The Early Erosive and Abrasive Challenge: A Profilometric, Electron Microscopic and Microhardness Study Using Human, Bovine and Ovine Enamel

Keywords

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Authors

Dr James Clark Field *
(BSc(Hons), BDS, MFGDP RCSEng, MFDS RCSEd, CertClinEd, MA(Ed), PhD, MPros RCSEd, FAcadMEd, PFHEA)

Dr Paula Jane Waterhouse *
(BEng(Hons), MSc, PhD)

Dr Matthew John German *
(BDS, FDS RCSEd, PhD, PGCAP)

Address for Correspondence

Dr James Clark Field *

Email: james.field@ncl.ac.uk

* *Newcastle University*

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ABSTRACT

Aim: This study aimed to test the null hypothesis that there are no significant differences in surface characteristics of eroded and abraded human, ovine and bovine enamel. Design: Twenty enamel slabs were prepared from bovine, human and ovine incisor crowns, and randomly assigned to one of 4 treatments: 30 second/4 minute immersion at 1%/6% w/v citric acid. Post-erosion, an oscillatory brush was used for 20 seconds. Roughness parameters, height change and scanning electron microscopy were measured at each stage. Results: Whilst the eroded surface became generally less rough after the abrasive challenge, there were significant tissue differences. Abraded microhardness was significantly increased compared to eroded values ($P < 0.001$), Surface loss was also significantly different between tissue types ($P < 0.001$). Bovine enamel showed similar trends to human enamel but was consistently harder and more resistant to surface change. Ovine enamel displayed little correlation with human enamel.Scanning electron microscopy images showed differences for each tissue that were consistent with the quantitative data. Conclusions: The null hypothesis was rejected: neither bovine nor ovine enamel can be reliably used interchangeably with human enamel for erosion/abrasion studies.

INTRODUCTION

As intact human teeth become increasingly difficult to obtain, and pressures from ethical and regulatory bodies continue to increase, researchers are turning to the use of alternatives for carrying out *in vitro* erosion and abrasion research.[1-3] Whilst the use of bovine enamel is perhaps the most frequent practice, disparate results are common.[4] Further, there are no reported studies on the use of ovine enamel. This paper follows on from previous laboratory studies that report the surface characteristics of human, bovine and ovine enamel after lapping.[5, 6] The previous studies demonstrated the use of bearing parameters, microhardness and scanning electron microscopy (SEM) as a useful series of triangulation tools to reliably qualify and quantify the enamel surface.

The latter study also highlighted the need for a standardised protocol for sample preparation and a baseline recording of surface features. Most significantly, the study also demonstrated a significant difference in characteristics of the lapped enamel surface across tissue types, despite being polished with the same protocol. When lapped with a 3 micron paste, ovine enamel was found to have the roughest and softest surface, whilst bovine enamel was the smoothest and hardest.

Whilst there are clear differences in surface hardness and roughness after lapping, this study aimed to test the null hypothesis that there are no significant differences in surface characteristics and tissue loss, of human, bovine and ovine enamel subjected to an erosive and a subsequent abrasive challenge.

MATERIALS AND METHODS

SPECIMEN COLLECTION AND PREPARATION

Teeth were collected and prepared in line with the previously published protocols.[5, 6]

Extracted human lower permanent incisor teeth were collected from Newcastle Dental Hospital and stored in 1% sodium p-toluenesulfonylchloramide (Chloramine-T) solution. Suitable teeth, showing no signs of coronal caries or tooth surface loss, were entered into the Newcastle University Tissue Bank (Human Tissue Act licence number 12534) and stored in the same solution at 4°C. Bovine permanent incisor teeth were obtained from Linden Foods abattoir, 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 obtained from the same abattoir. The sheep were North County Cheviots and were aged approximately 2-3 years. The bovine and ovine incisors were also stored in 1% Chloramine-T at 4°C.

20 bovine, human and ovine incisor crowns were sectioned axially 1 mm from the cemento-enamel junction (in an incisal direction) using a low-speed water-cooled diamond wheel saw (Testbourne 650 CE). The crowns were 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 and cast in acrylic resin (Bonda). Once set, the base was ground down 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 to a depth of 100 µm using 3 µm aluminium oxide paste. 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 0.7MPa. After lapping the samples were rinsed with HBSS and stored in the salt solution face-up in individual vials.

Two further samples sets from each species were prepared for SEM and microhardness testing (2 crowns from each species for SEM, 8 crowns from each species for microhardness testing).

SAMPLE MEASUREMENT AND ANALYSIS

Samples were measured and analysed in line with the previously published protocols.[5, 6]

The baseline surfaces were profiled using a stylus profilometer (Mitutoyu Surftest SV-500 and Surfpak-SV V1.600). The instrument range was 800 µm with a contact force of 4mN. 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 (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 of the reference layer) and were Gaussian filtered prior to analysis.

For SEM, samples from each tissue subset were rinsed with distilled water, dried and mounted onto aluminium stubs with Acheson silver DAG and then coated with a 15 nm thick layer of gold, using a Polaron SEM coating unit. The samples were then examined using an SEM (Steroscan 240). Images were taken at 3 levels of magnification (approximately x2250, x525 and x125) in order to assess for changes in surface structure and captured with Orion software (version 6.60.6).

Microhardness testing was then carried out on the enamel of the second subset using a Z2.5 hardness tester and associated software, TestXpert V11.02. Measurements were taken at baseline, post-erosion and post-erosion and abrasion. Three readings were taken per tooth on each occasion (n=24 per species), at a spacing of 1 mm down the long axis of the crown. A loading protocol of 100g for 15 seconds was used with a Vickers square indenter.

One way Analysis of Variance (ANOVA) was used to compare baseline surface characteristics between tissue types. All pairwise multiple comparisons were then made using the Holm-Šidák method with a significance level of 0.05 (mean values reported). Where normality failed, a Kruskal-Wallace ANOVA was carried out, and all pairwise multiple comparisons were made using the Tukey test with a significance level of 0.05 (median values reported).

EROSION

Citric acid solutions were formulated at 1% and 6% w/v and the pH was measured using a Thermo Orion 4 Star. After lapping, the samples were randomly assigned to one of 4 treatments (below). All treatments were undertaken at 30° Celsius in order to replicate a temperature similar to that of a human mouth retaining a cooled beverage:

i) 30 second immersion at 1% w/v citric acid

ii) 4 minute immersion at 1% w/v citric acid

iii) 30 second immersion at 6% w/v citric acid

iv) 4 minute immersion at 6% w/v citric acid

ABRASION

Previously eroded samples were stored in a balanced salt solution prior to testing. The samples were air dried and insulation tape was placed across the acrylic reference layer to protect it from any possible abrasive forces *(Figure 1)*. The samples were then abraded using an oscillatory brush Colgate Actibrush™ (model 3418KE) powered by 2 batteries (AAA alkaline 1.5V) was used with a force of 200g with non-fluoridated toothpaste, Euthymol® for 20 seconds on each enamel sample.

Figure 1: Insulation tape placed across the acrylic reference area to protect against toothbrush abrasion.

After treatment the tape was removed, and the samples were rinsed with a balanced salt solution and profiled again (as described previously). The maximum height change in the profile, measured as the lowest point on the profile 0.5-1.0 mm in from the acrylic reference level was also recorded. Subset microhardness testing was carried out following the protocol described above.

After both the erosive and abrasive treatments, the samples were rinsed with a balanced salt solution and profiled again (as described previously). The maximum height change in the profile, measured as the lowest point on the profile 0.5-1.0 mm in from the acrylic reference level was also recorded. A subset of samples were chosen for microhardness and SEM testing. Images were taken at 3 levels of magnification (approximately x2000, x1000 and x50).

ANALYSIS

One way Analysis of Variance (ANOVA) was used to compare eroded and abraded surface characteristics between tissue types. Pairwise multiple comparisons were then made using the Holm-Šidák method with a significance level of 0.05. Where normality failed, a Kruskal-Wallace ANOVA was carried out, and all pairwise multiple comparisons were made using the Tukey test with a significance level of 0.05. Finally, forward stepwise multiple linear regressions were carried out with abraded height change (representing tooth surface loss) as the dependent variable. A Spearman rank order correlation was then carried out between significant independent variables and the dependent variable (tooth surface loss) in order to further qualify the predictor variables. Significance for correlation was set at 0.05.

RESULTS

Human, bovine and ovine roughness averages (Ra) were significantly different to one another at baseline ($P<0.001$) and this data set was reported previously.[6] A summary is presented in Table 1.

A summary of post-erosion parameters by tissue and treatment type is shown in Table 2.

Table 1. Mean roughness and bearing parameters of human, bovine and ovine enamel at baseline, reported previously.[6] Standard deviations are within brackets. Values with differing superscripts are significantly different between tissues.

Tissue	Roughness average (µm)	Peak roughness (µm)	Core roughness (µm)	Valley roughness (µm)	Material ratio of peaks (%)	Material ratio of troughs (%)	Micro hardness
Human	0.15^{a} (0.02)	0.22^{a} (0.07)	0.49^{a} (0.08)	0.26^{a} (0.05)	9^{a} (1)	88^{a} (1)	412^{a} (100)
Bovine	0.13^{b} (0.02)	0.24^{a} (0.08)	0.44^{b} (0.07)	0.20^{b} (0.04)	10^{b} (1)	89^{b} (1)	532^{b} (102)
Ovine	0.19^{c} (0.02)	0.2^{a} (0.1)	0.62^{c} (0.09)	0.30^{c} (0.07)	9^{a} (2)	88^{a} (2)	293^{c} (74)

Table 2. A summary of post-erosion parameters by tissue and treatment type. Mean values are reported and standard deviations are in brackets. Acid tested was citric acid. Key: Ra (roughness average), Rpk (peak roughness), Rk (core roughness), Rvk (valley roughness), MR1 (material ratio of peaks), MR2 (material ratio of troughs), ΔH (maximum height change within the profile).

Tissue	Acid concentration	Immersion time	Ra (μm)	Rpk (μm)	Rk (μm)	Rvk (μm)	MR1 (%)	MR2 (%)	ΔH (μm)	Micro hardness
Human	1%	30 seconds	0.14 (0.03)	0.18 (0.05)	0.49 (0.11)	0.23 (0.06)	9 (1)	89 (2)	1.57 (0.14)	291 (12)
		4 minutes	0.16 (0.01)	0.26 (0.36)	0.51 (0.04)	0.26 (0.03)	9.0 (0.7)	88 (2)	2.02 (0.16)	314 (42)
	6%	30 seconds	0.15 (0.02)	0.16 (0.06)	0.49 (0.07)	0.24 (0.04)	9 (1)	88 (1)	1.80 (0.16)	374 (14)
		4 minutes	0.19 (0.03)	0.21 (0.05)	0.67 (0.12)	0.37 (0.22)	9 (1)	88 (2)	3.59 (0.34)	365 (47)
Bovine	1%	30 seconds	0.13 (0.01)	0.14 (0.02)	0.45 (0.05)	0.22 (0.04)	9 (01)	89 (1)	1.21 (0.30)	377 (31)
		4 minutes	0.12 (0.01)	0.14 (0.02)	0.44 (0.06)	0.19 (0.03)	9 (1)	89 (1)	1.06 (0.15)	441 (27)
	6%	30 seconds	0.12 (0.02)	0.13 (0.03)	0.40 (0.05)	0.19 (0.04)	9 (1)	88 (1)	1.22 (0.25)	528 (23)
		4 minutes	0.14 (0.02)	0.16 (0.04)	0.50 (0.06)	0.26 (0.06)	8.5 (0.8)	88 (1)	2.48 (0.27)	331 (34)
Ovine	1%	30 seconds	0.18 (0.03)	0.18 (0.06)	0.58 (0.12)	0.29 (0.07)	8 (1)	88 (2)	1.72 (0.27)	198 (33)
		4 minutes	0.19 (0.03)	0.25 (0.11)	0.62 (0.09)	0.27 (0.05)	10 (2)	90 (2)	2.48 (0.72)	186 (16)
	6%	30 seconds	0.17 (0.02)	0.17 (0.02)	0.55 (0.05)	0.26 (0.04)	9.2 (0.7)	88 (1)	1.72 (0.43)	273 (16)
		4 minutes	0.17 (0.02)	0.16 (0.03)	0.56 (0.07)	0.30 (0.08)	8 (1)	88 (2)	3.81 (0.89)	147 (34)

Table 3: Post-abrasion parameters by tissue type. Mean values are reported and standard deviations are in brackets. Values with differing superscripts are significantly different between tissues.

Tissue	Roughness average (μm)	Peak roughness (μm)	Core roughness (μm)	Valley roughness (μm)	Material ratio of peaks (%)	Material ratio of troughs (%)	Surface loss (μm)	Micro hardness
Human	0.16^{a} (0.02)	0.18^{a} (0.08)	0.6^{a} (0.1)	0.24^{a} (0.05)	9^{a} (1)	88^{a} (2)	4^{a} (2)	387^{a} (77)
Bovine	0.13^{b} (0.02)	0.13^{b} (0.04)	0.4^{b} (0.1)	0.21^{b} (0.09)	8^{b} (1)	88^{a} (2)	2^{b} (1)	546^{b} (104)
Ovine	0.16^{a} (0.02)	0.17^{a} (0.07)	0.6^{a} (0.1)	0.25^{a} (0.06)	8^{b} (1)	89^{a} (2)	8^{c} (2)	284^{c} (47)

Eroded roughness average (Ra) values were not significantly different to baseline values for this early erosive challenge (P=0.051). However, all tissues were significantly different to one another (P<0.001; Ra mean human 0.158, bovine 0.129, ovine 0.174). Ra was also significantly affected by time (P<0.001; Ra mean 30s 0.148, 4m 0.162) but not concentration (P=0.217; Ra mean 1% 0.153, 6% 0.157).

There were significant interactions between tissue, concentration and time (P=0.007) suggesting that the effects of each factor were not consistent at all combinations. There was a significant interaction between tissue and concentration (P<0.001; the effects of concentration were only significant within bovine and human tissues at longer immersion times), tissue and time (P=0.004; for shorter immersion times at 1% no significant difference between human and bovine P=0.312, at 6% no significant difference between ovine and human P=0.050) and concentration and time (P=0.003; within each concentration, longer exposure times resulted in a more rough surface – within the shorter exposure time, a higher concentration reduced the roughness, whereas the opposite occurred within the longer exposure time).

The proportions of eroded profile peaks (MR1) were significantly different to baseline (P=0.004), with a reduction in the number of profile peaks post-erosion. MR1 was significantly affected by concentration (P=0.035; MR1 mean 1% 9.072, 6% 8.661) but not tissue (P=0.911; MR1 mean human 8.794, bovine 8.890, ovine 8.784) or time (P=0.643; MR1 mean 30s 8.821, 4m 8.911).

The maximum height changes of the eroded profiles were significantly affected by tissue (P<0.001; mean human 2.238, bovine 1.493, ovine 2.433), concentration (P<0.001; mean 1% 1.677, 6% 2.432) and time (P<0.001; mean 30s 1.536, 4m 2.572).

The microhardness of the eroded surfaces were significantly different to baseline (P<0.001; MH base 412.468 vs. eroded 318.799). MH was only significantly affected by tissue type at the extremes, (between bovine and ovine, P=0.011; mean bovine 419.275 vs. ovine 201.096).

Typical SEM images of eroded enamel with 6% citric acid for 4 minutes' duration at high magnification are shown in Figure 2. The images show significantly different erosion patterns on the enamel surface. The eroded human enamel shows considerably more relief than the bovine and ovine profiles, and a characteristic 'keyhole' pattern, with raised areas of inter-prismatic enamel. The ovine enamel appears to show a laminar sheet-like structure with prisms overlapping one another. The bovine enamel appears less regular in form than the ovine or human tissues, displaying a number of pits with raised, rolled edges.

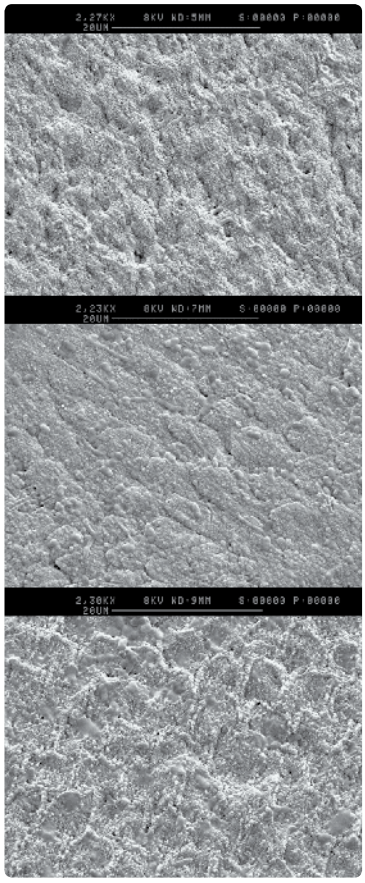

Figure 2: High magnification SEM (approximately 2,250X) of the eroded bovine (top), ovine (centre) and human (bottom) enamel. At high magnification it is possible to more accurately assess the eroded enamel surfaces. The eroded human enamel shows considerably more relief than the bovine and ovine profiles, and a characteristic 'keyhole' pattern, with raised areas of inter-prismatic enamel. The ovine enamel appears to show a laminar sheet-like structure with prisms overlapping one another. The bovine enamel appears less regular in form than the ovine or human tissues, displaying a number of pits with raised, rolled edges.

A summary of post-abrasion parameters by tissue type is shown in Table 3.

Abraded roughness average (Ra) and peak roughness (Rpk) were significantly lower than baseline eroded values (P = 0.002 and P=0.0018 respectively) with significant differences between tissue types (P < 0.001; whilst bovine was the smoothest surface, there was no significant difference between ovine and human values).

Abraded proportion of profile peaks (MR1) were significantly less than baseline eroded values (P < 0.001). MR1 ratios were significantly different between tissue types (P = 0.005; whilst human enamel recorded more profile peaks, there was no significant difference between bovine and ovine values).

Abraded microhardness was significantly increased compared to baseline eroded values (P < 0.001). MH values were significantly different between tissue types (P < 0.001) with bovine enamel recording the hardest enamel (546 VHN) and ovine the softest (284 VHN).

Surface loss was significantly different between tissue types ($P < 0.001$) with ovine enamel recording the largest amount of surface loss (8 µm), and bovine the smallest (2 µm).

Forward stepwise multiple linear regression analysis showed that the eroded profile height change ($P < 0.001$; correlation coefficient 0.652) and eroded microhardness ($P = 0.015$; correlation coefficient -0.76) were found to be significant predictors of tooth tissue loss.

Typical SEM images of eroded and then subsequently abraded human, ovine and bovine enamel at high magnification are shown in Figure 3. With the human enamel, there is a noticable loss of interprismatic enamel and surface roughness in the abraded profile, whilst it retains much the same general surface form. With the ovine enamel, there is a loss of prism ridging and roughness, resulting in a surface with less obvious form. The bovine enamel shows a loss of enamel ridges and surface roughness, whilst retaining much the same general surface form.

DISCUSSION

This experiment was carried out in order to determine differences in the effects of erosive and abrasive processes on human, bovine and ovine enamel. The dental pellicle and the presence of saliva during the erosive or abrasive challenges were unaccounted for; and although this reinforces the simple *in vitro* nature of the experiment, it allows more meaningful conclusions to be drawn about the direct interaction between erosion and abrasion.

Post-abrasion, the enamel surface was generally less rough than the initial eroded surface, displaying a lower proportion of profile peaks (MR1). There are no comparable reports within the literature, with most studies reporting a combination of surface loss and microhardness only.[3] This experiment demonstrated significant loss of the eroded surface post-abrasion. Despite measuring early surface change, a synergistic effect between erosion and abrasion is evident. Eroded ovine enamel demonstrated the greatest surface loss after abrasion (7.9 µm) and bovine enamel demonstrated the least surface loss (2.2 µm). Human enamel loss was in-between at 4.2 µm. Direct comparisons with existing literature are difficult, given the number of treatment parameters within the erosive/abrasive model. Nonetheless a similar amount of tissue loss for bovine incisors has been demonstrated,[7, 8] ranging from 1.3 µm to 3.80 µm. Despite the more aggressive acidic challenges used in the studies (submersion for up to 10 minutes in cola 4 times per day), the samples were protected by pellicle prior to immersion, and were remineralised by natural saliva in situ. Slightly higher bovine tissue loss (3.01 µm) was reported by Levy[9] and despite a more consistent erosive challenge, the abrasive weight applied during abrasion was greater than 1 Kg. Other bovine studies using large or unaccounted loading weights have also reported higher values (up to 7.3 µm) for bovine tissue loss.[10,11] Lower values (up to 1.8 µm) have been

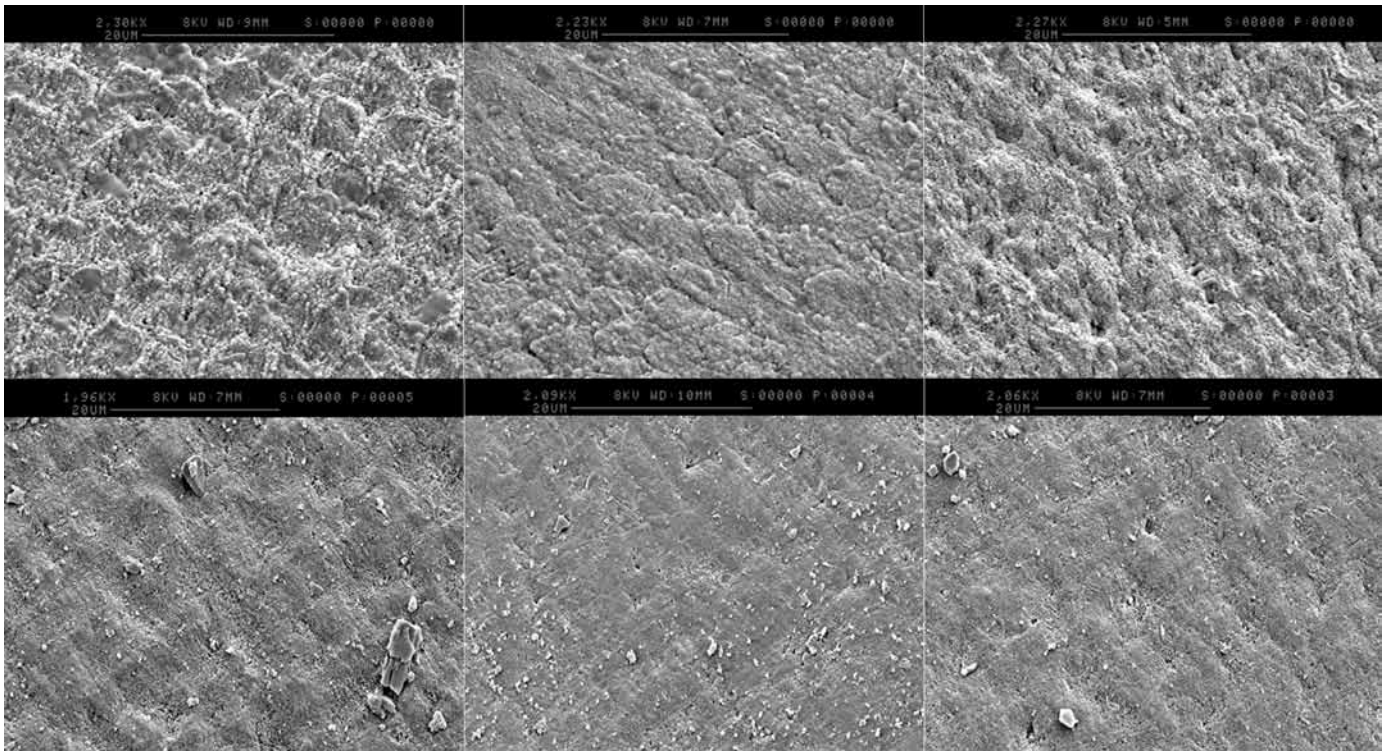

Figure 3: High magnification SEM (approximately 2000X) of the eroded enamel (top) and the subsequently abraded surface (bottom). For human enamel (left), note the loss of interprismatic enamel and surface roughness post-abrasion, whilst retaining much the same general surface form. For ovine enamel (centre) note the loss of prism ridging and surface roughness, resulting in a somewhat amorphous surface. For bovine enamel (right) note the loss of enamel ridges and surface roughness, whilst retaining much the same general surface form.

reported for human enamel surface loss following erosion and abrasion (Hooper *et al*., 2003a); however this was an in situ study using fluoridated toothpaste, and therefore allowed for the presence of saliva and pellicle. Two studies report human tissue loss at significantly higher values (up to 32 µm)[12, 13] and although the abrasive challenges were very similar to this experiment, the erosive challenges were significantly more aggressive (pH 2.3 for up to 12 minutes). Further, the study by Ganss subjected the samples to agitation during erosion which can significantly increase the treatment effect.[14] Finally, the relative enamel abrasivity (REA) of the toothpastes used within studies is often not reported. It has also been shown that whilst REA has a positive correlation with surface loss when the native enamel surface is abraded, there is a negative correlation with surface loss for eroded and then abraded enamel.[15] Clearly the comparison between studies is difficult given the number of treatment parameters and interactions that exist. Hooper[15] concludes that a surface softened layer of enamel is readily removed by most mechanical interactions and so the REA of toothpaste is not relevant. This further reinforces the need for a simpler, standardised, model to study surface change. No data exists regarding ovine tissue loss following erosion and abrasion.

Enamel microhardness was significantly greater following the abrasive challenge. This is a commonly reported finding.[16,17] Vieira[18] describes the eroded and softened enamel layer being abraded away to reveal a harder, less demineralised enamel surface underneath. Between tissues, bovine enamel remained the hardest post-abrasion whilst ovine remained the softest. This pattern mirrors the baseline trends that have been present throughout this series of studies, suggesting that microhardness is a good predictor for surface change. Indeed stepwise regression analysis showed that the only significant predictors of abraded tooth surface loss were eroded microhardness and eroded profile height change. A similar relationship between microhardness and abrasive loss was described by Attin *et al* (1997).

SEM ANALYSIS IN CONJUNCTION WITH OTHER PARAMETERS

Within each tissue type it was possible to see a significant reduction in surface features post-abrasion. This effect was particularly noticeable within the human enamel. Initially (post erosion), human enamel showed significantly more relief. The surface 'form' remained much the same post-abrasion but fine surface details were lost. The same, albeit lessened, effect was evident within the bovine and ovine enamel samples. Unlike the abrasion-only experiment, this degree of surface loss corresponded with a reduction in peak and core roughness, as well as a reduced proportion of peaks. It is purported that on the softened, eroded enamel surface, the action of the toothbrush bristles is potentiated; an action is apparent not just on the fine enamel peaks at the outermost region of the surface, but also the upper two thirds of the surface profile. It is unclear to what degree the toothpaste potentiated these effects, and this warrants further investigation. The apparent surface features identified with SEM correspond well to roughness parameters when measured by profilometry. As such, SEM analysis is confirmed as being useful in order to reinforce profilometric data.

CONCLUSION

In summary, the null hypothesis is rejected: there are highly significant differences in surface characteristics and tissue loss, of human, bovine and ovine enamel subjected to an erosive and a subsequent abrasive challenge. The measurement of roughness parameters, surface microhardness and SEM were confirmed as useful triangulation tools for quantifying and qualifying early surface changes. Microhardness was shown to be a significant predictor for tooth surface loss. Ovine enamel displayed little correlation with human enamel whereas bovine enamel showed similar trends but was consistently harder and more resistant to surface change. Differences in surface features between each tissue at baseline continued to be apparent throughout the erosive and then abrasive experiments. As such, a standard baseline polishing protocol for erosive and abrasive studies does not mean that final surface outcomes in bovine, human and ovine enamel can be directly compared. This must be accounted for when bovine enamel is used as a substitute for human enamel in erosion and abrasion studies.

MANUFACTURERS' DETAILS

- Chloramine-T (Sigma-Aldrich, UK)
- Testbourne saw 650 CE (outh Bay Technologies Inc. USA)
- Sticky wax (Kemdent, Associated Dental Products Ltd.)
- Bonda (Bondaglass-Voss Ltd.)
- Logitech PM2A precision lapping and polishing machine (Logitech, Glasgow)
- Aluminium oxide paste (Kemet, Kent)
- Mitutoyu Surftest SV-500 and Surfpak-SV (Mitutoyo Corp)
- Acheson silver DAG (Agar Scientific, UK)
- Steroscan 240 (Cambridge Instruments, Cambridge, UK)
- Zwick/Roell Z2.5 hardness tester and associated software, TestXpert V11.02 (Zwick testing machines Ltd, Herefordshire)
- Thermo Orion 4 Star (Fisher Scientific, Leicestershire)
- Colgate Actibrush™ model 3418KE (Colgate-Palmolive (UK) Ltd.)
- 2 batteries (Energiser®)
- Euthymol® (Johnson & Johnson, New Jersey, USA)

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