

# The Effect of Demineralization and Remineralization Cycles on the Bond Strength of Resin Composite to Enamel

## Keywords

Toothwear  
Erosion  
Enamel  
Demineralisation  
Remineralising Agent  
Shear Bond Testing

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## ABSTRACT

*Toothwear is a growing concern, particularly in young adults. Prevention strategies remain of utmost importance and may involve the use of remineralising agents. If restorative treatment is indicated, adhesive techniques are recommended. It is unknown whether remineralising agents affect adhesion. Method: 77 bovine incisors were subjected to simulated toothwear by erosion (0.3% citric acid) and abrasion (oscillating toothbrush). Samples were randomly arranged into 5 test and 2 control groups (n=11). Each test group had a remineralising agent applied. Subsequently, composite was bonded to each sample and subjected to shear bond testing. Shear bond strength, mode of failure and enamel surface changes were analysed. Results: There was no statistically significant difference for bond strength between groups (p=0.262). Mode of failure was statistically significant between groups (p<0.0001). Qualitative analysis showed a surface layer on samples remineralised by calcium silicate and stannous fluoride. Both groups had more adhesive failures. Conclusion: Within the limitations of this study, it can be concluded that the remineralising agents tested do not affect shear bond strength though surface layers created on the enamel influence the mode of failure. These 'sacrificial' surface layers have the potential to protect the underlying enamel structure.*

## INTRODUCTION

Toothwear in younger people is on the increase and becoming a growing concern.<sup>1</sup> A systematic review in 2015 of children and adolescents, estimated that 30.4% of people aged 8-19 years had signs of erosive toothwear.<sup>2</sup> Similarly, between 1998 and 2009, the UK adult dental health survey also showed an increase of toothwear in young adults (aged 16 to 24) from 35% to 50%. In addition, there was an increase in wear from 58% to 68% in 25-34 year olds.<sup>3</sup> There are a number of reasons why we may be seeing these increases. Firstly, the growing trend of healthy lifestyles and consumption of fruit drinks, nutrient waters and sports drinks.<sup>4</sup> It has also been identified that there is an increased intake of soft drinks and energy drinks in younger age groups.<sup>5</sup> Of these, the 5 most popular energy drinks in the UK have a pH ranging from 2.72 to 3.37 as the highest.<sup>6</sup> This is of concern, as consumption of these drinks risk a drop in intraoral pH. It has been proposed that the critical pH values for demineralisation of enamel, in an erosive challenge, ranges from 3.9 to 6.5<sup>7</sup>.

Received: 27.08.2020  
Accepted: 20.10.2020

doi: 10.1922/EJPRD\_2198Patel09

Whilst toothwear occurs as a natural process throughout life, pathologic tooth wear should be differentiated from physiological tooth wear. In one study, the rate of wear considered physiological was estimated at 25-38mm per year in premolar and molars respectively.<sup>8</sup> A separate study investigating the wear rate of permanent incisors, showed that the incisal length reduced by an average of 1.01mm over a 60 year period, from 11.94mm in a 10 year old to 10.93mm in a 70 year old.<sup>9</sup> Importantly, toothwear is cumulative throughout life and therefore, to establish if the degree of tooth wear is physiologic or pathologic, the patients' age should be taken into account. It is deemed pathological toothwear if the wear is greater than expected for the patient's age.<sup>10</sup>

Pathologic toothwear resulting in severe tooth structure loss is multifactorial, though acid erosion is considered to play a major role.<sup>11</sup> The source of the acid may be either intrinsic or extrinsic or in some instances, both. The process of dental acid erosion is complex and not yet fully understood.<sup>12</sup> Acids are first required to penetrate the acquired pellicle prior to dissolving the outermost enamel crystals. Once this occurs, the outer softened enamel becomes more vulnerable to friction such as toothbrushing<sup>12</sup> or bruxism.<sup>1</sup> These types of toothwear are described as abrasion and attrition, respectively.

Restorative management of toothwear may be driven by the patient and can include situations where there may be symptoms or aesthetic concerns. The clinician may also take a leading role when the toothwear is more advanced and further loss of tooth structure may complicate restorative treatment.<sup>13</sup> Nevertheless, the push towards minimally invasive strategies for restoring patients with toothwear is well documented.<sup>14-16</sup> Minimally invasive treatment uses adhesive techniques and a defect orientated restoration. Consequently, composite resin has been considered a material of choice.<sup>15,17,18</sup> Furthermore, it has been demonstrated that the use of composite resin has favourable outcomes.<sup>17-19</sup> Though retention of the material is dependent on adhesion to good quality dental enamel as this has been shown to provide the most durable and reliable bond.<sup>20</sup>

Bonding of composite resin to enamel relies on micromechanical retention between the enamel surface and the resin material. The pre-treatment of enamel using a phosphoric acid etchant results in selective dissolution of hydroxyapatite crystals to a depth of 10-40mm.<sup>21</sup> The resultant is an enhanced enamel surface topography due to the differing angulations of the enamel prisms at a surface level, leaving some areas more readily demineralised compared to others creating microroughness.<sup>22</sup> The resultant is a morphologically porous layer of 5-50mm and a surface that is more favourable for bonding and which a resin can infiltrate.<sup>23</sup> Consequently, any adhesive approach benefits from an optimal enamel surface in both quality and quantity.

As part of a management plan, prevention strategies remain of utmost importance and play a pivotal role when treating toothwear, particularly where early pathological wear is noticed.<sup>24</sup>

For many years, fluoride has been considered important for prevention of toothwear. There are many different types and concentrations of fluorides and these are available in different forms such as mouthwashes, toothpastes, gels and varnishes. Additionally, there are also non fluoride product options available, though the data of their efficacy is sparse.<sup>25</sup> The challenge for the clinician is choosing the right medicament for each patient and having confidence that these are effective. Furthermore, the clinician needs to be assured that the product does not have any impact on any future restorative treatment, particularly when enamel adhesion is required.

Fluoride application encourages remineralisation of enamel due to its lower solubility compared to calcium phosphate phases, resulting in fluorapatite formation. This is more stable at a lower pH compared to hydroxyapatite, giving a more resilient enamel surface to acid attack.<sup>26</sup> This results in less demineralisation. The fluoride ions adsorb onto the crystal surface offering direct protection.<sup>27</sup>

Various fluoride concentrations have been investigated where it has been concluded that the use of highly concentrated preparations such as 5000ppm in intensive regimes has been of benefit when aiming to remineralise tooth structure.<sup>28</sup> The result of this is a more acid resistant surface to demineralisation and abrasion compared to a conventional fluoride toothpaste containing 1450ppm. Despite this, fluoride will not prevent erosion in its entirety.<sup>28</sup> In addition, there are several different formulations of fluoride such as sodium fluoride and stannous fluoride. Fluoride compounds can also be combined with others such as calcium silicate in an attempt to improve its effectiveness.<sup>29</sup>

Sodium fluoride is most commonly available in regular toothpaste. A sodium lauryl sulphate (SLS) free preparation is also available in combination with potassium nitrate. It has been suggested that SLS can inhibit fluoride uptake by reducing the amount of fluoride deposited on the surface.<sup>30</sup> Consequently, the lack of SLS may therefore, increase the bioavailability of fluoride. *In vitro* studies have demonstrated the effectiveness of this formulation at protecting against acid erosion using an acid-erosion model, though these were compared to casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and fluoride free toothpaste.<sup>31,32</sup> The potassium nitrate is included to reduce sensitivity that may be a result of pathological toothwear.

Stannous fluoride preparations have also been recommended.<sup>25</sup> Stannous fluoride reacts with hydroxyapatite forming precipitates  $\text{CaF}_2$ ,  $\text{Sn}_2\text{OHPO}_4$ ,  $\text{Sn}_3\text{F}_3\text{PO}_4$ , and  $\text{Ca}(\text{SnF}_3)_2$ .<sup>33</sup> The tin ions form a barrier resulting in an enamel surface that is more resistant to acid erosion when compared to pure  $\text{CaF}_2$ .<sup>34</sup> Furthermore, this deposited tin barrier is also able to attach to the pellicle surface and may stay for several hours. Consequently, stannous fluoride is perceived to have greater ability to protect against erosion of dental enamel when compared to sodium fluoride solution.<sup>35</sup>

CPP-ACP is an alternative, non-fluoride product demonstrated to reduce demineralisation and promote remineralisation of enamel.<sup>36</sup> CPP stabilises high concentrations of calcium and phosphate ions with fluoride ions at the tooth surface by binding to the pellicle and plaque. The ions are then freely enabled to diffuse down concentration gradients thereby promoting remineralisation.<sup>37</sup> The high levels of calcium and phosphate ions prevent the precipitation of calcium and phosphate by their supersaturation within saliva.<sup>38</sup>

A novel toothpaste launched in 2014 has the active ingredients of calcium silicate and sodium phosphate salts (monosodium phosphate and trisodium phosphate) with 1450ppm sodium fluorophosphate.<sup>39</sup> It has been proposed that a rapid release of calcium occurs which results in a calcium silicate deposition on the enamel surface. As a result, a network, upon which a layer that is comparable to hydroxyapatite with respect to crystal structure and chemical composition forms.<sup>40</sup> From *in vitro* studies, the resultant hydroxyapatite layer that forms on enamel has been measured at 0.25-0.35mm after one day of incubation in artificial saliva. The thickness increased to 1.7-1.9mm after 7 days of incubation.<sup>41</sup> The surface hardness has also been shown to increase significantly over time in bovine samples when this toothpaste was applied.<sup>39</sup>

The use of agents to promote remineralisation is important in the initial phases of managing a patient with toothwear. The purpose is to reduce the ongoing loss of tooth structure, though this phase is often continuous and maintained long term. When restorative treatment is provided, this should be carried out using a minimally invasive approach utilising adhesive techniques.<sup>42</sup> The predictability of such methods involves reliance on a good quantity and quality of enamel to provide an ideal substrate for bonding. This may be affected by the application of different remineralising agents onto the tooth surface.

The aim of this study was to, therefore, investigate the effect of remineralising agents on the composite resin bond strength to bovine enamel and mode of failure following simulated toothwear. In addition, to determine whether there are surface changes to the enamel following remineralisation.

The null hypotheses were: a) there is no difference between the shear bond strength of composite resin on bovine enamel that have been put through acid demineralisation and abrasion followed by remineralisation; b) there is no difference in the enamel structure following acid demineralisation and abrasion and; c) there is no difference in the mode of failure following shear bond testing between groups.

## MATERIALS AND METHODS

This study used 77 mandibular bovine lower incisors acquired from a local abattoir. The crowns were stored in 1% chloramine-T trihydrate solution for 5 days and kept in and distilled water thereafter at 4° as recommended by ISO 11405:2015.<sup>43</sup>

The lower incisors were encased in epoxy resin mixed in accordance with the manufacturer's instructions at a ratio of 7:1 by weight. The labial surface was exposed at the surface. The resin was left for 24 hours to set. The samples (n=77) were polished flat, remaining within the enamel, using 500 and 1000 grit paper.

Figure 1 provides a flowchart of the experimental process. Initially, eleven samples were randomly selected and assigned to the negative control group (group 7). The remaining samples underwent an erosion-abrasion cycle. Samples were placed into a warmed 0.3% citric acid solution at 37° which was agitated for 10 minutes. The pH of 2.6 was monitored throughout using a pH probe. Each sample was rinsed with and stored in distilled water.

Simulated abrasion involved mounting each sample onto a jig placed on top of an electronic weighing scale. An oscillating electric toothbrush was held by a clamp and stand and positioned to apply a force of 200g. Each sample was brushed for 2 minutes with a slurry of non-fluoridated toothpaste in a 3:1 (dentifrice:distilled water) ratio. Each sample was rinsed and placed back into distilled water. The cycle was carried out three times a day for 5 days in total.

The samples were then randomly allocated to groups 1-6, with 11 per group (n=11). Group 6 (positive control) received no further treatment and was stored in artificial saliva in an incubator at 37°.

The samples from groups 1-5 were subjected to the remineralisation process. Application of the remineralising agents was carried out as per the manufacturers' recommended instructions. Table 1 lists the groups, the remineralising agent and its active ingredient. Prior to application of the remineralising agent, samples were rinsed with distilled water. In between applications, the samples were stored in artificial saliva in an incubator at 37°. The artificial saliva was prepared as described by McKnight-Hanes<sup>44</sup> and changed daily. This phase lasted a total of 10 days.

Ten samples from each group were then randomly selected and prepared for composite bonding. The samples were removed from the artificial saliva, rinsed with distilled water and dried. Each sample was etched with 40% phosphoric acid for 15 seconds, thoroughly rinsed and dried. The bonding agent was applied as per the manufacturer's instructions. A jig made from clear silicone enabled packing of composite onto the incisal enamel. This resulted in a 3.4mm diameter composite resin cylinder being bonded to the incisal third of each sample in preparation for shear bond testing, creating a bonding area of 9.08mm<sup>2</sup>.

The shear bond testing was carried out using a Shimadzu universal material testing machine. The specimens were loaded with the flat edge placed against each sample. The stroke was set to 1mm/minute. The maximal force required to shear the composite resin off the bovine enamel was recorded. This was determined by recording the maximal force required to shear the composite resin sample from the bovine enamel.

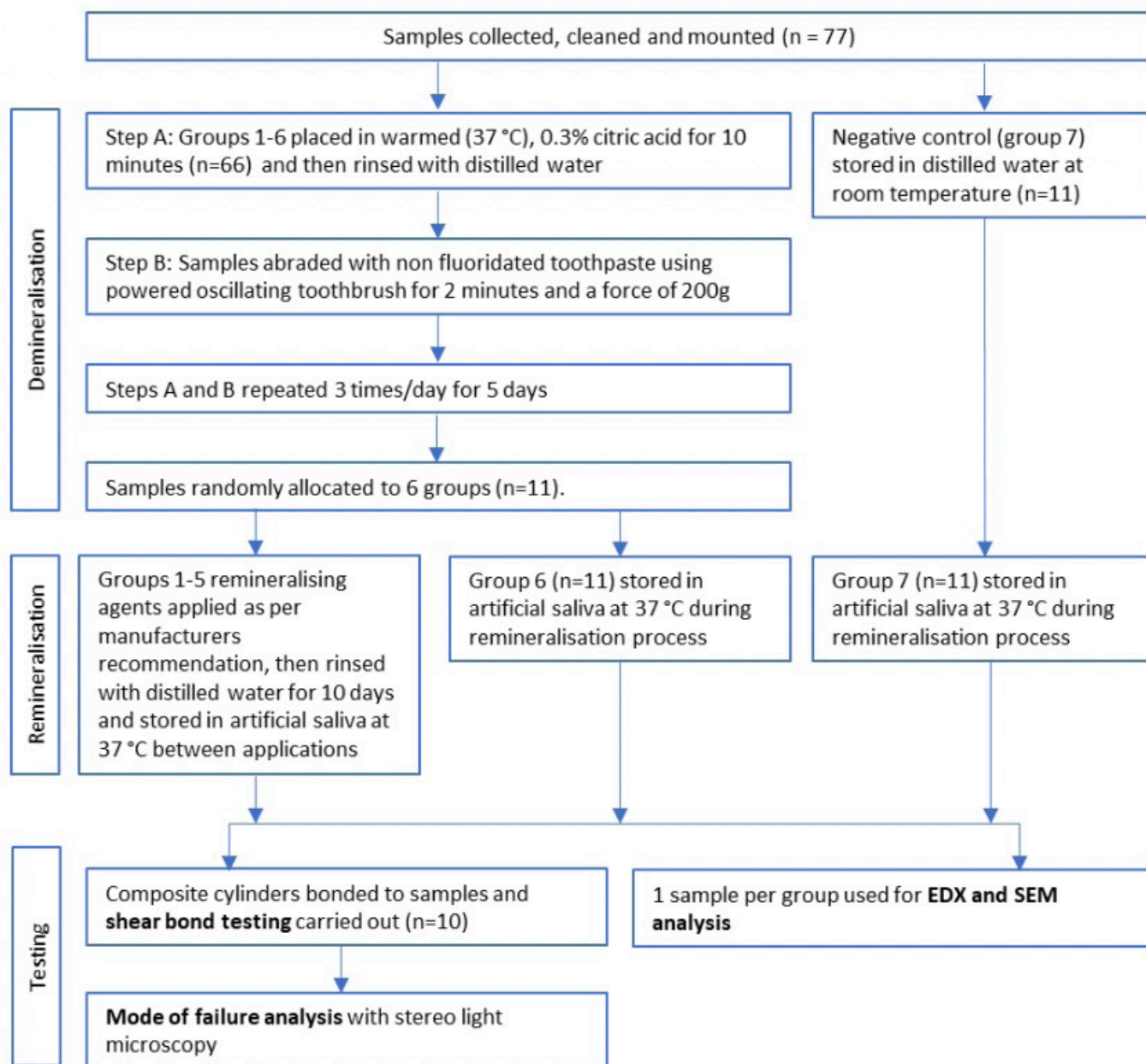


Figure 1: Diagrammatic representation of the workflow during the experimental phase of the study

Table 1: Test groups with the corresponding remineralising agent and active ingredient

Group	Product	Active ingredient
1	GC Tooth Mousse™	CPP-ACP: Casein-phosphopeptide amorphous calcium sulphate
2	Colgate® Duraphat® 5000	Sodium fluoride (5000ppm)
3	Oral-b Pro Expert Gum and Enamel Repair	Sodium hexametaphosphate, stannous fluoride (1100ppm), sodium fluoride (350ppm)
4	Regenerate™	Calcium silicate, monosodium phosphate, trisodium phosphate, sodium monofluorophosphate (1450ppm)
5	Sensodyne® Pronamel®	Potassium nitrate (5%), sodium fluoride (1450ppm)

Each sample was then viewed using a stereo light microscope at 10x magnification to classify the mode of failure. Mode of failure analysis categorised the fracture site as adhesive, cohesive or mixed failure. Adhesive failure involved clean shearing of the composite from the enamel or less than 10% composite remaining, cohesive failure was noted when the fracture was within the composite resin and 100% was remaining on the tooth surface and mixed failure was categorised as more than 10% composite resin, but less than 100% left on the surface. Any sample which was determined to have cohesive failure was excluded from the study.

The sample from each group not used in the shear bond test was prepared for scanning electron microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) by applying gold-palladium (80:20) sputter in a vacuum and mounted on an aluminium stub. The samples were viewed at 1000x and 5000x magnification to evaluate the effect of treatment on the surface of the enamel.

Analysis of the detectable elements upon the enamel surface using EDX analysis was carried out using INCA Suite version 4.15.

## STATISTICAL METHODS

The results from the shear bond strength test were input into SPSS® software for analysis. The shear bond strength data was not normally distributed and thus shear bond strength was summarised by medians, minimum and maximum values. Non-parametric Kruskal Wallis test was used to compare their distributions. The non-parametric Mann-Whitney *U* test was used to compare the distributions of shear bond strength in the two control groups.

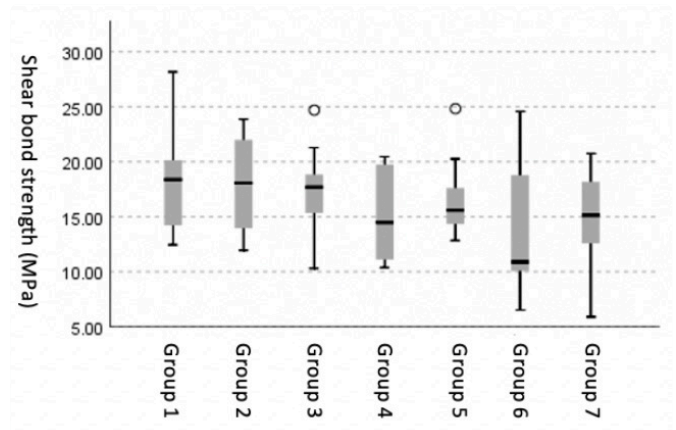
Contingency tables were created, and Fisher's exact test was used to compare the mode of failure in the different groups. The significance level was set at 0.05 for all significance tests apart from when multiple pairwise comparisons between groups were performed for the mode of failure. In this instance, a significance level of 0.01 was used to avoid spuriously significant results arising from multiple testing.

## RESULTS

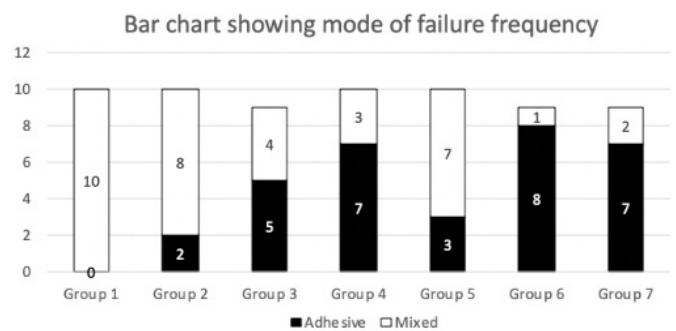
One sample from groups 6 and 7 recorded zero for the shear bond test. One sample from group 3 had full cohesive failure when viewed under a stereo light microscope. Consequently, the statistical analysis was carried out with only 9 samples in those groups.

The median shear bond strength across all groups is illustrated in Figure 2. Statistical analysis showed no statistically significant difference between test or control groups. Subsequently, the null hypothesis that there is no difference in the shear bond testing across the groups is therefore accepted.

Figure 3 illustrates the mode of failure between the different groups. There was no statistically significant difference between the mode of failure between the two control groups



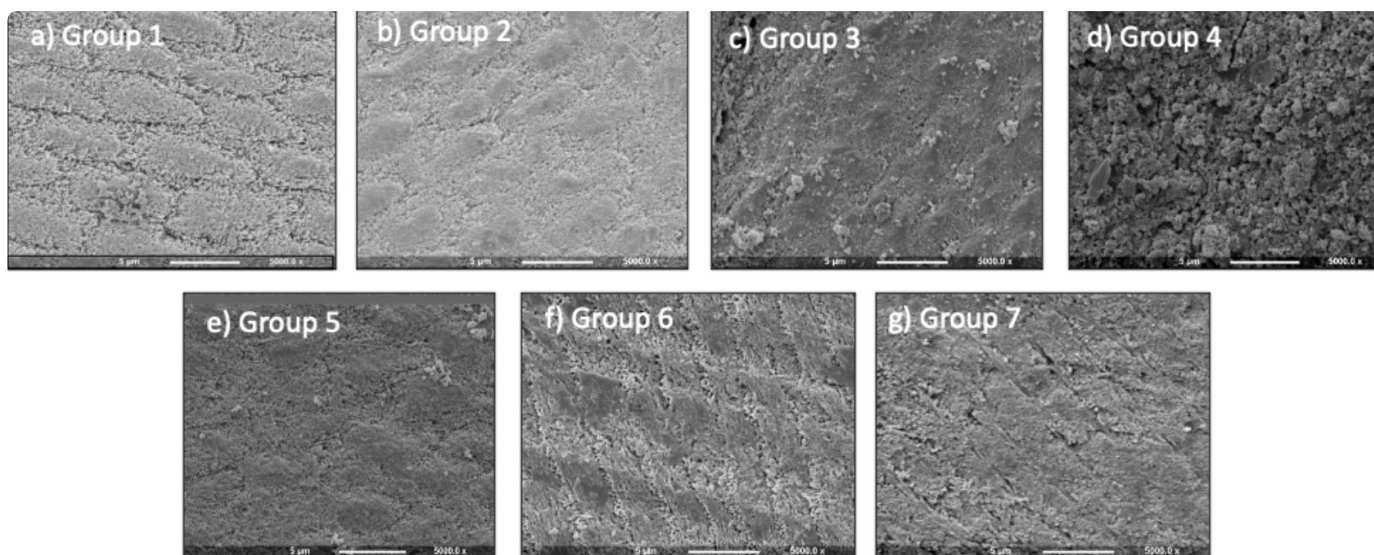
**Figure 2:** Box plot showing the median shear bond strength values. The horizontal black line within each bar represents the median shear bond strength. The highest and lowest values are represented by the vertical black lines. The circles in Group 3 and 5 represent outliers.



**Figure 3:** Graph showing the frequency of failure mode within each group. Failure was defined as either adhesive, mixed or cohesive. Only one sample showed a total cohesive failure in Group 3 which was eliminated from the statistical analysis. A single specimen from groups 3, 6 and 7 were eliminated leaving 9 specimens.

( $p > 0.999$ ). Group 3 and 4 had more samples fail adhesively than mixed. In contrast, groups 1, 2 and 5 had more mixed failures. Analysis of all groups with the mode of failure showed a statistically significant difference ( $p < 0.001$ ). The null hypothesis that there is no difference in the mode of failure following shear bond testing across the groups is therefore rejected.

Qualitative analysis was carried out using scanning electron microscopy images at 5000x (see Figure 4) and 1000x magnification. Groups 1, 2, 5, 6 and 7 showed an enamel surface with prisms clearly visible, comparable to etched enamel. Group 3 and 4 showed surface changes suggestive of a layer forming. EDX analysis was carried out for all groups. Groups 1, 2, 5, 6 and 7 showed peaks for calcium (Ca) and phosphorus (P). In comparison, group 3 and 4 showed a signal not only for Ca and P, but also tin (Sn) (see Figure 5) and silicon (Si) respectively. The null hypothesis that there is no difference in the enamel structure following a demineralisation-abrasion cycle and application of a remineralising agent is therefore rejected.



**Figure 4:** SEM micrograph of each group following simulated toothwear and remineralisation viewed at 5000x, a) Group 1 – remineralised by CPP-ACP showing a clear prism structure of the enamel, b) Group 2 – remineralised using 5000ppm NaF, with enamel prisms visible, but slightly less defined than group 1, c) Group 3 – remineralised by SnF showing a less clearly defined prism structure with a deposit on the enamel surface, d) Group 4 – remineralised by calcium silicate with a precipitate on the surface covering the prism structure, e) Group 5 – remineralised with SLS free NaF showing the outline of enamel prisms, f) Group 6 – positive control – visible etching pattern, g) Group 7 – negative control with prism outline but largely intact.

## DISCUSSION

Patients often go through a preventative phase prior to any restorative treatment carried out in the management of toothwear. Preventative regimes will involve the use of remineralisation agents in an attempt to harden the tooth surface through remineralisation and increase the resistance to acid erosion. This may influence the surface structure of dental enamel, which is relied upon during restorative treatment with a minimally invasive, adhesive philosophy.

For this study, the choice of remineralising agents used were those that have been advised by dental professionals and marketed by pharmaceutical companies to prevent and treat loss of enamel by dental erosion.

The highest recorded median shear bond strength was within group 1. In addition, specimens from this group also had the highest shear bond strength recorded. Another *in vitro* study has also demonstrated that the use of CPP-ACP to remineralise enamel over time enhances the bond strength between enamel and composite.<sup>45</sup> A possible explanation for this is due to the mode of action of CPP-ACP whereby there is an increased concentration of calcium and phosphate, resulting in a supersaturated surface preventing the precipitation of these ions from the enamel. The resultant is a more favourable substrate to bond to.

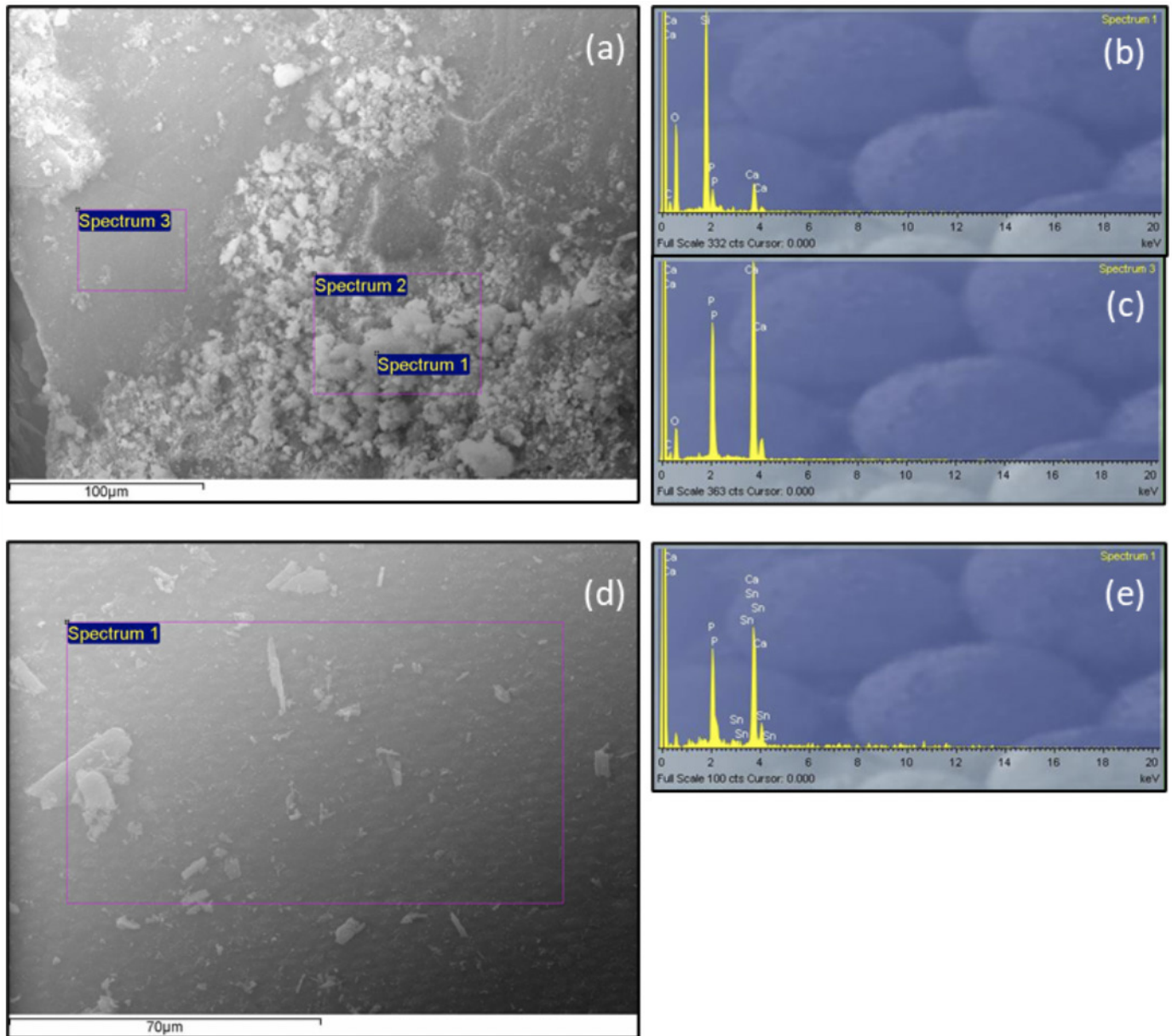
The lowest recorded median shear bond strength was within group 6 (positive control). When compared to the experimental groups, this may be due to the wear and reduced protection of the enamel substrate which was not remineralised with an active agent. When compared to the negative control (group 7) the action of an erosive challenge followed by abrasion

results in an increased loss of hydroxyapatite crystals. Despite there being a difference in the median bond strength across these two groups, it was not statistically significant. Alteration in the enamel surface can affect resin tag formation and bond strength.<sup>46</sup>

In this study, despite there being a difference in the median shear bond strength between the groups, this was not found to be statistically significant. This may have been due to the limited sample size. In addition, there were a number of mixed failures within the samples tested. It has been proposed that 30 adhesive failures are necessary to estimate shear bond strength,<sup>47</sup> therefore a higher sample size may have mitigated this limitation.

The mode of failure analysis showed that groups 3, 4, 6 and 7 had the most adhesive failures, whilst groups 1, 2 and 5 had more mixed failures. Variations in the enamel surface can affect the mode of failure.<sup>46</sup> When visualised under SEM, groups 1, 2 and 5, despite being exposed to remineralising agents, had a similar surface appearance to that of etched enamel.<sup>48,49</sup>

In contrast the stannous fluoride (group 3) and calcium silicate (group 4) based remineralising agents resulted in a surface layer (see Figure 4c, 4d and 5) consistent with results from other studies.<sup>39-41,50,51</sup> It is possible that these surface layers affect the action of phosphoric acid by limiting the penetration of the acid etch on the enamel surface compromising the optimal formation of resin tags, typical of enamel bonding, required to form adequate micromechanical retention. Alternatively, the different elements present on the surfaces (Ca, Si and Sn) may affect the surface solubility and chemistry, affecting the formation of an optimal adhesive layer. Consequently, this may have contributed to the more



**Figure 5:** a) EDX analysis of enamel surface following remineralisation with calcium silicate, b) Spectrum 1 and 2 show peaks of Si, Ca and P where precipitate layer is present, c) Spectrum 3 show a protected region and shows no evidence of a surface layer with peaks of Ca and P, d) surface following remineralisation by stannous fluoride, e) The peaks show a presence of Sn, P and Ca.

adhesive nature of failure within these groups. The resilience of these surface layers formed is unknown. Further research into how they may affect the acid etch process would prove useful when utilising adhesive techniques. Further analysis with fractographical analysis may give further understanding into the failure mechanism.

Within the limitations of this study, there appears to be no difference in how the remineralising agents impacted on the shear bond strength of composite to enamel. However, given that the calcium silicate dentifrice and stannous fluoride containing toothpaste demonstrated the formation of a surface layer, these products may be advocated for a purely prevention approach. Potentially, the capacity to produce a surface layer during a prevention phase of treatment could be utilised as a 'sacrificial' layer. Both remineralising agents have been shown to have their deposit layer resistant to rinsing.<sup>40,50</sup>

Whilst the overall integrity, resilience and the strength of this layer is still unknown, interestingly, in this study, it formed after only twenty applications over a ten-day experimental process. It is yet to be seen how this layer will develop over time with repeated demineralisation attacks and remineralisation cycles and to determine the robustness of this layer. A further unknown includes whether there is a peak duration of application which would result in the optimum surface topography and characteristics to give the desired properties, after which the layer does not develop further.

Correspondingly, if restorative management follows a preventive phase, one of the products which does not provide a superficial layer such as CPP-ACP or sodium fluoride may be more appropriate to ensure maximal effectiveness of enamel etching may provide a more favourable outcome.

## CONCLUSIONS

Within the limitations of this study it can be concluded that:

- The tested remineralising agents do not affect the shear bond strength of composite resin to bovine enamel when subjected to a demineralisation-abrasion-remineralisation cycle.
- A precipitate layer is produced on the surface of enamel after the application of a calcium silicate based remineralising agent or the application of stannous fluoride. This layer could be regarded as a sacrificial layer and play a role in prevention against acid erosion, though further research is needed.

## ACKNOWLEDGEMENTS

Dr Nicky Mordan and Dr George Georgiou for their invaluable help with imaging and sample preparation. Dr Aviva Petrie for help with the statistical analysis.

## MANUFACTURER'S DETAILS

- GC Tooth Mousse™ (GC, Japan)
- Colgate® Duraphat® 5000 (Colgate-Palmolive, UK)
- Oral-b Pro Expert Gum and Enamel Repair (Procter and Gamble, UK)
- Regenerate™ (Unilever, UK)
- Sensodyne® Pronamel® (GlaxoSmithKline, UK)
- Epoxy resin (Struers Resin, Struer, Denmark)
- Polishing – Struers Polisher
- Non-fluoridated toothpaste (Aloe Dent Whitening Toothpaste, Optima Naturals, Italy)
- Bonding agent – Optibond FL (Kerr™, USA)
- Clear silicone (Memosil 2, Kulzer, Germany)
- Composite resin (Gradia® direct, GC, Tokyo)
- SEM – Philips XL-330 FEG

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