Agency, Department of Environment, Food and Rural Affairs). The cattle were Beef Shorthorn cattle and were aged approximately 18–20 months.

Ovine permanent incisor teeth were also harvested in March 2010 from the same abattoir. The sheep were North Country Cheviots and were aged approximately 2–3 years. The ovine and bovine incisors were also stored in a 1% Chloramine-T solution at 4 °C.

Twenty bovine, human and ovine incisor crowns were sectioned coronally 1 mm from the cemento-enamel junction (in an incisal direction) using a low-speed water-cooled diamond wheel saw (Testbourne 650 CE, South Bay Technologies Inc., USA).

The crowns were then positioned into individual casting moulds with the labial surface facing down and the sectioned surface perpendicular to the base. They were held in place with sticky wax (Kemdent, Associated dental products Ltd.) and cast in acrylic resin (Bonda, Bondaglass-Voss Ltd.) Once set, the casts were removed from the moulds. The base was ground down using a Metaserv rotary pregrinder at grit size 600 (C200/RB, Metallurgical services Ltd.) to ensure that the relatively flat portion of enamel near the edge of the sample was exposed. The samples were then lapped further on a Logitech PM2A precision lapping and polishing machine (Logitech, Glasgow) to a depth of 100 μm using 3 μm aluminium oxide paste (Kemet, Kent). A depth of 100 μm was chosen to ensure that the prepared surface involved prismatic enamel, and that previous surface effects were minimised. Samples were held onto glass slides using sticky wax, and the slides were in turn held in place using an Edwards vacuum (E-LAB2) at 0.7 MPa. After lapping the samples were rinsed with HBSS and stored in the salt solution face-up in individual vials.

A further sample set from each species was prepared for microhardness testing (8 crowns from each species for microhardness testing).

3.2. *Sample measurement and analysis*

The baseline surfaces were profiled using a stylus profilometer and its associated software (Mitutoyo Surftest SV-2000 and Surfpak-SV Mitutoyo Corp V1.600). The instrument range was 800 μm with a contact force of 4 mN. The stylus was a diamond cone tip held at 90° to the surface, with a 5 μm radius. Average roughness values, and bearing area parameters[2] (Rk, Rvk, Rpk, MR1 and MR2) were recorded 3 times for each sample 0.5 mm apart. Each evaluation length included 5 readings with a 0.3 mm cut-off (1.5 mm total evaluation length, starting within the body acrylic reference layer) and were Gaussian filtered prior to analysis.

For SEM, samples from each tissue subset were isolated, rinsed with distilled water, dried and mounted onto aluminium stubs with Acheson silver DAG (Agar Scientific, U.K.) and then coated with a 15 nm thick layer of gold, using a Polaron SEM coating unit.

The specimens were then examined using an SEM (Stereoscan 240, Cambridge Instruments, Cambridge, U.K.).

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