

An in vitro evaluation of selective demineralised enamel removal using bio-active glass air abrasion

Avijit Banerjee · Hiten Pabari · George Paolinelis · Ian D. Thompson · Timothy F Watson

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Abstract Unnecessary over-preparation of carious enamel often occurs clinically during operative caries management. The working hypothesis to be investigated in this study is the potential for bio-active glass air abrasion to remove selectively only demineralised enamel in artificial enamel lesions when compared to equivalent alumina air abrasion, so potentially minimising cavity over-preparation. Bisected artificial, paired smooth surface enamel lesions on ethics-approved, extracted sound human molars were created and subsequently air abraded with 27 μm alumina ($n=19$) and bio-active glass ($n=19$). The difference between pre-operative lesion boundary and post-operative cavity margin was calculated following optical confocal fluorescent assessment of the lesion boundary. Data indicated mean% over-preparation (sound enamel removal) of 176% with alumina and 15.2% for bio-active glass ($p=0.005$). Bio-active glass abrasion removed completely the demineralised enamel from artificial lesions with clinically insignificant over-preparation of sound tissue, indicating technique selectivity towards grossly demineralised enamel. Alumina

air abrasion resulted in substantial enamel removal in both sound and demineralised tissues indicating the operator selectivity required to use the techniques effectively in clinical practice.

Keywords Enamel · Demineralisation · Air abrasion · Bio-active glass · Alumina · Minimally invasive dentistry

Introduction

Air abrasion is an operative technique used in dentistry since 1945 for the removal of sound and carious enamel and dentine during cavity preparation [1–3]. The abrasive powder commonly used intra-orally is 27 μm aluminium oxide (α -alumina) [4]. It has been reported that air abrasion units are capable of minimally invasive tooth preparation [5–7]. However, dentists are used to the parameters of tactile feedback and an appreciation of finite cutting depth when using rotary tooth-cutting techniques, both of which the end-cutting alumina air abrasive jet lacks. This makes the use of alumina air abrasion highly operator-sensitive and requires careful education of dentists to realise its potential for minimally invasive preparation and the prevention of cavity over-preparation [2, 8–10]. Studies have been published which characterise the efficacy of alumina air abrasion and its cutting characteristics on both sound and carious enamel and dentine and collectively these show the technique to be efficient if specific operating parameters (e.g., air pressure, powder flow rate and reservoir volume, nozzle diameter, and working distance) are regulated judiciously by the operator [11–15]. Clinical studies have indicated good patient acceptance of the technology, in terms of the lack of vibration, no heat generation, and the reduced need for local analgesia [8, 16].

A. Banerjee · G. Paolinelis · I. D. Thompson · T. F. Watson
Department of Biomaterials, King's College London Dental
Institute at Guy's, King's College and St. Thomas' Hospitals,
King's College London,
London, UK

A. Banerjee · H. Pabari · T. F. Watson
Unit of Conservative Dentistry, King's College London Dental
Institute at Guy's, King's College and St. Thomas' Hospitals,
King's College London,
London, UK

A. Banerjee (✉)
King's College London Dental Institute at Guy's Hospital,
Floor 26, Tower Wing, St Thomas' Street,
London SE1 9RT, UK
e-mail: avijit.banerjee@kcl.ac.uk

An important clinical use of air abrasion is obtaining suitable enamel access in minimally invasive cavity preparations. Meticulous cleaning of the occlusal surface prior to visual examination using a rotary brush or air polishing is essential for caries detection [17], followed by the use of a dental bur or alumina air abrasion for the removal of the carious, demineralised enamel. The microscopically roughened enamel surface created by alumina air abrasion is devoid of weakened prisms and is therefore better adapted for adhesive bonding. However, lack of substrate selectivity and no self-limiting operator feedback when using these operative technologies can result in cavity over-preparation. Following operative exploration and subsequent caries removal, an adhesive restoration may be placed, the retention and seal of which is dependent on a reliable bond to the underlying peripheral sound enamel surface. The aprismatic surface layer of enamel, however, might compromise this bond because of its resistance to etching [18].

The aim of this study was to assess the tissue selectivity for demineralised enamel removal using crushed, melt-derived bio-active glass powder (45S5: 46.1 mol% SiO₂, 26.9 mol% CaO, 24.4 mol% Na₂O and 2.5 mol% P₂O₅) air abrasion. Bio-active glasses, invented by Hench et al. [19], react with an aqueous solution where chemical and structural changes occur that result in the formation of hydroxycarbonate apatite surface layer and eventual dissolution of the powder particle. Bio-active glass (45S5) is used currently in the oral cavity as a bone-graft material (Bioglass®) and as a tooth cleaning, desensitising prophylaxis powder [20]. It is significantly softer than alumina: 420 Knoop hardness number (KHN) compared to 2,100 KHN for alumina [21, 22] and offers the potential for it to remove a softer substrate selectively, whilst leaving harder, histologically sound tissue intact [23, 24].

The objective of this *in vitro* study was to investigate the effect of bio-active glass powder air abrasion on artificial smooth-surface enamel lesions. The null hypothesis investigated was 45S5 melt-derived bio-active glass vs. alumina air abrasion of teeth resulted in no difference in the removal of sound or carious demineralised enamel.

Materials and methods

With Guy's and St. Thomas' Hospitals research ethics committee approval (04/Q0704/57), extracted human permanent molars were stored hydrated at 4°C no longer than 4 weeks after being cleaned using a pumice slurry on a rotating bristle brush and washed.

The roots of 19 extracted sound molars were sealed with red modelling wax and the teeth coated with an acid-resistant nail varnish (Night Shade-Fast Finish, Boots,

Nottingham, UK), leaving only a pair of 2×5 mm windows exposed opposite each other on the smooth buccal and lingual aspects of the coronal enamel ($n=38$). The teeth were submerged in 15 mL of an acidified hydroxy-ethyl-cellulose gel buffered to a pH of 3.5, within an airtight glass vial in an incubator at 37.5°C. The pH was monitored every 3 days using a pH metre (Hydrus 100, Fisherbrand, UK) and the solution was refreshed as required in order to maintain the pH<4. Histological microscopic analysis of hemisected demineralised samples in a pilot study assessing the demineralisation time required, confirmed a 21-day demineralisation protocol was sufficient to create suitably sized enamel lesions penetrating towards the middle/inner third of enamel but still leaving sound enamel at their advancing fronts, towards the enamel–dentine junction (EDJ). Following the creation of the artificially demineralised lesions, the teeth were plane sectioned bucco-lingually through the lesion pair using a water-cooled, diamond-coated blade at 100 rpm (Labcut 1010, Agar Scientific, Stansted, UK). Lesion allocation per tooth to either alumina and bio-active glass air abrasion experimental groups was randomised using the toss of a coin. Three fixed reference marks were cut just remote to the lesions (in enamel or dentine) using the tip of a fine tungsten carbide bur (0.5 mm fissure, Proxon, USA) in an air turbine handpiece. This permitted later image superimposition, before and after air abrasion. The varnished, sectioned teeth were submerged in 20 mL of 0.25% Rhodamine B solution (Sigma, Poole, UK) for 60 s and the emitted red fluorescent signal from the lesions examined using a tandem scanning confocal microscope (Noran Instruments, Middleton, WI, USA) with 546 nm excitation and >600 nm emission filters. Systematic adjustment of the microscope stage was required to image the whole lesion with a 5/0.01 objective lens (Nikon, Japan). Calibration of the analytical software (Lucida Analyse, Andor Technology, Northern Ireland, UK) was performed imaging a 1 mm² calibration slide.

A commercially available air abrasion unit (Abradent, Crystalmark, USA) using 60 psi air pressure, 90° nozzle with a 0.6-mm internal diameter and a tooth nozzle distance of 15 mm was used to abrade each lesion for 10 s initially. The details of this technique have been previously published [15]. The nozzle was then repositioned and the process repeated twice to cover the whole lesion, resulting in a total of 30 s abrasion for each lesion. The powder flow was adjusted to deliver the same rate (3 g/min) for both alumina (27 µm (range 10–55 µm), Crystalmark, USA) and bio-active glass powder (95%, 10–40 µm) groups. The resultant cavities were examined using the confocal microscope as described above in reflectance mode. Individual fields of view were stitched together using Adobe Photoshop Elements (Adobe Systems Inc. 2002, USA). The post-operative images were overlaid, aided by

the fixed reference bur marks on the reflection mode images, upon which the cross-sectional areas of the original lesions and post-operative cavities were mapped and recorded using a stylus pad (Adesso, California, USA) and Lucida Analyse software.

Statistical analysis was performed using Stata 4 (Stata Co, TX, USA). Descriptive analysis and a Wilcoxon-signed ranked test were carried out.

Results

The percentage difference in lesion size pre-operatively and cavity size post-operatively between the two abrasion groups are presented in Table 1. As the data was paired, a Wilcoxon-signed rank test was used to assess the statistical significance between the pre-operative lesion and post-operative cavity sizes for the alumina treatment group and the bio-active glass group separately, with *p* values of 0.002 and 0.005, respectively, indicating significant differences within each group. Statistical analysis between the two treatment groups indicated the paired percent excess tooth tissue removed was statistically significantly more in the alumina group than the bio-active glass group, with mean values of 176% vs. 15.2% (*p*=0.005).

Representative images of the pre-operative lesions and post-operative cavities can be seen in Figs. 1 (bio-active glass air abrasion) and 2 (alumina air abrasion). These examples indicate the representative over-preparation of the alumina group versus the appropriate preparation of the bio-active glass group. Figure 3 graphically depicts the raw

percentage increases in cavity size when compared to the original lesion, for all 38 lesions included in the study.

Discussion

The results from this study indicate that bio-active glass air abrasion selectively removes demineralised enamel better than equivalent alumina air abrasion (Fig. 4). The use of paired, bisected artificial lesions allowed the internally controlled quantification of enamel removal by alumina and bio-active glass air abrasion. A previously published technique was followed to limit the influence of the operating variables on the outcome of each air abrasion group [15]. These included controlling the air pressure, nozzle diameter, angle, and working distance, all of which have been shown to influence the effectiveness of the air abrasion procedure {11–15}. This study was designed to assess the differences offered by the two powders alone, proving the selectivity of bio-active glass air abrasion under controlled conditions and the potential for this technology to be used in a clinical setting, as a minimally invasive tooth preparation technology.

Table 1 shows that there were no significant differences in the original lesion sizes created for both experimental groups and that their range of depths were broadly equivalent. A 21-day demineralisation protocol was chosen to ensure lesion depths well into the middle/inner thirds of enamel, so emulating the clinical scenario where carious enamel may need to be excavated, whilst still maintaining healthy prisms at the advancing front of the lesions to

Table 1 Data showing the differences between original lesion and final cavity size between both experimental groups and the equivalent percentage increases

	Aluminium oxide air-abrasion (n=19)	Bio-active glass air-abrasion (n=19)
Pre-op lesion cross-sectional area (mm²) – median (IQR)	2.56 (1.21 - 3.44)	2.84 (1.89 - 3.68)
Post-op cavity cross-sectional area (mm²) – median (IQR)	5.89 (2.67 - 6.92)	3.01 (2.38 - 3.85)
% increase in cross-sectional area size – median (IQR)	111.3 (76.5 -141.6)	9.83 (5.7 – 18.1)

Shaded cells indicate statistically significant differences, pre- and post-operatively within each group (alumina, *p*=0.002; bio-active glass, *p*=0.005) as well as between the two groups (*p*=0.005)

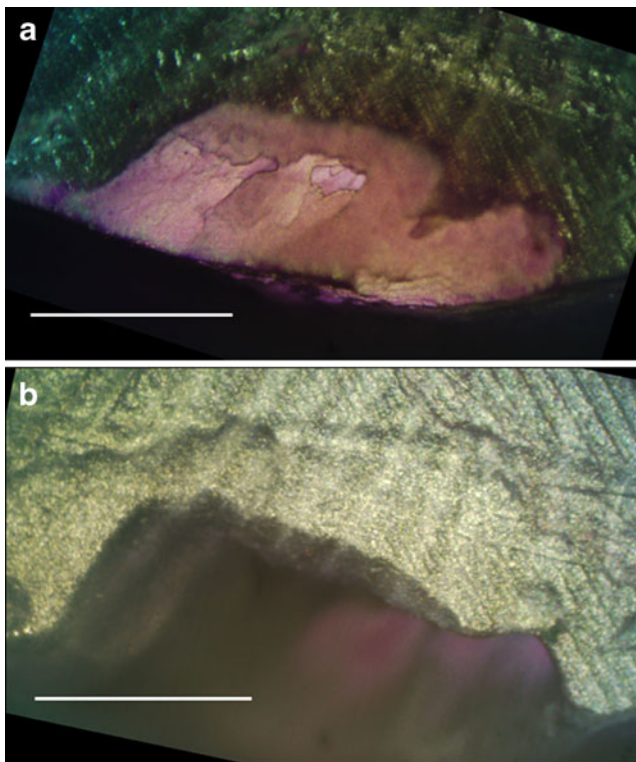


Fig. 1 **a** Photomicrograph in reflection mode of original artificial enamel lesion (white; scale bar 1 mm). **b** Lesion (**a**) after bio-active glass air abrasion for 30 s. Note how the rounded outline of the cavity is clearly demarcated and conforms to the original lesion boundary (scale bar 1 mm). The evenly distributed parallel lines running obliquely across the sample surface are the pattern of the saw cut when the samples were hemi-sectioned

enable detection of any cavity over-preparation. Initial experimentation indicated that a 30-s episode of air abrasion permitted complete removal of demineralised enamel using the slower bio-active glass air abrasion of 21-day-old lesions, whilst ensuring there would still be time to spare to potentially over-prepare the remaining healthy enamel cavity walls. Alumina air abrasion was then compared to this. It is intriguing to note that four teeth (numbers 8, 11, 14, and 15—Fig. 3) showed gross alumina over-preparation of the lesion when compared to the paired equivalent lesion using bio-active glass. In these cases, the artificial lesions were at the smaller end of the volumetric size spectrum and required a shorter clinical period of air abrasion to render them free from demineralised enamel than the standardised time period used in this study. This highlighted the problem of a lack of intrinsic tissue selectivity of alumina, creating over-prepared cavities well in excess of double their original lesion size whereas the bio-active glass abrasion was more minimally invasive in these cases.

The reason for the apparent selectivity of bio-active glass may be explained by the different physical properties of

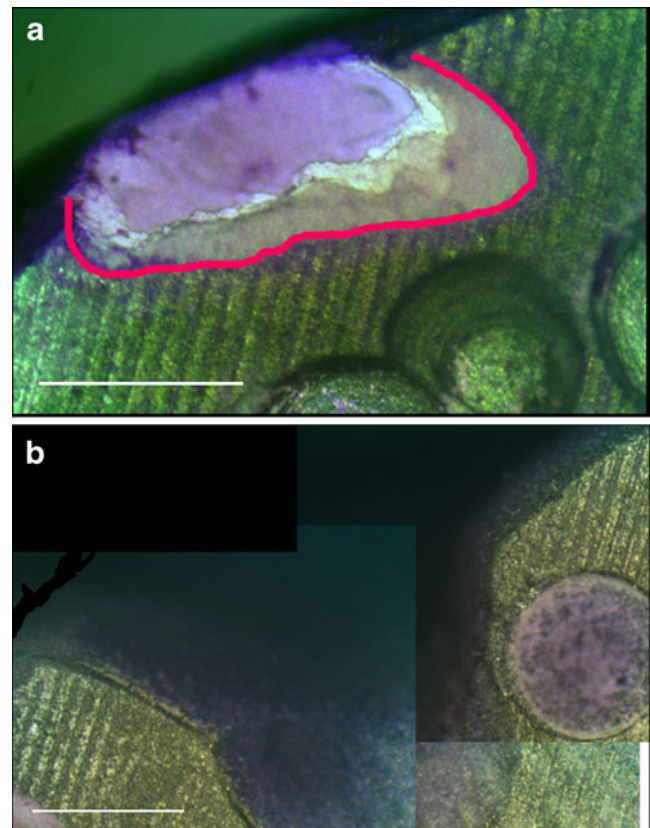


Fig. 2 **a** Photomicrograph of an artificially demineralised enamel lesion (white), the boundary of which has been highlighted in red. Note the three circular reference marks in the lower right corner of the field, cut into sound enamel away from the lesion using the tip of a fine bur (scale bar 1 mm). **b** The final cavity prepared using 30 s alumina air abrasion. Note the clearly significant over-preparation of the cavity in comparison with (**a**) (the partial far right reference mark in **a** only can be seen on this image) (scale bar 1 mm)

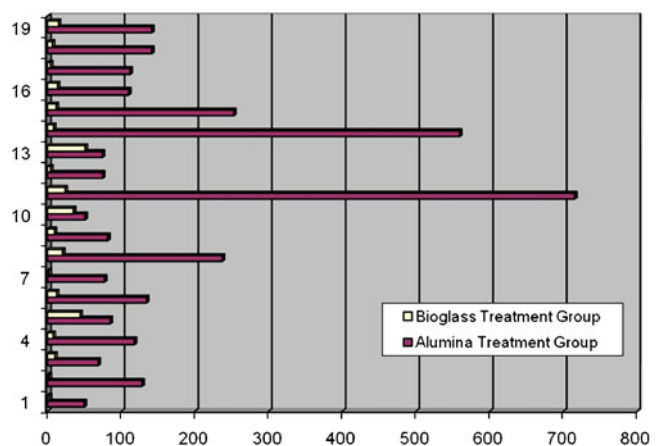
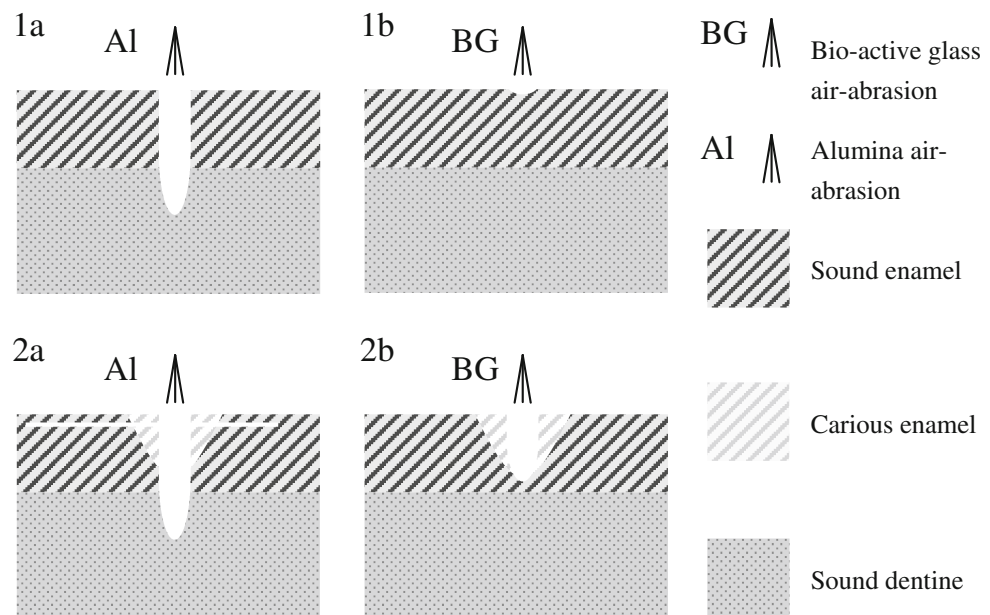


Fig. 3 Histogram highlighting the overall percentage increase in cavity size after 30-s air abrasion (x-axis) for each lesion created (y-axis) in both experimental groups. Note: a change in final cavity size of 10% translates to a clinical difference in depth of approximately 100 μ m. All paired lesions suffered significant lesion over-preparation in the hands of alumina air abrasion (IQR inter-quartile range)

Fig. 4 Diagram summarising the effects of alumina and bio-active glass air abrasion on sound and carious enamel and dentine following air abrasion. Alumina air abrasion will remove sound enamel and dentine (1a) indiscriminately, while bio-active glass air abrasion causes only surface modification of sound enamel (1b) for the equivalent abrasion time. Bio-active glass air abrasion removes carious enamel preferentially to sound enamel and dentine (2b), while alumina air abrasion does not (2a)



demineralised and sound enamel. The minimal organic content and brittle prismatic nature of enamel means that it is cut by subsurface cracking prior to detachment of a chip of the material. Demineralised enamel is more porous and softer than sound enamel so altering these mechanical properties. It is thought that the selectivity of the bio-active glass powder is due to the closer matching of the physical properties of the abrasive with those of the substrate. Horiguchi et al. [25] showed a similar effect in dentine using polyacrylic beads. The abrasives used in that study however, cannot be used clinically.

Air abrasion can produce conservative preparations because the end-cutting air-abrasive stream can negotiate a much narrower path through the enamel than the narrowest rotary bur. Operator judgement may be its limitation, however, as it is almost impossible to judge the quality of enamel at the depth of an ultraconservative, minimally invasive preparation. The use of bio-active glass air abrasion can significantly minimise the removal of sound enamel and offers a new, self-limiting technology for the clinician. Sound enamel is not removed at a rate quick enough to create a cavity in a clinical scenario. It is important to note that although data has showed that bio-active glass air abrasion does affect sound enamel to a degree, increasing the final cavity size by 9.83%, this clinically equates to an insignificant change of <100 μm within the cavity itself, a clinically indiscernible amount that would not affect treatment outcome. This implies that the doubtful occlusal fissure in a high caries risk individual where disease control and lesion prevention has not worked may be cleaned using bio-active glass air abrasion, removing the stain and debris, and following diagnosis,

explored and if necessary, minimally invasive cavity preparation carried out, restored with a sealant restoration (Fig. 4). Sound enamel would not be affected by this process apart from the surface stain and debris removal [20, 24].

Bio-active glass has been used in a range of soft and hard tissue applications for over 25 years. When applied to all oral soft tissues, the bioactive glass initially incorporates into the collagen structure aiding in soft tissue repair, but ultimately breaks down into its base components and is excreted. Dust inhalation has also been evaluated and shown to be non-toxic [26].

The rounded internal angles created by air abrasion (Figs. 1b and 2b) are ideal for resin restorative materials ensuring better marginal adaptation and reduction of voids [27]. The price to be paid for the advantages of bio-active glass air abrasion is that, because of its less aggressive cutting nature, when compared to alumina, it takes longer for cavities to be prepared. It is envisaged that in the dental surgery, the operator could use this tool both to aid detection of early fissure lesions and minimally invasively treat cavitated carious lesions in patients whose high caries risk status cannot be changed easily.

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