The investigation of enamel subjected to early erosive and abrasive challenges

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For Claire, Caroline, and my Mum and Dad

Abstract

Tooth surface loss is an increasingly recognised clinical problem and so there are an increasing number of *in vitro* studies investigating factors that modify effects at the tooth surface. A literature review found that erosion and abrasion studies often quantify the amount of tooth loss; this means that conditions used to simulate acidic and abrasive challenges are relatively aggressive. There are few attempts to describe or qualify early effects of these challenges. This is necessary in order to gain a greater understanding of the initial mechanisms of tooth surface loss.

A literature review also identified inconsistent reports of surface changes both between human and bovine enamel, and across treatments for erosive and abrasive challenges. No data are reported for ovine enamel. Therefore a simple *in vitro* model was developed in order to measure early surface changes in human, bovine and ovine enamel using profilometry, SEM and microhardness testing. Using these methods the study aimed to investigate the surface effects on enamel of early erosive and abrasive challenges, and to compare the enamel surfaces of human, bovine and ovine enamel.

Results indicated that there were significant differences between the enamel surfaces of each tissue at baseline, and different characteristics were recorded for different methods of polishing. Bovine enamel was often the hardest and the smoothest, demonstrating the least surface loss after the abrasive challenge; ovine enamel was often the softest and the roughest. Recording of bearing area parameters yielded significant differences for a number of surfaces that were not identified through the roughness average alone.

Bovine enamel may be able to substitute for human enamel for the *in vitro* testing of early erosive and abrasive challenges, showing similar surface effects to human enamel. After an erosive challenge, abrasive tooth surface loss could be predicted from a linear combination of the surface microhardness and the maximum height change within the eroded profile.

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Contents

Chapter 1. Introduction

Tooth surface loss (TSL) is an increasingly recognised clinical problem (Barbour and Rees, 2006). Consequently there are an increasing number of *in vitro* studies investigating factors that modify effects at the tooth surface. Often studies aim to demonstrate an amount of tooth surface *loss*; this means that conditions used to simulate acidic and abrasive challenges are relatively aggressive. There are few attempts to describe or qualify early effects of relatively mild erosive and abrasive challenges before surface loss occurs. This is necessary in order to gain a greater understanding of the *initial* mechanisms of tooth surface loss. An *in vitro* study was set-up in order to investigate these early surface changes. Eroded enamel, abraded enamel and abrasion of the eroded enamel surface were investigated using profilometry, scanning electron microscopy and microhardness testing.

Quantified results are often considered fundamental in order to compare laboratory measurements. Surface quality is reported less often, but has the potential to account for particular surface characteristics that determine how *future* loss may progress. This study was designed to include scanning electron microscopy assessment of the enamel surfaces, but also to record a number of profilometric variables not normally used within the biological sciences. Primarily used for the assessment of machined surfaces in engineering, the usefulness of recording the 'bearing parameters' was assessed.

Whilst the collection of intact human teeth for *in vitro* testing becomes more difficult (primarily due to increased oral health, and a subsequent increase in retention times

for teeth) researchers are turning to more accessible and consistent sources as substitutes such as bovine or ovine incisor teeth. This study involved the use of human, bovine and ovine enamel in order to judge whether either or both of these two tissues could be reliably used as a substitute for human enamel in *in vitro* erosive and abrasive studies.

Chapter 2. Literature review

In this chapter the structure of enamel and dentine, particularly that of human and bovine, will be discussed. The concept of tooth surface loss will be introduced followed by factors that influence this process and methods that are used to measure erosive surface changes. Finally, the factors taken into account when planning an *in vitro* model will be discussed.

2.1 Tooth structure

Mammalian teeth comprise a complex union of two highly mineralised tissues, enamel and dentine. Micro-computed-tomography of bovine incisors shows that a layer of wear-resistant enamel coats the remaining bulk of fracture-resistant dentine (Stock *et al*., 2008) (Figure 1). The thickness of mammalian enamel over tooth cusps can range from 0.05 mm in marmosets (Rosenberger, 1978) to 5 mm in some extinct species of ape (Olejniczak *et al*., 2008). The first published work investigating the enamel thickness of 40 human molar teeth reported a range of between 0.5 and 1.5 mm when sections were viewed by light microscopy (Gillings and Buonocore, 1961). A more recent transmission light microscopy study of 69 juvenile unworn molars (Mahoney, 2010) reported similar findings, with enamel thickness often around 1 mm in human molars; there are, however, wide variations over any given tooth crown.

The enamel layer is the most highly calcified and hardest tissue in the human body (Berkovitz *et al*., 1999). Three-dimensional modelling (Wroe *et al*., 2010) has shown that teeth are able to withstand masticatory forces of over 700N in humans (Hagberg,

1987) and 2000N in some nut and seed-eating primates (Lucas, 2004). The main constituent of enamel is calcium apatite (96-98% w/v), either as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) or fluorapatite ($Ca_{10}(PO_4)_6F_2$), and the mineral content of the enamel lessens further away from the tooth surface, as protein and water become more abundant (Hall *et al*., 2000). The underlying dentine is around 70% w/v carbonated apatite with the remainder being mostly collagen and water.

2.1.1 Human enamel microstructure

Microscopically, enamel comprises a series of rods or 'prisms' deposited by the Tomes processes of the advancing ameloblasts (Figure 1). The direction of movement of the ameloblasts cells dictates the prism orientation within mature enamel. The Tomes processes are lost near the surface, resulting in an aprismatic enamel layer, around 100 µm thick (Boyde, 1989). Each prism is about 5 µm wide by 9 µm long, coated with a nanometre-thick layer of enamelin (a secretory peptide used to regulate enamel deposition) and containing millions of individual crystals of hydroxyapatite (Ten Cate, 1994). Pioneering SEM studies of rat incisor amelogenesis showed that the crystals grow into a hexagonal shape (Selvig and Halse, 1972) and fill the space available within each prism. Transmission electron microscopy (TEM) studies also confirm that each crystal of mature human enamel is approximately 70 nm wide and between 25 nm and 40 nm thick (Kerebel *et al*., 1979; Jongebloed *et al*., 1975). Length has been reported to vary between 25 nm and several micrometres; however this measurement seems to be particularly dependent on the method of sectioning and orientation. The prisms typically have a wedge-shaped tail cervically and are often described to have a key-hole shape or fish-scale appearance in occlusal ground sections (Low *et al*., 2008).

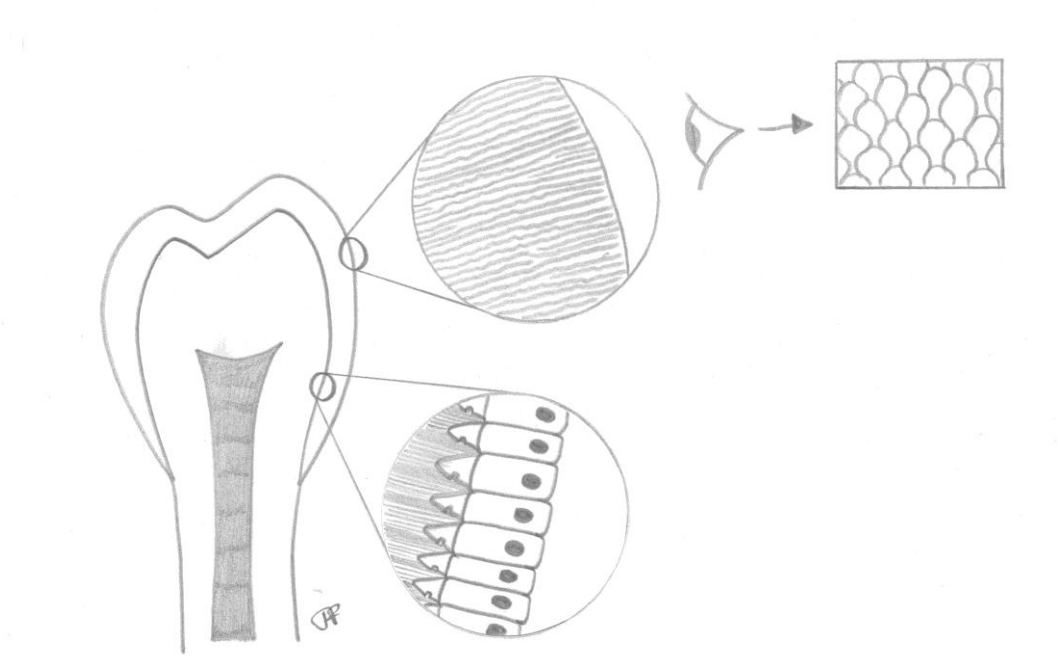


Figure 1 – A stylised drawing of a human premolar tooth, highlighting the orientation of enamel prisms. The lower magnified circle shows the ameloblast cell layer with the triangular Tomes processes depositing both prismatic and inter-prismatic enamel.

They extend from the amelodentinal junction to the outer surface. Enamel has a greater resistance to deformation (it is stiffer) along the axis of the prisms (Lucas, 2004) although crossing of the prisms does occur and confers particular resistance to fracture; this feature is typically confined to the amelodentinal junction (ADJ) in humans. Inciso-apically, the angle between the ADJ and the prisms is around 70 degrees, increasing to around 90 degrees in the cervical region. The prism orientation can have implications for tooth surface loss; prisms perpendicular to the enamel surface have been shown to be more resistant to polishing than those that lie parallel to the prepared surface (Osborn, 1965). The prisms have been previously categorised using Scanning Electron Microscopy (SEM) into 3 main patterns (Boyde, 1989) (Figure 2):

i) Rows of prisms with complete boundaries separated by a well-defined interprismatic region (particularly at the ADJ and near the outer surface of human enamel).
ii) Prisms with incomplete outlines arranged in columns with sheets of interprismatic enamel separating the columns (often bovine and ovine enamel (Maas, 1991; Grine *et al*., 1987; Grine *et al*., 1986)).
iii) Alternating rows of prisms with horseshoe shaped boundaries (typical in human and elephant enamel) where interprismatic enamel is mainly confined to the tail regions.

Prism diameter is shown to be consistent from the enamel surface through to the ADJ (Radlanski *et al*., 1995), yet the peripheral surface can be up to 30% larger than the inner surface of enamel. Several explanations have been proposed (Figure 3) to explain

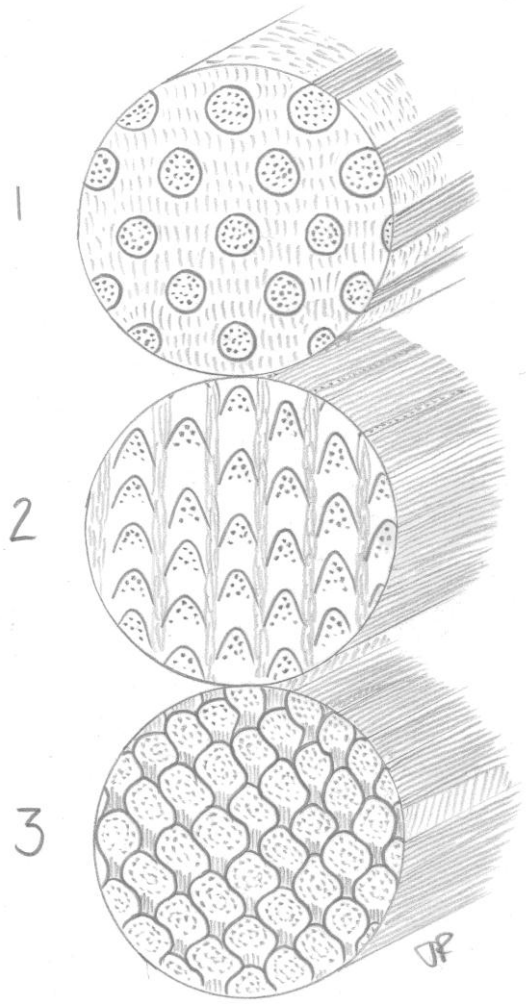


Figure 2 – A stylised drawing of the three main presentations of enamel: Type 1 shows rows of prisms with incomplete boundaries, separated by a well-defined interprismatic region; Type 2 shows columns of prisms with incomplete outlines, and sheets of interprismatic enamel separating the columns; Type 3 shows alternating rows of prisms with horseshoe-shaped boundaries where interprismatic enamel is mainly confined to the tail areas. Adapted from the original figure by Maas (Maas, 1991).

this phenomenon (Radlanski *et al*., 1995), including:

i) an increase in 'inter-prismatic substance' as the prisms move peripherally
ii) the propagation of new prisms or prism branches towards the surface in order to fill the volume
iii) a change in angulation of the prisms towards the periphery resulting in an increase in volume of the enamel, with an oblique presentation

In recent years, several nanoindentation studies have characterised the enamel surface in terms of elastic modulus and hardness (Low *et al*., 2008; Ge *et al*., 2005; Cuy *et al*., 2002). Hardness values are often investigated by the use of an indenter that is driven into the sample surface with a known force. The area of the indentation at a known force is measured over time. Two main types are the Knoop and the Vickers indenters; Knoop are most frequently used for brittle materials or thin sections due to their relatively superficial indentations. Some researchers warn against the use of a metric evaluation of hardness (Brennecke and Radlanski, 1995) because the calculation of a Vickers hardness number assumes that all geometrical surfaces of the indenter interact equally with the sample surface; SEM evaluation of probe indents revealed that a clear square impression outline was often lacking – however, the authors did not report accurately the forces used. Knoop hardness values between 272 and 440 have been reported for human enamel (He *et al*., 2010; Meredith *et al*., 1996) and the enamel appears to harden towards the outermost layers (Roy and Basu, 2008). Within the enamel layer, deformation beneath the probe tip seems to be accommodated by sliding within the inter-prismatic protein, with the prisms themselves remaining intact (He and Swain, 2007). This protein layer is one of several structural organic elements causing enamel to have appreciable porosity at prism boundaries and within enamel

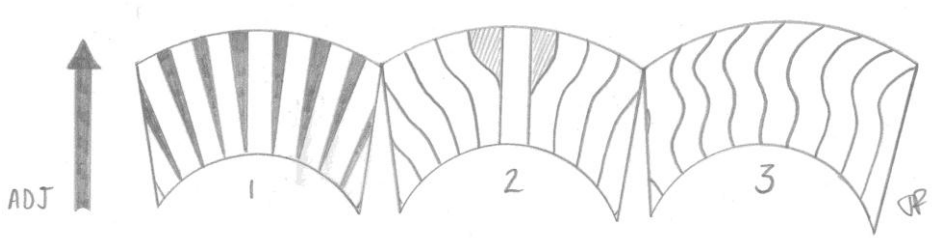


Figure 3 – A stylised drawing of three methods by which the enamel area at the occlusal surface may be larger than near the ADJ: 1) an increase in interprismatic substance; 2) the propagation of new prisms; 3) a change in angulation of the prisms with an oblique presentation at the surface. Original figure by Radlanski (Radlanski *et al*., 1995).

tufts adjacent to the ADJ. SEM and methacrylate replica comparison has shown this organic material to have a raised solubility when subjected to acetate buffer solutions (Shellis, 1996). Furthermore, incremental growth lines and cross-striations (which represent circadian variation in secretory activity) provide further localised porosities. Being able to differentiate areas of altered solubility is important because it may allow the pattern of early surface change to be explained and predicted.

2.1.2 Bovine & Ovine enamel microstructure

The extraction of 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. Although a number of studies (discussed below) have compared the structure and composition of bovine and human enamel, very few have compared or investigated the structure of ovine enamel; even then, these studies are often chiefly investigating goat or deer enamel structure (Kierdorf *et al*., 1991; Grine *et al*., 1987; Grine *et al*., 1986).

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 (Fonseca *et al*., 2008). 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) (Arends and Jongebloed, 1978). SEM analysis of ovine enamel (Grine *et al*., 1986) 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 (1987), and it has been concluded that enamel structure is least-variable mid-way between the surface and the ADJ.

Investigation of enamel matrix proteins was pioneered by Eastoe as early as 1960 (Eastoe, 1979). Improved chromatographic and electrophoretic analysis techniques have since allowed the matrix proteins of the developing enamel to be studied more closely (Fincham *et al*., 1982); the major protein found in human, bovine and ovine enamel has a similar weight (30kDa). However bovine enamel shows a deficiency in the amino acid alanine, and ovine enamel appears relatively enriched with glycine and aspartic acid resulting in a more 'enamelin-like' composition. Enamelin is a protein that drives enamel crystal formation and elongation, and it represents part of the organic component of enamel, and therefore areas of altered solubility. An increased 'enamelin-like' composition may therefore result in an enamel surface that is more susceptible to dissolution or wear.

Electron microprobe analysis of bovine and human enamel suggests similar calcium content by weight (37.9 % and 36.8% respectively), although bovine calcium distribution was more homogeneous compared to human (Davidson *et al*., 1973). Calcium/phosphate ratios of demineralised enamel surfaces (inferred through microhardness testing) also suggest a similar composition between human and bovine specimens (Feagin *et al*., 1969) reporting Knoop hardness values between 244 and 337 (Tantbirojn *et al*., 2008). An infrared spectroscopy study comparing human and bovine enamel at a number of developmental stages found no significant difference in

carbonate content (Sydney-Zax *et al*., 1991); it is unclear whether the latter study used primary or permanent bovine teeth. However, differences between human and bovine enamel are reported; a study comparing calcium release of stored human and bovine samples treated with calcium hydroxide found significantly higher calcium ion release for the bovine samples (Camargo *et al*., 2006).

2.1.3 The use of bovine & ovine tooth tissue in experimental erosive/abrasive models

Numerous studies have used bovine enamel as a substitute for human enamel. To date, no published work could be found that has utilised ovine enamel within an erosive or abrasive model. It is important to explore why bovine enamel is considered to be suitable as a substitute (Yassen *et al*., 2011):

i) A larger number of unaffected bovine samples are often easier to obtain; human teeth are often extracted due to extensive caries or tooth surface loss.

ii) The bovine sample age and source can be controlled to a greater extent, and the environment is likely to be more consistent (such as fluoride exposure between animals) (Zero, 1995). Sex, geography and individual metabolism have been shown to have significant effects on dental hard tissue composition (Lane and Peach, 1997; Vernois *et al*., 1989). Despite this, modern animal husbandry systems will undoubtedly provide some form of metabolic control for cattle (Wright *et al*., 2008); significantly greater control than could ever be ethically achieved with a human cohort.

iii) More same-tooth and same-subject samples can be prepared from a bovine incisor crown; human incisor teeth are relatively small (Zero, 1995).

iv) The ethical rigour of studies has increased, making the application for use of human tooth tissue a more involved process (Skene, 2002).

When considering bovine enamel as a substitute for demineralisation (erosion) studies, there are a number of conflicting studies. Under relatively mild acidic challenges (submersion between 2 and 60 seconds in citric acid at pH 3.2), no significant difference in microhardness between human and bovine enamel was measured (White *et al*., 2010). This was confirmed recently by Turssi who after an erosive challenge obtained Knoop surface microhardness readings of 425 and 413 for human and bovine enamel respectively (Turssi *et al*., 2010). Microradiography studies have shown significantly lower mineral loss and lesion depth for human than bovine enamel (Amaechi *et al*., 1999) and profilometric analysis shows that bovine erodes faster (up to 30%) at longer exposure times (White *et al*., 2010); qualitative comparison using SEM has failed to display a difference (Meurman and Frank, 1991) although it is unclear how much surface change must occur before it becomes obvious using methods such as SEM. It has been suggested that this greater response of bovine enamel to an erosive challenge is due to greater porosity (Amaechi *et al*., 1999; Arends *et al*., 1989).

When considering abrasive studies (the movement of a foreign body over the enamel surface, such as a toothbrush, toothpaste, food slurry or opposing tissue) there is a high positive correlation between the surface shear of human and bovine tissues (Reeh

et al., 1995) – frictional coefficients were recorded over a series of different lubricants for human and bovine enamel. Although this suggests that the bovine and human samples may be similarly affected by lubricants, or even with simple abrasive challenges (Attin T, 2007), it does not necessarily mean that the surfaces will behave similarly when subjected to a combination of erosive and abrasive treatments. Indeed, when abraded with a toothbrush and toothpaste slurry (Attin *et al*., 2007; Rios *et al*., 2006a), human eroded enamel has been shown using profilometry to offer better resistance against brushing than bovine enamel. A similar finding with microhardness testing and surface profilometry was also obtained; bovine enamel displayed significantly lower microhardness and higher wear (Rios *et al*., 2006b). However, when SEM was used to qualify this difference, no difference was seen (Rios *et al*., 2008a).

Radiodensity and hardness testing has shown that age can significantly affect the hardness of bovine enamel (Fonseca *et al*., 2008). Five 1 mm-thick specimens were obtained from cattle aged 20, 30, 38 and 48 months old, and from 20-30 year-old human third molars. The study reported a difference in Knoop hardness between the 20-30 month-old bovine enamel and the older bovine and human enamel, with the former perhaps due to a slow, continued and cumulative topical effect of fluoride. The authors therefore recommend the use of older bovine teeth due to a better chance of finding greater similarity with human samples.

2.2 Tooth surface loss

2.2.1 Types of tooth surface loss

The concept of tooth wear was first reported in the early 19th century (Royston, 1808).

Since then, the existence of three main types of tooth 'wear' are widely referenced by the dental profession; attrition, abrasion and erosion:

1. Attrition, also known as two-body wear, results from tooth-to-tooth contact (Soames and Southam, 1998).Typically the edges of the lower incisors become worn first of all, followed by the occlusal surfaces of the posterior teeth.

2. Abrasion results from the introduction of a foreign body onto the tooth surface, typically a toothbrush and/or toothpaste (Soames and Southam, 1998). Abrasion can also be caused by holding objects between the teeth such as ceramic pipes, paper clips or hair clips.

3. Erosion results from an acidic challenge of non-bacterial origin (which distinguishes it from dental caries). Erosion is often a localised process, whereby the tooth structure enters a cycle of demineralisation and remineralisation. If the balance is altered, then excess demineralisation will result in loss of tooth structure (Moss, 1998).

A fourth type of tooth surface loss known as 'abfraction', describes the loss of hard tissues within the cervical portion of teeth; this loss is thought to be due to excessive cyclical loading resulting in cuspal flexure (Michael *et al*., 2009). Although abfraction is often reported clinically, there is little scientific evidence for its existence.

More recently the term 'tooth surface loss' (Kelleher and Bishop, 1999) has become

favoured, mainly because the older term 'tooth wear' suggested some form of abrasive component. In reality, although tooth surface loss does often involve abrasion, it is more likely that several forms of mechanical and chemical insult are working in combination. The widely used definitions above are useful as descriptors, but it is purported that clinically the process of tooth surface loss is more complex. Although erosion is nearly always attributed to an acidic challenge, tooth surface loss due to alkaline compounds has also been reported. Taube *et al* (2010) described two cases detailing damage to tooth structure due to exposure to alkaline aerosols such as potassium hydroxide, the mechanism of which is described below.

2.2.2 Mechanism of erosion

Acidic dissolution of the mineral content in enamel results in a weaker, softened surface (Eisenburger, 2009) that is more susceptible to physical challenges (Jaeggi and Lussi, 1999). SEM work investigating the pattern of enamel erosion suggests that the erosion of prisms and prism junctions generally happens earlier than the inter-prismatic enamel (Xiao *et al*., 2009), resulting in a honeycomb appearance (Meurman and Ten Cate, 1996; Shulin, 1989). The erosive process is centripetal, proceeding from the outer surface inwards, initially without any bulk tissue loss (Eisenberger *et al*., 2004).

Characteristics of the eroded surface depend on the mode of acidic challenge; with high liquid velocity, prism junctions have been reported to appear narrow and the enamel surface is generally smoother. At low velocities, with agitation, and longer exposure times, prism junctions appear relatively broad and deep and the eroded

surface shows considerably more relief (Shellis *et al*., 2005; Nekrashevych and Stosser, 2003; Çehreli and Altay, 2000; Oliver, 1988). SEM study has shown that although there is evidence that the erosive process may be different towards the cervical portion of a tooth, there is little evidence that there is a difference between human tooth types (Hobson *et al*., 2005).

Erosion due to *alkaline* compounds does not follow the same pattern of surface change; degradation of the organic components rather than the apatite crystals happens first. This results in tooth structure literally 'collapsing' as the protein rich matrix holding the prisms together is dissolved (Taube *et al*., 2010).

2.3 The measurement of tooth surface loss

Despite a range of different study designs, it may be suggested that few relate closely to a clinical model. In the early stages of erosion, there is softening at the surface as mineral is lost. At this early stage repair is still possible as the tissue scaffold remains. This collagen scaffold may then act as a barrier to further erosion unless it is removed either enzymatically (bacterial or induced) or abraded (Gregg *et al*., 2004). The way in which the mineral is lost may confer unique surface characteristics that determine how further tooth surface loss may follow with a subsequent abrasive challenge.

2.3.1 Measurement techniques and the effects of scale

Biological tissues are a complex class of materials because their structures contain important features at the nanometre scale (Ebenstein and Pruitt, 2006). On this scale, structures such as enamel and dentine are composites based on the interdigitation of

collagen, apatite and other various inorganic components. Scale considerations are therefore significant in determining the mechanical behaviour of dental hard tissues (Katz *et al*., 2007). Direct visualisation of the tooth surface *in vivo* makes accurate tissue loss very difficult to determine. Traditional microscopy techniques may allow surface change to be identified (Nirmala and Subba Reddy, 2011), but describing or 'qualifying' the change may prove to be difficult. Recently, Hughes (Hughes *et al*., 2009) and Hua (Hua *et al*., 2009) suggested the use of focussed ultrasound to detect and monitor the thickness of remaining enamel covering a tooth. This is accurate to within 10% of the total enamel thickness. More advanced indirect measurement techniques such as stereo-imagery (Grenness *et al*., 2009) or using reference markers (Schlueter *et al*., 2005) may provide a more accurate explanation of the patterns of tooth surface loss. Even when these more accurate methods are used, the surface change cannot be accurately described, only quantified. Although quantified results are often considered fundamental in order to compare laboratory measurements, the ability to further 'describe' a surface may have the potential to account for particular surface characteristics that determine how *future* loss may progress.

2.3.2 Micro and nanoscopic techniques

A review of the literature shows a wide range of techniques used to study dental hard tissues. The following list details the techniques that will be discussed:

- Polarised light microscopy
- White light inferometry
- Quantitative light fluorescence

- Laser-induced fluorescence
- Microradiography
- Surface profilometry
- Scanning electron microscopy
- Optical coherence tomography
- Cumulative calcium release
- Nanoindentation
- Iodide permeability
- Confocal scanning laser microscopy
- Atomic force microscopy

Polarised light microscopy (PLM) was first used to image dental enamel in 1967 (Gustafson, 1967) and thereafter, it was used to investigate the progression of caries using thin tooth sections prepared *in vitro* (Mor and Rodda, 1980). A form of differential interference contrast microscopy, the technique uses a split beam of polarised light to form a highly magnified and detailed image of the prepared tooth surface. The results should be interpreted carefully, as the image relief produced may not actually resemble the true surface.

A more recently-developed technique known as *White light interferometry* (WLI) is based on the same principle. With the development of computer and software-aided systems, this can now be used quickly and effectively to map 3-D surface images. It has recently been used to successfully demonstrate the protective effects of novel

copolymers in an anti-erosion mouthwash (Gracia *et al*., 2009) and to investigate the surface effects of different splint removal techniques (Cehreli *et al*., 2008). PLM has been used successfully *in vitro* on primary teeth (Smith and Shaw, 1987) to estimate the amount of sub-surface loss due to erosion (mineral loss presents as a colour change on the image). Unlike WLI which can also record textural characteristics, the main barrier for assessing surface changes with PLM is that it can only really determine trends rather than actually quantify tooth loss. This drawback is shared with another technique used to assess demineralisation, *quantitative light or laser-induced fluorescence (QLF/LIF)*. Teeth naturally fluoresce, and when light waves of a particular wavelength and intensity are directed at the tooth surface, atoms within the enamel or dentine become excited. A fraction of a second later the tooth surface returns to normal, but emits light at a wavelength larger than the absorbed light. Changes in the intensity of this fluorescent light are measured and used as an indicator for mineral loss (de Jong *et al*., 2009). Images are analysed digitally using dedicated scanners and software. Lesion assessment is considered as 'semi-automatic' (Inspektor Research Systems, 2009), in that after scanning, the operator must define the lesion boundaries on-screen prior to analysis. Tools within the software help to standardise this process. The most important result parameters reported are lesion size, depth and volume. QLF is often preferred due to less safety concerns and portable units have now been developed for safe chair-side use. LIF is still used, however, and has been successful for investigating early mineral loss (Thomas *et al*., 2008). Originally used as an adjunct for caries detection, Thomas *et al* (Thomas *et al*., 2008) were able to modify the LIF technique to monitor demineralisation changes. The major strengths of the fluorescence techniques is that they are non-destructive (and therefore surfaces can

be monitored over time, *in vivo* or *in vitro*) and can provide information relating to sub-surface mineral loss (Elton *et al*., 2009).

Microradiography came into use in the mid-1970s (Groeneveld and Arends, 1974). It involves the penetration of thin specimens by low energy X-rays to produce an enlarged image of the surface. Unlike PLM, this technique allows the total mineral loss to be quantified, and is useful where large amounts of surface loss are anticipated. Mineral loss is often computed using a microdensitometer (Ganss *et al*., 2009a), although modern camera and software systems are becoming more popular (Higham *et al*., 2009). The technique will allow *in vitro* assessment of significantly higher depths than can be measured by a stylus (known as *surface profilometry*), and has been used recently to assess levels of abrasion and erosion (Ablal *et al*., 2009b). West (West *et al*., 1998) suggests that the technique cannot characterise early erosive changes. In contrast, it has been shown to allow discrimination between erosion times of less than an hour (Hall *et al*., 1997), but during this *in vitro* study, the tooth tissue was subjected to relatively acidic conditions (pH3), and the protective effects of saliva or pellicle were not accounted for. Despite the potential for disruption of the specimen (Anderson *et al*., 1998), it has been recognised as a useful and acceptable tool for assessing early tooth tissue loss from thin sections of tissue. In common with PLM, microradiography can also indicate subsurface demineralisation (Klimek *et al*., 1996); a feature which QLF/LIF cannot. Although useful, microradiography still does not provide information relating to the remaining surface quality.

Scanning Electron Microscopy (SEM) was one of the first techniques used for

measuring the *in vitro* resorption of dental hard tissues (Boyde and Lester, 1967) and it was thereafter adopted for the investigation of cavity preparations using indirect replicas (Barnes, 1978). Still widely used today, (Ferrazzano *et al*., 2008; Sorvari *et al*., 1996) (Schmidlin *et al*., 2003a) SEM involves the scattering of electrons at the sample surface and the resulting received signal provides information about the surface topography and composition. SEM images have a large depth of field (all parts of the image are in focus, despite being at different depths), and can therefore yield high resolution 3-D images. For conventional SEM, the surface must be coated with a material that is electrically conductive, to prevent the accumulation of electrostatic charge. This material is usually gold, and the specimens will undoubtedly be irreversibly altered during the desiccation and sputtering process. Biological samples can be impregnated with Osmium instead, or imaged in an uncoated manner using environmental SEM, which works within a pressurised container. Casts can also be made of the specimen if there is a risk of sample destruction, but these may suffer from potential dimensional inaccuracies (Faria *et al*., 2008).

Optical coherence tomography (OCT) is a more recently developed technique (Warren *et al*., 1998) that uses near-infra-red light to produce a cross-sectional image of the sample surface. OCT works in a similar way to ultrasound, but uses high frequency light (around 820 nm (Fernando Arevalo *et al*., 2009)) instead of high frequency sound. It can therefore penetrate significantly deeper into samples than other sub-surface techniques, providing an extremely high quality 3-D image that is non-destructive to the sample surface. OCT can assess enamel thickness, reflectivity and absorbance, which can then be related to levels of demineralisation (Wilder-Smith *et al*., 2009).

Although it has been used successfully *in vivo* (Amaechi *et al*., 2001) and *in vitro* (Jones *et al*., 2006) to monitor caries severity and depth, it does not allow further analysis of the remaining surface in terms of textural characteristics.

Cumulative calcium release (CCR) of the tissues has only recently been documented in the dental literature for demineralisation studies (Willershausen *et al*., 2009; Hannig *et al*., 2008; Wiegand *et al*., 2008a). Based on the principle of continuum source atomic absorption spectroscopy (at 422.7 nm) (Wiegand *et al*., 2009c), it can be related to certain levels of demineralisation. Willershausen (Willershausen *et al*., 2009) showed that whereas roughness average values were similar between red wine and white wine erosive challenges, when compared using CCR, white wines resulted in a higher loss of calcium down to a depth of 60µm.

Nanoindentation quickly became a popular mechanical test after its first documentation in the late 1980s (Bhushan *et al*., 1988). It has been used successfully in the biological testing of mineralised tissues (Sales-Peres *et al*., 2007; Rios *et al*., 2006a; Pethica *et al*., 1983). Due to the small volume requirement for each indentation, it can be used to examine micro-structural features that are only several micrometres or less in dimension. This is useful in order to characterise the way in which a surface has been affected, but the results are of limited use other than to quantify surface softening. Further, in erosion/abrasion studies, the indenter is essentially the abrasive force and this may not relate entirely to hard tissue loss by an erosive attack. Some investigations on human enamel have shown a load-dependent behaviour of nanoindentation (He and Swain, 2007) and this can result in aberrant

features on the force curves that are produced. Subsurface damage may also directly influence the reported surface hardness. The loading time of the tip can be varied, although several studies report no significant effect on hardness testing (Chuenarrom *et al*., 2009; de Marsillac *et al*., 2008). Nanoindentation also has the potential to change the sample surface, making re-measurement in the same location inaccurate or impossible (Angker and Swain, 2006).

Iodide permeability was first used to describe the effects of abrasion on enamel in 1982 (Brudevold *et al*., 1982). It is still a useful technique (Attin, 2006) and involves the non-destructive penetration of potassium iodide into the enamel surface. Permeability is assessed by measuring the back-diffusion of iodide. A change in permeability can suggest a level of demineralisation or surface softening, but fails to qualify the actual surface change that is taking place. It has been suggested that this technique may be complementary with nanoindentation tests previously described (West *et al*., 2000).

Confocal scanning laser microscopy (CSLM) is a non-destructive 3-D technique, capable of producing high resolution images. Originally confocal microscopy was carried out using visible light, but in recent years (since the late 1980s) the light has been replaced with a laser source. Further, specimens are now often labelled with fluorescent markers. In contrast to wider-field microscopy techniques, the microscope filters away 'out of focus' fluorescent information. Surface images of samples can be produced which are similar in character to those of the SEM, but without many of the problems of specimen preparation. The improved resolution and removal of 'out-of-focus' blur allows much more information to be gained from fluorescence microscopy techniques,

with the ability to reconstruct the sample in 3-D using appropriate software (Min *et al*., 2011; Duschner *et al*., 2000). As with the other microscopy techniques, CSLM fails to record textural details which would serve to further qualify the remaining surface.

Atomic force microscopy (AFM) can measure height changes as little as one atom (10^{-10}m). It is a very high resolution form of microscopy. Essentially the AFM comprises a cantilever that is used to scan the specimen surface. The constant force mode is useful for recording surface topography and also due to torsional flexion, gives information on the frictional properties of a surface. With very soft surfaces, frictional effects are not always desirable so the tapping mode is used. No surface preparation is required but the images can be subject to artefacts caused by blunt or dirty tips, problems with the piezoelectric scanner or sample movement. Soft hydrated structures must be immobilised in a fluid cell, however the fluid cell can allow the real-time recording of surface events. AFM has been used to evaluate erosion in human enamel (Marshall *et al*., 2004; Marshall *et al*., 2001; Finke *et al*., 2000) and this has proved to be a suitable tool for measuring the early stages of enamel demineralisation.

Surface profilometry involves surface measurement directly using a contacting stylus or indirectly, using a laser profilometer (Figure 4). Contact stylus profilometry is an older and, therefore, more established technique; indeed the current national standards on measuring surface texture (Number 5436-1, International Standards Organisation, 2001) are defined using stylus profilometry. *Stylus profilometry* involves traversing the surface with a diamond-tipped stylus. The tip is usually of a fixed radius 1.5-2.5µm (Stachowiak *et al*., 2004), however, the shape of the tip can vary (Bhushan, 2001).

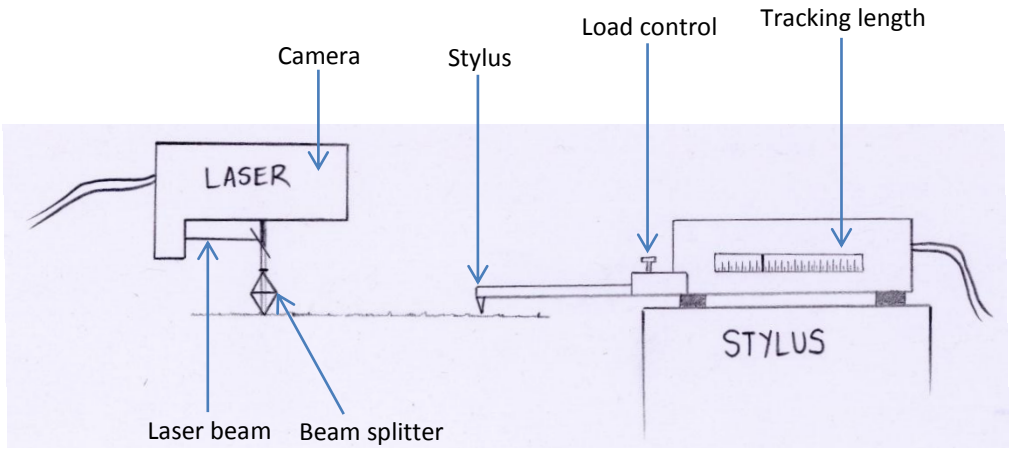


Figure 4 – Schematic of stylus and laser profilometry. Note the stylus tip that contacts the sample surface (right) compared to the optical beam that indirectly scans the sample surface (left).

Chisel-point (0.25µmx2.5µm) tips may be used for detecting raised areas in a surface whereas conical tips are almost exclusively used for micro-roughness measurements (International Standards Organisation, 1975). The loading weight on the stylus can range from 0.05-100mg (Stachowiak *et al*., 2004). The vertical motion as the stylus is dragged across the surface is transformed into an analogue/digital signal. Due to the nature of the technique, the stylus is in almost perpetual contact with the surface that is being measured. Although this is often an advantage in that a large vertical range is possible (typically 2µm to 250µm), there is a risk of the diamond tip causing damage to the specimen. A finite tip radius is also unable to record detail of concave radii smaller than the tip and so finer surface detail may be filtered out (See Figure 5). Although the lateral resolution is dependent on stylus radius, the accuracy of the vertical resolution can be affected by external vibrations and electrical interferences. In order to minimise these effects, the recording speed is maintained at around 1 mm/s or less. The vertical resolution for smooth surfaces can be as low as 0.1 nm, up to 1 nm for rough surfaces or large steps (Bhushan, 2001).

Laser profilometers can overcome many of the drawbacks of contact profilometry (namely lateral resolution and problems related to recording speed) because they do not directly contact the surface. A light spot is directed at the surface, typically less than 100µm in diameter (Rodriguez *et al*., 2008). The laser profilometer can profile surface topography either by measuring the deflection of the laser beam, or (with white light) by using the confocal principle (McBride and Maul, 2004). A recognised problem with laser profilometry on dental hard tissues is that the results can be affected by colour and transparency, with lighter and more transparent surfaces

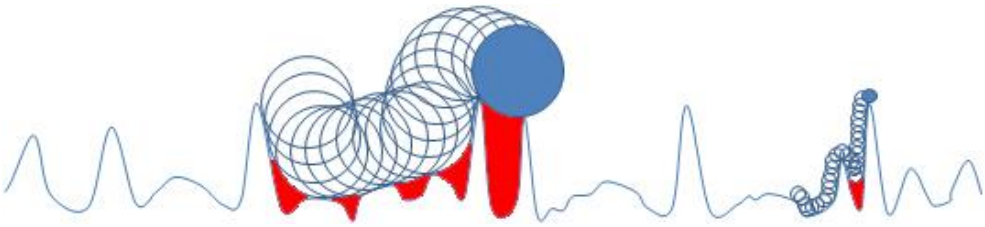


Figure 5 – Limitations of stylus profilometry – the radius of the tip will determine how accurate the profile reading is. A larger tip (left) will not record voids that are smaller than the tip diameter (shown in red). A smaller tip results in a more detailed recording (right).

recording a lower roughness average (Rodriguez *et al*., 2008; McBride and Maul, 2004); often it is necessary to record a polyvinyl siloxane impression of the sample which is then scanned by the laser profilometer in order to overcome translucencies on the surface. However, the laser profile is still affected by the surface colour; studies using laser profilometers at light wavelengths of 785 nm have shown that darker coloured impression materials show a higher roughness and, furthermore, if an impression material absorbs colour at the same wavelength of the laser, then the surface will not be scanned (DeLong *et al*., 2001). Notwithstanding the potential inherent dimensional errors in replicating a surface by taking an impression (Chun *et al*., 2008), it is also suggested that the filler particles within impression materials can result in aberrant profile results from the laser (Rodriguez *et al*., 2008).

Surface profilometry still remains one of the most frequently used methods of assessing surface change in dental hard tissue studies (Giles *et al*., 2009; Magalhães *et al*., 2009a; Ranjitkar *et al*., 2009b; Wiegand *et al*., 2008b; Sales-Peres *et al*., 2007; Hemingway *et al*., 2006c; Hemingway *et al*., 2006b; Rios *et al*., 2006a; Vieira *et al*., 2006; Hooper *et al*., 2003b; Schmidlin *et al*., 2003a; Attin *et al*., 2001; Attin *et al*., 1999; Jaeggi and Lussi, 1999). The parameters that these studies report are often limited, allowing little qualification of the tooth surface. It has been suggested that no method is suitable for all stages of the eroded lesion (Schlueter *et al*., 2011). Some investigators have modified the information collated from profilometric results in order to provide a more descriptive account of surface change (Nasution *et al*., 2008). Chadwick (Chadwick *et al*., 1997) describes a system of 'novel mapping', which uses a computer to create a 3D plot of the surface of a replica model. The software

compared plots over time, and the lesion depths and actual dissolution rates can be quantified; this method required the impression to be sprayed with a nickel spray prior to casting up the impression. Accuracy was reported to be around 2.8µm. Other informative techniques can be used as an adjunct to profilometry in order to further understand the surface dynamics – microhardness testing using a standardised Knoop indent has been used successfully as a method of determining surface loss after an abrasive challenge (Jaeggi and Lussi, 1999). Recording values of microhardness is useful in itself, as it can infer information regarding the level of mineralisation of the hard tissue. Values for enamel hardness however, have been shown to depend on the specific measurement technique and should not be thought of as constant (Collys *et al*., 1992). It was shown that values obtained by Knoop indenters were inversely related to the load applied– for sound enamel, Knoop hardness values of 431 and 339 at loads of 50g and 200g respectively were reported (Collys *et al*., 1992). Within a tooth, Knoop hardness may vary between 280 and 390 using a loading weight of 100g (Lussi *et al*., 2011). This may be due to the chemical composition and physical properties changing with depth (Meredith *et al*., 1996; Weatherell *et al*., 1974) (density and hardness decrease with increasing distance from the surface and solubility increases). It is suggested that mechanical properties of calcified tissues are generally linked to the mineral content (Kinney *et al*., 1996). Mineral content measured by light microscopy and microradiography has been shown to reach a maximum in areas where enamel is thickest, and decreases toward the cervical region (Theuns *et al*., 1983). These details are noteworthy for more sensitive experiments. Not unlike stylus profilometry, the microhardness indenters have the potential to deform the native surface. A Knoop indenter penetrates about 1.5 µm and Vickers

around 5 µm at similar loads. That said, indentations in enamel are relatively stable and are not vulnerable to time-dependent changes in their morphology, since enamel shows low elasticity (Herkstroter *et al*., 1989). It has been suggested that correctly performed hardness measurements have proved to be robust (Schlueter *et al*., 2011) and it is purported that microhardness testing can still provide a worthwhile adjunct to profilometric data.

Potentially these techniques should be used in combination in order to maximise the way in which surface characteristics are reported (Attin, 2006). Indeed, some studies do use a combination of these techniques, such as profilometry (reporting surface roughness) and levels of calcium release (Willershausen *et al*., 2009). In reality, using multiple methods is often unfeasible due to the specialist nature of the techniques, particularly the equipment and skills required in order to accurately perform and interpret the results of the tests.

2.3.3 Citric acid and its relevance to fruit acid erosion

Fruit acids have a significant erosive potential (O'Sullivan and Milosevic, 2007). The most abundant is citric acid; whilst occurring naturally in citrus fruits, it is also used as a food additive to produce acidity and sour tastes. Citrate salts of various metals are also used to deliver minerals in supplements and medicines. Alcopops, introduced in 1995, also have a high citric acid content (Ablal *et al*., 2009b).

Citric acid is most concentrated in lemons and limes; recent ion chromatography studies (Penniston *et al*., 2008) suggest content of nearly 5% of the fresh fruit weight.

It is present at significant levels in more frequently consumed fruit juices and fruit-base drinks (2% w/v, Tropicana® ready-to-drink orange juice) and lemonades (5% w/v, Tropicana® sugar-free lemonade) (Penniston *et al*., 2008) but also in tomato juice (1.6% w/v measured with enzyme spectrophotometry).

Citric acid is a weak tri-basic carboxylic acid (2-OH-1,2,3-Propane Tricarboxylic Acid) which causes enamel dissolution by reaction of the hydroxyapatite with the acid following the equation below (Dorozhkin, 1997):

$Ca_{10}(PO_4)_6(OH)_2 + 2H^+ \leftrightarrow 6PO_4^{3-} + 2H_20 + 10Ca$

Equation 1 – the dissolution reaction of hydroxyapatite and acid

Investigation using SEM and Infra-red reflection spectroscopy shows that there is a definite sequence of ionic loss from the surface of the apatite. Initially the hydroxyl ions are lost from the hydroxyapatite, followed by calcium and then phosphate ions (Dorozhkin, 1997).

Whilst various studies exist to support the erosive potential of citric acid, (West *et al*., 2001; Zero, 1996) there is no published information about the citric acid content of commercially-available beverages in the United Kingdom or The United States (NAL, 2011). Simple laboratory tests have been used to determine the acidity of fruits and fruit juices. Titratable acidity gives an indication of the strength of the acidic solution and can be reported as strength (Molarity) or a percentage of the volume as weight (%w/v). The titratable acidity is a measure of the acid's buffering capacity – a higher

buffering capacity means that the acidic solution will remain at a lower pH for longer. However, although citric acid is the major acid in fruit juices, when a titratable acidity is converted into an acid content, this may overestimate the content of citric acid due to contributions of other minor organic acids such as malic or lactic acid. This effect is compounded if manufacturers add ascorbic acid (as an antioxidant or as a vitamin supplement) to the juice or if the juice is modified to make it more palatable or microbiologically stable. Such modifications may include the addition of trisodium citrate or carbonating the drink; further complicating the chemical dynamics with carbonic acid (West *et al*., 2001).

Enzymatic tests may therefore be considered to be more accurate than titration for determining the specific fruit content of juices. However enzymatic tests require specific kits for each organic acid. They also use large amounts of reagents and are relatively time-consuming (Yilmaz *et al*., 2008). A faster method involves ion exclusion chromatography (separation of individual acids based on their ionic charge) but this can be subject to interference by the water soluble pigments in fruits (anthocyanins) (Lin *et al*., 2011), and does not always allow separation of minor organic acids. Chromatography often requires preventative purification techniques to eliminate the interference of sugars and phenol compounds (Saccani *et al*., 1995). The measurement process is further complicated by the fact that acid content changes according to the state of the juice; for example, the level of maturity or oxidation state (Sturm *et al*., 2003), whether the fruit has been peeled or not (Velterop and Vos, 2001) and at what temperature it has been stored (Oh *et al*., 2008). Despite the drawbacks of each technique, the evidence suggests that chromatography has perhaps been used the

most successfully for determining the micronutrient content of juices (Shui and Leong, 2002) and even subtle maturation effects in strawberries (Sturm *et al*., 2003) and plums (Garcia-Marino *et al*., 2008).

2.3.3.1 Fruit acids

There are a significant number of *in vivo* experiments and case reports that serve to highlight the problem of fruit acids on the functional dentition; the first published work on the clinical effects of fruit acids was by Levine in 1973 (Levine, 1973). Since 1956 there had been a four-fold increase in the consumption of fruit juice and Levine felt that the severity of the problem was related to the level of an individual's fluid intake and their salivary gland function. Thereafter a number of papers were published detailing the effects specifically on the primary dentition (Asher and Read, 1987; Smith and Shaw, 1987), but also identifying localised effects such as sensitivity at the gingival margins (Touyz, 1983). In 1997, a study identified how acidic fruit-based lozenges could exacerbate erosion on abraded hard tissues (Lussi *et al*., 1997). A series of *in vitro* and *in situ* experiments in 1999 (Hughes *et al*., 1999b; Hughes *et al*., 1999a; West *et al*., 1999) suggested that low pH fruit-based drinks were capable of causing a 1 mm loss of enamel in periods from as little as 2 years up to 20 years. More recent papers detail the clinical effects of sports drinks based on fruit juices (Foster and Readman, 2009) and the longer-term effects of habitually drinking carbonated soft-drinks (Cheng *et al*., 2009a).

A number of *in vitro* experiments also exist that investigate the effects of fruit acids on tooth structure (Ablal *et al*., 2009b; Ren *et al*., 2009b; Wagoner *et al*., 2009; Zheng *et*

al., 2009; Hunter *et al*., 2008; Reis *et al*., 2008; Wang *et al*., 2008; Davis *et al*., 2007; Attin *et al*., 2003; Barbour *et al*., 2003a; Eisenburger *et al*., 2001; Marshall *et al*., 2001; Hughes *et al*., 2000; Hunter *et al*., 2000; Marshall *et al*., 2000; West *et al*., 2000; Addy *et al*., 1998; Sorvari *et al*., 1988). The effects *in vitro* are often assessed using nanoindentation, atomic force microscopy or scanning electron micrograph techniques. *In vitro* testing often fails to relate closely to the clinical situation, with samples immersed in fruit acids for many hours (Willershausen *et al*., 2009; Eisenburger *et al*., 2001). Some interesting findings from the aforementioned studies serve to highlight the complexity of fruit acid erosion; nanoindentation studies suggest that increasing the pH of a drink will not significantly reduce enamel dissolution (Barbour *et al.*, 2003a; Barbour *et al*., 2003b). The recommendation is made that drinks should be modified in other ways such as with the addition of calcium salts. Further, profilometric results suggest that further diluting fruit-based drinks may not reduce their erosive potential (Hunter *et al*., 2008). In addition, reducing the frequency of exposure to a fruit-based drink does not appear to result in a proportional decrease in tissue loss (Hunter *et al*., 2000). Citric acid in particular is considered to be a complex acid with respect to erosive potential (Gambon *et al*., 2006). At a low pH, the citric acid releases $[H]^+$ ions that attack the surface enamel; at higher values of pH, the citrate anion is able to chelate calcium from the enamel surface. The potential for demineralisation is therefore not solely dependent on pH, and studies have shown that the effects of citric acid on enamel are greater than for hydrochloric or phosphoric acid (when surface loss was compared using stylus profilometry (West *et al*., 2000) and calcium and phosphate release (Hannig *et al*., 2005)).

2.3.4 The induction of erosion and abrasion within a study

A variety of methods have been used previously to simulate an erosive challenge. The most reported method is using fruit acids or actual fruit juices and fruit-based products (Ren *et al*., 2011; Beyer *et al*., 2010; Cheng *et al*., 2009b; Elton *et al*., 2009; Gracia *et al*., 2009; Hara *et al*., 2009; Magalhães *et al*., 2009a; Ranjitkar *et al*., 2009a; Rees and Fowler, 2009; Ren *et al*., 2009a; Xiao *et al*., 2009; Yu *et al*., 2009a; Zheng *et al*., 2009; Willershausen *et al*., 2008; Hooper *et al*., 2003a). Whilst using actual fruits juices, there is a potential inconsistency in terms of acidity (Scaramucci *et al*., 2012). The next most reported type of challenge is based on cola and cola-type carbonated drinks (Magalhães *et al*., 2009a; Poggio *et al*., 2009; Quartarone *et al*., 2008; Sales-Peres *et al*., 2007a; Rios *et al*., 2006a; Attin *et al*., 2001). Less reported erosive challenges include wine (Willershausen *et al*., 2009) and sports drinks (Sorvari *et al*., 1988) or laboratory-based preparations such as acidified gel (Attin T, 1999), hydrochloric acid (Ganss *et al*., 2009a; Ranjitkar *et al*., 2009a; Bakar and McIntyre, 2008; Wiegand *et al*., 2008b) lactic acid (Rees and Fowler, 2009) and sucrose solution (Spiguel *et al*., 2009). The advantage of laboratory-based preparations over using actual drinks is that of consistency. The disadvantage is that of relevance, and there are currently no studies that investigate how laboratory-based preparations are able to replicate the erosive effects of commercially available fruit-based drinks.

Methods used to simulate an abrasive element include tooth brushing *ex vivo* (outside the mouth) (Sales-Peres *et al*., 2007; Hooper *et al*., 2003b; Attin *et al*., 1999) and *in vivo* (Jaeggi and Lussi, 1999), administering a rough diet *in vivo* (Sorvari *et al*., 1988), and introducing a micro-abrasive slurry such as Opalustre™ (a polishing slurry

containing 6.6% hydrochloric acid and silicon carbide micro-particles in a water-soluble paste) (Schmidlin *et al*., 2003a).

2.3.5 Brushing and dentifrice abrasivity

Stylus profilometry studies have shown that several individual tooth brushing variables can influence the abrasion of dental hard tissues during brushing (De Boer *et al*., 1985; Saxton and Cowell, 1981); brushing rate, number of bristles, bristle stiffness, and particle size all significantly affected the amount of surface loss. A linear correlation was seen between number of brush strokes and lesion depth. Further, a firmer brush resulted in up to 1.4-times greater lesion depth than a softer one, and larger particles resulted in higher abrasion rates. It is recognised that the abrasivity of toothpaste can have a significantly higher influence on levels of abrasion than the features of the brush alone (Dyer *et al*., 2000; Absi *et al*., 1992). Epidemiological studies (818 adults in Stockholm, Sweden) confirm a relationship between brushing and abrasion (Bergstrom and Lavstedt, 1979) – in particular, a horizontal brushing technique correlated with a greater level of abrasion.

Work by Mellberg (Mellberg, 1979) demonstrated the need to categorise the relative abrasivity of prophylactic pastes for both enamel and dentine, and confirmed that toothpaste abrasivity is an important modulator of erosive surface loss in both enamel and dentine, when compared by laser profilometry (Hara *et al*., 2009). Modern toothpastes are available in numerous different formulations and with differing dentine abrasivity values. Despite the early work by Mellberg (Mellberg, 1979), relative enamel abrasivity (REA) values are rarely reported. This may be because most

toothpastes, in isolation, have little or no effect on enamel (Hunter *et al*., 2002), and REA values do not correlate with levels of dentine abrasivity (Joiner *et al*., 2004). *In vitro* studies using laser interferometry at the cemento-enamel junction (Azevedo *et al*., 2008) have shown that soft, medium and hard brushes are not capable of abrading the native enamel surface. In contrast, dentine *can* be abraded by medium and hard bristle brushes. For instance, interferometry enabled detailed 3D surface analysis to be carried out, however the toothbrush abrasion with the hard bristle brush exceeded 500 µm and was unable to be recorded; although no value was therefore reported, clearly the harder bristles were capable of causing a greater amount of tooth surface loss. *In vivo* it is difficult to standardise the force applied to a toothbrush. Modern electric toothbrushes do not allow forces in excess of 1-2N to be applied (either by sounding a warning tone or stopping themselves from oscillating) and some modern manual brushes now have highly flexible heads to prevent overloading.

The aim of the toothpaste manufacturers is to optimise the cosmetic and oral health benefits, but to limit the physical damage to the oral environment. Often calcium carbonate, dicalcium phosphate, silica and alumina oxide are used to provide an abrasive action. The action of these compounds however is complex; chemically identical components can result in different abrasion characteristics, and synergism may exist between formulations. It is also possible that enamel abrasivity values may differ significantly without major changes in dentine abrasivity (Wulknitz, 1997). Stain removal properties of toothpastes are often assessed based on a method developed at Indiana University in 1982 by Stookey *et al* (Stookey *et al*., 1982); bovine slabs are conditioned and stained and then mechanically brushed for stain removal.

The effects are then assessed visually. Correlations between cleaning power and dentine abrasion have been found to be low and this could be due to different abrasive types, particle surface and size and the chemical influence of other ingredients (Wulknitz, 1997). The staining procedure involved treatment with several different chemical solutions (hydrochloric acid, sodium carbonate and phytic acid) in order to maximise stain uptake and care should be taken when interpreting these results. The chemical action during the staining process may have inadvertently affected the efficacy of one compound over another.

2.3.6 The synergy of erosion and abrasion

The synergy of erosion and abrasion in relation to tooth surface loss was suggested as early as 1980 (Davis and Winter, 1980). Studies have shown an increased susceptibility of primary tooth enamel to erosion, when measured using profilometry (Hunter *et al*., 2000) and transverse microradiography (Amaechi *et al*., 1999), but others (using AFM) have failed to do so (Lippert *et al*., 2004). *In vitro* models have also demonstrated synergy between erosive slurries and *attrition* against the opposing tooth (measured using a profilometer), again with synergy in primary enamel but not permanent enamel (Correr *et al*., 2007). This, and similar study (Hemingway *et al*., 2006a) took no account of the buffering effect of saliva or pellicle formation, both of which have been shown to protect against erosive challenges (Amaechi *et al*., 1999). Nonetheless, there seems to be a general consensus that the eroded enamel surface is more susceptible to abrasion. The suggestion is that erosive challenges may make the tooth surface more susceptible to tissue loss. This is confirmed by profilometry *in vitro* (Wiegand *et al*., 2007b; Wiegand *et al*., 2006) where the effects of manual, rotary and ultrasonic

toothbrushes on eroded enamel have been investigated. Further, the abrasion resistance of eroded enamel increases proportionally with the remineralisation time.

2.3.7 In situ testing

In vivo measurements would accommodate the *intra oral* factors mentioned above, and a randomised controlled trial (RCT) would undoubtedly be the most robust model when testing the erosivity or preventive properties of products. The main problem is that when using fixed reference points intra-orally, optical instruments are unable to measure small surface changes (Huysmans *et al*., 2011a). West (West *et al*., 2011) described further problems such as uncertainty of patterns of disease progression, lack of control with respect to abrasive forces and minimising extraneous sources of erosion. Despite these problems, and given the ethical problems of carrying out RCTs, intra-oral appliances are the next-best option so that samples can be removed from the oral environment and tested *in vitro*. These are known as *in situ* models, and although it has been suggested that an intra-oral appliance may change the ecology of the mouth (Brill *et al*., 1977) the design allows a degree of standardisation to be built into the study, with the stepwise introduction or analysis of variables. Participants can be prescribed removable-appliances (West *et al*., 2004; West *et al*., 2003), or apparatus that is fixed to their teeth (Amaechi *et al*., 2010; Amaechi and Higham, 2001). Both allow the enamel samples to be exposed to the oral environment. The latter, fixed, option overcomes potential problems of compliance but does not easily allow intermittent removal for measurement or treatment; it may be desirable to remove samples in order to subject them to abrasion *in vitro* - despite accounting for salivary presence and formation of the dental pellicle, the variability of individual tooth

brushing techniques (such as brush type, paste type and quantity, brushing time, consistency of brushing, compliance with protocol) is difficult to control. Nonetheless an *in situ* study by Pickles *et al* (2005) used enamel and dentine samples embedded into the polished surface of complete denture wearers in order to compare the abrasivity of different toothpastes. The authors came to a reasonable conclusion, that whilst *in vitro* tests have value in understanding differences in abrasivity between products, *in situ* tests are more appropriate for predicting *in vivo* effects. Ultimate control of intra-oral factors relies on animal models, where the issue of compliance can be avoided, and diet more closely controlled. An *in vivo* study in rats (Sorvari *et al*., 1996) allowed complete control over intra-oral factors, and showed a synergistic effect of erosion and abrasion – enamel loss measured by scanning electron microscopy was greater for the rats that were given more fibrous food with an acidic sports drink. It is unclear whether the findings of this and other animal studies are valid in the context of the human population.

2.3.8 Modifying factors and surface effects

2.3.8.1 Fluoride

The protective effect of fluoride has been investigated in a number of erosion and abrasion studies. Samples are typically subjected to repeated cycles of de- and re-mineralisation prior to abrasion and surface measurement. *In vitro* and *ex vivo* studies report using carbonated drinks such as Sprite® (Vieira *et al*., 2007; Larsen and Richards, 2002; Attin *et al*., 1999) or Coca Cola® (Rios *et al*., 2008b; Magalhães *et al*., 2007), orange juice (Ren *et al*., 2011) or acids (Huysmans *et al*., 2011b; Wiegand *et al*., 2009a) in order to simulate an erosive challenge.

Currently the most common form of fluoride delivery is within toothpaste as sodium fluoride (NaF). Magalhães (Magalhães *et al*., 2007) reported the protective effects of fluoride on eroded human enamel that was subjected to brushing abrasion. Samples brushed with dentifrice containing fluoride showed significantly less surface loss (5.38 µm vs. 6.84 µm for the control, P = 0.04) and less percentage change in surface microhardness (45.7% vs. 54.6% for the control, P = 0.04). The findings contradict those of Rios (Rios *et al*., 2008b) who later failed to show a protective effect for fluoride in toothpaste up to 5000 ppm (surface loss of 4.40 µm for 1,100 ppm Fluoride vs. 4.26 µm for 5000 ppm Fluoride, P>0.05). The study design was very similar to that used by Magalhães, however bovine enamel was used instead of human, and the immersion time in cola was for only for 1 minute, 4 times per day compared to 5 minutes, 4 times per day. The authors suggest that this negative finding may be due to the limited effects of fluoride at shorter immersion times, or the differences in abrasivity of toothpastes used between the studies. Ren (Ren *et al*., 2011) showed, using a double-blind randomised controlled crossover study, that a 5000 ppm fluoride toothpaste confers significantly more protection against erosion than a 1450 ppm fluoride toothpaste; orange juice was used to erode the human enamel samples for ten minutes (n=16) and the surfaces were assessed using 3D scanning microscopy. Interestingly this longer immersion time also showed a protective effect for fluoride (Fluoride present median surface loss 5.7 µm vs. control surface loss 12.6 µm, P < 0.05). Despite the evidence for a protective effect, it has been suggested that fluoride toothpastes alone are not capable of *completely* inhibiting tooth wear (Moretto *et al*., 2010); even the use of 5000 ppm fluoride toothpastes can still result in detectable tooth surface loss in bovine enamel blocks after *in vitro* cycles of demineralisation in

Sprite™ and remineralisation in artificial saliva. Further, it has been shown at below pH3 the protective effect of fluoride is diminished (Larsen and Richards, 2002).

The formulation of fluoride has also been shown to significantly alter the protective effects; Wiegand (Wiegand *et al*., 2009a) investigated the effects of 0.5% and 1% sodium, amine and stannous fluoride solutions (NaF, AmF and SnF_2, respectively) in protecting against erosive enamel loss *in vitro* when subjected to hydrochloric acid (pH 2.6) for 90 seconds. Samples were allowed to remineralise for one hour in artificial saliva following each erosive challenge, and surface change was measured using profilometry. Only SnF_2 (at both 0.5% and 1%) and AmF were capable of significantly reducing enamel erosions (Enamel loss SnF_2 0.6 µm, AmF 0.9 µm, control 2.3 µm, P,0.05). Huysmans (Huysmans *et al*., 2011b) also showed a greater protective effect for SnF_2 toothpaste than NaF paste; 12 participants wore acrylic palatal appliances containing human enamel slabs for this *in situ/ex vivo* trial. Immersion for 5 minutes in citric acid (pH 2.3) was used as the erosive challenge before brushing. Surface changes were assessed using optical profilometry and showed a significant reduction in erosive wear for the SnF_2 paste compared to the NaF paste (reduction in erosive wear for SnF_2 35%, NaF control 7%, $P < 0.05$).

Toothpastes are not the only mode of fluoride delivery; fluoride gels, varnishes and rinses have also been studied. Attin (Attin T, 1999) exposed 64 slabs of bovine enamel to Sprite™ for 5 minutes *in vitro* after treatment with an acidified fluoride gel. Attin observed a higher toothbrush-abrasion resistance compared to non-fluoridated or pH neutral gels when measured using stylus profilometry. This study used artificial saliva,

and the model did not allow for pellicle and buffering effects at the same time as the erosive or abrasive challenges.

Tetra fluoride rinses (ZrF_4, HfF_4, TiF_4) have also been shown to be beneficial, conferring a protective effect against erosion when assessed by cumulative calcium release, SEM, micro hardness and profilometry (Vieira *et al*., 2011; Wiegand *et al*., 2008a; Hove *et al*., 2007a). These studies did allow for formation of a salivary pellicle *in vitro*, but saliva was not present during the erosive or abrasive challenge.

Fluoride may also be applied as a surface varnish, which has been shown to confer protective effects against erosion (5 minutes in Sprite™) when measured by optical profilometry (Vieira *et al*., 2007). The degree to which protection is conferred by a varnish alone is not reported in the literature.

The effects of fluoride have been attributed to the formation of a CaF_2 precipitate on the tooth surface (Ganss *et al*., 2004). Multiple studies have shown that increasing the concentration of fluoride further improves the resistance to erosion (Gracia *et al*., 2010; Zero *et al*., 2006), and it is thought that this effect is due to a thicker, more stable, layer of the precipitate (Wiegand *et al*., 2009a).

In summary the ability of fluoride to modify the effects of erosion at the tooth surface is well-documented. When investigating erosive and abrasive challenges *in situ*, there may be a need to account for the presence of Fluoride in order to ensure that the model is clinically relevant. In contrast it is purported that *in vitro* study investigating

only the early mechanisms of erosion and abrasion should be carried out in the absence of the modifying effects of Fluoride.

2.3.8.2 Electrolytes and other additives

Although saliva is around 99% water by volume, the remaining components are important to ensure an individual's oral health. These remaining components comprise electrolytes (such as calcium, phosphate, bicarbonate and potassium) and proteins (in the form of enzymes, immunoglobulins, glycoproteins and proline-rich proteins). Their main functions in relation to the integrity of dental hard tissues relate to:

i) modulating remineralisation and demineralisation of enamel

ii) buffering of acidic challenges, either endogenous from intra-oral bacteria or exogenous from dietary or environmental components.

The protective importance of the electrolytes has been shown in various studies; Davis (Davis *et al*., 2007) reported the beneficial effect of calcium when added to pure fruit juices. In this *in vitro* study, 64 human enamel specimens were submersed for 25 hours at room temperature before analysis with polarised light microscopy. Calcium-fortified juices were sufficient to prevent erosion occurring – the mean enamel surface loss for natural juice made with apple was 106 µm, orange 69 µm and grapefruit 187 µm. There was no detectable surface loss for the same juices containing calcium ($P < 0.001$). These findings were also supported by Magalhães (Magalhães *et al*., 2009a) who found that modification of carbonated fruit juice-based drinks with calcium reduced their erosive potential. 90 bovine enamel samples were immersed in erosive

solutions for 1 minute prior to remineralisation in artificial saliva for 59 minutes. This cycle was repeated 6 times over 24 hours, and enamel loss was measured by profilometry. Attin (Attin *et al*., 2003) modified 1% citric acid (pH 2.21) with calcium, phosphate and fluoride and subjected 156 bovine enamel specimens to demineralisation for 1 minute followed by remineralisation in artificial saliva for one minute. The procedure was cycled 5 times prior to surface measurement with laser profilometry and microhardness testing. Despite showing a protective effect whilst using relatively low levels of fluoride (maximum 0.9 ppm) enamel dissolution could not be completely prevented.

Despite the demonstrated protective effects of electrolytes, it has been suggested that consumers may reject the altered palatability and texture of the modified drinks (Ramalingam *et al*., 2005).

The protective effects of milk (McDougall, 1977) and cheese (Glabska *et al*., 2007; GedaliaI, 1991) have been attributed to a high content of calcium and phosphate ions. Recent studies have shown the protective effects of a commercially available product, Tooth Mousse®. Tooth Mousse® consists of a casein phosphopeptide and calcium phosphate solution (CPP-ACP). Casein phosphopeptides (CPP) are phosphorylated casein-derived peptides. Their protective activity is attributed to their ability to stabilise high levels of the amorphous calcium phosphate at the surface of the tooth. This prevents demineralisation and promotes remineralisation. This was confirmed in a study that sectioned 30 human enamel incisors into treatment and control halves (Poggio *et al*., 2009); specimens were immersed in Cola® for 2 minutes *in vitro* with

and without the presence of CPP-ACP. The surfaces were measured using AFM showing a reduced roughness and lesion depth for the specimens treated with CPP-ACP. Ranjitkar (Ranjitkar *et al*., 2009a) investigated the modifying effects of CPP-ACP for tooth wear under severely erosive conditions; 36 human enamel specimens were subjected to 10,000 wear cycles at a load of 100N and pH 1.2. For one experimental group, the machine was stopped every 2 minutes to allow CPP-ACP paste to be applied for 5 minutes. The resultant surfaces were analysed by 3D profilometry and stereomicroscopy, showing a reduction in tooth wear for the group treated with CPP-ACP paste. Quartarone (Quartarone *et al*., 2008) also showed a protective effect against erosion for CPP-ACP paste using 24 human enamel samples exposed to Cola® and Gatorade™ for 10 minutes *in vitro*. The paste was applied to a sub group of the samples for 5 minutes after the second immersion (out of a total of 20). The resultant surfaces were assessed by AFM and showed that the CPP-ACP paste significantly reduced the depth of erosion cavities caused by the drinks. Although the above studies involved the introduction of CPP-ACP paste, natural CPPs are found in yoghurt; *in vitro* testing has shown a significant inhibitory effect for these natural casein phosphopeptides on demineralisation, and promotion of remineralisation at the tooth surface (Ferrazzano *et al*., 2008). 80 human enamel specimens were demineralised for 96 hours in 0.1M lactic acid. Sub groups had 50ml of natural yoghurt added. Resultant surfaces were assessed with quantitative weight analysis and SEM, showing a remineralisation effect of CPPs in the yoghurt.

Some researchers have added UHT milk to carbonated drinks and observed less reduction in surface micro hardness compared to the unmodified drinks, but no

significant difference in loss of tooth structure when measured by profilometry (Syed and Chadwick, 2009). A perhaps more palatable option involves the single addition of calcium to carbonated drinks (Magalhães *et al*., 2009a), described previously. Calcium added to other potentially erosive products such as sour candies has also resulted in a decrease in the erosive potential (Jensdottir *et al*., 2006b) when measured by the degree of the participants' salivary saturation with respect to hydroxyapatite.

The addition of calcium to fruit juice drinks and carbonated drinks has been shown to reduce erosive potential *in vitro* and *in situ* (Davis *et al*., 2007; Hughes *et al*., 1999a; West *et al*., 1999). The latter study was a blinded, randomised placebo-controlled crossover design which concluded that a similar level of erosion in the same drinks modified with added calcium would take in excess of 100 years.

The addition of other organic components has also been investigated. Hemingway (Hemingway *et al*., 2008) reported the modifying effect of ovalbumin on citric acid-based erosion; *in vitro*, an addition of 0.2% w/v ovalbumin reduced the dissolution rate of hydroxyapatite discs in citric acid by around 50% when measured by changes in calcium concentration. It has been reported that a 0.02% w/v addition of casein to citric acid solutions can significantly reduce the hydroxyapatite dissolution rate (between 50-60%) when measured by changes in pH (Barbour *et al*., 2008); this effect was also shown in the presence of a salivary pellicle. This experiment subjected hydroxyapatite discs to citric acid solutions, but not in the direct presence of the protective effects of saliva. When assessed by calcium and phosphate release, 0.5% sodium dodecyl phosphate has been shown to be as effective as 0.03% fluoride at

inhibiting dietary-acid-mediated demineralisation *in vitro* (Rees and Fowler, 2009). Some researchers have investigated the efficacy of metals and metallic compounds to modify enamel erosion; profilometric results from *in vitro* tests show that iron-containing gels (Bueno *et al*., 2010) and matrix metalloproteinase inhibitors (MMPI) (Kato *et al*., 2010) are capable of interfering with the dissolution of the enamel and dentine respectively. Schlueter *et al* (Schlueter *et al*., 2010) studied tin-containing solutions *in situ* and found that a $SnCl_2$ (1900 ppm) and F^- (1000 ppm) solution was effective in reducing enamel erosion when assessed by profilometry.

The modifying effects of various polymers added to citric acid solutions *in vitro* has also been investigated (Beyer *et al*., 2010). SEM and AFM nanohardness tests concluded that the addition of alginate and gum arabic polymers to citric solutions resulted in a significantly harder surface compared to standard citric solutions. The authors hypothesised that the polymers adsorb to the eroded enamel surface. These results should be interpreted with caution, as the samples were stored in deionised water after preparation until use, and it is purported that this has the potential to disrupt levels of mineralisation within the samples (deionised water is under-saturated with respect to calcium and phosphorus ion that may cause precipitation from the enamel surface) . Other recent *in vitro* studies investigating the addition of various minerals to citric acid solutions have failed to show a protective effect (Magalhães *et al*., 2010), yet the addition of shrimp paste (high in calcium) seems to reduce the erosive potential of tamarind juice and potentially re-harden already softened enamel (Chuenarrom and Benjakul, 2010) when measured by profilometry and microhardness testing.

2.3.8.3 Saliva and soft tissue effects

The total volume of intra-oral saliva present at any one time amounts to approximately 1ml. The clearance, buffering capacity and remineralisation capacity of saliva are well documented (De Almeida *et al*., 2008; Berkovitz *et al*., 1999). Protective effects of saliva were demonstrated by Jaeggi (1999) and Attin (2001) who both found that toothbrush abrasion of enamel was significantly lower after a 60-minute exposure to the oral environment than after no exposure. In these and similar studies (Rios *et al*., 2006a), the samples were held and brushed *in situ*, but no saliva was present during the *ex vivo* acidic challenges. There were also potential issues with compliance, as the intra-oral appliances within these studies often had no protection against soft tissue abrasion and subjects were asked not to touch their appliance with their tongue. Frictional forces of the tongue have been shown previously to significantly abrade eroded enamel (Vieira *et al*., 2006; Gregg *et al*., 2004). One study (Amaechi *et al*., 2003) did attempt to prevent soft tissue and food abrasion and concluded that erosion is a combined effect of acidic softening and soft tissue and food effects. Jensdottir (Jensdottir *et al*., 2006a) found that the presence of salivary proteins *in vitro* can reduce the erosive potential of a cola drink by up to 50%; however, the protective effect of the proteins was dependent on pH, having a peak benefit around pH2.5.

2.3.8.4 Fluid dynamics, surface chemistry and the dental pellicle

The presence of saliva itself can also have significant surface effects which act either as:

i) a shear force, displacing an acidic challenge that may be in contact with the hard tissues or

ii) a barrier to prevent the contact of an acidic challenge.

The ability for saliva to act as a barrier to an acidic challenge will, in part, be a function of the viscosity of the saliva (Ireland *et al*., 1995); the work required by a drink to displace a film of saliva depends primarily on the thermodynamic work of adhesion. This is the energy required to break bonds between the saliva and the tooth surface. The work of adhesion in turn depends on a number of factors such as the surface tension of the saliva, and the angle at which the drink is approaching the tooth surface. Ireland suggested that it should be easier to displace a thin coating of saliva by a soft drink than vice versa, but this phenomenon doesn't seem to obey surface thermodynamical analyses. It is thought that this may be due to other variables such as the temperature of the drink, potential miscibility of the two fluids and chemical composition of the drink. The rate at which an erosive liquid flows over the enamel surface is also an important factor (Shellis *et al*., 2005); *in vitro* this has been demonstrated using citric acid as the erosive solution. The levels of erosion increased with increasing velocity of the liquid. Eisenberger (Eisenberger and Addy, 2003) also simulated laminar flow (by passing fluids through a straw) and confirmed a higher degree of enamel softening with increasing flow rate. Shellis (Shellis *et al*., 2005) suggests that because of the potential effects of flow rate "reproducible stirring is essential for precision and inter-comparison of *in vitro* studies". In reality there will always be *some* movement that occurs within a fluid, for example, when the samples are introduced or removed from the medium. Controlling the dynamics of this fluid

movement would be almost impossible; however some deliberate stirring may help to standardise the process. Fluid movement is essential within a clinical model; indeed it is necessary in order to prevent inhibition under a saturated layer - agitation or stirring will enhance the dissolution process because the semi-static layer surrounding the tooth surface (Nernst layer) is replaced frequently before reaching saturation. Barbour (Barbour *et al*., 2003b) subjected specimens to rapid stirring, in order to simulate subjects 'swishing' fluids around the mouth. It was found that this treatment significantly increased the erosive effects. This study also reinforced the clear relationship between increasing temperature and levels of erosion demonstrated in other studies (Barbour *et al*., 2006; Barbour and Rees, 2004; West *et al*., 2000).

Despite the complex surface dynamics, the ability of saliva to form a protective dental pellicle is highlighted in a number of studies. The dental pellicle (sometimes called the acquired pellicle) is a protein layer which adsorbs selectively (Lendenmann *et al*., 2000) to the dental hard tissues when exposed to saliva (Young *et al*., 2000). The layer is thought to form relatively quickly (within a few seconds), and transmission electron microscopy studies suggest that globules (Hannig, 1997) or knots (Busscher *et al*., 1989) around 200-300 nm in diameter are formed. It is thought that the globules are similar in structure to casein micelles in milk (Rollema, 1992) (a collection of molecules dispersed in a liquid colloid; particularly stable and containing relatively high levels of calcium and phosphate). The layer acts as a 'dynamic biofilm' which can modify events at the tooth surface (Lendenmann *et al*., 2000); the solubility of the enamel surface is reportedly reduced by proteins and mucins that display selective permeability for minerals and acidic solutions (Featherstone *et al*., 1993).

Some studies do account for the salivary pellicle, usually allowing development *in situ* within an appliance (Giles *et al*., 2009), but also in artificial saliva *in vitro* (Attin T, 1999). Most studies do not expose samples to saliva during the erosive (Attin *et al*., 2001) or abrasive (Hooper *et al*., 2003a) challenge phases; for example *in situ* tests with bovine enamel slabs have shown a protective effect against enamel erosion (Hara, 2006; Hannig, 2003), but these tests failed to account for salivary presence during the erosive challenge. An *in situ* randomised, blinded and split-mouth study by Giles (Giles *et al*., 2009) did account for pellicle and salivary presence whilst comparing the abrasivity of different toothpastes. Although the study was investigating effects on dentine, an important finding from the study showed that samples *within* treatment groups varied significantly; it is purported that the ability to control for individual salivary types and buffering capacities, pellicle formation and oral hygiene regimes/techniques is likely to be very difficult. This highlights the need for a robust *in vitro* model.

Finke (Finke *et al*., 2002) has shown that the thickness of the acquired pellicle *in situ* can be affected by drink consumption, time and individual host factors when measured by atomic force microscopy. A drink with a relatively high ionic state, and higher molecular weight polymers, such as xanthan gum, will result in a thicker pellicle (Rykke and Rolla, 1992). A thicker pellicle has been shown to be beneficial with a suggested inverse relationship between enamel dissolution and pellicle thickness (Amaechi *et al*., 1999). The thickness of the pellicle generally reaches a plateau after 30-90 minutes (Skjorland *et al*., 1995). Pellicles of different ages of maturity have also been studied (Hannig and Balz, 1999; Amaechi *et al*., 1998; Featherstone *et al*., 1993) with a general

consensus that the main protective effects of the pellicle are established relatively early on (within 60 minutes of the enamel being exposed to saliva *in situ*); 24-hour and 7-day pellicles do not seem to confer a significantly greater protective effect against erosive challenges. Toothbrushing can remove the pellicle layer, but the electron-dense basal layer (the most important layer with respect to enamel protection) forms again almost immediately (Hannig *et al*., 2004).

2.4 Tribology

Tribology is the study of the way in which surfaces interact in motion. In order to understand the mechanisms of surface change, it is important to consider certain tribological parameters:

2.4.1 Roughness

Roughness is a measure of surface texture and, as such, it can affect the way in which a surface wears (Stachowiak *et al*., 2004). It is quantified by the vertical deviations of the surface from its ideal form. Greater deviations will result in a rougher surface. However, the way in which this is reported can lead to a misinterpretation of surface features. The profiles shown in Figure 6 have the same average roughness, yet differ markedly with respect to their actual surfaces.

Since the original International Standards were published in 1984 (International Standards Organisation, 1984), over 100 variants to measure roughness have evolved.

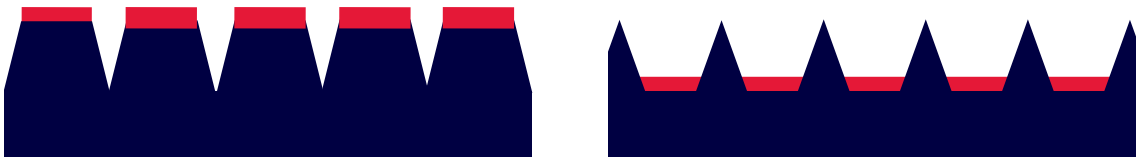


Figure 6 – Two profiles with the same R_a value but surface characteristics that differ markedly (red line indicates the 'ideal' form of the surface). The profiles have the same R_a value because they deviate from the ideal form by the same magnitude.

Within engineering systems, these variants often deal with average distances between the highest peaks and lowest valleys of the profile. They often also truncate certain outlying peaks and valleys, depending on the engineering system being used. These systems are increasingly complex and serve to qualify the effects of surface change in the best possible way for the system being tested. The main challenge is that, to the untrained investigator, the parameters are difficult to interpret in tabular form.

2.4.1.1 Roughness average (Ra) and Root mean square (Rq)

Within dentistry, the most common forms of reporting roughness are still the surface roughness average (R_a) or the root mean square (R_q) (McCabe *et al*., 2002). Both parameters measure the profile height deviations from the mean line across a profile. Ra denotes the *arithmetic* mean roughness whereas Rq denotes the *geometric* mean roughness (ASME, 2009). Each value uses the same individual height measurements of a profile's peaks and valleys; however whilst Ra calculates the average deviations from the mean line, Rq calculates the root mean of the square of those deviations. This is shown schematically in Figure 7. One can infer from the definitions that a single large peak or flaw within the microscopic surface texture will affect (raise) the Rq value more than the Ra value.

2.4.2 The bearing curve

Ra or Rq values convey no information about the textural characteristics of a profile, the likelihood of future wear or wear-resistance, the rate of future wear or the potential of a surface to retain fluids/lubricants. This makes the qualitative assessment

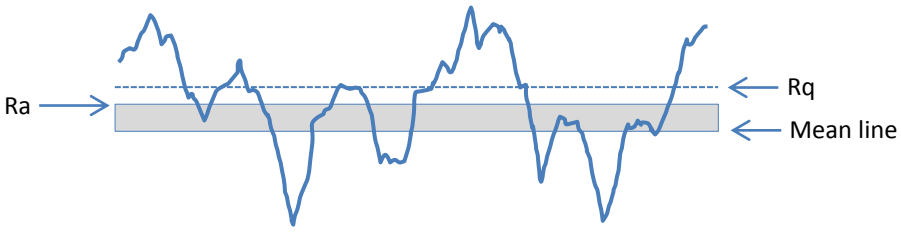


Figure 7 – Schematic of the difference between Ra and Rq – note that when amplitudes are squared, the Rq value becomes raised relative to the Ra value.

of the surface relatively limited, and empirical, and only relays information about fixed heights at regular intervals. Another way of describing the profile is to consider plotting the surface features in a curve that can be compared both quantitatively and qualitatively with other surface profiles. Abbott and Firestone defined the 'bearing area fraction' (Abbott and Firestone, 1933); this fraction represents the cumulative distribution of the lengths of individual plateaux that would result if the surface were abraded down to a level plane at that height. Essentially, a profile is examined on a level plane from the highest peak to the lowest valley; at each level of the profile, the amount of material intersected is reported as a proportion (or ratio) of the total surface area. The curve is normalised by the total assessment length. If this ratio was plotted against sample height, the bearing area curve results (Figure 8). The original use of the bearing curve was to assess newly-machined surfaces (Abbott and Firestone, 1933). This was important because it affected the 'run-in' of the surface (how it would perform in the early function). Outlying peaks (which represent early material loss) were then removed in order to 'finish' the surface, leaving a predictable and more resistant machined part. The profile also provided information about inherent valleys (which represent a reservoir for lubrication or pooling of fluid) (Anderberg *et al*., 2008; Jourani *et al*., 2005). Although the bearing curve would not be used in this way in order to grind down a tooth, a bearing curve measured *after* a surface challenge may provide a more extensive qualification of how the surface has been affected and how it is likely to change following future erosive, abrasive and synergistic challenges. If so, information obtained will relate to the life of the tooth surface, such as early tooth tissue loss, longer-term resistance and the ability of the tooth surface to retain fluid components under differing loading

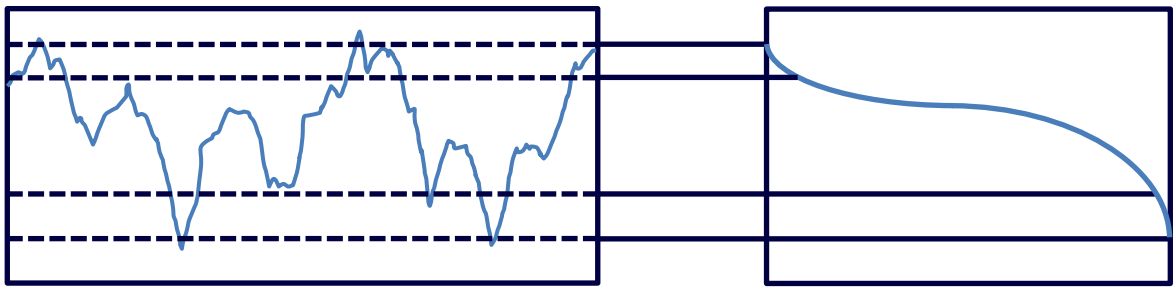


Figure 8 – The bearing curve that results as a cumulative distribution of plateaux lengths at varying heights. The curve is normalised by the total assessment length and when this ratio is plotted against sample height, the bearing area curve results. The x-axis represents the percentage of material across the assessment length (0-100%) and the y-axis represents the distance down through the profile (usually in micrometres).

conditions and after being subjected to a variety of mechanical and chemical insults.

2.4.2.1 Describing the bearing curve

A variety of sources describe how to interpret the bearing curve (Pawlus and Grabon, 2008; Schmähling and Hamprecht, 2007; Petropoulos *et al*., 2003; International Standards Organisation, 1996). The total height of the curve is known as the roughness total. The width of the curve represents the percentage of material at different profile amplitudes (Figure 9). Five parameters are determined from the curve – peak roughness (Rpk), core roughness (Rk), valley roughness (Rvk), material ratio of peaks (MR1) and material ratio of troughs (MR2). These bearing parameters are defined using German standard DIN 4776 and more recently ISO 13565-2. The value of Rk is found by software searching the curve for its 'flattest' portion. This is defined as the portion bounded by a chord spanning a 40% bearing ratio, which has the lowest slope. The chord is then expanded to reach the 0% and 100% axes. The point at which the chord hits the y axis is then traced across to the curve. The corresponding y values equate to Mr1 and Mr2. The Rpk value represents the roughness of the material peaks – the area that will come into contact with an opposing surface first; in engineering it indicates the amount of material that will wear away during early contact of the machined surface. The Rk value represents the roughness of the material core – the area that will support most of the contact load (in engineering this represents the longer-term running surface). The Rvk value represents the roughness of the material troughs/valleys – the area that will retain fluid or lubricant. These are known as the reduced peak height, core roughness and reduced trough depth respectively. The curve can also relate Rpk and Rvk to a proportion of the material's surface (Figures 9

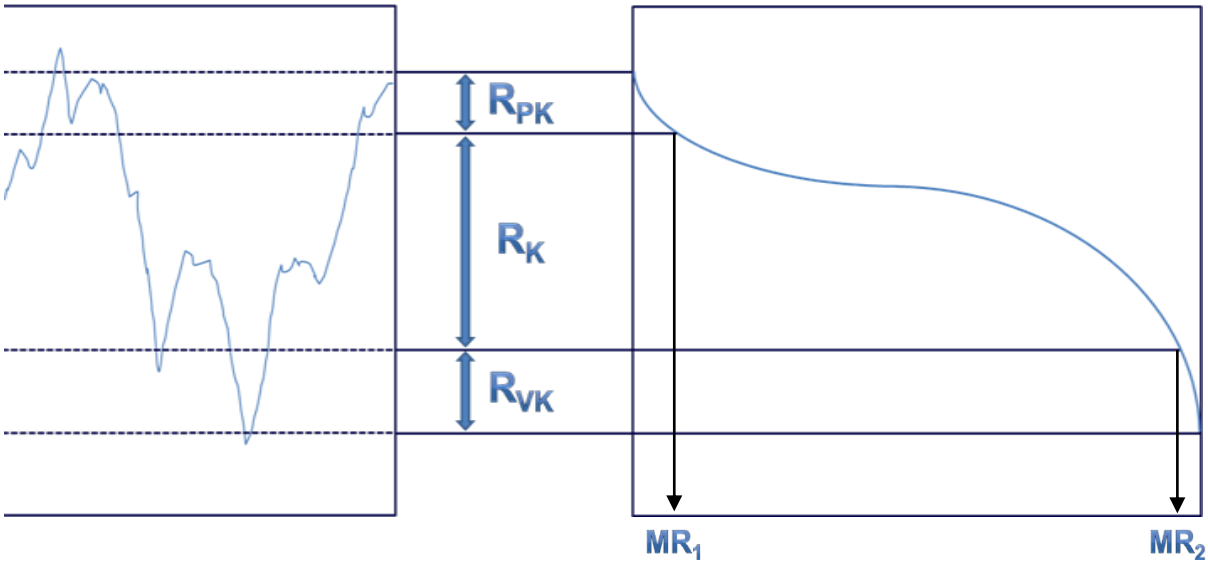


Figure 9 - Quantifying parameters on the bearing curve. R_{pk}, R_k and R_{vk} values relate to peak, core and trough average roughness values. Mr_1 and Mr_2 relate to the percentage of the surface occupied by peaks or troughs respectively.

and 10). The latter are known as the *material ratios* – MR1 relates to the *proportion* of peaks within the sample, and MR2 relates to the *proportion* of troughs.

It has been suggested that the bearing curve is better for assessing surfaces that are subjected to several different machining processes than Ra (Pawlus and Grabon, 2008; Bačová and Draganovská, 2004; Torrance, 1997). One process may remove the peaks but then a finer texture may be superimposed onto the resulting plateaux by a subsequent process; the deep valleys may remain unaffected. The resulting surfaces are known as 'multi-stratified' (Thomas, 1999). Characteristically, these surfaces are negatively skewed which makes it difficult for a parameter such as Ra to represent them effectively. In relation to tooth tissue, the bearing curve could also be used to measure the surface effects of a synergistic process, like that of erosion and abrasion. It may be possible using the curve, to explain the bi-phasic pattern of tooth surface loss which has been previously identified and described as 'running-in wear' and 'steady-state wear' (Kaidonis *et al*., 1998).

The bearing curve has been used to assess engineered surfaces in medicine, particularly that of femoral stem wear (Howell *et al*., 2004). Its use to monitor and qualify labial erosion *in vivo* (via silicone models) was piloted in 1997 (Whitehead *et al*., 1997) and, more recently, it has been used to describe the native enamel surface (Las Casas *et al*., 2008). The latter study showed the potential use of the bearing curve in further qualifying the tooth surface; however data were presented in tabular form only. There may have been more value in displaying the bearing area curves for visual comparison.

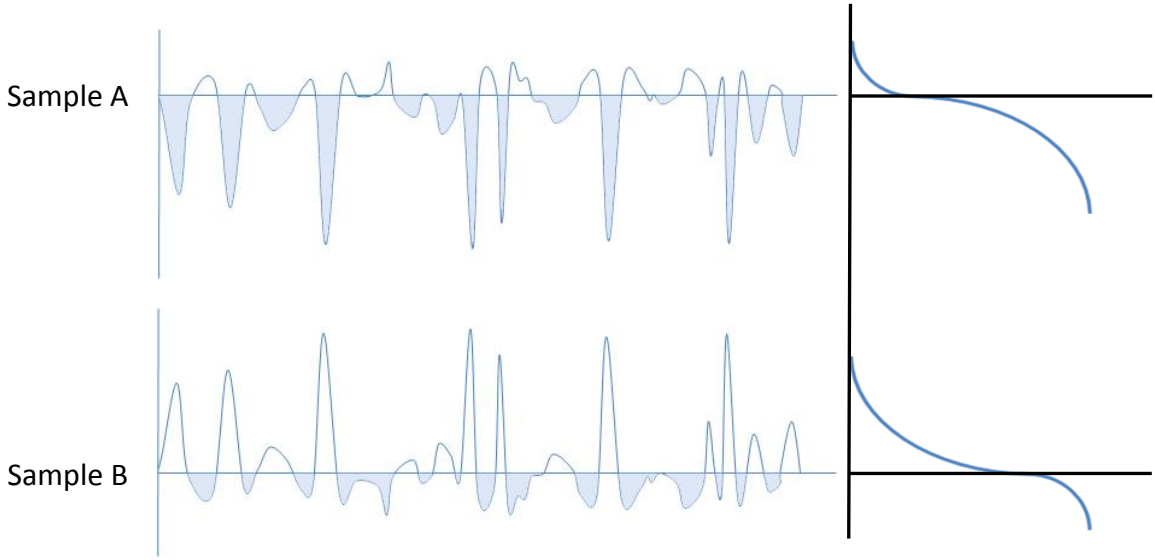


Figure 10 – Comparison of two different surfaces with similar R_a using the bearing curve. Surface A has significantly more, deeper troughs (higher R_{vk} and higher Mr_2). Surface A therefore has a much greater pooling/lubricative potential. Surface B has significantly more, higher peaks (higher R_{pk} and higher Mr_1) and is therefore likely to suffer significantly more early surface loss in the future.

One disadvantage of the bearing curve is that it does not describe spatial information pertaining to the number of isolated voids and peaks in the surface. Voids combined with their contour length and area could be used to describe the frictional behaviour of the surface (Schmähling and Hamprecht, 2007), relating to roughness, fluid flow and connectedness of the peaks. The isolated voids and peaks can be described using the Euler characteristic, which counts the number of objects (peaks) minus the number of holes (voids). The Euler characteristic (χ) is defined for a surface according to Formula 1 (Schmähling and Hamprecht, 2007) (Figure 11). A negative Euler characteristic would indicate predominantly holes and *vice versa*. This can result in fluid being trapped in the holes and would potentially be of great interest in wear studies.

2.5 Concepts of *in vitro* testing

2.5.1 General design

It is clear that there are significant variations in study design for erosion and abrasion testing of dental hard tissues; most frequently the number of erosive/abrasive cycles, the particular erosive/abrasive condition(s) and exposure time(s) (Wiegand and Attin, 2011). At one end of the scale, studies use few repetitions with severe challenges, and at the other end, studies use many repetitions, but short erosive or abrasive challenges. Because of this level of variation it is not uncommon for studies to show exaggerated results (Ganss *et al*., 2007b; Hughes *et al*., 1999a). There is often a degree of standardisation within experiments, but this inter-experimental variability can become a problem when different experiments need to be compared. *In vitro* testing allows a controlled exposure, the study of various combinations of agents, a controlled tissue type, and control over temperature and the buffering capacity of

$$'\chi = V - E + F'$$

Formula 1 - V, E, and F are respectively the numbers of vertices (corners), edges and faces in the given surface.

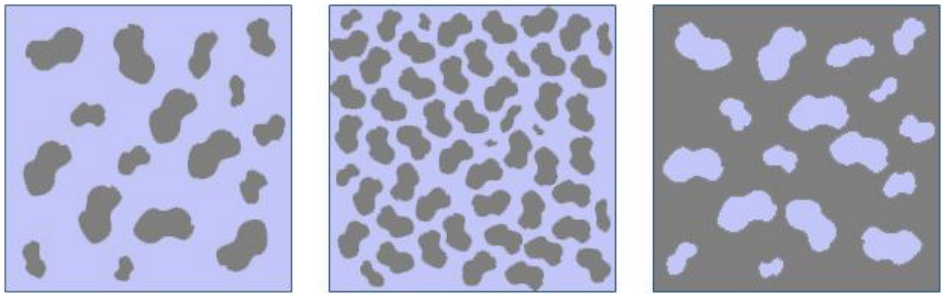


Figure 11 – Surfaces displaying different Euler characteristics (Left, high; middle, equal; right, low). Blue represents materials, grey represents voids.

saliva. Several combinations of factors can be investigated, and the concept of *in vitro* testing is therefore useful for demonstrating wear 'propensity' (Lambrechts *et al*., 2006). Despite this level of control, *in vitro* testing will not fully replicate the oral environment. To this end, only trends and indications can be obtained (Lambrechts *et al*., 2006).

2.5.2 Sourcing samples

Ideally teeth used in clinical research should be representative of the population. However this makes interpretation of the findings more difficult - the environment that surrounds human teeth can vary in terms of diet, anomalies during growth or eruption, oral hygiene, salivary composition, and interventions such as fluoride therapy. Thus bovine teeth are often used based on the assumption that their environment will have been more consistent; they are also easier to acquire in bulk at an abattoir. If cattle are culled aged 18-36 months, all 6 permanent incisors will be available.

2.5.3 Storage techniques

The logistics of *in vitro* testing mean that samples must often be stored prior to use. This may mean storage of intact teeth, or that of the prepared sample or surface. A number of different storage media are reported in the literature such as distilled water (Ren *et al*., 2011; Yu *et al*., 2009; Zheng *et al*., 2009), formalin (Moretto *et al*., 2010; Ranjitkar *et al*., 2009a; Ferrazzano *et al*., 2008), thymol (Turssi *et al*., 2010; Hara *et al*., 2009; Ranjitkar *et al*., 2009b) and saline (Ren *et al*., 2009b; Willershausen *et al*., 2008).

Although many *in vitro* measurement techniques only examine a thin surface layer (<1 µm), the assumption is made that the measurements are representative of the bulk material. A change in *surface* structure is therefore an important consideration (Habelitz *et al*., 2002). It has been recommended that hard tissue samples are stored in wet conditions at all times to prevent desiccation (Attin *et al*., 2009). It has also been demonstrated that storage of enamel and dentine specimens in de-ionised water, or solutions of acidic pH, for one day reduces mechanical properties (as measured by elastic modulus and hardness) by up to 30% (Habelitz *et al*., 2002). Further storage up to one week reduced mechanical properties to below half of the original values. The study recommends storage in Hanks' Balanced Salt Solution (HBSS), which displayed no significant effects on mechanical properties over a period of 2 weeks. The choice of storage medium may therefore have important implications for surface changes, and although a number of *in vitro* studies exist to investigate the effects of storage on bonding characteristics, no published study describes the effects of fluid storage on enamel samples used in abrasive/erosive studies.

2.5.4 Disinfection/Sterilisation techniques

In order to maintain a safe and controlled research-environment, all biological hard tissue specimens should be adequately disinfected. In reality this means that specimens should be treated so that the risk of infection from viruses, bacteria, spores and prions is removed. Cleaning with sterile gauze prior to storage is not considered to be adequate; such practise was demonstrated by Ferrazzano *et al* (2008).

Often hard tissue samples that are to be disinfected in solution must remain immersed

for a reasonable amount of time (at least over an hour, often up to several days). The concentration of the disinfectant must also be prepared appropriately. In such cases, the process of infection control may be combined with the storage technique – for example, storage in Chloramine T or Thymol. Even so, some solutions (2% glutaraldehyde (Dominici *et al*., 2001), 2.6% sodium hypochlorite (Dominici *et al*., 2001) and 3% hydrogen peroxide (Kumar *et al*., 2005)) have failed to remove microorganisms even after 1 week of storage. The use of greater concentrations of sodium hypochlorite have been reported (5% for one hour (Poggio *et al*., 2009) or 24h (Giles *et al*., 2009; Quartarone *et al*., 2008) or 11% for several days (Cheng *et al*., 2009b)), however the use of these and other stronger solutions (10% formalin and 7.5% povidone iodine) may affect the structural integrity of the samples. There have been similar findings for steam autoclaving (typically at 134°C for 3 minutes) (Kumar *et al*., 2005; Dominici *et al*., 2001). Ethylene oxide is often favoured due to its gaseous form that leaves no residue on the samples. Samples are exposed for 12 hours (Turssi *et al*., 2010) followed by immersion in 0.5% chlorhexidine plus 70% ethanol for 30mins (Ren *et al*., 2011). The process can take several hours, and at room temperature ethylene oxide is flammable, carcinogenic and mutagenic (Bruce *et al*., 1985). A number of studies have used gamma radiation (typically 25 kGy), either from the radio nucleotide Cobalt-60 or Caesium-137 (Currey *et al*., 1997). This method is highly effective, as the Gamma radiation completely sterilises the sample without altering its structural properties (Brauer *et al*., 2008; Moscovich *et al*., 1999; Amaechi *et al*., 1998; DeWald, 1997) or its resistance to demineralisation (Rodrigues *et al*., 2004) (when measured by surface microhardness). Barriers to the use of gamma radiation include the cost of the equipment and the lengthy sterilisation process.

More recently microwave irradiation (650 Watts for 3 minutes) has been shown to be successful for the disinfection of tooth tissue samples with no effect on surface microhardness for sound, remineralised or demineralised enamel (Viana *et al*., 2010).

2.5.5 Preparation and reference areas

The types of preparation, with respect to surface finish or choice of sample area, can have implications for how the tested surface will behave – erosive mineral loss increases with distance from the amelo-dentinal junction (Creanor, 2009). This is an important consideration given that the most common forms of surface analysis require a surface that has been lapped flat. Whilst there is a strong association between the particle size of the abrasive and the resulting surface striations within aprismatic enamel (Maas, 1991), no such association has been displayed for the remaining bulk of prismatic enamel. It has been suggested that the *shape* and *hardness* of the individual grit particles has more of an influence than the actual *size* in terms of striation morphology (Kay and Covert, 1983). Furthermore smaller grit size silicon carbide particles such as with ISO 400 and 600 (23 µm and 16 µm) respectively (International Standards Organisation, 1998) have been shown to be more uniform in shape than a larger grit size such as ISO 240 (53 µm) (Walker *et al*., 1978).

When enamel specimens are prepared for erosion and abrasion testing, the assumption is often made that specimens prepared in the same way will have the same surface characteristics. From published literature, it appears that investigators do not routinely report baseline surface characteristics – atypically, some have inspected specimens under the light microscope for surface defects, or carried out pre-

testing in order to select specimens of similar mechanical properties (Turssi *et al*., 2010). Care should be taken between tissues, given the previously mentioned lack of association between grit size and surface finish in prismatic enamel.

2.5.6 The erosive challenge

A number of factors contribute to the make-up of the erosive challenge, including the composition and delivery of the acidic solution, immersion time, flow rate and temperature (Zero, 1996). Models that determine the method of exposure to the acidic solution vary in complexity. The simplest method is to allow submersion of the sample into the acidic solution, without any other form of mechanical interference (Cheng *et al*., 2009b). The method by which a drink is imbibed will have implications for the acidity of the oral environment; Johansson (Johansson *et al*., 2004) showed that holding solutions in place caused the largest change in pH; gulping failed to display a significant change in intra-oral pH. The more complex models allow immersion, swilling/stirring and flushing either in isolation or in combination. A number of methods have been used to agitate fluids such as magnets and overhead or rotary stirrers (Hooper *et al*., 2007; Barbour *et al*., 2005; Hughes *et al*., 1999b). Regardless, fluid and surface dynamics become increasingly difficult to control and account for with increasing levels of mechanical intervention. Although this is undoubtedly also the case for increasing temperatures, there is a strong relationship between temperature and erosive potential of solution. The use of a water bath is recommended to ensure a consistent and accurate approach (Barbour *et al*., 2006) and although there is a risk of exaggerating levels of erosion, a temperature similar to that of the oral environment (around 30° Celsius) will provide a more accurate model.

It has been reported that the sipping of soft drinks can only cause the pH of the oral environment to drop significantly for between 2 and 3 minutes (Millward *et al*., 1997); the mean drinking time for 100ml has been shown to be around 18 seconds (Millward *et al*., 1997). Care should be taken with erosion studies that the chosen immersion time does not significantly exaggerate the clinical situation. Studies often over-expose samples (Bueno *et al*., 2010; Ehlen *et al*., 2008; Jensdottir *et al*., 2006a), whereas others choose more realistic immersion times (Poggio *et al*., 2009). This makes comparison of inter-study results difficult and certain models will relate more closely to a clinical scenario than others.

Fruit acids have a high buffering capacity, which sustains their pH (Touyz, 1983). Replication of the chemical composition of erosive agents (especially fruit juices, wines or carbonated soft drinks) can be extremely difficult. Because of this, researchers often choose a quantifiable indicator such as pH and titratable acidity (Ablal *et al*., 2009b; Ganss *et al*., 2009a; Ranjitkar *et al*., 2009a; Zheng *et al*., 2009; Willershausen *et al*., 2008; Barbour *et al*., 2003b) or percentage weight to volume acid (Reis *et al*., 2008; Bashir *et al*., 1995) in order to characterise the solution. Some solutions are even buffered in order to maintain the acidic challenge at a constant level for the duration of the experiment (Hara *et al*., 2009). This becomes further complicated when saliva (having its own natural buffering capacity) is added to the model. It is at this point that the use of an actual erosive agent rather than a standardised solution has more value. Interestingly, some are of the opinion (even recently) that saliva should not be added, as it would limit the demineralisation process and buffer the acidity of the drink (Tantbirojn *et al*., 2008). Clearly it is this paradox that may prevent some studies from

creating a more relevant clinical model. In contrast, other studies *have* accounted for the effects of saliva *in vitro* (which do confirm that its presence can significantly reduce levels of erosion) (Amaechi *et al*., 1999). There is some concern about particular components of artificial salivas such as carboxymethylcellulose (CMC) or mucins that may alter the re-hardening potential of the formulation (Vissink, 1985). The rehardening potential of CMC-containing salivas has been shown to be better than those containing mucin (Gelhard *et al*., 1983).

2.5.7 Saliva & Pellicle

It is purported that the use of natural saliva would provide the most realistic model but there are problems of collection, disinfection and consistency of sampling/inter-subject variability. Further, no data are available to detail for how long collected (pooled) human saliva can be stored before suffering any amount of degradation. A number of artificial saliva formulations could be used and these would allow a degree of standardisation within the models. Artificial salivas containing mucins have been shown to be as effective as pooled human saliva in protecting enamel against an erosive challenge (Hara *et al*., 2008). When *any* form of saliva is used it must be remembered that salivary clearance is specific to the individual (Bashir *et al*., 1995); the importance of saliva can be accounted for but care must be taken when extrapolating the findings to the general population.

When samples are exposed to natural or artificial saliva prior to the erosive or abrasive challenge, the enamel surface will immediately develop a pellicle layer. As discussed previously, the maturity of the pellicle is time-dependent (Finke *et al*., 2002). Some

pellicle will form within a matter of seconds. However there is no consensus within the literature in terms of study design - some allow only minutes for pellicle formation (Wetton *et al*., 2006; Hannig *et al*., 2004; Amaechi *et al*., 1999) and others up to several hours or longer (Magalhães *et al*., 2008; Wiegand *et al*., 2008a; Hannig *et al*., 2007; Hove *et al*., 2007b; Hara, 2006). Some studies allow the development of a pellicle *in situ* by seating the sample within a removable oral appliance (West *et al*., 2011). Indeed the pellicle formed *in situ* has been shown to be different to that formed *in vitro* (Carlen *et al*., 1998). Other methods include bathing the sample in saliva prior to the challenge; despite the environment under which the pellicle is formed, studies may then also allow for the presence of saliva *during* the challenge in order to mimic the complex dynamics of pellicle growth versus degradation during the experiment (Wiegand *et al*., 2010a; Pontefract *et al*., 2001; Lussi *et al*., 1997).

2.5.8 The abrasive challenge

Given the variety in dietary and oral hygiene habits of a population, there is value in investigating a number of abrasive challenges under a range of different conditions. This is reflected in the literature, with little standardisation between models. A number of studies seek to investigate the abrasive effect of toothbrushes and toothpastes (Voronets and Lussi, 2010; Wiegand *et al*., 2009b; Wiegand *et al*., 2008b; Magalhães *et al*., 2007; Wiegand *et al*., 2006; Hooper *et al*., 2003b), but often the abrasive challenge immediately follows the erosive challenge (Hunter *et al*., 2000). Although this scenario may present clinically, dentists will often advise patients against brushing immediately after an erosive challenge. There is still value in following this model but care must be taken to extrapolate its value only to those individuals who *do* brush immediately

after. For example, patients who are conscientious in terms of oral hygiene but unaware of the detrimental effects, or individuals suffering from reflux or vomiting who would prefer to practise oral hygiene procedures immediately after the acidic attack. Some studies consider delaying the abrasive challenge and allowing a period of remineralisation either by saliva, or other commercial products that have been developed specifically for the purpose of limiting the effects of erosion (Gracia *et al*., 2009). There is no doubt that abrasion resistance is significantly increased by storage in artificial saliva (Attin *et al*., 2000) or natural saliva intra-orally (Rios *et al*., 2006b). A time delay before brushing might model the clinical scenario more effectively. A number of studies (Wiegand *et al*., 2010a; Magalhães *et al*., 2009b; Ganss *et al*., 2007a) introduce a cyclical nature to the model that alternates between erosion, remineralisation, abrasion and remineralisation; however few studies incorporate less abrasive than erosive challenges, the reasons for which are not reported. It is unclear how this relates to a clinical scenario. It has also been suggested that *in vitro* brushing is generally more severe than *in situ* (Ganss *et al*., 2009b); indeed typically each tooth within the dental arch will only receive 10-15 strokes (or 5 seconds of activity for powered brushes) on each oral hygiene episode. In the majority of studies, samples are brushed manually and are subjected to much greater levels of abrasion than occurs clinically (Wiegand and Attin, 2011).

Studies that investigate the effects of brushing *in situ* (Lussi *et al*., 2004; Jaeggi and Lussi, 1999) are able to choose the location(s) at which the erosive/abrasive conditions are investigated. If the samples are placed anteriorly and palatally, it is purported that they will receive less contact with saliva, but be more exposed to beverages. At the

same time they may well receive less brushing force than the buccal aspects of more posterior teeth. In any case the appliances should be designed in order to control other abrasive effects that cannot be accounted for, such as those caused by the tongue or mucosa. Again, findings should only be extrapolated to the specific surfaces that were studied, and to those individuals practising the same oral hygiene procedures.

Studies have shown than brush type, technique and force can all affect tooth surface loss – the use of ultrasonic brushes results in less tissue loss than manual brushes when the same loading force was used (Wiegand *et al*., 2007a), and softer, narrower filaments result in greater tissue loss in dentine (Dyer *et al*., 2000). The latter is thought to be due to an increased contact of filaments with the surface, coupled with an increased retention of toothpaste by smaller diameter bristles. Individual brushing behaviour can also significantly affect which surfaces are visited (Macgregor and Rugg-Gunn, 1979). It is purported that *in vitro* study will allow more specific control to be placed over the abrasive factors, especially if the brushing is carried out by a dedicated machine. Nonetheless a brushing force will need to be chosen in order to programme the machine appropriately. Ganss (Ganss *et al*., 2009b) digitally recorded brushing force in 103 subjects that were uneducated in terms of oral hygiene practise. The average brushing force was 2.3N, with a maximum recorded value of 4.1N.

2.5.9 Simulated soft tissue effects

Some studies purposefully include the abrasive effects of soft tissues such as the tongue (Vieira *et al*., 2006; Gregg *et al*., 2004) in order to replicate the effects of the

oral environment to a greater degree. Care must be taken to ensure that the fine particles produced by the replicated soft tissue (for example a chamois leather over a toothbrush (Ablal *et al*., 2009a)) don't produce an element of three-body wear. Data show that the tongue is capable of increasing the rate of enamel loss, but this effect was not seen in dentine (Gregg *et al*., 2004). Studies into the effects of three-bodied wear (true mastication) involving a simulated food bolus are less common (Amaechi *et al*., 2003; Sorvari *et al*., 1996). Table 1 outlines some of the machines that have been reported within the literature. Each machine will operate within a specific range of loading force, brushing frequency and total number of cycles. The significant difference between the machines relates to the type of masticatory movement – for example sliding or striking the components together. Further, each machine is designed to operate with a specific abrasive component such as a grit suspension, plastic beads or a range of toothpastes. Models using these machines are also not without their complicating factors; the extent to which a rigid mechanical model can relate to a dynamic occlusion (with teeth able to move several millimetres within their periodontal ligament) is debatable. A number of studies have estimated typical chewing frequencies to be between 1.2Hz and 1.7Hz (Suzuki, 2004; Krejci *et al*., 1992), but less is known about the duration or method of tooth-to-tooth contact. More complex models have been devised, such as the 'Bristol mechanical jaw'. The jaw was constructed using a Stewart platform - a 3-D mechanism with six linear actuators that are able, in theory, to reproduce the specific motion and forces typically sustained by teeth within a human mouth. The authors reported that the mechanical jaw had the potential to dramatically improve the process of *in vitro* testing, although there is currently no evidence to support this statement.

In summary, there is little evidence in the literature of studies that describe or qualify the early surface effects of dental erosion, occurring before tooth surface structure is lost. There are conflicting reports on the suitability of using bovine enamel as a substitute for human enamel; there is a need to investigate this further, ideally also including enamel from other species that are, to date, unreported such as ovine enamel. A simple *in vitro* model is required that can reproducibly and reliably simulate early enamel erosion and abrasion, whilst allowing subtle surface changes to be recorded.

Name	Force	Frequency	Cycles	Movement type	Abrasive component/ wetting agent	Stylus	Other notes
ACTA (Nihei et al., 2008)	15-20N Spring	1.0Hz	100-200k	Sliding	Rice/millet seed suspension	-	-
BIOMAT (Yap et al., 1999)	2N Weights	-	-	Sliding and impact	Water	Counter-body	Shock-absorbing layer & temperature controlled
Minnesota MTS (Sakaguchi et al., 1986)	13.35N Hydraulic	-	120k-1.2m	Sliding	Water	Tooth	-
OHSU (Condon and Ferracane, 1996)	10-70N Electro Magnetic loading	1.2Hz	50-100k	Sliding and impact	Poppy seeds & PMMA beads	Enamel	-
University of Alabama Wear simulator (Leinfelder et al., 1989)	75.6N vertical	1.2Hz	100-400k	Sliding and impact	PMMA beads	Plastic	-
Willytech Munich (Kunzelmann et al., 2001; Sakaguchi et al., 1986)	50N Weights	-	120k	Gnashing, slippage or striking	Water, although other fluids could be used	Enamel	Thermo-cycling programmable
Zurich computer controlled masticator (Schmidlin et al., 2003b)	49N Electro Magnetic loading	1.7Hz	120k-1.2M	Sliding and impact	Water, alcohol and toothpaste	Enamel	0.2 mm lateral shift

Table 1 - Three-body wear machines adapted from (Lambrechts *et al*., 2006).

Chapter 3. Aims and objectives

3.1 Main aims

The review of literature identified inconsistent reports of surface changes both between human and bovine enamel, and across treatments for erosive and abrasive challenges. No data are reported for ovine enamel. Therefore a simple *in vitro* model was developed in order to measure early surface changes in human, bovine and ovine enamel using profilometry, SEM and microhardness testing, with the following aims:

- To investigate the surface effects on enamel of early erosive and abrasive challenges.
- To compare the enamel surfaces of human, bovine and ovine enamel after lapped, erosive and abrasive challenges.

3.1.1 Objectives

In order to achieve the above aims, it was important to meet the following objectives:

- To determine the citric acid content of a range of fruit-based solutions in order to accurately represent clinical challenges *in vitro*.
- To determine the dimensional stability of reference layers stored within a fluid medium to ensure that storage of the samples would not affect the outcome.
- To determine the longevity of a battery-powered rotary toothbrush to ensure that that life of the brush would not impact on the outcomes of the tests.

- To determine whether ovine and bovine enamel can be used as suitable substitutes for human enamel in erosion and abrasion studies.
- To determine if characteristics of the eroded surface may be used to predict future abrasive tooth tissue loss.

3.2 Null hypotheses

- There are no differences in surface characteristics between lapped human, bovine or ovine enamel.
- There are no differences in surface characteristics between human, bovine or ovine enamel when subjected to erosive and abrasive challenges.
- There are no differences in surface characteristics of enamel subjected to erosive challenges of differing pH or immersion time.

Chapter 4. Methods

This chapter details the methods of investigation used to compare bovine, human and ovine enamel before and after erosive and abrasive challenges. An outline is presented in Figure 12.

4.1 Part 1 - Development

When investigating the surface changes in enamel it was important to be able to measure the degree of surface loss. This required a flat, stable, reference layer within enamel that could be reliably and accurately measured following erosive and abrasive challenges. Initially, time was spent developing such a layer. It was also necessary to use erosive challenges that were clinically relevant, and so the fruit acid content of several commercially available juices was determined. Findings from these initial investigations allowed a small study to be carried out to assess the surface effects of erosion on human and bovine enamel. Stylus profilometry was later used in preference to laser profilometry due to problems that presented with replication and scanning of the samples; nonetheless a series of qualification experiments, including laser profilometry, were undertaken in order to check calibration of the measuring instruments and consistency of the results. An abrasive experiment was also carried out on human, bovine and ovine enamel with an electric toothbrush in order to determine the forces necessary to cause demonstrable surface change. These experiments allowed a robust *in vitro* technique to be developed for erosion and abrasion studies of human, bovine and ovine enamel. These methods are described in detail below:

4.1.1 Determining the citric acid content of fruits and fruit juices

Commercial products were chosen in order to provide consistency between tests. A mainstream market brand was chosen for testing a pure orange juice. A natural lemon was tested in order to provide an indication of the upper limit of acidity whilst a fruit smoothie that also contained yoghurt was tested in order to provide an indication of a more moderate level of acidity. The fruit juices tested were:

i) Tropicana smooth®
ii) Fresh lemon juice and
iii) Sainsbury's® strawberry and raspberry smoothie (containing yoghurt)

Based on the literature reviewed, this experiment assumed that citric acid was the major acid present in the fruit juices tested. The concentration of citric acid was determined by titration with 0.1M sodium hydroxide (NaOH). The NaOH was dispensed from a 50ml burette. 10mL of each of the juices was dispensed into a flask along with 10mL of deionised water and 3 drops of phenolphthalein solution as an indicator. NaOH was titrated into the flask, and the end point reached when the solution turned to a pink colour for 20 seconds. The amount of NaOH titrated was read from the burette. This process was repeated three times for each juice. The average acidity in % weight/volume was then calculated using Equation 2.

$$\%w{:}\,v = \left(\frac{ATV}{3}\right) 192.123$$

Equation 2 derived from Penniston (2008) – calculating the %w/v of citric acid. ATV (average titration value). Division by 3 accounts for the tribasic nature of citric acid. Numerical value relates to molecular mass of citric acid

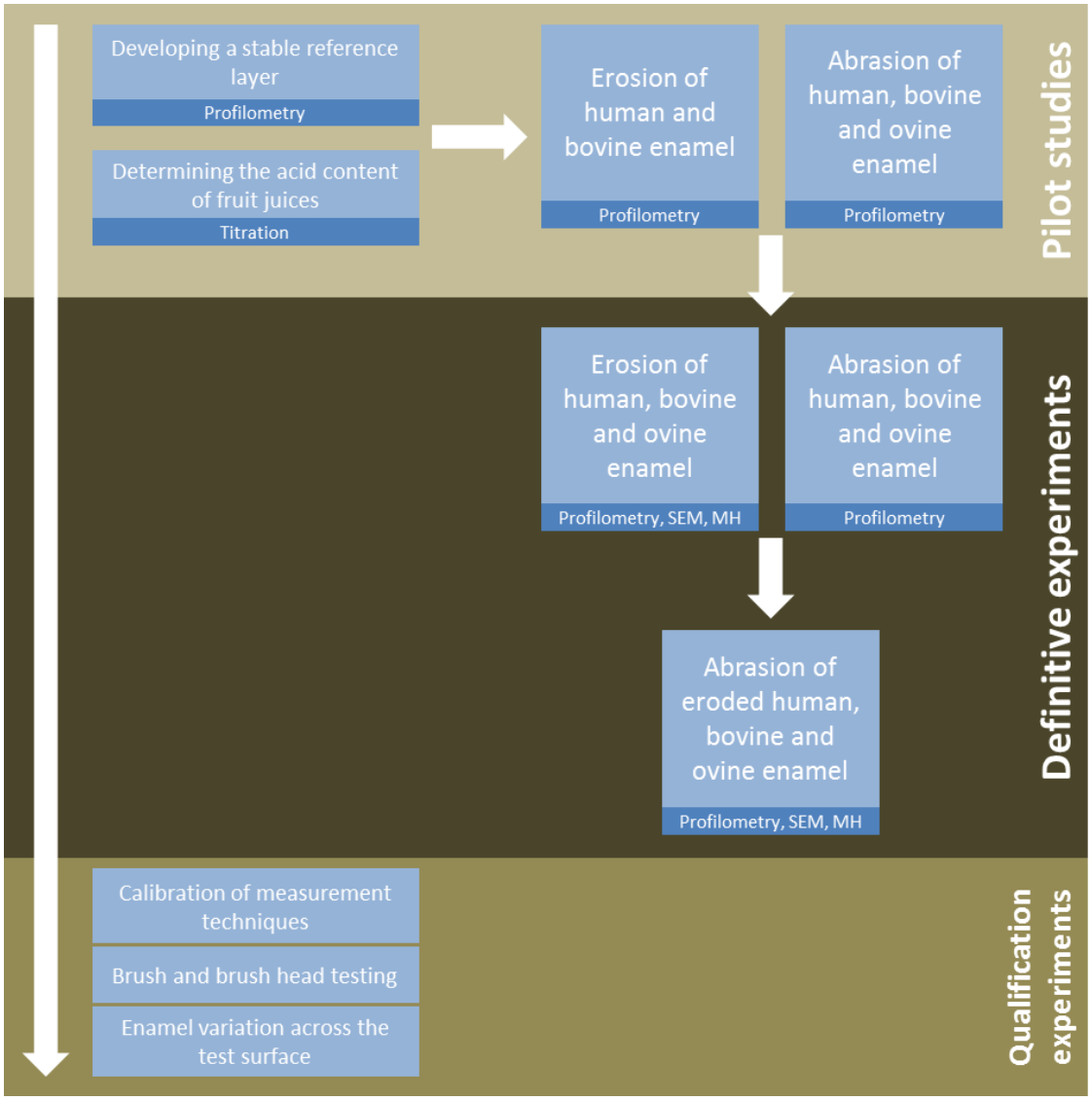


Figure 12 – A flow diagram outlining the methods used – pilot studies, definitive experiments and qualification experiments.

4.1.2 Collection of human, bovine and ovine incisor teeth

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) 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° Celsius in a fresh solution of Ch-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 male 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 degrees Celsius.

4.1.3 Developing a reference layer

It was necessary to develop a reference layer in order to accurately measure abrasive surface changes. This measurement is known as the ‘step height’ and it is measured

from the surface of the reference layer to the deepest valley of the abraded surface. The reference layer needed to be completely flat without detectable voids or porosities after lapping, and stable when stored in a fluid medium.

Initially it was assumed that a layer of cyanoacrylate glue may be suitable as a reference layer. A human molar crown was sectioned longitudinally using a low-speed diamond wheel saw (Testbourne 650 CE, South Bay Technologies Inc, US.) and then glued back together again with cyanoacrylate (Loctite® 401, Henkel Ltd). Once set, the sample was sectioned in the coronal plane to produce a flat disc with the cyanoacrylate as a reference layer across its diameter. The disc was then attached to a resin holder using sticky wax (Kemdent, Associated dental products Ltd) (Figure 13).

The reference disc was ground flat with a Metaserv rotary pregrinder at successive grit sizes from 80 up to 1200 (C200/RB, Metallurgical services Ltd) and further polished with a Metaserv universal polisher (C200/5V, Metallurgical services Ltd) using a 0.05 µm polishing paste.

The disc was then rinsed with a balanced salt solution (HBSS, Gibco® Invitrogen™) and profiled using a stylus profilometer and its associated software (Mitutoyo Surftest SV-2000 and Surfpak-SV Mitutoyo Corp V1.600) in order to examine the relationship between the reference layer and the enamel surface. The instrument height range was 800 µm with a force of 4mN. The stylus was a diamond cone tip held at 90 degrees to the surface, with a 5 µm radius.

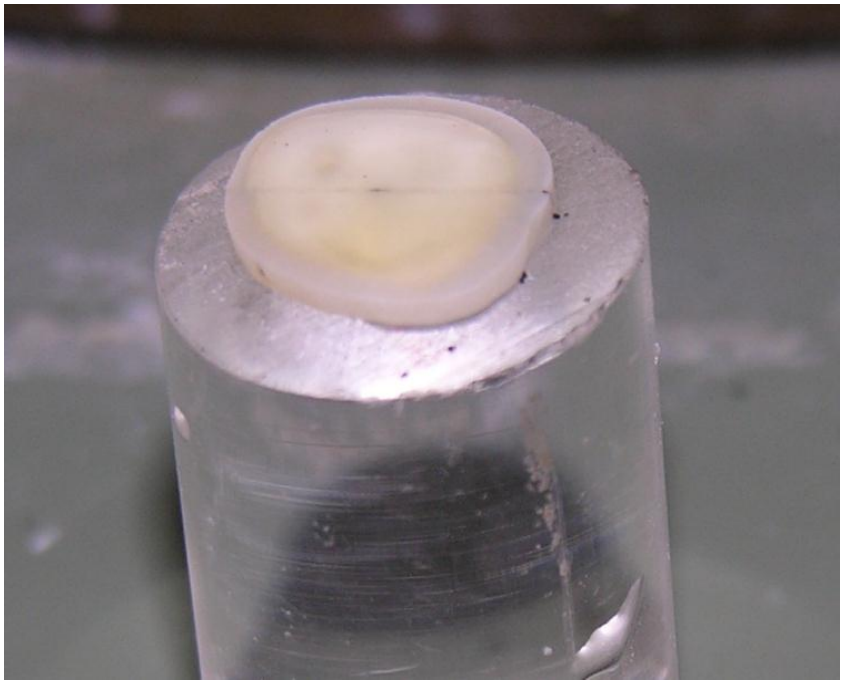


Figure 13 – A flat coronal disc with a central reference layer, attached to a resin holder using sticky wax.

An impression was then taken of the disc surface with a polyether material (Impregum™, 3M ESPE) and an addition cured silicone (Take 1®, Kerr Sybron) in order to form an indirect profile. The impression and the native disc surface were then assessed under laser profilometry (Uniscan Optical Surface Profiler, Model OSP100-HD with Control Unit SCV100OA; software OSP100A v1.06RD 2001, Uniscan®).

An alternative technique for developing a reference layer was also considered, involving the labial surface of a bovine incisor crown. The crown was prepared with a number of undercut grooves using a pear-shaped high-speed diamond bur (Hi-Di® medium diamond bur ISO 237/012, Dentsply Ash instruments) as shown in Figure 14. The grooves were filled with cyanoacrylate again, but also amalgam and acrylic resin. The aim was to assess the response of each material to the lapping process, and also the relationship of each material to the lapped enamel surface. In particular, the materials used were:

Amalgam (Sybraloy®, Kerr Sybron) – 44.5% Hg by weight, 41% Ag, 31% Sn, 28% Cu, 100% spherical.

Cyanoacrylate – (Loctite® 401, Henkel Ltd.) – active ingredient Ethyl 2-cyanoacrylate 7085-85-0.

Acrylic resin (Bonda, Bondaglass-Voss Ltd.) – clear casting resin with catalyst.

The labial surface was ground flat with successive grit sizes up to size 1000 and further polished with a 0.05 µm polishing paste. The sample was then rinsed with HBSS and profiled. Due to problems with obtaining a satisfactory scan (both directly and indirectly) from the laser profilometer, the stylus profilometer was used in preference.

Any surface defects that were visible on the profile were noted.

4.1.4 The stability of reference layers stored in a fluid medium

A bovine incisor crown was prepared with three equidistant and parallel undercut reference grooves across the labial surface (mesio-distally) using a pear-shaped high-speed diamond bur. The reference grooves were filled with amalgam and acrylic resin as described previously. The reference layer was ground flat with successive grit sizes up to size 1000 and further polished with a 0.05 µm polishing paste. The flat labial surface was then sectioned into 2 mm slabs using a low-speed water-cooled diamond wheel saw. Two slabs were chosen and embedded onto the surface of sticky wax to facilitate handling and storage (Figure 15). The samples were rinsed with HBSS and profiled using a stylus profilometer. The samples were then immersed in a solution of Chloramine-T and re-profiled on the stylus profilometer at intervals of 2, 6, 24 and 32 months.

4.1.5 SEM, stylus and laser profilometer calibration

Horizontal calibration

A small round diamond bur (Hi-Di® small round diamond bur ISO 001/014, Dentsply Ash instruments) was used to make a depression in the enamel surface of one of the subset samples. The diameter of the depression was measured using the stylus profilometer, the laser profilometer (directly and indirectly as described previously) and then examined under the SEM at a magnification of approximately x45.

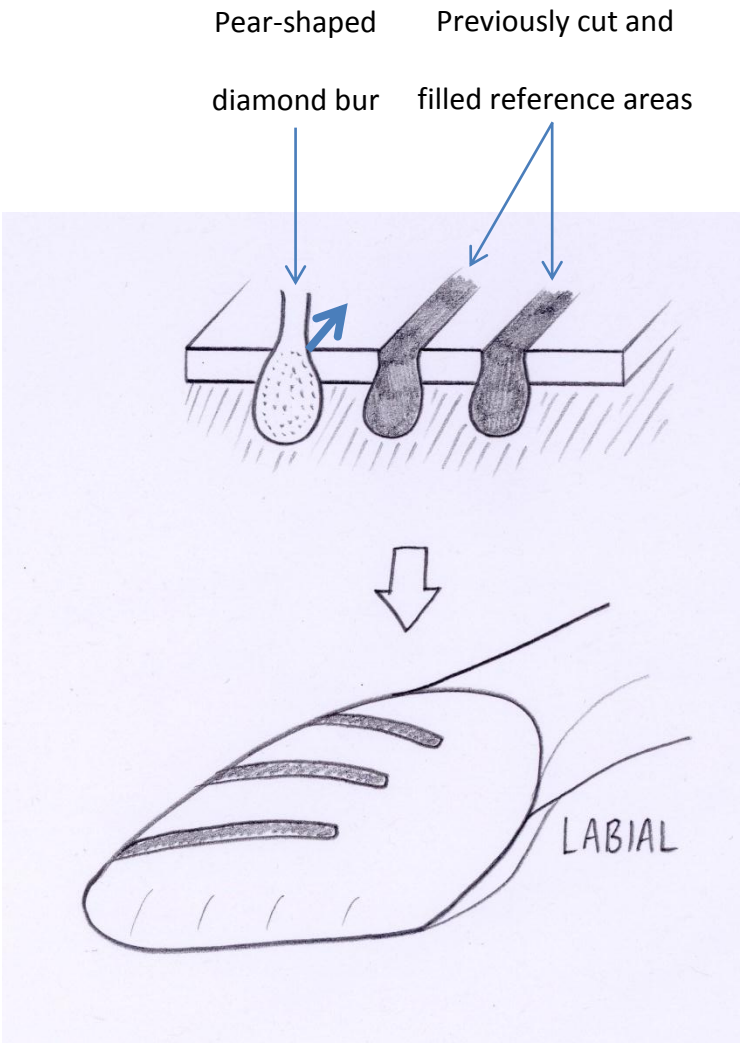


Figure 14 – Undercut grooves prepared into the labial surface of a bovine incisor crown using a pear-shaped high-speed diamond bur.

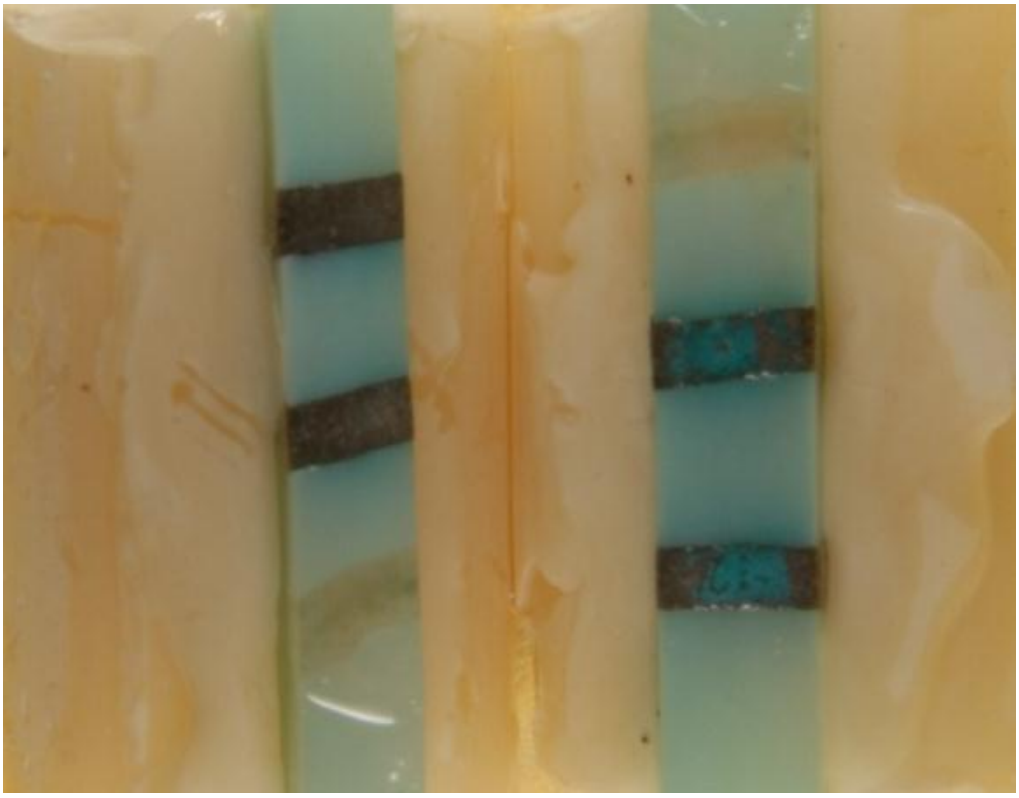


Figure 15 – Two samples of bovine enamel containing amalgam and resin reference areas, held in sticky wax.

Vertical calibration

An eroded and abraded sample was chosen at random, and the Ra value was measured using the stylus profilometer, the laser profilometer (directly and indirectly as described previously) and then examined under the SEM at a magnification of approximately x75.

4.1.6 The effects of in vitro testing on the toothbrush

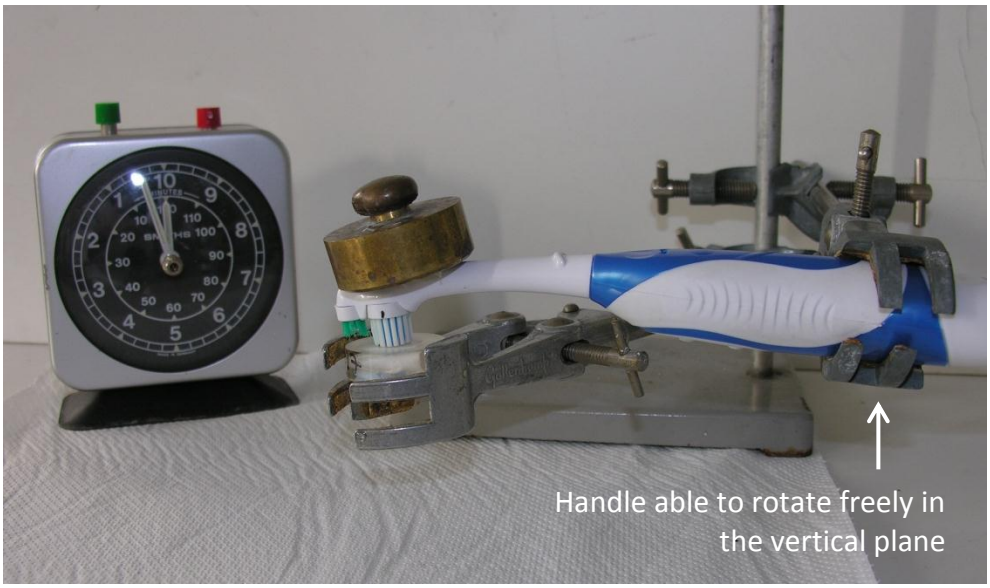
Toothbrush operating frequency

A strobe light (Griffin Xenon Stroboscope Model 60) was used to determine the operating frequency of a Colgate Actibrush™ (model 3418KE) powered by 2 batteries (AAA Energiser® alkaline 1.5V 03-2015). The same loading protocol that was to be used for the abrasive experiments was implemented (A new head was used for each of the 4 treatment groups; 15 seconds at 200g with toothpaste, no water). The apparatus was set up as shown in Figure 16.

The initial number of oscillations per second was recorded, and then at 11.25 minutes (representing the abrasion of one treatment group; 45 samples over 15 seconds), 20 minutes, 30 minutes, 140 minutes and 250 minutes.

Toothbrush head wear

Two brush heads (one new and one used, in accordance with the above loading protocol for 11.25 minutes) were isolated, rinsed with distilled water, dried and mounted onto aluminium stubs with Acheson's silver dag (Agar Scientific, Essex) (an air-drying silver based paint that electrically 'grounds' the sample to the metal base



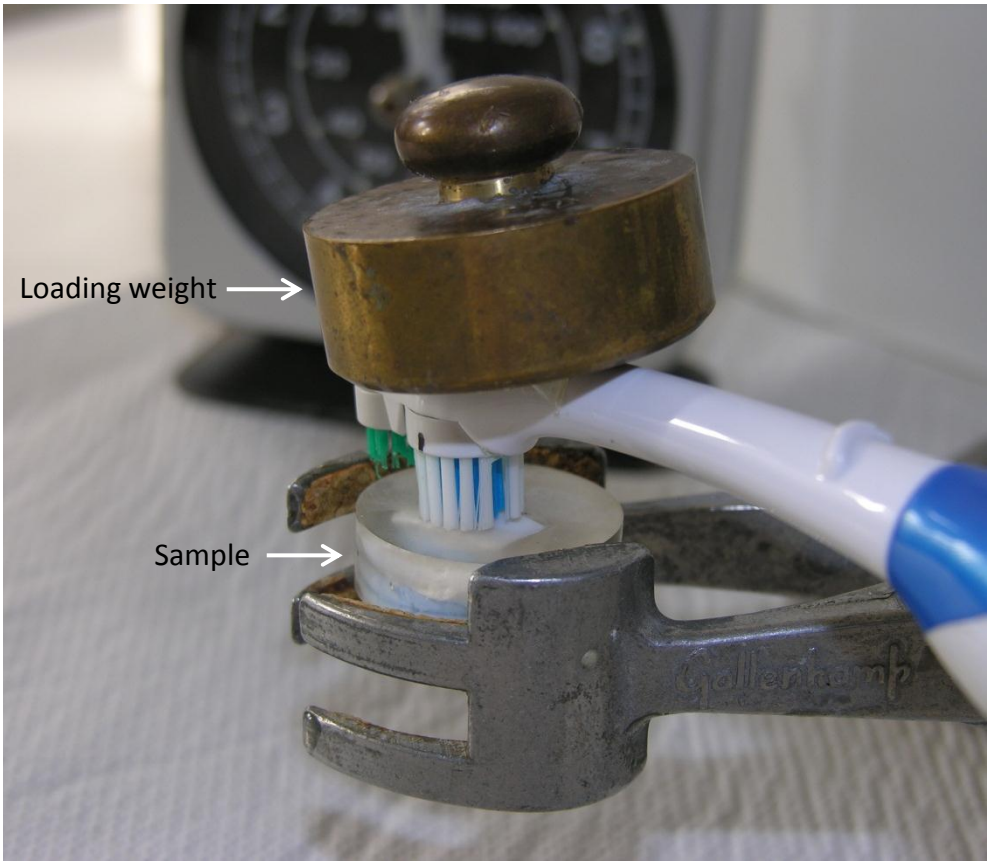


Figure 16 – Apparatus set-up for toothbrush operating frequency tests, showing loading protocol of 200g attached to the free end of the toothbrush. The handle was able to rotate freely in the vertical plane.

plate). The specimens were coated with a 15 nm thick layer of gold, using a Polaron SEM coating unit (Quorum Technologies, West Sussex).

The specimens were then examined using a Stereoscan 240 Scanning Electron Microscope, housed within EM Research Services at Newcastle University. Images were taken at 3 levels of magnification (approximately x245, x80, and x25) in order to assess for changes in bristle structure and captured with Orion software version 6.60.6.

4.2 Part 2 - Erosion

4.2.1 Early erosive surface change on human and bovine enamel

4 bovine and 2 human incisor crowns were prepared with two equidistant and parallel undercut reference grooves across the labial surface (mesio-distally) using a pear-shaped high-speed diamond bur. The crowns were then positioned into individual 30 mm casting moulds (Buehler, Germany) with the labial surface facing upwards and the cemento-enamel junction perpendicular to the base. They were held in place with sticky wax and cast in acrylic resin (Figure 17).

Once set, the casts were removed from the moulds. The surface was ground flat with a Metaserv rotary pregrinder (C200/RB, Metallurgical services Ltd) until the majority of the labial enamel surface was exposed. The sample was then further polished at successive grit sizes up to 1200 and then finished for 25 seconds (anticlockwise rotation at light pressure) with a Metaserv universal polisher (C200/5V, Metallurgical services Ltd) using 0.05 µm polishing paste. The samples were rinsed with HBSS, and inspected under the light microscope (Apophot, Nikon Europe BV) for visible defects

and machine marks. The polished crowns were then sectioned coronally using a low-speed water-cooled diamond wheel saw (Testbourne 650 CE, South Bay Technologies Inc, US.) to produce reference slabs (Figure 18). Some slabs became damaged during the sectioning process and if possible these were re-polished. Otherwise they were discarded. A slab holder was constructed (Figure 19) in order to prevent the polished surfaces from becoming damaged during storage. The slabs were coded in terms of tissue type and tooth origin and immersed in HBSS.

The baseline surfaces were profiled using a stylus profilometer and its associated software (Mitutoyo Surftest SV-2000 and Surfpak-SV Mitutoyo Corp V1.600), calibrated at Ra 2.90 µm using a calibration grid. Average roughness values, and bearing area parameters (Rk, Rvk, Rpk, MR1 and MR2) were recorded twice for each sample 0.5 mm apart. Each evaluation length included 5 samples at 0.3 mm each (1.5 mm total evaluation length, starting within the body of the left hand reference layer).

Citric acid solutions were formulated at 1% and 6% w/v and the pH was measured using a Thermo Orion 4 Star (Fisher Scientific, Leicestershire) at pH2.2 and pH1.8 respectively. The samples were randomly assigned to one of 4 treatments, all undertaken at 30° Celsius:

i) 15 second immersion at 1% w/v citric acid
ii) 2 minute immersion at 1% w/v citric acid
iii) 15 second immersion at 6% w/v citric acid
iv) 2 minute immersion at 6% w/v citric acid

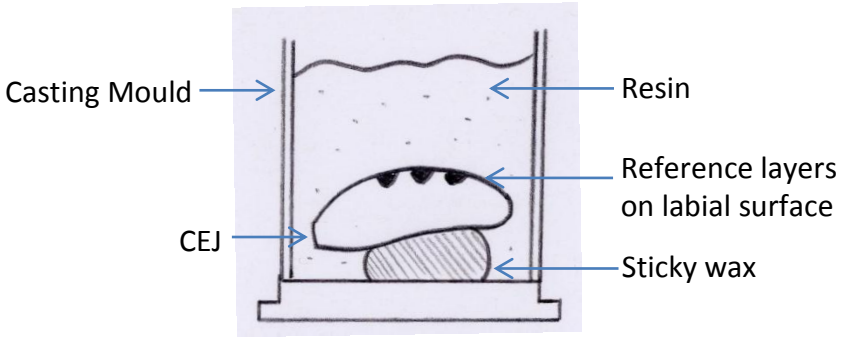


Figure 17 – Bovine incisor crown held labial surface-up inside the individual casting mould.

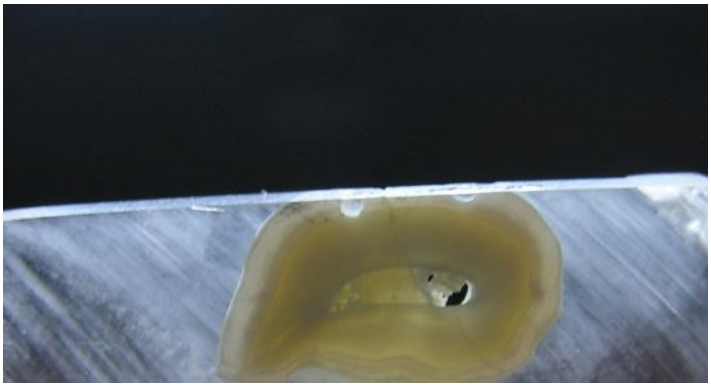


Figure 18 – Reference slab showing two acrylic reference layers and a polished flat enamel surface.

Figure 19 – Acrylic slab holder with samples immersed in HBSS.

Citric acid solutions were warmed using a water bath (Figure 20), and fresh solution was used for each sample. After treatment the samples were rinsed with a balanced salt solution and profiled again.

Analysis

All statistical analyses were carried out using Sigmaplot for Windows Version 11.0, Build 11.0.1.80 (Systat software 2008®). T-tests were carried out in order to compare baseline surface characteristics between tissue types (mean values reported). On the occasions that normality tests were failed, the Mann Whitney Rank Sum Test was used (median values reported).

Paired t-tests were used to compare baseline surface characteristics of each tooth surface to the same surface post-erosion (mean values reported). Where normality tests failed, a Wilcoxon Signed Rank Test was used (median values reported).

3-way Analyses of Variance (ANOVA) were used to test (using a general linear model) the individual effects of the variables: tissue type (bovine or human), concentration (1% or 6% w/v citric acid) and time (15 seconds or 2 minutes). The model also tested for significance of variable interaction (tissue*concentration, tissue*time, concentration*time and tissue*concentration*time). Where all 3 variables interacted significantly, this indicated that the effect of one variable was not consistent at all combinations of the other 2 variables – in these cases, individual significant interactions were reported using the Holm-Šidák pairwise multiple comparison procedure with significance at the 0.05 level (least square mean values reported).

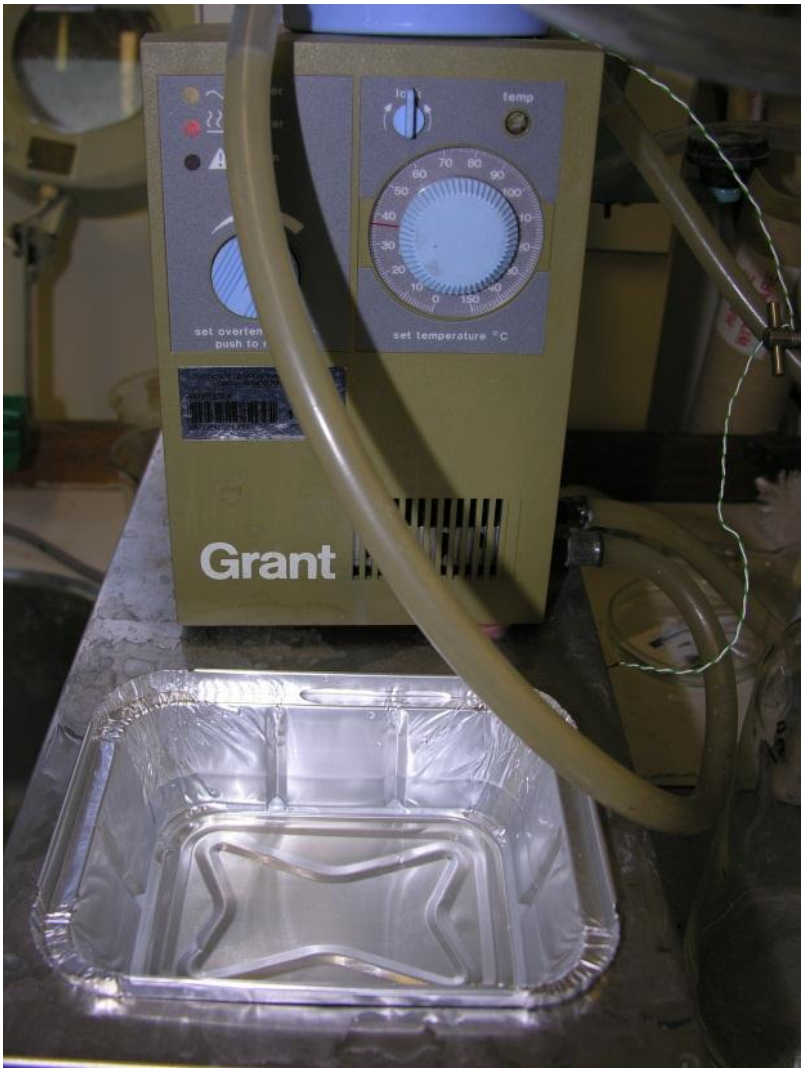


Figure 20 – Water bath ready to set-up at 30 degrees Celsius for the erosive challenges.

4.2.2 Human, bovine and ovine sample preparation and baseline testing

20 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. 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 and cast in acrylic resin.

Once set, the casts were removed from the moulds. 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 (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 0.7MPa. After lapping the samples were rinsed with HBSS and stored in the salt solution face-up in individual vials.

Three further samples sets from each species were prepared for baseline and post-erosion SEM, abrasion pilot testing and microhardness testing (2 crowns from each species for SEM, 8 crowns from each species for microhardness testing and 2 crowns from each species for abrasion pilot testing).

Sample measurement and analysis

The baseline surfaces were profiled using a stylus profilometer. Average roughness values, and bearing area parameters (Rk, Rvk, Rpk, MR1 and MR2) were recorded 3 times for each sample 0.4 mm apart. Each evaluation length included 5 samples at 0.3 mm each (1.5 mm total evaluation length, starting 1 mm into the body of the acrylic, down the long axis of the crown).

For SEM, samples from each tissue subset were prepared and examined as described previously. Microhardness testing was then carried out on the enamel of the second subset using a Zwick/Roell Z2.5 hardness tester and associated software, TestXpert V11.02 (Zwick testing machines Ltd, Herefordshire). 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).

4.2.3 The early erosive challenge on human, bovine and ovine enamel

Citric acid solutions were formulated at 1% and 6% w/v and the pH was measured as described previously. Prior to baseline testing, the samples had been randomly assigned to one of 4 treatments, all undertaken at 30° Celsius:

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

Citric acid solutions were warmed using a water bath, and fresh solution was used for each sample. The previously measured samples were subjected to their allocated treatment.

After treatment the samples were rinsed HBSS and profiled again (as described previously). The maximum height change in the profile, measured as the lowest point on the profile 0.5-0.75 mm in from the acrylic reference level was also recorded.
A subset of samples that were subjected to treatment 4 (considered to be the most severe erosive challenge; 4 minutes at 6%) were chosen for SEM testing, following the protocol described above. Images were taken at 3 levels of magnification (approximately x2000, x1000 and x50). A subset of microhardness testing was carried out following the protocol described above.

Analysis

Paired t-tests were used to compare baseline surface characteristics to those post-erosion (mean values reported). Where normality failed, a Wilcoxon Signed Rank Test was used (median values reported). One way Analysis of Variance (ANOVA) was used to compare eroded 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). 3-way ANOVA was used to test (using a general linear model) the individual effects of the variables: tissue type (bovine, human or ovine), concentration (1% or 6% w/v citric acid) and time (30 seconds or 4 minutes). The model also tested for significance of variable interaction (tissue vs. concentration, tissue vs. time, concentration vs. time and tissue vs. concentration vs. time). Where all 3 variables interacted significantly, this indicated that the effect of one variable was not consistent at all combinations of the other 2 variables – in these cases, individual significant interactions were reported using the Holm-Šidák pairwise multiple comparison procedure with significance at the 0.05 level (least square means reported for time and concentration).

4.3 Part 3 - Abrasion

4.3.1 The early abrasive challenge on human, bovine and ovine enamel

Pilot

An oscillatory brush Colgate Actibrush™ (model 3418KE, Colgate-Palmolive (UK) Ltd.) powered by 2 batteries (AAA Energiser® alkaline 1.5V 03-2015) was used with a force

of 200g with non-fluoridated toothpaste, Euthymol® (Johnson & Johnson, New Jersey, USA) for both 5 and 20 seconds on enamel samples eroded for 4 minutes in 6% w/v citric acid (see section 4.2.2).

One crown was used for each treatment (5s or 20s) for each species (bovine, human and ovine). 3 measurements were taken for each crown (n=18).

Paired t-tests were used to compare baseline eroded surface characteristics to those post-abrasion. Where normality failed, a Wilcoxon Signed Rank Test was used.

Abrasion of human, bovine and ovine enamel

6 bovine, human and ovine incisor crowns were prepared and baseline tested as previously described. The abrasion regime described above was carried out for 20 seconds on each of the lapped samples. A 20 second regime was chosen instead of 5 seconds, because the 20 second exposure resulted in significant changes in bearing parameters, an increased data range and reduced skewness of the data. After treatment the samples were rinsed with a balanced salt solution and profiled again.

Analysis

Paired t-tests were used to compare baseline (lapped) surface characteristics to those post-abrasion. Where normality failed, a Wilcoxon Signed Rank Test was used. One way Analysis of Variance (ANOVA) was used to compare abraded surface characteristics between tissue types. All 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.

4.4 Part 4 – Erosion and abrasion

4.4.1 Abrasion of the early-eroded lesion on human, bovine and ovine enamel

Previously eroded samples were stored in a balanced salt solution prior to abrasion testing in order to minimise the risk of surface change.

The samples were air dried and insulation tape was placed across the acrylic reference layer to protect it from any possible abrasive forces (Figure 21). The samples were then abraded using the same abrasive protocol described above.

After treatment the tape was removed, and the samples were rinsed with HBSS 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.

Analysis

Paired t-tests were used to compare baseline eroded surface characteristics to those post-abrasion (mean values reported). Where normality failed, a Wilcoxon Signed Rank Test was used (median values reported). One way Analysis of Variance (ANOVA) was used to compare abraded surface characteristics between tissue types. All pairwise multiple comparisons were then made using the Holm-Šidák method with a

Figure 21 – Insulation tape placed across the acrylic reference area to protect against toothbrush abrasion.

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).

Finally, forward stepwise multiple linear regressions were carried out with abraded height change (representing tooth surface loss) as the dependent variable. The inclusion level was set at P = 0.049 and the exclusion level was set at P = 0.052. The findings were compared to backward stepwise multiple linear regressions using the same criteria. 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. Spearman rank order correlation was used in preference to the Pearson product moment, because it is less sensitive to outliers. Significance for correlation was set at 0.05.

4.5 Part 5 - Further qualification experiments

4.5.1 Surface effects of microhardness testing on enamel

Previously described preparatory and microhardness testing procedures were employed for one bovine incisor. Several microhardness indents were made across the enamel surface, and SEM images were taken at magnifications of approximately x1250 and x40 in order to assess surface deformation.

4.5.2 Changes in surface parameters of polished samples away from the CEJ

20 baseline surface profiles chosen randomly from each tissue type were split into thirds (away from the cemento-enamel-junction) using the Surfpak software. Data

were collected relating to the bearing parameters. Roughness average could not be calculated accurately once the profiles had been split.

One way Analysis of Variance (ANOVA) was used to compare bearing surface characteristics between surface thirds. 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).

Chapter 5. Results

5.1 Development

5.1.1 Determining the citric acid content of fruits and fruit juices

Table 2 shows the recorded citric acid content of lemon juice and two commercially available fruit drinks. The squeezed lemon juice recorded a citric acid content of approximately 7.5% weight/volume. Freshly squeezed orange juice (Tropicana Smooth®) recorded a mean acidity of 0.65% w/v and the Sainsbury's® strawberry and raspberry smoothie recorded a mean acidity of 0.69%.

5.1.2 Developing a reference layer

The surface profile of the coronal reference disc recorded on the stylus profilometer can be seen in Figure 22. The enamel and dentine areas are identifiable, and also the central cyanoacrylate reference layer. The surface profiles of the lapped reference areas are shown in Figures 23 and 24. Amalgam showed minor voids at the enamel interface of around 6 µm. Cyanoacrylate showed significant deficiencies across the whole reference area of around 23 µm. Acrylic resin showed no signs of deficiency either across the reference area *or* at the enamel interface. The resin remained virtually undetectable by stylus profilometry.

5.1.3 The stability of reference layers stored in a fluid medium

A typical surface profile of the reference layers after 2 months of storage is shown in Figure 25. The amalgam reference area displays a large amount of expansion whilst the resin layer is still almost undetectable. A minor deficiency adjacent to one of the resin

Juice/smoothie	***n***	**Titration (mL)**	**Mean (mL)**	**Citric Acid content (w/v%)**
Tropicana® smooth	1	10.1	10.2	**0.653%**
	2	10.2		
	3	10.2		
Lemon juice	1	11.8	11.7	**7.492%**
	2	11.6		
	3	11.7		
Sainsbury's® Strawberry and raspberry smoothie	1	10.7	10.7	**0.686%**
	2	10.5		
	3	10.7		

Table 2 – mean citric acid content (w/v%) of two commercially available fruit drinks and freshly-squeezed lemon juice.

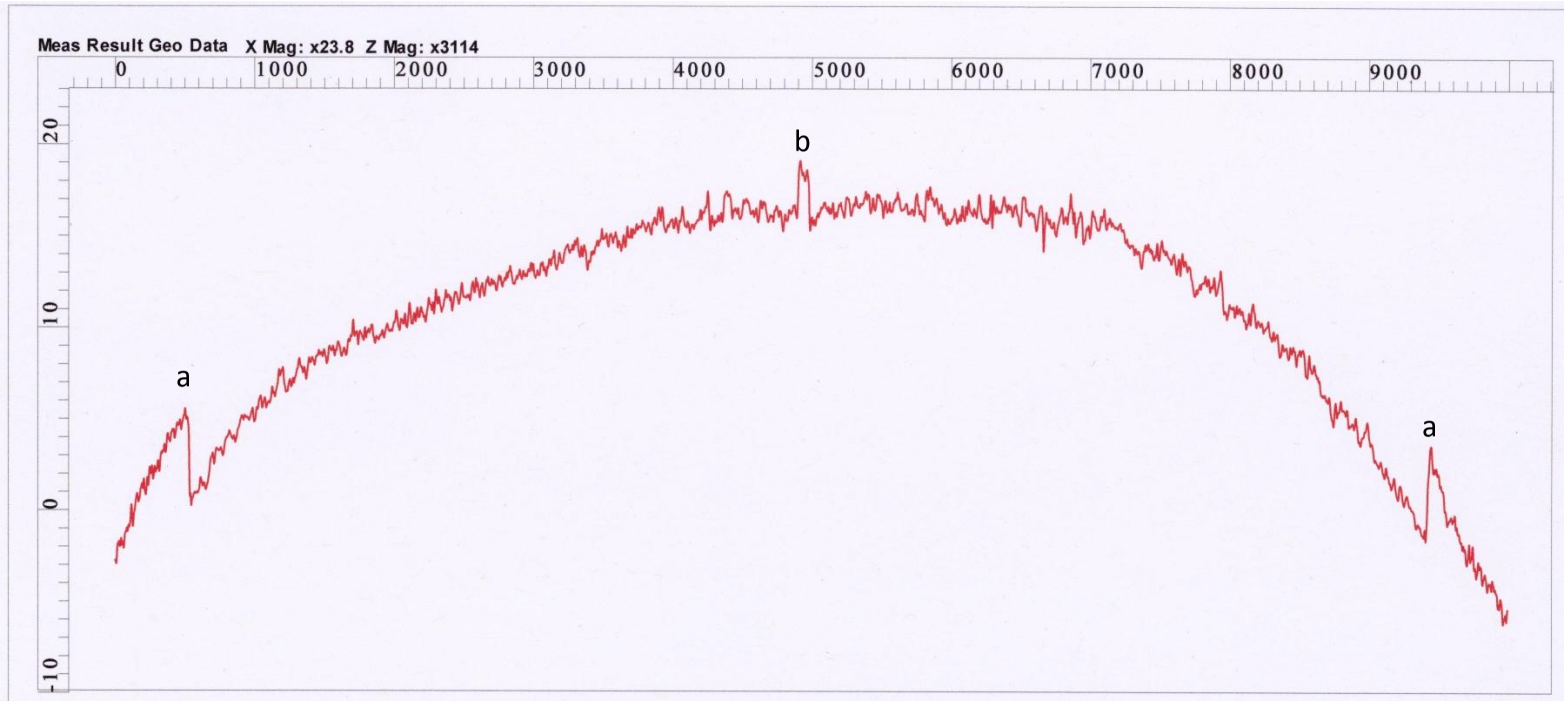


Figure 22 – Surface profile of the coronal reference disc after lapping - scale in micrometres. Note the obvious steps between enamel and dentine at the peripheries (a), and dentine and the cyanoacrylate reference layer at the centre of the profile (b). Also note the distinct curvature on the sample as an artefact of the initial polishing technique.

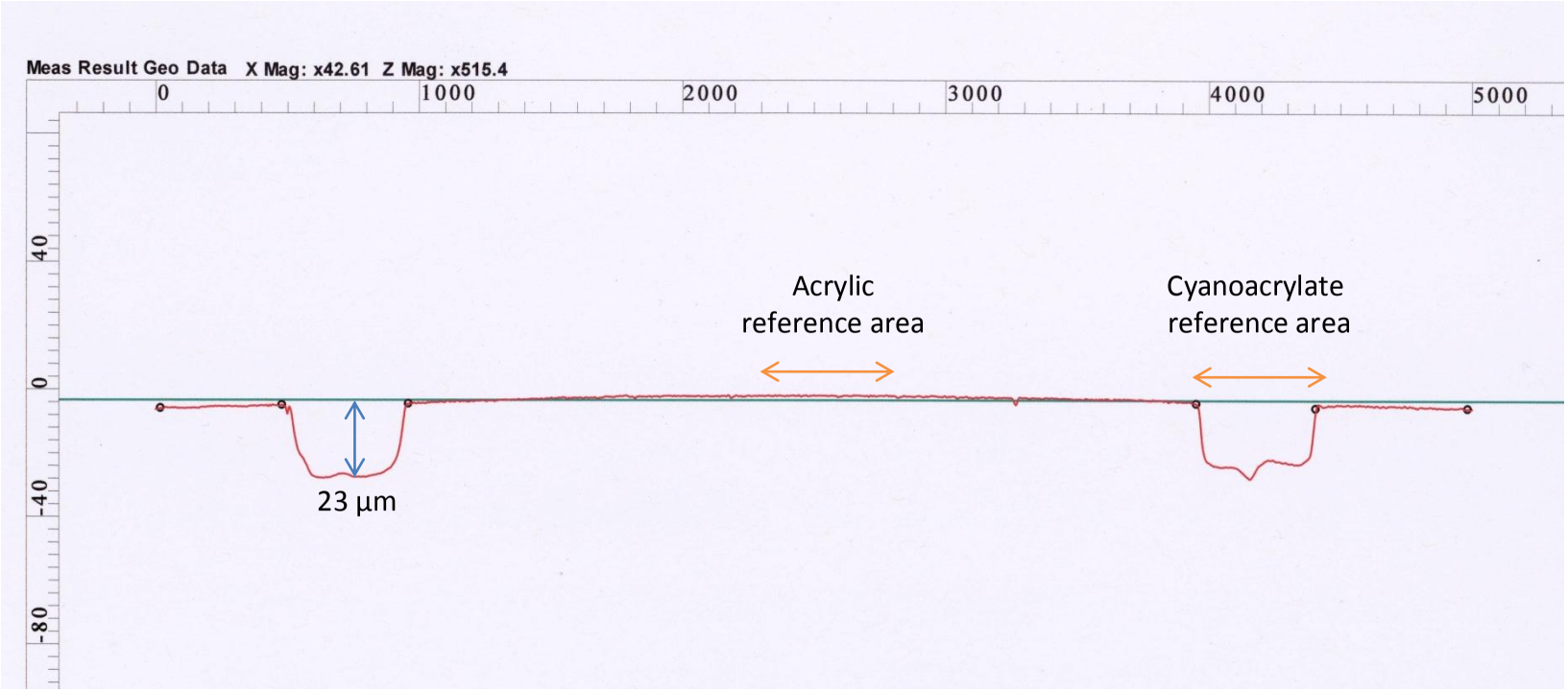


Figure 23 – Typical profile of the lapped tooth surface with an acrylic reference area in-between two cyanoacrylate reference areas – the scale is in micrometres. Note the significant amount of surface loss across the cyanoacrylate reference areas, typically around 23 µm in depth (blue arrow). The acrylic reference layer remains undetectable to stylus profilometry.

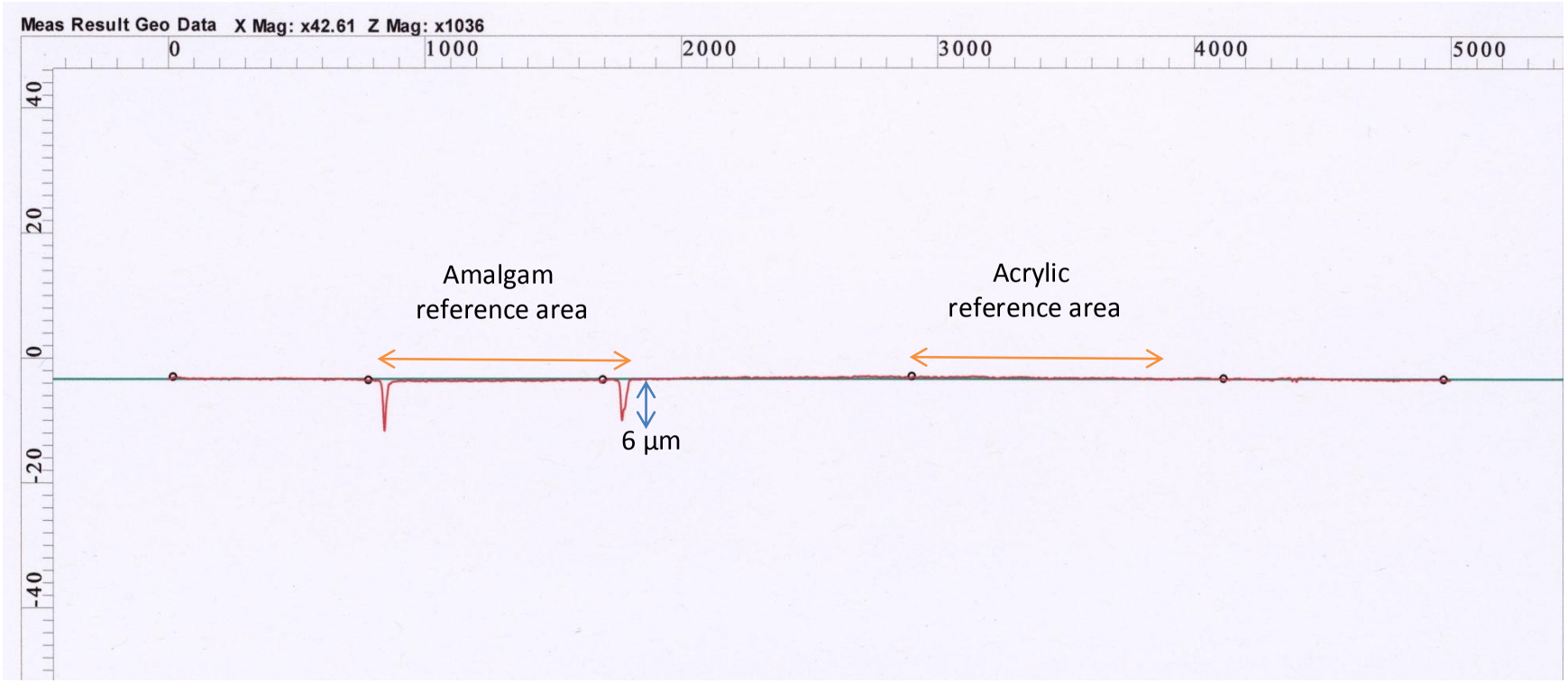


Figure 24 – Typical profile of the lapped tooth surface with an acrylic reference (right) adjacent to an amalgam reference area (left) – the scale is in micrometres. Note the significant amount of surface loss at the peripheries of the amalgam reference area, typically around 6 µm in depth (blue arrow). The acrylic reference layer remains undetectable to stylus profilometry.

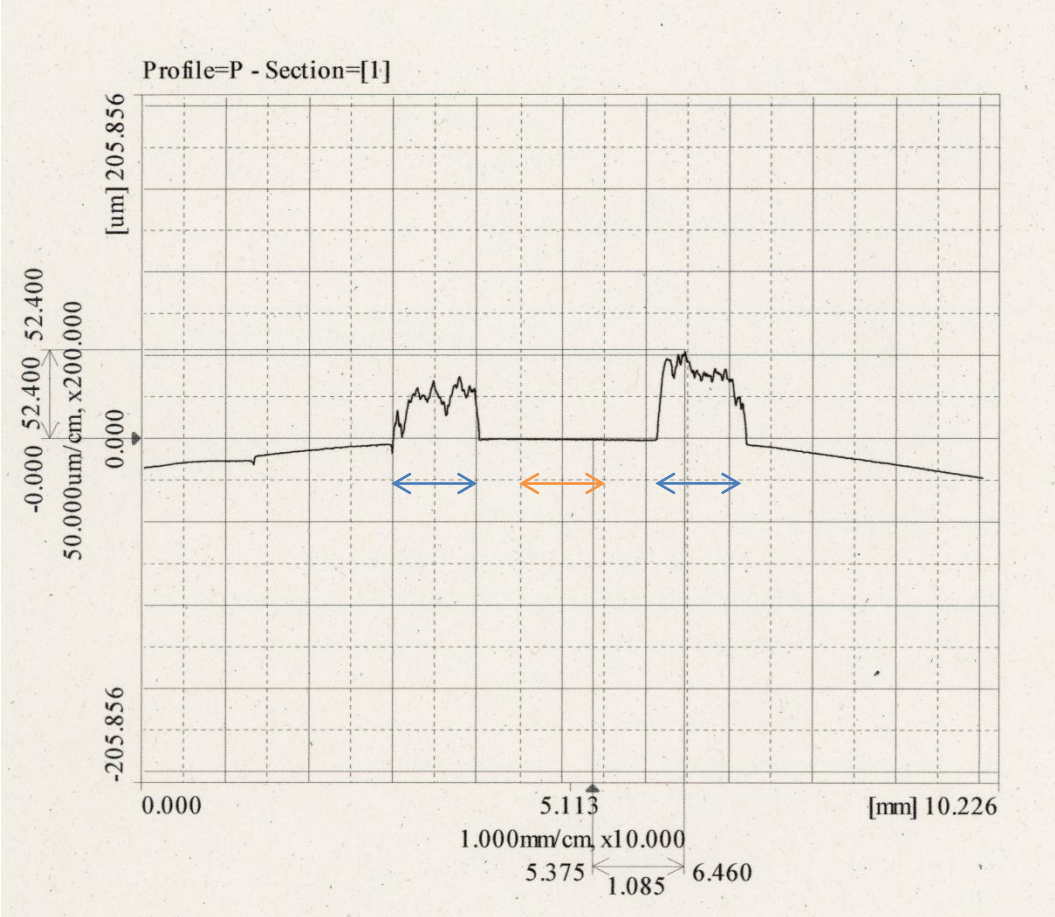


Figure 25 - A typical surface profile of the reference layers after 2 months of storage in a balanced salt solution. The amalgam reference areas (blue line) display a large amount of expansion (exceeding 50 µm) whilst the resin layer in the centre (orange line) is still almost undetectable.

reference layers was noted at 6 months, but the profile then remained unchanged at 24 and 32 months.

5.1.4 SEM, stylus and laser profilometer calibration

Despite problems of obtaining a satisfactory laser profile in relation to a stable reference area, it was necessary to assess the calibration between the three modes of profile measurement (SEM, stylus and laser profilometry).

Horizontal calibration

For the depression created by the small round diamond bur, the stylus profilometer recorded a width of 1.14 mm; this and the SEM image of the depression can be seen in Figure 26. Note the visible profilometer mark on the SEM image, which allowed a more accurate calibration. The SEM recorded a width of 1.18 mm. The laser profilometer was unable to focus on the native enamel surface and so a polyether impression was taken. The laser profilometer indirectly recorded a width of 1.18 mm but unlike the SEM image, the stylus profilometer marks were not visible under laser profilometry (Figure 27).

Vertical calibration

When measuring a random ovine sample from treatment group 4, the stylus profilometer recorded Ra at 0.176 µm. The laser profilometer indirectly recorded Ra of the same surface at 0.228 µm. Similarly to the horizontal calibration tests, the stylus profilometer marks are visible on the SEM of the native enamel surface (Figure 28) but not on the laser profiles (Figures 29 & 30).

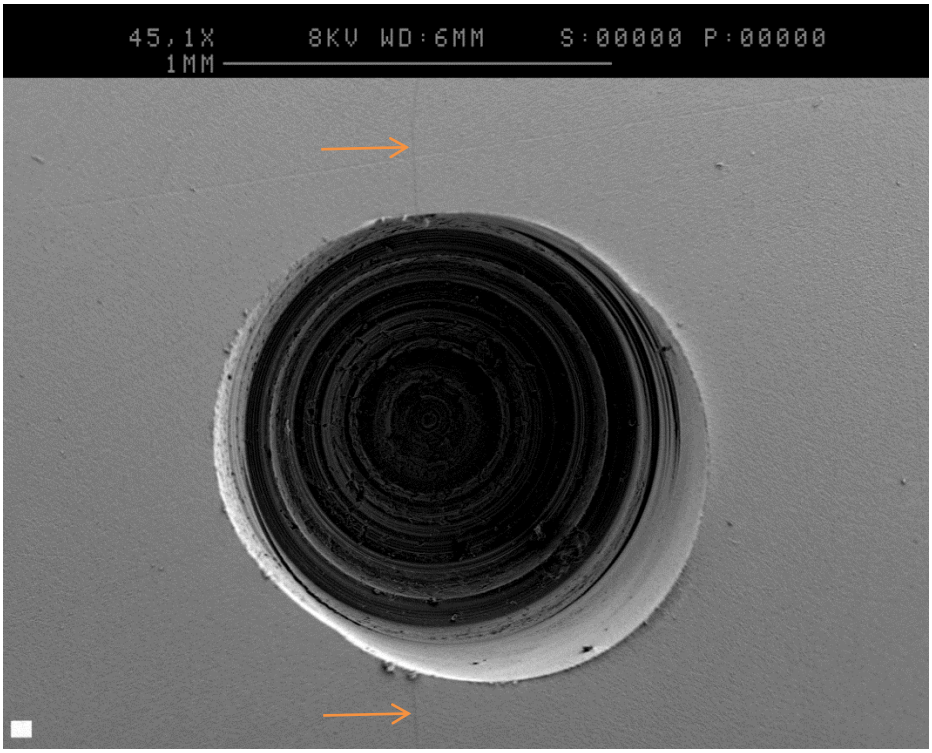


Figure 26 – Above – stylus profilometer profile measuring the width of the bur depression created with the high-speed handpiece at 1.14 mm. Note the flat bottom (red line) where the vertical limit of the stylus was reached. Below - SEM image (45X magnification) of the same depression. Note the visible stylus profilometer mark (orange arrows). The SEM recorded a width across this area of 1.18 mm.

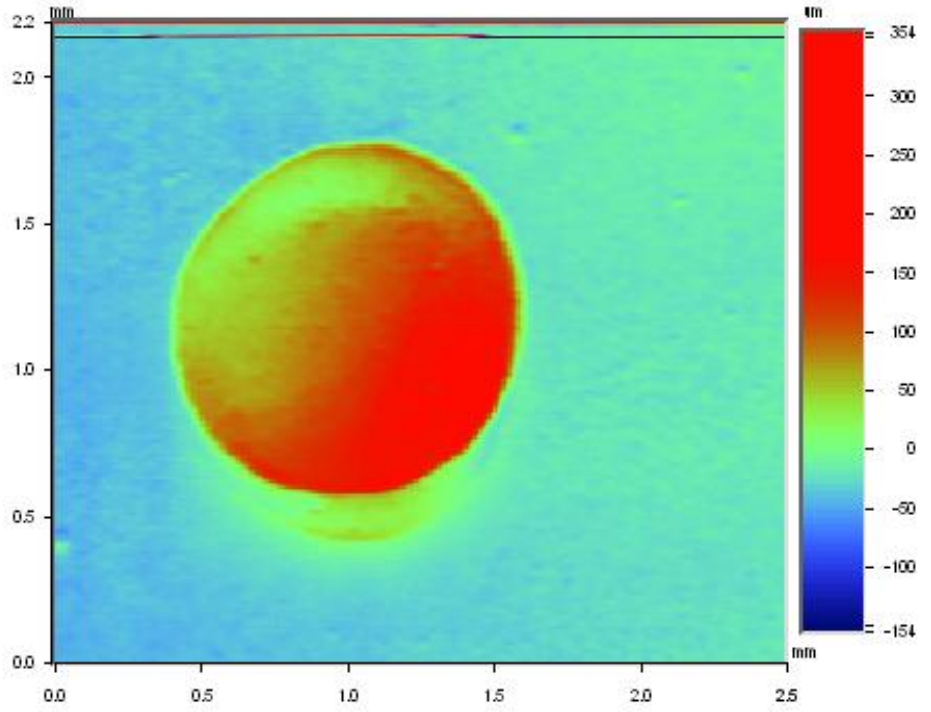


Figure 27 - Laser profilometer scan of the impression of the bur depression created with the high-speed handpiece. The laser profilometer recorded a width of 1.18 mm but unlike the SEM image, the stylus profilometer marks were not visible.

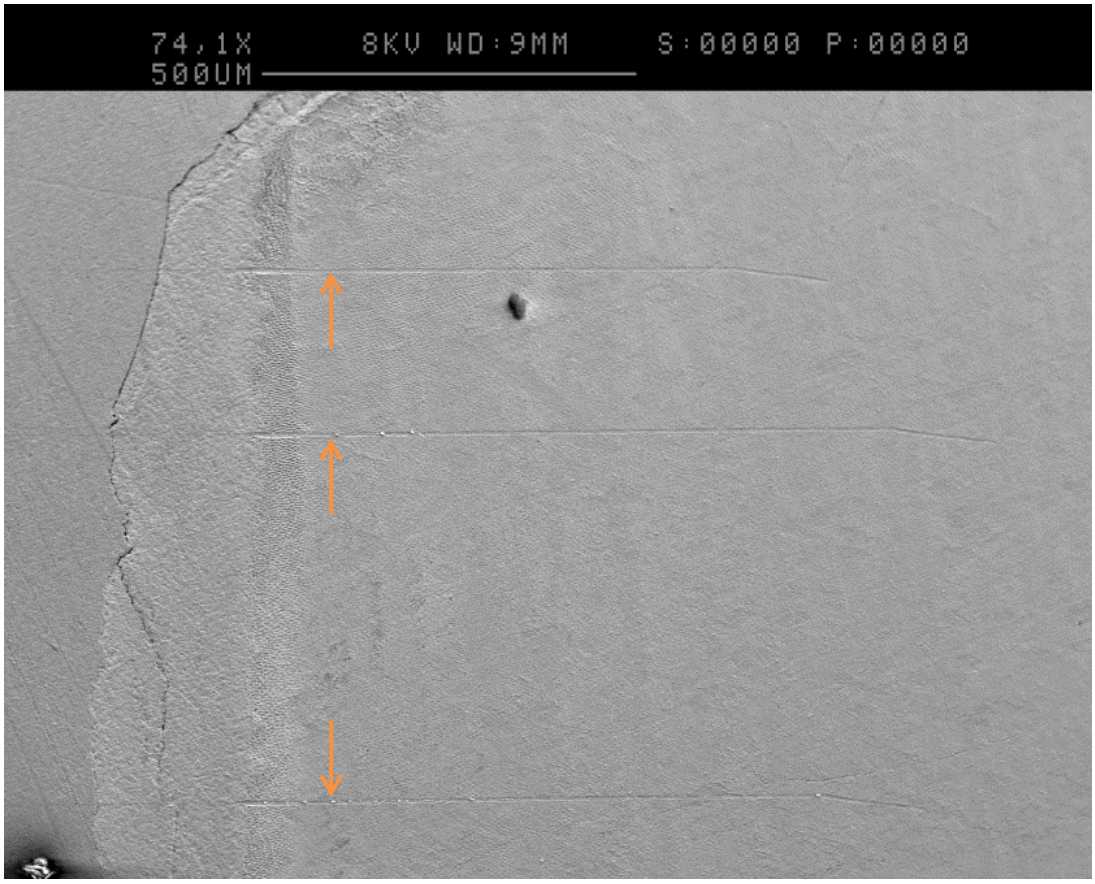


Figure 28 - Similarly to the horizontal calibration tests, the stylus profilometer marks (orange arrows) were visible on a typical SEM (74X magnification) of the native enamel surface.

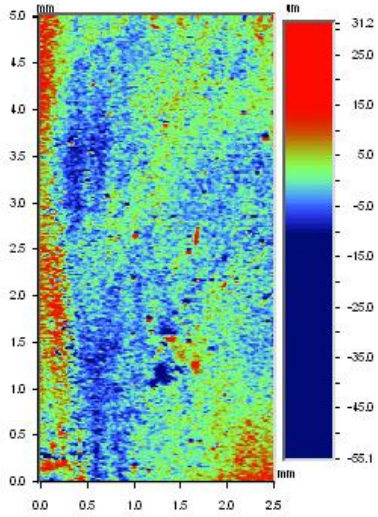


Figure 29 – Typical laser profilometry scan of the eroded enamel surface – note the higher protected reference area (red) to the left of the image, with no evidence of profilometer marks horizontally across the profile.

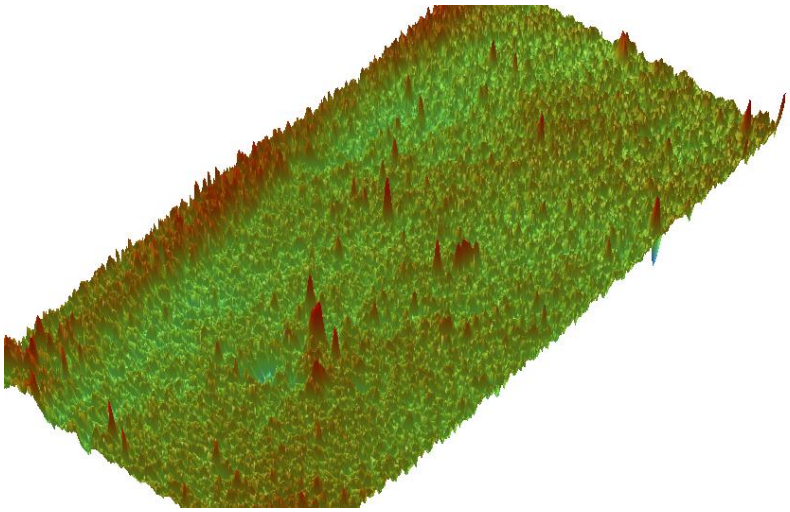


Figure 30 – Typical laser profilometry 3D scan of the same eroded enamel surface – again note the higher protected reference area (red) to the upper left corner of the scan, with no evidence of profilometer marks across the profile.

5.1.5 The effects of in vitro testing on the toothbrush

Toothbrush operating frequency

The operating frequencies recorded for the loading protocol (200g, toothpaste, no water) are displayed in Table 3.

	Rotations per second (RPS)						
Time	**Test 1**	**Test 2**	**Test 3**	**Test 4**	**Test 5**	**Mean RPS**	**SD**
Initial RPS	34	34	33	34	34	34	0
11.5 minutes	34	33	33	33	33	33	0
20 minutes	31	32	32	33	32	32	0
30 minutes	31	31	31	31	31	31	0
140 minutes	30	30	31	31	32	31	1
240 minutes	30	29	29	30	30	30	1
250 minutes	28	27	28	26	26	27	1
260 minutes	9.9	10.1	9.7	10.3	8.9	9.8	1

Table 3 – operating frequencies (in rotations per second, RPS) determined with a strobe light at time intervals up to 260 minutes.

Toothbrush head wear

The SEM images of the toothbrush heads before and after the abrasive challenges are shown in Figures 31-33. Minor surface features on the native, unused, filaments are lost once the brush has been used to simulate an abrasive challenge. The general form of the filaments and integrity of the brush head remain unchanged when viewed at a lower magnification.

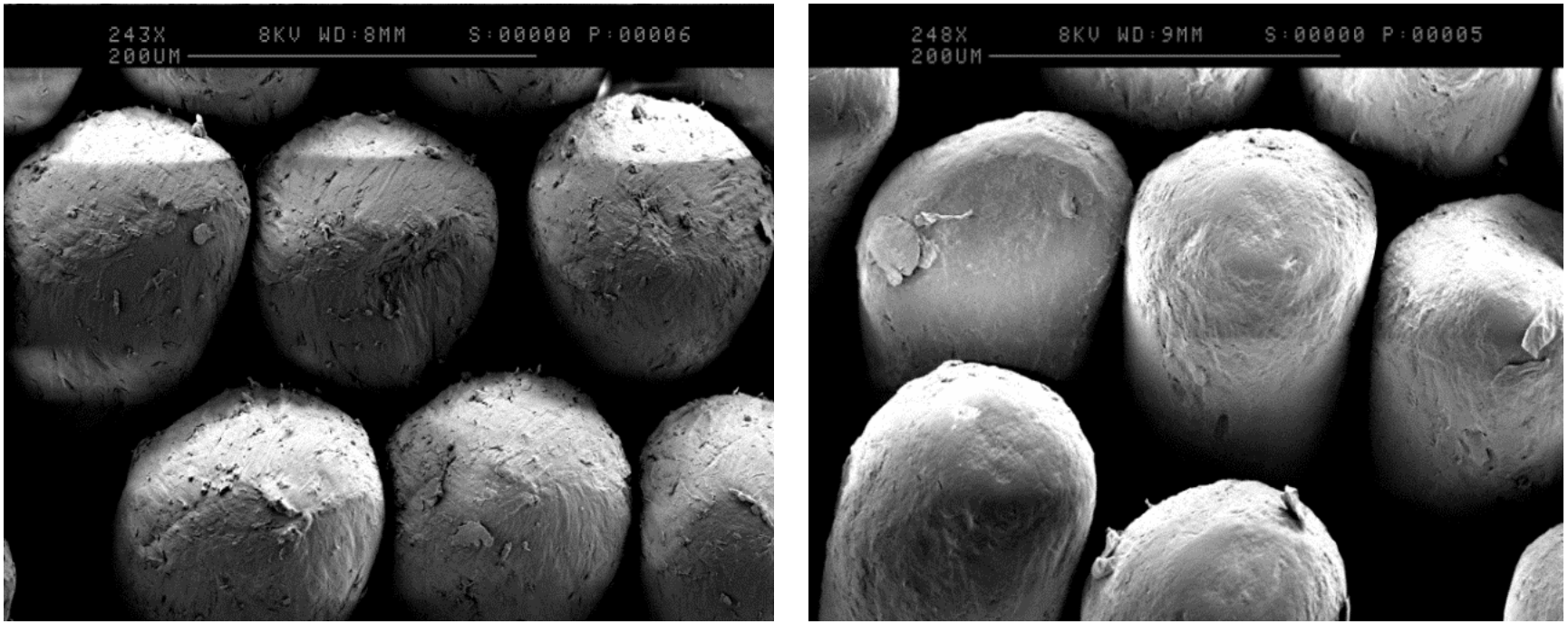


Figure 31 – High magnification SEM of toothbrush bristles before (left) and after (right) the abrasive challenge. Note the twisted finish to the filaments on the virgin surface, and the loss of this subtle characteristic after use.

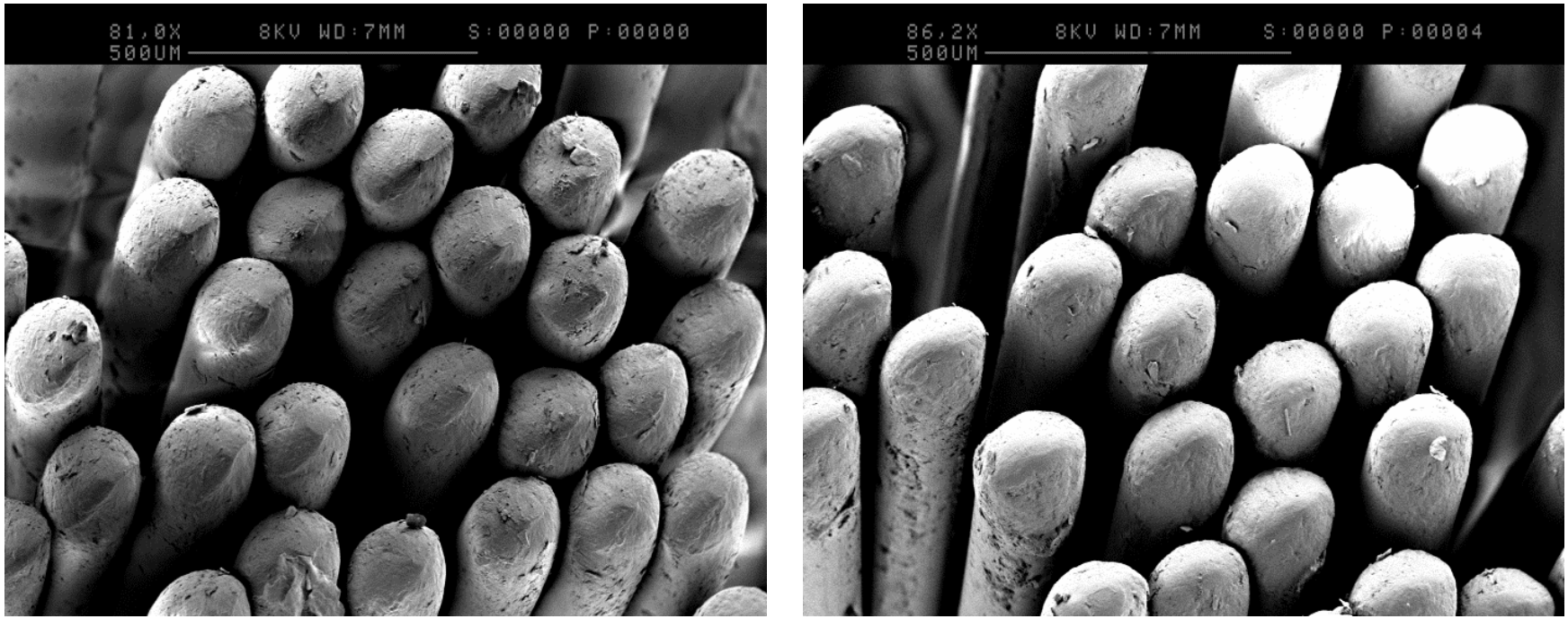


Figure 32 – Medium magnification SEM of toothbrush bristles before (left) and after (right) the abrasive challenge. Note the twisted finish to the filaments on the virgin surface, and the loss of this subtle characteristic after use.

Figure 33 – Low magnification SEM of toothbrush bristles before (left) and after (right) the abrasive challenge. It is possible to observe the relationship of the individual bristles that comprise the brush 'tufts'. Post-exposure, there is slight divergence from the tufts by a number of peripheral bristles. However, the integrity and form of the brush head was not markedly different.

5.2 Erosion

5.2.1 Early erosive surface change on human and bovine enamel

Baseline

Baseline parameters are shown in Table 4. A typical profile and bearing area curve are shown in Figure 34.

Tissue	Roughness average (µm)	Peak roughness (µm)	Core roughness (µm)	Valley roughness (µm)	Material ratio of peaks (%)	Material ratio of troughs (%)
Human	0.11^{a} (0.02)	0.20^{a} (0.03)	0.31^{a} (0.07)	0.36^{a} (0.10)	21^{a} (3)	92^{a} (3)
Bovine	0.12^{a} (0.11)	0.16^{a} (0.08)	0.33^{a} (0.05)	0.30^{a} (0.09)	7^{b} (2)	81^{b} (9)

Table 4 – Mean roughness and bearing parameters of human and bovine enamel at *baseline*. Standard deviations are within brackets. Values with differing superscripts are significantly different between tissues. The full data set for profilometric data can be found in Appendix A and statistical analyses in Appendix B.

Human and bovine enamel roughness (Ra) was not significantly different at baseline ($P = 0.176$). Human and bovine core roughness (Rk), valley roughness (Rvk) and peak roughness (Rpk) were also not significantly different at baseline (Rk $P = 0.280$; Rvk $P = 0.066$; Rpk $P = 0.177$). Significant differences were measured for the proportions of profile peaks (Mr1) and troughs (Mr2) (Mr1 $P < 0.001$; Mr2 $P < 0.001$); human enamel displayed significantly more peaks and less troughs than bovine enamel.

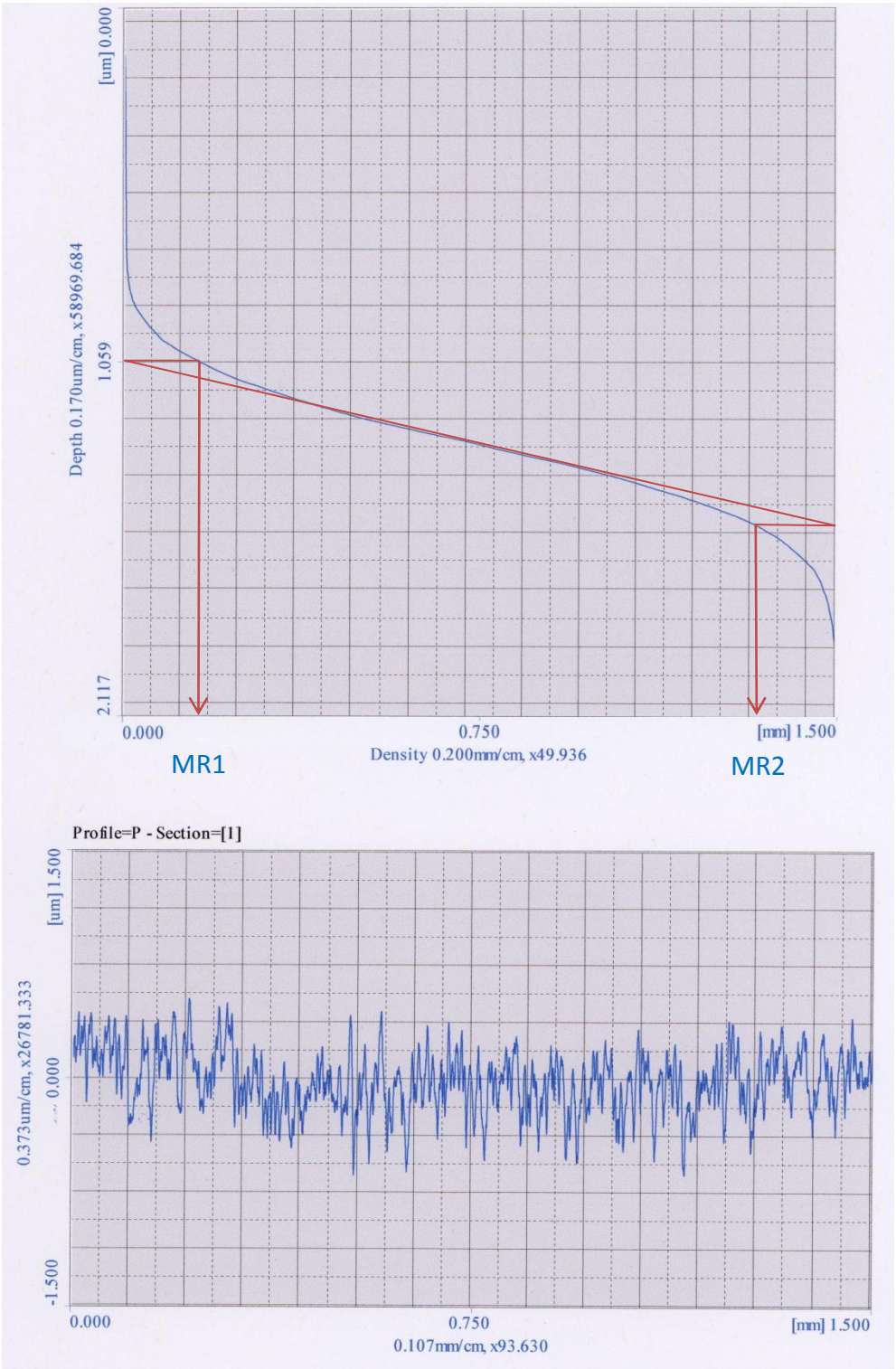


Figure 34 – Typical stylus profilometry profile (Ra 150 µm) and bearing curve (MR1 11%, MR2 89%) for baseline lapped bovine enamel.

Post acidic challenge

Initially it was important to consider the overall effects of the erosive challenges, irrespective of tissue, concentration and immersion time (Table 5). The literature reports general and definite trends in roughness parameters post-erosion and it was important to assess whether similar trends were apparent in this study. There were significant differences within these roughness parameters post-erosion and these are considered below. A summary of these parameters in relation to individual tissue, concentration and immersion time is then shown in Tables 6 and 7. A typical stylus profile and bearing curve are shown in Figure 35.

Stage	Roughness average (µm)	Peak roughness (µm)	Core roughness (µm)	Valley roughness (µm)	Material ratio of peaks (%)	Material ratio of troughs (%)
Baseline	0.13^{a} (0.10)	0.31^{a} (0.10)	0.31^{a} (0.07)	0.32^{a} (0.06)	10^{a} (3)	92^{a} (6)
Eroded	0.18^{b} (0.07)	0.40^{b} (0.08)	0.26^{b} (0.13)	0.34^{b} (0.17)	8^{a} (2)	80^{b} (7)

Table 5 –Mean roughness and bearing parameters (for species combined) at baseline and *post-erosion*. Standard deviations are within brackets. Values with differing superscripts are significantly different. The full data set for profilometric data post-erosion can be found in Appendix C and statistical analyses in Appendix D.

Eroded roughness average (Ra) values were significantly different to baseline values ($P < 0.001$); becoming more rough post-erosion. Ra was not significantly affected individually by tissue type ($P = 0.085$) or time ($P = 0.099$), but was significantly affected by concentration of citric acid ($P = 0.006$; Ra mean 1% 0.192, 6% 0.137). There were no significant *interactions* between tissue and concentration ($P = 0.915$) or tissue and time

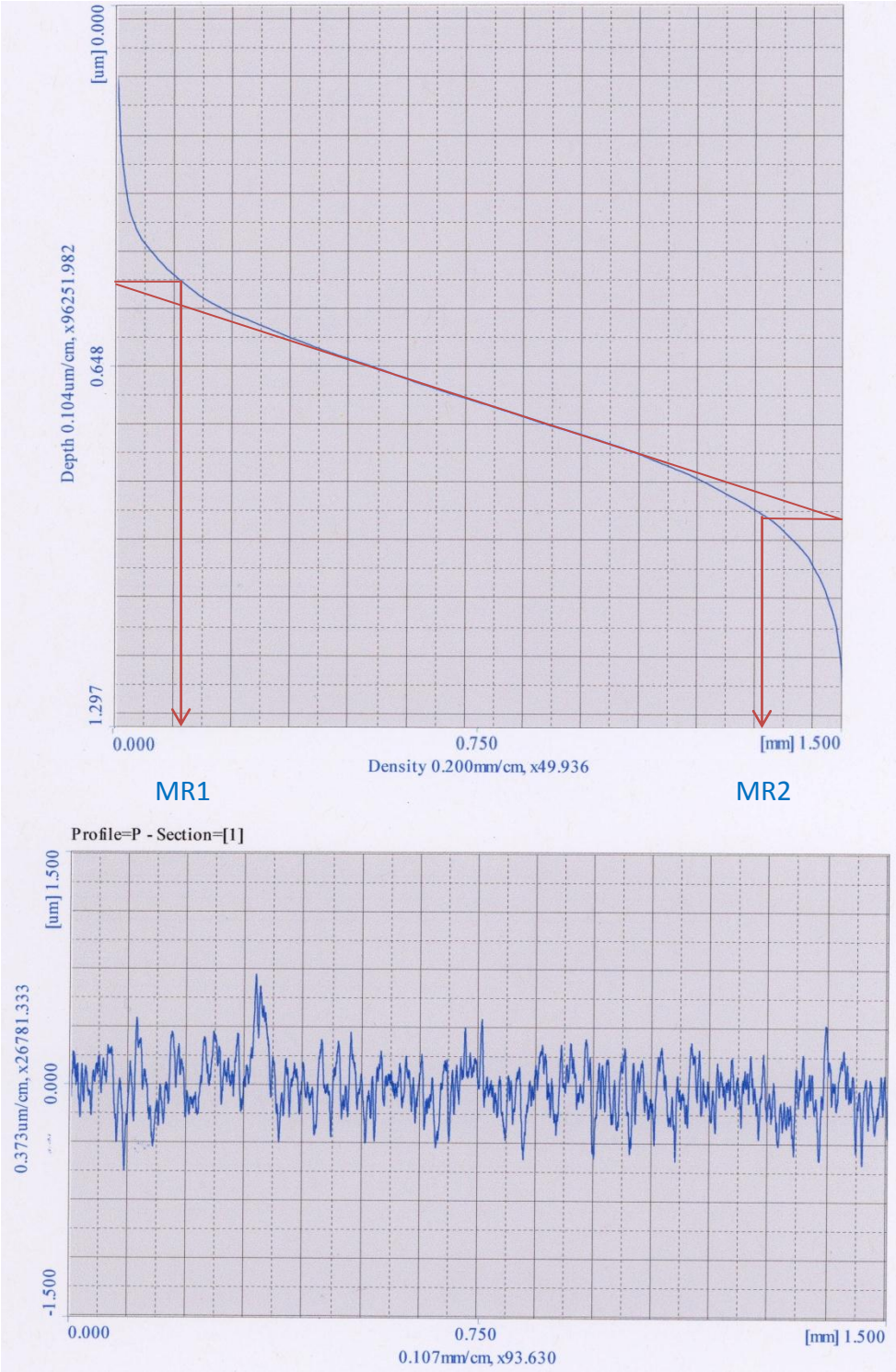


Figure 35 – Typical stylus profilometry profile (Ra 129 µm) and bearing curve (Material ratio 1, MR1 9.5%, Material ratio 2, MR2 90%) for eroded human enamel (1% citric acid for 2 minutes).

Tissue	Acid concentration	Immersion time	Ra (µm)	Rpk (µm)	Rk (µm)	Rvk (µm)	MR1 (%)	MR2 (%)	ΔH (µm)
Human	1%	15 seconds	0.23 (0.08)	0.50 (0.05)	0.21 (0.03)	0.59 (0.05)	12 (1)	93 (2)	0.7 (0.2)
		2 minutes	0.12 (0.02)	0.46 (0.03)	0.24 (0.06)	0.17 (0.01)	10.6 (0.6)	90.4 (0.8)	1.1 (0.2)
	6%	15 seconds	0.12 (0.01)	0.34 (0.02)	0.53 (0.02)	0.18 (0.02)	11.1 (0.6)	92.0 (0.7)	1.0 (0.1)
		2 minutes	0.126 (0.005)	0.306 (0.008)	0.352 (0.006)	0.15 (0.01)	6.9 (0.3)	89.4 (0.8)	1.2 (0.2)
Bovine	1%	15 seconds	0.2 (0.1)	0.43 (0.05)	0.12 (0.01)	0.54 (0.10)	8.01 (0.80)	71 (2)	0.41 (0.20)
		2 minutes	0.18 (0.03)	0.42 (0.08)	0.17 (0.04)	0.42 (0.02)	7.31 (0.58)	75 (52)	1.04 (0.20)
	6%	15 seconds	0.14 (0.05)	0.42 (0.04)	0.39 (0.08)	0.27 (0.05)	7.98 (0.54)	79 (1)	0.58 (0.20)
		2 minutes	0.17 (0.04)	0.29 (0.06)	0.32 (0.08)	0.13 (0.03)	5.8 (0.6)	82 (2)	1.4 (0.2)

Table 6 – 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.

Parameter	Tissue	Concentration	Time
Ra		●	
Rk	●	●	●
Rpk	●	●	●
Rvk		●	●
MR1	●	●	●
MR2	●	●	
ΔH	●	●	●

Table 7 –Individual experimental factors affecting roughness parameters of human and bovine enamel post-erosion. 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. ● denotes a significant effect ($P < 0.05$).

(P = 0.387). There was a significant interaction between concentration and time (P = 0.008) – the effects of different levels of concentration were only significant within the shorter exposure time (15s P = 0.003; 2m P = 0.441) and the effect of different times were only significant at the lower concentration (1% P < 0.001; 6% P = 0.965).

Eroded core roughness (Rk) values were significantly different to baseline values (P = 0.007), becoming significantly less rough post-erosion. Rk was significantly affected by tissue (P < 0.001; Rk mean human 0.332, bovine 0.250), concentration (P < 0.001; Rk mean 1% 0.183, 6% 0.399) and time (P = 0.027; Rk mean 15s 0.312, 2m 0.270). There were no significant interactions between tissue and concentration (P = 0.915) or tissue and time (P = 0.094). There was a significant interaction between concentration and time (P < 0.001) – for combined tissue data the effects of time were significant at both concentrations but the effects of concentration were only significant within the longer exposure time.

Eroded peak roughness (Rpk) values were significantly different to baseline values (P < 0.001), becoming significantly more rough post-erosion. Rpk was significantly affected by tissue (P < 0.001; Rpk mean human 0.272, bovine 0.338), concentration (P < 0.001; Rpk mean 1% 0.427, 6% 0.183) and time (P < 0.001; Rpk mean 15s 0.394, 2m 0.216). There were significant interactions between tissue, concentration and time (P < 0.001) suggesting that the effects of each factor are not consistent at all combinations. There was a significant interaction between concentration and time (P < 0.001) and tissue and time (P = 0.007) but not tissue and concentration (P = 0.088). The effects of concentration and tissue were only significant at the longer exposure time (P = 0.002, P < 0.001 respectively, with a positive interaction for bovine enamel, and negative

interaction for the higher concentration).

Eroded valley roughness (Rvk) values were significantly different to baseline values ($P < 0.001$), becoming significantly more rough post-erosion. Rvk was significantly affected by concentration ($P < 0.001$; Rvk mean 1% 0.454, 6% 0.340) and time ($P = 0.005$; Rvk mean 15s 0.424, 2m 0.369) but not tissue ($P = 0.679$; Rvk mean human 0.401, bovine 0.393). There were significant interactions between tissue, concentration and time ($P = 0.080$) suggesting that the effects of each factor are not consistent at all combinations. There are significant interactions between tissue and concentration ($P = 0.029$) but not tissue and time ($P = 0.413$) or concentration and time ($P = 0.166$). The effects of tissue were significant at both concentrations ($P < 0.001$, Rvk mean 1% human 0.478, bovine 0.429 6% human 0.323, bovine 0.357) however the effects of concentration for each tissue were not significant (Human $P = 0.064$, Bovine $P = 0.206$).

When all eroded surfaces were compared to baseline, irrespective of tissue type or treatment condition, the proportion of eroded profile peaks (MR1) was not significantly different ($P = 0.118$). However, MR1 was significantly affected by tissue ($P < 0.001$; MR1 mean human 10.253, bovine 7.265), time ($P < 0.001$; MR1 mean 15s 9.870, 2m 7.648) and concentration ($P < 0.001$; MR1 mean 1% 9.580, 6% 7.937). There were significant interactions between tissue and concentration ($P < 0.001$; lower values for bovine and higher concentration), tissue and time ($P < 0.001$; lower values for bovine and longer time) and concentration and time ($P < 0.001$; negative interaction for both).

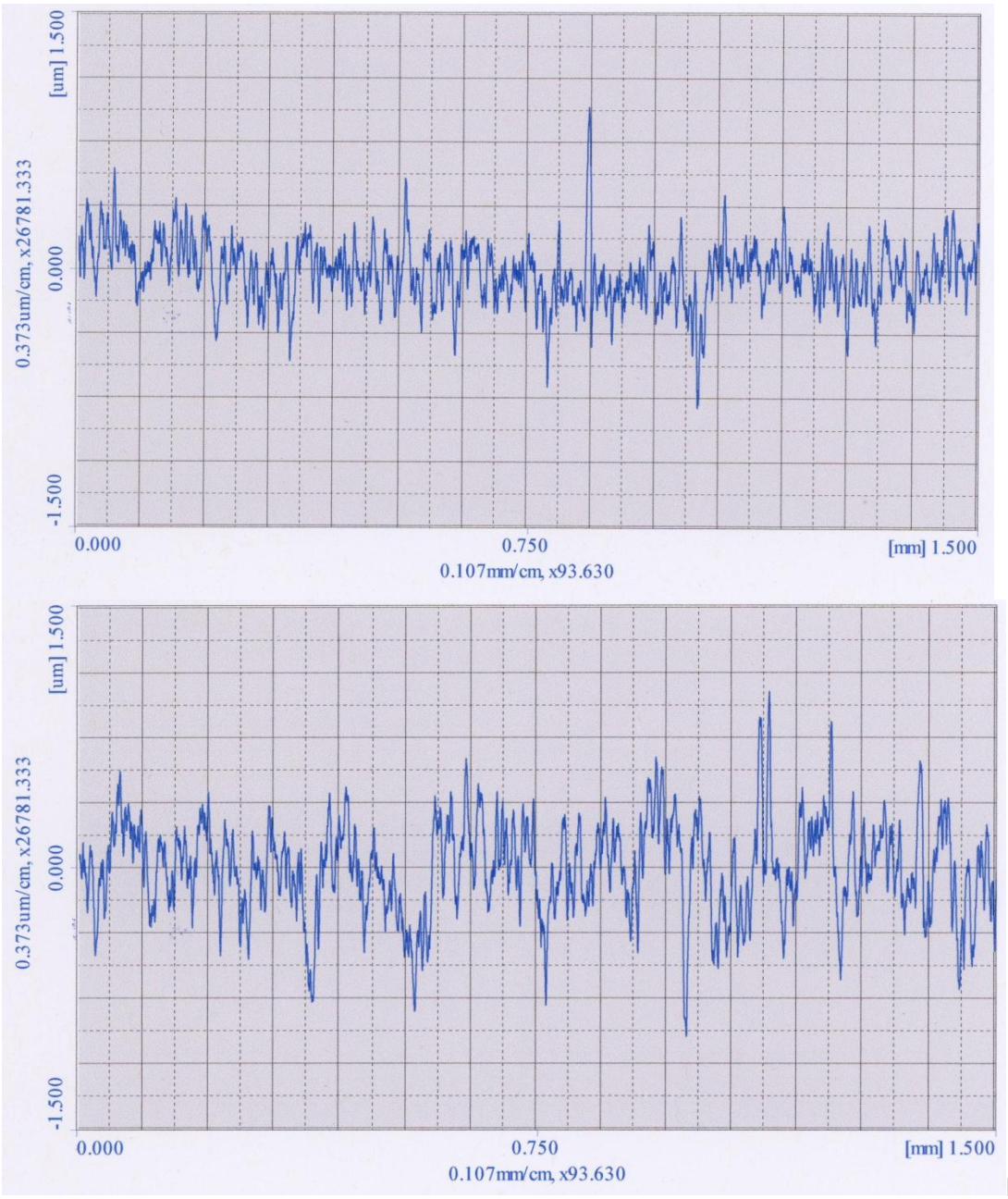
The proportion of eroded profile troughs (MR2) were significantly different to baseline values (P = 0.013), with significantly more troughs post-erosion. MR2 was significantly affected by tissue (P < 0.001; MR2 mean human 91.114, bovine 77.071) and concentration (P < 0.001; MR2 mean 1% 82.454, 6% 85.731) but not time (P = 0.668). There were significant interactions between tissue and concentration (P < 0.001) and tissue and time (P < 0.001; human negative interaction, bovine positive) but not concentration and time (P = 0.631). The effects of tissue were only significant for the higher concentration (6% P < 0.001; MR2 mean human 90.725, bovine 80.737, 1% P = 0.520). The effects of concentration were significant for both tissues (P < 0.001).

The maximum height changes of the eroded profiles were significantly affected by tissue (P = 0.028; mean human 0.996 vs. bovine 0.852), concentration (P < 0.001; mean 1% 0.800 vs. 6% 1.047) and time (P < 0.001; mean 15s 0.667 vs. 2m 1.180). There were significant interactions between tissue and time (P = 0.003) but not tissue and concentration (P = 0.991) or concentration and time (P = 0.911). The effects of time were only significant within the human samples (human P < 0.001; mean 15s 0.841, 2m 1.151, bovine P = 0.520). The tissue effects were significant at both exposure lengths (human values higher at shorter exposures, bovine values higher for longer exposures).

5.2.2 Early erosive surface change on human, bovine and ovine enamel

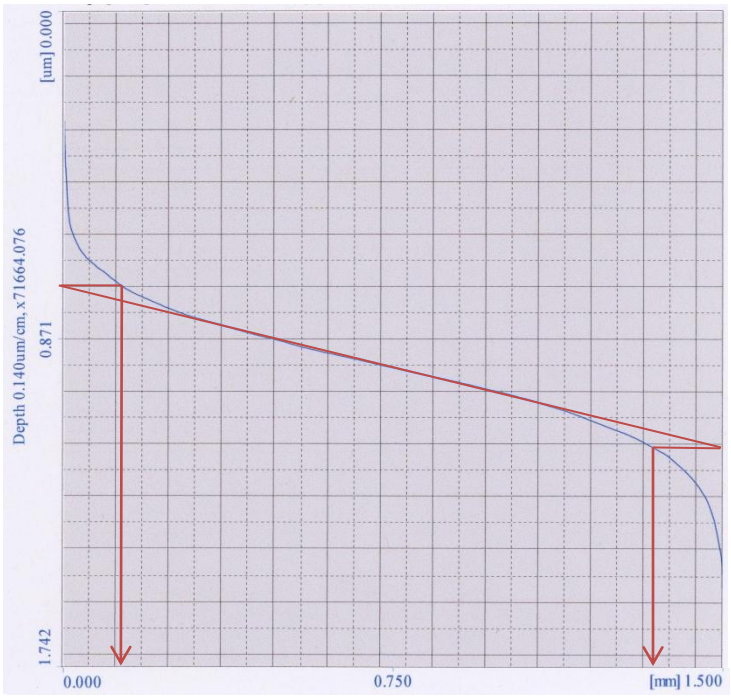
Baseline

Baseline parameters are shown in Table 8, and in contrast to the initial experiment, the data now include ovine parameters. A typical profile and bearing area curve for bovine and ovine enamel are shown in Figures 36 and 37:



Ra

Figure 36 – Typical stylus profilometry profiles for baseline lapped enamel. Bovine enamel (top profile) displays a lower roughness average (126 µm) than ovine enamel (bottom profile, 204 µm).



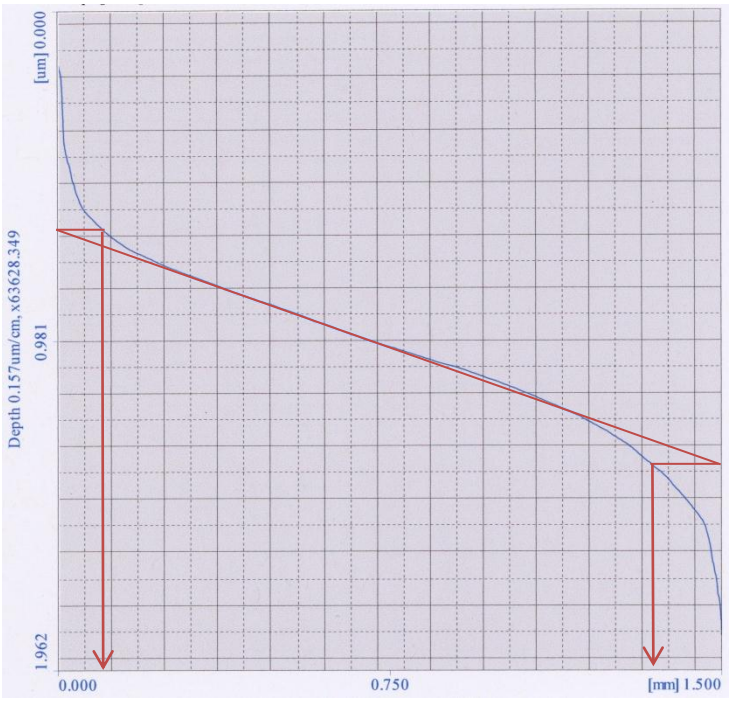


Figure 37 – Typical bearing curves for baseline lapped enamel. Bovine enamel (top curve) shows a higher Material ratio of peak (MR1) value (10%) than the ovine enamel (lower curve, 7.5%).

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 8 – Mean roughness and bearing parameters of human, bovine and ovine enamel at *baseline*. Standard deviations are within brackets. Values with differing superscripts are significantly different between tissues. The full data set for profilometric data can be found in Appendix E and statistical analyses in Appendix F.

Human, bovine and ovine roughness averages (Ra) were significantly different to one another at baseline ($P < 0.001$); ovine enamel was the roughest and bovine enamel was the smoothest. There were similar findings for the core roughness (Rk) ($P < 0.001$), valley roughness (Rvk) ($P < 0.001$) and microhardness ($P < 0.001$). The proportions of profile peaks (MR1) for bovine enamel were also significantly higher than ovine and human enamel ($P = 0.006$) but ovine and human values did not differ significantly. There were similar findings for profile troughs (MR2) ($P < 0.001$). Peak roughness was not significantly different at baseline ($P = 0.634$).

SEM images of each baseline tissue at low, medium and high magnification are shown in Figures 38-40 respectively.

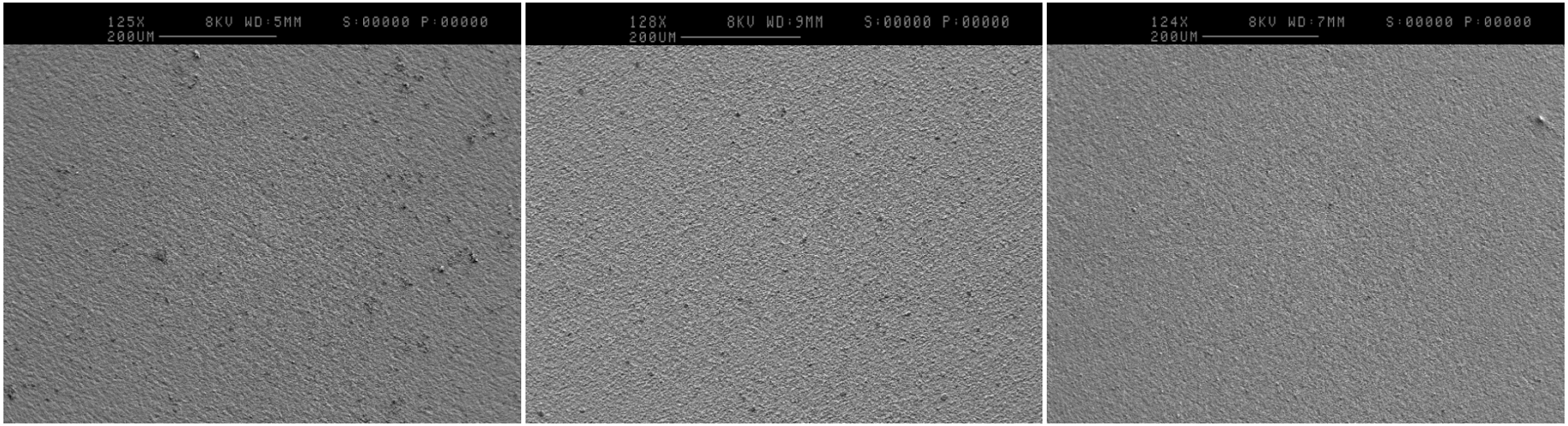


Figure 38 – Low magnification SEM (approximately 125X) of the lapped surfaces at baseline of bovine (left), ovine (centre) and human (right) enamel. Although difficult to discriminate between the tissues at this level of magnification, the ovine enamel (centre) appears more particulate than the bovine or human enamel.

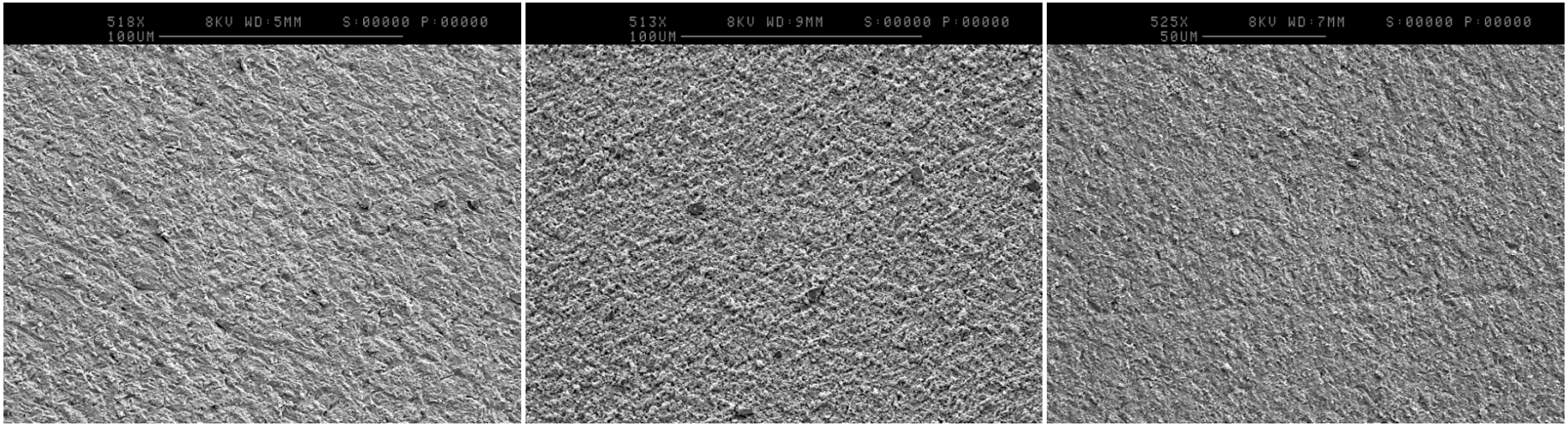


Figure 39 – Medium magnification SEM (approximately 525X) of the lapped surfaces at baseline of bovine (left), ovine (centre) and human (right) enamel. A similar presentation to that of the low magnification images is apparent, with ovine enamel (centre) appearing more particulate than the bovine or human enamel.

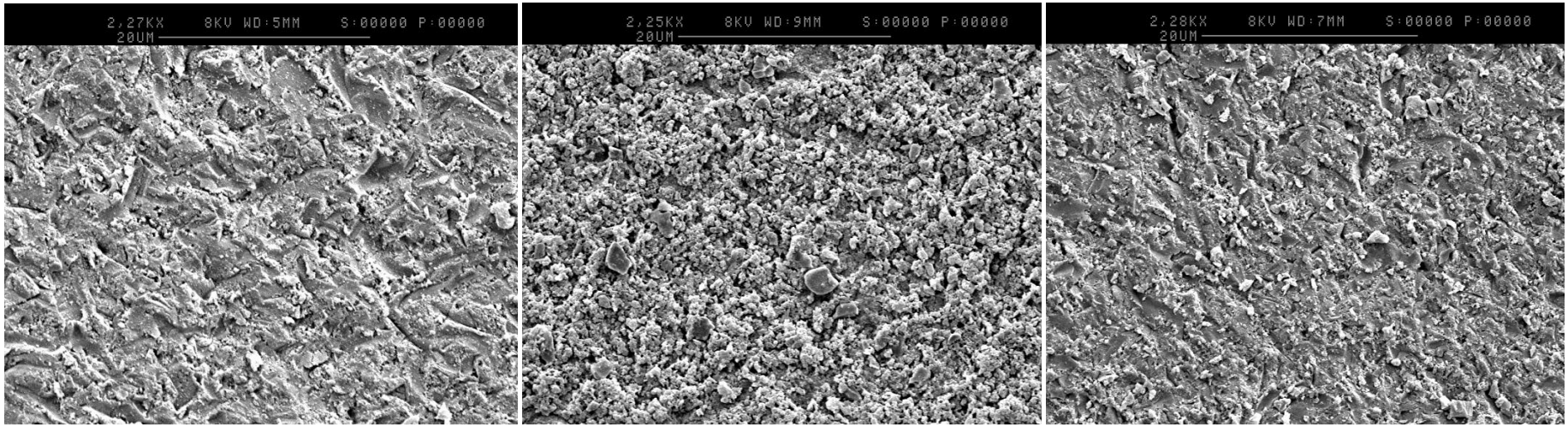


Figure 40 – High magnification SEM (approximately 2,250X) of the lapped surfaces at baseline of bovine (left), ovine (centre) and human (right) enamel. At high magnification it is possible to more accurately assess the lapped enamel surfaces. The ovine enamel (centre) appears to comprise smaller particulate matter, and appears the most rough. Bovine enamel (left) looks to be the smoothest surface but with significant ridges and voids.

At low and medium magnification (x125, x525) is it difficult to see significant differences between the lapped surfaces of human, bovine or ovine enamel. The ovine enamel surface appears to be more particulate in nature and this is confirmed with the higher magnification image (x2,250) within which the ovine surface appears relatively rough. The bovine enamel surface looks to be the smoothest, yet contains relatively large ridges whilst the human enamel's appearance is somewhere in-between the other two.

Post acidic challenge

Again, it was important to consider the overall effects of the erosive challenges, irrespective of tissue, concentration and immersion time (Table 9). The literature reports general and definite trends in roughness parameters post-erosion and it was important to assess whether similar trends were apparent despite the novel inclusion of ovine tissue. There were significant differences within these roughness parameters post-erosion and these are considered below. A summary of these parameters in relation to individual tissue, concentration and immersion time is then shown in Tables 10 and 11. A typical stylus profile and bearing curve are shown in Figure 41.

Ra was significantly affected by tissue, and 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).

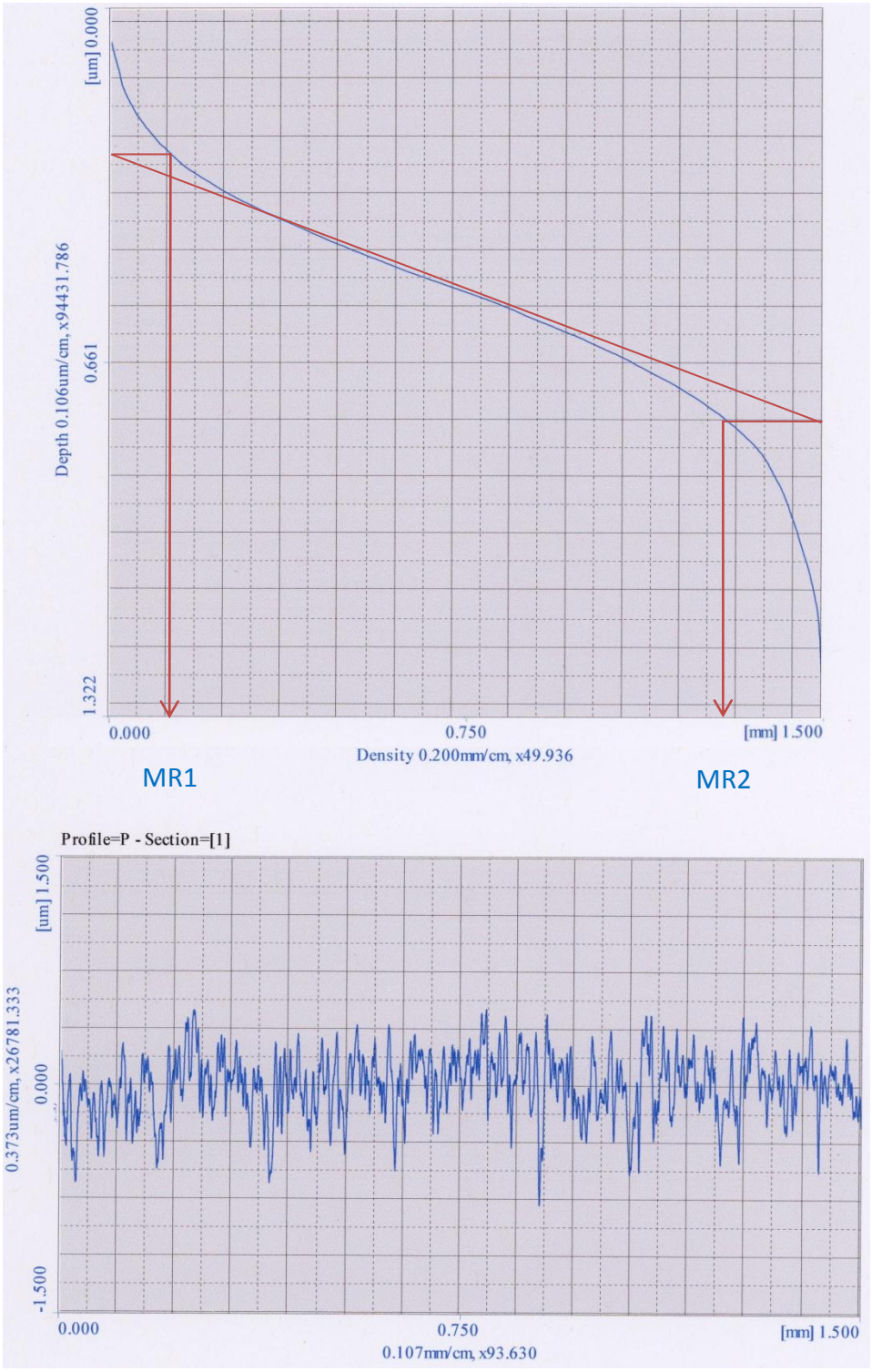


Figure 41 – Typical stylus profilometry profile (Ra 161 µm) and bearing curve (Material Ratio of peaks, MR1, 8.8%, Material ratio of troughs, MR2, 88%) for eroded ovine enamel (6% citric acid for 30 seconds).

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 10 – 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).

Parameter	Tissue	Concentration	Time
Ra	●		●
Rk	●		●
Rpk	●		●
Rvk	●	●	●
MR1		●	
MR2		●	
ΔH	●	●	●
MH			

Table 11 –Individual experimental factors affecting roughness parameters of human, bovine and ovine enamel post-erosion. 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, MH (microhardness). ● denotes a significant effect ($P < 0.05$).

Stage	Roughness average (µm)	Peak roughness (µm)	Core roughness (µm)	Valley roughness (µm)	Material ratio of peaks (%)	Material ratio of troughs (%)
Baseline	0.16[a] (0.03)	0.23[a] (0.09)	0.52[a] (0.11)	0.25[a] (0.07)	9.3[a] (1.4)	88[a] (2)
Eroded	0.16[a] (0.03)	0.18[b] (0.12)	0.52[a] (0.11)	0.26[a] (0.09)	8.9[b] (1.3)	88[a] (2)

Table 10 – Combined species mean roughness and bearing parameters at baseline and *post-erosion*. Standard deviations are within brackets. Values with differing superscripts are significantly different between tissues. The full data set for profilometric data post-erosion can be found in Appendix G and statistical analyses in Appendix H.

Eroded roughness average (Ra) values were not significantly different to baseline values (P = 0.051). 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).

Eroded core roughness (Rk) values were not significantly different to baseline (P = 0.755). Rk was significantly affected by tissue (P < 0.001; Rk mean human 0.516, bovine

0.448, ovine 0.576) and time (P < 0.001; Rk mean 30s 0.494, 4m 0.550) but not concentration (P = 0.295; Rk mean 1% 0.516, 6% 0.528).

There were significant interactions between tissue, concentration and time (P = 0.003) 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; this was only apparent with human tissue at the longer exposure time), tissue and time (P = 0.035; tissue differences were only apparent with the shorter exposure time) and concentration and time (P < 0.001; concentration differences were only apparent with the longer exposure time).

Eroded peak roughness (Rpk) values were significantly different to baseline (P < 0.001), becoming significantly smoother post-erosion. Rpk was significantly affected by tissue (P = 0.012; Rpk mean human 0.166, bovine 0.139, ovine 0.172 - however ovine values were not significantly different to human) and time (P = 0.036; Rpk mean 30s 0.160, 4m 0.196) but not concentration (P = 0.160; Rpk mean 1% 0.190, 6% 0.166). There were no significant interactions between the factors.

Eroded valley roughness (Rvk) values were not significantly different to baseline (P = 0.444). Rvk was significantly affected by tissue (P < 0.001; Rvk mean human 0.257, bovine 0.204, ovine 0.282 – however ovine values were not significantly different to human), concentration (P = 0.026; Rvk mean 1% 0.242, 6% 0.269) and time (P = 0.003; Rvk mean 30s 0.238, 4m 0.274).

The only significant interaction was between concentration and time ($P < 0.001$; concentration effects were only apparent at the longer immersion time, and time effects were only apparent at the higher concentration).

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).

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 concentration and time ($P = 0.023$; concentration effects were only apparent at the longer immersion time)

The proportions of eroded profile troughs (MR2) were not significantly different to baseline ($P = 0.887$). MR2 was significantly affected by concentration ($P = 0.007$; MR2 mean 1% 88.730, 6% 88.118) but not tissue ($P = 0.512$; MR2 mean human 88.260, bovine 88.579, ovine 88.434) or time ($P = 0.322$; MR2 mean 30s 88.312, 4m 88.536).

There were significant interactions between tissue, concentration and time ($P = 0.016$) suggesting that the effects of each factor were not consistent at all combinations. There was a significant interaction between tissue and time ($P = 0.024$; some tissue differences were apparent but only at the lower concentration for longer immersion)

and concentration and time (P = 0.005).

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).

There were significant interactions between tissue and time (P < 0.001) and concentration and time (P < 0.001; the effects of concentration were only significant with the longer exposure).

The microhardness of the eroded surfaces were significantly different to baseline (P < 0.001; MH base 412.468 vs. eroded 318.799). MH was not significantly affected by any individual factor (apart from tissue between bovine and ovine, P = 0.011; mean bovine 419.275 vs. ovine 201.096).

There were significant interactions between tissue and concentration (P < 0.001) and tissue and time (P < 0.001).

Typical SEM images of eroded enamel with 6% citric acid for 4 minutes' duration at low, medium and high magnification are shown in Figures 42-44 respectively. Little difference can be identified between the low magnification images, yet the medium and high magnification 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.

5.3 Abrasion

5.3.1 The early abrasive challenge on human, bovine and ovine enamel

Pilot

Eroded (base) and post-abrasion parameters is shown in Table 12. Given that no evidence existed for species-specific abrasion-only effects, all samples were initially considered together in order to determine evidence of an overall effect.

	Mean base values			Mean abraded values			
Abrasion time	**Roughness average (µm)**	**Material Ratio of peaks (%)**	**Material Ratio of troughs (%)**	**Roughness average (µm)**	**Material Ratio of peaks (%)**	**Material Ratio of troughs (%)**	**Surface loss (µm)**
5 seconds	0.16^{a} (0.02)	9^{a} (2)	89^{a} (1)	0.15^{A} (0.01)	9.2^{a} (0.8)	89^{a} (1)	3.8 (0.9)
20 seconds	0.17^{a} (0.02)	10^{a} (1)	89^{a} (1)	0.16^{a} (0.02)	8.0^{A} (1.2)	88^{a} (2)	5.9 (2.4)

Table 12 – Combined species mean roughness and material ratios at baseline and *post-abrasion* for abrasion times of 5 seconds and 20 seconds. Levels of abraded surface loss are also reported. Standard deviations are within brackets. Significant differences between base and abraded values are indicated with differing cases. The full data set for profilometric data at baseline and post-abrasion can be found in Appendix I and statistical analyses in Appendix J.

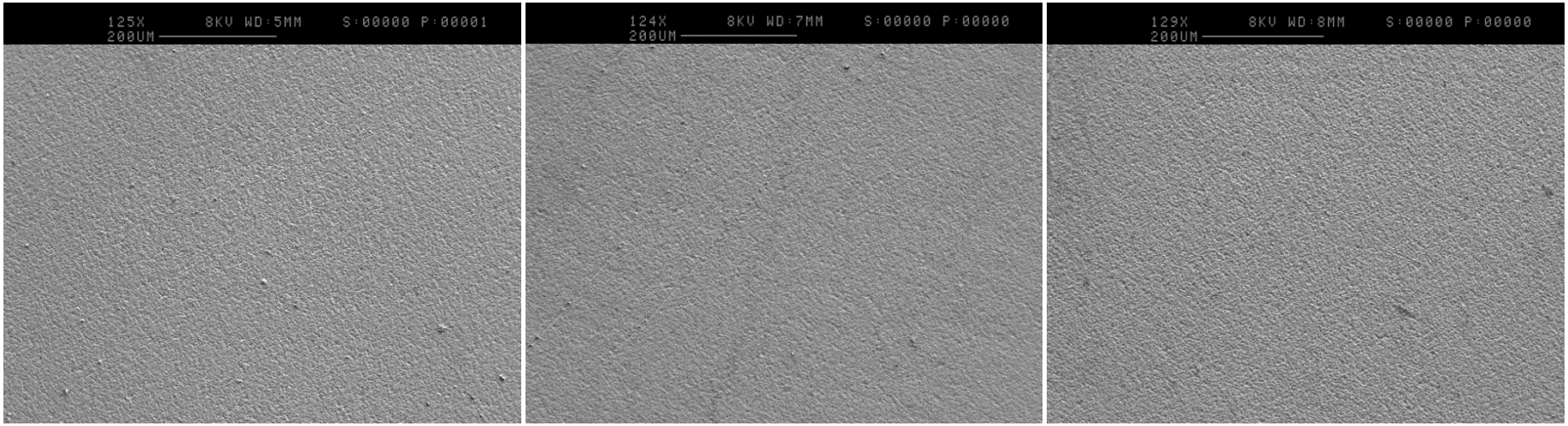


Figure 42 – Low magnification SEM (approximately 125X) of the eroded bovine (left), ovine (centre) and human (right) enamel.

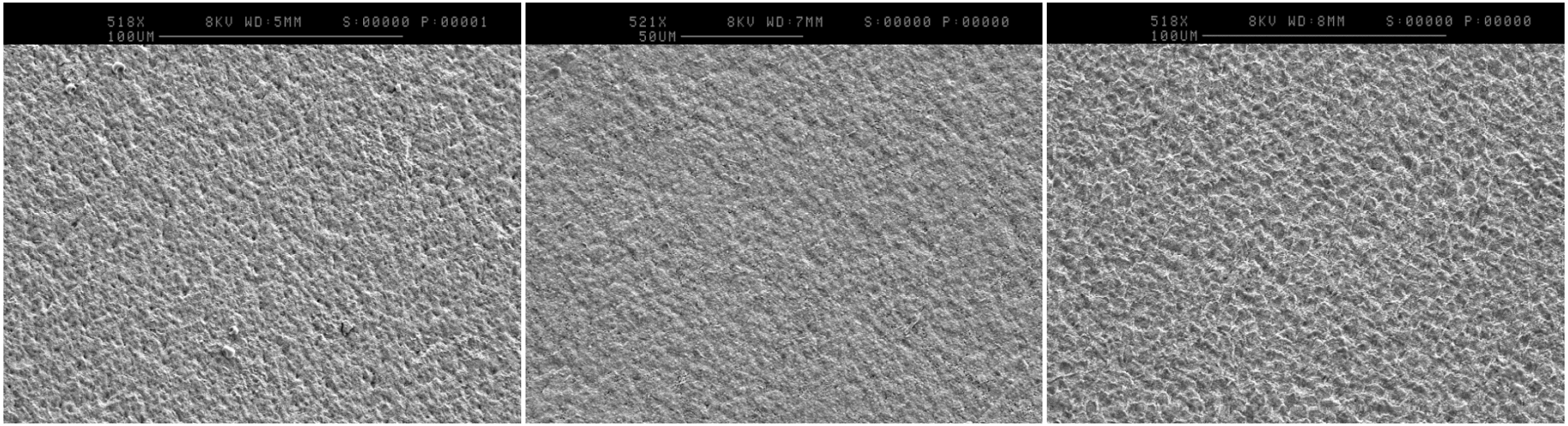


Figure 43 – Medium magnification SEM (approximately 525X) of the eroded bovine (left), ovine (centre) and human (right) enamel. Significantly different erosion patterns are visible on each enamel surface.

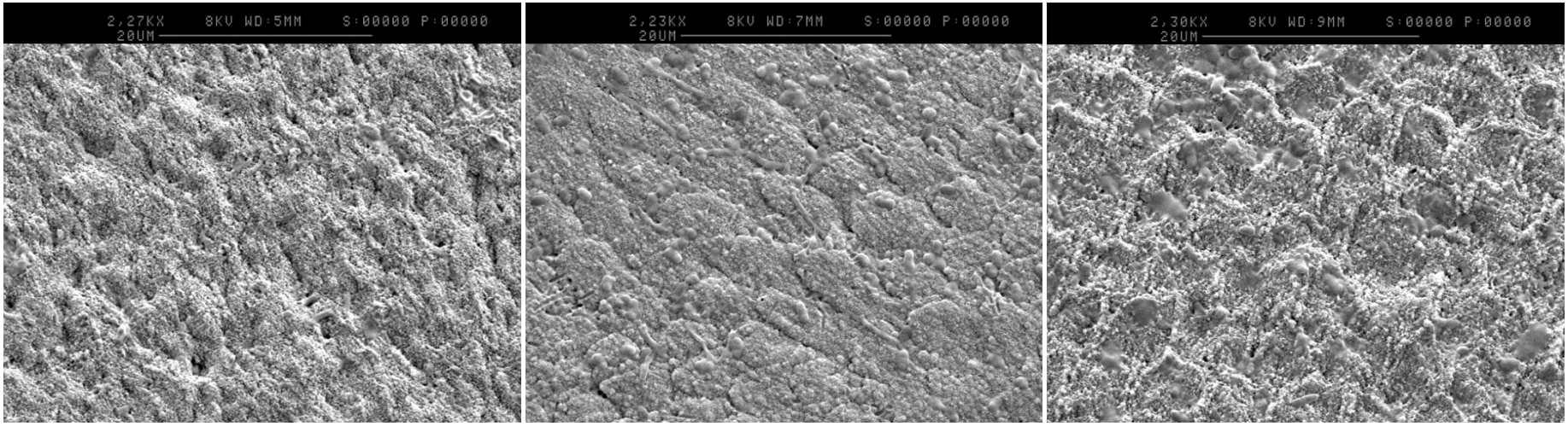


Figure 44 – High magnification SEM (approximately 2,250X) of the eroded bovine (left), ovine (centre) and human (right) enamel. At high magnification it is possible to more accurately assess the eroded enamel surfaces. The eroded human enamel shows considerably more relief than the bovine and ovine profiles, 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.

The 5-second abrasion resulted in a significant reduction in Ra (P = 0.048) and a demonstrable change in profile height (mean 3.790 µm, range 2.159, skewness -0.414). There were no significant changes in the proportions of material peaks or troughs (MR1 P = 0.531; MR2 P = 0.535).

The 20 second exposure resulted in a slightly larger profile height change (mean 5.291 µm, range 5.819, skewness -0.218) but also a significant decrease in the proportions of peaks within the profile (MR1 P = 0.001). The 20 second exposure was therefore considered to be more valuable given the indicated effect on the material ratio, increased range (more platykurtic) and reduced skewness of the data.

Abrasion only experiment

A summary of baseline and abraded parameters is shown in Table 13:

Stage	Roughness average (µm)	Peak roughness (µm)	Core roughness (µm)	Valley roughness (µm)	Material ratio of peaks (%)	Material ratio of troughs (%)
Baseline	0.16[a] (0.03)	0.25[a] (0.05)	0.52[a] (0.09)	0.25[a] (0.07)	9.4[a] (1.4)	87[a] (3)
Abraded	0.16[a] (0.03)	0.24[a] (0.05)	0.52[a] (0.10)	0.25[a] (0.07)	9.2[b] (1.3)	87[a] (3)

Table 13 – Combined species mean roughness and material ratios of enamel at baseline and post-abrasion. Standard deviations are within brackets. Values with differing superscripts are significantly different between tissues. The full data set for profilometric data can be found in Appendix K and statistical analyses in Appendix L.

There was no significant difference in average roughness (P = 0.598), core roughness (P = 0.552), peak roughness (P = 0.612) or valley roughness (P = 0.884) between the lapped and abraded surfaces. Although there was also no significant difference in the proportions of profile troughs (P = 0.542) there were significantly less profile peaks on the abraded enamel surface (P = 0.033).

A summary of post-abrasion parameters by tissue are shown in Table 14.

Tissue	Roughness average (µm)	Peak roughness (µm)	Core roughness (µm)	Valley roughness (µm)	Material ratio of peaks (%)	Material ratio of troughs (%)
Human	0.16^{a} (0.02)	0.23^{a} (0.03)	0.49^{a} (0.05)	0.24^{a} (0.03)	9.0^{a} (0.9)	86.0^{a} (2.4)
Bovine	0.14^{b} (0.01)	0.25^{a} (0.05)	0.45^{a} (0.08)	0.19^{b} (0.02)	9.1^{a} (0.6)	88.1^{b} (2.7)
Ovine	0.19^{c} (0.02)	0.24^{a} (0.06)	0.62^{b} (0.06)	0.31^{c} (0.06)	9.5^{a} (2.0)	87.6^{a} (2.7)

Table 14 – 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.

There were significant differences in roughness average (P < 0.001) and valley roughness (P < 0.001) between all-tissue types post-abrasion, with ovine enamel recording the roughest surface, and bovine the smoothest. Core roughness was also significantly different (P < 0.001); ovine enamel was significantly rougher than bovine and human enamel. The proportions of troughs between bovine and human enamel post-abrasion was significantly different (P = 0.026); bovine enamel recorded

significantly less troughs. There were no significant differences between tissue types for peak roughness (P = 0.360) or proportions of profile peaks (P = 0.745) post-abrasion.

5.4 Erosion and abrasion

5.4.1 Abrasion of the early-eroded lesion on human, bovine and ovine enamel

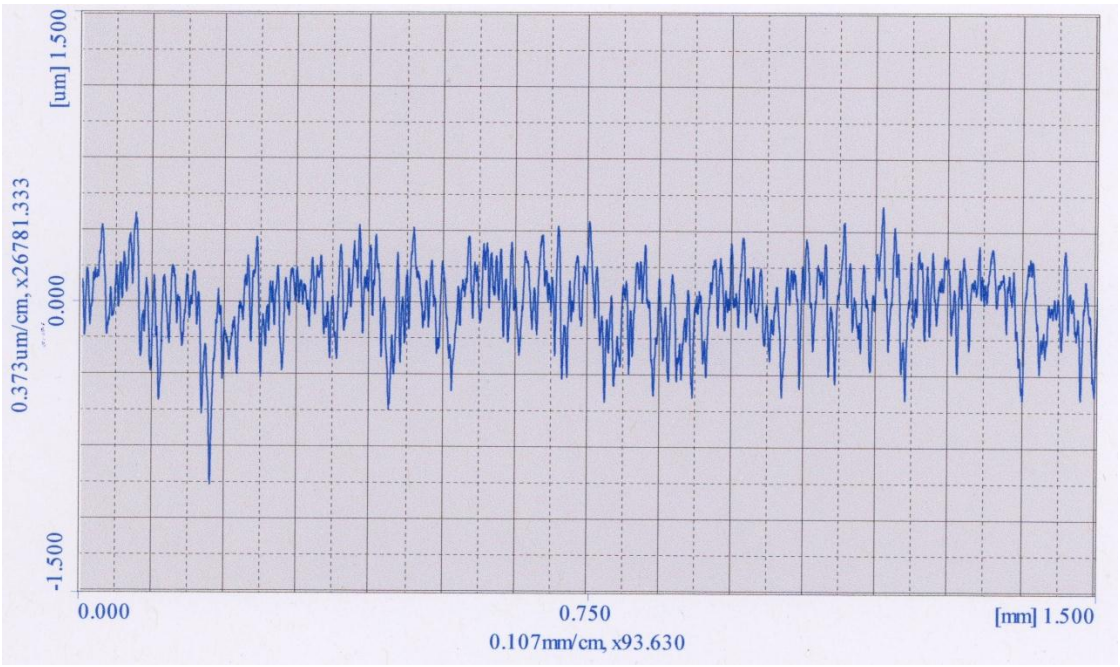
A summary of eroded and post-abrasion parameters is shown in Table 15:

Stage	Roughness average (µm)	Peak roughness (µm)	Core roughness (µm)	Valley roughness (µm)	Material ratio of peaks (%)	Material ratio of troughs (%)	Micro hardness
Eroded Baseline	0.16[a] (0.03)	0.2[a] (0.1)	0.5[a] (0.1)	0.26[a] (0.09)	8.9[a] (1.3)	88[a] (2)	319[a] (109)
Abraded	0.15[b] (0.03)	0.16[b] (0.07)	0.5[a] (0.1)	0.23[b] (0.07)	8.3[b] (1.4)	89[a] (2)	406[b] (134)

Table 15 –Mean roughness and bearing parameters at baseline and *post-abrasion*. Standard deviations are within brackets. Values with differing superscripts are significantly different between tissues. The full data set for profilometric data post-abrasion can be found in Appendix M and statistical analyses in Appendix N.

A summary of post-abrasion parameters by tissue type is shown in Table 16. A typical stylus profile and step height measurement is shown in Figure 45.

Abraded roughness average (Ra) was significantly lower than baseline eroded values (P = 0.002). Eroded and abraded Ra was significantly different between tissue types (P < 0.001; whilst bovine was the smoothest surface, there was no significant difference between ovine and human values).



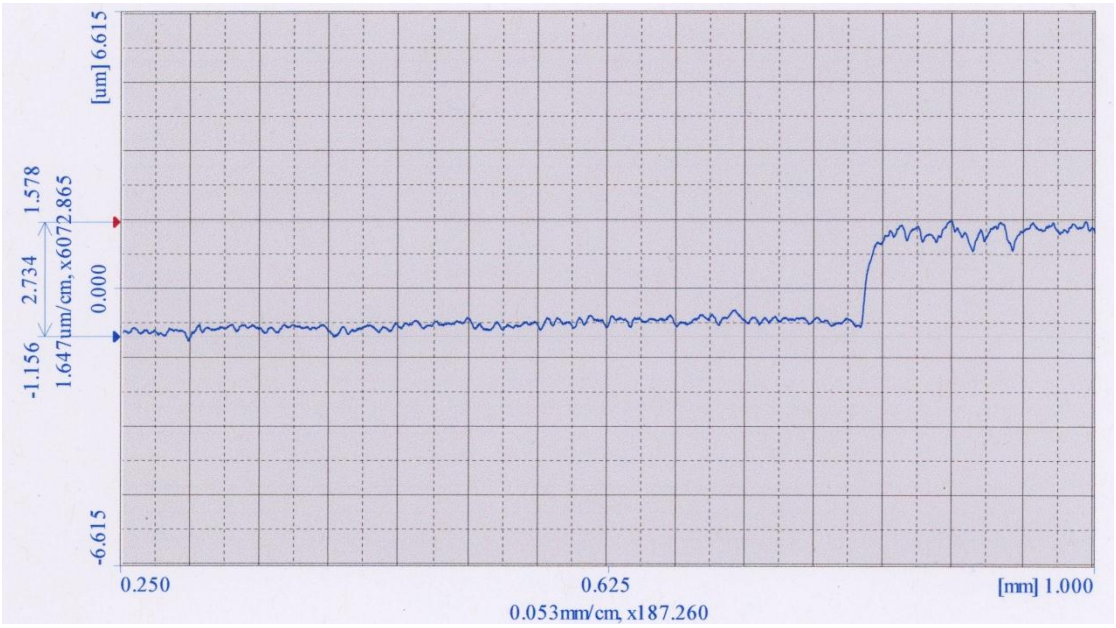


Figure 45 – A typical stylus profile and step height measurement profile for eroded and abraded bovine enamel (Ra 149, Surface loss 2.734 µm).

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)

Table 16 –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.

Abraded core roughness (Rk) was significantly lower than baseline eroded values ($P = 0.258$). Eroded and abraded Rk was significantly different between tissue types ($P < 0.001$; whilst bovine was the smoothest surface, there was no significant difference between ovine and human values).

Abraded peak roughness (Rpk) was significantly lower than baseline eroded values ($P = 0.018$). Eroded and abraded Rpk was significantly different between tissue types ($P < 0.001$; whilst bovine was the smoothest surface, there was no significant difference between ovine and human values).

Abraded valley roughness (Rvk) was significantly lower than baseline eroded values ($P < 0.001$). Eroded and abraded Rvk was significantly different 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 proportion of profile troughs (MR2) were not significantly different to baseline eroded values ($P = 0.524$) and MR2 ratios were not significantly different between tissue types ($P = 0.427$).

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 and ovine the softest.

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

Appendix O details the forward stepwise multiple linear regression analysis that was carried out with abraded height change (representing tooth surface loss) as the dependent variable. 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. Variables not included in a final model (removed stepwise due to insignificance) were Eroded Ra ($P = 0.420$), Eroded Rk ($P = 0.185$), Eroded Rvk ($P = 0.951$), Eroded Rpk ($P = 0.232$), Eroded MR1 ($P = 0.769$) and Eroded MR2 ($P = 0.203$).

Typical SEM images of eroded and then subsequently abraded human, ovine and bovine enamel at high magnification are shown in Figures 46-48 respectively. 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.

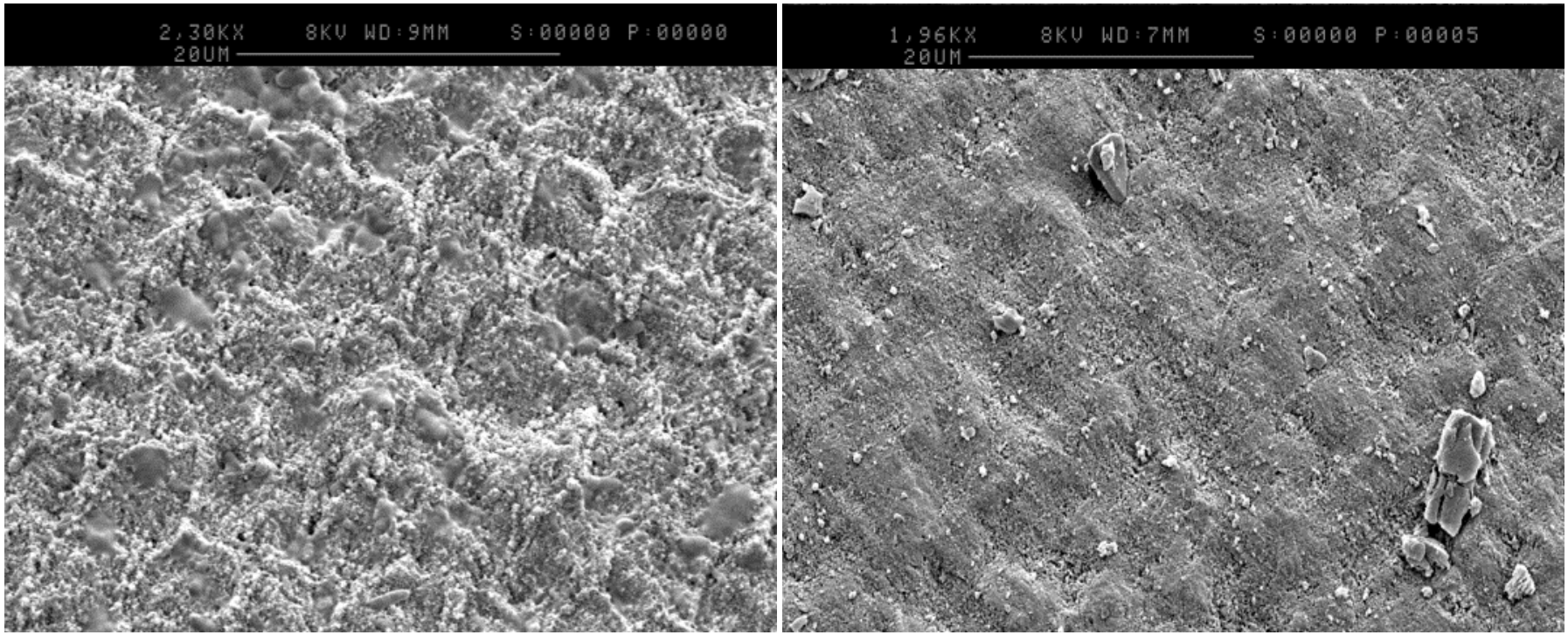


Figure 46 – High magnification SEM (approximately 2000X) of the eroded human enamel (left) and the subsequently abraded surface (right). Note the loss of interprismatic enamel and surface roughness, whilst retaining much the same general surface form.

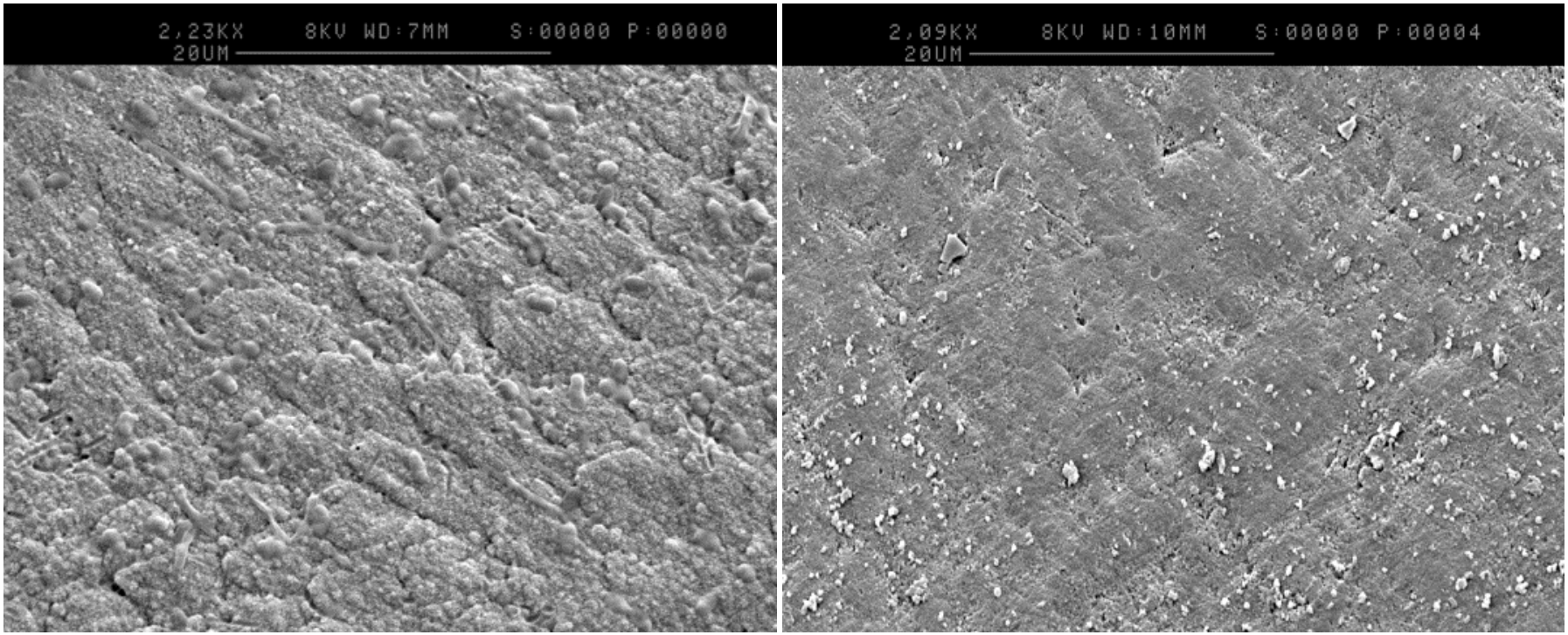


Figure 47 – High magnification SEM (approximately 2000X) of the eroded ovine enamel (left) and the subsequently abraded surface (right). Note the loss of prism ridging and surface roughness, resulting in a somewhat amorphous surface.

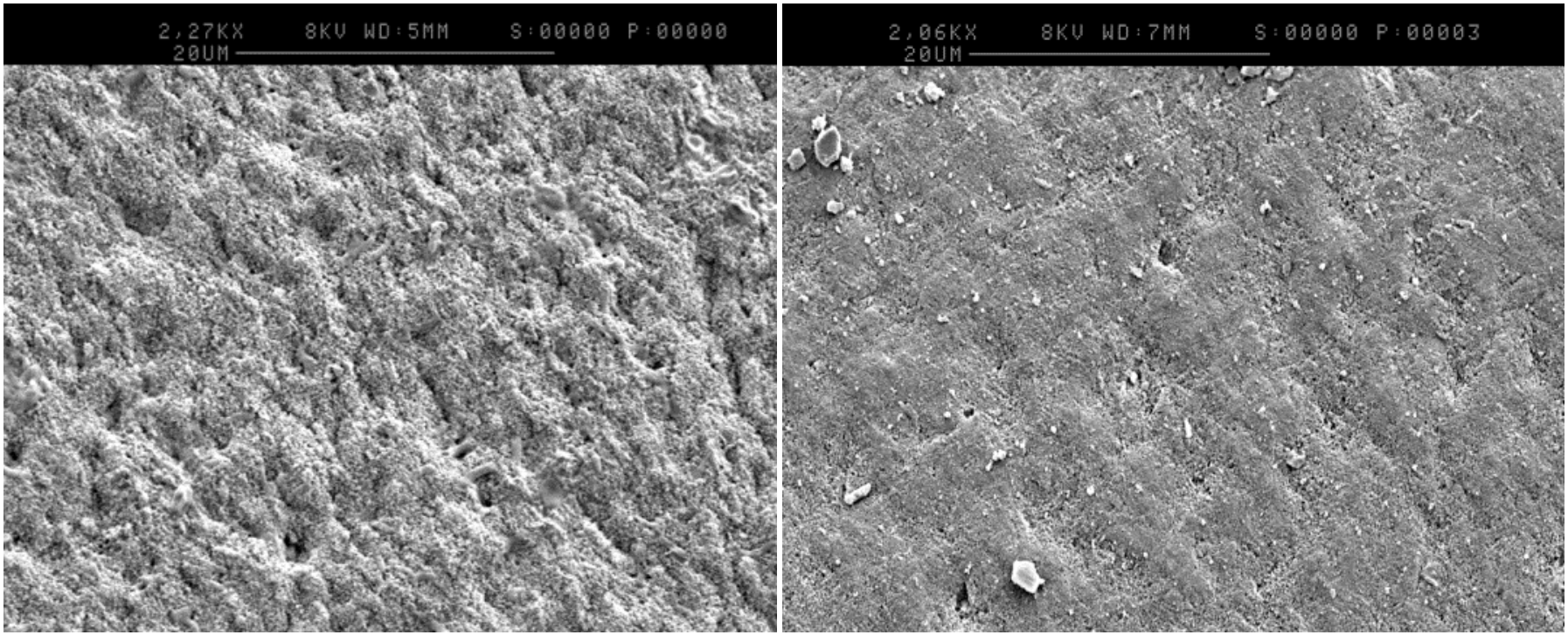


Figure 48 – High magnification SEM (approximately 2000X) of the eroded bovine enamel (left) and the subsequently abraded surface (right). Note the loss of enamel ridges and surface roughness, whilst retaining much the same general surface form.

5.5 Further qualification experiments

5.5.1 Surface effects of microhardness testing on enamel

Figure 49 shows high (128,000X) and low (40X) magnification SEM images of the surface effects caused by the microhardness indenter on the enamel surface. Note the clear geometrical indent made by the tip. No obvious deformations propagate from the indent margins or corners, and the indenter appears to contact the enamel surface completely. This suggests that the chosen loading protocol of 100g for 15 seconds was appropriate, representing enough weight for the indenter to fully contact the surface whilst avoiding structural failure or crack propagation within the enamel.

5.5.2 Changes in surface parameters of polished samples away from the CEJ

There were no significant differences between bearing surface parameters across surface thirds at baseline (after lapping) for Rk ($P = 0.922$), Rvk ($P = 0.851$), Rpk (0.733), MR1 ($P = 0.456$) or MR2 ($P = 0.522$).

5.6 Summary of Principal findings

- Citric acid accounts for between 0.5% and 8% of fruit juice weight by volume.
- Clear casting resin provides a more stable reference layer after lapping, and after being stored in a salt solution, than amalgam or cyanoacrylate.
- Significant differences in roughness parameters and microhardness exist between human, bovine and ovine enamel after lapping, erosion and abrasion of the eroded surface.
- There are significant differences between human, bovine and ovine enamel with respect to tooth surface loss.

- Bearing parameters provide a more critical comparison of the enamel surface than the roughness average alone.
- Immersion time and concentration correlate positively with several roughness parameters.
- Eroded surface loss and microhardness correlate positively with abraded surface loss.

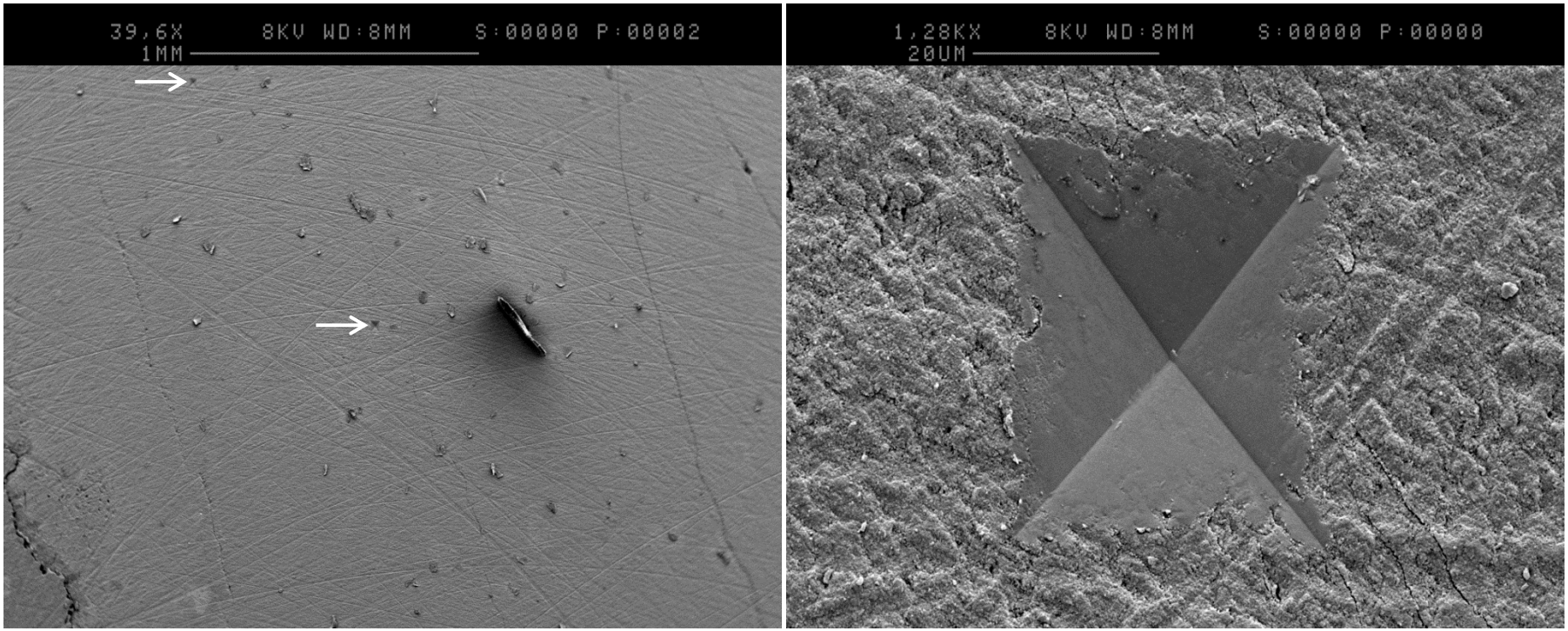


Figure 49 – High (128,000X, right) and low (40X, left) magnification SEM images of the surface effects caused by the Vickers microhardness indenter on the enamel surface. The white arrows indicate the low magnification indents. With high magnification, note the clear geometrical indent made by the tip. No obvious deformations propagate from the indent margins or corners, suggesting that the chosen loading protocol was appropriate.

Chapter 6. Discussion

6.1 Part 1 - Development

6.1.1 Citric acid content of fruit juices

In order to accurately represent an erosive challenge *in vitro,* it was necessary to determine the citric acid content of several fruit-based juices. Although a relatively simple laboratory test was used as an indicator for the citric acid content, the results are not dissimilar to previously reported findings. Ion chromatography studies (Penniston *et al*., 2008) report a citric acid content of nearly 5% of the fresh fruit weight of lemons and limes; the result obtained here through titration was higher, at nearly 7.5% of the fruit weight. This is unsurprising given that other minor acids such as malic or lactic acid will be contributing to the overall acidity of the juice. Interestingly the acid content of the orange juice was lower than previously reported chromatography values (0.65% w/v vs. 2% w/v) for the same brand. However ion chromatography tests can be subject to interference by unrelated compounds (Saccani *et al*., 1995), and indeed the accuracy of measurement can be further complicated by the age of the juice, storage conditions and variations in fruit harvesting and processing. The strawberry and raspberry smoothie recorded similar levels of acidity to the fresh orange juice, and this may be due to the ascorbic acid that is added as an antioxidant. This finding is supported within the literature (Blacker *et al*., 2011). In light of these results, concentrations of 1% and 6% citric acid w/v were used for the erosion experiments; these represented a relatively mild and an aggressive erosive challenge, respectively.

6.1.2 Developing a reference layer

Profilometry of the lapped human reference disc highlighted two main problems:

i) The sample had a distinct curvature which made the profile difficult to interpret quantitatively.

ii) Across the sample surface, the enamel and dentine demonstrated wear at different rates, causing significant lipping at each end of the profile.

The differential wear rate of enamel and dentine is unsurprising given the difference in reported hardness values. Knoop hardness values for enamel have been reported in the range of 355-431 (Collys *et al*., 1992) and dentine hardness values can be five to six times lower (Craig and Peyton, 1958). Knoop hardness is measured in Kilograms of force per square millimetre, but is often reported as an empirical measurement, without units. Cyanoacrylates are not reportedly tested with Knoop indenters – instead a hardness test is used that relates to elasticity such as Shore or Rockwell, and so a direct comparison of hardness is not possible. However, the wear evidence from the profile suggests hardness above that of dentine.

Laser profilometry was used to directly scan the sample surface, but this was unsuccessful due to laser scatter from the hard tissues. This has been reported elsewhere, without apparent explanation (Diaci, 2008). The effect may be due to the translucent nature of the enamel surface. An impression was taken in both polyether and addition-cured silicone so that an indirect scan could be carried out from a plaster cast; however the cyanoacrylate reference layer retarded the setting reaction of both

materials. This effect is not reported elsewhere; the inhibition may be due to sulphur compounds or rubber components such as isoprene, present in the set cyanoacrylate material.

In order to reduce the curvature on the profile it was necessary for the sample to be lapped in a circular motion *counter* to the polisher rotation, rather than being held statically. This revised polishing method was successful in reducing the curvature of the sample, but material towards the periphery still suffered from a significant 'drop-off'. In order to also eliminate the peripheral surface curvature, the sample was embedded *within* an acrylic block, with a *border* of acrylic (Figure 50). This further revision resulted in a completely flat reference surface.

After lapping, acrylic resin suffered from the least surface defects, being almost undetectable to stylus profilometry. Amalgam suffered from voids at the reference-tooth interface, and cyanoacrylate suffered significant surface loss across the whole reference area of around 23 µm in depth. Amalgam Knoop hardness values have been reported between 165 and 191 (Yamada and Fusayama, 1981) and as low as 20 for acrylic resin (Rizzatti-Barbosa and Ribeiro-Dasilva, 2009). Some modified cyanoacrylates have been hardness tested with comparable systems such as Vickers hardness; equating to Knoop hardness of around 120 (Tomlinson *et al*., 2007). As such, hardness is apparently not a direct indicator of a material's suitability as a reference area. Instead, other mechanical properties of the material should also be taken into consideration. Although amalgam should have a compressive strength after 24 hours of 300MPa or greater (International Standards Organisation, 1995), it has a

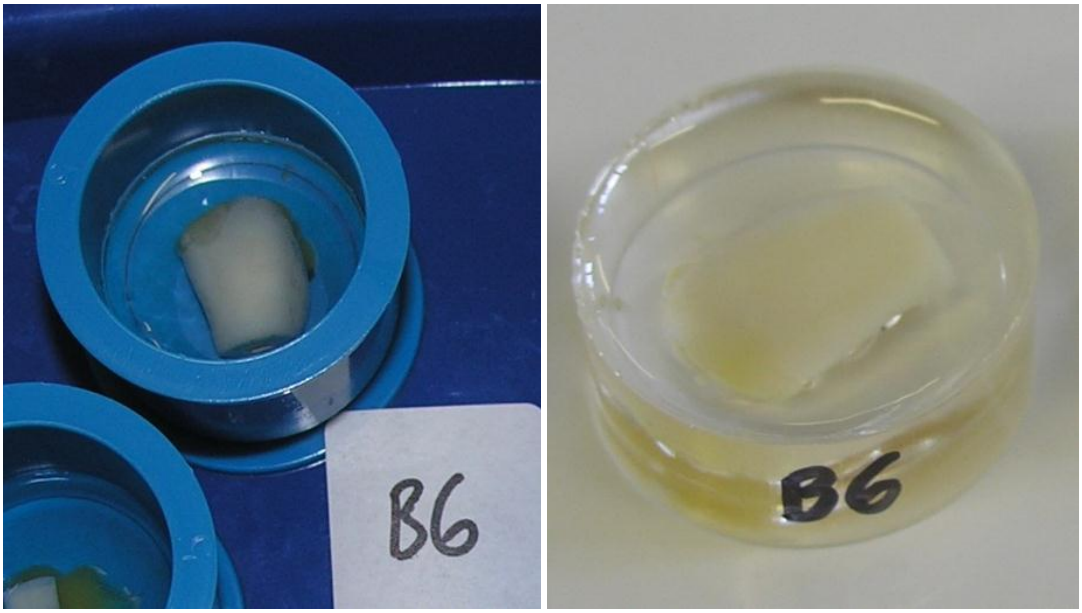


Figure 50 – Bovine crowns embedded in acrylic resin using a cylindrical mould, leaving a definite acrylic border around the edge.

significantly lower tensile strength (McCabe and Walls, 2000). Amalgam is therefore weak in thin-section; it is likely that the rotating action of the lapping discs placed excessive lateral forces onto the amalgam causing marginal failure, especially if the material had started to expand outside the confines of the enamel cavity. Acrylic resin displays significantly different properties – whilst the resin is softer than amalgam, it also displays much more flexibility (modulus of elasticity for resin is 2GPa c.f. 30GPa for amalgam). The features that make resin unfavourable for use as a cavity filling material may indeed make it favourable for use as a lapped reference material; when differentially loaded, the flexibility of resin may lead to marginal breakdown at the cavity margin. However, these results suggest that when loaded evenly and lapped on a flat surface, the low modulus of elasticity results in a low marginal failure rate. This type of experiment is unreported within the literature.

The ability of the material to fully cure before being stored in a fluid medium will have implications for its suitability. Manufacturer instructions do not recommend storing setting or cured cyanoacrylate in humid conditions; indeed a recent forensic study demonstrated the detrimental effect of excessive humidity (over 80%) on the effectiveness of cyanoacrylate to reproduce fingerprints (Paine *et al*., 2011). In this study the samples were stored for over half a day in a balanced salt solution prior to lapping, and this may account for the high wear-rate of the cyanoacrylate reference layer.

Based on the results reported above, acrylic resin displays the characteristics of a stable reference layer, showing a consistently flat surface without significant voids or

defects after lapping. The prepared samples were due to be stored in a balanced salt solution, and so it was important to also understand how the reference layers behaved when stored in a fluid medium.

6.1.3 The stability of reference layers stored in a fluid medium

This study has confirmed that cold-cured acrylic resin provides a more stable reference layer than dental amalgam or cyanoacrylate when samples are to be lapped flat and then stored in a fluid medium for longer than 24 hours.

The amalgam reference area displayed significant expansion (exceeding 50 µm) after only two months of storage in fluid, whilst the acrylic area displayed no detectable surface change by surface profilometry. These findings remained the same for the test period of 3 years. During the 30 minutes of placement, amalgam undergoes a contraction; this is followed by expansion, the extent to which is dependent on the type of alloy used, the particle size and shape and the pressure with which the amalgam was placed (McCabe and Walls, 2000). Initially the amalgam will expand into the tiny deficiencies at the tooth interface, and thereafter it will begin to extrude from the preparation. Data describing the long-term (>24 hours) dimensional change that occurs with dental amalgam is limited but ISO standards limit dimensional change within the first 24 hours to between -0.1% and +0.2% (International Standards Organisation, 1995). 5-year expansion levels for dry-stored amalgam in 4 mm (diameter) by 8 mm (length) cylinders of up to 31 µm have been reported (Paffenbarger *et al*., 1982). This degree of expansion is not dissimilar to that seen after 3 years in this study, and unless amalgam is contaminated with moisture at placement

there is no evidence to suggest that the presence or absence of water will affect the dimensional change that occurs within the set material. In-keeping with other research (Perez *et al*., 2004) this study has confirmed the lack of dimensional change that occurs with acrylic resin stored in water.

6.1.4 The effects of in vitro testing on the toothbrush

Toothbrush operating frequency

The loading protocol of 200g with non-fluoridated toothpaste did not cause a significant change in brush performance over the experimental duration of 11.5 minutes. Thereafter, a significant decrease was noted after 250 minutes. An extra set of tests were carried out in order to record oscillation frequency just prior to this length of time (240 minutes). The oscillation frequency at 260 minutes (9.8 oscillations per second) was significantly lower than the initial frequency of 34 rotations per second. The performance was considered to be severely affected with the brush also delivering, albeit subjectively, a significantly lower oscillatory tone and magnitude of vibration. When a loading protocol of 200g is used, it is assumed that the useful life of the brush prior to battery replacement is 250 minutes. For an individual brushing twice a day for 2 minutes this would equate to a battery life of approximately 2 months. This relates closely to the manufacturer's recommended battery regime (Colgate 2012).

This test did not investigate the effects of a dynamic loading protocol, and indeed the way in which a battery behaves will in many cases be determined by the way in which the device is used (Lahiri *et al*., 2002). Greater loading may result in a greater rate of discharge, and a reduced operating time; the force with which individuals brush their

teeth is variable (Ganss *et al*., 2009b) and therefore the operating time of the brush is likely to vary.

Toothbrush head wear

Early reports of brush wear measurement (Rawls *et al*., 1989) investigated the 'splay' of the brush head, in relation to brush width. This was found to be affected by bristle length and the way in which the brush head was applied to the tissues (often producing asymmetrical bending of the bristles). More recent study has used macroscopic analysis, SEM (Van Nüss *et al*., 2010) and light microscopy concurrently to compare brush head wear; the authors reported that the results from the different test methods differed markedly from one another when comparing brush heads with clear signs of use. With no clear consensus on which method was most suitable for assessing brush head wear, SEM was chosen as an appropriate method of analysing *early* brush head wear in this study due to the ability to view the specimens at a wide range of magnification.

SEM analysis at low, medium and high magnification showed subtle effects from the 11.5-minute brushing regime. High magnification of the bristle tips showed a relatively rough surface with twisted ends to the fibres; post exposure, the form of the fibre tips looked much the same but they presented with a smoother surface. The same effect could be seen at medium magnification. At lower magnification it was possible to observe the relationship of the individual bristles that comprise the brush 'tufts'. Post-exposure, there was slight divergence from the tufts of a number of peripheral bristles. However, the integrity and form of the brush head was not significantly different and it

is assumed that the experiment(s) were not influenced by this factor. Early abrasive effects on rotary brush heads are currently unreported in the literature and this work is unique in being able to report any such effects.

6.2 Part 2 - Erosion

6.2.1 Early erosive surface change on human and bovine enamel

Baseline

This relatively small (n=5) initial study was carried out in order to develop experimental methods with respect to sample preparation, erosion and measurement.

When polished with a 0.05 µm paste, the resultant surfaces of human and bovine enamel showed roughness average values of 0.11 µm and 0.12 µm respectively. These values were not significantly different to one another and this may be due to the small sample size used. No published studies to date report a direct comparison between human and bovine incisor teeth after lapping.

Few studies report baseline human enamel roughness. Quartarone (Quartarone *et al*., 2008) reported a range of values for human enamel of between 0.05 µm and 0.120 µm, and although Rq was reported rather than Ra, the upper limits of the reported values are similar to the findings from this work; unfortunately the study did not detail any particular technique for enamel preparation and so further comparison is not possible. Higher values have been reported by Ren (Ren *et al*., 2009a) who reported baseline Ra between 1.20 µm and 1.40 µm after polishing buccal and lingual surfaces of human third molars with 0.3 µm paste. Azrak (Azrak *et al*., 2010) also recorded

similar Ra values (median 1.57 µm) for the labial surfaces of human lower incisors; the enamel surfaces were prepared using a pumice slurry and then polished with silicon carbide and aluminium oxide discs (to unreported particle and grit sizes). Given that pumices can contain particles up to 50 µm in diameter (Lutz *et al*., 1993), it is unlikely that any amount of fine polishing will remove surface defects obtained due to treatment with pumice slurry, and this may explain the significantly higher roughness values obtained. More importantly, the remaining surface defects may have affected the results of the study, which was investigating the influence of different bleaching agents on surface roughness. Even fewer studies report *bovine* baseline enamel roughness; Gerbo *et al* (1993) reported values between 3.4 µm and 3.8 µm for bovine incisors but this was of the native enamel surface only. No surface preparation had been carried out. Fuji (Fujii *et al*., 2011) reported baseline Ra values for bovine incisors of between 4.7 nm to 6.4 nm. These values are significantly lower than those obtained in this study. The enamel samples were polished with a 0.25 µm paste and then cleaned ultrasonically to remove paste debris before measurement with a stylus tip of radius 2.5 µm; it is unknown whether the stylus tip was able to accurately measure roughness in the nanometre range, and it may be the case that the values reported do not relate to the surface finish. Although the paste used in this study was of a smaller particle size, it is unlikely that this would be responsible for the significantly smoother surface finish; it has already been demonstrated that grit size does not correlate well with prismatic enamel roughness (Maas, 1991). Further, it is unknown what effect the ultrasonic instrumentation had on the enamel surface; shearing of enamel peaks may have significantly reduced the surface roughness average, and this would warrant further investigation. Fuji also measured roughness average values using focus

variation 3D microscopy, which gave higher Ra values than the reported profilometry values, but still significantly lower than expected (15 nm-17 nm).

Although in the current experiment the majority of baseline roughness parameters were not significantly different between human and bovine tissue, two parameters *were* significantly different. There were significantly more peaks within the human samples (higher MR1 value) and significantly more troughs within the bovine samples (lower MR2 value). To date these parameters have not been used to compare the lapped enamel surface and it is unknown to what degree the underlying enamel morphology affected the lapping process. These findings suggest that if Ra alone is measured, subtle differences in surface characteristics may be missed. Surface changes may be able to be modelled more effectively if these extra baseline characteristics are recorded.

Post-erosion

After the erosive challenge the resultant surfaces showed an increased roughness average (0.175 µm vs. 0.131 µm at baseline). At the nano-level, this increase in roughness may be explained by the erosive challenge preferentially 'punching out' the centre of the apatite crystals (Arends *et al*., 1992). On a micro-scale, others describe the resultant 'honeycomb' pattern typically seen in human enamel (Meurman and Ten Cate, 1996; Shulin, 1989), resulting from early erosion of prism and prism junctions leaving behind steep ridges of inter-prismatic enamel (Eisenburger, 2009; Xiao *et al*., 2009). These findings are in keeping with other published work; Quartarone (Quartarone *et al*., 2008) reported a 'remarkable' increase in enamel roughness after

acidic exposure (over 500%) and although the current experiment demonstrated a much more modest increase in roughness (32%), the total submersion times were significantly shorter (2 minutes vs. 280 minutes).

In this study, the eroded roughness average of human and bovine enamel was not significantly different. This mirrors the baseline trend. Despite similar roughness averages, human samples exhibited a greater profile depth range after the erosive challenge. This is in keeping with recent work which reported that human enamel loss was significantly greater than bovine enamel loss when exposed to citric acid (pH 3.2) for up to 5 minutes (White *et al*., 2010). This difference may be due to the tissue-specific proportions of prismatic to interprismatic enamel; it has already been reported that bovine tissue contains a greater proportion of interprismatic enamel than human tissue, and this interprismatic enamel is more resistant to erosion than prismatic (Xiao *et al*., 2009). The conclusion that bovine enamel has the potential to be more resistant to erosion than human enamel mirrors the results from the current study. A number of studies contradict these findings (Attin *et al*., 2007; Featherstone and Mellberg, 1981), suggesting that bovine enamel is more susceptible to erosion than human. However, the acidic challenges used were more aggressive (at greater concentrations and lower pH, for longer periods of time). Indeed the work by White *et al* (2010) has also shown that as the acid-exposure time increases beyond 5 minutes, bovine enamel then begins to display a greater surface loss than human enamel. Also, less aggressive erosive challenges result in more contrast in relief between prismatic and aprismatic enamel (Meurman and Frank, 1991). In conclusion, the behaviour of bovine and human enamel may change significantly depending on the type of erosive insult. This has

implications when attempting to compare results from different research groups or even between similar experiments utilising disparate erosive solutions/regimes; ultimately it adds further complexity to whether bovine enamel is a suitable model for erosion on human teeth, and the results from the initial experiment suggest that it is not.

In relation to the bearing parameters, post-erosion there was an increase in the proportion of troughs within the samples. The proportion of peaks remained the same as at baseline. Within each profile the peak and valley roughness were also increased, whilst the core roughness showed an overall decrease. There is an expectation that all surfaces will become rougher post-erosion - the reduced core roughness may be a consequence of the increasing proportion of troughs within the profiles.

Despite the eroded Ra values being similar between tissues, there were again significant differences in bearing parameters between the human and bovine samples The eroded human samples displayed more profile peaks and less profile troughs than bovine, which closely mirrors the baseline trends.

In agreement with previous research (Fujii *et al*., 2011; West *et al*., 2000), higher concentrations of acid and longer exposure times resulted in larger profile height changes. However these results show that the situation is more complex when the surface roughness parameters are considered; a higher concentration of citric acid left a significantly less rough surface than a lower concentration. A similar trend was also seen for the number of profile troughs. This implies that an increased concentration of

citric acid isn't likely to result in a *rougher* enamel surface. These observations could be caused by the stronger erosive challenge eroding less selectively; however this would still have been associated with a larger degree of surface loss. This phenomenon can be further explained by Barbour (Barbour *et al*., 2011) in relation to dissolution rates and fluid dynamics – an erosive solution with a lower pH develops a higher concentration of calcium and phosphate ions at the site of erosion. This can result in a slower rate of dissolution. Barbour reports that the level of erosion also depends on the rate of stirring during the erosive challenge; stirring will disrupt the static layer immediately adjacent to the erosive site, reducing the saturation of calcium and phosphate ions and promoting further dissolution. This study did not stir or agitate the samples during the erosive challenge and this may explain the limited effect of the more concentrated erosive challenges. The results also suggest that the effects of concentration are more pronounced at shorter exposure times, and it may be that more extensive levels of erosion are either too destructive to allow subtle changes in surface parameters to be monitored, or are completely obliterating the enamel surface.

6.2.2 Early erosive surface change on human, bovine and ovine enamel

Baseline

The results suggest that when lapped on a glass plate with 3 µm slurry, the roughness average is significantly different between the three tissue types. Ovine enamel was the roughest (185 µm) and bovine the smoothest (132 µm); human enamel was in-between (151 µm). These baseline roughness average values are different to the findings for the initial experiment that found no significant difference between human

and bovine enamel at baseline. This anomaly has also been reported by Putt (Putt *et al*., 1980) when polishing human and bovine enamel incisors with a variety of abrasive pastes. Degree of polish was measured using a reflectometer; although the ranking of the abrasives was identical for human and bovine enamel, those abrasives resulting in a poorer surface finish (larger grit size) resulted in greater differences between the human and bovine enamel. This finding is mirrored in the results from the current experiments, and has implications for the ways in which enamel samples are ground down and lapped for surface studies. There is a need for a standardised protocol to ensure that grit size and paste characteristics do not differentially abrade species-specific enamel.

There were significant differences in the bearing parameters after lapping. Bovine enamel exhibited the most peaks and the least troughs (human and ovine values were not significantly different), whereas it was the human enamel in the initial study that displayed these characteristics. Although Ra was different for each species, there was no significant difference in the peak roughness (Rpk), and this may be the result of the method of polishing. This experiment employed a uniform, standardised approach using a glass slab and a mechanical polishing arm. The earlier experiment relied on a hand-held sample pressed onto a polishing cloth with an unstandardized force. The core roughness and valley roughness for each species followed the same trend as the roughness average. The importance of polishing parameters such as load applied, tool condition, feed state and lubricant type or grit size should not be underestimated (Maas, 1991). Clearly these factors had a significant impact on the results that were obtained at baseline, and perhaps even subsequently.

For this and subsequent experiments, the microhardness of a sample subset was recorded. Few studies report actual micro-hardness values post-erosion or abrasion, reporting changes only. Bovine enamel was the hardest at baseline (Vickers 532), whilst ovine enamel was the softest (Vickers 293). The data collected for the human samples (Vickers 412) is complemented in the literature, reporting baseline human enamel Vickers microhardness from 304 (Chuenarrom *et al*., 2009) to 409 (Turssi *et al*., 2010). In the former study, no details were given about how the teeth were prepared. Despite reporting similar baseline human microhardness values, the studies by Turssi *et al* (2010) and White *et al* (2010) reported that human and bovine microhardness readings were not significantly different at baseline (human 425 vs. bovine 413). This contradicts the findings from this study, and again highlights the possible complications of a multitude of preparation methods. Turssi used human third molars, not incisors. Further, after lapping, samples were polished with paste containing particles of 0.3 µm diameter, treated ultrasonically and then stored in deionised water prior to testing. It is recognised that storage in deionised water may reduce the mineral content of the enamel surfaces and therefore prevent an accurate comparison of the two tissues (Habelitz *et al*., 2002). The study by White *et al* (2010) polished with 0.25 µm grit and also used ultrasonication after polishing, but in tap water. No detail was given about the mineral content of the water used on the newly exposed enamel surface, or details pertaining to the types of human teeth used. Nanohardness values were reported in GPa with a Berkovich tip on an AFM (human 4.30 vs. bovine 4.65). Note that although both studies recorded no significant difference between bovine and human tissues, the actual mean values followed different patterns for each study. In agreement with this experiment, White *et al* (2010) reported a higher mean

microhardness value for bovine enamel.

SEM images were also obtained for each tissue at baseline. At low (x125) and medium (x525) magnification it was difficult to discriminate between the tissue types, although the ovine enamel surface did appear noticeably more particulate than human or bovine enamel. At high magnification (x2250), the particulate ovine surface was even more noticeable, and this is in keeping with previous research – Grine *et al* (1986, 1987) reports ovine enamel as more 'irregular' than human. The bovine enamel appeared to be the smoothest surface, but with significant voids and ridges. These subjective findings correspond to significantly different roughness parameters when measured by profilometry. As such, SEM analysis served as a useful visual technique to confirm the numerical bearing parameters.

Post-erosion

After the erosive challenge there was no statistically significant change in roughness average from baseline. These findings contradict the initial erosive experiment and earlier published work that show an increase in roughness average post-erosion (Shellis *et al*., 2005; Nekrashevych and Stosser, 2003; Çehreli and Altay, 2000; Oliver, 1988). This experiment subjected samples to erosive challenges for twice as long as the initial experiment, and the studies reported above subjected samples to notably more aggressive acidic challenges (both with respect to concentration and time). It is unknown to what extent these factors may have influenced the difference in results; indeed immersion time has already been demonstrated to show a non-linear relationship with erosion depth (Gregg *et al*., 2004). Nonetheless the aim within this

study was to investigate *early* surface changes and to develop a suitable model to *measure* early surface change; and although Ra values were not statistically significantly different post-erosion, analysis of the bearing parameters did reveal statistically significant differences. Post-erosion the samples displayed a significant reduction in the number of profile peaks, and this was in-keeping with the initial experiment. No significant changes in valley or core roughness were demonstrated; similarly there was no significant change in the proportions of profile troughs. The roughness average and core roughness values of the eroded samples were significantly different between tissues (ovine enamel was the roughest, bovine the smoothest), and these findings also mirror the baseline data. Tissue type alone did not significantly affect the proportions of peaks or troughs within the profiles but this finding contradicts that of the initial experiment. These disparate findings may be due to longer immersion times within the second experiment, or a different surface finish after lapping.

When the individual features of the erosive challenge are considered, the findings are similar to the initial experiment; a longer immersion time resulted in a more roughened surface. Citric acid concentration alone did not affect the roughness average but it was responsible for causing significantly more profile troughs and less profile peaks. The effects of citric acid concentration were apparent only at *longer* immersion times, showing that a *lower* concentration resulted in the roughest surface, and this latter observation has already been discussed.

In relation to the depth range within the eroded profiles, the findings were also similar

to the results from the initial experiment; human samples exhibited a greater profile depth range than bovine after the erosive challenge. This is in contrast to other reported findings that mineral loss and lesion depth is less for human than bovine enamel (Rios *et al*., 2006b; Amaechi *et al*., 1999a). However, often these findings are reported at *longer* exposure times, resulting in actual surface *loss*. Both Amaechi *et al* (1999) and Arends *et al* (1989) explain that their increased susceptibility of bovine enamel is likely due to the presence of larger porosities than human enamel. With long exposure times it may well be the case that these topographical features promote tissue loss. It is important to remember however that early erosive challenges will not result in bulk tissue loss (Eisenberger *et al*., 2004). As discussed previously, bovine enamel has the potential to be more resistant to erosion than human, given a reduced susceptibility of, and greater proportion of interprismatic enamel. Further, the behaviour of bovine and human enamel may change significantly depending on the type of erosive challenge. In this experiment, the ovine enamel samples exhibited the largest depth range post-erosion and this may imply that ovine enamel is the most susceptible to surface change. No literature exists to support or refute this statement. Further, no literature exists that directly details the structure of ovine enamel. Few studies mention ovine enamel and even then, these studies are often chiefly investigating goat or deer enamel structure (Kierdorf *et al*., 1991; Grine *et al*., 1987; Grine *et al*., 1986). Boyde (1989) described ovine enamel as columns of prisms with incomplete outlines, and sheets of interprismatic enamel separating the columns. There are no reports of the proportions of prismatic to interprismatic enamel, although the results from this experiment suggest an abundance of prismatic enamel. These results also confirm the analysis of ovine matrix proteins by Fincham *et al* (1982)

who reported that ovine enamel displayed a more 'enamelin-like' composition, resulting in a surface that may be more susceptible to dissolution or wear.

The present study also recorded the micro-hardness of the samples post-erosion. A significant reduction in micro-hardness was observed (Vickers 413 base, Vickers 319 eroded, $P < 0.001$). This finding is confirmed by other studies that directly investigated the effects of demineralisation on enamel micro-hardness (Ren *et al*., 2009a; de Marsillac *et al*., 2008; Tantbirojn *et al*., 2008; Collys *et al*., 1992; Feagin *et al*., 1969). It is proposed that the reduction in microhardness is due to erosive mineral loss, resulting in a more delicate enamel substructure. Bovine and human enamel remained the hardest post-erosion whilst ovine enamel remained the softest. This pattern is consistent with baseline data. Despite other significant surface differences between bovine and human enamel, previous work (Turssi *et al*., 2010; White *et al*., 2010) also supports the finding from this study that with a relatively mild erosive challenge (fresh orange juice and citric acid pH3.2 for up to 5 minutes) there was no significant difference in micro-hardness between human and bovine enamel.

SEM analysis

Within the eroded human sample it was possible to see the heads of the enamel prisms that had been preferentially eroded before the interprismatic enamel, resulting in this classical 'keyhole' appearance (Xiao *et al*., 2009; Meurman and Ten Cate, 1996; Shulin, 1989). This appearance is typical to Type 3 enamel described previously by Boyde (1989). The head of the prisms measured approximately 8 µm which is in keeping with previously published data (Ten Cate, 1994). Within the eroded ovine

sample it was possible to see laminar sheets of prisms arranged into oblique columns. Despite no previous SEM work to compare these findings, this appearance is typical to Type 2 enamel (Boyde, 1989). This appearance is also seen in similar species such as deer and goat, with plate-like prisms and incomplete outlines (Maas, 1991; Grine *et al*., 1987; Grine *et al*., 1986). Despite obvious patterns of erosion with the human and ovine enamel, these results fail to show an obvious pattern of erosion for bovine enamel. The appearance of eroded bovine enamel seems rather amorphous, although 'pits' can be identified across the enamel surface at regular intervals. Boyde described bovine enamel as Type 2 (similar to ovine enamel) yet the appearance from this study seems to contradict this. Instead, the eroded bovine enamel appears more like Type 1 (relatively thin rows of prisms with incomplete boundaries, separated by an obvious interprismatic region) which typically contains a greater proportion of interprismatic enamel. Indeed, bovine enamel is reported to contain more interprismatic enamel than human enamel (Fonseca *et al*., 2008) which may explain the greater resistance to erosion.

Again, the apparent surface features identified with SEM correspond well to roughness parameters when measured by profilometry. As such, SEM analysis has been shown to be useful in confirming profilometric data.

6.3 Part 3 - Abrasion

6.3.1 The early abrasive challenge on human, bovine and ovine enamel

Investigating how abrasion *alone* affects the lapped enamel surface was important for two reasons; primarily it allowed more meaningful analysis of the 'erosion and

abrasion' experiment, providing an insight into how the erosive process may *modify* typical abrasive effects. Further, this experiment provided another opportunity to record the bearing area parameters and assess their usefulness in discriminating surface features that Ra alone may not have identified.

These results suggest that when enamel is abraded for 20 seconds with a loading weight of 200g and a medium abrasivity non-fluoridated paste, the roughness average is not significantly altered. Azevedo (Azevedo *et al*., 2008) reported similar findings despite exposing human enamel samples to an abrasive challenge for longer (10 minutes). No other studies report changes in roughness average after abrasion-only enamel challenges. Instead, it is more common for studies to report the amount of surface loss. The abrasion pilot experiment failed to demonstrate a measurable surface loss and as such, this parameter was not recorded within this experiment. A review from 2002 also concludes that abrasion with pastes of low relative enamel abrasivity have little or no effect on enamel (Hunter *et al*., 2002). Further published work shows that bovine enamel also failed to demonstrate surface loss when subjected to abrasion for 3 minutes (Vieira *et al*., 2006). The study by Vieira *et al* (2006) used a fluoridated paste, and despite evidence to show a protective effect on eroded enamel, at present it is unclear how fluoride may modulate a purely abrasive challenge on the enamel surface. Several studies (Franzò *et al*., 2010; Vieira *et al*., 2006; Philpotts *et al*., 2005; Joiner *et al*., 2004) *have* recorded enamel surface loss due to abrasion only, reporting values ranging from 0.01 µm to 0.11 µm; in all cases the samples were subjected to extensive periods of abrasion (up to 85 times longer), with greater loading forces (375g).

To date no studies report changes in bearing parameters due to abrasion. Despite no change in surface roughness average, this experiment identified a significant reduction in the number of profile peaks (-2.2%). Although there were still significant tissue differences between roughness averages of the abraded enamel, there were no differences between tissues for the proportion of profile peaks. It is purported that even though the toothbrush bristles alone were too large to directly interfere with the roughness of core, valley (and perhaps even peak) portions of the profile, the reduction in profile peaks may be the result of a shearing effect by the bristles as they pass over the enamel surface; because the large bristles fail to interact with the remainder of the tooth surface, no measurable tissue-specific changes occurred.

Clinically, the presence of saliva and the dental pellicle may modify these results, and this warrants further investigation. However the subtle differences within the enamel surface would not have been identified if roughness average and surface loss had been measured in isolation. This experiment has again shown the usefulness of the bearing parameters in discriminating surface changes over and above using Ra alone.

6.4 Part 4 - Erosion and abrasion

6.4.1 Abrasion of the early-eroded lesion on human, bovine and ovine enamel

This experiment was carried out in order to determine the synergistic 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; 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 less rough than the initial eroded surface. There are no comparable reports within the literature; most published works describe a combination of surface loss and microhardness (West *et al*., 2011). The proportions of profile peaks (MR1) were also significantly less post-abrasion. This mirrors the effect seen in the abrasion-only experiment, which suggests that a similar pattern of abrasion is occurring. However, the actual reduction in abraded profile peaks was greater for the previously eroded enamel, and it is purported that this is because the softened enamel surface was more susceptible than the lapped enamel surface.

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 (no surface loss was measured for the abrasive or erosive experiments in isolation). 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, (Wiegand *et al*., 2010b; Rios *et al*., 2006a) 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 (Levy *et al*., 2012) 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 (Rochel *et al*., 2011; Rios *et al*., 2008b). Lower values (up to 1.8 µm) have been 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) (Ganss *et al*., 2011; Yu *et al*., 2009a) 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 (Barbour *et al*., 2003b). 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 (Hooper *et al*., 2003a). Clearly the comparison between studies is difficult given the number of treatment parameters and interactions that exist. Hooper *et al* (2003a) conclude 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 (Moretto *et al*., 2010; Sales-Peres *et al*., 2007a). Vieira

et al (2006) 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 experiments, 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.

Again, 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.

6.5 Other qualification experiments

6.5.1 Surface effects of microhardness testing on enamel

This study used a microhardness testing protocol with indents 250 µm apart across the enamel surface. It was important to ensure that the loading of the indenter was not damaging the enamel surface and affecting adjacent readings. It was also important to ensure that the indenter was fully contacting the enamel surface. Some researchers warn against the use of a metric evaluation of hardness particularly when using a Vickers probe (Brennecke and Radlanski, 1995) because the probe tip may not completely contact the surface; SEM evaluation of probe indents revealed that a clear square impression outline was often lacking. No detail of the method of analysis was given, so a direct comparison is not possible. However this experiment suggests that under a loading protocol of 200g for 15 seconds, a Vickers square indenter is making full contact with the enamel surface (Figure 49), and therefore makes an accurate representation of surface microhardness. No cracks were seen radiating from the Vickers indent, and spacing of 250 µm is adequate to ensure that there is no interference with other indents (Figure 49).

6.5.2 Changes in surface parameters of polished samples away from the CEJ

It was necessary to ensure that the recorded surface characteristics did not change significantly away from the cemento-enamel junction (the junction between the

enamel and the acrylic reference area). Although the samples had been polished 100 µm from the native enamel surface, the different crown sizes and curvature meant that parts of the samples would have suffered a greater degree of preparation than others, and consequently may display different surface features. Analysis of the bearing area parameters showed no significant differences across the surface thirds for each tissue type.

6.5.3 SEM, stylus and laser profilometer calibration

During SEM study of the eroded and abraded surface, it became apparent that the stylus profilometer tip was damaging the enamel surface (Figure 51). This effect has been reported elsewhere (Heurich *et al*., 2010; Ren *et al*., 2009b). Ren *et al* (2009b) measured scratch mark depth using focus variation 3D scanning microscopy on human enamel discs. Scratch marks were displayed that measured 0.3 µm at baseline and 3.5 µm on eroded enamel surfaces, suggesting that the eroded enamel surface is more susceptible to stylus damage. This was also reported by Heurich *et al* (2010). These *in vitro* models used immersion times of 10 minutes or longer. Although the current experiment did not ascertain the scratch depths, it was necessary to ensure that the phenomenon was not providing altered surface measurements. Heurich *et al* (2010) reported no significant difference in surface height change between a confocal scanning microscope, AFM and a FormTalysurf Series 2 stylus profilometer (Taylor Hobson, Leicester). However the way in which roughness average was recorded was not reported. In the current experiment, horizontal calibrations were carried out in the form of bur width measurements, and stylus profilometry provided a similar measurement when compared to laser profilometry and SEM. Vertical calibrations

were carried out in the form of roughness analysis measurements, and stylus profilometry provided a similar measurement when compared to laser profilometry. It is purported that despite visible surface damage to the eroded enamel, the stylus profilometer is able to record almost all surface detail before causing collapse of the enamel structure. This is reinforced by the fact that the roughness average recorded by the stylus was slightly lower than that recorded by the laser profilometer.

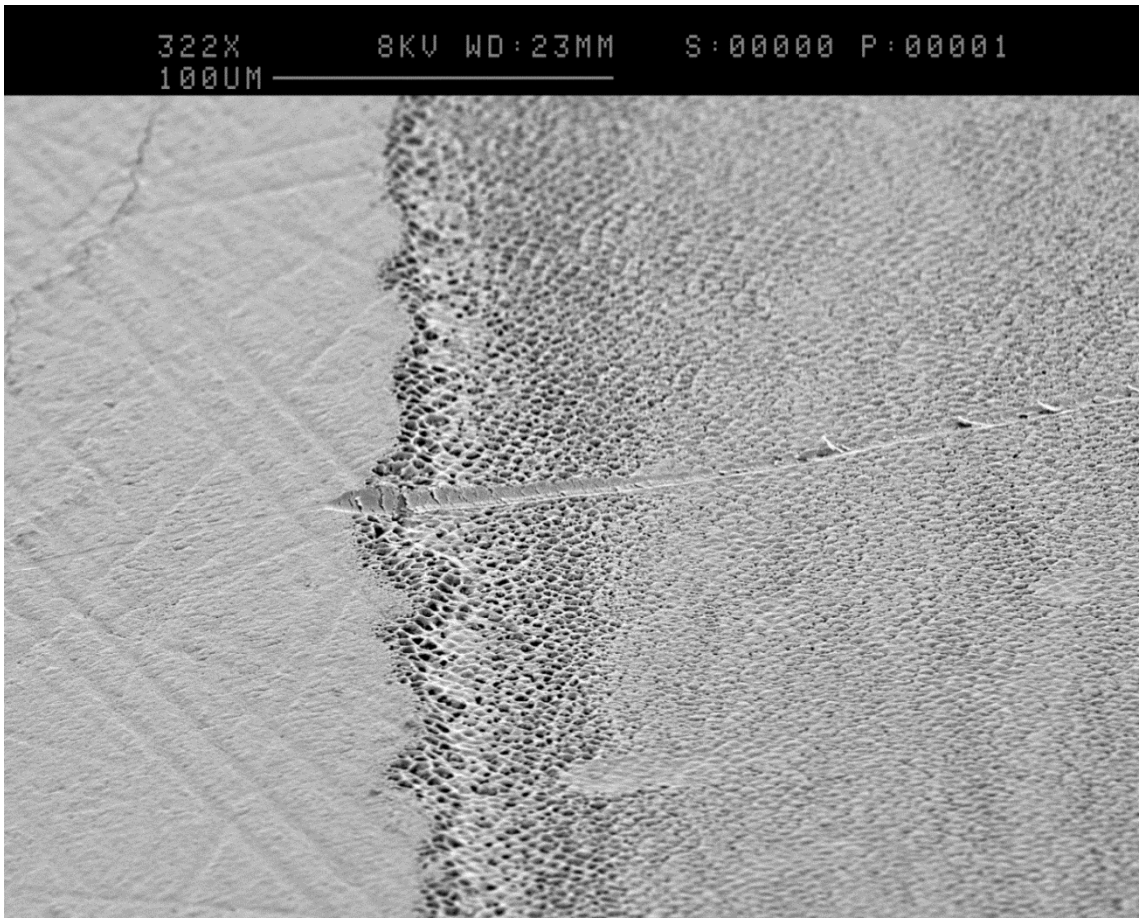


Figure 51 – Medium magnification SEM (x322) of eroded and abraded ovine enamel, showing the effects of the profilometer stylus as it moves into softer, eroded enamel. Note the reference layer (left) previously protected by tape.

Chapter 7. Summary and Conclusions

7.1 Introduction

The present study, primarily divided into two main sections mainly considered:

i) The surface effects of erosive and abrasive challenges on human, bovine and ovine enamel, measured using SEM, stylus profilometry and surface microhardness

ii) Whether there were significant differences between human, bovine and ovine enamel at each treatment stage, quantified and qualified using profilometry, SEM and surface microhardness

The review of the literature showed variable surface changes both between human and bovine enamel, and across treatment models for erosive and abrasive challenges. There is a general consensus that eroded enamel is rougher and softer, whilst abraded enamel shows little surface change. Abrasion of the eroded lesion leads to measureable surface loss and an increase in microhardness. A simple *in vitro* model was developed in order to measure *early* surface changes, recording bearing parameters in order to better understand these processes.

7.2 Main aims

- To investigate the surface effects on enamel of early erosive and abrasive challenges
- To compare the enamel surfaces of human, bovine and ovine enamel

7.3 Conclusions and recommendations

Within the limitations of this study, the data are inconsistent with the null hypotheses and so the following are rejected:

i) *There are no differences in surface characteristics between lapped human, bovine or ovine enamel.*

Surface studies should lap and polish samples with a consistent approach, ensuring that baseline data are recorded. The assumption should not be made that the same preparation techniques will result in consistent baseline roughness or surface characteristics.

ii) *There are no differences in surface characteristics between human, bovine or ovine enamel when subjected to erosive and abrasive challenges.*

Ovine enamel displayed little correlation with human enamel when subjected to erosive and abrasive challenges. Bovine enamel showed similar trends to human enamel but was consistently harder and more resistant to surface change. Therefore bovine enamel cannot be reliably used interchangeably with human enamel for erosion and abrasion studies.

iii) *There are no differences in surface characteristics of enamel subjected to erosive challenges of differing pH or immersion time.*

Immersion time and pH correlated positively with several roughness parameters. However the relationship was not simple; effects of pH were modulated by immersion time and this should be investigated further.

7.4 Summary

Notwithstanding the limitations from this *in vitro* study, it can be concluded that enamel from differing species does not behave in the same manner, even when prepared using the same procedures. As erosion and abrasion models become more complex it is increasingly difficult to compare results between studies and to isolate individual treatment effects. This simple *in vitro* model allows demonstrable and measurable *early* erosive surface change without surface *loss*, using MH, stylus profilometry and SEM. Predictors of abraded surface loss after erosion include the surface microhardness and the depth range of the eroded profile.

7.5 Further study

The baseline testing of enamel specimens has identified that the roughness parameters of each species are not consistent. Further, there is no correlation between enamel surface finish and grit-size. It is necessary to develop a reference set of expected roughness parameters for each species using a number of preparation techniques, including ultrasonication of the prepared surface.

The relationship between citric acid concentration and immersion time to surface parameters is complex. Further work is required investigating a greater range of concentration and immersion times to unpick this complex interaction.

Most profilometric studies to date report Ra or Rq alone. This study has shown that inclusion of the bearing parameters will provide greater insight into surface changes that would ordinarily be overlooked. It is strongly recommended that recording of the

bearing parameters is considered as part of the standard measurement set for these types of surface study.

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Appendix A – Baseline values for human and bovine enamel

Tissue	Sample	RaBase	Rk base	Rpk base	Rvk base	MR1 base	MR2 base
H	H1a1	0.140	0.336	0.291	0.195	20.150	90.340
H	H1a2	0.144	0.326	0.333	0.190	21.220	92.320
H	H1a3	0.135	0.423	0.278	0.213	26.210	89.540
B	B1a1	0.116	0.289	0.375	0.260	7.440	80.211
B	B1a2	0.120	0.299	0.333	0.298	8.420	81.265
B	B1a3	0.118	0.312	0.411	0.314	6.590	76.590
B	B2a1	0.147	0.503	0.073	0.121	5.410	86.620
B	B2a2	0.142	0.326	0.211	0.168	6.589	88.564
B	B2a3	0.138	0.426	0.131	0.133	8.598	79.879
B	B3a1	0.112	0.253	0.369	0.341	7.720	80.790
B	B3a2	0.114	0.316	0.279	0.211	8.843	85.680
B	B3a3	0.110	0.378	0.311	0.187	8.598	81.369
B	B4a1	0.124	0.311	0.218	0.170	10.140	86.950
B	B4a2	0.129	0.299	0.311	0.222	9.872	88.000
B	B4a3	0.111	0.318	0.316	0.198	7.859	81.265
H	H1b1	0.113	0.311	0.332	0.178	18.590	92.620
H	H1b2	0.117	0.366	0.523	0.198	20.210	90.780
H	H1b3	0.114	0.289	0.381	0.222	21.590	93.780
B	B1b1	0.125	0.259	0.363	0.160	8.430	81.440
B	B1b2	0.119	0.316	0.391	0.211	8.590	85.610
B	B1b3	0.120	0.354	0.561	0.185	6.890	84.540
B	B2b1	0.119	0.357	0.304	0.106	12.980	92.760
B	B2b2	0.123	0.299	0.264	0.164	4.689	66.688
B	B2b3	0.123	0.314	0.197	0.125	5.485	71.459
B	B3b1	0.106	0.227	0.376	0.132	4.940	79.410
B	B3b2	0.110	0.378	0.287	0.087	5.122	71.268
B	B3b3	0.113	0.333	0.320	0.149	5.648	68.222
B	B4b1	0.118	0.207	0.370	0.245	4.400	65.310
B	B4b2	0.116	0.338	0.312	0.145	5.476	60.265
B	B4b3	0.120	0.356	0.278	0.111	6.795	68.164
H	H2a1	0.103	0.210	0.286	0.213	22.610	95.620
H	H2a2	0.112	0.185	0.300	0.128	20.130	96.840
H	H2a3	0.110	0.199	0.296	0.198	20.990	92.650
B	B1c1	0.117	0.350	0.295	0.261	8.250	85.860
B	B1c2	0.121	0.298	0.295	0.125	5.487	88.598
B	B1c3	0.120	0.358	0.345	0.097	4.798	79.845
B	B2c1	0.123	0.333	0.329	0.118	8.660	89.940
B	B2c2	0.121	0.378	0.326	0.132	9.950	64.587
B	B2c3	0.135	0.349	0.300	0.108	5.789	71.648
B	B3c1	0.127	0.363	0.421	0.097	3.260	87.960
B	B3c2	0.122	0.337	0.345	0.120	5.134	88.598
B	B3c3	0.087	0.287	0.312	0.118	6.498	74.548
B	B4c1	0.115	0.341	0.301	0.007	3.460	82.195
B	B4c2	0.120	0.297	0.411	0.111	5.946	88.597
B	B4c3	0.100	0.311	0.315	0.120	9.498	70.165
H	H2b1	0.103	0.326	0.303	0.201	24.890	90.390
H	H2b2	0.112	0.379	0.578	0.198	26.540	97.620
H	H2b3	0.098	0.326	0.367	0.256	20.130	92.220
B	B1d1	0.106	0.329	0.287	0.218	6.730	87.410
B	B1d2	0.840	0.411	0.187	0.087	12.870	55.648
B	B1d3	0.116	0.312	0.311	0.311	6.897	88.975
B	B2d1	0.125	0.240	0.069	0.313	7.990	73.770
B	B2d2	0.129	0.315	0.152	0.319	5.684	88.850
B	B2d3	0.122	0.333	0.111	0.297	9.865	70.165
B	B3d1	0.104	0.297	0.296	0.245	6.070	86.720
B	B3d2	0.110	0.369	0.346	0.198	5.495	88.496
B	B3d3	0.109	0.312	0.315	0.215	6.794	72.465

Appendix B – Statistical analyses of human and bovine enamel at baseline

T-test for base comparison
Dependent Variable: ***RaBase***
Normality Failed (P < 0.050)

Mann-Whitney Rank Sum Test

Group	N	Median	25%	75%
H	12	0.113	0.106	0.126
B	45	0.120	0.113	0.123

Mann-Whitney U Statistic= 200.500
T = 278.500 n(small)= 12 n(big)= 45 (**P = 0.176**)

T-test for base comparison
Dependent Variable: ***RkBase***
Normality Test: Passed (P = 0.066)
Equal Variance Test: Passed (P = 0.210)

Group	N	Mean	Std Dev	SEM
H	12	0.306	0.0741	0.0214
B	45	0.326	0.0513	0.00764

t = -1.092 with 55 degrees of freedom. (**P = 0.280**)
95 percent confidence interval for difference of means: -0.0569 to 0.0168

T-test for base comparison
Dependent Variable: ***RvkBase***
Normality Test: Passed (P = 0.060)
Equal Variance Test: Passed (P = 0.966)

Group	N	Mean	Std Dev	SEM
H	12	0.356	0.0971	0.0280
B	45	0.298	0.0933	0.0139

t = 1.872 with 55 degrees of freedom. (**P = 0.066**)
95 percent confidence interval for difference of means: -0.00402 to 0.118

T-test for base comparison
Dependent Variable: ***RpkBase***
Normality Test: Passed (P = 0.050)
Equal Variance Test: Failed (P < 0.050)

Mann-Whitney Rank Sum Test

Group	N	Median	25%	75%
H	12	0.198	0.193	0.213
B	45	0.164	0.120	0.228

Mann-Whitney U Statistic= 200.500
T = 417.500 n(small)= 12 n(big)= 45 (**P = 0.177**)

T-test for base comparison
Dependent Variable: ***Mr1Base***
Normality Test: Failed (P < 0.050)

Mann-Whitney Rank Sum Test

Group	N	Median	25%	75%
H	12	21.105	20.140	23.750
B	45	6.794	5.486	8.592

Mann-Whitney U Statistic= 0.000
T = 618.000 n(small)= 12 n(big)= 45 (**P = <0.001**)

T-test for base comparison
Dependent Variable: ***Mr2Base***
Normality Test: Failed (P < 0.050)

Mann-Whitney Rank Sum Test

Group	N	Median	25%	75%
H	12	92.470	90.585	94.700
B	45	81.265	71.601	87.547

Mann-Whitney U Statistic= 9.000
T = 609.000 n(small)= 12 n(big)= 45 (**P = <0.001**)

Appendix C – Post-erosion values for human and bovine enamel

Rx	Time	Conc	Tissue	Sample	DH max	Eroded Ra	Eroded Rk	Eroded Rvk	Eroded Rpk	Eroded MR1	Eroded MR2
1	15s	1.00%	H	H1a1	0.873	0.218	0.235	0.450	0.574	13.256	92.325
1	15s	1.00%	H	H1a2	0.749	0.312	0.201	0.546	0.645	11.265	90.265
1	15s	1.00%	H	H1a3	0.425	0.159	0.186	0.510	0.555	12.589	95.362
1	15s	1.00%	B	B1a1	0.410	0.209	0.114	0.429	0.504	7.578	75.987
1	15s	1.00%	B	B1a2	0.289	0.312	0.102	0.456	0.316	8.596	72.322
1	15s	1.00%	B	B1a3	0.781	0.421	0.144	0.561	0.521	8.222	70.569
1	15s	1.00%	B	B2a1	0.531	0.231	0.102	0.389	0.512	7.945	70.265
1	15s	1.00%	B	B2a2	0.348	0.159	0.111	0.412	0.498	9.262	71.211
1	15s	1.00%	B	B2a3	0.712	0.264	0.109	0.399	0.501	8.562	71.985
1	15s	1.00%	B	B3a1	0.426	0.102	0.132	0.498	0.612	6.523	69.659
1	15s	1.00%	B	B3a2	0.319	0.095	0.128	0.412	0.602	7.256	68.569
1	15s	1.00%	B	B3a3	0.515	0.215	0.111	0.444	0.712	7.000	70.132
1	15s	1.00%	B	B4a1	0.251	0.321	0.124	0.398	0.523	8.526	72.326
1	15s	1.00%	B	B4a2	0.165	0.361	0.132	0.412	0.612	8.698	72.396
1	15s	1.00%	B	B4a3	0.197	0.210	0.101	0.400	0.520	7.989	74.236
2	2m	1.00%	H	H1b1	1.073	0.142	0.213	0.465	0.164	10.258	90.265
2	2m	1.00%	H	H1b2	1.265	0.111	0.200	0.421	0.177	10.333	89.568
2	2m	1.00%	H	H1b3	0.849	0.102	0.302	0.479	0.154	11.285	91.236
2	2m	1.00%	B	B1b1	1.120	0.137	0.119	0.426	0.397	7.234	76.589
2	2m	1.00%	B	B1b2	1.231	0.215	0.231	0.459	0.401	7.345	77.465
2	2m	1.00%	B	B1b3	0.920	0.165	0.102	0.349	0.399	7.111	75.648
2	2m	1.00%	B	B2b1	1.231	0.111	0.231	0.458	0.412	6.899	77.654
2	2m	1.00%	B	B2b2	0.987	0.189	0.201	0.457	0.423	6.948	76.598
2	2m	1.00%	B	B2b3	1.021	0.215	0.198	0.359	0.444	7.012	77.102
2	2m	1.00%	B	B3b1	1.131	0.189	0.165	0.399	0.409	7.012	72.265
2	2m	1.00%	B	B3b2	0.865	0.175	0.164	0.654	0.423	6.789	71.625
2	2m	1.00%	B	B3b3	0.648	0.165	0.157	0.419	0.491	7.231	71.899
2	2m	1.00%	B	B4b1	1.365	0.213	0.159	0.369	0.389	7.564	78.548
2	2m	1.00%	B	B4b2	1.020	0.219	0.149	0.379	0.401	7.648	76.512
2	2m	1.00%	B	B4b3	0.980	0.154	0.157	0.351	0.397	8.954	70.165
3	15s	6.00%	H	H2a1	1.126	0.128	0.543	0.349	0.186	11.745	91.254
3	15s	6.00%	H	H2a2	1.026	0.111	0.502	0.312	0.199	10.588	92.658
3	15s	6.00%	H	H2a3	0.845	0.105	0.543	0.356	0.158	11.021	92.111
3	15s	6.00%	B	B1c1	0.867	0.109	0.346	0.387	0.248	7.458	80.245
3	15s	6.00%	B	B1c2	0.648	0.152	0.302	0.412	0.236	8.524	81.265
3	15s	6.00%	B	B1c3	0.666	0.120	0.359	0.399	0.201	8.111	80.078
3	15s	6.00%	B	B2c1	0.518	0.114	0.325	0.412	0.216	8.334	79.485
3	15s	6.00%	B	B2c2	0.438	0.102	0.301	0.401	0.208	8.548	76.522
3	15s	6.00%	B	B2c3	0.798	0.156	0.334	0.423	0.312	8.719	77.555
3	15s	6.00%	B	B3c1	0.466	0.098	0.540	0.485	0.324	7.598	80.251
3	15s	6.00%	B	B3c2	0.615	0.108	0.520	0.444	0.365	7.489	79.844
3	15s	6.00%	B	B3c3	0.602	0.230	0.487	0.512	0.285	6.899	80.255
3	15s	6.00%	B	B4c1	0.514	0.126	0.412	0.401	0.285	7.954	79.844
3	15s	6.00%	B	B4c2	0.555	0.095	0.401	0.388	0.245	8.045	78.237
3	15s	6.00%	B	B4c3	0.216	0.235	0.399	0.402	0.299	8.051	80.299
4	2m	6.00%	H	H2b1	1.246	0.121	0.345	0.302	0.139	6.554	89.634
4	2m	6.00%	H	H2b2	1.468	0.132	0.354	0.302	0.159	7.122	90.133
4	2m	6.00%	H	H2b3	1.002	0.126	0.357	0.315	0.154	7.015	88.561
4	2m	6.00%	B	B1d1	1.345	0.135	0.242	0.289	0.129	4.598	84.265
4	2m	6.00%	B	B1d2	1.589	0.130	0.215	0.312	0.112	5.562	85.648
4	2m	6.00%	B	B1d3	1.235	0.155	0.248	0.333	0.156	5.999	80.165
4	2m	6.00%	B	B2d1	1.560	0.235	0.312	0.287	0.131	5.265	79.658
4	2m	6.00%	B	B2d2	1.235	0.232	0.298	0.255	0.189	6.011	80.132
4	2m	6.00%	B	B2d3	0.989	0.198	0.321	0.143	0.123	5.598	84.222
4	2m	6.00%	B	B3d1	1.666	0.162	0.410	0.333	0.111	6.625	82.388
4	2m	6.00%	B	B3d2	1.213	0.133	0.444	0.315	0.129	6.013	81.258
4	2m	6.00%	B	B3d3	1.549	0.136	0.398	0.357	0.122	6.134	80.123

Appendix D – Statistical analyses of human and bovine enamel post-erosion

General Linear Model
Dependent Variable: ***Eroded Ra***

Source of Variation	DF	SS	MS	F	P
Tissue	1	0.0111	0.0111	3.099	0.085
Concentration	1	0.0290	0.0290	8.113	**0.006**
Time	1	0.0101	0.0101	2.821	0.099
Tissue x Conc	1	0.0000415	0.0000415	0.0116	0.915
Tissue x Time	1	0.00273	0.00273	0.762	0.387
Conc x Time	1	0.0278	0.0278	7.763	**0.008**
Tissue x Conc x Time	1	0.000491	0.000491	0.137	0.713
Residual	49	0.175	0.00358		
Total	56	0.283	0.00506		

General Linear Model
Dependent Variable: ***Eroded Rk***

Source of Variation	DF	SS	MS	F	P
Tissue	1	0.0625	0.0625	19.645	**<0.001**
Concentration	1	0.440	0.440	138.364	**<0.001**
Time	1	0.0165	0.0165	5.198	**0.027**
Tissue x Conc	1	0.0000365	0.0000365	0.0115	0.915
Tissue x Time	1	0.00927	0.00927	2.914	0.094
Conc x Time	1	0.0655	0.0655	20.603	**<0.001**
Tissue x Conc x Time	1	0.00411	0.00411	1.293	0.261
Residual	49	0.156	0.00318		
Total	56	0.998	0.0178		

General Linear Model
Dependent Variable: ***Eroded Rvk***

Source of Variation	DF	SS	MS	F	P
Tissue	1	0.000574	0.000574	0.174	0.679
Concentration	1	0.122	0.122	36.999	**<0.001**
Time	1	0.0289	0.0289	8.730	**0.005**
Tissue x Conc	1	0.0167	0.0167	5.034	**0.029**
Tissue x Time	1	0.00226	0.00226	0.683	0.413
Conc x Time	1	0.00655	0.00655	1.981	0.166
Tissue x Conc x Time	1	0.0106	0.0106	3.205	0.080
Residual	49	0.162	0.00331		
Total	56	0.372	0.00664		

General Linear Model
Dependent Variable: ***Eroded Rpk***

Source of Variation	DF	SS	MS	F	P
Tissue	1	0.0417	0.0417	13.765	**<0.001**
Concentration	1	0.560	0.560	184.840	**<0.001**
Time	1	0.299	0.299	98.867	**<0.001**
Tissue x Conc	1	0.00918	0.00918	3.029	0.088
Tissue x Time	1	0.0238	0.0238	7.869	**0.007**
Conc x Time	1	0.0859	0.0859	28.352	**<0.001**
Tissue x Conc x Time	1	0.0995	0.0995	32.835	**<0.001**
Residual	49	0.148	0.00303		
Total	56	1.586	0.0283		

General Linear Model
Dependent Variable: ***Eroded MR1***

Source of Variation	DF	SS	MS	F	P
Tissue	1	84.296	84.296	205.218	**<0.001**
Concentration	1	25.490	25.490	62.056	**<0.001**
Time	1	46.620	46.620	113.495	**<0.001**
Tissue x Conc	1	6.777	6.777	16.498	**<0.001**
Tissue x Time	1	5.466	5.466	13.308	**<0.001**
Conc x Time	1	9.428	9.428	22.952	**<0.001**
Tissue x Conc x Time	1	0.539	0.539	1.312	0.258
Residual	49	20.127	0.411		
Total	56	181.869	3.248		

General Linear Model
Dependent Variable: ***Eroded MR2***

Source of Variation	DF	SS	MS	F	P
Tissue	1	1862.168	1862.168	429.244	**<0.001**
Concentration	1	101.388	101.388	23.371	**<0.001**
Time	1	0.807	0.807	0.186	0.668
Tissue x Conc	1	155.273	155.273	35.792	**<0.001**
Tissue x Time	1	69.964	69.964	16.127	**<0.001**
Conc x Time	1	1.014	1.014	0.234	0.631
Tissue x Conc x Time	1	0.349	0.349	0.0805	0.778
Residual	49	212.574	4.338		
Total	56	2868.734	51.227		

Paired t-test for base **Ra** comparison post-erosion
Normality Test: Failed (P < 0.050)

Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
RaBase	57	0.118	0.112	0.123
Eroded Ra	57	0.156	0.121	0.215

W= 1235.000 T+ = 1415.500 T-= -180.500
Z-Statistic (based on positive ranks) = 5.037
(P = **<0.001**)

Paired t-test for base **Rk** comparison post-erosion
Normality Test: Passed (P = 0.082)

Treatment Name	N	Mean	Std Dev	SEM
RkBase	57	0.322	0.0567	0.00750
Eroded Rk	57	0.264	0.134	0.0177
Difference	57	0.0584	0.158	0.0209

t = 2.789 with 56 degrees of freedom. (**P = 0.007**)
95 percent confidence interval for difference of means: 0.0164 to 0.100

Paired t-test for base **Rpk** comparison post-erosion
Normality Test: Failed (P < 0.050)

Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
RpkBase	57	0.311	0.287	0.350
Eroded Rpk	57	0.401	0.350	0.446

W= 1321.000 T+ = 1487.000 T-= -166.000
Z-Statistic (based on positive ranks) = 5.248
(P = **<0.001**)

Paired t-test for base **Rvk** comparison post-erosion
Normality Test: Failed (P < 0.050)

Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
RvkBase	57	0.187	0.124	0.219
Eroded Rvk	57	0.316	0.174	0.493

W= 1206.000 T+ = 1429.500 T-= -223.500
Z-Statistic (based on positive ranks) = 4.791
(**P = <0.001**)

Paired t-test for base **MR1** comparison post-erosion
Normality Test: Failed (P < 0.050)

Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Mr1Base	57	7.859	5.675	10.822
Eroded MR1	57	7.578	6.936	8.570

W= -395.000 T+ = 629.000 T-= -1024.000
Z-Statistic (based on positive ranks) = -1.569
(**P = 0.118**)

Paired t-test for base **MR2** comparison post-erosion
Normality Test: Failed (P < 0.050)

Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Mr2Base	57	85.680	74.353	89.116
Eroded MR2	57	79.658	73.776	84.233

W= -625.000 T+ = 514.000 T-= -1139.000
Z-Statistic (based on positive ranks) = -2.483
(**P = 0.013**)

Appendix E – Baseline data for ovine, human & bovine enamel

Tissue	Tooth	Sample	RaBase	RkBase	RvkBase	RpkBase	Mr1Base	Mr2Base
H	1	H1a	0.189	0.608	0.322	0.171	7.320	87.220
H	1	H1b	0.170	0.568	0.309	0.173	6.730	87.590
H	1	H1c	0.180	0.598	0.269	0.219	8.080	87.650
H	2	H2a	0.106	0.368	0.166	0.165	12.150	89.896
H	2	H2b	0.117	0.362	0.144	0.258	10.113	89.558
H	2	H2c	0.101	0.349	0.126	0.137	9.505	90.206
H	3	H3a	0.125	0.458	0.201	0.162	9.058	89.600
H	3	H3b	0.136	0.679	0.201	0.331	10.254	91.934
H	3	H3c	0.112	0.615	0.263	0.248	15.169	90.057
H	4	H4a	0.145	0.467	0.216	0.157	9.495	87.663
H	4	H4b	0.153	0.488	0.244	0.202	9.004	88.752
H	4	H4c	0.156	0.471	0.254	0.244	8.786	87.268
H	5	H5a	0.142	0.430	0.246	0.152	8.863	86.512
H	5	H5b	0.138	0.452	0.219	0.147	9.467	87.754
H	5	H5c	0.139	0.434	0.226	0.181	9.371	88.799
B	6	B6a	0.150	0.448	0.206	0.224	11.040	89.410
B	6	B6b	0.161	0.524	0.238	0.347	9.810	88.720
B	6	B6c	0.163	0.563	0.214	0.265	8.850	89.390
B	7	B7a	0.112	0.394	0.204	0.158	9.230	88.260
B	7	B7b	0.121	0.393	0.191	0.139	10.010	87.260
B	7	B7c	0.122	0.406	0.238	0.137	8.770	88.560
B	8	B8a	0.126	0.404	0.197	0.185	10.121	88.304
B	8	B8b	0.139	0.453	0.207	0.241	8.050	87.258
B	8	B8c	0.141	0.457	0.194	0.276	7.923	88.132
B	9	B9a	0.148	0.485	0.203	0.225	9.913	88.639
B	9	B9b	0.150	0.509	0.219	0.193	9.547	88.970
B	9	B9c	0.149	0.422	0.177	0.340	12.029	89.021
B	10	B10a	0.155	0.478	0.225	0.331	10.247	88.291
B	10	B10b	0.155	0.491	0.233	0.446	11.035	89.601
B	10	B10c	0.170	0.501	0.238	0.437	11.325	88.195
O	11	O11a	0.150	0.513	0.237	0.166	6.940	89.430
O	11	O11b	0.139	0.438	0.198	0.186	9.790	87.870
O	11	O11c	0.140	0.437	0.240	0.165	9.420	87.390
O	12	O12a	0.198	0.528	0.416	0.191	8.657	81.374
O	12	O12b	0.204	0.668	0.292	0.268	7.508	88.097
O	12	O12c	0.199	0.577	0.366	0.188	7.913	83.871
O	13	O13a	0.214	0.748	0.285	0.185	6.485	89.814
O	13	O13b	0.216	0.705	0.420	0.154	6.682	88.670
O	13	O13c	0.237	0.721	0.433	0.240	8.463	84.623
O	14	O14a	0.171	0.546	0.223	0.175	9.664	88.073
O	14	O14b	0.173	0.548	0.227	0.174	9.428	87.658
O	14	O14c	0.172	0.548	0.235	0.172	9.160	88.012
O	15	O15a	0.174	0.595	0.220	0.241	7.220	89.279
O	15	O15b	0.165	0.524	0.277	0.162	8.605	86.603
O	15	O15c	0.201	0.634	0.303	0.358	10.955	87.828
H	16	H16a	0.149	0.523	0.271	0.195	7.616	88.537
H	16	H16b	0.157	0.474	0.240	0.205	10.029	86.422
H	16	H16c	0.148	0.498	0.272	0.224	9.702	87.530
H	17	H17a	0.133	0.450	0.225	0.181	7.952	90.052
H	17	H17b	0.145	0.552	0.290	0.195	7.192	87.985
H	17	H17c	0.163	0.528	0.289	0.202	9.332	88.381
H	18	H18a	0.148	0.430	0.286	0.170	9.028	85.947
H	18	H18b	0.146	0.469	0.242	0.156	8.556	89.079
H	18	H18c	0.167	0.542	0.266	0.241	8.280	87.899
H	19	H19a	0.151	0.482	0.221	0.233	7.317	87.510
H	19	H19b	0.153	0.481	0.236	0.257	10.259	89.507
H	19	H19c	0.134	0.434	0.196	0.155	9.068	89.508
H	20	H20a	0.161	0.511	0.243	0.240	7.734	88.565
H	20	H20b	0.159	0.521	0.296	0.229	7.666	88.107
H	20	H20c	0.159	0.495	0.285	0.313	12.402	88.492

Tissue	Tooth	Sample	RaBase	RkBase	RvkBase	RpkBase	Mr1Base	Mr2Base
B	21	B21a	0.110	0.382	0.161	0.131	8.375	88.868
B	21	B21b	0.115	0.396	0.196	0.178	10.920	89.961
B	21	B21c	0.123	0.588	0.185	0.221	8.807	93.618
B	22	B22a	0.137	0.446	0.153	0.160	9.347	89.134
B	22	B22b	0.142	0.463	0.215	0.226	9.280	88.298
B	22	B22c	0.139	0.437	0.229	0.233	8.378	89.378
B	23	B23a	0.148	0.446	0.246	0.363	9.970	89.311
B	23	B23b	0.156	0.521	0.199	0.420	9.469	89.681
B	23	B23c	0.146	0.465	0.204	0.362	11.912	88.145
B	24	B24a	0.126	0.394	0.199	0.195	9.307	87.687
B	24	B24b	0.121	0.376	0.170	0.237	11.307	88.289
B	24	B24c	0.120	0.376	0.191	0.236	10.473	89.026
B	25	B25a	0.113	0.368	0.171	0.149	9.480	88.217
B	25	B25b	0.114	0.380	0.169	0.142	9.821	89.920
B	25	B25c	0.117	0.364	0.172	0.218	9.837	88.672
O	26	O26a	0.195	0.632	0.275	0.243	8.259	88.632
O	26	O26b	0.200	0.640	0.319	0.225	8.235	87.518
O	26	O26c	0.192	0.637	0.319	0.154	8.010	87.829
O	27	O27a	0.180	0.629	0.304	0.268	11.034	87.835
O	27	O27b	0.181	0.629	0.318	0.291	13.372	88.320
O	27	O27c	0.177	0.627	0.323	0.250	12.964	88.373
O	28	O28a	0.216	0.820	0.272	0.353	12.670	90.856
O	28	O28b	0.212	0.843	0.273	0.357	12.411	90.397
O	28	O28c	0.213	0.789	0.334	0.347	11.727	89.953
O	29	O29a	0.201	0.797	0.665	0.226	7.378	80.659
O	29	O29b	0.193	0.750	0.289	0.165	9.934	89.240
O	29	O29c	0.193	0.809	0.290	0.150	7.904	89.975
O	30	O30a	0.167	0.591	0.244	0.195	8.865	85.226
O	30	O30b	0.165	0.617	0.258	0.198	7.520	85.645
O	30	O30c	0.166	0.596	0.248	0.169	7.655	84.603
H	31	H31a	0.177	0.536	0.266	0.251	10.515	87.769
H	31	H31b	0.176	0.531	0.301	0.292	10.241	87.344
H	31	H31c	0.173	0.553	0.251	0.217	8.930	88.380
H	32	H32a	0.149	0.441	0.370	0.354	9.455	88.529
H	32	H32b	0.141	0.420	0.189	0.252	9.991	87.929
H	32	H32c	0.132	0.413	0.262	0.144	8.266	87.542
H	33	H33a	0.144	0.420	0.242	0.304	10.895	87.393
H	33	H33b	0.130	0.403	0.232	0.183	8.860	87.912
H	33	H33c	0.129	0.382	0.241	0.178	9.939	86.734
H	34	H34a	0.141	0.427	0.213	0.260	10.427	88.541
H	34	H34b	0.148	0.451	0.225	0.208	9.792	87.468
H	34	H34c	0.144	0.461	0.195	0.254	8.177	89.308
H	35	H35a	0.173	0.498	0.286	0.413	9.351	87.237
H	35	H35b	0.180	0.531	0.282	0.418	9.299	87.494
H	35	H35c	0.169	0.565	0.279	0.323	7.398	89.926
B	36	B36a	0.093	0.378	0.136	0.172	11.115	88.306
B	36	B36b	0.089	0.417	0.155	0.136	8.775	90.623
B	36	B36c	0.099	0.340	0.148	0.157	9.723	88.058
B	37	B37a	0.121	0.382	0.144	0.355	9.840	89.760
B	37	B37b	0.111	0.391	0.145	0.252	9.330	89.380
B	37	B37c	0.114	0.371	0.162	0.285	8.560	89.680
B	38	B38a	0.133	0.412	0.198	0.271	8.651	88.208
B	38	B38b	0.131	0.376	0.172	0.310	10.532	87.805
B	38	B38c	0.130	0.423	0.151	0.366	11.535	90.083
B	39	B39a	0.128	0.447	0.209	0.160	8.574	89.098
B	39	B39b	0.121	0.428	0.214	0.213	11.314	89.836
B	39	B39c	0.119	0.414	0.155	0.227	11.180	90.621
B	40	B40a	0.122	0.409	0.176	0.119	8.400	89.340
B	40	B40b	0.125	0.452	0.201	0.152	9.490	90.710
B	40	B40c	0.128	0.483	0.226	0.126	7.030	90.340

Tissue	Tooth	Sample	RaBase	RkBase	RvkBase	RpkBase	Mr1Base	Mr2Base
O	41	O41a	0.164	0.544	0.296	0.233	8.323	88.106
O	41	O41b	0.172	0.528	0.277	0.217	6.721	85.368
O	41	O41c	0.173	0.560	0.281	0.204	9.594	89.287
O	42	O42a	0.195	0.602	0.256	0.340	11.433	89.092
O	42	O42b	0.173	0.568	0.350	0.311	12.461	89.759
O	42	O42c	0.180	0.595	0.272	0.177	8.244	89.015
O	43	O43a	0.228	0.759	0.338	0.187	6.410	86.786
O	43	O43b	0.212	0.718	0.371	0.373	6.696	86.625
O	43	O43c	0.204	0.660	0.341	0.361	9.479	87.359
O	44	O44a	0.183	0.589	0.290	0.189	7.093	87.646
O	44	O44b	0.172	0.564	0.283	0.142	8.503	88.708
O	44	O44c	0.174	0.578	0.279	0.139	7.938	89.203
O	45	O45a	0.198	0.569	0.239	0.708	10.712	89.522
O	45	O45b	0.163	0.475	0.267	0.360	11.226	87.675
O	45	O45c	0.224	0.604	0.246	0.786	10.275	88.907
H	46	H46a	0.166	0.589	0.229	0.205	7.740	89.780
H	46	H46b	0.180	0.641	0.361	0.218	7.540	88.190
H	46	H46c	0.198	0.644	0.349	0.192	8.040	85.500
H	47	H47a	0.155	0.527	0.239	0.175	7.661	88.823
H	47	H47b	0.149	0.467	0.308	0.176	8.736	88.073
H	47	H47c	0.169	0.564	0.455	0.353	8.992	82.982
H	48	H48a	0.126	0.426	0.230	0.151	8.801	88.898
H	48	H48b	0.134	0.390	0.269	0.255	10.337	89.134
H	48	H48c	0.117	0.378	0.174	0.187	9.149	87.847
H	49	H49a	0.172	0.587	0.214	0.200	8.887	89.884
H	49	H49b	0.178	0.548	0.301	0.268	9.222	88.858
H	49	H49c	0.170	0.564	0.242	0.157	9.550	87.355
H	50	H50a	0.155	0.490	0.257	0.166	8.080	86.767
H	50	H50b	0.148	0.478	0.264	0.159	8.473	87.646
H	50	H50c	0.158	0.510	0.302	0.177	7.319	87.035
B	51	B51a	0.164	0.558	0.231	0.348	9.850	90.560
B	51	B51b	0.155	0.590	0.277	0.233	9.150	86.820
B	51	B51c	0.163	0.684	0.330	0.294	9.820	87.770
B	52	B52a	0.146	0.471	0.198	0.205	8.103	88.632
B	52	B52b	0.152	0.465	0.203	0.257	9.637	87.166
B	52	B52c	0.152	0.481	0.190	0.283	10.136	89.710
B	53	B53a	0.097	0.335	0.194	0.134	9.630	88.400
B	53	B53b	0.092	0.365	0.123	0.154	11.540	90.080
B	53	B53c	0.095	0.324	0.157	0.125	10.520	89.120
B	54	B54a	0.142	0.479	0.179	0.211	9.077	89.248
B	54	B54b	0.135	0.421	0.197	0.184	8.835	87.599
B	54	B54c	0.128	0.399	0.182	0.209	9.390	87.874
B	55	B55a	0.145	0.486	0.238	0.223	9.097	89.123
B	55	B55b	0.164	0.539	0.245	0.262	9.189	89.534
B	55	B55c	0.160	0.532	0.243	0.244	9.151	89.957
O	56	O56a	0.146	0.526	0.276	0.205	8.930	88.200
O	56	O56b	0.154	0.497	0.309	0.151	8.600	87.110
O	56	O56c	0.154	0.524	0.209	0.200	9.510	88.170
O	57	O57a	0.181	0.548	0.362	0.238	9.968	88.040
O	57	O57b	0.198	0.610	0.403	0.263	10.290	88.786
O	57	O57c	0.194	0.605	0.371	0.191	9.341	88.456
O	58	O58a	0.199	0.711	0.222	0.191	6.738	90.484
O	58	O58b	0.184	0.585	0.255	0.287	8.904	86.871
O	58	O58c	0.179	0.568	0.263	0.226	8.248	87.035
O	59	O59a	0.193	0.562	0.325	0.296	10.523	86.635
O	59	O59b	0.188	0.559	0.306	0.280	10.166	86.573
O	59	O59c	0.184	0.559	0.316	0.228	9.625	86.808
O	60	O60a	0.174	0.706	0.313	0.177	8.843	90.677
O	60	O60b	0.179	0.665	0.235	0.167	8.130	90.514
O	60	O60c	0.188	0.623	0.321	0.271	10.024	87.877

Appendix F – Statistical analyses for ovine, human & bovine enamel at baseline

One Way Analysis of Variance for base comparison
Dependent Variable: **Rk base**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.485	0.434	0.545
B	60	0.433	0.392	0.482
O	60	0.595	0.554	0.663

H = 83.942 with 2 degrees of freedom. (**P = <0.001**)

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	5138.500	12.731	Yes
O vs H	3410.000	8.449	Yes
H vs B	1728.500	4.283	Yes

One Way Analysis of Variance for base comparison
Dependent Variable: **MH base**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	399.975	331.140	523.970
B	60	489.350	467.335	609.535
O	60	285.375	241.405	323.675

H = 38.123 with 2 degrees of freedom. (**P = <0.001**)

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
B vs O	892.500	8.705	Yes
B vs H	385.500	3.760	Yes
H vs O	507.000	4.945	Yes

One Way Analysis of Variance for base comparison
Dependent Variable: **Ra base**
Normality Test: Passed (P = 0.263)
Equal Variance Test: Passed (P = 0.846)

Group	Name	Mean	Std Dev
H	60	0.0205	0.00265
B	60	0.0207	0.00267
O	60	0.0214	0.00276

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.0862	0.0431	99.099	**<0.001**
Residual	177	0.0770	0.000435		
Total	179	0.163			

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05
Comparisons for factor: Tissue

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Sig?
O vs. B	0.0529	13.880	<0.001	0.017	Yes
O vs. H	0.0342	8.978	<0.001	0.025	Yes
H vs. B	0.0187	4.903	<0.001	0.050	Yes

One Way Analysis of Variance for base comparison
Dependent Variable: **Rvk base**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.249	0.225	0.285
B	60	0.198	0.172	0.217
O	60	0.287	0.256	0.322

H = 83.993 with 2 degrees of freedom. (**P = <0.001**)

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	5174.500	12.821	Yes
O vs H	1923.500	4.766	Yes
H vs B	3251.000	8.055	Yes

One Way Analysis of Variance for base comparison
Dependent Variable: **Rpk base**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.204	0.172	0.253
B	60	0.225	0.160	0.279
O	60	0.211	0.176	0.276

H = 0.911 with 2 degrees of freedom. (**P = 0.634**)

One Way Analysis of Variance for base comparison
Dependent Variable: **Mr1 base**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	9.016	8.080	9.747
B	60	9.588	8.963	10.360
O	60	8.884	7.926	10.095

H = 10.253 with 2 degrees of freedom. (**P = 0.006**)

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
B vs H	1624.500	4.025	Yes
B vs O	1537.500	3.809	Yes
O vs H	87.000	0.216	No

One Way Analysis of Variance for base comparison
Dependent Variable: **Mr2 base**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	88.029	87.481	88.988
B	60	89.023	88.239	89.680
O	60	88.057	86.953	89.148

H = 17.550 with 2 degrees of freedom. (**P = <0.001**)

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
B vs O	2161.000	5.354	Yes
B vs H	1967.000	4.874	Yes
H vs O	194.000	0.481	No

Appendix G – Post-erosion data for ovine, human & bovine enamel

Time	Conc	Tissue	Sample	ΔHmax	MH	Eroded Ra	Eroded Rk	Eroded Rvk	Eroded Rpk	Eroded Mr1	Eroded Mr2
30s	1%	H	H1a	1.736	297.170	0.186	0.597	0.336	0.179	7.870	87.100
30s	1%	H	H1b	1.391	310.870	0.176	0.569	0.279	0.184	7.790	88.130
30s	1%	H	H1c	1.806	293.200	0.175	0.540	0.275	0.208	10.910	87.490
30s	1%	H	H2a	1.412	286.670	0.112	0.372	0.148	0.137	9.463	88.568
30s	1%	H	H2b	1.517	282.750	0.113	0.366	0.219	0.115	9.163	89.017
30s	1%	H	H2c	1.382	277.090	0.101	0.326	0.155	0.120	11.139	89.463
30s	1%	H	H3a	1.679	-	0.124	0.437	0.198	0.228	11.613	86.825
30s	1%	H	H3b	1.384	-	0.116	0.692	0.157	0.231	11.535	93.752
30s	1%	H	H3c	1.619	-	0.119	0.405	0.239	0.141	8.091	88.754
30s	1%	H	H4a	1.613	-	0.142	0.471	0.209	0.131	8.360	89.309
30s	1%	H	H4b	1.594	-	0.154	0.511	0.182	0.164	9.068	88.851
30s	1%	H	H4c	1.585	-	0.135	0.432	0.227	0.160	7.721	88.170
30s	1%	H	H5a	1.682	-	0.155	0.506	0.242	0.165	7.486	87.323
30s	1%	H	H5b	1.682	-	0.157	0.548	0.244	0.259	10.547	88.177
30s	1%	H	H5c	1.525	-	0.158	0.638	0.304	0.231	10.128	89.384
30s	1%	B	B6a	1.790	410.960	0.150	0.479	0.260	0.143	9.010	87.670
30s	1%	B	B6b	1.708	408.010	0.142	0.458	0.262	0.131	9.010	87.170
30s	1%	B	B6c	1.703	393.040	0.161	0.546	0.262	0.138	8.870	90.000
30s	1%	B	B7a	0.967	351.260	0.116	0.428	0.193	0.136	8.840	89.520
30s	1%	B	B7b	0.976	342.190	0.124	0.426	0.221	0.114	7.730	88.590
30s	1%	B	B7c	1.017	356.130	0.126	0.449	0.177	0.131	9.020	90.280
30s	1%	B	B8a	0.947	-	0.129	0.398	0.234	0.138	9.073	86.380
30s	1%	B	B8b	1.105	-	0.121	0.395	0.208	0.189	8.579	87.927
30s	1%	B	B8c	1.184	-	0.119	0.371	0.183	0.114	8.345	87.158
30s	1%	B	B9a	0.973	-	0.142	0.448	0.308	0.162	10.716	88.294
30s	1%	B	B9b	1.155	-	0.129	0.422	0.175	0.176	9.535	90.151
30s	1%	B	B9c	0.943	-	0.143	0.501	0.205	0.166	8.221	90.570
30s	1%	B	B10a	1.283	-	0.137	0.458	0.174	0.105	8.782	89.384
30s	1%	B	B10b	1.155	-	0.141	0.486	0.207	0.147	7.313	88.555
30s	1%	B	B10c	1.263	-	0.131	0.428	0.228	0.164	9.795	87.124
30s	1%	O	O11a	1.316	200.420	0.126	0.414	0.208	0.116	8.600	88.510
30s	1%	O	O11b	1.263	210.800	0.134	0.435	0.186	0.118	9.100	87.990
30s	1%	O	O11c	1.367	225.170	0.128	0.396	0.214	0.120	8.590	86.970
30s	1%	O	O12a	1.977	194.090	0.191	0.539	0.330	0.166	9.843	82.409
30s	1%	O	O12b	1.836	224.250	0.185	0.531	0.325	0.262	9.959	84.501
30s	1%	O	O12c	2.100	135.150	0.173	0.589	0.302	0.187	7.157	88.204
30s	1%	O	O13a	1.412	-	0.224	0.710	0.408	0.159	6.807	87.825
30s	1%	O	O13b	1.736	-	0.216	0.832	0.225	0.176	6.055	92.236
30s	1%	O	O13c	1.789	-	0.229	0.734	0.362	0.305	9.719	85.418
30s	1%	O	O14a	2.048	-	0.178	0.550	0.295	0.231	7.591	86.246
30s	1%	O	O14b	1.947	-	0.184	0.607	0.351	0.124	6.928	86.713
30s	1%	O	O14c	1.579	-	0.175	0.634	0.394	0.208	6.473	88.085
30s	1%	O	O15a	1.838	-	0.159	0.574	0.210	0.168	8.640	89.414
30s	1%	O	O15b	1.891	-	0.165	0.584	0.233	0.177	10.063	89.640
30s	1%	O	O15c	1.712	-	0.174	0.612	0.269	0.151	6.938	90.166
4m	1%	H	H16a	2.017	380.490	0.158	0.542	0.299	0.149	7.864	88.238
4m	1%	H	H16b	2.178	334.320	0.157	0.523	0.281	0.126	7.793	89.970
4m	1%	H	H16c	2.247	254.270	0.153	0.492	0.234	0.172	9.870	88.009
4m	1%	H	H17a	1.758	316.050	0.151	0.500	0.218	0.166	9.003	90.054
4m	1%	H	H17b	1.926	303.850	0.155	0.488	0.260	0.192	10.605	88.379
4m	1%	H	H17c	1.894	293.410	0.157	0.501	0.241	0.185	9.102	88.528
4m	1%	H	H18a	1.976	-	0.184	0.591	0.326	0.165	8.574	86.778
4m	1%	H	H18b	2.179	-	0.159	0.511	0.265	0.137	9.159	87.677
4m	1%	H	H18c	1.998	-	0.178	0.567	0.253	0.180	8.248	86.102
4m	1%	H	H19a	2.022	-	0.161	0.511	0.277	0.159	8.846	88.276
4m	1%	H	H19b	2.261	-	0.156	0.470	0.281	1.560	9.705	86.348
4m	1%	H	H19c	1.912	-	0.144	0.451	0.201	0.165	9.509	87.115
4m	1%	H	H20a	2.210	-	0.159	0.490	0.241	0.146	8.742	86.327
4m	1%	H	H20b	1.799	-	0.148	0.498	0.228	0.190	9.348	88.320
4m	1%	H	H20c	1.909	-	0.150	0.520	0.216	0.192	9.123	91.577
4m	1%	B	B21a	0.944	460.290	0.108	0.347	0.180	0.095	8.147	88.074
4m	1%	B	B21b	0.913	465.960	0.103	0.438	0.162	0.120	9.077	90.123

Rx	Time	Conc	Tissue	Sample	ΔHmax	MH	Eroded Ra	Eroded Rk	Eroded Rvk	Eroded Rpk	Eroded Mr1	Eroded Mr2
2	4m	1%	B	B21c	0.895	464.030	0.106	0.412	0.172	0.138	9.537	91.432
2	4m	1%	B	B22a	1.178	409.260	0.139	0.449	0.167	0.159	9.702	89.398
2	4m	1%	B	B22b	1.052	440.260	0.136	0.481	0.195	0.151	11.425	89.930
2	4m	1%	B	B22c	1.367	407.570	0.134	0.468	0.258	0.128	7.863	90.094
2	4m	1%	B	B23a	1.261	-	0.134	0.460	0.191	0.144	8.133	88.450
2	4m	1%	B	B23b	1.263	-	0.129	0.595	0.189	0.136	7.320	88.261
2	4m	1%	B	B23c	1.002	-	0.137	0.511	0.217	0.157	9.145	88.743
2	4m	1%	B	B24a	1.134	-	0.118	0.437	0.181	0.139	9.901	89.280
2	4m	1%	B	B24b	1.025	-	0.124	0.388	0.225	0.126	9.758	87.791
2	4m	1%	B	B24c	1.025	-	0.124	0.420	0.206	0.113	8.099	90.095
2	4m	1%	B	B25a	0.895	-	0.115	0.423	0.200	0.106	7.595	87.879
2	4m	1%	B	B25b	0.920	-	0.117	0.399	0.174	0.139	10.871	88.824
2	4m	1%	B	B25c	0.948	-	0.111	0.376	0.185	0.168	9.733	89.964
2	4m	1%	O	O26a	2.707	183.780	0.169	0.603	0.214	0.210	9.651	90.060
2	4m	1%	O	O26b	2.937	209.000	0.173	0.557	0.228	0.174	10.775	90.684
2	4m	1%	O	O26c	1.937	175.850	0.177	0.677	0.321	0.185	7.833	92.200
2	4m	1%	O	O27a	2.732	202.740	0.186	0.637	0.190	0.290	12.566	92.142
2	4m	1%	O	O27b	2.778	174.400	0.176	0.592	0.272	0.193	7.818	91.117
2	4m	1%	O	O27c	3.093	171.930	0.172	0.539	0.252	0.179	9.721	87.044
2	4m	1%	O	O28a	1.781	-	0.226	0.771	0.320	0.203	7.004	89.398
2	4m	1%	O	O28b	4.416	-	0.230	0.786	0.330	0.217	6.010	88.641
2	4m	1%	O	O28c	1.576	-	0.226	0.758	0.302	0.226	6.942	88.656
2	4m	1%	O	O29a	2.769	-	0.185	0.571	0.181	0.393	12.043	90.299
2	4m	1%	O	O29b	2.263	-	0.213	0.579	0.308	0.507	15.292	91.639
2	4m	1%	O	O29c	2.367	-	0.196	0.608	0.243	0.454	11.925	92.114
2	4m	1%	O	O30a	1.977	-	0.157	0.564	0.310	0.177	10.677	89.486
2	4m	1%	O	O30b	1.733	-	0.159	0.533	0.293	0.155	10.268	89.249
2	4m	1%	O	O30c	2.130	-	0.156	0.547	0.286	0.162	9.927	89.508
3	30s	6%	H	H31a	1.839	375.650	0.174	0.581	0.286	0.160	8.712	87.090
3	30s	6%	H	H31b	1.550	366.100	0.183	0.618	0.278	0.178	7.442	89.101
3	30s	6%	H	H31c	1.992	371.640	0.178	0.609	0.272	0.137	6.565	88.807
3	30s	6%	H	H32a	1.738	396.480	0.120	0.394	0.176	0.124	9.108	89.680
3	30s	6%	H	H32b	1.779	378.860	0.132	0.443	0.210	0.142	8.460	90.417
3	30s	6%	H	H32c	1.628	353.030	0.128	0.414	0.198	0.126	9.446	88.603
3	30s	6%	H	H33a	1.800	-	0.137	0.434	0.242	0.152	10.926	88.332
3	30s	6%	H	H33b	1.608	-	0.146	0.455	0.234	0.117	8.550	86.458
3	30s	6%	H	H33c	1.929	-	0.142	0.458	0.215	0.125	8.438	87.672
3	30s	6%	H	H34a	1.524	-	0.145	0.471	0.204	0.156	8.676	89.146
3	30s	6%	H	H34b	1.604	-	0.146	0.455	0.207	0.136	8.104	86.445
3	30s	6%	H	H34c	1.946	-	0.147	0.486	0.231	0.163	8.975	88.673
3	30s	6%	H	H35a	1.708	-	0.164	0.502	0.294	0.224	9.805	88.200
3	30s	6%	H	H35b	1.867	-	0.176	0.526	0.282	0.338	8.867	87.632
3	30s	6%	H	H35c	2.026	-	0.171	0.558	0.297	0.159	6.771	88.667
3	30s	6%	B	B36a	1.646	507.650	0.105	0.355	0.174	0.091	7.580	88.145
3	30s	6%	B	B36b	1.157	522.680	0.102	0.439	0.164	0.139	8.930	88.696
3	30s	6%	B	B36c	1.145	528.820	0.097	0.363	0.171	0.125	9.538	89.429
3	30s	6%	B	B37a	1.226	513.900	0.101	0.346	0.157	0.082	6.810	87.570
3	30s	6%	B	B37b	1.513	523.780	0.096	0.334	0.127	0.096	7.860	87.680
3	30s	6%	B	B37c	1.345	572.540	0.093	0.304	0.151	0.101	9.490	88.730
3	30s	6%	B	B38a	1.161	-	0.119	0.372	0.201	0.113	9.008	85.870
3	30s	6%	B	B38b	1.168	-	0.117	0.390	0.186	0.145	9.948	88.669
3	30s	6%	B	B38c	0.999	-	0.116	0.412	0.188	0.135	6.905	90.132
3	30s	6%	B	B39a	0.920	-	0.122	0.381	0.203	0.169	9.895	86.236
3	30s	6%	B	B39b	0.691	-	0.130	0.456	0.231	0.181	8.066	89.327
3	30s	6%	B	B39c	1.105	-	0.123	0.390	0.196	0.185	10.358	88.966
3	30s	6%	B	B40a	1.511	-	0.128	0.455	0.181	0.136	9.120	90.250
3	30s	6%	B	B40b	1.502	-	0.142	0.470	0.263	0.133	8.830	89.380
3	30s	6%	B	B40c	1.271	-	0.141	0.470	0.230	0.157	8.910	87.310
3	30s	6%	O	O41a	1.157	262.690	0.162	0.587	0.268	0.212	9.317	86.976
3	30s	6%	O	O41b	1.791	279.210	0.156	0.563	0.257	0.142	9.624	86.199
3	30s	6%	O	O41c	1.889	294.900	0.161	0.565	0.268	0.200	8.534	87.475
3	30s	6%	O	O42a	1.366	248.030	0.164	0.547	0.241	0.167	8.642	88.463
3	30s	6%	O	O42b	1.105	273.090	0.174	0.596	0.272	0.147	7.653	88.970
3	30s	6%	O	O42c	1.420	278.970	0.158	0.543	0.217	0.164	8.869	89.744
3	30s	6%	O	O43a	1.946	-	0.184	0.578	0.284	0.190	10.199	87.299
3	30s	6%	O	O43b	1.683	-	0.194	0.629	0.289	0.185	9.593	88.953
3	30s	6%	O	O43c	1.473	-	0.194	0.640	0.274	0.182	9.983	89.329
3	30s	6%	O	O44a	1.473	-	0.176	0.537	0.306	0.168	9.482	86.723

Rx	Time	Conc	Tissue	Sample	ΔHmax	MH	Eroded Ra	Eroded Rk	Eroded Rvk	Eroded Rpk	Eroded Mr1	Eroded Mr2
3	30s	6%	O	O44b	1.576	-	0.173	0.540	0.310	0.163	8.696	87.506
3	30s	6%	O	O44c	1.724	-	0.166	0.524	0.306	0.147	8.699	88.522
3	30s	6%	O	O45a	2.645	-	0.141	0.434	0.203	0.156	10.352	90.479
3	30s	6%	O	O45b	2.314	-	0.146	0.501	0.177	0.133	9.094	89.705
3	30s	6%	O	O45c	2.236	-	0.158	0.492	0.252	0.130	8.914	87.025
4	4m	6%	H	H46a	4.101	362.690	0.205	0.674	0.352	0.260	10.300	86.830
4	4m	6%	H	H46b	3.946	334.820	0.259	0.985	1.154	0.302	8.370	83.780
4	4m	6%	H	H46c	4.102	394.720	0.192	0.655	0.400	0.302	8.350	88.030
4	4m	6%	H	H47a	3.687	312.510	0.182	0.597	0.265	0.149	7.486	88.350
4	4m	6%	H	H47b	3.786	344.750	0.182	0.605	0.253	0.175	8.681	86.849
4	4m	6%	H	H47c	3.839	443.260	0.192	0.621	0.318	0.181	7.103	86.929
4	4m	6%	H	H48a	3.746	-	0.171	0.594	0.385	0.144	7.331	87.946
4	4m	6%	H	H48b	3.560	-	0.150	0.512	0.209	0.173	8.201	88.689
4	4m	6%	H	H48c	3.573	-	0.167	0.529	0.299	0.154	8.142	87.387
4	4m	6%	H	H49a	3.314	-	0.204	0.690	0.285	0.228	9.376	90.162
4	4m	6%	H	H49b	3.006	-	0.201	0.678	0.307	0.210	10.061	88.364
4	4m	6%	H	H49c	3.423	-	0.198	0.657	0.307	0.193	8.592	88.544
4	4m	6%	H	H50a	3.028	-	0.198	0.662	0.360	0.265	11.617	87.893
4	4m	6%	H	H50b	3.506	-	0.179	0.704	0.261	0.217	9.686	90.060
4	4m	6%	H	H50c	3.205	-	0.219	0.883	0.333	0.221	5.358	88.833
4	4m	6%	B	B51a	2.233	304.040	0.145	0.509	0.236	0.139	8.730	88.710
4	4m	6%	B	B51b	2.653	310.770	0.145	0.555	0.260	0.173	9.480	88.210
4	4m	6%	B	B51c	2.299	346.990	0.149	0.646	0.318	0.157	7.040	87.080
4	4m	6%	B	B52a	2.114	331.360	0.157	0.557	0.287	0.267	10.066	89.687
4	4m	6%	B	B52b	2.412	389.960	0.161	0.537	0.248	0.152	7.948	89.013
4	4m	6%	B	B52c	2.377	301.150	0.154	0.480	0.274	0.143	8.819	87.240
4	4m	6%	B	B53a	2.176	-	0.139	0.500	0.251	0.113	7.700	88.070
4	4m	6%	B	B53b	2.540	-	0.133	0.448	0.265	0.111	8.060	86.140
4	4m	6%	B	B53c	2.499	-	0.138	0.487	0.250	0.126	8.050	87.220
4	4m	6%	B	B54a	2.308	-	0.128	0.458	0.197	0.126	8.246	89.604
4	4m	6%	B	B54b	2.761	-	0.116	0.421	0.215	0.129	8.797	89.394
4	4m	6%	B	B54c	2.258	-	0.117	0.394	0.176	0.145	9.142	87.167
4	4m	6%	B	B55a	2.967	-	0.145	0.455	0.218	0.182	9.008	86.573
4	4m	6%	B	B55b	2.949	-	0.169	0.536	0.445	0.241	9.481	86.534
4	4m	6%	B	B55c	2.685	-	0.137	0.502	0.214	0.167	7.470	90.597
4	4m	6%	O	O56a	2.496	165.600	0.141	0.431	0.290	0.143	7.310	86.450
4	4m	6%	O	O56b	2.760	194.520	0.138	0.457	0.199	0.152	9.170	87.390
4	4m	6%	O	O56c	2.496	140.550	0.134	0.484	0.199	0.129	8.440	89.900
4	4m	6%	O	O57a	4.996	143.960	0.183	0.547	0.368	0.180	9.505	86.605
4	4m	6%	O	O57b	5.384	91.290	0.190	0.663	0.400	0.193	8.006	89.337
4	4m	6%	O	O57c	5.257	145.920	0.188	0.587	0.371	0.171	8.481	87.733
4	4m	6%	O	O58a	3.376	-	0.177	0.603	0.180	0.210	9.674	89.891
4	4m	6%	O	O58b	3.754	-	0.165	0.580	0.243	0.161	7.748	90.071
4	4m	6%	O	O58c	3.803	-	0.170	0.525	0.231	0.184	8.303	87.720
4	4m	6%	O	O59a	3.737	-	0.177	0.592	0.339	0.145	8.058	88.681
4	4m	6%	O	O59b	3.944	-	0.180	0.531	0.366	0.139	10.219	85.292
4	4m	6%	O	O59c	3.950	-	0.179	0.555	0.386	0.129	7.017	87.723
4	4m	6%	O	O60a	3.364	-	0.181	0.663	0.318	0.224	5.924	86.981
4	4m	6%	O	O60b	3.991	-	0.174	0.581	0.281	0.161	8.129	87.592
4	4m	6%	O	O60c	3.843	-	0.179	0.579	0.338	0.146	9.087	84.718

Appendix H – Post-erosion statistical analysis for ovine, human & bovine enamel

General linear model
Dependent Variable: **Eroded DH max**

Source of Variation	DF	SS	MS	F	P
Tissue	2	29.503	14.751	88.460	**<0.001**
Concentration	1	25.696	25.696	154.090	**<0.001**
Time	1	48.283	48.283	289.538	**<0.001**
Tissue x Concentration	2	0.383	0.192	1.150	0.319
Tissue x Time	2	5.935	2.967	17.795	**<0.001**
Concentration x Time	1	21.212	21.212	127.201	**<0.001**
Tiss x Conc x Time	2	0.0130	0.00651	0.0390	0.962
Residual	168	28.015	0.167		
Total	179	159.040	0.888		

General linear model
Dependent Variable: **MH eroded**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.000	0.000	0.000	1.000
Concentration	1	0.000	0.000	0.000	1.000
Time	1	0.000	0.000	0.000	1.000
Tissue x Concentration	2	1477905.217	738952.608	9.015	**<0.001**
Tissue x Time	2	1510924.027	755462.013	9.216	**<0.001**
Concentration x Time	1	261424.403	261424.403	3.189	0.079
Tiss x Conc x Time	2	0.000	0.000	0.000	1.000
Residual	60	4918302.372	81971.706		
Total	71	844678.321	11896.878		

General linear model
Dependent Variable: **Eroded Ra**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.0687	0.0344	84.457	**<0.001**
Concentration	1	0.000623	0.000623	1.533	0.217
Time	1	0.00926	0.00926	22.766	**<0.001**
Tissue x Conc	2	0.00983	0.00491	12.081	**<0.001**
Tissue x Time	2	0.00455	0.00228	5.597	**0.004**
Concentration x Time	1	0.00382	0.00382	9.387	**0.003**
Tiss x Conc x Time	2	0.00413	0.00207	5.080	**0.007**
Residual	168	0.0683	0.000407		
Total	179	0.169	0.000946		

General linear model
Dependent Variable: **Eroded Rk**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.569	0.284	46.569	**<0.001**
Concentration	1	0.00673	0.00673	1.102	0.295
Time	1	0.141	0.141	23.001	**<0.001**
Tissue x Conce	2	0.121	0.0607	9.938	**<0.001**
Tissue x Time	2	0.0417	0.0208	3.410	**0.035**
Concentration x Time	1	0.0702	0.0702	11.494	**<0.001**
Tiss x Conce x Time	2	0.0740	0.0370	6.060	**0.003**
Residual	168	1.026	0.00611		
Total	179	2.050	0.0115		

General linear model
Dependent Variable: **Eroded Rvk**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.153	0.0767	11.769	**<0.001**
Concentration	1	0.0328	0.0328	5.024	**0.026**
Time	1	0.0579	0.0579	8.880	**0.003**
Tissue x Conce	2	0.0300	0.0150	2.300	0.103
Tissue x Time	2	0.0364	0.0182	2.792	0.064
Concentration x Time	1	0.0772	0.0772	11.844	**<0.001**
Tiss x Conc x Time	2	0.00392	0.00196	0.301	0.741
Residual	168	1.095	0.00652		
Total	179	1.487	0.00831		

General linear model
Dependent Variable: **Eroded Pk**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.120	0.0601	4.544	**0.012**
Concentration	1	0.0264	0.0264	1.996	0.160
Time	1	0.0588	0.0588	4.447	**0.036**
Tissue x Conc	2	0.0230	0.0115	0.870	0.421
Tissue x Time	2	0.0247	0.0124	0.934	0.395
Concentration x Time	1	0.00615	0.00615	0.465	0.496
Tiss x Concn x Time	2	0.0217	0.0108	0.820	0.442
Residual	168	2.222	0.0132		
Total	179	2.503	0.0140		

General linear model
Dependent Variable: **Eroded Mr1**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.316	0.158	0.0937	0.911
Concentration	1	7.576	7.576	4.499	**0.035**
Time	1	0.363	0.363	0.215	0.643
Tissue x Conc	2	1.101	0.551	0.327	0.722
Tissue x Time	2	3.155	1.577	0.937	0.394
Concentration x Time	1	8.909	8.909	5.290	**0.023**
Tiss x Conce x Time	2	17.078	8.539	5.070	**0.007**
Residual	168	282.931	1.684		
Total	179	321.428	1.796		

General linear model
Dependent Variable: **Eroded Mr2**

Source of Variation	DF	SS	MS	F	P
Tissue	2	3.067	1.534	0.671	0.512
Concentration	1	16.828	16.828	7.364	**0.007**
Time	1	2.255	2.255	0.987	0.322
Tissue x Concen	2	2.937	1.469	0.643	0.527
Tissue x Time	2	17.398	8.699	3.807	**0.024**
Concentration x Time	1	18.414	18.414	8.058	**0.005**
Tiss x Conc x Time	2	19.328	9.664	4.229	**0.016**
Residual	168	383.911	2.285		
Total	179	464.138	2.593		

Paired t-test for **Ra** comparison to baseline
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Ra base	180	0.155	0.135	0.176
Eroded Ra	180	0.156	0.133	0.176

W= -2594.000 T+ = 6315.500 T-= -8909.500
Z-Statistic (based on positive ranks) = -1.949
P = 0.051

Paired t-test for **Rk** comparison to baseline
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Rk base	180	0.510	0.434	0.586
Eroded Rk	180	0.511	0.448	0.585

W= 434.000 T+ = 8272.000 T-= -7838.000
Z-Statistic (based on positive ranks) = 0.313
P = 0.755

Paired t-test for **Rpk** in comparison to baseline
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Rpk base	180	0.217	0.172	0.267
Eroded Pk	180	0.160	0.137	0.185

W= -11129.000 T+ = 2580.500 T-= -13709.500
Z-Statistic (based on positive ranks) = -7.949
P = <0.001

Paired t-test for **Rvk** in comparison to baseline
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Rvk base	180	0.241	0.202	0.286
Eroded Rvk	180	0.242	0.203	0.291

W= 1046.000 T+ = 8399.500 T-= -7353.500
Z-Statistic (based on positive ranks) = 0.766
P = 0.444

Paired t-test for **MR1** in comparison to baseline
Normality Test: Passed (P = 0.323)

Treatment Name	N	Mean	Std Dev	SEM
Mr1 base	180	9.306	1.446	0.108
Eroded Mr1	180	8.866	1.340	0.0999
Difference	180	0.440	2.043	0.152

t = 2.887 with 179 degrees of freedom. **P = 0.004**

Paired t-test for **MR2** in comparison to baseline
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Mr2 base	180	88.346	87.623	89.359
Eroded Mr2	180	88.457	87.316	89.422

W= 200.000 T+ = 8245.000 T-= -8045.000
Z-Statistic (based on positive ranks) = 0.143
P = 0.887

Paired t-test for **MH** in comparison to baseline
Normality Test: Passed (P = 0.052)

Treatment Name	N	Mean	Std Dev	SEM
MH base	180	412.468	134.390	15.838
MH eroded	180	318.799	109.073	12.854
Difference	180	93.669	83.413	9.830

t = 9.529 with 71 degrees of freedom. **P = <0.001**

One Way Analysis of Variance eroded tissue comparison
Dependent Variable: **MH eroded**
Normality Test: Passed (P = 0.610)
Equal Variance Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	334.570	295.290	373.645
B	60	408.635	349.125	486.805
O	60	197.470	168.765	236.600

H = 51.623 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
B vs O	1027.000	10.017	Yes
B vs H	362.000	3.531	Yes
H vs O	665.000	6.486	Yes

One Way Analysis of Variance eroded tissue comparison
Dependent Variable: **Eroded Ra**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.158	0.146	0.178
B	60	0.129	0.117	0.141
O	60	0.174	0.160	0.185

H = 81.010 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	4996.500	12.380	Yes
O vs H	1465.500	3.631	Yes
H vs B	3531.000	8.749	Yes

One Way Analysis of Variance eroded tissue comparison
Dependent Variable: **Eroded Rk**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.516	0.471	0.601
B	60	0.448	0.397	0.480
O	60	0.576	0.538	0.607

H = 61.112 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	4361.500	10.806	Yes
O vs H	1365.500	3.383	Yes
H vs B	2996.000	7.423	Yes

One Way Analysis of Variance eroded tissue comparison
Dependent Variable: **Eroded Rvk**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.257	0.217	0.295
B	60	0.204	0.180	0.242
O	60	0.282	0.230	0.321

H = 40.099 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	3480.500	8.623	Yes
O vs H	896.500	2.221	No
H vs B	2584.000	6.402	Yes

One Way Analysis of Variance eroded tissue comparison
Dependent Variable: **Eroded Rpk**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.166	0.145	0.209
B	60	0.139	0.126	0.158
O	60	0.172	0.149	0.202

H = 33.734 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	2976.000	7.373	Yes
O vs H	223.500	0.554	No
H vs B	2752.500	6.820	Yes

One Way Analysis of Variance eroded tissue comparison
Dependent Variable: **Eroded Mr1**
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	8.794	8.123	9.598
B	60	8.890	8.063	9.512
O	60	8.784	7.783	9.782

H = 0.0715 with 2 degrees of freedom. **(P = 0.965)**

One Way Analysis of Variance eroded tissue comparison
Dependent Variable: **Eroded Mr2**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	88.298	87.355	88.842
B	60	88.683	87.620	89.562
O	60	88.516	87.035	89.725

H = 2.168 with 2 degrees of freedom**. (P = 0.338)**

One Way Analysis of Variance eroded tissue comparison
Dependent Variable: **Eroded DH max**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	1.910	1.654	2.633
B	60	1.205	1.010	1.952
O	60	2.013	1.718	2.857

H = 43.104 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	3418.000	8.469	Yes
O vs H	378.500	0.938	No
H vs B	3039.500	7.531	Yes

Appendix I – Abrasion-data for 5s and 20s abrasion regimes

Time	Sample	Tissue	Ra base (post erosion)	Mr1	Mr2	Abraded Ra	Abraded Mr1	Abraded Mr2	Abraded surface loss
5s	B1	B	0.173	9.015	87.986	0.164	8.159	87.861	2.575
5s	B1	B	0.192	9.457	86.693	0.157	8.729	87.612	2.629
5s	B1	B	0.187	9.696	88.012	0.154	9.919	88.390	2.981
20s	B2	B	0.186	8.966	90.976	0.177	4.886	86.675	3.018
20s	B2	B	0.189	8.309	89.989	0.159	7.931	91.233	2.822
20s	B2	B	0.191	9.266	89.264	0.162	7.782	90.718	3.336
5s	H1	H	0.156	10.718	89.387	0.162	8.983	90.158	4.722
5s	H1	H	0.163	8.039	90.486	0.161	9.250	89.754	4.585
5s	H1	H	0.164	8.623	89.373	0.160	9.174	89.784	4.734
20s	H2	H	0.170	10.659	86.412	0.190	8.835	87.076	5.927
20s	H2	H	0.171	10.878	87.745	0.186	8.688	87.107	6.181
20s	H2	H	0.172	9.306	88.054	0.186	8.830	86.852	6.446
5s	O1	O	0.149	8.055	90.057	0.131	7.874	86.438	3.746
5s	O1	O	0.141	13.464	88.495	0.139	10.317	89.885	4.045
5s	O1	O	0.149	8.294	90.100	0.145	10.010	87.605	4.089
20s	O2	O	0.148	12.291	90.730	0.133	8.373	86.175	8.345
20s	O2	O	0.150	10.352	88.994	0.143	8.446	87.288	8.574
20s	O2	O	0.141	10.254	88.529	0.138	8.139	85.697	8.641

Appendix J – Abrasion-only statistical analyses for 5s and 20s abrasion regimes

5-second abrasion

Paired t-test: **Ra**
Normality Test: Passed (P = 0.100)

Treatment Name	N	Mean	Std Dev	SEM
RaBase (PE)	9	0.164	0.0175	0.00582
Abraded Ra	9	0.153	0.0116	0.00386
Difference	9	0.0112	0.0144	0.00480

t = 2.336 with 8 degrees of freedom. **(P = 0.048)**

Paired t-test: **MR1**
Normality Test: Passed (P = 0.923)

Treatment Name	N	Mean	Std Dev	SEM
Mr1	9	9.485	1.729	0.576
Abraded Mr1	9	9.157	0.831	0.277
Difference	9	0.327	1.500	0.500

t = 0.654 with 8 degrees of freedom. **(P = 0.531)**

Paired t-test: **MR2**
Normality Test: Passed (P = 0.103)

Treatment Name	N	Mean	Std Dev	SEM
Mr2	9	88.954	1.244	0.415
Abraded Mr2	9	88.610	1.325	0.442
Difference	9	0.345	1.677	0.559

t = 0.617 with 8 degrees of freedom. **(P = 0.555)**

20-second abrasion

Paired t-test: **Ra**
Normality Test: Passed (P = 0.417)

Treatment Name	N	Mean	Std Dev	SEM
RaBase (PE)	9	0.169	0.0186	0.00620
Abraded Ra	9	0.164	0.0221	0.00738
Difference	9	0.00489	0.0184	0.00614

t = 0.797 with 8 degrees of freedom. **(P = 0.449)**

Paired t-test: **MR1**
Normality Test: Passed (P = 0.262)

Treatment Name	N	Mean	Std Dev	SEM
Mr1	9	10.031	1.203	0.401
Abraded Mr1	9	7.990	1.223	0.408
Difference	9	2.041	1.289	0.430

t = 4.750 with 8 degrees of freedom. **(P = 0.001)**

Paired t-test: **MR2**
Normality Test: Passed (P = 0.466)

Treatment Name	N	Mean	Std Dev	SEM
Mr2	9	88.966	1.472	0.491
Abraded Mr2	9	87.647	1.955	0.652
Difference	9	1.319	2.249	0.750

t = 1.759 with 8 degrees of freedom. **(P = 0.117)**

Appendix K – Abrasion-only data for ovine, bovine and human enamel

Tissue	Tooth	Sample	RaBase	RkBase	RvkBase	RpkBase	Mr1Base	Mr2Base	Abraded Ra	Abraded Rk	Abraded Rvk	Abraded Rpk	Abraded Mr1	Abraded Mr2
H	1	H11	0.147	0.444	0.222	0.202	8.645	88.123	0.151	0.486	0.222	0.190	9.435	88.170
H	1	H12	0.152	0.486	0.223	0.195	9.461	88.174	0.159	0.465	0.248	0.240	8.095	84.265
H	1	H13	0.160	0.465	0.250	0.246	8.164	84.562	0.147	0.443	0.221	0.200	8.640	88.165
H	2	H21	0.158	0.501	0.213	0.197	7.891	85.523	0.158	0.500	0.213	0.195	7.862	85.645
H	2	H22	0.162	0.520	0.256	0.232	8.162	85.998	0.161	0.520	0.256	0.231	8.056	85.999
H	2	H23	0.152	0.497	0.198	0.278	10.784	86.978	0.152	0.497	0.198	0.265	10.687	86.784
H	3	H31	0.180	0.555	0.213	0.275	10.012	90.122	0.160	0.560	0.245	0.232	9.823	89.101
H	3	H32	0.163	0.562	0.235	0.245	9.852	88.512	0.181	0.556	0.213	0.263	10.002	89.978
H	3	H33	0.170	0.521	0.263	0.231	8.412	88.526	0.170	0.521	0.250	0.260	8.398	88.526
H	4	H41	0.155	0.478	0.280	0.256	8.467	88.165	0.156	0.478	0.280	0.250	8.365	87.948
H	4	H42	0.144	0.399	0.270	0.211	7.946	84.516	0.150	0.390	0.300	0.207	8.212	85.231
H	4	H43	0.152	0.478	0.298	0.207	8.256	85.247	0.143	0.399	0.269	0.210	7.845	83.998
H	5	H51	0.131	0.398	0.275	0.196	9.846	88.500	0.130	0.399	0.272	0.196	9.823	86.201
H	5	H52	0.134	0.501	0.213	0.187	9.887	84.578	0.133	0.498	0.212	0.185	9.813	83.978
H	5	H53	0.126	0.498	0.214	0.243	10.001	87.050	0.126	0.494	0.215	0.240	10.000	87.135
H	6	H61	0.198	0.523	0.260	0.250	9.444	80.598	0.177	0.500	0.254	0.265	8.401	82.562
H	6	H62	0.175	0.502	0.254	0.266	8.412	86.491	0.160	0.548	0.214	0.265	8.549	83.697
H	6	H63	0.165	0.555	0.214	0.271	8.550	83.916	0.197	0.513	0.266	0.250	9.411	81.002
B	7	B11	0.140	0.512	0.172	0.215	9.100	89.200	0.132	0.489	0.197	0.201	8.654	88.162
B	7	B12	0.132	0.482	0.197	0.200	8.750	88.121	0.130	0.411	0.180	0.199	9.230	85.945
B	7	B13	0.129	0.412	0.180	0.195	9.520	86.945	0.140	0.511	0.171	0.205	8.989	89.131
B	8	B21	0.150	0.465	0.201	0.213	9.236	87.555	0.149	0.456	0.198	0.233	9.587	90.121
B	8	B22	0.148	0.487	0.200	0.185	9.415	86.845	0.149	0.466	0.200	0.210	9.101	87.545
B	8	B23	0.149	0.456	0.198	0.235	9.897	90.101	0.148	0.487	0.200	0.184	9.342	86.845
B	9	B31	0.135	0.391	0.197	0.229	8.125	89.871	0.129	0.456	0.177	0.320	9.899	89.578
B	9	B32	0.142	0.388	0.165	0.298	8.465	88.165	0.134	0.401	0.197	0.229	7.948	89.847
B	9	B33	0.129	0.456	0.177	0.321	10.384	89.995	0.142	0.388	0.164	0.298	8.541	88.161
B	10	B41	0.148	0.478	0.178	0.265	9.456	84.103	0.148	0.478	0.172	0.265	9.632	84.101
B	10	B42	0.159	0.489	0.197	0.312	9.112	81.589	0.159	0.491	0.199	0.301	9.023	86.584
B	10	B43	0.161	0.499	0.201	0.277	8.945	90.256	0.160	0.501	0.201	0.277	8.416	81.021
B	11	B51	0.152	0.439	0.216	0.255	10.111	89.956	0.121	0.439	0.212	0.245	9.131	90.001
B	11	B52	0.149	0.523	0.231	0.201	9.815	90.056	0.150	0.512	0.201	0.313	10.001	85.915
B	11	B53	0.153	0.512	0.198	0.333	10.945	89.817	0.148	0.525	0.234	0.198	9.711	89.874
B	12	B61	0.130	0.399	0.156	0.365	9.623	90.369	0.123	0.411	0.165	0.251	8.494	92.222
B	12	B62	0.121	0.411	0.165	0.250	8.998	91.233	0.122	0.401	0.124	0.290	8.920	89.901
B	12	B63	0.119	0.400	0.123	0.288	8.917	89.959	0.129	0.198	0.158	0.354	9.623	91.123
O	13	O11	0.160	0.599	0.243	0.201	8.584	81.259	0.160	0.598	0.241	0.201	8.558	81.260
O	13	O12	0.166	0.591	0.260	0.199	7.948	86.549	0.166	0.590	0.261	0.201	7.694	86.549
O	13	O13	0.171	0.612	0.248	0.175	8.121	85.222	0.170	0.612	0.248	0.175	7.948	85.213

Tissue	Tooth	Sample	RaBase	RkBase	RvkBase	RpkBase	Mr1Base	Mr2Base	Abraded Ra	Abraded Rk	Abraded Rvk	Abraded Rpk	Abraded Mr1	Abraded Mr2
O	14	O21	0.201	0.623	0.262	0.156	11.645	91.021	0.222	0.700	0.280	0.366	10.024	90.687
O	14	O22	0.222	0.712	0.283	0.366	10.948	90.687	0.201	0.623	0.262	0.157	11.021	91.021
O	14	O23	0.203	0.775	0.294	0.284	12.010	90.002	0.203	0.775	0.294	0.284	11.948	90.021
O	15	O31	0.231	0.652	0.310	0.275	8.112	88.347	0.211	0.687	0.321	0.312	7.312	86.165
O	15	O32	0.211	0.687	0.321	0.311	7.315	86.635	0.212	0.689	0.258	0.297	8.254	88.598
O	15	O33	0.212	0.598	0.258	0.297	8.495	88.598	0.233	0.652	0.310	0.278	7.638	88.337
O	16	O41	0.165	0.623	0.294	0.258	8.915	86.152	0.166	0.623	0.299	0.258	8.773	88.487
O	16	O42	0.155	0.545	0.283	0.211	8.514	87.770	0.155	0.545	0.283	0.201	8.547	87.714
O	16	O43	0.159	0.523	0.211	0.201	9.611	88.701	0.161	0.525	0.212	0.201	8.304	88.701
O	17	O51	0.179	0.557	0.311	0.234	10.012	89.025	0.179	0.558	0.315	0.234	9.908	89.002
O	17	O52	0.201	0.611	0.451	0.296	9.888	89.060	0.211	0.590	0.381	0.222	8.715	88.561
O	17	O53	0.202	0.594	0.381	0.214	8.997	88.595	0.202	0.611	0.451	0.296	9.408	89.102
O	18	O61	0.189	0.628	0.411	0.198	10.248	87.485	0.182	0.630	0.391	0.256	12.341	88.234
O	18	O62	0.182	0.633	0.391	0.254	12.658	88.369	0.189	0.628	0.398	0.198	10.024	87.485
O	18	O63	0.168	0.598	0.357	0.258	15.222	81.294	0.165	0.597	0.358	0.258	15.021	81.278

Appendix L – Abrasion-only statistical analyses for ovine, human & bovine enamel

One Way Analysis of Variance
Dependent Variable: **Abraded Ra**
Normality Test: Passed (P = 0.549)
Equal Variance Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	18	0.157	0.147	0.161
B	18	.141	0.129	0.149
O	18	0.186	0.166	0.211

H = 32.467 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	535.500	8.023	Yes
O vs H	309.000	4.630	Yes
H vs B	226.500	3.393	Yes

One Way Analysis of Variance
Dependent Variable: **Abraded Rk**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	18	0.498	0.465	0.520
B	18	0.461	0.411	0.491
O	18	0.617	0.590	0.652

H = 35.343 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	534.500	8.008	Yes
O vs H	415.000	6.218	Yes
H vs B	119.500	1.790	No

One Way Analysis of Variance
Dependent Variable: **Abraded Rvk**
Normality Test: Passed (P = 0.070)
Equal Variance Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	18	0.247	0.214	0.266
B	18	0.197	0.171	0.200
O	18	0.296	0.261	0.358

H = 37.713 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	575.500	8.622	Yes
O vs H	228.500	3.423	Yes
H vs B	347.000	5.199	Yes

One Way Analysis of Variance
Dependent Variable: **Abraded Rpk**
Normality Test: Passed (P = 0.242)
Equal Variance Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	18	0.236	0.200	0.260
B	18	0.248	0.205	0.298
O	18	0.245	0.201	0.284

H = 2.043 with 2 degrees of freedom. **(P = 0.360)**

One Way Analysis of Variance
Dependent Variable: **Abraded Mr1**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	18	8.595	8.212	9.823
B	18	9.116	8.654	9.623
O	18	8.744	8.254	10.024

H = 0.588 with 2 degrees of freedom. **(P = 0.745)**

One Way Analysis of Variance
Dependent Variable: **Abraded Mr2**
Normality Test: Failed (P < 0.050)
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	18	86.100	83.998	88.165
B	18	88.647	86.584	89.901
O	18	88.412	86.549	89.002

H = 7.287 with 2 degrees of freedom. **(P = 0.026)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
B vs H	239.000	3.581	Yes
B vs O	43.000	0.644	No
O vs H	196.000	2.937	No

Paired t-test **Ra**
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
RaBase	54	0.159	0.147	0.175
Abraded Ra	54	0.159	0.143	0.177

W= -85.000 T+ = 409.000 T-= -494.000
Z-Statistic (based on positive ranks) = -0.533
(P = 0.598)

Paired t-test: **Rk**
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
RkBase	54	0.507	0.465	0.594
Abraded Rk	54	0.506	0.465	0.590

W= -103.000 T+ = 443.500 T-= -546.500
Z-Statistic (based on positive ranks) = -0.601
(P = 0.552)

Paired t-test: **Rvk**
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
RvkBase	54	0.233	0.198	0.280
Abraded Rvk	54	0.237	0.200	0.280

W= -26.000 T+ = 482.000 T-= -508.000
Z-Statistic (based on positive ranks) = -0.152
(P = 0.884)

Paired t-test: **Rpk**
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
RpkBase	54	0.244	0.201	0.275
Abraded Rpk	54	0.240	0.201	0.265

W= -88.000 T+ = 451.000 T-= -539.000
Z-Statistic (based on positive ranks) = -0.514
(P = 0.612)

Appendix M – Post-erosion abrasion data for ovine, human & bovine enamel

Tissue	Tooth	Sample	Abraded surface loss	MH abraded	Abraded Ra	Abraded Rk	Abraded Rvk	Abraded Rpk	Abraded Mr1	Abraded Mr2
H	1	H1a	2.946	337.050	0.143	0.453	0.228	0.145	10.882	89.100
H	1	H1b	2.629	321.180	0.138	0.495	0.148	0.164	9.641	89.316
H	1	H1c	1.315	326.140	0.139	0.600	0.242	0.252	8.529	90.799
H	2	H2a	2.631	278.920	0.115	0.486	0.185	0.150	9.194	89.133
H	2	H2b	2.575	333.820	0.147	0.471	0.227	0.153	9.009	86.890
H	2	H2c	2.892	288.930	0.146	0.463	0.227	0.178	9.120	86.332
H	3	H3a	1.315	-	0.134	0.407	0.189	0.137	10.366	86.676
H	3	H3b	0.945	-	0.137	0.417	0.189	0.139	9.056	86.010
H	3	H3c	2.473	-	0.135	0.427	0.191	0.133	10.786	87.050
H	4	H4a	3.272	-	0.127	0.451	0.200	0.159	10.892	88.565
H	4	H4b	1.945	-	0.155	0.467	0.277	0.186	10.390	85.097
H	4	H4c	2.403	-	0.130	0.460	0.169	0.185	10.003	91.031
H	5	H5a	2.579	-	0.123	0.346	0.257	0.129	9.932	85.765
H	5	H5b	2.485	-	0.131	0.415	0.217	0.101	7.242	87.354
H	5	H5c	1.446	-	0.157	0.618	0.249	0.257	12.417	92.057
B	6	B6a	1.473	409.560	0.137	0.509	0.156	0.163	9.864	90.359
B	6	B6b	1.581	456.410	0.138	0.509	0.158	0.165	9.103	90.717
B	6	B6c	0.921	436.790	0.131	0.513	0.187	0.157	8.971	89.483
B	7	B7a	1.182	447.640	0.139	0.459	0.219	0.163	8.841	86.586
B	7	B7b	1.579	437.220	0.132	0.448	0.255	0.131	10.075	86.107
B	7	B7c	1.183	357.230	0.127	0.422	0.206	0.105	8.033	87.252
B	8	B8a	1.575	-	0.125	0.418	0.221	0.114	8.053	88.215
B	8	B8b	1.525	-	0.115	0.362	0.172	0.109	9.481	88.290
B	8	B8c	1.421	-	0.122	0.342	0.273	0.128	9.811	85.870
B	9	B9a	0.841	-	0.132	0.467	0.188	0.122	6.592	88.671
B	9	B9b	0.920	-	0.127	0.411	0.179	0.253	5.378	88.892
B	9	B9c	1.052	-	0.144	0.511	0.207	0.170	8.515	89.487
B	10	B10a	1.314	-	0.130	0.666	0.770	0.105	4.588	89.106
B	10	B10b	1.578	-	0.121	0.380	0.216	0.094	6.081	87.248
B	10	B10c	1.447	-	0.131	0.398	0.203	0.141	9.388	86.687
O	11	O11a	5.916	254.790	0.171	0.685	0.273	0.191	8.148	88.942
O	11	O11b	6.442	256.090	0.187	0.584	0.318	0.160	9.772	88.100
O	11	O11c	7.757	285.640	0.176	0.577	0.240	0.153	8.642	88.439
O	12	O12a	7.231	262.780	0.176	0.585	0.294	0.155	8.345	89.320
O	12	O12b	6.838	171.430	0.205	0.769	0.215	0.159	7.149	90.811
O	12	O12c	7.886	302.690	0.170	0.541	0.261	0.151	7.520	87.823
O	13	O13a	9.339	-	0.172	0.547	0.239	0.198	8.901	88.428
O	13	O13b	7.626	-	0.171	0.524	0.244	0.190	10.059	86.750
O	13	O13c	6.571	-	0.172	0.526	0.242	0.197	9.968	86.721
O	14	O14a	4.272	-	0.177	0.571	0.348	0.166	8.706	87.658
O	14	O14b	4.785	-	0.178	0.607	0.351	0.167	7.989	89.845
O	14	O14c	4.054	-	0.177	0.604	0.323	0.165	8.304	89.236
O	15	O15a	4.232	-	0.185	0.549	0.277	0.580	8.531	88.347
O	15	O15b	4.652	-	0.169	0.888	0.217	0.190	6.511	93.443
O	15	O15c	5.689	-	0.173	0.926	0.208	0.243	7.076	93.871
H	16	H16a	5.069	399.150	0.153	0.472	0.294	0.125	8.455	82.228
H	16	H16b	4.077	355.760	0.154	0.534	0.197	0.145	7.943	91.693
H	16	H16c	4.882	344.310	0.150	0.517	0.191	0.127	7.358	90.783
H	17	H17a	3.986	375.250	0.140	0.455	0.208	0.157	8.910	86.518
H	17	H17b	5.248	374.030	0.157	0.622	0.244	0.157	7.299	88.636
H	17	H17c	4.683	343.240	0.159	0.619	0.251	0.160	6.976	87.933
H	18	H18a	4.001	-	0.172	0.862	0.311	0.141	6.511	89.069
H	18	H18b	3.946	-	0.173	0.722	0.234	0.187	6.412	90.605

Tissue	Tooth	Sample	Abraded surface loss	MH abraded	Abraded Ra	Abraded Rk	Abraded Rvk	Abraded Rpk	Abraded Mr1	Abraded Mr2
H	18	H18c	5.246	-	0.146	0.488	0.239	0.196	8.598	87.474
H	19	H19a	3.902	-	0.145	0.496	0.254	0.188	8.830	88.875
H	19	H19b	4.811	-	0.144	0.500	0.188	0.188	8.790	90.596
H	19	H19c	4.273	-	0.140	0.480	0.199	0.143	8.647	89.708
H	20	H20a	5.010	-	0.120	0.396	0.232	0.111	6.542	88.458
H	20	H20b	4.901	-	0.119	0.392	0.233	0.099	6.785	88.317
H	20	H20c	4.766	-	0.152	0.472	0.220	0.157	9.870	87.340
B	21	B21a	2.312	573.690	0.119	0.382	0.184	0.180	9.530	87.117
B	21	B21b	1.841	576.470	0.119	0.385	0.180	0.161	9.685	86.988
B	21	B21c	2.056	566.500	0.103	0.359	0.149	0.118	7.581	88.851
B	22	B22a	2.264	477.680	0.104	0.328	0.162	0.095	9.438	87.545
B	22	B22b	2.577	489.890	0.096	0.339	0.146	0.111	8.709	89.155
B	22	B22c	2.414	491.790	0.092	0.295	0.146	0.102	8.045	84.422
B	23	B23a	3.308	-	0.129	0.423	0.209	0.145	10.346	88.149
B	23	B23b	2.470	-	0.129	0.450	0.176	0.135	7.850	88.647
B	23	B23c	2.634	-	0.110	0.356	0.168	0.099	8.435	88.661
B	24	B24a	2.890	-	0.110	0.349	0.176	0.110	8.739	88.465
B	24	B24b	2.945	-	0.100	0.318	0.163	0.088	9.245	89.106
B	24	B24c	2.734	-	0.113	0.370	0.167	0.125	8.230	89.020
B	25	B25a	2.580	-	0.119	0.385	0.181	0.140	8.274	89.460
B	25	B25b	3.744	-	0.081	0.279	0.117	0.097	9.813	89.956
B	25	B25c	3.149	-	0.102	0.329	0.152	0.085	7.458	84.096
O	26	O26a	7.100	225.270	0.170	0.547	0.308	0.138	7.181	85.127
O	26	O26b	10.649	269.670	0.172	0.606	0.291	0.225	7.284	89.657
O	26	O26c	15.364	269.290	0.185	0.704	0.323	0.210	6.633	87.633
O	27	O27a	11.701	209.550	0.201	0.678	0.285	0.206	7.177	88.264
O	27	O27b	9.466	296.730	0.154	0.508	0.220	0.189	9.947	90.668
O	27	O27c	9.598	201.260	0.149	0.593	0.201	0.121	6.752	90.794
O	28	O28a	12.492	-	0.151	0.506	0.215	0.130	7.851	90.234
O	28	O28b	12.233	-	0.149	0.538	0.171	0.121	5.736	91.957
O	28	O28c	11.964	-	0.149	0.517	0.198	0.120	7.396	91.620
O	29	O29a	10.911	-	0.180	0.710	0.217	0.199	6.116	90.645
O	29	O29b	9.861	-	0.177	0.695	0.223	0.214	6.898	89.920
O	29	O29c	10.640	-	0.177	0.709	0.224	0.233	7.570	90.420
O	30	O30a	6.836	-	0.156	0.525	0.285	0.199	7.283	88.314
O	30	O30b	6.995	-	0.158	0.522	0.295	0.156	8.178	87.339
O	30	O30c	7.100	-	0.175	0.590	0.258	0.171	6.346	89.372
H	31	H31a	3.370	481.060	0.157	0.546	0.239	0.190	8.954	91.867
H	31	H31b	3.732	389.750	0.159	0.338	0.248	0.158	10.362	90.433
H	31	H31c	3.679	331.000	0.158	0.535	0.250	0.179	10.400	90.258
H	32	H32a	3.158	327.570	0.145	0.493	0.208	0.156	8.755	88.574
H	32	H32b	2.768	379.280	0.158	0.842	0.247	0.209	9.520	92.478
H	32	H32c	2.503	400.500	0.155	0.502	0.194	0.167	9.546	88.855
H	33	H33a	3.522	-	0.163	0.655	0.429	0.207	8.711	86.426
H	33	H33b	3.108	-	0.154	0.489	0.233	0.176	9.874	89.303
H	33	H33c	3.215	-	0.168	0.533	0.224	0.156	9.064	87.411
H	34	H34a	3.623	-	0.150	0.539	0.216	0.139	7.467	90.684
H	34	H34b	3.310	-	0.154	0.554	0.215	0.154	7.097	89.448
H	34	H34c	3.250	-	0.150	0.476	0.235	0.164	8.271	88.025
H	35	H35a	3.944	-	0.167	0.552	0.240	0.179	9.310	90.687
H	35	H35b	2.643	-	0.145	0.474	0.239	0.143	9.865	88.798
H	35	H35c	3.948	-	0.144	0.485	0.205	0.147	8.086	88.333
B	36	B36a	1.844	707.480	0.115	0.360	0.206	0.101	6.815	85.148
B	36	B36b	1.709	616.440	0.121	0.389	0.188	0.182	9.429	87.362
B	36	B36c	1.313	655.770	0.115	0.481	0.188	0.231	12.081	89.713

Tissue	Tooth	Sample	Abraded surface loss	MH abraded	Abraded Ra	Abraded Rk	Abraded Rvk	Abraded Rpk	Abraded Mr1	Abraded Mr2
B	37	B37a	2.002	670.430	0.129	0.469	0.220	0.138	6.838	90.403
B	37	B37b	1.550	690.530	0.119	0.367	0.268	0.104	9.418	88.173
B	37	B37c	2.105	720.720	0.106	0.369	0.160	0.111	9.464	87.915
B	38	B38a	2.630	-	0.122	0.417	0.170	0.129	8.044	90.293
B	38	B38b	1.578	-	0.126	0.416	0.180	0.138	8.932	90.231
B	38	B38c	1.841	-	0.113	0.349	0.184	0.115	8.764	87.390
B	39	B39a	1.262	-	0.113	0.335	0.179	0.116	9.800	85.607
B	39	B39b	1.210	-	0.116	0.363	0.180	0.116	7.471	87.630
B	39	B39c	1.473	-	0.117	0.366	0.177	0.109	7.859	86.989
B	40	B40a	1.263	-	0.119	0.391	0.167	0.102	8.251	88.679
B	40	B40b	1.327	-	0.119	0.387	0.175	0.098	8.400	88.668
B	40	B40c	1.526	-	0.117	0.386	0.161	0.147	7.085	89.168
O	41	O41a	6.573	332.210	0.168	0.585	0.277	0.217	7.958	88.731
O	41	O41b	5.776	298.630	0.197	0.639	0.286	0.196	9.186	85.192
O	41	O41c	6.046	305.290	0.166	0.552	0.201	0.144	8.359	88.492
O	42	O42a	6.985	287.930	0.167	0.542	0.223	0.150	8.918	86.745
O	42	O42b	6.447	299.040	0.164	0.544	0.287	0.150	8.033	90.480
O	42	O42c	6.056	305.950	0.161	0.523	0.268	0.159	8.311	89.869
O	43	O43a	6.441	-	0.162	0.697	0.375	0.191	9.607	87.790
O	43	O43b	6.967	-	0.170	0.534	0.249	0.176	9.366	87.147
O	43	O43c	7.494	-	0.170	0.523	0.252	0.174	10.585	86.849
O	44	O44a	6.573	-	0.172	0.663	0.224	0.228	8.799	89.875
O	44	O44b	6.836	-	0.167	0.636	0.317	0.265	11.679	91.199
O	44	O44c	6.314	-	0.163	0.573	0.249	0.145	8.401	89.611
O	45	O45a	6.836	-	0.166	0.553	0.246	0.152	9.419	87.354
O	45	O45b	6.310	-	0.165	0.540	0.241	0.150	9.260	86.645
O	45	O45c	6.178	-	0.179	0.641	0.528	0.284	7.009	87.180
H	46	H46a	6.044	583.430	0.184	0.687	0.329	0.200	7.338	85.746
H	46	H46b	6.179	554.360	0.182	0.594	0.258	0.171	7.743	86.496
H	46	H46c	6.183	405.140	0.189	0.630	0.240	0.241	9.856	90.131
H	47	H47a	5.916	466.800	0.181	0.603	0.223	0.214	9.226	89.816
H	47	H47b	5.784	468.470	0.183	0.554	0.270	0.575	7.963	86.626
H	47	H47c	4.998	426.430	0.209	0.653	0.349	0.320	11.271	87.767
H	48	H48a	5.916	-	0.185	0.887	0.268	0.258	9.804	89.197
H	48	H48b	8.322	-	0.184	0.861	0.253	0.233	9.844	88.071
H	48	H48c	9.710	-	0.180	0.619	0.263	0.168	8.517	88.737
H	49	H49a	6.700	-	0.182	0.656	0.251	0.176	6.786	90.393
H	49	H49b	6.966	-	0.170	0.541	0.264	0.150	8.832	88.498
H	49	H49c	5.914	-	0.206	0.624	0.390	0.527	10.745	83.707
H	50	H50a	5.916	-	0.207	0.717	0.347	0.159	7.579	87.178
H	50	H50b	7.362	-	0.231	0.770	0.316	0.173	4.590	85.655
H	50	H50c	7.047	-	0.195	0.668	0.241	0.175	7.028	89.572
B	51	B51a	3.044	652.780	0.149	0.463	0.226	0.136	7.114	85.906
B	51	B51b	2.787	626.360	0.174	0.788	0.257	0.190	6.279	92.302
B	51	B51c	3.101	539.380	0.154	0.539	0.221	0.136	6.871	90.215
B	52	B52a	2.314	565.640	0.165	0.575	0.193	0.159	7.654	90.056
B	52	B52b	4.000	486.550	0.162	0.575	0.195	0.140	7.461	90.452
B	52	B52c	3.893	462.090	0.140	0.445	0.332	0.125	7.527	86.337
B	53	B53a	2.313	-	0.134	0.470	0.190	0.109	6.706	88.437
B	53	B53b	2.629	-	0.133	0.487	0.188	0.107	6.140	90.080
B	53	B53c	2.830	-	0.134	0.470	0.206	0.119	7.339	88.035
B	54	B54a	3.952	-	0.160	0.489	0.362	0.087	5.582	85.784
B	54	B54b	5.258	-	0.175	0.904	0.208	0.235	7.978	93.751
B	54	B54c	3.892	-	0.180	0.635	0.263	0.192	6.995	90.308
B	55	B55a	2.734	-	0.124	0.416	0.178	0.115	6.189	88.361

Tissue	Tooth	Sample	Abraded surface loss	MH abraded	Abraded Ra	Abraded Rk	Abraded Rvk	Abraded Rpk	Abraded Mr1	Abraded Mr2
B	55	B55b	3.785	-	0.123	0.408	0.188	0.097	6.403	88.097
B	55	B55c	3.465	-	0.127	0.417	0.178	0.115	6.490	87.412
O	56	O56a	10.256	348.370	0.132	0.479	0.151	0.158	9.084	89.349
O	56	O56b	9.855	371.530	0.138	0.530	0.177	0.182	8.694	90.834
O	56	O56c	9.732	317.560	0.117	0.411	0.162	0.100	5.822	88.522
O	57	O57a	9.203	310.780	0.134	0.464	0.219	0.119	8.085	89.559
O	57	O57b	9.464	302.040	0.133	0.459	0.218	0.121	7.848	88.690
O	57	O57c	6.049	321.780	0.135	0.483	0.225	0.122	6.083	88.907
O	58	O58a	9.684	-	0.133	0.460	0.239	0.118	7.748	86.387
O	58	O58b	8.678	-	0.132	0.455	0.240	0.121	7.546	86.184
O	58	O58c	8.742	-	0.133	0.494	0.214	0.123	8.022	88.457
O	59	O59a	8.023	-	0.131	0.450	0.230	0.117	7.859	86.149
O	59	O59b	8.286	-	0.127	0.421	0.197	0.141	8.659	87.229
O	59	O59c	9.072	-	0.132	0.448	0.190	0.118	8.941	87.718
O	60	O60a	9.463	-	0.131	0.483	0.217	0.131	6.661	90.961
O	60	O60b	8.083	-	0.138	0.504	0.240	0.149	7.383	88.430
O	60	O60c	6.682	-	0.138	0.492	0.258	0.121	7.362	87.551

Appendix N – Post-erosion abrasion statistical analysis for ovine, human & bovine enamel

General linear model
Dependent Variable: **Abraded surface DH**

Source of Variation	DF	SS	MS	F	P
Tissue	2	1001.553	500.777	442.849	**<0.001**
Concentration	1	10.805	10.805	9.556	**0.002**
Time	1	275.985	275.985	244.060	**<0.001**
Tissue x Conc	2	33.262	16.631	14.707	**<0.001**
Tissue x Time	2	21.120	10.560	9.338	**<0.001**
Concentration x Time	1	0.265	0.265	0.234	0.629
Tissue x Conc x Time	2	14.958	7.479	6.614	**0.002**
Residual	168	189.975	1.131		
Total	179	1547.924	8.648		

General linear model
Dependent Variable: **MH abraded**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.000	0.000	0.000	1.000
Concentration	1	0.000	0.000	0.000	1.000
Time	1	0.000	0.000	0.000	1.000
Tissue x Conc	2	2144785.681	1072392.841	8.118	**<0.001**
Tissue x Time	2	2163085.966	1081542.983	8.187	**<0.001**
Concentration x Time	1	477307.109	477307.109	3.613	0.062
Tissue x Concn x Time	2	0.000	0.000	0.000	1.000
Residual	60	7926273.011	132104.550		
Total	71	1276746.463	17982.345		

General linear model
Dependent Variable: **Abraded Ra**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.0449	0.0224 1	52.280	**<0.001**
Concentration	1	0.00270	0.00270	18.320	**<0.001**
Time	1	0.000110	0.000110	0.750	0.388
Tissue x Conc	2	0.0211	0.0105	71.506	**<0.001**
Tissue x Time	2	0.0170	0.00848	57.555	**<0.001**
Concentration x Time	1	0.00329	0.00329	22.300	**<0.001**
Tissue x Conc x Time	2	0.0121	0.00606	41.159	**<0.001**
Residual	168	0.0247	0.000147		
Total	179	0.126	0.000703		

General linear model
Dependent Variable: **Abraded Rk**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.645	0.3233	8.623	**<0.001**
Concentration	1	0.0265	0.0265	3.176	0.077
Time	1	0.0146	0.0146	1.752	0.187
Tissue x Conc	2	0.300	0.1501	7.970	**<0.001**
Tissue x Time	2	0.240	0.1201	4.353	**<0.001**
Concentration x Time	1	0.0691	0.0691	8.276	**0.005**
Tissue x Conc x Time	2	0.199	0.0997	11.933	**<0.001**
Residual	168	1.403	0.00835		
Total	179	2.897	0.0162		

General linear model
Dependent Variable: **Abraded Rvk**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.0777	0.0389 1	0.011	**<0.001**
Concentration	1	0.00483	0.00483	1.243	0.266
Time	1	0.00549	0.00549	1.414	0.236
Tissue x Conc	2	0.0213	0.0107	2.746	0.067
Tissue x Time	2	0.0461	0.0231	5.944	**0.003**
Concentration x Time	1	0.0101	0.0101	2.593	0.109
Tissue x Conc x Time	2	0.0493	0.0247	6.355	**0.002**
Residual	168	0.652	0.00388		
Total	179	0.867	0.00484		

General linear model
Dependent Variable: **Abraded Rpk**

Source of Variation	DF	SS	MS	F	P
Tissue	2	0.0904	0.0452 1	3.197	**<0.001**
Concentration	1	0.00219	0.00219	0.640	0.425
Time	1	0.00112	0.00112	0.325	0.569
Tissue x Con	2	0.0520	0.0260	7.594	**<0.001**
Tissue x Time	2	0.0442	0.0221	6.456	**0.002**
Concentration x Time	1	0.0117	0.0117	3.409	0.067
Tissue x Conc x Time	2	0.0273	0.0137	3.987	**0.020**
Residual	168	0.576	0.00343		
Total	179	0.805	0.00449		

General linear model
Dependent Variable: **Abraded Mr1**

Source of Variation	DF	SS	MS	F	P
Tissue	2	20.261	10.130	7.116	**0.001**
Concentration	1	0.454	0.454	0.319	0.573
Time	1	46.389	46.389	32.586	**<0.001**
Tissue x Conc	2	12.996	6.498	4.565	**0.012**
Tissue x Time	2	4.331	2.166	1.521	0.221
Concentration x Time	1	1.246	1.246	0.875	0.351
Tissue x Conc x Time	2	26.292	13.146	9.234	**<0.001**
Residual	168	239.160	1.424		
Total	179	351.128	1.962		

General linear model
Dependent Variable: **Abraded Mr2**

Source of Variation	DF	SS	MS	F	P
Tissue	2	6.162	3.081	0.879	0.417
Concentration	1	0.171	0.171	0.0490	0.825
Time	1	0.0245	0.0245	0.00701	0.933
Tissue x Conc	2	22.497	11.248	3.211	**0.043**
Tissue x Time	2	6.653	3.327	0.950	0.389
Concentration x Time	1	1.803	1.803	0.515	0.474
Tissue x Conc x Time	2	18.367	9.183	2.621	0.076
Residual	168	588.545	3.503		
Total	179	644.223	3.599		

Paired t-test **Ra**
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Eroded Ra	180	0.156	0.133	0.176
Abraded Ra	180	0.148	0.129	0.170

W= -4176.000 T+ = 5700.000 T-= -9876.000
Z-Statistic (based on positive ranks) = -3.085
(P = 0.002)

Paired t-test **Rk**
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Eroded Rk	180	0.511	0.448	0.585
Abraded Rk	180	0.501	0.422	0.585

W= -1570.000 T+ = 7270.000 T-= -8840.000
Z-Statistic (based on positive ranks) = -1.131
(P = 0.258)

Paired t-test **Rvk**
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Eroded Rvk	180	0.242	0.203	0.291
Abraded Rvk	180	0.223	0.189	0.256

W= -5894.000 T+ = 5108.000 T-= -11002.000
Z-Statistic (based on positive ranks) = -4.245
(P = <0.001)

Paired t-test **Rpk**
Normality Test: Failed (P < 0.050)
Wilcoxon Signed Rank Test

Group	N	Median	25%	75%
Eroded Pk	180	0.160	0.137	0.185
Abraded Rpk	180	0.153	0.122	0.184

W= -3276.000 T+ = 6417.000 T-= -9693.000
Z-Statistic (based on positive ranks) = -2.359
(P = 0.018)

Paired t-test **MR1**
Normality Test: Passed (P = 0.056)

Treatment Name	N	Mean	Std Dev	SEM
Eroded Mr1	180	8.866	1.340	0.0999
Abraded Mr1	180	8.322	1.401	0.104
Difference	180	0.544	1.970	0.147

t = 3.707 with 179 degrees of freedom. **(P = <0.001)**

Paired t-test **MR2**
Normality Test: Passed (P = 0.437)

Treatment Name	N	Mean	Std Dev	SEM
Eroded Mr2	180	88.424	1.610	0.120
Abraded Mr2	180	88.544	1.897	0.141
Difference	180	-0.120	2.514	0.187

t = -0.639 with 179 degrees of freedom. **(P = 0.524)**

One Way Analysis of Variance abraded tissue comparison
Dependent Variable: **MH abraded**
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	374.640	332.410	415.785
B	60	552.510	459.250	639.570
O	60	297.680	259.435	308.365

H = 51.557 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
B vs O	1041.000	10.153	Yes
B vs H	507.000	4.945	Yes
H vs O	534.000	5.208	Yes

One Way Analysis of Variance abraded tissue comparison
Dependent Variable: **Abraded Ra**
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.154	0.143	0.172
B	60	0.124	0.115	0.134
O	60	0.167	0.144	0.175

H = 68.571 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	4345.000	10.765	Yes
O vs H	563.000	1.395	No
H vs B	3782.000	9.370	Yes

One Way Analysis of Variance abraded tissue comparison
Dependent Variable: **Abraded Rk**
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.525	0.472	0.621
B	60	0.413	0.365	0.470
O	60	0.546	0.507	0.607

H = 55.756 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	4019.000	9.958	Yes
O vs H	781.000	1.935	No
H vs B	3238.000	8.023	Yes

One Way Analysis of Variance abraded tissue comparison
Dependent Variable: **Abraded Rvk**
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.239	0.212	0.256
B	60	0.186	0.171	0.208
O	60	0.240	0.217	0.285

H = 46.762 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
O vs B	3572.000	8.850	Yes
O vs H	424.000	1.051	No
H vs B	3148.000	7.800	Yes

One Way Analysis of Variance abraded tissue comparison
Dependent Variable: **Abraded Rpk**
Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Median	25%	75%
H	60	0.164	0.146	0.188
B	60	0.121	0.106	0.146
O	60	0.159	0.135	0.197

H = 39.932 with 2 degrees of freedom. **(P = <0.001)**

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
H vs B	3294.000	8.161	Yes
H vs O	375.000	0.929	No
O vs B	2919.000	7.232	Yes

One Way Analysis of Variance abraded tissue comparison
Dependent Variable: **Abraded Mr1**

Group Name	N	Mean	Std Dev	SEM
H	60	8.796	1.454	0.188
B	60	8.092	1.422	0.184
O	60	8.078	1.213	0.157

Source of Variation	DF	SS	MS	F	P
Between Groups	2	20.261	10.130	5.419	**0.005**
Residual	177	330.867	1.869		
Total	179	351.128			

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: Tissue

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
H vs. O	0.719	2.880	0.004	0.017	Yes
H vs. B	0.704	2.821	0.005	0.025	Yes
B vs. O	0.0146	0.0584	0.953	0.050	No

One Way Analysis of Variance abraded tissue comparison
Dependent Variable: **Abraded Mr2**

Group Name	N	Mean	Std Dev	SEM
H	60	88.476	2.044	0.264
B	60	88.359	1.808	0.233
O	60	88.796	1.835	0.237

Source of Variation	DF	SS	MS	F	P
Between Groups	2	6.162	3.081	0.855	**0.427**
Residual	177	638.061		3.605	
Total	179	644.223			

Appendix O – Post-erosion abrasion multiple linear regression

Forward Stepwise Regression:

Dependent Variable:Abraded surface DH
F-to-Enter: 4.000 P = 0.049
F-to-Remove: 3.900 P = 0.052

Analysis of Variance:

Group	DF	SS	MS	F	P
Residual	71	658.395	9.273		

Variables in Model

Group	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
Constant	4.815		0.359		

Variables not in Model

Group	F-to-Enter	P
Eroded DH max	22.778	<0.001
Eroded Ra	23.757	<0.001
MH eroded	99.024	<0.001
Eroded Rk	17.785	<0.001
Eroded Rvk	4.363	0.040
Eroded Pk	14.763	<0.001
Eroded Mr1	0.162	0.689
Eroded Mr2	0.0169	0.897

Step 1: **MH eroded** Entered
R = 0.765 Rsqr = 0.586 Adj Rsqr = 0.580
Standard Error of Estimate = 1.974

Analysis of Variance:

Group	DF	SS	MS	F	P
Regression	1	385.726	385.726	99.024	**<0.001**
Residual	70	272.669	3.895		

Variables in Model

Group	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
Constant	11.628		0.723		
MH eroded	-0.0214	-0.765	0.00215	99.024	**<0.001**

Variables not in Model

Group	F-to-Enter	P
Eroded DH max	6.264	0.015
Eroded Ra	4.265	0.043
Eroded Rk	5.889	0.018
Eroded Rvk	1.226	0.272

Eroded Pk	4.533	0.037
Eroded Mr1	0.287	0.594
Eroded Mr2	0.450	0.505

Step 2: **Eroded DH max** Entered
R = 0.788 Rsqr = 0.620 Adj Rsqr = 0.609
Standard Error of Estimate = 1.903

Analysis of Variance:

Group	DF	SS	MS	F	P
Regression	2	408.417	204.209	56.367	**<0.001**
Residual	69	249.977	3.623		

Variables in Model

Group	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
Constant	9.582	1.074			
Eroded DH max	0.606	0.205	0.242	6.264	**0.015**
MH eroded	-0.0189	-0.677	0.00229	68.115	**0.001**

Variables not in Model

Group	F-to-Enter	P
Eroded Ra	0.658	0.420
Eroded Rk	1.797	0.185
Eroded Rvk	0.00383	0.951
Eroded Pk	1.457	0.232
Eroded Mr1	0.0870	0.769
Eroded Mr2	1.651	0.203

The dependent variable Abraded surface DH can be predicted from a linear combination of the independent variables:

	P
Eroded DH max	**0.015**
MH eroded	**<0.001**

The following variables did not significantly add to the ability of the equation to predict Abraded surface DH and were not included in the final equation:
Eroded Ra Eroded Rk Eroded Rvk Eroded Pk Eroded Mr1 Eroded Mr2 .