Evaluation of Remineralization of Artificial White Spot Lesions on Teeth Treated with Bioactive Glass Air Abrasion via SEM-EDX

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INTRODUCTION

Patients undergoing orthodontic treatment have greater difficulty maintaining adequate oral hygiene.[1] There is an increased tendency for plaque accumulation around the brackets creating an environment conducive to growth of acid-forming bacteria.[2] This acid subsequently results in the demineralization of the enamel resulting in WSLs.[4] In severe cases, restorative treatment may be necessary after orthodontic treatment.

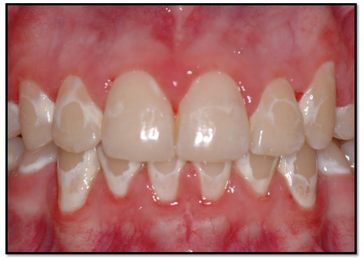

Fig. 1 White spot lesions post-orthodontic treatment as a result of acidogenic bacteria

Ever since the introduction of bioactive glass into the market, researchers have attempted to incorporate it into various types of restorative materials like adhesives and resin restoratives.[3] Researchers have demonstrated that they are capable of devising a formulation for orthodontic adhesive incorporating bioactive glass that has clinically accepted bond strength while simultaneously increase the local pH.[3] By increasing local pH, this bioactive glass resin has shown promise in mitigating the damage done by acid-forming bacteria and creating an environment more conducive to remineralization.[3-4]

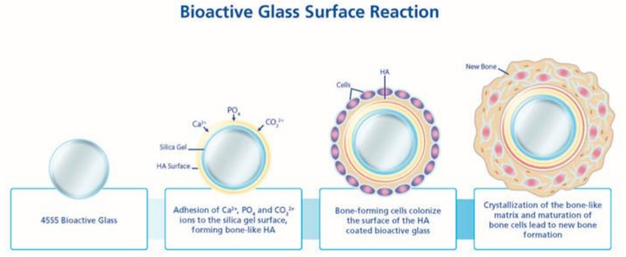

Fig. 2 Mechanism of bioactive glass induced bone regeneration[5]

MATERIALS

- 40 Extracted Human Third Molars
- Chemicals (Acetic Acid, Calcium Chloride, Potassium Chloride, Potassium Hydroxide, Potassium Phosphate, Sodium Phosphate)
- 45S5 Bioactive Glass
- Inert Epoxy
- Diamond Polishing Paste
- Air Abrasion Unit
- Water Bath
- Low Speed Saw
- Variable Speed Grinder-Polisher
- Sputter Coater
- SEM-EDX
- Electron Probe Microanalyzer
- Scanning Electron Microscope

METHODS

Preparation of Samples

1. 40 extracted human third molars were randomly divided into 4 groups (n=10/group)
   1. Group 1 (Control), Group 2 (Demineralized), Group 3 (Air Abrasion), Group 4 (Remineralized)
2. Group 1 was stored in Remineralization solution made of 1.5 mM calcium chloride (Sigma-Aldrich, US), 0.9 mM sodium phosphate (Sigma-Aldrich, US) and 0.15 M potassium chloride (Sigma-Aldrich, US) adjusted to a pH of 7.0 at intraoral temperature of 37°
3. Group 2-4 were demineralized in 2.2. mM calcium chloride, 2.2 mM monopotassium phosphate (Sigma-Aldrich, US), 0.05 mM acetic acid (Sigma-Aldrich, US) adjusted to a pH of 4.4 using 1 M potassium hydroxide (Sigma-Aldrich, US) at intraoral temperature of 37° C for 96 hours in a laboratory waterbath (Fisher-Scientific, US).
4. Group 3-4 were then treated with bioactive glass (Velopex, UK) air abrasion.
   1. Air abrasion was delivered at 2.6 bars at ¼ media output and ¾ water output from a distance of 5 mm via an Aquacare air abrasion unit (Velopex, UK).
   2. 2 passes over treated surface Δ5 seconds.
5. Group 4 was finally stored in remineralization solution consisting of 1.5 mM calcium chloride, 0.9 mM sodium phosphate and 0.15 M potassium chloride adjusted to a pH of 7.0 at intraoral temperature of 37° C for 7 days.

Scanning Electron Microscope

1. Forty enamel specimens were prepared one from each of the extracted molars.
2. Cut via low-speed diamond discs (Buehler, US) and refined with carbide burs (Komet, US) to 4 mm x 3 mm x 1 mm pieces.
3. Specimens were then gold coated for SEM-EDX to allow for electron dispersion. Gold coating was done via a Cressington Sputter Coater 108 Auto (Cressington Scientific Instruments, UK).
4. SEM-EDX was carried out via a Jeol JSM-5600 Scanning Electron Microscope (Jeol, USA). Measuring time was 60 seconds at resolution of 4.954 keV.
5. Elemental mapping was carried out in 1024x800 pixel matrix with 512 frames using INCA Microanalysis Suite (Oxford Instruments, UK). A 1 mm x 1 mm area analysis was done on each specimen.

Electron Probe Micro-Analyzer

1. One extracted human third molar from the demineralized and remineralized group each were selected
2. Samples were mounted in inert epoxy (Buehler, US)
3. Samples were bisected via low-speed diamond discs (Buehler, US)
4. Samples were verified under light microscopy for smooth polish
5. Carbon coating was done with a Denton DV-502A Vacuum
6. Samples were mounted into a JXA-8900R Electron Probe Micro-Analyzer (Jeol, US)
7. Configuration was set for analysis of Ca, P, F and Si
8. Analysis was ran for 20 hours to complete mapping

Fig. 3 Aquacare Air Abrasion Handpiece (Velopex, UK)

Fig. 4 JXA-8900R Electron Probe Microanalyzer (Jeol, USA)

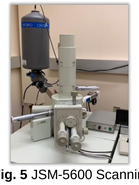

Fig. 5 JSM-5600 Scanning Electron Microscope(Jeol, USA)

RESULTS AND DISCUSSION

A statistically significant amount of remineralization was detected between the remineralized group and demineralized group, (t18)=-2.441, p=0.025.

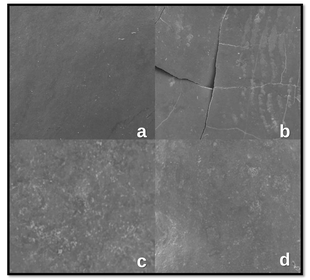

Fig 6: SEM images of various samples (1 mm by 1mm images) a. Control, b. Demineralized, c. Air Abrasion, d. Remineralized

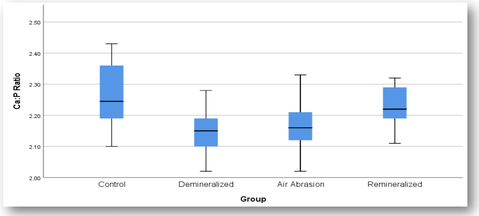

Table 1: Boxplot of Ca:P content by groups: Control, Demineralized, Air Abrasion, Remineralized. The average ratio decreases with demineralization but increases through the course of treatment.

Bioglass air abrasion treatment was able to remove thin layer of demineralized enamel. However, the Glass beads ranging from 30-90 microns could not be retained on the surface of the enamel. Because of the mechanism by which bioactive glass functions; the inability for retention therefore means that delivery via air abrasion is an ineffective means for initiating remineralization.

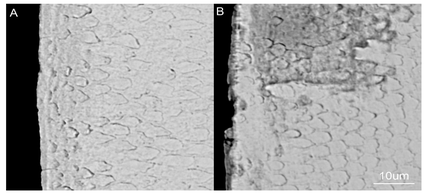

Fig 7: EPMA images of Enamel Specimens. A. Remineralized, B. Demineralized

However, as a coincidental finding, teeth treated with air abrasion were noted to be less susceptible to enamel fracture after the desiccation process necessary for microscopy. The additional therapeutic effect if curious and may have clinical value.

CONCLUSION

While remineralization did occur during our study, it did not seem to be a direct result of bioactive glass treatment. The enamel remineralization potential via air abrasion delivery does not seem to be significant due to its inability to be retained on the surface of enamel. Remineralization seen was more a result of the natural process and removal of a thin demineralized layer.

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