

**ANTI-EROSIVE POTENTIAL OF COMMERCIAL
BIOACTIVE GLASSES
ON DENTAL HARD TISSUES**

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**DISSERTATION SUBMITTED IN FULFILMENT OF THE
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ABSTRACT

Aims and Objectives: This study aimed to investigate the potential of two commercial bioactive glasses NUPRO® Sensodyne® Prophylaxis Paste and Sylc® Prophy Powder (Novamin®) in the secondary prevention of dental erosion.

Methodology: This *in-vitro* study utilized 30 enamel and 30 dentine specimens that were prepared from human premolars and molars. The specimens were flattened and polished and randomly divided into six groups having ten specimens in each group, of which equal proportions of enamel and dentine specimens were further assigned to three groups: Control, Nupro, and Sylc. All specimens were subjected to 10 minutes of demineralisation in 0.3% citric acid at a pH of 3.2 ± 0.1 . Baseline surface microhardness (SMH) and surface roughness (R_a) measurements were made using a Knoop indenter (HVM-2 Shimadzu Corporation, Japan) and the Infinite FocusG4 microscope (IFM, Alicona Imaging, Grambach/Graz, Austria). SMH was measured for only enamel specimens. Nupro and Sylc were applied on the enamel and dentine. Specimens were stored in remineralisation solution at 37°C and SMH measurements were made for enamel specimens and R_a was measured for all enamel and dentine specimens again 24 hours later. The specimens in all 6 groups were subjected to daily cycles of acid challenge for 10 minutes and stored in remineralisation solution at 37°C for six days. SMH (enamel) and R_a (enamel and dentine) measurements were made every day before acid challenge. Additional 24 specimens were prepared for SEM analysis at Baseline, Intervention and Day 6 of demineralisation. To determine the elemental gain or loss analysis was done by Energy dispersive x-ray spectroscopy (EDX) on specimens of Intervention and Day 6. The difference in SMH and R_a at the

various time points from Baseline ΔSMH & ΔR_a , was the outcome measure used. The data were analysed using SPSS version 22

Results:

All three groups of enamel showed a general trend of decrease in SMH over time. A significant difference in ΔSMH was observed in all three groups. Furthermore, there were significant differences in ΔSMH between the test groups and the controls ($P < 0.05$). However, significant differences in ΔSMH were found only between Baseline and Intervention. No significant differences were observed between the other measurement time points and Baseline. In the Sylc group, significant differences in ΔSMH were observed between Baseline and Intervention and up to Day 3 demineralisation. Regarding R_a , there was a significant difference in ΔR_a over time. A significant difference in ΔR_a existed between the test groups and the Control group as well as between Nupro and Sylc ($P < 0.05$). All six groups showed a net increase in ΔR_a over the study period but with varying amounts and patterns. SEM-EDX showed remineralisation tendency of both Sylc® Prophy Powder and NUPRO® prophylaxis paste.

Conclusion: Sylc® Prophy Powder and NUPRO® prophylaxis paste were able to reduce the rate of dental erosion. Sylc® Prophy Powder showed better reduction than Nupro.

ABSTRAK

Objektif: Kajian ini bertujuan untuk menyelidik potensi dua gelas bioaktif komersial NUPRO® Sensodyne® Profilaksis Tampakan dan Sylc® Prophy Powder (Novamine®) dalam pencegahan sekunder hakisan gigi.

Metodologi: Kajian in vitro ini menggunakan 30 enamel dan 30 dentin spesimen yang telah disediakan daripada gigi geraham kecil dan geraham manusia. Spesimen telah diratakan dan digilap dan secara rawak dibahagikan kepada enam kumpulan, tiga kumpulan enamel (Kawalan, Nupro dan Sylc) dan tiga kumpulan dentin (Kawalan, Nupro dan Sylc). Semua spesimen dikenakan 10 minit demineralisasi dengan 0.3 % asid sitrik pada pH 3.2 ± 0.1 . Pengukuran '*Surface microhardness*' (SMH) dan kekasaran permukaan (R_a) dibuat menggunakan pelekuk Knoop (HVM-2 Shimadzu Corporation, Jepun) dan mikroskop Infinite FocusG4 (IFM, Alicona Imaging, Grambach / Graz, Austria). SMH digunakan untuk mengukur spesimen enamel sahaja. Selepas Nupro dan Sylc telah diletakkan pada permukaan enamel dan dentin, spesimen-spesimen disimpan dalam larutan pemineralan pada 37 °C. Ukuran SMH dilakukan bagi spesimen enamel dan ukuran SMH dan R_a diukur bagi kedua-dua spesimen enamel dan dentin 24 jam kemudian. Spesimen dalam kesemua 6 kumpulan dikenakan kitaran harian asid cabaran selama 10 minit dan disimpan dalam larutan pemineralan pada 37 °C selama enam hari. Ukuran SMH (enamel) dan R_a (enamel dan dentin) dilakukan setiap hari sebelum cabaran asid. Spesimen tambahan telah disediakan untuk analisis '*Scanning Electron Microscope*' pada peringkat Permian, Intervensi dan Hari-6 demineralisasi. Tambahan 24 spesimen telah disediakan untuk analisis SEM pada garis dasar, Intervensi dan Hari 6 demineralisation. Menentukan unsur keuntungan atau

kerugian analisis itu dilakukan oleh Tenaga serakan spektroskopi x -ray (EDX) pada spesimen Intervensi dan Hari 6 .

Perbezaan SMH dan R_a (ΔSMH & ΔR_a) di antara peringkat Permulaan dan setiap hari demineralisasi dikira. Data dianalisis dengan menggunakan perisian SPSS versi 22.

Keputusan:

Ketiga-tiga kumpulan enamel menunjukkan trend umum penurunan SMH. Satu perbezaan ketara dalam ΔSMH diperhatikan bagi ketiga-tiga kumpulan. Tambahan pula, terdapat perbezaan yang ketara (*significant*) dalam ΔSMH antara kumpulan ujian dan kawalan ($P < 0.05$). Walau bagaimanapun, perbezaan yang signifikan dalam ΔSMH ditemui hanya antara peringkat Permulaan dan Intervensi. Tiada perbezaan yang ketara telah diperhatikan antara mata masa ukuran lain dan peringkat Permulaan. Dalam kumpulan Sylc itu, perbezaan ketara dalam ΔSMH diperhatikan antara peringkat Permulaan dan Intervensi dan sehingga Hari 3 demineralisasi. Mengenai R_a , terdapat perbezaan yang ketara dalam ΔR_a . Satu perbezaan ketara dalam ΔR_a wujud antara kedua-dua kumpulan ujian dan kumpulan Kawalan dan juga antara Nupro dan Sylc ($P < 0.05$). Kesemua enam kumpulan menunjukkan peningkatan bersih dalam ΔR_a sepanjang tempoh kajian tetapi dengan jumlah yang berbeza dan corak. SEM - EDX menunjukkan pemineralan kecenderungan kedua-dua Sylc® Prophy Powder dan NUPRO® profilaksis tampal.

Kesimpulan: Nupro dan Sylc dapat mengurangkan kadar hakisan gigi. Sylc menunjukkan pengurangan lebih baik daripada Nupro.

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Erum Saleem Khan

September, 2014

DECLARATION

I certify that this research is based on my own independent work, except where acknowledged in the text or by reference.

No part of this work has been submitted for any degree or diploma to this or any other university.

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CONTENTS

ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGMENTS	v
DECLARATION	vi
LIST OF FIGURES	x
LIST OF TABLES	xiv
CHAPTER 1	1
INTRODUCTION	1
1.1 Background of the study:	2
1.2 Scope of the study:	5
1.3 Aim of the Study:	6
1.4 Research objectives:	6
1.5 Null Hypothesis:	7
CHAPTER 2	8
LITERATURE REVIEW	8
2.1 Basic Tooth Structure	9
2.1.1 Enamel	10
2.1.2 Dentine	11
2.2 Demineralisation	12
2.3 Dental Erosion	13
2.3.1 Prevalence of Dental Erosion	14
2.3.2 Aetiology of Dental Erosion	16
2.3.3 Management of Dental Erosion	22
2.4 Remineralisation in Dental Erosion	26
2.4.1 Saliva	27
2.4.2 Fluoride	29
2.5.1 History and development	32
2.5.2 Composition	33
2.5.3 Variants	34
2.5.4 Properties	34
2.5.5 Mechanism of Action	35
2.5.6 Uses of Bioactive Glass	36

2.5.7 Products	38
2.6 Methods of Assessing Demineralisation and Remineralisation of Dental Hard Tissue	45
2.6.1 Quantitative Methods.....	46
2.6.2 Qualitative and Semi-quantitative Methods	52
CHAPTER 3	55
MATERIALS AND METHODS	55
3.1 Sample Collection	56
3.2 Disinfection, Cleaning, and Storage.....	56
3.3 Specimen Preparation.....	56
3.3.1 Sectioning	56
3.3.2 Mounting	57
3.3.3 Grinding and Polishing	57
3.3.4 Work window Preparation.....	57
3.3.5 Specimen Identification	58
3.4 Specimen Randomization:.....	59
3.5 Testing:.....	60
3.6 Surface Roughness (R_a) Measurements and Outcome Measure	67
3.7 Surface Microhardness (SMH) Measurements and Outcome Measure:	69
3.7.1 Indentation depth	71
3.8 Scanning Electron Microscope (SEM) Imaging & Elemental analysis (EDX): ..	72
3.9 Statistical Analysis:	73
CHAPTER 4	75
RESULTS	75
4.1 Surface Microhardness (Δ SMH) of Enamel:	76
4.1.1 Control Group.....	76
4.1.2 Nupro Group.....	79
4.1.3 Sylc Group:.....	81
4.1.4 Comparison between groups:	83
4.1.5 Indentation depth:	85
4.2 Surface Roughness of Enamel:	86
4.2.1 Control Group.....	86
4.2.2 Nupro Group.....	89

4.2.3 Sylc Group	92
4.2.4 Comparison of Surface Roughness (ΔR_a) among the three groups (Enamel):	94
4.3 Surface Roughness of Dentine:	97
4.3.1 Control Group	97
4.3.2 Nupro Group	99
4.3.3 Sylc Group:	102
4.3.4 Comparison of Surface Roughness (ΔR_a) among the three groups (Dentine):	104
<i>Figure 4.12</i>	106
4.4 Qualitative analysis of SEM images:	107
4.4.1 Enamel	107
4.4.2 Dentine	112
4.5 Elemental analysis by Energy dispersive X-ray Spectroscopy (EDX)	116
4.6 Cross Sectional SEM	118
4.6.1 Enamel Nupro	118
4.6.2 Enamel_Sylc	119
4.6.3 Dentine Nupro	120
4.6.4 Dentine_Sylc	121
CHAPTER 5	122
DISCUSSION	122
5.1 Discussion of Methods:	123
5.2 Discussion of Results:	128
CHAPTER 6	138
CONCLUSIONS	138
&	138
RECOMMENDATIONS	138
6.1 Conclusions for Enamel :	139
6.2 Conclusions for Dentine:	139
6.3 Recommendations:	139
Reference List	142

LIST OF FIGURES

Figure Number	Description	Page
Figure 2.1	The tooth and surrounding tissues [Aiello and Dean, 1990(45)].	10
Figure 2.2	Interactions of the factors involved in the development of dental erosion (Lussi, 2006) (59).	17
Figure 2.3	Salivary factors associated with the control of dental erosion in Enamel and dentin (Buzalaf, Hannas, & Kato, 2012) (152).	28
Figure 3.1A	Dentine specimen with circular work window made by wallpaper.	58
Figure 3.1B	Enamel specimen with circular work window made by nail varnish.	58
Figure 3.2	Erosion induction apparatus	61
Figure 3.3A	Showing Nupro® prophylaxis paste, hand piece, and rubber cups.	62
Figure 3.3B	Application of Nupro® prophylaxis paste on a specimen.	62
Figure 3.4	Sylc® prophy powder and polishing device	63
Figure 3.5	Sylc application on specimen	73
Figure 3.6	Flow chart of experimental cycle	66
Figure 3.7	White paper sheet marked with cardinal points, pasted on the stage of infinite focus microscope g4, Alicona.	68
Figure 3.8	Infinite focus microscope g4, Alicona, surface texture analyser.	68
Figure 3.9	For repositioning of specimens a white paper sheet with cardinal points marked on it, pasted on working stage of surface microhardness tester.	70
Figure 3.10	Specimen ready for surface microhardness test	71
Figure 4.1A	SMH reading of Enamel control specimen at intervention Day	78

Figure 4.1B	SMH reading of Enamel Nupro specimen at Day 6	78
Figure 4.2A	SMH reading of Enamel Nupro specimen at Intervention day.	80
Figure 4.2B	SMH reading of Enamel Nupro specimen at Day 6.	80
Figure 4.3A	SMH reading of Enamel Sylc specimen at Intervention Day	82
Figure 4.3B	SMH reading of Enamel Sylc specimen at Day6.	82
Figure 4.4	Change in Δ SMH from baseline to demineralisation Day 6	84
Figure 4.5A	Surface roughness of Enamel control specimen on Intervention day.	87
Figure 4.5B	Surface roughness of Enamel control specimen on Day 6.	88
Figure 4.6A	Surface roughness of Enamel Nupro specimen on Intervention day.	90
Figure 4.6B	Surface roughness of Enamel Nupro specimen on Day 2.	90
Figure 4.6C	Surface roughness of Enamel Nupro specimen on Day 6.	91
Figure 4.7A	Surface roughness of Enamel Sylc specimen on Intervention day.	93
Figure 4.7B	Surface roughness of Enamel Sylc specimen on Day 6.	93
Figure 4.8	Changes in Surface Roughness of Enamel from Baseline to Demineralisation Day 6.	96
Figure 4.9A	Surface roughness of Dentine control specimen on Intervention day.	98
Figure 4.9B	Surface roughness of Dentine control specimen on Day 6.	98
Figure 4.10A	Surface roughness of Dentine Nupro specimen on intervention Day.	100
Figure 4.10B	Surface roughness of Dentine Nupro specimen on Day 2.	100
Figure 4.10C	Surface roughness of Dentine Nupro specimen on Day 6.	101
Figure 4.11A	Surface roughness of Dentine Sylc specimen on Intervention Day.	103
Figure 4.11B	Surface roughness of Dentine Sylc specimen on Day 6.	103

Figure 4.12	Change in ΔRa from baseline to demineralisation Day 6 (Dentine).	106
Figure 4.13(A-a)	Enamel control specimen surface subjected to one acidic challenge.	107
Figure 4.13(B-b)	Enamel control specimen surface after 6-Days Demineralisation – Remineralisation cycle.	107
Figure 4.14(A-a)	Enamel Nupro specimen surface after application of material.	108
Figure 4.14(B-b)	Enamel Nupro specimen surface at the end of 6-Days experimental cycle.	108
Figure 4.15(A-a)	Enamel Sylc specimen surface after application of material at Intervention stage.	110
Figure 4.15(B-b)	Enamel Sylc specimen surfaces at the end of 6-Days Demineralisation-Remineralisation cycle.	110
Figure 4.16(A-a)	Dentine control specimen surface subjected to one acidic challenge.	112
Figure 4.16(B-b)	Dentine control specimen at the end of 6-days Demineralisation-Remineralisation cycle.	112
Figure 4.17(A-a)	Dentine Nupro specimen surface after application of Nupro prophylaxis paste.	113
Figure 4.17(B-b)	Dentine Nupro specimen surface after 6-Days experimental cycle.	113
Figure 4.18(A-a)	Dentine Sylc specimen surface after application of Sylc prophylaxis powder.	115
Figure 4.18(B-b)	Dentine Sylc specimen surface at the end of 6-Days Demineralisation-Remineralisation cycle.	115
Figure 4.19	Elemental weight percentage in enamel groups at Intervention and Day 6 of experimental cycle.	117
Figure 4.20	Elemental weight percentage in dentine groups at Intervention and Day 6 of experimental cycle.	117
Figure 4.21A	Cross-section SEM image of enamel Nupro specimen at Intervention.	118
Figure 4.21 B	Cross-section SEM image of enamel Nupro specimen at Day 6.	118

Figure 4.22 A	Cross-section SEM image of enamel Sylc specimen at Intervention.	119
Figure 4.22 B	Cross-section SEM image of enamel Sylc specimen at Day 6.	119
Figure 4.23 A	Cross-section SEM image of dentine Nupro specimen at Intervention.	120
Figure 4.23 B	Cross-section SEM image of dentine Nupro specimen at Day 6.	120
Figure 4.24 A	Cross-section SEM image of dentine Sylc specimen at Intervention.	121
Figure 4.24 B	Cross-section SEM image of dentine Sylc specimen at Day 6.	121

LIST OF TABLES

Table Number	Description	Page
Table 2.1	Prevalence of dental erosion across different geographic locations in children aged 11 to 14 years	16
Table 2.2	Common commercially available bioactive glass products. (39)	41
Table 2.3	Common Amplitude Parameters for Surface Measurement [adapted from Field et al. (2010) (255)]	50
Table 3.1	Composition and manufacturer's details of materials used in this study.	60
Table 4.1	Results of Multivariate test for $\Delta SMH(t_0)$	77
Table 4.2	Post-hoc test (Dunnett) for intergroup comparison of ΔSMH	83
Table 4.3	Post-hoc test (Bonferroni) for intergroup comparison of ΔSMH	84
Table 4.4	Comparison of Thickness of BAG layer with "h" value of SMH.	86
Table 4.5	Results of Multivariate test for $\Delta Ra(t_0)$ (Enamel)	87
Table 4.6	Post-hoc test (Dunnett) for intergroup comparison ΔRa (Enamel)	95
Table 4.7	Post-hoc test (Bonferroni) for intergroup comparison ΔRa (Enamel)	95
Table 4.8	Results of Multivariate test for $\Delta Ra(t_0)$ (Dentine)	97
Table 4.9	Post-hoc test (Dunnett) for intergroup comparisons of ΔRa (Dentine)	105
Table 4.10	Post-hoc test (Bonferroni) for intergroup comparison of ΔRa (Dentine)	105

LIST OF SYMBOLS AND ABBREVIATIONS

<	Less than
>	More than
%	Percentage
°C	Degree Celsius
n	Number of patients
µm	Micrometre
mm	Millimetre
cm	Centimetre
g	Gram
mg	Milligram
ml	Millilitre
nm	nanometre
ppm	part per million
MPa	mega Pascal
N/A	Not Applicable
wt	weight
Ca	Calcium
Si	Silicone
H	Hydrogen
F	Fluoride
P	Phosphorus
O	Oxygen

LIST OF SYMBOLS AND ABBREVIATIONS (continued)

CT	Computed tomography
SE	Standard Error
PI	Plaque index
Ra	Surface Roughness
KHN	Knoop Hardness Number
SMH	Surface microhardness
HCA	Hydroxy carbonated apatite
Rpm	Rotations per minute
HA	Hydroxyapatite
GBI	Gingival bleeding index
BAC	Bearing area curve
XRD	Xray-diffraction
SEM	Scanning electron microscopy
AFM	Atomic force microscope
IFM	Infinite focus microscope
BAGs	Bioactive glasses
WHO	World Health Organization
SPSS	Statistical Package for Social Sciences
ANOVA	Analysis of variance
Lc	Lambda C

CHAPTER 1

INTRODUCTION

1.1 Background of the study:

Bioactive glasses (BAGs) were introduced in the early 1970s when Hench et al. (1) discovered that rat bone could chemically bind to some silicate-based glass compositions in an aqueous environment. These glasses were later termed “bioactive” because they elicited a specific biological response at the material surface, resulting in the creation of a bond between tissues and the materials (1). The oldest BAG composition, 45S5 Bioglass® consists of a silicate network (45 wt. % silicon dioxide [SiO_2]) incorporating 24.5 wt. % sodium oxide (Na_2O), 24.5 wt. % calcium oxide (CaO), and 6 wt. % di phosphorus pent oxide (P_2O_5) (2).

Nowadays BAGs are produced in different shapes and forms and have a wide range of applications in medicine and dentistry. The form, 45S5, is frequently used in bone grafts (3). In otorhinolaryngology BAGs are produced as solid plates for reconstructing orbital floor fractures (4, 5) and as microspheres for frontal sinus obliteration (6, 7). They are also used as implants for contour restoration of facial skeleton (8) and have been used as adjuncts to conventional surgery for treating osseous periodontal defects (9).

Bioactive glasses are considered to be a major development in dental remineralisation technology (10). In aqueous solution, BAGs release bioavailable calcium, sodium, and phosphate ions, which contribute to the remineralisation process (11). Furthermore, the potential of commercial BAGs has been demonstrated in a study that assessed their capacity to remineralise bleached enamel (12). In their study, Gjorgievska and Nicholson (12) found that treatment with commercially marketed toothpastes that contained the BAG NovaMin® resulted in the formation of a protective layer—which consisted of BAG deposits—on the enamel surface, and application of

these dentifrices caused an increase in the calcium and phosphorus content of enamel, returning it to that of undamaged enamel. Furthermore, BAGs have the potential to seal dentine tubules by forming hydroxycarbonate apatite (12).

Dental erosion is the loss of dental tissue through chemical etching and dissolution by acids of non-bacterial origin or by chelation (13). It was reported as early as the 19th century (14), and since then the incidence and prevalence of dental erosion is increasingly being reported (15). Clinically, early dental enamel erosion appears as a smooth shiny glazed surface (13). In erosions of the facial aspects of teeth, there is usually a ridge of enamel that delineates the defect from the marginal gingiva. On the other hand, occlusal erosion typically presents as rounded cusps and concavities, and further progression of occlusal erosion causes a distinct grooving of the cusps (16).

The management of dental erosion is an area of clinical practice that is undoubtedly expanding (13). Treatment depends on the underlying cause owing to the multi-etiological character of dental erosion (17). Restorative therapy is necessary for advanced cases of dental erosion (18). This includes resin composites, resin ionomers, placement of bridges and crowns, and use of composite or porcelain veneers (17). Desensitizing agents and toothpastes may be used to treat sensitive teeth (17).

Bioactive glasses have been used in the treatment of dental erosion, and they have the advantage of being safe (19). Various compositions of silicate-based BAGs of the family $\text{SiO}_2\text{--CaO--P}_2\text{O}_5$ (45S, 58S, and 77S) have been evaluated for their remineralising effect on etched human enamel, and it has been demonstrated that 45S dentifrice had the best remineralisation capacity, highest mechanical strength, and satisfactory surface roughness (20). Furthermore, Dong Z et al. (20) showed that the level of silicon in BAGs played a major role in remineralising enamel.

Several studies (21, 22) have been conducted to compare the effect of different classes of bioactive glasses and other dentifrices in combatting dental erosion. In one study that compared commercial prophylactic pastes and air-polishing powders, Sauro et al. (23) found that Sylc® prophylactic powder and sodium bicarbonate were the most efficient in decreasing the permeability of dentine specimens. On the other hand, air-polishing procedures performed with Sylc BAG were the most efficient in decreasing the permeability of dentine specimens etched with phosphoric acid. The permeability of dentine specimens were reduced by 81.1% and 88.8% by Sylc Bioactive Glass® powder and Sylc bioactive glass H₂O, respectively. Colgate Sensitive Pro-Relief and Nupro NU-Solution each decreased dentine permeability by 69.8% and 66.9%, respectively (23). In another study, Milleman et al. (24) found that subjects who had received NovaMin-containing prophylaxis pastes (with and without fluoride) had statistically lower dentine hypersensitivity compared to those who had received Nupro classic prophylactic paste without fluoride immediately after the procedure. Banerjee et al. (25), in a study that compared the clinical effectiveness of sodium bicarbonate and Sylc powder, found that Sylc air-polishing was more clinically and statistically efficient at desensitizing both good and poor oral hygiene groups. In addition to providing better overall patient comfort during the procedure, Sylc was more effective at removing stain in the poor oral hygiene patient subgroup. However, the findings of these authors, Banerjee et al. (25) were limited by the relatively low number of patients in the study.

Despite major technological advances in oral health care over the last five decades, the increasing incidence and prevalence of dental erosion (26, 27) suggests that current prevention and treatment strategies do not suffice to control the progress of this condition. This increase is mainly attributed to dietary lifestyles, notably with the increased consumption of acidic beverages (26, 28). In addition, patients can hardly

detect early enamel erosion due to the smooth and shiny appearance of teeth (13). Even when detected, patients do not, in most cases, seek treatment until the erosion progresses to an advanced stage when they develop symptoms or the teeth become etched (13). Clinical management becomes crucial in the care of such advanced cases. Thus, a range of BAGs, either in the form of powders or pastes have been marketed for the treatment of dental erosion (19). Most research has focused on the efficacy of Novamin-based products on dental erosion and hypersensitivity. Prophylactic powders such as Sylc are relatively new and have proven safe in a few studies (23, 29). The BAG-containing products used in this study were Sylc and Nupro. Sylc was selected as a remineralising agent mainly because it contains 100% weight SiO_2 , Na_2O , CaO .

1.2 Scope of the study:

During the last few decades, Western and Asian countries have experienced a considerable increase in the consumption of acidic drinks, which has subsequently led to an increase in the incidence and prevalence of dental erosion (30-33). Unfortunately, most reports are primarily based on anecdotal evidence, hence it is difficult to get to the true incidence of dental erosion. Most prevalence studies have been conducted in children, and results show that almost 52% of five-year olds have significant dental erosion of primary teeth and 25% of teenagers have steady dental erosion (30). Limited data show that between 76 and 100% of adults have erosive tooth wear (32, 34). In one study conducted by Isaksson (35) it was reported that 75% of Swedish young adults had dental erosion. Another study reported that the prevalence of dental erosion in a sample of adolescents was 41% in the United States and 37% in the United Kingdom. A lower rate (27.3%) was reported in a sample of 12-13-year-old school children in Southern China (36). Furthermore, one report suggests that dental erosion is significantly more

common in Caucasian than Asian children (37) . However, prevalence data may be influenced by socio-economic, cultural, and geographic factors.

The increase in the incidence and prevalence of dental erosion has prompted research in the development of effective preventive and therapeutic options. To date, several researches (23, 25, 38-43) have investigated BAGs in treating patients with dental erosion, especially in cases of dentine hypersensitivity, and results have demonstrated that BAGs have the potential to decrease dentine sensitivity in patients with moderate to advanced dental erosion.

1.3 Aim of the Study:

The aim of this study was to determine the anti-erosive potential of two commercial bioactive glass products, i.e. Sylc® Prophy powder and NUPRO® prophylaxis paste on eroded enamel and dentine surfaces.

1.4 Research objectives:

1. To investigate the anti-erosive potential of Sylc® Prophy Powder and NUPRO® Prophylaxis Paste, two bioactive glass products, when subjected to multiple acidic challenge on enamel and dentine surfaces.
2. To compare and characterise the anti-erosive effect of Sylc® Prophy Powder and NUPRO® Prophylaxis Paste on enamel and dentine respectively.
3. To investigate the remineralising potential of Sylc® Prophy Powder and NUPRO® Prophylaxis Paste on dmineralised enamel.

1.5 Null Hypothesis:

1. Sylc® Prophy Powder and NUPRO® prophylaxis paste has no anti-erosive effect on demineralised enamel.
2. Sylc® Prophy Powder and NUPRO® prophylaxis paste has no anti-erosive effect on demineralised dentine.
3. There is no significant difference in the anti-erosive effect between Sylc® Prophy Powder and NUPRO® prophylaxis paste on demineralised enamel and dentine surfaces.

CHAPTER 2

LITERATURE REVIEW

This review is focused on dental demineralisation and remineralisation as well as advances in the development of biomaterials to restore diseased tooth tissues. The scope of this research is noteworthy to dental professionals as it provides essential information relevant to their daily practice. Given that information is regularly updated, our consideration was limited to peer-reviewed information published in the last decade, wherever possible.

However, because the body of knowledge regarding dentistry and oral surgery is vast and numerous technological advances are occurring in this field of science, we systemically followed the literature where it leads us to appropriate explanations and details. In order to achieve this, we examined case and longitudinal studies. The summation that appears at the conclusion of this review will incorporate reflection and synthesis of the information contained in this study. An extensive bibliography appears at the end of this review to allow readers to examine the references that were consulted in preparing this chapter.

2.1 Basic Tooth Structure

The dental professional must master all aspects of tooth anatomy, histology, morphology, and physiology. A basic understanding of tooth structure will enable dental professionals to effectively detect disease processes that affect teeth.

The tooth comprises three basic layers — the inner pulp, middle dentine, and outer enamel (Figure 2.1). The soft inner layers of teeth provide nutrients for the growth and function of teeth, while the outer hard layers are designed for structure and to confer protection and chewing functions. However, for the purpose of this review, much emphasis was placed on enamel and dentine, as these are the primary tissues that are involved in demineralisation.

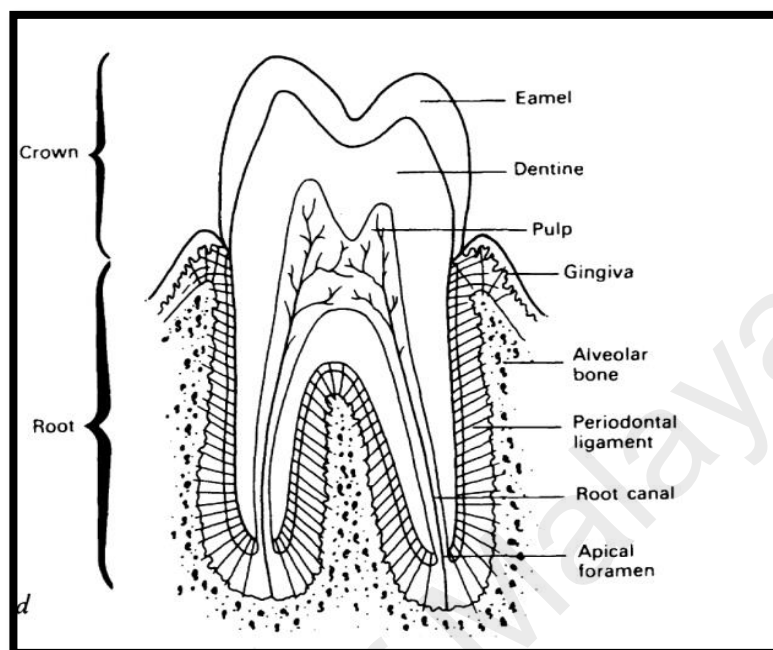


Figure 2.1: The tooth and surrounding tissues (Aiello and Dean, 1990)(44).

2.1.1 Enamel

Enamel is the visible layer of tooth. It is the calcified substance that covers the anatomic crown of the tooth and protects the dentine and pulp (45). As it has a semi-translucent nature, the materials that lie underneath it—alongside the dentine—affect its overall colour and appearance. Enamel is formed by epithelial cells known as ameloblasts in a process called amelogenesis. Enamel formation starts at the cusp tip(s) of a tooth and proceeds in a cervical direction. Once fully developed, enamel does not contain any sort of nerves or blood vessels, which explains why it has no power to grow further or to be repaired after it is formed; rather, it can only gain or lose minerals.

Enamel is the hardest tissue in the human body, and it is the most mineralized among all other components (46). It is composed mainly of inorganic minerals (approximately 97.0% of enamel by weight), mainly calcium and phosphorus as

hydroxyapatite; the remainder is made up of 1.5% organic materials and 1.5% water. Structurally, enamel is made up of millions of rods, which consist of tightly packed masses of hydroxyapatite crystals (47). Each rod consists of an 'occlusally' directed head and a 'cervically' directed tail (48). The rods are aligned in rows along the tooth, such that the long axis of each rod is, in general, perpendicular to the underlying dentine. However, rods are aligned differently in permanent teeth—the rods near the cement enamel junction are slightly tilted toward the root of the teeth. On radiographs, enamel appears radiolucent in comparison to dentine or pulp because the crystalline structure of the salts in enamel makes it denser and more radiopaque (2).

2.1.2 Dentine

Dentine is a calcified tissue situated in the tooth's second layer. It is usually covered by enamel and consists of various small tubules. Dentine makes up most of the structure of the tooth as it also covers the pulp completely. Due to the fact that it is a lot denser than bone, its colour ranges from grey to pale yellow and this differs from one person to another, depending on the overall state of their teeth and other factors (49).

Dentine consists of approximately 45% mineral, 33% organic material (mainly Type I collagen) and 22% water by volume (50). The calcified intercellular substance that makes up dentine is penetrated by dentinal tubules, which contain odontoblasts, the bodies of which lie close to the periphery of the pulp. The tubules vary in diameter, based on their location. Their diameters vary between 2.5 μm for those close to the pulp and to less than 1 μm for tubules located close to enamel (50).

Dentine is categorized into two main structural types: peritubular and intertubular dentine. Peritubular dentine is a layer of dentin that surrounds dentine

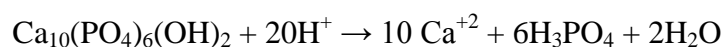
tubules mainly in the crown of the tooth. Intertubular dentine, on the other hand, is surrounded by a peritubular envelope and it is located between the tubules. It is chiefly found in the roots of the teeth (51).

Ultra structurally, peritubular dentine is denser than intertubular dentine (52). The crystals in peritubular dentine are parallelepiped in structure and measure approximately $36 \times 26 \times 10$ nm (51). Peritubular dentine consists primarily of hydroxyapatite, but it is also rich in magnesium and carbonate, which makes it very soluble. It consists of plate-shaped crystals that are arranged in layers. However, it only consists of a small quantity of collagen. Intertubular dentine, on the contrary, is a fibrous network of collagen with deposited mineral crystals (52). Only 9% of calcium, phosphorus, and magnesium make up the mineral content of intertubular dentine. Structurally, the crystals in intertubular dentine are typically larger than those in peritubular dentine and are arranged as hexagonal crystalline plates (44).

2.2 Demineralisation

Dental demineralisation is the loss of minerals from the tooth surface. The chemical process associated with erosion is complex. Dental hard tissues are mainly composed of mineral crystals of hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$). Dental hydroxyapatite is usually described as "carbonated" and "calcium deficient" owing to the fact that sodium, magnesium and potassium can substitute some calcium ions in the mineral, and carbonates can substitute some phosphate ions, making the mineral more susceptible to acid dissolution. Conversely, fluoride can substitute some hydroxyl groups to form fluoro-hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{FOH})_2$, which has a greater crystalline stability and is less susceptible to acid dissolution during acidic challenge, as

compared to hydroxyapatite (53). Thus solutions that are under saturated in calcium, phosphate, and fluoride will encourage dental erosion. The dissolution of hydroxyapatite is summarized in the equation below:

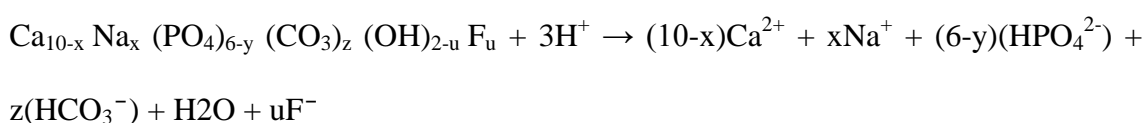


2.3 Dental Erosion

Dental erosion is the loss of dental tissue through chemical etching and dissolution by acids of non-bacterial origin or by chelation (13).. Clinically, erosion usually co-exists with attrition (direct tooth-to-tooth wear) and/or abrasion (movement of particles on tooth surface as a result of contact), but one of these factors may be predominant over the others (54), making differential diagnosis difficult.

When dental tissue is exposed to an acidic environment for long, a clinically visible defect occurs. The original lustre of the tooth dulls on smooth surfaces and subsequently, the convex areas flatten or shallow concavities become evident (18) . The cusps on occlusal surfaces become rounded or cupped and edges of restorations seem to rise above the level of the adjacent tooth surfaces. In more advanced cases, the entire tooth morphology disappears and the vertical crown height can be substantially decreased (18).

At the biochemical level, dental erosion occurs when hydrogen ions from strong or weak acids (citric and acetic acid) or anions bind with calcium. As acids dissociate in water, hydrogen ions are formed, which then attack the minerals crystals in teeth and directly dissolve the teeth by combining with calcium or phosphate ions as shown in the equation below: (55)



The crystal surface becomes etched as a result of the direct attack of the hydrogen ions that combine with carbonate and or phosphate, leading to the release of all of the ions from that portion of the crystal.

The process is different in the case of weak acids such as citric acids, which have a more complex interaction. In water, these acids exist as a mixture of hydrogen ions, citrate ions, and undissolved molecules (55). When released from citric acid, the hydrogen ions directly attack the crystal surface of teeth. In addition, citrate ions may bind with calcium ions, thus removing calcium from the crystal surface. Since each acid anion has different calcium complexation strength, which depends on the molecule structure and how readily it can form complexes with the calcium ion, citric acid has a double action and it is very damaging to the dental surface (56).

2.3.1 Prevalence of Dental Erosion

Several longitudinal and cross-sectional studies have been performed to assess the epidemiology of dental erosion, and the results show that the prevalence of erosion varies substantially across geographical locations and age groups (Table 2.1). There are also studies that report the increasing prevalence of erosion in both children and adults (32, 57) although the findings differ significantly among studies. The prevalence of erosion in deciduous teeth was reported in one study (58) to vary between 2 and 57%. In a recent systematic review, Kreulen et al. (57) reported that the prevalence of dentine erosion ranged between 0 and 82% for deciduous teeth in children up to 7 years old, while it ranged from 0 and 54% in permanent dentition. In adolescents, Ganns et al. (59) reported an increase in erosive damage between 1977–87 and 1990–99, with an approximate doubling in the frequency of lesions during this period of time. They found that dentine erosion (on at least one deciduous tooth) increased from 18 to 32%; erosion

into dentine on the first mandibular molars, on the other hand, increased from 4 to 9% (59). Similar results were obtained by Nunn et al. (60) who conducted a review based on cross-sectional studies in British adolescents. According to another report by Dugmore and Rock (37), 27% of the 12-year-olds in their study had developed new or more advanced dentine erosion at the age of 14. The authors observed that 5% of 12-year-old children had developed lesions into the dentine. By the age of 14, the percentage of children with dental damage had increased to 13%. Erosion into enamel was observed in 56% and 64% of the children at the ages of 12 and 14 years, respectively (37). Recent findings from a study conducted in the Netherlands (61) found that the incidence of new dental erosion decreased during the three-year period that the children were followed up. Conversely, the prevalence of deep enamel/dentine erosion increased from 2% to 24% in children who already had signs of dental erosion at age 12 (61).

In adults, erosion is significantly associated with age, increasing from 3% at age 20 years to 17% at 70 years (34). More recent research shows that in many countries, dental erosion, especially damage of the palatal surface of the upper front teeth, is frequent in children and young adults (62). Changing dietary habits, mainly as a result of an increase in the consumption of soft drinks, sweets, and fruits by children and adolescents, has been linked to an increase in the prevalence of erosion (63).

Table 2.1: Prevalence of dental erosion across different geographic locations in children aged 11 to 14 years

Study	Country	Age (years)	Prevalence	Teeth examined
Deery (2000)(44)	United Kingdom and United States	11-13	37.0%	Upper permanent incisors
Ganss et al. (2001)(59)	Germany	11.4	11.6%	All permanent teeth
Al-Majed et al. (2002)(64)	Saudi Arabia	12-14	95.0%	Upper permanent incisors and first molars
Dugmore and Rock (2004)(65)	United Kingdom	12	59.7%	Permanent incisors and first molars
Caglar et al. (2005)(27)	Turkey	11	28.0%	Permanent dentition
EL Karim et al. (2007)(66)	Sudan	12-14	66.9%	Permanent dentition
Waterhouse et al. (2008)(67)	Brazil	13-14	34.1%	Permanent dentition
Talebi et al. (2009)(68)	Iran	12	38.1%	Upper permanent incisors
Waag et al. (2010)(36)	China	12-13	27.3%	Permanent incisors and first molars

2.3.2 Aetiology of Dental Erosion

The aetiology of dental erosion is multifactorial and may involve intrinsic or extrinsic factors (Figure 2.2). Intrinsic factors include those that are caused by the presence of gastric acid in the mouth, while extrinsic factors are mainly linked to the ingestion of foods, drinks and medications.

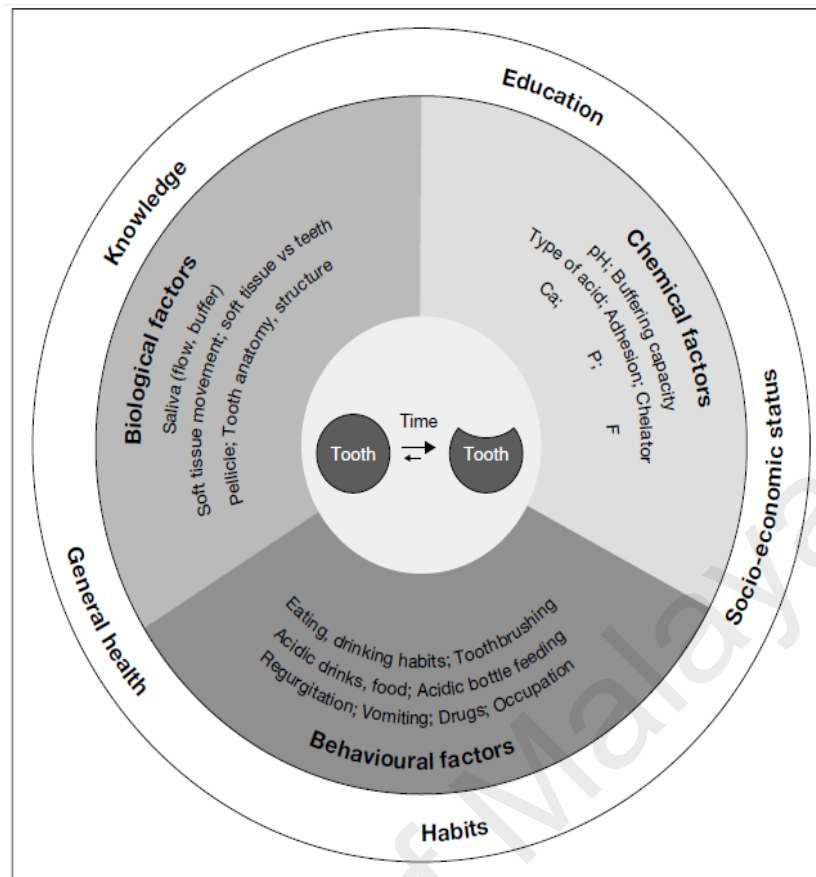


Figure 2.2: Interactions of the factors involved in the development of dental erosion (59).

2.3.2.1 Intrinsic Factors of Dental Erosion

a) Gastro-oesophageal Reflux Disease

Gastro-oesophageal reflux disease (GORD) is a chronic condition that occurs when refluxed acid moves upward through the oesophagus into the oropharynx (69). Common oesophageal complications that have been reported to occur in patients with GORD include reflux esophagitis, haemorrhage, stricture, Barrett's oesophagus and adenocarcinoma (70). Dental erosion is an extra-oesophageal manifestation of GORD. The median prevalence of dental erosion in adult and paediatric patients with GORD

was estimated respectively at 32.5% (range, 21-83%) and 17.0% (range, 14-87%) (71). Its prevalence has been reported in up to 42% of the general population.

Dental erosions involve enamel loss in facial, occlusal, and lingual surfaces in children and adolescents with GORD (72). In addition, it was reported that children with GORD also had an increased risk of dental caries (73). While findings from a large case control study (74) demonstrated that there was no significant association between GORD and either dental erosion or tooth sensitivity, a recent systematic review by Marsicano et al. (75) found a significant association between GORD and dental erosion.

In adults, a few reports (76, 77) evaluate the efficacy of GORD treatment in the reduction of dental erosions. Recently, one randomized clinical trial (78) demonstrated quantitative suppression of tooth erosion after the treatment with a proton pump inhibitor. However, further research has to be conducted given the controversial findings in the literature (79). The most accepted mechanism for dental erosion in patients with GORD is the presence of decalcifying acid or chelating agents in the oral cavity, which destroy the pellicle, dissolve the tooth's organic substrate, and cause demineralisation of the surface of the tooth (80). The damaged dental hard tissue surface is then vulnerable to mechanical friction during processes of chewing, swallowing, movement of soft tissues, or brushing. The erosive lesions due to intrinsic acid regurgitation are different from those due to extrinsic acid (81). The eroded teeth in patients with acid reflux appear to have broad concavities within smooth surface enamel, and in most cases, the enamel "cuff" is preserved in gingival crevice (82).

b) Vomiting

Vomiting episodes can affect dental health. Vomiting may be spontaneous or self-induced as in the case of patients suffering from anorexia and bulimia nervosa. Gandara et al. (82) suggested that weekly vomiting in the presence of other risk factors,

was associated with dental erosion. The emergence of gastric contents in the mouth results in a decrease in the pH to about 3.8, as is typically the case in bulimic patients (83). Furthermore, a decrease in saliva secretion, which is a common finding in bulimic patients, potentiates the erosive process (82).

c) Regurgitation

Regurgitation is the movement of partially digested food from the stomach into the throat or mouth (84). Several factors predispose the movement of gastric acid from the stomach to the buccal cavity, namely obesity, genetic factors, such as inherited congenital hiatus hernia (85), pregnancy, fatty foods, alcohol consumption, smoking, non-steroidal anti-inflammatory drugs, and sleeping position (86). The prevalence of dental erosion due to regurgitation is also reportedly higher in bulimic than in non-bulimic persons (87). The most commonly reported sign of the presence of gastric juice in the mouth is the development of erosive lesions. In some cases, it might be easier to identify the cause of dental erosion, while in others, diagnosis is not straightforward. As a result, an in-depth and thorough clinical examination is needed to establish and confirm the diagnosis. However, even with the use of an oesophageal pH test, the proper diagnosis might not be made.

2.3.2.2 Extrinsic Factors of Dental Erosion

a) Beverage

There is mounting evidence of a considerable increase in the consumption of soft drinks, sport drinks, fruit teas and fruit juices which are potentially erosive (88). A recent study (89) showed that there were significant associations between carbonated drink consumption and dental erosion (89). Results of one meta-analysis (90) revealed

that soft drinks were associated with approximately a 2.4 fold increased risk of dental erosion. In this report, Li et al. (90) suggested that soft drinks could damage the teeth in the following two ways:

- i. by their low pH and high titrable acidity, causing dental erosion and
- ii. by their sugar content, which are metabolized by bacteria to produce organic acids that could cause caries.

The erosive potential of erosive beverages is principally due to their pH and buffering capacity. In previous reports (65, 91, 92), it was demonstrated that carbonated drinks had lower pH than fruit juices. The buffering capacities of the drinks were in the following order: fruit juices > fruit-based carbonated drinks > non-fruit-based carbonated drinks (92). Other researchers showed that the greater the buffering capacity of the drink, the longer it will take for the saliva to neutralize the acid (88, 93).

Dental erosion is also associated with drinking methods. Frothing acidic drinks around the mouth, for example, increases the risk of acid erosion (94). Drinking at an increased flow rate and with a decreased outlet diameter has also been reported to increase erosion depth (95). Furthermore, the effect is potentiated when the temperature of the drink is higher (96).

b) Food

The consumption of acidic foods in children and adults has been extensively researched, but the findings are inconsistent. While some authors reported that acidic foods such as citrus fruits and drinks cause tooth erosion (64, 97-100), other authors found that there was no relationship between tooth erosion and acidic food consumption (101). This could be because most of these studies were limited by their cross-sectional design, where the dietary patterns during data collection could have been different from that experienced by the participants during the occurrence of dental erosion or an

erosive dietary habit had only just begun. Dental erosion could be a long process resulting from frequent and prolonged insults by acidic food.

The amount of acidic fruits consumed is also reportedly not associated with the occurrence of dental erosion (102, 103) (104). In case-control studies, (105, 106) the association between dental erosion and fruit consumption was reported only when fruit ingestion was excessive. Specifically an increased risk of erosion was reported when citrus foods were eaten more than twice daily (105, 106).

There is less evidence of the effect of covert acids in food stuff such as brown sauce, tomato ketchup, vinaigrette, and crisps on dental erosion in teenagers (97).

c) Supplements

Vitamin C and hydrochloric acid supplements, which have a low pH and high titrability, may also cause tooth erosion (107, 108). A meta-analysis showed that when chewed, the ingestion of vitamin C tablets was associated with a 1.16 higher odds of having erosion (90) .

d) Lifestyle

Certain lifestyle be it leisure or fashion trends had been associated with a greater risk of tooth erosion. Previous studies showed that prolonged exposure to a gas-chlorinated swimming pool was associated with dental erosion, especially in competitive swimmers (109, 110). The use of mood enhancing drugs such as Ecstasy has also been reported to increase the risk of erosion (111).

e) Environment

Several case reports have reported dental erosion in workers of lead storage battery manufacturing plants (112-116). It is believed that workers in an environment that have high concentration of cadmium or sulphuric ions such as battery and galvanizing factories, have an increased risk of dental erosion.

2.3.3 Management of Dental Erosion

In general, if no effective intervention occurs at an early stage of erosive damage, the lesions will subsequently lead to severe loss of dental tissue. In practice, clinicians have attempted to develop indices to record and monitor erosion severity.

A dentist has to consider a number of factors when assessing the need to treat a patient with dental erosion, as assessment must always be made on an individual basis. Hence, one patient might need treatment while another does not although they have the same degree of damage. Certainly, the dentist cannot make such complex decisions from a scoring system only. When a case of dental erosion is diagnosed, the patient should be followed up with individualized recall periods. Further, the patient should be assessed for possible progression of erosive damage. In some cases, the dentist may need to request a medical consultation and/or complementary investigation.

2.3.3.1 Preventive Therapy

Preventive strategies constitute the first line of treatment of erosion. These require lifestyle modifications, such as avoiding acidic foods and beverages, which is of greater benefit than recommending treatment with fluoride-containing products (59,

117-120). Antacids have been shown to increase intra buccal pH after an acidic challenge (121). Sodium bicarbonate solution, when used as an oral rinse, has also been shown to decrease tooth surface loss after artificially induced erosion (122). However, despite the wide range of dental care products that are marketed for the prevention of dental erosion, there is currently no product that offers adequate protection against dental erosion (123).

A modern preventive strategy that was proposed by Ganss and Lussi (18) involves the training of dental professionals in early detection and monitoring of the erosive process, as there is no diagnostic device that can clinically detect most of the dental erosion once dissolution has started. Besides, it is a challenging task to diagnose erosion at an early stage and it appears difficult to determine whether dentine is exposed or not (124). The use of fluoride products is recommended in patients who are at risk for dental erosion (56). However, they should be advised to avoid tooth brushing immediately after an acid challenge (vomiting or acidic diet) (56). Patients at risk should also be advised to use a soft toothbrush, low abrasion fluoride-containing toothpastes, and mouthwashes with a very low pH.

Fluoride therapy has been shown to be beneficial, especially in patients with dentine hypersensitivity (56). Furthermore, the capacity of bioactive glasses to occlude dentinal tubules has been explored in order to treat dental erosion (39). This approach—which we also investigated in our study—is non-invasive and involves the application of bioactive glass using a slow-speed hand piece or an air-polishing device.

Other preventive methods, which are not frequently used at present, involve the addition of products such as calcium or phosphate to drinks that had an erosive

potential. It is suggested that these chemicals, when added to drinks, modify the pH and hence decrease their erosive potential (125, 126).

It is challenging to manage erosion in children with primary tooth wear. However, it may serve as a means to preclude erosion in the permanent dentition. In addition, giving advice and information about dental erosion can be a challenging task, as it might be efficient in some patients, while in others it might be unsuccessful. Nevertheless, advice and prophylaxis have been shown to decrease the risk of dental erosion even in cases of severe erosion (127).

2.3.3.2 Restorative Therapy

The restorative treatment of dental erosion may vary from minimally invasive therapies to multidisciplinary interventional procedures. It is generally indicated when tooth integrity is threatened or when dentinal hypersensitivity is present (36). The decision thus belongs to the dentist, who has to judge whether the benefits of restorative therapy outweigh the risks of the treatment options being considered. Though restoration may be necessary, it is not always indicated to restore all cases of tooth surface loss due to erosion. In addition, there is no evidence that supports appropriate restorative treatment for tooth erosion, coupled to the fact that no strong evidence exists regarding the long-term benefits of any form of restorative treatment (41).

In cases of localized tooth wear in the posterior tooth, composite restorations and crowns can be offered to restore the condition (128). When the tooth is extensively worn, these may cause changes in the occlusal vertical dimension. In cases of severe erosion of the dentist should consider a multidisciplinary approach (41). In children with dental erosion, composite restorations are the cornerstone of restorative interventional procedures, while expensive conventional fixed and removable

prosthodontics was, and is still used in rehabilitating extensively worn adult teeth, in cases where treatment is indicated (129). Besides being complex, the treatment is also very invasive and involves additional removal of dental substance for retentive needs in a patient who already has erosive-diminished dentition. Thus, enough emphasis cannot be placed on prevention as the most efficient measure.

For aesthetic reasons, restoration is mainly done to the teeth in the lower jaw, especially in children (62). However, the procedure is clearly hindered when there is little available inter occlusal space in worn anterior teeth. As a result, less invasive strategies are necessary to complete occlusal reconstruction by combining forced intrusion of anterior teeth and supra-eruption of posterior teeth, as initially described by Dahl et al. (130). Multiple clinical studies by Poyser et al.(132), Chana et al.(133), and Renner (134) have consistently shown the reliability of this method (131-133). Dahl's approach can be applied in children and adolescents who are eligible to have restorative therapy. The technique has been modified as described in a previous report (130), and it includes the use of single- or multiple-bonded restorations at increased vertical dimension of occlusion.

Most children and adolescents deemed to require restorative treatment can be treated with a Dahl approach, or modifications thereof. Several such modifications have been described following the original report, including the use of a removable metal bite platform to create inter-occlusal space to favour the placement of restorations on worn anterior teeth (131). Thus, there is currently a shift toward the use of direct and indirect resin composite restorations (134, 135), which have been described to be aesthetically and functionally very satisfactory (62). This method can also be applied to older patients. In one case-control study, Bartlett and Sundaram (136) found a shorter life-

span with both direct and indirect resin composite restoration in patients with erosion compared to controls. Similar results were shown in other clinical studies conducted by Attin et al. and Hemmings et al. (137, 138).

With the advent of innovative approaches, advances have been made in treating patients with dental erosion. Notably, the use of ceramics, with an adhesive, has been shown to produce good results (62). Thus, nowadays, clinicians have more possibilities to offer better and more reliable strategies for managing their patients with dental erosions. However, for management to be effective, clinicians have to recognize that early diagnosis and prevention should be considered in at-risk patients in order to avoid progression to severe dental wear.

2.4 Remineralisation in Dental Erosion

Although dental erosion is readily observable, it is relatively difficult to measure, model and treat (54, 93). Much research in dentistry has uncovered the processes of demineralisation and remineralisation; however, dentists worldwide do not agree on the details regarding the mechanism of mineral deposition in surface-softened enamel. This section focuses on remineralisation of eroded enamel lesions through natural processes as well as dentifrice application. The latter is currently the most common form of anti-erosion treatment. Nevertheless, some researchers (139) are sceptical about the clinical relevance of exposing the teeth to routine fluoride application in order to prevent tooth erosion given that the incidence of erosion is generally on the rise.

In fact, the process of demineralisation and remineralisation of the tooth occur constantly either simultaneously or alternately (140). Acids, from intrinsic or extrinsic sources, lower the surface pH and diffuse into the tooth, leaching calcium and

phosphate from the enamel. The pH of the oral cavity may be approximately 4.0 – 4.5 at this time (141). As alluded to earlier, saliva has the capability to neutralize acids in the oral cavity; however, this process usually takes up to two hours. The time for saliva to neutralize the acids is crucial to control the pH in the oral cavity as well as calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ion concentrations in saliva owing to the fact that apatite in enamel is susceptible to destruction by acids (142). The subsequent process, remineralisation, is practically the opposite.

After the pH in the oral cavity returns to near neutral values, Ca^{2+} and PO_4^{3-} ions in saliva are deposited into the depleted mineral layers of enamel as new apatite. The demineralized areas in the tooth function as nucleation sites for the deposition of new mineral. The use of fluoride, especially at high concentrations, causes the tooth to lose its initial carbonated hydroxyapatite, which is replaced with a combination of hydroxyapatite and Fluor apatite (142). The process of mineral loss and gain basically depends on the solubility of enamel and ion gradients. In practice, after a meal, there is a rapid fall in the pH of the oral cavity, resulting in the low saturation of Ca^{2+} and PO_4^{3-} ions in saliva as compared with that of enamel. Because of this difference in ion gradient, Ca^{2+} and PO_4^{3-} ions are lost from the enamel into saliva, leading to the dissolution of tooth enamel. When the pH in the oral cavity rises, the resulting ionic super saturation shifts the equilibrium the other way, causing the deposition of mineral in the tooth.

2.4.1 Saliva

Saliva is perhaps the single most important and natural defence mechanism against dental erosion. It provides protection against acid erosion in various ways (88, 143-145). First, saliva forms a thin protective membrane or pellicle of varying degrees

and thickness around the teeth (at different locations within the mouth), depending on an individual's health status (146). Second, it dilutes the acids in the oral cavity. Third, saliva clears the oral cavity and gradually eliminates acids through swallowing. Fourth, the buffering capacity of saliva plays a key role in impeding changes in pH within the oral cavity. Fifth, saliva is supersaturated in fluoride, Ca^{2+} , and PO_4^{3-} —which are necessary in remineralisation—with respect to dental mineral content (147). Sixth, the numerous proteins in saliva and the acquired pellicle play a key role in tooth erosion (Figure 2.3) by inhibiting the precipitation of calcium and phosphate, binding to dissolution sites and covering crystallites by specific adsorption (148).

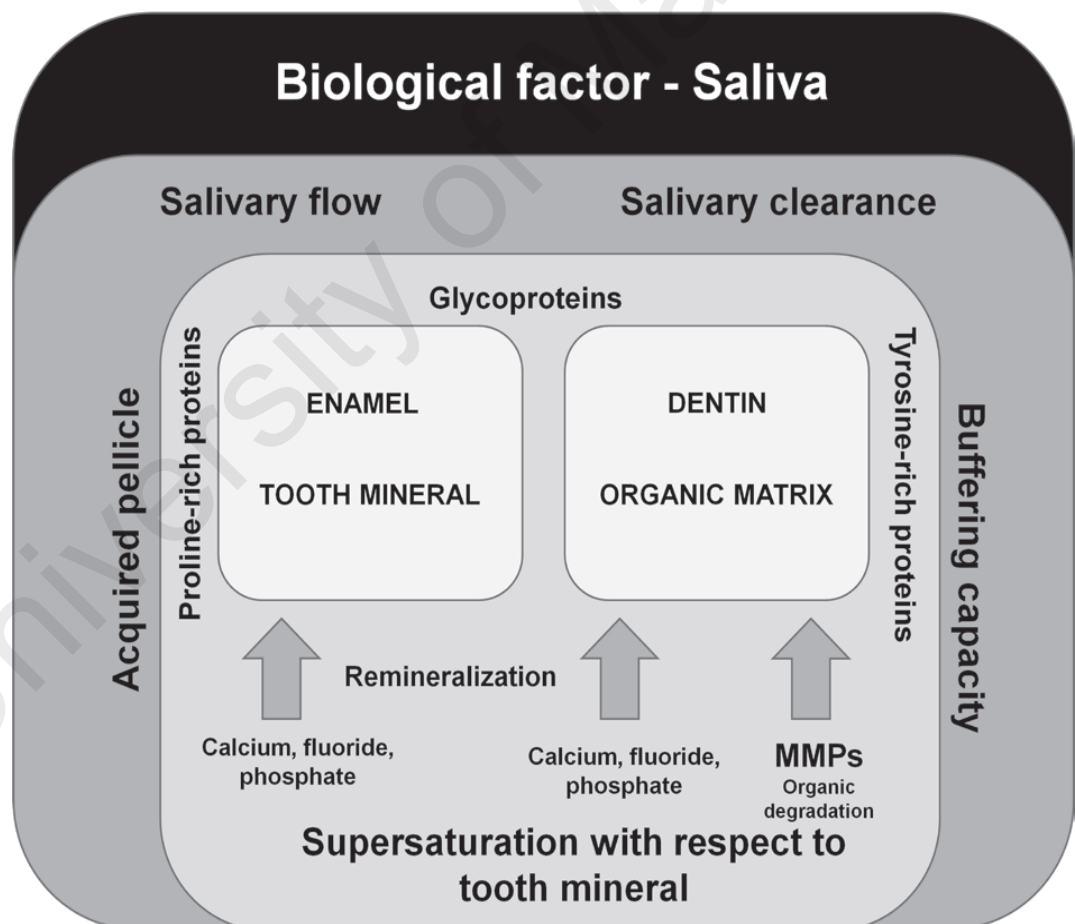


Figure 2.3: Salivary factors associated with the control of dental erosion in enamel and dentin (Buzalaf, Hannas, & Kato, 2012(148)).

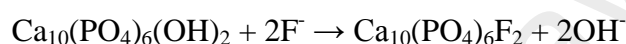
The above-mentioned factors, either individually or in combination, impact the ability of saliva to combat erosion. For example, a person's ability to swallow and his or her salivary secretion rate will both directly affect erosion. Additionally, the effectiveness of the pellicle may also be constrained by existing challenges to the dentine and enamel surface. In fact, in cases where the surface has been recently eroded, the pellicle may actually hinder the process of remineralisation.

Acids (from acidic beverages and/or sugary foods) temporarily cause pH drops within the mouth, during which the process of demineralisation is increased. Bicarbonates in saliva are known to play a major role in elevating low oral pH, while Ca^{2+} and PO_4^{3-} maintain dental mineral integrity (148). Other organic constituents of saliva, namely proteins and glycoproteins may influence several aspects of oral health, including tooth erosion (149). In particular, statherin, a tyrosine-rich acidic peptide, is known to allow saliva to maintain its state of super saturation with respect to calcium and phosphate ions. Thus, saliva largely contributes to the maintenance of an intact dentition by binding and inhibiting spontaneous calcium phosphate precipitation and crystal growth (148). In addition, histamines group of small multifunctional proteins present in saliva have been suggested to possess anti-demineralisation properties when phosphorylated (150).

2.4.2 Fluoride

Recently, findings from studies have shown the impact of fluoride as a contributor to the overall beneficial effect for the prevention of tooth erosion (151, 152). Although the systemic action of fluoride is secondary to a topical application, brushing after an acid challenge causes considerable complications where softened enamel is easily worn through toothbrush/toothpaste abrasion (153).

Over the course of time, fluoride ions ingested from fluoridated water and milk, fluoride supplements, and foods processed with fluoridated water can become integrated into the tooth. This results in a constant exchange of mineral ions between crystals of the enamel surface and the fluid bathing the tooth surfaces. Through the process of brushing with toothpastes, using mouth rinses, and drinking fluoridated water, topical fluorides are regularly applied to the teeth surface. The fluorides interact with the mineral component of teeth and produce fluoro-hydroxyapatite by the substitution of fluoride ions for hydroxyl ions as shown in the equation below:



This leads to increased hydrogen bonding, smaller crystal lattice, and a general decrease in tooth solubility (53). Thus, it is plausible that even a dental surface that is poorly mineralized can be gradually improved by the fluoride exchange equilibrium described above (154).

When incorporated in the enamel surface as Fluoro apatite, fluoride makes enamel more resistant to demineralisation than hydroxyapatite during acid challenge. In addition, fluoridated saliva besides decreasing critical pH also hinders demineralisation of the deposited calcium fluoride at the tooth surface. It has also been reported that fluoride at the enamel surface attracts and “binds” to calcium ions (155) , thus augmenting nucleation of new mineral at particular demineralised areas of the tooth (142). However, the exact mechanism of fluoride in the surface lesions is still not well understood.

Researchers are still debating over the exact role of fluoride in tooth remineralisation. It was reported that enamel dissolution was primarily a function of two external properties, namely the pH and fluoride concentration of surrounding solution (156). In another study, it was found that under simple pH cycling regimen,

low concentrations of fluoride, ambient 0.06 ppm, and toothpaste 1100 ppm, significantly reduced enamel mineral loss by 5% and 9%, respectively (156). Further analyses showed that after tooth wear lesions were remineralised with fluoride, the new enamel crystallites were dimensionally larger than the original sound enamel. Gate et al. (156) observed that the crystallites lacked organization, which rendered the enamel slightly less dense. Nevertheless, the probability for enamel to dissolve in subsequent acid challenges is decreased by the capacity for fluoride to bind to free Ca^{2+} and PO_4^{3-} . Because Fluoro apatite is far less soluble and it has a considerably lower buffering capacity, lower pH values will be required to force the dissolution of enamel (157).

2.5 Bioactive glass

Bioactive glass is a silica-based melt-derived glass that comprises of a silicon dioxide (SiO_2) content of less than 60%, a high disodium oxide (Na_2O) and calcium oxide (CaO) content, and a high calcium oxide to phosphorus pent oxide ($\text{CaO}:\text{P}_2\text{O}_5$) ratio (158). It is being used extensively in producing materials for human implants because it is a highly biocompatible material (120).

On their own, the constituents of bioactive glass are of little use in oral health. However, in the presence of saliva and water, a calcium phosphate layer forms and crystallizes to form hydroxyapatite, which is similar both chemically and structurally to the minerals in teeth (159). Bioactive glass was initially used in clinical practice as a biomaterial to replace lost bony tissue in the body. It bonds strongly with bone, and unlike metals and polymers that had been originally used as implants, bioactive glass is resistant to the aggressive defence mechanisms and corrosive fluids in the human body.

Currently, with novel technological advances in tissue engineering, bioactive glass has been credited with nearly a million clinical applications in the repair of osseous defects throughout the skeletal system in only a single decade (160). Research continues to confirm that bioactive glass is a feasible and an extremely promising alternative in bone grafts where its use as an adjunct in regenerative procedures is confirmed by multiple human (153, 161, 162) and animal studies (163-165). Bioactive glass is also known to possess superior homeostasis, good osteoconduction, bone resorbability and biocompatibility.

2.5.1 History and development

Bioactive glass was created by Professor Larry Hench. In 1967, he was an assistant professor at Florida University, where he mainly focused on materials made from glass and how they interacted with nuclear radiations. He was also researching effective treatment methods to alleviate injuries that soldiers sustained at the warfront. In 1969, he created small rectangles from 45S5 glass and with the collaboration of his colleagues they implanted the materials into rat femurs. They noticed that the glass materials resisted removal from the implant site--he said, *"These ceramic implants will not come out of the bone. They are bonded in place. I can push on them, I can shove them, I can hit them and they do not move. The controls easily slide out."* (166).

The first effective bioactive glass device was created to replace middle ear bones in patients with conductive hearing loss. It was beneficial in that it had the ability to successfully bond with bone and soft tissue, and it could effectively transmit sound waves from the tympanic membrane to the cochlear (167). The second device that was created using bioactive glass was the ridge maintenance implant, which was approved

for use in 1988 (168). It was designed to support lingual plates in the roots of teeth and provide a stable ridge for constructing dentures after the process of tooth extraction. The ridge maintenance implant consists of 45S5 bioactive glass, which is placed in extraction sites of fresh tooth. The materials then bond to bone and have proven to be very stable with low rates of failure (39).

Since its introduction by Hench in 1969, various compositions of bioactive glass have been researched. Xie et al. (169) used Vivoxid (S53P4), which is characterized by the following: di phosphorus dioxide (P_2O_2), 4%; calcium oxide (CaO), 20%; sodium oxide (Na_2O), 23%; and silicon dioxide (SiO_2), 53%. In another study, Vollenweider et al.(170), used NBG (45S4), which was characterized by: P_2O_2 , 4.9%; SiO_2 , 44.7%; Na_2O , 22.8%; and CaO, 27.6%. They also used Perioglass (Nova Bone or 45S5) and micron-sized particles. The current study involves the use of two bioactive glass products, Sylc[®] Prophy Powder and NUPRO[®] Prophylaxis Paste.

2.5.2 Composition

Bioactive glass has the following chemical composition and weight proportions: 45% SiO_2 , 24.5% Na_2O , 6% P_2O_5 , and 24.5% CaO. When implanted in the human body, a chemical reaction starts immediately. In an aqueous environment, bioactive glass starts surface reaction in three phases, namely leaching and exchange of ions, dissolution of the SiO_2 structure, and precipitation of calcium and phosphate ions to form a layer of apatite.

2.5.3 Variants

Bioactive glasses are categorized into two different classes, class A and B. Class A bioactive glass (Bioglass 45S5) is osteoproliferative. It can easily bond to soft tissues or bones and is used regularly in dental implants. Class B bioactive glass (Bioglass 8625) is osteoconductive, and it does not readily bond to soft tissues unlike Class A bioactive materials. Bioglass 8625 is used for encapsulating implanted devices and is mainly used in radiofrequency identification transponders in animal or human microchip implants. Owing to its incapacity to bond to bone and soft tissues, bioactive glass is fixed in place using fibrous tissues (171).

2.5.4 Properties

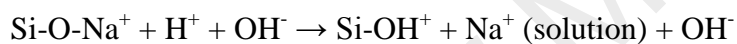
Bioactive glass is highly reactive in aqueous mediums, including saliva and other body fluids such as synovial fluid. This property is mainly due to its composition in SiO_2 and its high Na_2O content. This property of bioactive glass makes it very beneficial in clinical practice. However, it is limited by its low mechanical strength and fracture toughness (172). It has a fracture toughness of approximately $1.2\text{--}2.25 \text{ MN/m}^{3/2}$, which is lower than that for bone, estimated at $2.2\text{--}5.7 \text{ MN/m}^{3/2}$ (173). Its bending strength, which is approximately $40\text{--}60 \text{ MPa}$, also makes it unsuitable in the application of glass scaffolds for the repair of load-bearing bone defects (174). Implants of bioactive glass are also commonly used for human body implants that are buried and slightly loaded.

Another benefit of bioactive glass is its incapacity to easily form fibrous tissue, which makes it very practical for use in jaw implantations after tooth extractions (175).

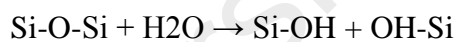
2.5.5 Mechanism of Action

The high biocompatibility and bonding features of bioactive materials have prompted much interest in the past few decades. Extensive studies over the past 30 years have demonstrated the series of reaction steps involved in bioactive glass bonding mechanisms within the body. After implantation of bioactive glass in the human body, a string of reactions lead to the formation of an appetite layer. Five critical steps have been described for surface reactions (2):

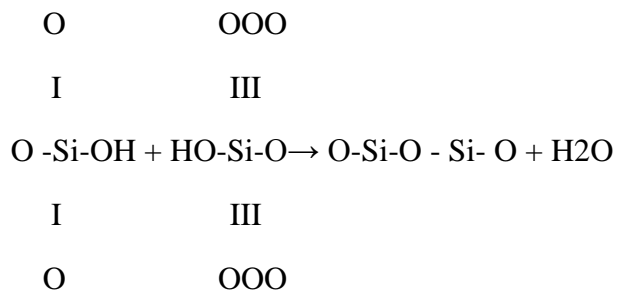
- 1) Rapid exchange of sodium or potassium ions with hydrogen or hydronium ions from solution:



- 2) The process of hydrolysis occurs, which includes the breakdown of Si-O-Si bridges to form independent groups of Si-OH (silanol). This leads to the disruption of the glass network.



- 3) Silanol condensation and re-polymerization takes place on the surface depleted alkalis and alkaline-earth cations.



- 4) Ca^{2+} and PO_4^{3-} groups migrate to the surface through the SiO_2 -rich layer forming a layer rich in $\text{CaO-P}_2\text{O}_5$ on the previously formed SiO_2 layer.

Subsequently, the amorphous $\text{CaO-P}_2\text{O}_5$ rich layer grows by the incorporation of soluble calcium and phosphates from solution.

- 5) The amorphous $\text{CaO-P}_2\text{O}_5$ layer is crystallized by incorporation of OH^- , CO_2 , or fluoride anions from solution to form a Fluor apatite layer.

2.5.6 Uses of Bioactive Glass

Bioactive glasses have been used in clinical practice for approximately 40 years. They were initially created to replace damaged tissues. First generation bioactive materials were designed to be bio-inert for the purpose of minimizing adverse effects in the host. It was not until the year 1969 that bioactive glasses were subject to a full fledged discovery. Owing to several years of research and investigations, the uses of the bioactive glasses have gone through a number of evolutionary stages. Now a days, billions of dollars are being invested in producing and applying bioactive glasses in the clinical setting.

2.5.6.1 Graft Materials

In order to minimize rejection, materials that are selected for grafting have to be biocompatible, bio resorbable, and osteogenic. Because they possess these properties, bioactive glasses have been used in filling bone defects in periodontal surgeries. They have also been used in autogenous bone grafts and alloplasts (171).

2.5.6.2 Endosseous Implants

Resorption of alveolar bone has been reported in many patients after dental extraction (176). This results in ill-fitting dentures thereby compromising mastication and causing oral and systemic health problems. Dental implants are frequently used by clinicians with the main aim of providing aesthetic restorations (177). Over time, the concept of proper implementation has morphed from one in which the approach was to

originally ‘bring the implant to the bone’ to the current paradigm in which it is believed to be more appropriate to ‘bring the bone to the implant’ for optimal and effective aesthetics and prosthetic results. Today’s oral surgeon is inclined to always preserve the alveolar ridge by reconstructing the site of extraction through the use of bone grafts-- although other less traumatic options such as the use of growth factors, recombinant human bone morphogenetic protein-2 may also be considered (178).

2.5.6.3 Remineralising Agents

Bioactive glasses have been incorporated into dentifrices to aid in remineralization of teeth (179). Owing to their osteogenic properties, bioactive glasses have been used in occluding dentinal tubules. In one report, Gillam et al. (43) compared a new dentifrice formulation that contained a modified bioactive glass material with the original 45S5 bioactive glass and found that original bioactive glass was easily dislodged when compared with modified bioactive glass. This finding suggested that, when utilized with an appropriate vehicle, bioactive glasses can be excellent in treating dentine hypersensitivity.

In another study Forsback et al. (42) found that when compared with regular commercial glass, bioactive glass released a greater amount of silica and caused lesser decalcification, suggesting that the bioactive glass S53P4 was more efficient in treating dentinal hypersensitivity.

2.5.6.4 Antibacterial Agents

Bioactive glass was found to cause osteo integration in aqueous media, prompting scientists to investigate its antibacterial activity (180). In their study, Allan et al. (2001) found that particulate bioactive glass had an antibacterial effect on certain oral bacteria, namely *Streptococcus sanguis*, *S. mutans*, and *Actinomyces viscosus*.

Zhang et al. (181) proposed that bioactive glasses owed their antibacterial effect to its alkaline nature.

2.5.6.5 Drug Delivery Agents

A degradable and bioactive borate glass was investigated in one study by Xie et al. (182), is found to be an effective carrier of vancomycin in the treatment of osteomyelitis in rabbits.

2.5.6.6 Bone Tissue Engineering

Over the past few decades, scientists have faced a big challenge to create a material that could mimic the extracellular matrix. After extensive research, it was found that scaffolds built using bio composite nano fibres and nano hydroxyapatite were, by nature, very porous. This consequently eased good cell occupancy, vascularity, movement of nutrients and metabolic waste products (171). Great advances were made when Xynos et al.(183) and Venugopal et al. (183, 184) found that bioactive glass ceramic templates produced increased osteoblast proliferation and differentiation, which help human fetal osteoblasts to adhere, migrate, proliferate, and mineralize into bone.

2.5.7 Products

Over the last few years, researchers have developed several bioactive glass products, but they have traditionally kept the P_2O_5 constant while varying the SiO_2 content. It is believed that when bioactive glass is integrated into toothpaste and other oral care products, ions are released from the amorphous calcium phosphate layer that help to remineralises the tooth surface. Bioactive materials have been successfully used clinically in dentine remineralisation and in treating dentine hypersensitivity (23, 24, 38, 40), and they have been approved by the Food and Drug Administration for use in oral and orthopaedic bone grafting for nearly two decades (185, 186).

Bioactive glasses have been marketed worldwide under several trade names (Table 2.2). It was demonstrated that fine particulate bioactive glasses (<90 µm), when integrated into an aqueous dentifrice, had the ability to reduce tooth hypersensitivity by occluding dentine tubules through the formation of a calcium phosphate (187). Bioactive glass powders, solutions, and extracts have also been shown to have a significant antibacterial effect toward caries pathogens, such as *Streptococcus mutans* and *S. sanguis* (180, 188).

One of the bioactive glass products sold under the brand name NovaMin® was patented in 2002. It is used in dental care products for tooth remineralisation. It is the key technology in many products, including Sensodyne® Repair & Protect toothpaste. The active ingredient in NovaMin® is the inorganic chemical calcium sodium phosphosilicate.

Several studies have been conducted to examine the effects of Novamin®-containing products. In one study, Tai et al. (189) matched 100 volunteers for gingival bleeding index (GBI), plaque index (PI), age, and gender. Volunteers were offered supra gingival prophylaxis to remove all calculus, extrinsic stain, and plaque, after which they underwent a baseline examination. The volunteers were then instructed to use an assigned dentifrice for six weeks. The authors measured the PI and GBI at baseline and at six weeks and observed that there was a decrease in gingival bleeding and supra-gingival plaque in volunteers who used a NovaMin®-containing dentifrice as compared with those who used a negative dentifrice. In another study conducted by Wang et al. (190), 30 dentine discs were divided into three groups of 10 and each group was treated with no brush, distilled water, and NovaMin®-containing toothpaste, respectively. The NovaMin®-based toothpaste was used twice daily for seven days, and dentine permeability was measured after successive chemical challenges with acids.

Based on their findings the authors concluded that the NovaMin®-based toothpaste significantly decreased dentine permeability after the 7-day treatment and showed excellent resistance to acid challenge compared to the other groups.

Recent findings from a study by Gjorgievska and Nicholson (12) show that the application of 16% carbamide peroxide to the enamel surface causes distinct morphological changes to the surface, which may vary from mild to severe. After bleaching, treatment with two toothpastes containing the bioactive glass NovaMin® resulted in the formation of a protective layer on the enamel surface. The authors observed that there were only slight differences between the two brands. Furthermore, they found that the application of these dentifrices also caused increases in the Ca and P levels of the enamel layer, which is similar to that of intact enamel.

Conversely, the protective effect of four commercial novel agents against dental erosion has been tested in another study by Wang et al. (36). In their study, the authors used ninety human molars (divided into several groups), which they incubated in human saliva for 2 hours. After a pellicle was formed, the teeth were then submitted to demineralisation and remineralisation through the application of paste slurries with varied levels of NovaMin®, separately and with/without the use of fluoride. The authors found no significant difference in the surface nano hardness of the enamel specimens among the groups and concluded that tooth erosion could not be prevented or repaired by NovaMin, regardless of the fluoride content of the toothpaste.

Table 2.2: Common commercially available bioactive glass products. (Adapted from Sauro et al. 2011)(39)

Product	Manufacturer	Constituents	Application Mode
Biosilicate®	Vitrovita, São Carlos, Brazil	P ₂ O ₅ -Na ₂ O-CaO-SiO ₂	Applied to dentine using a slow-speed hand piece and a rotating rubber cup
Colgate® Sensitive Pro-Relief™	Colgate Palmolive, New York, US	Hydrated silica, calcium carbonate, glycerine, 8% arginine, water, bicarbonate, flavour, cellulose gum, sodium saccharin, FD&C blue no. 1 (CI 42090)	Applied to dentine using a slow-speed hand piece and a rotating rubber cup
NovaMin®	Sultan Healthcare Inc., Englewood, New Jersey, US	SiO ₂ -Na ₂ O-CaO-P ₂ O ₅	Applied to dentine using a slow-speed hand piece and a rotating rubber cup
NUPRO® NU Solutions™ Prophy Paste	DENTSPLY corp., London, UK	Hydrated silica, glycerine, water, bicarbonate, flavour, cellulose gum, sodium saccharin and bioactive glass NovaMin®	Applied to dentine using a slow-speed hand piece and a rotating rubber cup
Sylc bioactive glass® Powder	Sylc, OSspray Ltd, Cambridge, UK	SiO ₂ -Na ₂ O-CaO-P ₂ O ₅	Applied to dentine using an air-polishing device
Sylc bioactive glass®H2O	Sylc, OSspray Ltd, Cambridge, UK	SiO ₂ -Na ₂ O-CaO-P ₂ O ₅ and deionized water	Applied to dentine using an air-polishing device

Although the past 50 years have witnessed advances in oral health care, dental erosion and hypersensitivity are common problems worldwide, especially in young adults (191-195). In Europe, as well as in most countries, changes in dietary habits may have contributed to the increased prevalence of dental erosion during the last ten years (26, 28). The consumption of acidic fruits and beverages has increased probably as a result of economic development, and children in particular have been exposed to campaigns aimed at promoting these products. Besides, fewer efforts have been put in

to encourage the marketing and distribution of less erosive foods and beverages. The results of this study may be beneficial to regular consumers of potentially erosive beverages as well as persons who suffer from GORD, anorexia and bulimia nervosa, which are common risk factors for erosion (72, 82, 196) . In addition, patients who suffer from dental hypersensitivity have reportedly experienced relief from their symptoms after using dentifrices incorporated with bioactive glasses (24), which are the subject of this research.

Current strategies to prevent or slow down dental erosion and treat dental hypersensitivity include a change of lifestyle (197), dietary analysis and counselling (71), treatment of GORD and vomiting (71), use of fluoride mouth rinses, varnishes and desensitizing agents (198), high fluoride concentration toothpaste (71), use of toothpastes incorporated with bioactive glass materials (40), appropriate oral hygiene (71, 197), and low abrasive toothpaste (199). By determining and comparing the efficacies of Sylc® Prophy Powder and NUPRO® Prophylaxis Paste on demineralized tooth, it would be possible to offer patients the best option for the management of dental erosion and hypersensitivity.

2.5.7.1 Sylc® Prophy Powder

Sylc® Prophy Powder is one of the products used in this study. It is a therapeutic prophylactic powder that is reported by manufacturers to reduce dentinal hypersensitivity, repair dental tissues and whiten teeth. It is applied with the help of air-polishing equipment. A prior laboratory study by Banerjee et al. (25) on patients who underwent prophylaxis treatment demonstrated that Sylc, compared to bicarbonates, was more effective at removing enamel stain in patients with poor oral hygiene as well as desensitizing good and poor oral hygiene groups.

Similar studies have been conducted to show the effect of Sylc on dentinal hypersensitivity. In one study, Sauro et al. (38) compared the effect of prophylactic powders and pastes on dentine discs from human third molars. The authors observed that prophy-powders and pastes were able to significantly decrease the dentine permeability of acid-etched samples; however, air-polishing procedures performed with Sylc resulted in the formation of a dentine surface that was resistant to citric acid attack.

Further studies have evaluated changes in dentinal permeability after prophylactic use of Sylc. Amaechi et al. (38), for example, evaluated dentine remineralisation induced by bioactive substances that were commonly used in preventive and operative dentistry and found that Sylc was the sole substance that could decrease dentine permeability after immersion in remineralising solution. It was also able to show hydroxyapatite precipitation as a sign of dentine remineralisation. In another study, Sauro et al. (23) used several prophylactic pastes or air-polishing powders on dentine discs from human third molars. They found that prophylactic pastes or air-polishing powders caused varying reductions on dentine permeability. However, Sylc and sodium bicarbonate were the most efficient in decreasing dentine permeability of the phosphoric acid-etched specimens. The authors attributed this to the presence of a compact multi-layered smear layer that covered the dentine surface, formed during air-polishing procedures with Sylc.

2.5.7.2 NUPRO® Prophylaxis Paste

The other product used in this study, NUPRO® Prophylaxis Paste, is used in removing dental plaque and staining. NUPRO® Prophylaxis Paste is available in fluoride-containing and fluoride-free formulations, and it is indicated for professional use in cleaning and polishing procedures. It is also available in several grits: fine or

gold, which is indicated for use in young patients and prophylactic procedures that necessitate minimal tooth abrasion; medium or blue, which is indicated for most cleaning procedures that necessitate a high level of polish; coarse or red, which is indicated in patients with considerable staining of the enamel due to smoking or excessive consumption of teeth-staining drinks; and plus or green, which is indicated for heavy-duty cleaning of stain that is difficult to remove through conventional procedures (NUPRO® Prophylaxis Paste, Packet Insert). The formulation without fluoride is indicated for use prior to pit and fissure sealing. Prophylaxis Paste comprises pumice, glycerine, diatomite (present in fine grit only), sodium saccharin, water, sodium silicate, flavouring, colour, thickeners, and preservatives (NUPRO® Prophylaxis Paste, Packet Insert).

Over the last ten years, researchers have investigated the effectiveness of NUPRO® Prophylaxis Paste on patients with dentine hypersensitivity. In 2012, Hamlin et al. (200) studied the clinical efficacy of a single professional treatment with an in-office desensitizing paste followed by twice daily brushing with a desensitizing toothpaste and toothbrush for 24 weeks. They recruited 100 adult patients with dentine hypersensitivity whom they randomly assigned to two groups. The test group underwent dental scaling, followed by a single in-office treatment with a desensitizing paste containing 8% arginine and calcium carbonate and twice daily brushing for 24 weeks with desensitizing toothpaste composed of 8% arginine, calcium carbonate with 1450 ppm fluoride using the Colgate Sensitive Pro-Relief toothbrush. The negative control group, on the other hand, underwent dental scaling, followed by a single in-office treatment with Nupro-M pumice prophylaxis paste and 24 weeks of twice daily brushing twice with non-desensitizing toothpaste containing 1450 ppm fluoride using the Oral-B Indicator toothbrush. After in-office procedures and after 8 and 24 weeks of

twice daily brushing, the authors (200) found that patients in the test group showed statistically significant improvements in dentin hypersensitivity compared to those in the negative control group in tactile (49.8%, 57.5% and 32.9%, respectively) and air blast sensitivity (26.0%, 38.4% and 34.3%, respectively) scores.

Milleman et al.(24) conducted a study to test the dentin hypersensitivity reduction of NUPRO Sensodyne® Prophylaxis Paste with NovaMin®, with and without fluoride, as compared with a standard prophylaxis paste without fluoride following dental scaling and root planing. They (24) found that both NUPRO pastes were effective in reducing dentinal hypersensitivity immediately after one application following dental scaling and root planing, and there was no statistical difference between the pastes with and without fluoride.

2.6 Methods of Assessing Demineralisation and Remineralisation of Dental Hard Tissue

Dental tissue undergoes histological changes as a result of erosion. Because of the histological differences between dentine and enamel, these two tissues respond differently to erosion (201). When assessing dental erosion, the dentist should bear in mind that the choice of the technique depends mainly on the stage of erosion, the expected modifications in the structure of the eroded tissue, and the tissue studied. According to a previous report (202), profilometry was the most common quantitative technique to assess dentine and enamel *in vitro*, *in situ*, and in clinical studies. The next commonly applied technique was quantitative evaluation of surface hardness (enamel) and microradiography (dentine). Regarding qualitative studies, the authors reported

scanning electron microscopy (SEM) to be the most frequent technique used for qualitative assessment of erosion in dentine and enamel.

2.6.1 Quantitative Methods

2.6.1.1 Chemical Analysis of Dissolved Materials

Chemical analysis has been employed to investigate erosion by measuring the concentrations of calcium and phosphate ions that are released into the dissolving solution. Similarly, the pH and uptake or release of other constituents, including fluoride and magnesium ions have been measured (203). Calcium analysis is performed using an ion-selective electrode (204). However, the shortcoming of this technique involves errors caused by complexation by some acids (205). To overcome such errors, the atomic absorption spectrometry has been used. It has proven to be a reliable and sensitive method for analysing calcium (202), as interference by other solutes are circumvented. Another advantage of the atomic absorption spectrometry is the possibility of using it to quantify erosion in both dentine and enamel (204).

In an *in vivo* study, Young et al. (206) used extra-oral erosive challenge to analyse mineral release in healthy human teeth. However, this technique is limited in that the presence of saliva precludes erosive challenge. In addition, with this method, it is impossible to determine possible mineral gain or the occurrence of physical and morphological modifications.

2.6.1.2 Surface Hardness

Surface microhardness is measured with the use of a Knoop or Vickers diamond indenter, which determines the resistance of a substance to the indenter. The use of surface Microhardness is more appropriate during the early stages of erosion (202). It also has the added advantage of obtaining accurate information about erosion in its

early stages and of being relatively cheap. It is probably for these reasons that this method is commonly used in dentistry (207).

The depth of penetration by the Knoop and Vickers diamond indenters vary, with the Vickers diamond indenter having a greater penetration, estimated at 5 μm as compared with 1.5 μm for the Knoop diamond indenter (208). These differences arise due to the varying loads that are impressed by the indenters on dental tissue, typically 50 and 200 g for the Knoop and Vickers diamond indenter, respectively. Hence, it can be implied that sensitivity is higher with the Knoop indenter for changes that involve the most superficial layers of eroded tooth.

Surface hardness tests have the advantage of detecting modifications in the surface hardness of enamel within a few minutes of exposure to an erosive substance (145, 204). Nevertheless, these tests cannot clearly delineate the indentation boundaries in advanced erosion. Furthermore, when certain substances such as fluorides are deposited on tooth surface, these tests may not accurately determine surface hardness. This is because the application of fluorides causes the formation of precipitates on dental surface, making it difficult for indenters to penetrate the surface of the test material (202).

Further research shows that surface hardness tests may not be appropriate for assessing dentine erosion. Herkströter et al. (209) demonstrated that demineralized dentine sustained changes in length (of approximately 30% reduction) within 2 hours after indentation. The authors attributed this change to retraction of the exposed matrix after compression and shrinkage, which was caused by desiccation.

Nano indenters have also been successfully used in assessing enamel erosion (210). Nano indentation is a newer technique that uses the same principle as

microhardness indentation although at a smaller scale. This technique usually gives results as hardness and reduced elastic modulus in Pascal's (Nm^{-2}). While microhardness techniques usually yield indentation depths of micrometres or tens of micrometres in healthy enamel, Nano indentation yields depths in sub-micrometres (211). Nano indenters are better in that they penetrate dental tissue less deeply than microhardness indenters (212) and hence, they can better detect minimal changes on tooth surface. They are limited because they have to be combined with atomic force microscopy (AFM) to assess dentine erosion (213).

2.6.1.3 Surface Profilometry

Surface roughness is generally measured by using a profilometer, which can be contact (uses a diamond tip) or optical (uses a light beam). In contact profilometry, a diamond tip of fixed radius 1.5–2.5 mm is usually used (66); however, the shape of the tip can vary (67). While chisel-point ($0.25\ \mu\text{m} \times 2.5\ \mu\text{m}$) tips may be used for detecting bumps in a surface, conical tip are almost exclusively used for measuring surface micro roughness, with the load ranging from 0.05 to 100 mg (214). An analogue / digital signal is generated by the vertical movements of the stylus, as it is dragged across the surface of the material. A recording speed of approximately 1 mm/s is usually maintained in order to minimize the effect of external vibrations and electrical interferences on the accuracy of the lateral resolution (214). The vertical resolution can be as low as 0.1 nm for smooth surfaces or as high as 1 nm for rough surfaces. However, because the stylus is practically in constant contact with the surface that is being measured, there is a risk of the diamond tip causing damage to the specimen (207).

Many of the drawbacks of stylus profilometers can be overcome by laser profilometers, as they do not directly touch the surface of the specimen. In this technique, a light spot is directed at the surface of the specimen, typically below 100 mm in diameter. Surface topography can be profiled either by measuring the deflection of the laser beam, or (with white light) by utilizing the confocal principle (215). A main limitation of laser profilometry is that the results can be affected by colour and transparency (214). It is usually necessary to record a polyvinyl siloxane impression of the sample, which is then scanned by the laser profilometer to overcome translucencies on the surface. The surface colour of the specimen, however, affects the laser profile. In studies using laser profilometers at wavelengths of 785 nm, it was demonstrated that specimens with darker colours had a higher roughness. Further, the authors De Long et al.(216) showed if an impression material absorbed colour at a wavelength similar to that of the laser, then the surface will not be scanned.

Other parameters are also used to measure tooth surface changes (Table 2.3). These variants usually measure the average distance between the highest peaks and valleys of the profile and can also truncate some outlying peaks and valleys, depending on the engineering system employed for measurement (214). Although these systems measure the effects of surface change in the best possible way, it is very challenging for untrained or unqualified personnel to interpret the tabular form of the parameters.

Table 2.3: Common Amplitude Parameters for Surface Measurement [adapted from Field et al. (2010) (255)]

Parameter	Description
R_a	Arithmetic average of all deviations of the profile from the centreline
R_q	Geometric average of all deviations of the profile from the centreline
R_z	Mean of five roughness depths of five successive sample lengths of the profile
R_{max}	Largest of the five roughness depths
R_p	Height of the highest point above the centreline within the length of the profile
R_v	Depth of the lowest point below the centreline within the length of the profile
R_{pm}	Mean value of R_p in five consecutive sample lengths
R_t	Vertical height between the highest and lowest points of the profile within the evaluation length
R_{tm}	Mean value or R_{max} in five consecutive sampling lengths
R_{3z}	Similar to R_z except the individual roughness depth is the depth from the highest peak to the third lowest valley within the sample length

2.6.1.4 Microradiography

In this technique, X-ray beams are directed toward an enamel section, and a photographic plate (211) is used to record the penetrating radiation. The mineral content of enamel is thus quantified by measuring the attenuation of X-rays transmitted through a section of the tissue by comparing it with a reference value. There are three generations of micro radiography (207). These include: longitudinal micrography, which is used to assess erosion, abrasion, and erosion-abrasion in dentine and enamel *in vitro* and *in situ* (124, 217); transverse micrography, which is widely used in assessing carious lesions but has been modified for studying erosion (218); and wavelength-dependent micrography, which is used to measure mineral content in whole teeth (219).

2.6.1.5 Other Methods

Other methods have been used by researchers to assess erosion in dentine and enamel. These include quantitative light-induced fluorescence and optical coherence tomography, which are non-invasive techniques. Schlueter et al. and Bozkurt et al. (202, 220) suggested the use of ultrasound for assessing enamel thickness, while Huysmans and Thijssen (221) suggested that it may be a potential tool for studying dental erosion. However, the above-mentioned techniques are novel and their use has not yet been validated in clinical practice (202).

The iodide permeability test has also been used in assessing erosion. According to Attin (222), this technique is cheap and it can be used for the rapid detection of erosion in enamel but not dentine.

2.6.2 Qualitative and Semi-quantitative Methods

Changes to dental tissue that are brought about by erosive agents can be studied by qualitative methods. In most cases, microscopy, either alone or in combination with quantitative techniques can be employed to study erosion in dentine or enamel.

2.6.2.1 Transmitted Light Microscopy

In transmitted light microscopy, erosive lesion of dentine and enamel can be visualized and quantified (223). Saunders and McIntyre (120) found that polarized-light microscopy detected changes on erosive enamel to a lesser extent than on carious enamel. Conversely, it could accurately differentiate between partly and completely demineralized tissues in the case of eroded dentine.

2.6.2.2 Confocal Laser Scanning Microscopy

This technique is mostly employed to obtain qualitative data by using monochromatic laser light. It has been used to quantify loss of dental tissue brought about by erosion and softening depth (202). Although it is used in quantifying demineralisation in carious dentine, its use in dentine erosion has not yet been determined (202).

2.6.2.3 Transmission Electron Microscopy

Researchers have used transmission electron microscopy to assess the impact of acids on the salivary pellicle (143). It has also been used *in vivo* to study changes caused by early microbial colonization of human enamel (224).

2.6.2.4 Scanning Electron Microscopy

Scanning electron microscopy (SEM) is used to qualitatively estimate surface alterations after erosive attacks. It has also been used to evaluate the efficacy of salivary

acquired pellicle to protect underlying enamel surface from acidic attacks (143) to show superficially deposited precipitates that resulted from mineral dissolution with acids (225). This test can be performed on polished and unpolished surfaces after gold-sputtering, and the severity of surface alteration can be graded by using individually adopted scales.

In enamel, acid attacks due to immersion of specimens in erosive solutions cause the surface to have an etching pattern, and enamel prisms are exposed to an extent that depends on the severity of the erosive challenge. In dentine, treatment with an acidic solution may cause the dentin tubules to open (56). Common scanning electron microscopes could cause the specimens to lose moisture due to necessary preparation of the specimens for the SEM investigation. The loss of moisture may cause additional changes in the eroded surface. To prevent collapse of the fragile eroded enamel surface structure, freeze drying of samples was suggested (226). Precipitates formed by dissolved enamel mineral may block the enamel surface so that the eroded enamel prism structure might not be seen with SEM. Eisenburger et al. (226) recommended that before removing samples from the acidic bath, the acid should be neutralized to decrease the risk of artificial re-precipitation. The delicate surface should be impregnated with methacrylate or dentin adhesives to permit the production of resin replicas (56). After the enamel sample has been completely dissolved with hydrogen chloride, the resin replicas could then be examined with SEM, providing insight into structural surface and subsurface alterations.

Although SEM has been demonstrated to be an efficient tool for studying the ultra-structural changes associated with erosion in enamel and dentine (129, 227, 228), the investigation is limited because it causes drying artefacts, and specimens must be coated with metal or carbon to prevent charging. Serial measurements are not possible

with conventional SEM and hence, the effects of treatments cannot be studied using this test. Environmental SEM partially overcomes this limitation, as it permits the observation of specimens under humid conditions (202). Thus, specimens do not have to be dried or coated. While the resolution is lower than that of conventional SEM, environmental SEM is potentially useful for future investigation, particularly in cases where specimens have to be examined several times (202).

2.6.2.5 Scanning Probe Microscopies

Some examples of scanning probe microscopies include atomic force microscopy (AFM) and scanning tunnelling microscopy, which can give resolutions at a molecular or atomic level (202). Atomic force microscopy is advantageous in that artefacts can be decreased or circumvented and demineralisation can be assessed in both enamel (212) and dentine (229).

2.6.2.6 Energy-Dispersive X-ray Spectroscopy

When used in combination with environmental SEM, energy-dispersive X-ray spectroscopy can provide data about the constitution of a specimen from the nature of the X-rays that are given off after bombardment (202).

2.6.2.7 Secondary Ion Mass Spectroscopy

This is a semi-quantitative technique which has been used to study enamel erosion (207). However, there are no data in dental research that describe the use of this method in assessing dentine erosion.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample Collection

44 human molars and premolars were collected from the orthodontic and oral maxillofacial clinics, Faculty of Dentistry, University Malaya. The teeth had to be free of erosive lesions, caries, restorations, stains and cracks.

3.2 Disinfection, Cleaning, and Storage

The teeth were disinfected according to ISO/TS 11405:2003 by storing them in 0.5% chloramine-T trihydrate solution for seven days at room temperature. After disinfection, each tooth was cleaned with ultrasonic scaler (Peizon Master 400, Switzerland) to remove any calculus, debris, and soft tissue which were still remaining. The cleaned teeth were then placed in distilled water and stored in a refrigerator at 4°C. The water was changed weekly during the storage period.

3.3 Specimen Preparation

A total of 88 tooth specimens were prepared (n=80): 44 enamel and 44 dentine. The crown portions of teeth collected were used to prepare enamel specimens, while root dentine was used to prepare dentine specimens.

3.3.1 Sectioning

Under continuous water agitation, a low speed precision cutter (Metkon® Micracut 125 Bursa/Turkey) was used to separate the crown and root portion at the cemento-enamel junction. Standardized 5x5mm² enamel samples were prepared from the middle third of the buccal surface of the crowns. For dentine samples, 5x5mm² were cut cross-sectionally from the middle portion of the outer surface of roots using a low speed precision cutter (Metkon® Micracut 125 Bursa/Turkey) under continuous water flow.

3.3.2 Mounting

Square blocks of equal size of $8 \times 8 \text{ mm}^2$ were prepared from a modelling wax to fix the crowns and roots in order to prepare the enamel and dentine specimens. A thin uniform layer of separating media (Silicone mould release, PACE Technologies, USA) was applied on the silicone moulds with help of a small painting brush. Cylindrical moulds measuring $3 \times 3 \times 1 \text{ cm}$ were filled with epoxy resin (PACE technologies, Quick mount 2 Epoxy Resin, USA) according to the manufacturer's instructions, and the specimens were placed on the wax beds embedded inside these moulds. Specimens were kept untouched for 6 hours as per manufacturer's instructions to ensure the absolute setting of epoxy resin. For the easy removal of the specimens, they were momentarily dipped in boiling water for 10 seconds.

3.3.3 Grinding and Polishing

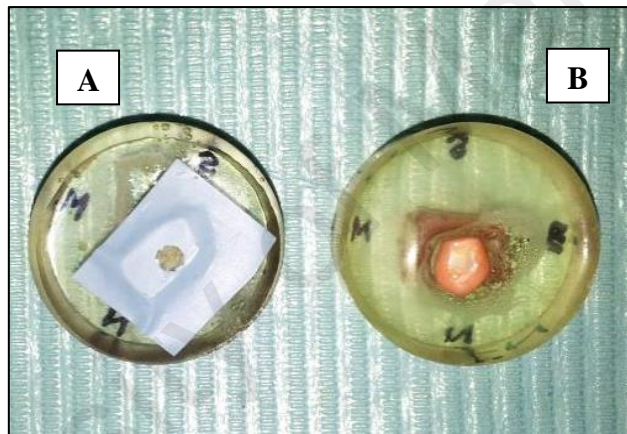
Proceeding towards the final preparations, the samples were ground under constant water coolant to achieve a flat surface using 420, 800 and 1200 grit silicon carbide paper (ExtecCorp, USA) followed by polishing with Microlux-R $0.3 \mu\text{m}$ polishing compound (Adolf Miller Company, Providence USA) on a polishing cloth (Extec Plano Cloth, ExtecCorp, USA) in order to produce a smooth working surface.

The polished samples were cleaned in ultrasonic cleaner (Wise Clean, Ultrasonic cleaner set, Model no: WUC-A02H, Korea) for 3 minutes in distilled water to ensure complete removal of any polishing material.

3.3.4 Work window Preparation

A standardized working window of 2 mm diameter was created on all enamel and dentine specimens using two different techniques. As the buccal surface of the

crown is convex from an anatomical point of view, it was not possible to stick and retain the cut wallpaper windows on enamel specimens during the 6-day experimental cycle. Therefore, to standardize the working area throughout the experimental cycle, the working windows on enamel and dentine specimens were created with different techniques. On all enamel specimens, the 2mm circular working window was created using acid resistant nail varnish (Maxfactor-Infinity[®], USA). On the dentine specimens, the windows were created by cutting a circle of 2 mm diameter from 2×2 cm² acid resistant and waterproof wallpaper (IKEA wallpaper, UK). The pieces of wallpaper were then stuck to the dentine specimens.



*Figure 3.1 : (A) Dentine specimen with circular work window made by wallpaper.
(B) Enamel specimen with circular work window made by nail varnish.*

3.3.5 Specimen Identification

To enable identification, each specimen was numbered on the mounting resin. Enamel specimens were numbered from 1 to 44, while dentine specimens were numbered from 45 to 88 using a permanent marker (OHP Marker-Art line 855, Malaysia).

To ensure exact repositioning of the specimens during testing, cardinal points were marked on the sides of the specimens with a permanent marker (OHP Marker-Art line 855, Malaysia).

3.4 Specimen Randomization:

Total of 44 enamel and 44 dentine specimens were prepared. 14 enamel and 14 dentine specimens were separated for qualitative analysis with a scanning electron microscope. The remaining (n=60) i.e. 30 enamel and 30 dentine specimens were separated for quantitative analysis and were randomly distributed into 6 treatment groups comprising 1 control and 2 test groups in both enamel and dentine and were labelled as follows:

Group 1(EC):	Enamel Control -Remineralising solution
Group 2(EN):	Enamel Test 1 -Nupro® prophylaxis paste
Group 3(ES):	Enamel Test 2 -Sylc® prophy powder
Group 4 (DC):	Dentine Control -Remineralising solution
Group 5(DN):	Dentine Test 1 -Nupro® prophylaxis paste
Group 6(DS):	Dentine Test 2 -Sylc® prophy powder

Table 3.1: Composition and manufacturer's details of materials used in this study.

Code	Material	Manufacturer	Lot	Composition
NU	NUPRO Sensodyne® Prophylaxis paste	Sensodyne, Novamin® DENTSPLY, USA.	13010301	Sodium Flouride 1.23%, Glycerine, Pumice, Calcium Sodium Phosphosilicate Novamin®, Titanium Dioxide, Methyl Salicyclate, Purified water, Sodium carboxymethyl cellulose, Sodium Saccharin, Flavour.
SY	Sylc® Prophy powder by Novamin®	OSSPRAY™, United Kingdom.	0040	Calcium Sodium Phosphosilicate, Novamin®.
DS	Demineralising solution	A&C American Chemicals Ltd, Montreal, Canada.	ZZJF 4- 5020	0.003 M citric acid at pH 3.2
RS	Remineralising solution	A&C American Chemicals Ltd, Montreal, Canada	ZZJF 3- 5020	KCL, 8.83mM MgCl ₂ .6H ₂ O, 0.29mM CaCl ₂ .2H ₂ O, 1.13mM K ₂ HPO ₄ , 4.62mM Fluoride, 0.022ppm

3.5 Testing:

After final preparation, the specimens were washed with distilled water and dried with a three-in-one air syringe for 10 seconds. For all 60 specimens (30 enamel and 30 dentine), the surface roughness (R_a) of pre-specimens were measured using the Infinite FocusG4 microscope (IFM, Alicona Imaging, Grambach/Graz, Austria). The 30 enamel specimens were also tested for surface microhardness (SMH) using microhardness tester equipment (HMV-2 series Shimadzu Corporation, Japan) with the Knoop indenter prior to any treatment. A scanning electron microscope (Quanta

250FEG, Japan) was used to capture the pre-specimen images of the surfaces of two enamel and dentine specimens each.

Each enamel and dentine specimen was immersed in 200 ml of citric acid at pH 3.2 and temperature of the solution was kept constant at $24 \pm 0.1^\circ\text{C}$ and stirred constantly at a speed of 400 rpm for 10 minutes. The specimens were retrieved from the solution and washed with distilled water. A three-in-one syringe was used to dry the specimens for 10 seconds. Similar to the pre-specimens, all enamel and dentine specimens were tested for surface roughness while enamel specimens were tested for surface microhardness as well. The data were recorded as baseline readings. To assess the effect of citric acid, 2 specimens of enamel and dentine each, were imaged using Scanning Electron Microscopy (SEM), and the findings were recorded as baseline images.

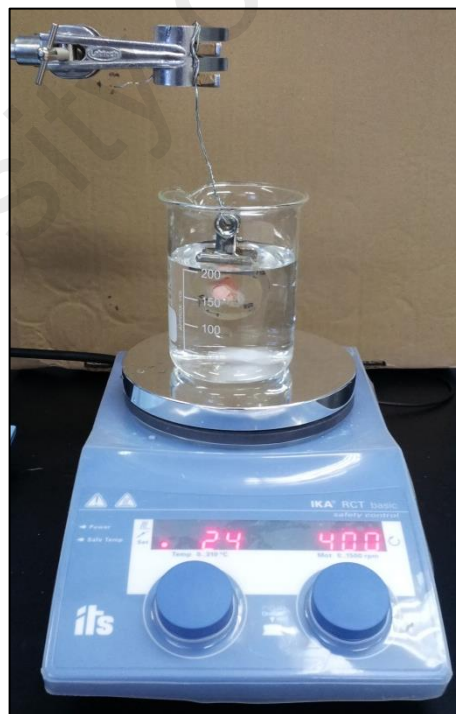


Figure 3.2: Erosion induction apparatus

Next, the test materials were applied on the enamel and dentine samples according to the previously determined test groups.

- Nupro® Prophylaxis paste (Nupro): The paste was applied on 10 enamel and dentine specimens (Group 2 and 5) using a rotating rubber cup attached to a slow speed contra-angled hand piece for 30 seconds according to manufacturer's instructions. The specimens were individually washed to remove Nupro and subsequently dried for 10 seconds using a three-in-one compressed air syringe. The specimens were then stored in a 50 mL glass container in remineralising solution in the incubator at 37°C.

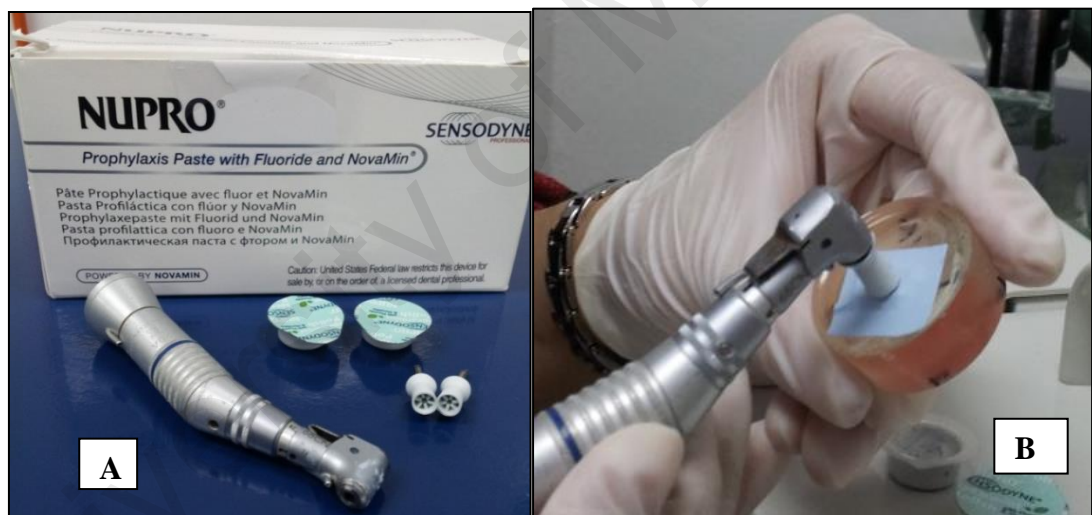


Figure 3.3: (A) showing Nupro® prophylaxis paste, hand piece, and rubber cups. (B) Application of Nupro® prophylaxis paste on a specimen.

- Sylc® Prophy Powder (Sylc): The powder was applied on 10 enamel and dentine specimens each (Group 3 and 6) using the prophylaxis air polishing device (Veloplex). A Sylc Smart Tip was attached to the polishing device and Sylc was air blasted onto the surface of each

specimen for 15 seconds at a constant distance of 5 mm according to manufacturer's instructions. Subsequently, all treated specimens were washed, dried, and stored in remineralising solution (RS) in the incubator at 37°C.



Figure 3.4: Sylc® prophy powder and polishing device



Figure 3.5: Sylc application on specimen

- Control group: All enamel and dentine specimens in the control groups (Group 1 and Group 4) were placed in remineralising solution immediately after erosive treatment and stored in the incubator at 37°C.

24 hours after Intervention, all the specimens in the test and control groups were retrieved and the remineralising solution was drained. The specimens were washed and dried for 10 seconds. The enamel and dentine specimens were imaged with a non-contact profilometer to record surface roughness readings and data was recorded as Intervention. The enamel specimens were further tested for surface microhardness. Ten specimens (5=enamel, 5=dentine) were imaged under SEM to evaluate the changes after Intervention and the elemental analysis EDX was also done. From control groups of both enamel and dentine only one specimen from each was imaged as there was no intervention done on the control group specimens. Whereas two specimens of each enamel and dentine were imaged from test groups.

Subsequently, all the remaining dentine and enamel specimens were again subjected to the erosive cycle as described above before immersing in fresh remineralising solution and eventually stored in an incubator.

24 hours later the specimens were again removed from the incubator washed and drained. All specimens were subjected to quantitative analysis with non-contact profilometer. The enamel specimens were further subjected to surface microhardness testing. These data was recorded as Day 1 of demineralisation as this is the first acidic exposure after application of materials on the specimens.

After the quantitative data was recorded, the enamel and dentine specimens were once again treated with the acid challenge as described previously before storing in the incubator with fresh remineralising solution for the next 24 hours.

All the samples were subjected to the same cycle of quantitative analysis (non-contact profilometer and SMH) followed by Demineralisation-Remineralisation cycle for 6 consecutive days and the data was recorded accordingly. As mentioned previously, only the enamel samples were tested for SMH.

SEM images of enamel and dentine samples were only imaged at Baseline, Intervention and Day 6 of the Demineralisation-Remineralisation cycle. The elemental analysis EDX was done on Intervention day and at the end of the experimental cycle.

The figure below shows a flowchart which details the distribution of the specimens and the experimental cycle (Fig 3.6).

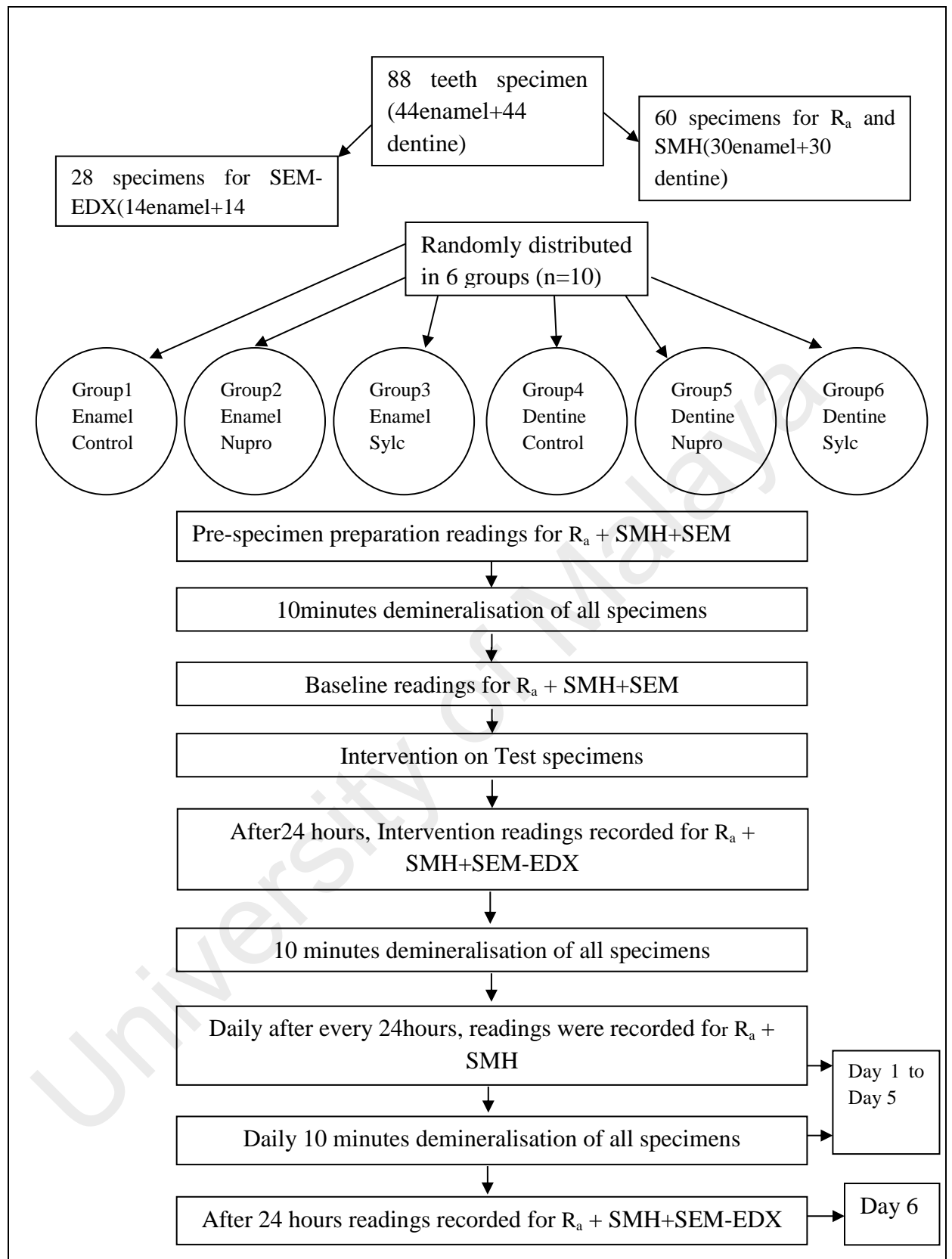


Figure 3.6: Flow chart of experimental cycle

3.6 Surface Roughness (R_a) Measurements and Outcome Measure

Surface roughness (R_a) indicates the area between the roughness profile and its mean line or the integral of the absolute value of the roughness profile height over the evaluation length. Roughness includes the finest irregularities of a surface. To measure R_a of specimens in this study, a novel Focus 3D scanning microscope (Infinite FocusG4 microscopy; IFM, Alicona Imaging, Grambach/Graz, Austria) was used as a non-contact profilometer to capture the 2D topography of enamel and dentine surfaces at every step. This microscope is based on a variation of focus procedures, which combine the functions of meteorology and microscopy in a single optical instrument. Its operating principle combines the small depth of focus of an optical system with vertical scanning. For profile analysis, IFM G4 software (Alicona imaging/ ISO 4287/4288) was used. The specimen to be measured was placed onto the motorized stage and was illuminated with modulated white light. The coaxial white light was provided by a light source delivered through a beam splitter. The specimens reflected light was projected through a beam splitter on to a colour digital sensor. An area of $350 \times 350 \mu\text{m}^2$ with no visual defects was chosen in the image field and measured for R_a within the work window of the specimen. In that selected area, five different scan lines of $1\mu\text{m}$ each were drawn to measure the R_a and their mean was recorded at each time point. The images were taken at magnification of 20X with vertical resolution of $0.02 \mu\text{m}$ and lateral resolution of $0.004 \mu\text{m}$. The Lambda C (L_c) was adjusted to $80\mu\text{m}$. The topographic information was registered to a 2D data file and then reconstructed by the software. To ensure the exact repositioning of specimens every time R_a was measured, a white sheet of paper measuring $210 \times 297\text{mm}$ was pasted on the motorized stage, which had the diagram of the base of the specimen. Cardinal points were marked on the

diagram. Similarly, the specimens were marked to define the cardinal points such that the points would be aligned with those on the paper (Figure 3.7).

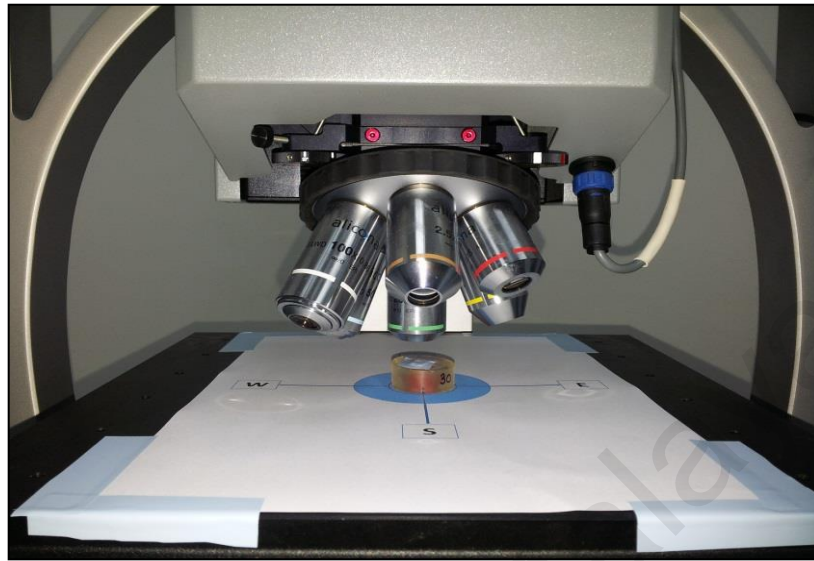


Figure 3.7: White paper sheet marked with cardinal points, pasted on the stage of Infinite focus microscope G4, Alicona. Specimen is ready for surface roughness analysis.

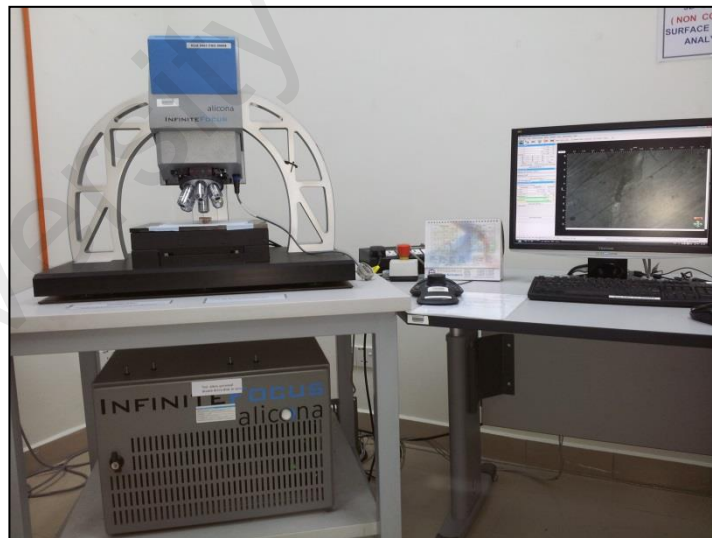


Figure 3.8: Infinite Focus Microscope G4, Alicona, Surface texture analyser.

The outcome measure for surface roughness is expressed as the percentage of surface roughness change (ΔR_a), calculated based on the differences between Surface Roughness reading at Baseline $R_a(t_0)$ or at the Intervention Day, $R_a(t_1)$ with that of the subsequent erosion intervals, $R_a(t)$, as shown below:

$$\Delta R_a(t_0) = 100 \left[\frac{Ra(t) - Ra(t_0)}{Ra(t_0)} \right]$$

Or

$$\Delta R_a(t_1) = 100 \left[\frac{Ra(t) - Ra(t_1)}{Ra(t_1)} \right]$$

3.7 Surface Microhardness (SMH) Measurements and Outcome Measure:

The Surface Microhardness (SMH) of the enamel specimens was determined using microhardness tester equipment (HMV-2 series Shimadzu Corporation, Kyoto, Japan) with Knoop diamond indenter. The Knoop indenter was diamond ground to a pyramidal form that produced a diamond-shaped indentation. Enamel specimens were placed on the electric XY stage. The test force was selected as 50 g and the force duration was adjusted as 10 seconds per indentation. Three indents were made at a distance of 50 μm from each other in a plane. After an indentation was made, the electric turret automatically revolved, making the objective lens with 40X magnification ready to measure the long diagonal length, L , of the indentations. The mean of the three values was calculated and recorded as surface hardness of the specimen each time. The Knoop Hardness Number (KHN) was calculated automatically through the software

installed in Shimadzu Microhardness Tester HMV-2 series, using the following formula:

$$KHN = \frac{\text{load(kgf)}}{\text{impression area(mm}^2\text{)}} = \frac{P}{C_p L^2}$$

Where L is length of indentation along its long axis, C_p correction factor related to the shape of the indenter ideally, 0.070279 and P is equal to load.

As described earlier, cardinal points were marked on the specimens to ensure their exact repositioning on the working stage.

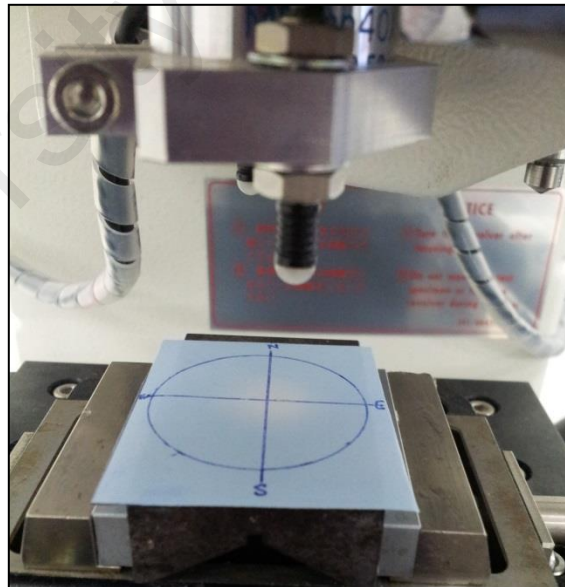


Figure 3.9: For repositioning of specimens a white paper sheet with cardinal points marked on it, pasted on working stage of surface microhardness tester.

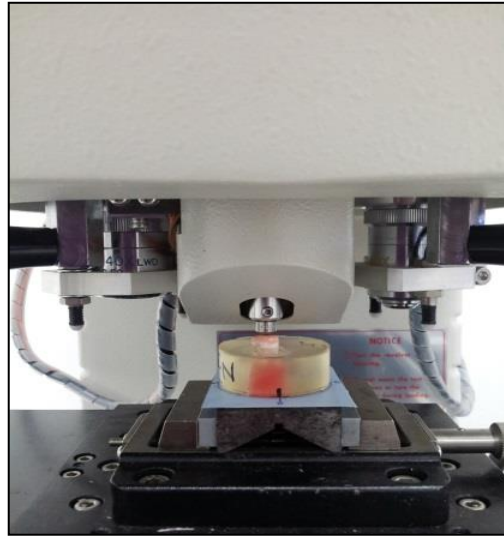


Figure 3.10: Specimen ready for surface microhardness test

The outcome measure for surface microhardness is expressed as the percentage of surface microhardness change (ΔSMH), calculated based on the differences between Knoop Hardness Numbers at Baseline, $\text{KHN}(t_0)$, or at Intervention Day, $\text{KHN}(t_1)$ with that of the subsequent erosion intervals, $\text{KHN}(t)$, as shown below:

$$\Delta\text{SMH}(t_0) = 100 \left[\frac{\text{KHN}(t) - \text{KHN}(t_0)}{\text{KHN}(t_0)} \right]$$

Or

$$\Delta\text{SMH}(t_1) = 100 \left[\frac{\text{KHN}(t) - \text{KHN}(t_1)}{\text{KHN}(t_1)} \right]$$

3.7.1 Indentation depth

Based on the known diamond pyramid geometry of the Knoop indenter (230) the depths (short diagonal) of the indentations, h , were calculated from the measurements of the long diagonals, L , as shown in the equation below:

$$h = \frac{L}{2 \tan(86.25)}$$

3.8 Scanning Electron Microscope (SEM) Imaging & Elemental analysis (EDX):

A total of 24 specimens (12 enamel and 12 dentine) were prepared for this part of the study. The specimens were imaged under a scanning electron microscope (Quanta 250FEG, Japan) at 2000X, 3000X, 5000X magnifications at different time points, namely pre-specimen preparation, baseline, Intervention and Day 6 of the experimental cycle.

For each time point, a separate specimen was prepared and imaged i.e. 2 specimens each for enamel and dentine were scanned under SEM at all three magnifications to capture the pre-specimen preparation images. 2 specimens each of enamel and dentine were exposed to acidic challenge and scanned for baseline images at all three magnifications. Similarly, six specimens were prepared: 3 enamel and 3 dentine, separately. The specimens were exposed to one erosive cycle as mentioned above and then the testing material was applied on test specimens leaving the control specimens as untreated. The specimens were left in remineralising solution for 24 hours and removed thereafter. The specimens were then dried and scanned under SEM and images were captured to see post intervention images of test groups. In order to get the images of Day 6, six specimens were prepared separately one from each group and they completed the experimental cycle of six days and scanned under SEM for the Day 6 images.

To determine the presence and thickness of layer of BAG on the surface of test group specimens after Intervention day and at the end of experimental cycle Day 6, eight additional specimens were prepared i.e. 4 enamel and 4 dentine. These 4 specimens of each enamel and dentine groups were divided into sets of two specimens each for enamel and dentine. One set of two enamel (1=Nupro, 1=Sylc) and two dentine

(1=Nupro, 1=Sylc) specimens was sectioned longitudinally and the cross sectioned surface was imaged to confirm the presence and thickness of applied test materials on these specimens after Intervention day. The second set of four (2=enamel, 2=dentine) specimens were sectioned on Day 6, after completing the whole experimental cycle. Day 6 specimens were then cut longitudinally and imaged cross sectionally, to confirm the presence and thickness of BAG layer and to determine how much resistant Nupro and Sylc layers were on enamel and dentine after six erosive challenges.

The qualitative elemental composition was investigated using an X-ray energy dispersive spectrometer (EDX) (Oxford Instruments XMAX 20). The EDX has a super thin window used for the detection of the lightest elements. It is integrated with SEM (Quanta 250FEG, Japan) with software being embedded in the SEM controller system. The detector is of silicone drift detector type and is a liquid nitrogen free system having sensor size of 20mm. EDX was carried out using an accelerating voltage of 20kV and low vacuum conditions (20Pa). The resolution was in accordance with ISO 15632:2002. The elemental analysis was expressed in weight percentage and atomic percentage. The elemental analysis was done for the specimens at Intervention day and Day 6, in order to check elemental gain and loss after application of test material and at the end of experimental cycle.

3.9 Statistical Analysis:

The data was collected and analysed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, US), version 21. Repeated Measure ANOVA was performed for each test group to determine whether there were any statistically significant time related changes in the outcome measures mentioned in Sub-sections 3.6

and 3.7. When a significant time related change was observed, pairwise comparison was then applied to determine at which time point statistically significant change was first observed. For inter-group comparisons the Dunnette and Bonfferroni Post-Hoc tests were used. The Dunnette test was used to compare the test groups with the control group while the Bonfferroni test was used to compare the two test groups.

University of Malaya

CHAPTER 4

RESULTS

Prior to any statistical analysis, the following assumptions were tested to determine whether a parametric or non-parametric analysis was to be used.

- i) Absence of outliers in any of the groups
- ii) The pattern of distribution of the data
- iii) The variances of the differences between the groups is equal

It was found that there were no outliers as assessed by box-plots. The data was normally distributed as assessed by the Shapiro-Wilks test of normality with $P > 0.05$ for Surface Microhardness change (ΔSMH) and Surface Roughness change (ΔR_a).

4.1 Surface Microhardness (ΔSMH) of Enamel:

4.1.1 Control Group

For the control group, there was a net decrease of 59.34 ± 8.00 (Mean \pm Standard Error (SE)) in the ΔSMH (t_0) over the period of 6 Days Demineralisation-Remineralisation cycle as shown in Figure 4.4.

There was a general decreasing trend in the mean ΔSMH (t_0) of specimens in the Control group from Baseline to demineralisation Day 6 as there was no intervention for this group.

It could also be noted that the decrease in ΔSMH (t_0) in the control group was relatively uniform across all the time points with the greatest decrease occurring between demineralisation Day 5 and demineralisation Day 6 of 10.46 ± 2.20 (Mean \pm SE). The smallest decrease of 5.24 ± 0.62 (Mean \pm SE) was between demineralisation Day 2 and Day 1.

Multivariate test for repeated measure showed that there was significant difference ($P = 0.035$) in ΔSMH (t_0) over time (Table 4.1). Pairwise comparison

between Δ SMH at various time points with baseline showed that Δ SMH (t_0) started to show significant difference from Day 3 of demineralisation.

Table 4.1: Results of Multivariate test for Δ SMH (t_0)

Effect (Pillai's trace)	Value	F	Hypothesis	Error	Sig.	Partial Eta Squared	Observed Power
Control	.964	11.476	7.000	3.000	<u>.035</u>	.964	.746
Nupro	.975	16.574	7.000	3.000	<u>.021</u>	.975	.874
Sylc	.966	12.010	7.000	3.000	<u>.033</u>	.966	.764

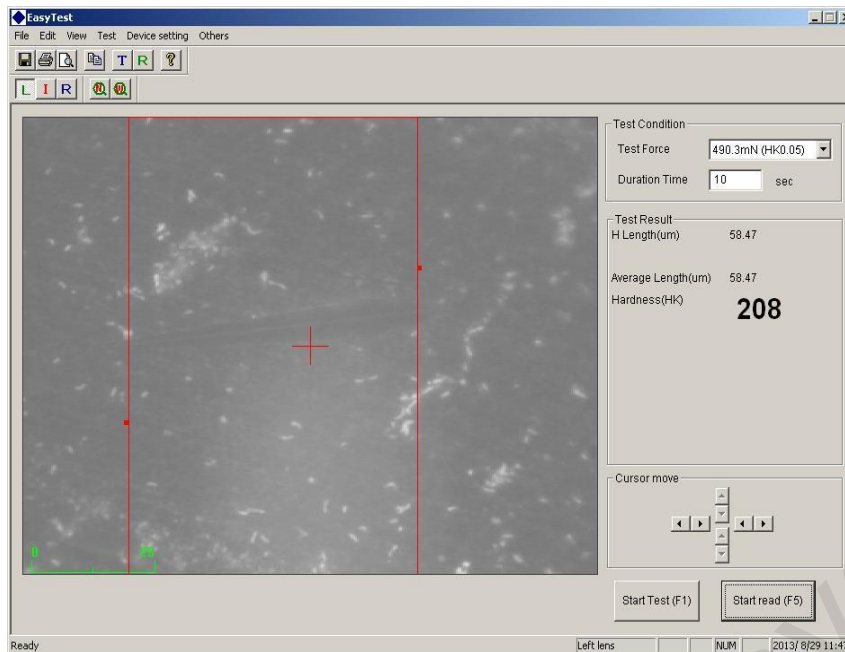


Figure 4.1A: This print screen image shows an example of SMH measurement on an enamel specimen in the Control group at Intervention

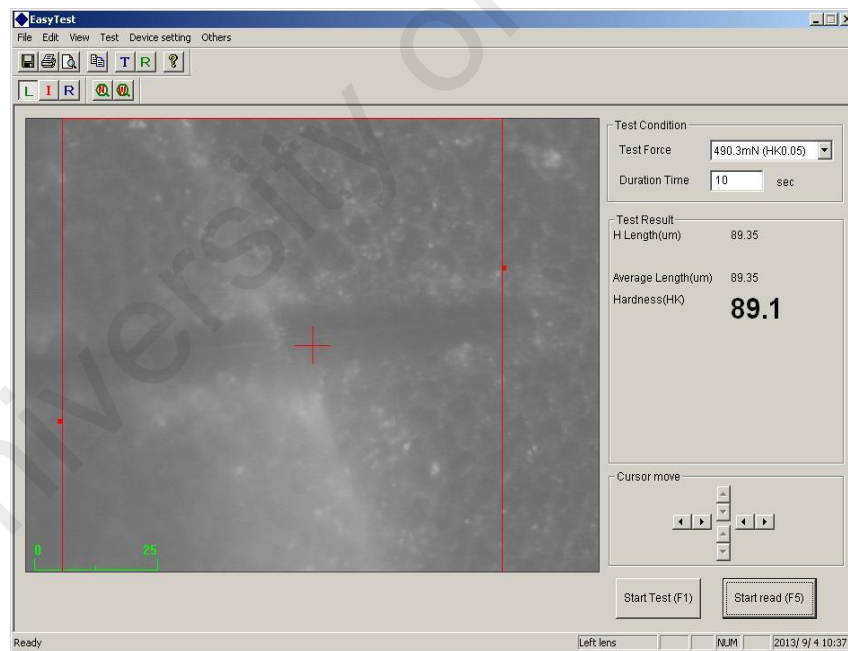


Figure 4.1B: Print screen image showing an example of SMH measurement of an enamel specimen in the Control group at Day 6 of demineralisation. The pyramidal indentation on the test surface can be seen on this image.

4.1.2 Nupro Group

Similar to the Control group, there was a net decrease of ΔSMH (t_0) but lesser than that of the control group. The net decrease of ΔSMH (t_0) was 24.13 ± 10.72 (Mean \pm SE) over the 6-Days Demineralisation-Remineralisation cycle, as shown in Figure 4.4.

However, there was a net decrease of 65.09 ± 7.26 (Mean \pm SE) from baseline in ΔSMH (t_1) observed at the end of the Demineralisation-Remineralisation cycle. This was due to an increase of 40.96 ± 8.42 (Mean \pm SE) in the mean ΔSMH (t_0) after Intervention, followed by a general decreasing trend. The largest decrease in ΔSMH was observed between Intervention and demineralisation Day 1 of 15.13 ± 3.10 (Mean \pm SE) whilst the smallest decrease was between demineralisation Day 3 and Day 4 with a decrease of 8.76 ± 1.73 (Mean \pm SE).

Multivariate test for repeated measure showed that there was also significant difference ($P=0.021$) in ΔSMH (t_0) over time (Table 4.1).

For both test groups, pairwise comparison was done for both ΔSMH (t_0) and ΔSMH (t_1). Pairwise comparison of ΔSMH (t_0) for Nupro showed significant difference only between Baseline and Intervention. Thereafter, there were no significant differences of ΔSMH (t_0) with the other days of demineralisation. However the pairwise comparison of ΔSMH (t_1) showed significant differences between Intervention and Day 1 up till Day 6 of demineralisation.

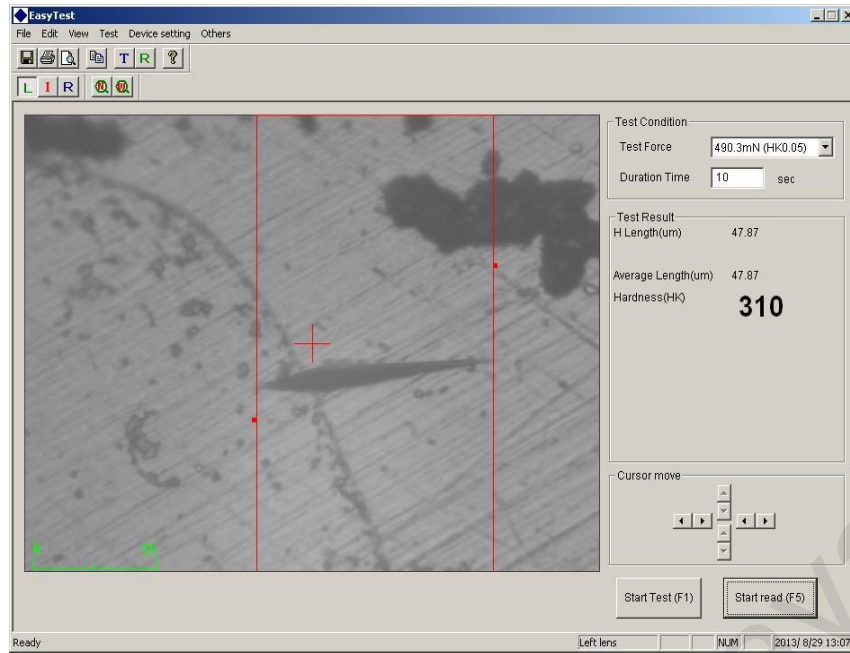


Figure 4.2A: This print screen image showing a pyramidal indent is a case of SMH measurement on an enamel specimen in the Nupro group at Intervention Day

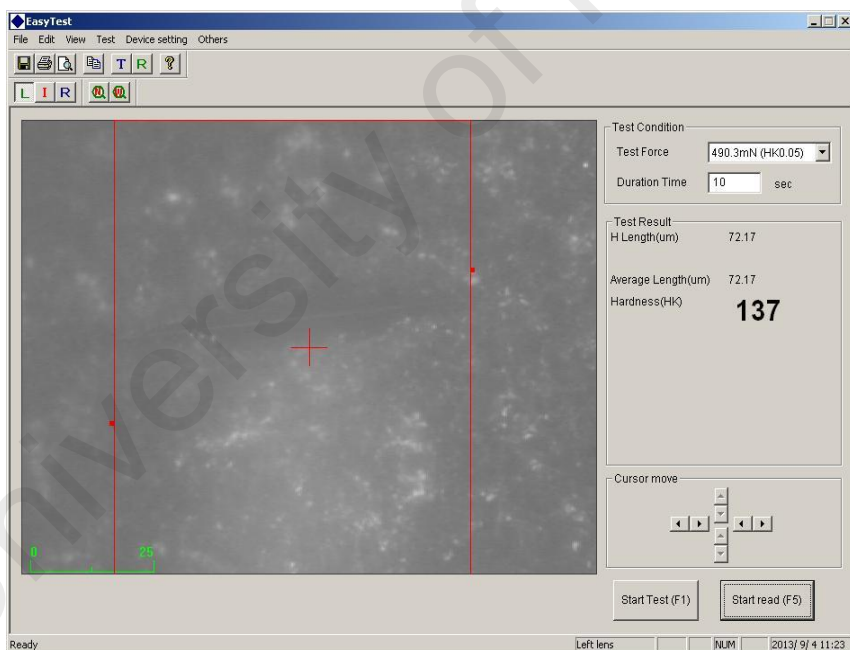


Figure 4.2B: Print screen image of SMH measurement on enamel specimen in the Nupro group at Day 6

4.1.3 Sylc Group:

From Figure 4.4, it could be observed that there was a similar trend in the Δ SMH of both Sylc and Nupro groups but for the Sylc group, rather than decrease, there was a net increase of 17.60 ± 10.65 (Mean \pm SE) in the Δ SMH(t_0) at the end of the 6-Days cycle.

Between Baseline and Intervention, there was an increase of 98.36 ± 7.08 (Mean \pm SE) in the Δ SMH (t_0). Thereafter a decreasing trend in the mean Δ SMH (t_0) was observed from demineralisation Day 1 to demineralisation Day 6, albeit still resulting in 17.60 ± 10.65 (Mean \pm SE) higher than Baseline. For Sylc, the SMH at end of the study was higher than that at Baseline whilst for the Nupro group it was lower than Baseline.

The trend of decrease in Δ SMH (t_1) in the Sylc group was also similar compared to Nupro group with the greatest decrease in Δ SMH occurring between Intervention and demineralisation Day 1 of 32.56 ± 7.18 (Mean \pm SE) whilst the smallest decrease of 6.17 ± 1.35 (Mean \pm SE) was also observed between demineralisation Day 3 and Day 4.

Multivariate test for repeated measures also showed that there was significant difference ($P=0.033$) in Δ SMH (t_0) over time (Table 4.1)

Pairwise comparison in Sylc group for Δ SMH (t_0) showed significant difference from Baseline up to demineralisation Day 3. Thereafter, there were no significant differences detected. However, pairwise comparison for Δ SMH (t_1) showed significant difference in Δ SMH between Intervention and all time points i.e. from demineralisation Day 1 up to demineralisation Day 6.

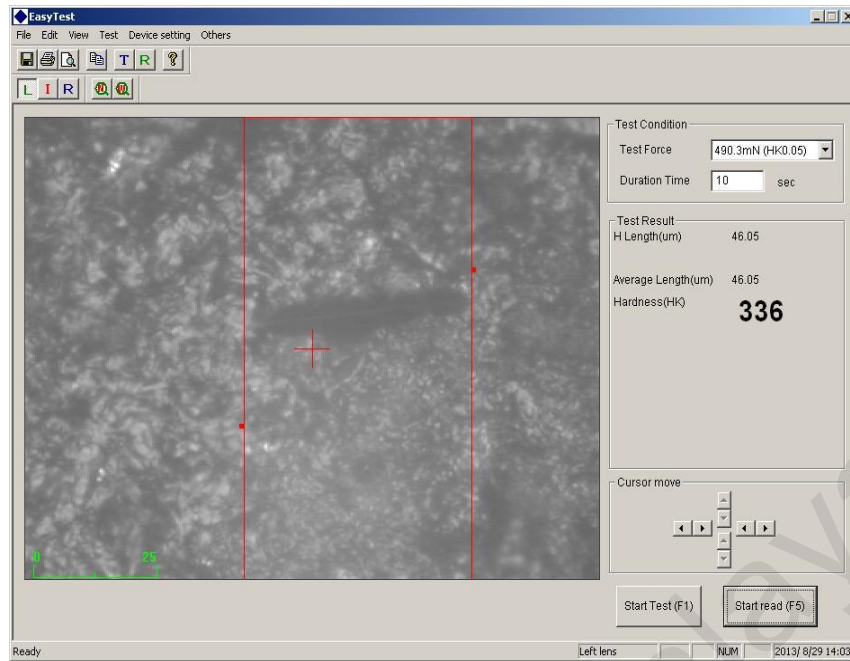


Figure 4.3-A: This image of SMH measurement of enamel Sylc specimen at Intervention Day is showing a clear small indent on enamel surface

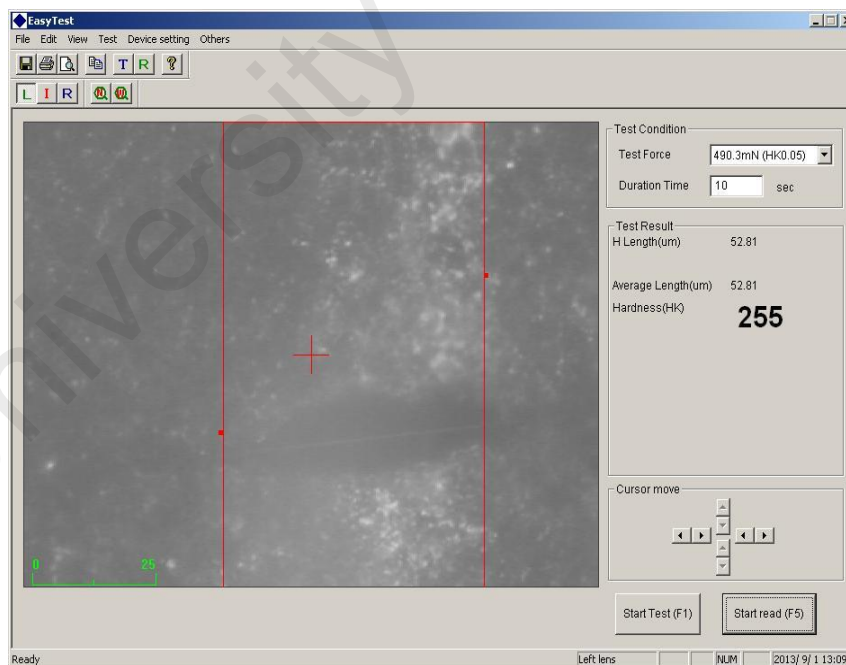


Figure 4.3B: Print screen image of SMH measurement of enamel Sylc specimen at Day 6 is showing a bigger indent as compared to Intervention Day indent.

4.1.4 Comparison between groups:

After Intervention, Δ SMH decreased from Baseline in the Control group although no intervention has been done on Control group specimens, while an increase was observed in both test groups. A progressive decrease in the mean Δ SMH was observed across all groups during the 6-Days acid challenge period (Figure 4.4). On demineralisation Day 1, the greatest decrease in mean Δ SMH was observed for the Control group 18.80 ± 5.17 (Mean \pm SE), followed by the Nupro 25.83 ± 10.84 (Mean \pm SE) and Sylc 65.80 ± 8.98 (Mean \pm SE) groups. The average Δ SMH values for the Sylc group remained above the Baseline value throughout the study while the Δ SMH values for Nupro stayed above the Baseline value only up to demineralisation Day 3.

Post-hoc analysis of Δ SMH (t_0) using Dunnett test was applied for comparison of each of the test group with the control group. Significant differences were found between both of the test groups with the control group ($P < 0.05$) as shown in Table 4.2. Bonferroni test was applied for comparison between the two tests groups and significant difference was also found between the two test groups ($P < 0.05$) (Table 4.3).

Table 4.2 Post-hoc test (Dunnett) for Intergroup Comparison of Δ SMH

groups	groups	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Enamel Nupro	Enamel control	35.3946	10.37607	.004	11.1829	59.6062
Enamel Sylc	Enamel control	73.1279	10.37607	.000	48.9163	97.3396

Table 4.3 Post-hoc test (Bonferroni) for Intergroup Comparison of ΔSMH

Groups	Groups	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Enamel Nupro	Enamel Syle	-37.7333	10.37607	.003	-64.2178	-11.2488

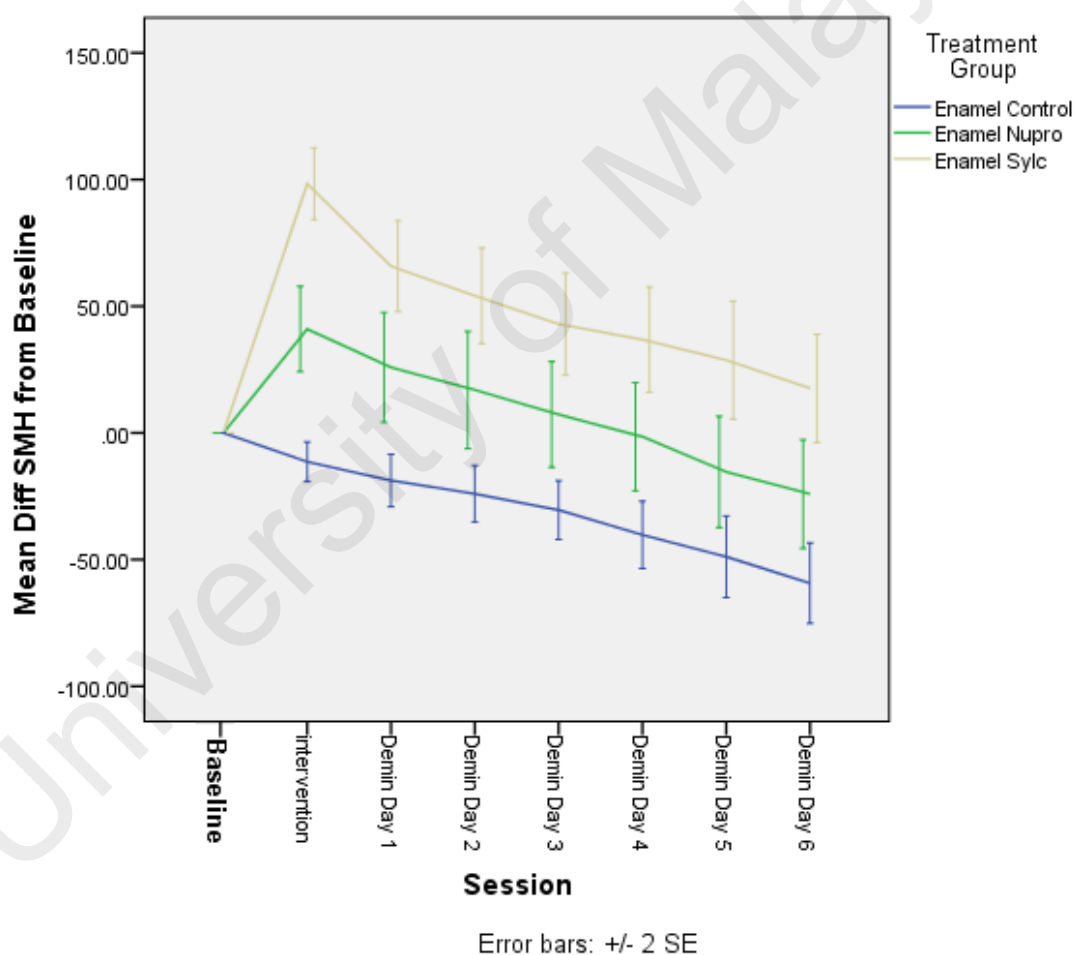


Figure 4.4: Change in ΔSMH from Baseline to Demineralisation Day 6

4.1.5 Indentation depth:

In the control group the average penetration depth at Intervention day was $6.2 \pm 0.7 \mu\text{m}$ (Mean \pm SD) as there was no Intervention done on the specimens of this group. While in the Nupro group on Intervention day the average penetration depth was calculated as $5.7 \pm 0.4 \mu\text{m}$ (Mean \pm SD). The value of “h” in Sylc group specimens on Intervention day was calculated as $4.9 \pm 0.2 \mu\text{m}$ (Mean \pm SD).

At the end of the experimental cycle on Day 6, the average “h” value for the control group specimens was $7.2 \pm 1 \text{ mm}$ (Mean \pm SD). The average “h” value for Nupro group specimens on Day 6 was $6.8 \pm 0.6 \mu\text{m}$ (Mean \pm SD) and for Sylc group specimens the “h” value was calculated as $5.7 \pm 0.2 \mu\text{m}$ (Mean \pm SD).

The penetration depth of the indenter “h” was calculated and compared with thickness of BAGs layer from cross sectional SEM images of test groups (Figure.4.21 & 4.22) on Intervention day and at the end of the experimental cycle in enamel specimens. It was noted that in Nupro group (Figure.4.21) the thickness of BAG layer on Intervention day was $2.64 \pm 0.24 \mu\text{m}$ (Mean \pm SD) while the h value was $5.7 \pm 0.4 \mu\text{m}$. On Day 6 the thickness of BAG layer on the specimen of this group was $1.40 \pm 0.05 \mu\text{m}$ (Mean \pm SD) whereas the h value at this time was calculated as $6.8 \pm 0.6 \mu\text{m}$ (Mean \pm SD). In the enamel Sylc group (Figure.4.22) the thickness of BAG layer on intervention day was $5.19 \pm 0.50 \mu\text{m}$ (Mean \pm SD) and the h value was calculated as $4.9 \pm 0.2 \mu\text{m}$ (Mean \pm SD). At the end of the experimental cycle on Day 6 the thickness of BAG layer was noted as $1.70 \pm 0.08 \mu\text{m}$ (Mean \pm SD) while the “h” value was $5.7 \pm 0.2 \mu\text{m}$ (Mean \pm SD) (Table 4.4).

Table 4.4 Comparison of Thickness of BAG layer with “h” value of SMH.

	Thickness of BAG layer on Intervention (μm)	Thickness of BAG layer on D6 (μm)	h (Intervention) mm (μm)	h (Day 6) mm (μm)
Enamel Nupro	$2.64 \pm 0.24\mu\text{m}$ (Mean \pm SD)	$1.40 \pm 0.05\mu\text{m}$ (Mean \pm SD)	$5.7 \pm 0.4\mu\text{m}$ (Mean \pm SD)	$6.8 \pm 0.6\mu\text{m}$ (Mean \pm SD)
Enamel Sylec	$5.19 \pm 0.50\mu\text{m}$ (Mean \pm SD)	$1.70 \pm 0.08\mu\text{m}$ (Mean \pm SD)	$4.9 \pm 0.2\mu\text{m}$ (Mean \pm SD)	$5.7 \pm 0.2\mu\text{m}$ (Mean \pm SD)

4.2 Surface Roughness of Enamel:

4.2.1 Control Group

There was a net increase of $0.086 \pm 0.005 \mu\text{m}$ (Mean \pm SE) in the surface roughness over the 6-Days of Demineralisation-Remineralisation cycle as shown in Figure 4.8. The graph also shows an increasing trend in the mean $\Delta R_a(t_0)$ from Baseline to demineralisation Day 6 as no intervention was included for this group.

The increase in the mean surface roughness $\Delta R_a(t_0)$ in the Control group was relatively uniform across all time points, with the greatest increase occurring between Intervention and demineralisation Day 1 of $0.015 \pm 0.002 \mu\text{m}$ (Mean \pm SE). The smallest increase of $0.009 \pm 0.001 \mu\text{m}$ (Mean \pm SE) was between demineralisation Day 5 and Day 6.

Multivariate test for repeated measures showed that there was significant difference ($P=0.009$) in ΔR_a over time (Table 4.5). Pairwise comparison showed significant differences in $\Delta R_a(t_0)$ for all the time points when compared from Baseline.

Table 4.5: Results of Multivariate Test for $\Delta R_a(t_0)$ (Enamel)

Effect (Pillai's trace) Enamel	Value	F	Hypothesis	Error	Sig.	Partial Eta Squared	Observed Power
Control	.986	29.873	7.000	3.000	<u>.009</u>	.986	.981
Nupro	.997	157.495	7.000	3.000	<u>.001</u>	.997	1.000
Sylc	.982	23.952	7.000	3.000	<u>.012</u>	.982	.956

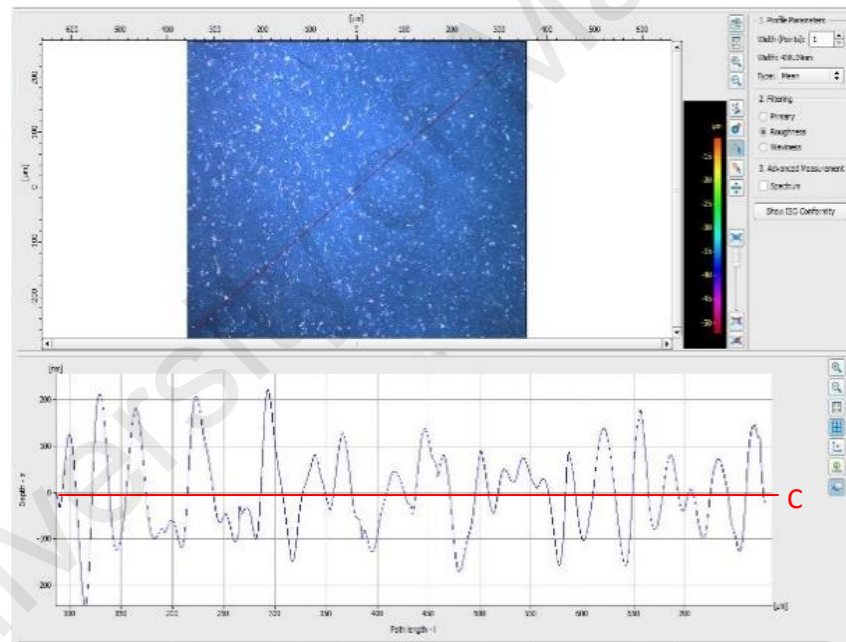


Figure 4.5A: The picture at the top is a surface view of an enamel **Control** specimen at **Baseline** stage. No obvious irregularity was seen on the enamel surface. The surface roughness profile graph (below the image) shows relatively equal number of positive (peaks) and negative (troughs) waves. The peaks and troughs were observed in relation to the central line, (C), which is depicted in red. The troughs were generally of smaller amplitudes than the peaks.

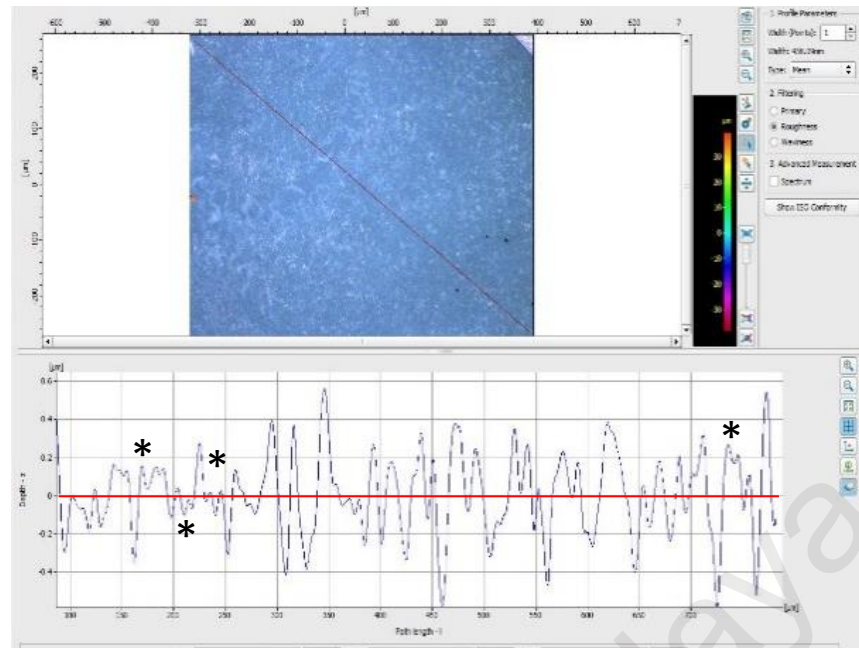


Figure 4.5-B: The picture at the top is a surface view of an enamel **Control** specimen at the end of **Day 6 demineralisation**. While the picture did not show obvious difference from Figure 4.5A, obvious difference of the surface roughness profile was observed on the graph. The troughs exhibited bigger amplitude and the wavelengths were smaller than those in Baseline (Figure 4.5A). There were also waves with very small wavelengths and amplitude (*) interspersed in

4.2.2 Nupro Group

For Nupro group, the ΔR_a demonstrated an unusual behaviour with an increase in ΔR_a between Baseline and Intervention, followed by a decrease between Intervention and demineralisation Day 3 before exhibiting an increase again between Day 3 to Day 6. However, at the end of the 6-Days Demineralisation-Remineralisation cycle, there was a net increase of $0.079 \pm 0.004 \mu\text{m}$ (Mean \pm SE) in ΔR_a .

During the decrease between Intervention to demineralisation Day 3, the greatest decrease in the mean ΔR_a was recorded between Intervention and demineralisation Day 1 of $0.025 \pm 0.007 \mu\text{m}$ (Mean \pm SE) and the smallest decrease was noticed between demineralisation Day 1 and Day 2 of $0.013 \pm 0.006 \mu\text{m}$ (Mean \pm SE) and the decreasing trend stopped at demineralisation Day 3 at $0.015 \pm 0.002 \mu\text{m}$ (Mean \pm SE).

This was followed by an increasing trend in the ΔR_a with the greatest increase of $0.040 \pm 0.005 \mu\text{m}$ (Mean \pm SE) occurring between demineralisation Day 3 and Day 4 and the least increase was noticed between demineralisation Day 4 and Day 5 of $0.019 \pm 0.001 \mu\text{m}$ (Mean \pm SE).

Multivariate test for repeated measure also showed that there was significant difference ($P = 0.001$) in ΔR_a over time (Table 4.5).

For both test groups, pairwise comparison was done for both $\Delta R_a(t_0)$ and $\Delta R_a(t_1)$.

Pairwise comparison showed significant differences in $\Delta R_a(t_0)$ between Baseline with Intervention and Day 1 demineralisation. No significant differences in $\Delta R_a(t_0)$ were detected for Day 2 and Day 3 demineralisation. Subsequently, significant

differences in $\Delta R_a(t_0)$ were detected again for Day 4 up to Day 6 demineralisation. Significant differences in $\Delta R_a(t_1)$ were found between Intervention with all-time points.

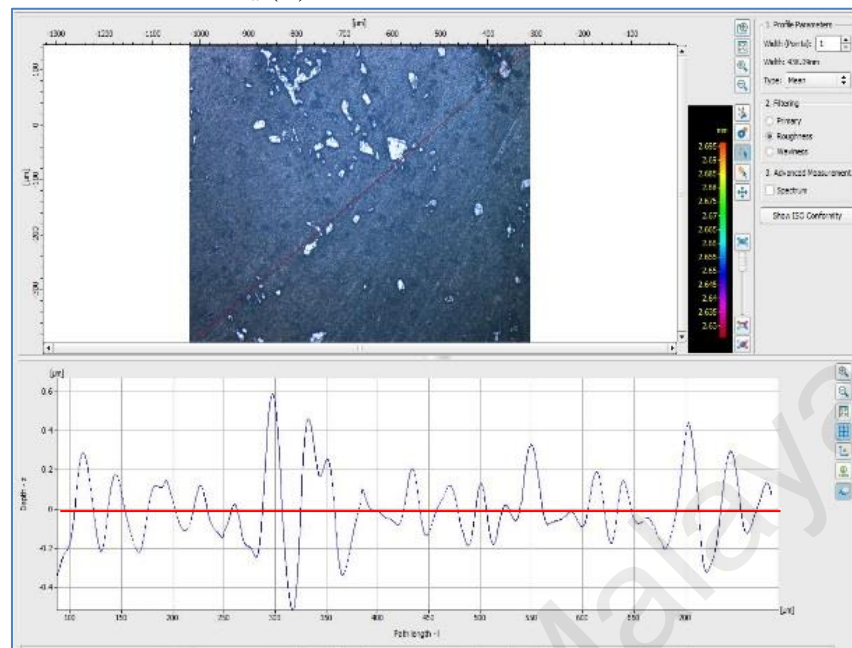


Figure 4.6-A: The picture at the top is a surface view of an enamel **Nupro** specimen at **Intervention stage**. Compared to surface at Baseline (Figure 4.5A), the enamel surface appears to display some irregularities. The surface roughness profile graph (below the image) shows the peaks and troughs are of smaller amplitudes than that in Baseline (Figure 4.5A). However, the wavelengths are similar to that in Baseline.

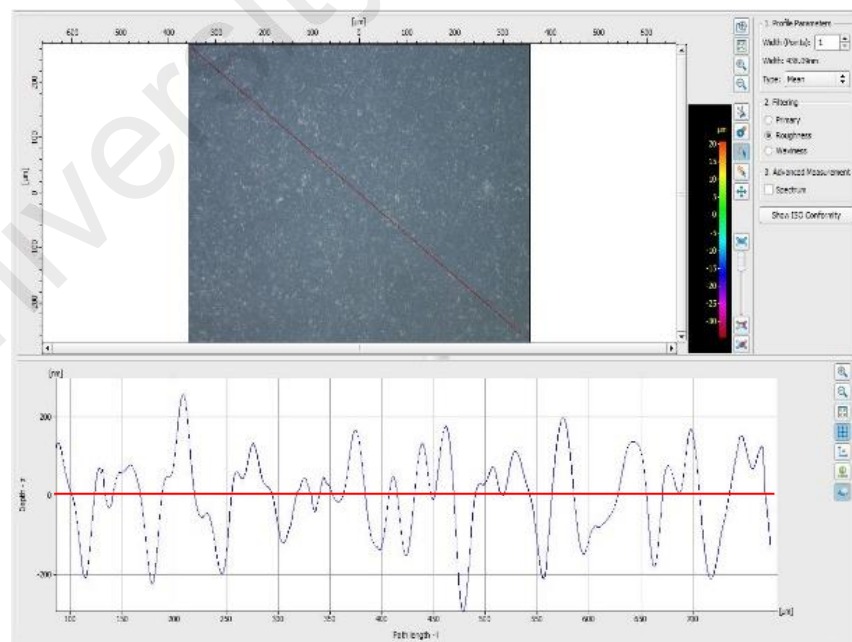


Figure 4.6-B: The picture at the top is a surface view of an enamel **Nupro** specimen at **demineralisation Day 2**. The surface roughness profile graph shows that the amplitude of the troughs had increased compared to the troughs at Intervention (Figure 4.6A). The troughs were also of greater amplitude than the peaks. However, the wavelengths are relatively similar to that of Baseline (Figure 4.5A).

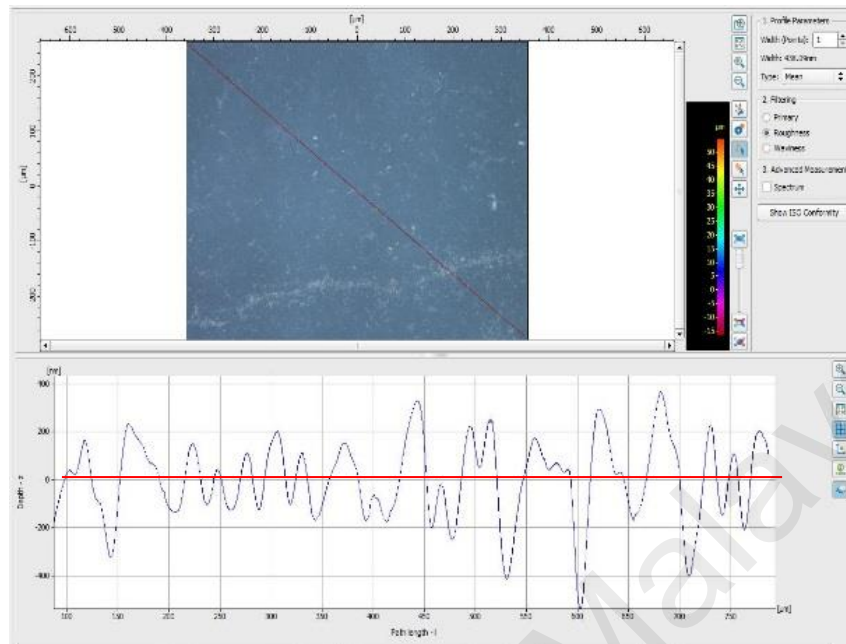


Figure 4.6-C: The picture at the top is a surface view of an enamel **Nupro** specimen at **Day 6 of demineralisation**. The surface roughness profile graph (below the image) shows that the troughs continue to have a similar pattern as demineralisation Day 2 (Figure 4.6B) where the troughs are of bigger amplitude than the peaks. The wavelengths on the other hand appear to be smaller than the wavelengths in Day 2 (Figure 4.6A) and very similar to Baseline (Figure 4.5A).

4.2.3 Sylc Group

A net increase of $0.118 \pm 0.008 \mu\text{m}$ (Mean \pm SE) in the surface roughness was observed in the Sylc group of this study over the 6-Days Demineralisation-Remineralisation cycle as seen in Figure 4.8.

The mean surface roughness increased considerably from Baseline to $0.152 \pm 0.008 \mu\text{m}$ (Mean \pm SE) after Intervention. Thereafter, a gradual decreasing trend was observed from demineralisation Day 1 to demineralisation Day 6 resulting in a final Ra value that remained higher than the Baseline. The pattern of an increase of Ra in Day 2 observed in the Nupro group did not occur in this group.

The drop in the mean surface roughness in the Sylc group was relatively uniform across all time points; the greatest decline in the mean surface roughness was recorded between demineralisation Day 2 and Day 3 of $0.007 \pm 0.002 \mu\text{m}$ (Mean \pm SE) whilst the smallest decrease of 0.004 ± 0.0001 (Mean \pm SE) μm occurred between demineralisation Day 1 and Day 2.

Multivariate test for repeated measure also showed that there was significant difference ($P=0.012$) in $\Delta R_a(t_0)$ over time (Table 4.5). Pairwise comparison showed that there was a significant difference in $\Delta R_a(t_0)$ across all time points with respect to Baseline. The second level of pairwise comparison also showed significant differences in $\Delta R_a(t_1)$ between Intervention with all other subsequent time points.

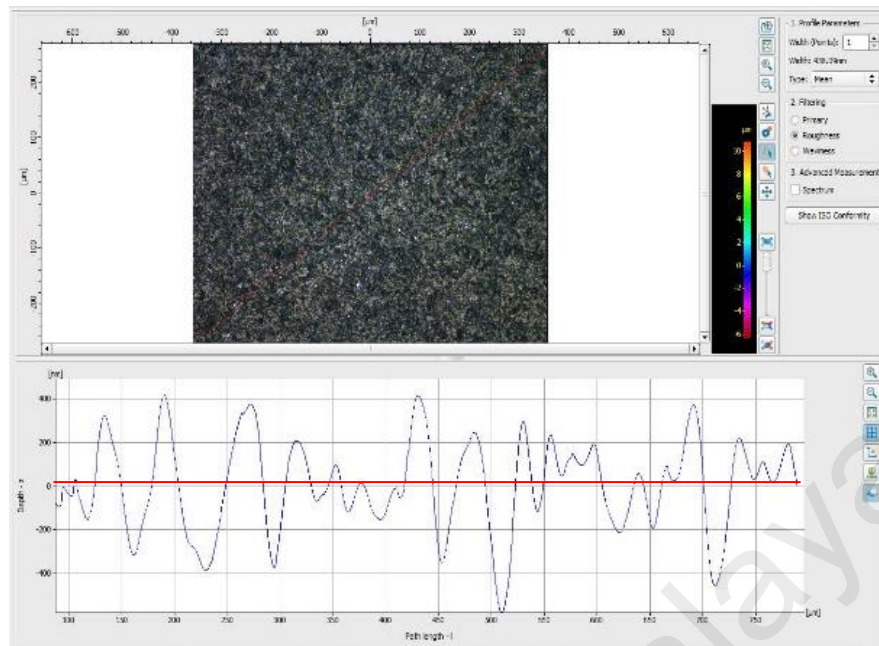


Figure 4.7-A: The picture at the top is a surface view of an enamel Sylc specimen at Intervention stage. The enamel surface appears homogenous and no obvious irregularities observed. The surface roughness profile graph (below the image) shows relatively equal number of peaks and troughs. The amplitude of the peaks and troughs are generally equally distributed and are similar to Baseline. However, the wavelengths are greater than that of Baseline (Figure 4.5A)

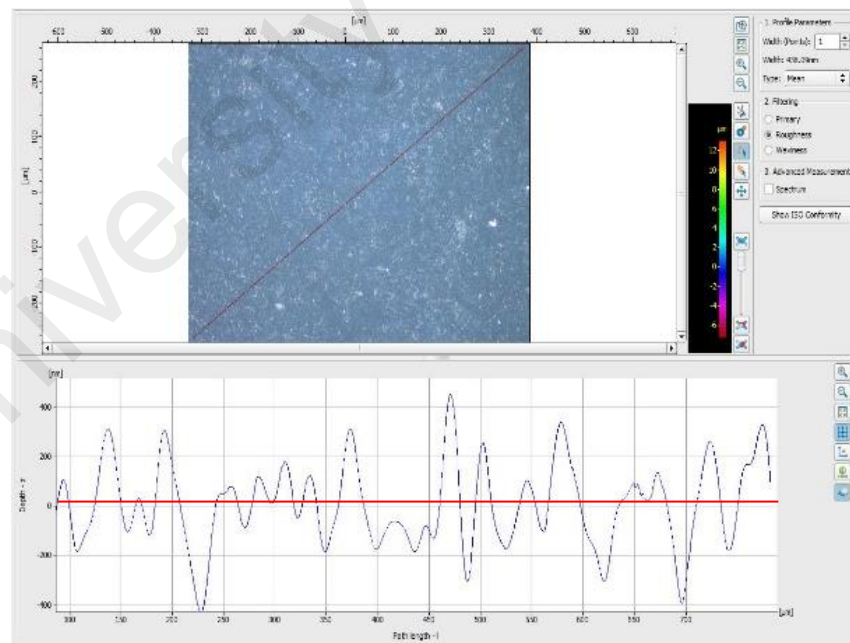


Figure 4.7-B: The picture at the top is a surface view of an enamel Sylc specimen at Day 6 of demineralisation. The surface roughness profile graph (below the image) shows high amplitudes of both the peaks and troughs but the peaks appear to be higher in number compared to the troughs. The wavelengths do not defer from that of Intervention (Figure 4.7A), i.e. it is still bigger than that of Baseline and it is also bigger than that of Nupro at Day 6 of demineralisation (Figure 4.6C)

4.2.4 Comparison of Surface Roughness (ΔR_a) among the three groups (Enamel):

The ΔR_a in all three groups of enamel showed different patterns. The increase of ΔR_a after Intervention, the ΔR_a (t_1) increased immensely in the Sylc group up to $0.152 \pm 0.008 \mu\text{m}$ (Mean \pm SE), followed by Nupro, which showed increase of $0.053 \pm 0.003 \mu\text{m}$ (Mean \pm SE); the least increase was recorded in the control group of $0.013 \pm 0.002 \mu\text{m}$ (Mean \pm SE).

After the first acidic challenge on all specimens, Day 1 reading was recorded and a further increase in surface roughness of $0.028 \pm 0.003 \mu\text{m}$ (Mean \pm SE) was observed in the Control group, while a decrease of $0.14 \pm 0.008 \mu\text{m}$ (Mean \pm SE) and $0.029 \pm 0.007 \mu\text{m}$ (Mean \pm SE) was noted in the Sylc and Nupro groups, respectively. On Day 1, the decrease in ΔR_a was greater in the Nupro group as compared with the Sylc group probably due to less resistance of Nupro against acidic exposure.

A further decrease in ΔR_a was observed in all three groups on Day 2 and Day 3. An interesting behaviour of change in surface roughness was noticed on Day 4, when the Nupro group showed a sudden increase in surface roughness of $0.040 \pm 0.005 \mu\text{m}$ (Mean \pm SE) while the Sylc group showed a slight decrease of $0.12 \pm 0.007 \mu\text{m}$ (Mean \pm SE). On Day 4, a constant increase in surface roughness of $0.06 \pm 0.005 \mu\text{m}$ (Mean \pm SE) was also observed in the control group of enamel.

The same rising pattern of increase was observed in both enamel control and Nupro groups on Day 5 and Day 6 showing further demineralisation of enamel surface while Sylc group was still showing a gradual and slow decrease in surface roughness on both Day 5 and Day 6.

Post-hoc test was done for intergroup comparison where both test groups were compared to control group. Dunnett test showed that there was no significant difference between Nupro test group with the Control group, while there was a significant difference in Sylc test group with Control group ($P < 0.05$). Bonferroni test was conducted between two test groups and showed that there was a significant difference between the two test groups ($P < 0.05$) (Table 4.6 and 4.7).

Table 4.6 Post-hoc test (Dunnett) for Intergroup Comparison of ΔR_a (Enamel)

Groups	Groups	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Enamel Nupro	Enamel Control	-.0118	.00648	.142	-.0269	.0034
Enamel Sylc	Enamel Control	.0722*	.00648	.000	.0571	.0873

Table 4.7 Post-hoc test (Bonferroni) for Intergroup Comparison of ΔR_a (Enamel)

Groups	Groups	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Enamel Sylc	Enamel Nupro	.0840	.00648	.000	.0674	.1005

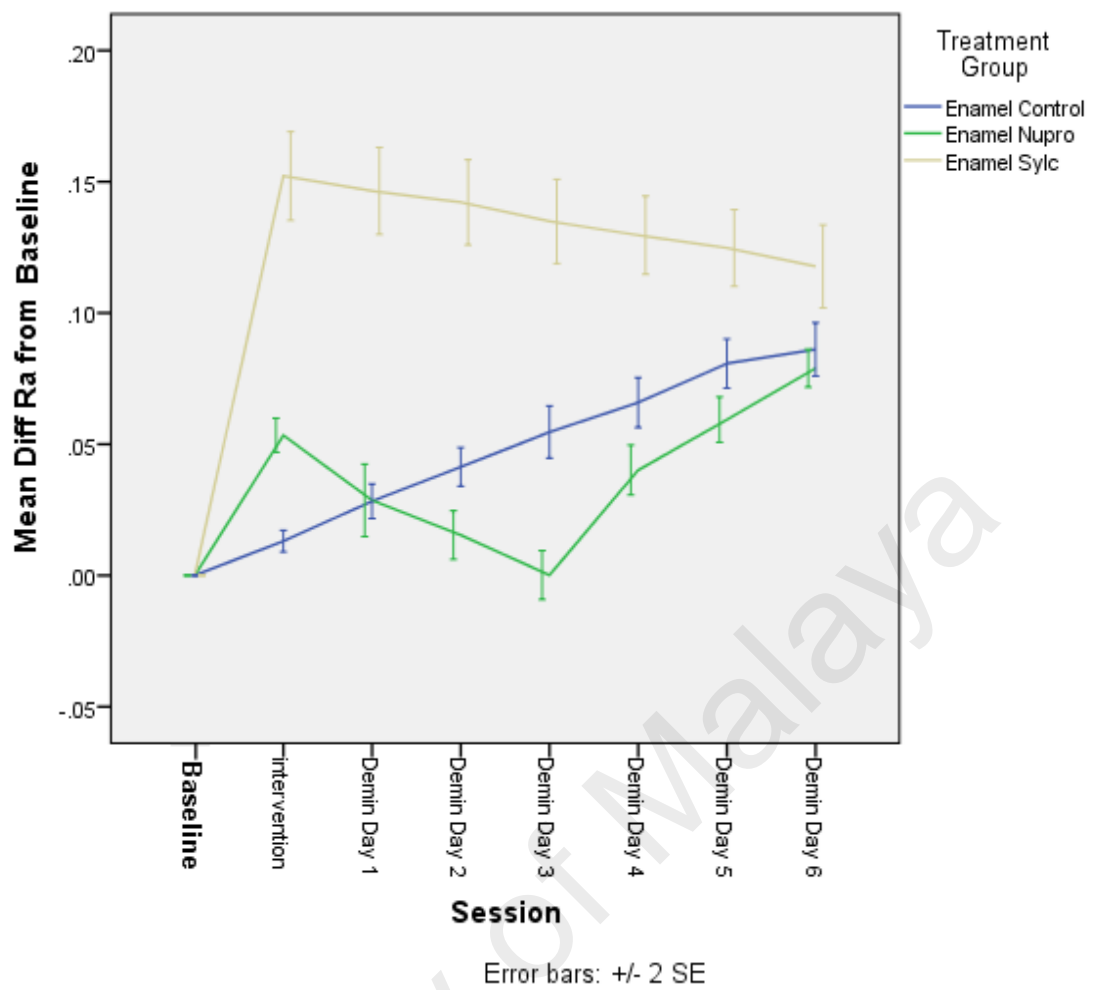


Figure 4.8 Changes in Surface Roughness of Enamel from Baseline to Demineralisation Day 6.

4.3 Surface Roughness of Dentine:

4.3.1 Control Group

The control group showed a steady increase in the $\Delta R_a(t_0)$ from Baseline to demineralisation Day 6 with a net increase of $0.073 \pm 0.004 \mu\text{m}$ (Mean \pm SE).

The largest increase was between Intervention and demineralisation Day 1 of $0.012 \pm 0.002 \mu\text{m}$ (Mean \pm SE) whilst the smallest increase was between demineralisation Day 3 and demineralisation Day 4 of 0.006 ± 0.001 (Mean \pm SE) μm .

Multivariate test for repeated measure showed that there was significant difference ($P=0.006$) in $\Delta R_a(t_0)$ over time (Table 4.8). Pairwise comparison showed that $\Delta R_a(t_0)$ also showed significant difference from Baseline at all-time points.

Table 4.8 Results of Multivariate Test for $\Delta R_a(t_0)$ (Dentine)

Effect (Pillai's trace)	Value	F	Hypothesis	Error	Sig.	Partial Eta Squared	Observed Power
Control	.989	39.770	7.000	3.000	.006	.989	.996
Nupro	.994	73.458	7.000	3.000	.002	.994	1.000
Sylc	.999	729.895	7.000	3.000	.000	.999	1.000

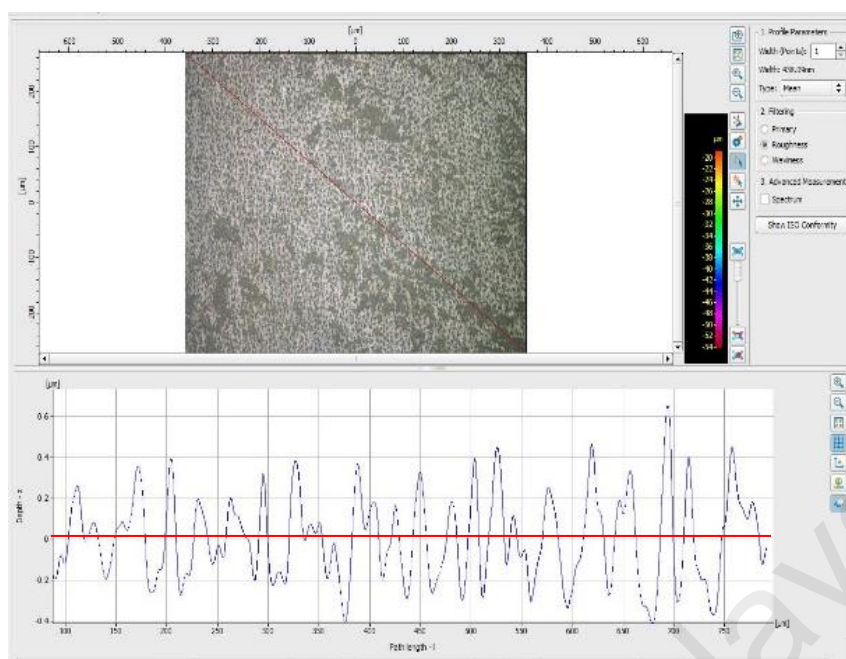


Figure 4.9-A: The picture at the top is a surface view of a dentine **Control** specimen at **Baseline**, showing an irregular surface of dentine. The surface roughness profile graph (below the image) shows relatively equal number of peaks and troughs. Generally, the amplitude of the peaks and troughs are also similar in dimension although there are some peaks exhibiting high amplitudes. The wavelength recorded

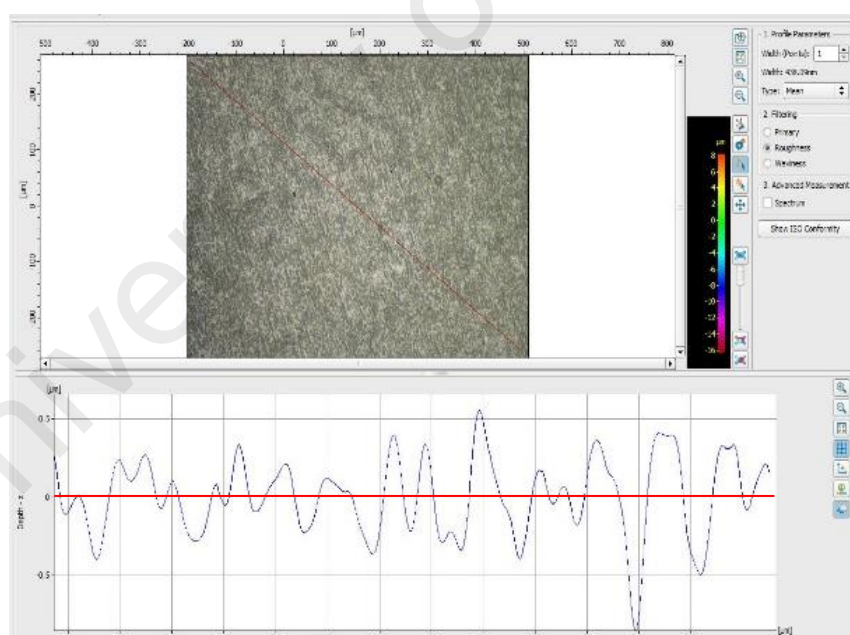


Figure 4.9B. The picture at the top is a surface view of a dentine **Control specimen** on **demineralisation at Day 6**. The picture appears to show relatively regular dentine surface. However, the surface roughness profile exhibits some apparent differences compared to Figure 4.9A. The wavelengths were obviously larger than that of Baseline (Figure 4.9A). The peaks and troughs have generally of similar amplitudes except for one trough which exhibits an amplitude almost double than that of the rest of the

4.3.2 Nupro Group

For the Nupro group, Figure 4.12 shows that there was a net increase in the surface roughness of $0.048 \pm 0.004 \mu\text{m}$ (Mean \pm SE) during the same 6-Day cycle.

The mean surface roughness for the dentine specimens showed similar trend to the enamel specimens with an increase in ΔR_a between Baseline and Intervention, followed by a decrease between demineralisation Day 1 and 3, and finally exhibiting an increase from demineralisation Day 4 to Day 6.

A decreasing-increasing trend in ΔR_a was exhibited by the dentine specimens, whereby the greatest decrease in the ΔR_a occurred between Intervention and Day 1 of $0.002 \pm 0.004 \mu\text{m}$ (Mean \pm SE). The least decrease in ΔR_a was recorded between Day 1 and Day 2 of $0.011 \pm 0.002 \mu\text{m}$ (Mean \pm SE), whilst the greatest increase was recorded between demineralisation Day 3 and Day 4 of $0.015 \pm 0.002 \mu\text{m}$ (Mean \pm SE) and the least rise in ΔR_a was observed between Day 4 and Day 5 of $0.012 \pm 0.002 \mu\text{m}$ (Mean \pm SE).

Multivariate test for repeated measure also showed that there was significant difference ($P=0.002$) in ΔR_a over time (Table 4.8).

Pairwise comparison showed significant differences in ΔR_a at different time points between Baseline to Intervention and Day 1 demineralisation and then on Day 5 and Day 6 of demineralisation. Thereafter, there were no significant differences of ΔR_a between Day 2, Day 3 and Day 4 of demineralisation with Baseline. However the second level of pairwise comparison showed that there were significant differences in ΔR_a (t_1) between Intervention up to Day 4 demineralisation after that no significant difference can be observed on Day 5 and Day 6 of demineralisation with Intervention.

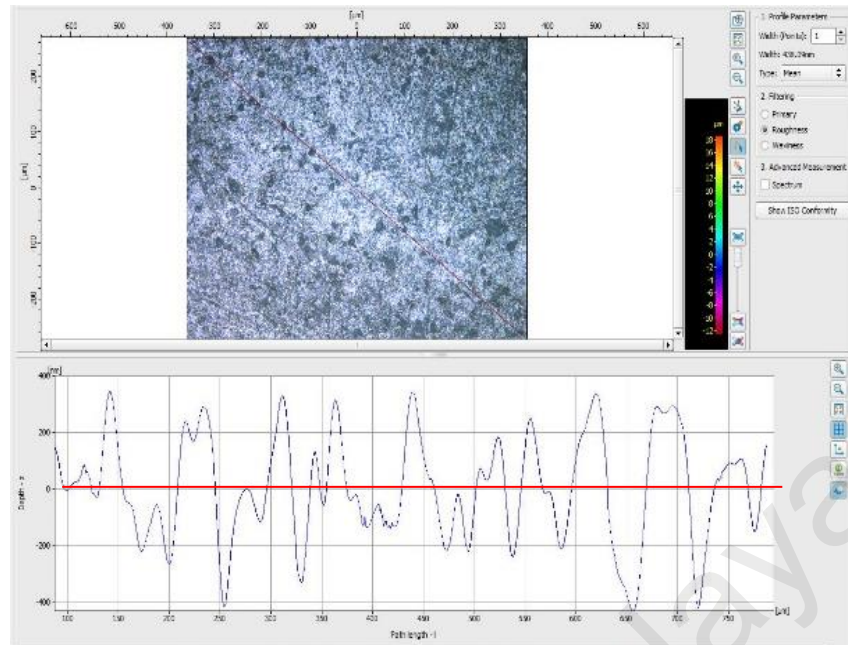


Figure 4.10A: The picture at the top is a surface view of a **dentine Nupro** specimen at **Intervention** stage, showing a relatively irregular dentine surface. Generally, the surface roughness profile graph (below the image) shows equal number of peaks and troughs. Both the peaks and troughs also exhibited relatively similar amplitude dimensions.

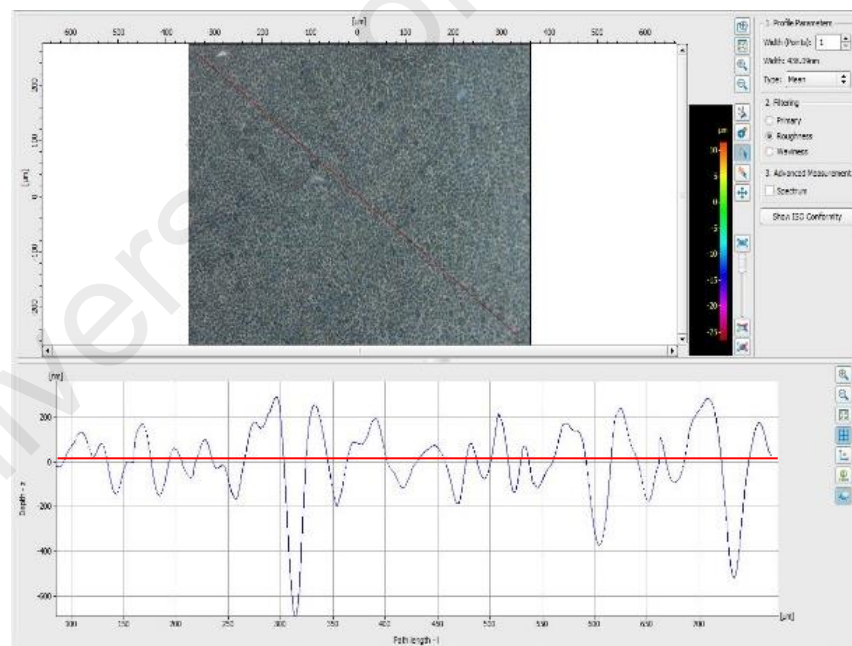


Figure 4.10-B: The picture at the top is a surface view of a **dentine Nupro** specimen at **Day2** of demineralization-remineralisation cycle, showing a moderately irregular dentine surface. The surface roughness profile graph (below the image) shows both the peaks and troughs were of relatively similar amplitude dimensions except for a few troughs that presented with high amplitude. The peaks and troughs were of smaller amplitude when compared to Intervention (Figure 4.10A) but with approximately similar wavelengths.

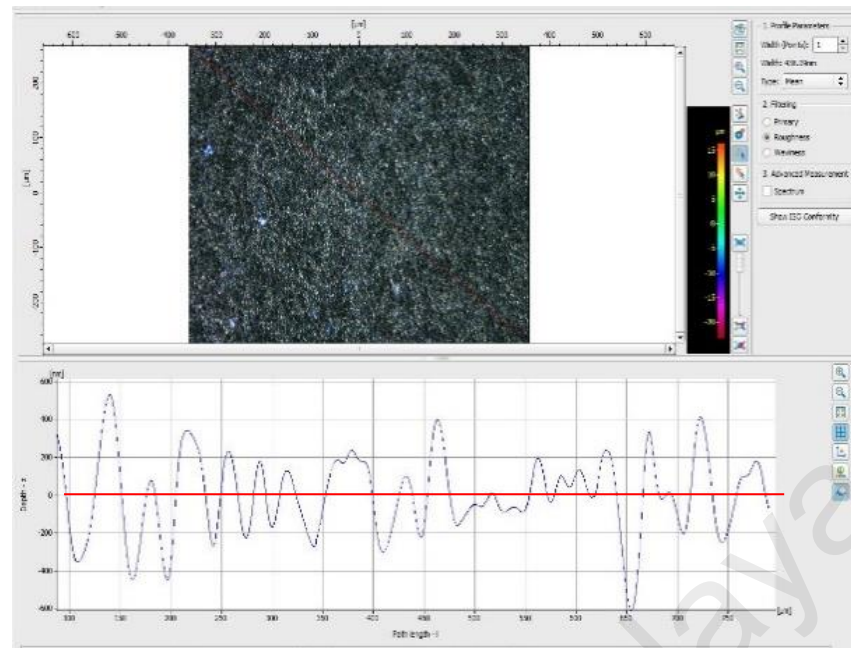


Figure 4.10-C: The picture at the top is a surface view of a **dentine Nupro** specimen at **demineralisation Day 6**. The dentine surface appears irregular. The peaks and troughs were of higher amplitudes than that of Day 2 (Figure 4.11B) demineralisation but with similar wavelengths. When compared to Day 6 demineralisation of the control group (Figure 4.9B), the amplitudes were similar but the wavelengths were smaller.

4.3.3 Sylc Group:

A net increase of $0.310 \pm 0.004 \mu\text{m}$ (Mean \pm SE) in the surface roughness was observed in the dentine Sylc group of this study over the 6-Days Demineralisation-Remineralisation cycle as seen in Figure 4.12.

The mean surface roughness increased considerably from Baseline to $0.347 \pm 0.004 \mu\text{m}$ (Mean \pm SE) after Intervention. Thereafter, a gradual decreasing trend was observed from demineralisation Day 1 to demineralisation Day 6 resulting in a final SMH value that remained higher than the Baseline. The pattern of an increase of SMH in Day 2 observed in the Nupro group did not occur in this group.

The drop in the mean surface roughness in the Sylc group was relatively uniform across all time points; the greatest decline in the mean surface roughness was recorded between demineralisation Day 4 and Day 5 of $0.008 \pm 0.002 \mu\text{m}$ (Mean \pm SE) whilst the smallest decrease was between intervention and demineralisation Day 1 of $0.004 \pm 0.000 \mu\text{m}$ (Mean \pm SE).

Multivariate test for repeated measure also showed that there was significant difference ($P=0.000$) in $\Delta R_a(t_0)$ over time (Table 4.8). Pairwise comparison showed that there was a significant difference in ΔR_a across all time points with respect to Baseline. The second level of pairwise comparison also showed significant differences in $\Delta R_a(t_1)$ between Intervention with all other subsequent time points

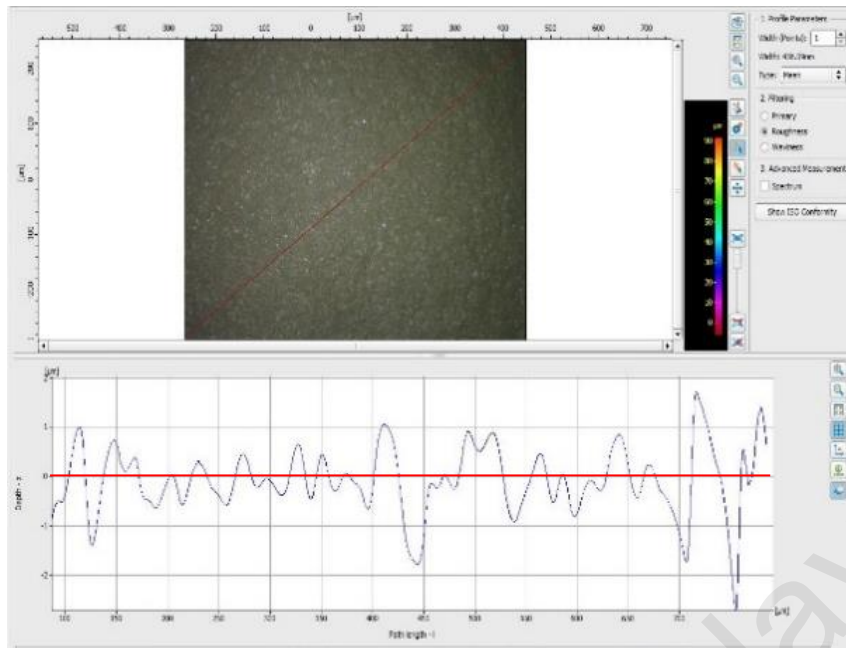


Figure 4.11-A: The picture at the top is a surface view of a dentine Sylc specimen at Intervention stage. The surface roughness profile graph (below the image) shows relatively equal number of peaks and troughs of amplitudes that are approximately double that of Baseline (Figure 4.9A). Some of the troughs were longer compared to the peaks. The wavelengths of the waves were bigger compared to Baseline.

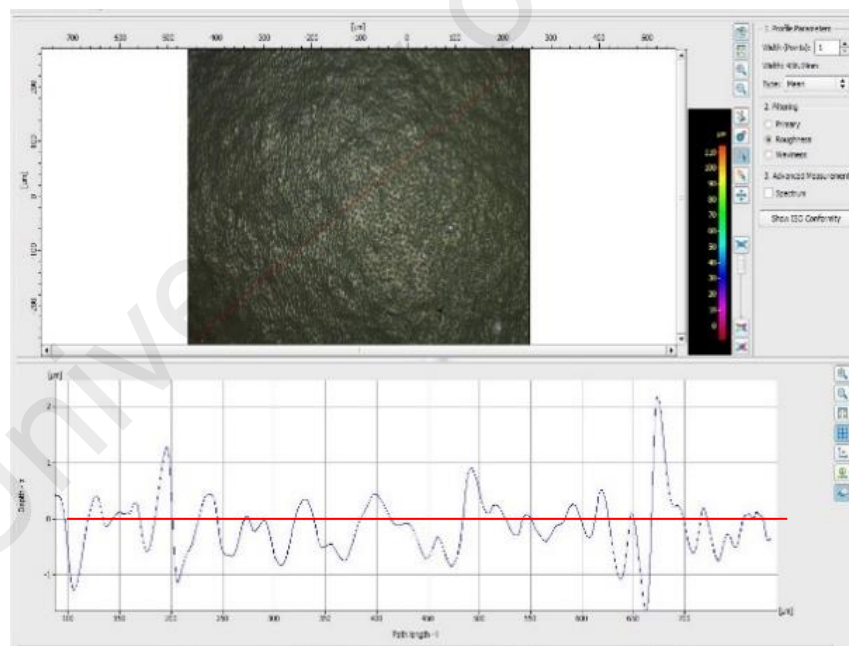


Figure 4.11-B: The picture at the top is a surface view of a dentine Sylc specimen on demineralisation Day 6. The picture shows obvious homogenous irregular surface of dentine. The surface roughness profile (below the image) shows peaks and troughs with similar amplitude as that at Intervention (Figure 4.11A). However, the wavelengths are slightly wider than at Intervention

4.3.4 Comparison of Surface Roughness (ΔR_a) among the three groups (Dentine):

The mean surface roughness in all three groups of dentine showed similar trend of change with time as in enamel groups but with higher values. The mean surface roughness difference from Baseline after Intervention indicates that mean surface roughness increased greatly in Sylc group up to $0.310 \pm 0.004 \mu\text{m}$ (Mean \pm SE) followed by Nupro which showed an increase of $0.0427 \pm 0.004 \mu\text{m}$ (Mean \pm SE) and in control group the raise was recorded to be the least at $0.022 \pm 0.002 \mu\text{m}$ (Mean \pm SE).

Following the first acidic challenge given to all specimens Day 1 reading was recorded, which is the mean difference in surface roughness on Day 1 from baseline. A further increase in surface roughness was observed in dentine control group at $0.034 \pm 0.003 \mu\text{m}$ (Mean \pm SE) μm while a non-significant decrease in ΔR_a was noted in Sylc group at $0.342 \pm 0.004 \mu\text{m}$ (Mean \pm SE) and in Nupro group at $0.031 \pm 0.005 \mu\text{m}$ (Mean \pm SE). The decline was greater in Nupro as compared to Sylc group on Day 1 possibly due less resistance of Nupro against acidic exposure.

A further decrease in surface roughness was observed in all three groups on Day 2 and Day 3 as well. The drop in ΔR_a of Nupro group was recorded at $0.011 \pm 0.006 \mu\text{m}$ (Mean \pm SE) on Day 3, after which, an interesting behaviour of change in ΔR_a was noticed on Day 4 in the Nupro group when there was a sudden increase of $0.026 \pm 0.006 \mu\text{m}$ (Mean \pm SE) in surface roughness was noted, which was greater than the reading of Day 3. The Sylc group showed a slight decrease in ΔR_a again of $0.323 \pm 0.004 \mu\text{m}$ (Mean \pm SE) on Day 4 and a constant increase in ΔR_a was still observed in dentine control group which was $0.055 \pm 0.003 \mu\text{m}$ (Mean \pm SE) on Day 4.

The similar increasing pattern was observed in both dentine control and Nupro groups on Day 5 and Day 6 showing further demineralisation of dentine surface while Sylc group was still showing a gradual and slow decrease in ΔR_a on both Day 5 and Day 6.

Post-hoc test was done for intergroup comparison. Dunnett test was applied for comparison of test groups with control group which showed that there were significant differences between both test groups with the control group ($P < 0.05$). Bonferroni test was used to assess the difference between test groups, hence it showed that there was also significant difference ($P < 0.0001$) between the two test groups (Table 4.9 and 4.10).

Table 4.9 Post-hoc test (Dunnett) for Intergroup Comparison of ΔR_a (Dentine)

groups	groups	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Dentine Nupro	Dentine Control	-.0155	.00471	.005	-.0264	-.0045
Dentine Sylc	Dentine Control	.2455	.00471	.000	.2345	.2565

Table 4.10 Post-hoc test (Bonferroni) for Intergroup Comparison of ΔR_a (Dentine)

groups	groups	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Dentine Sylc	Dentine Nupro	.2610	.00471	.000	.2490	.2730

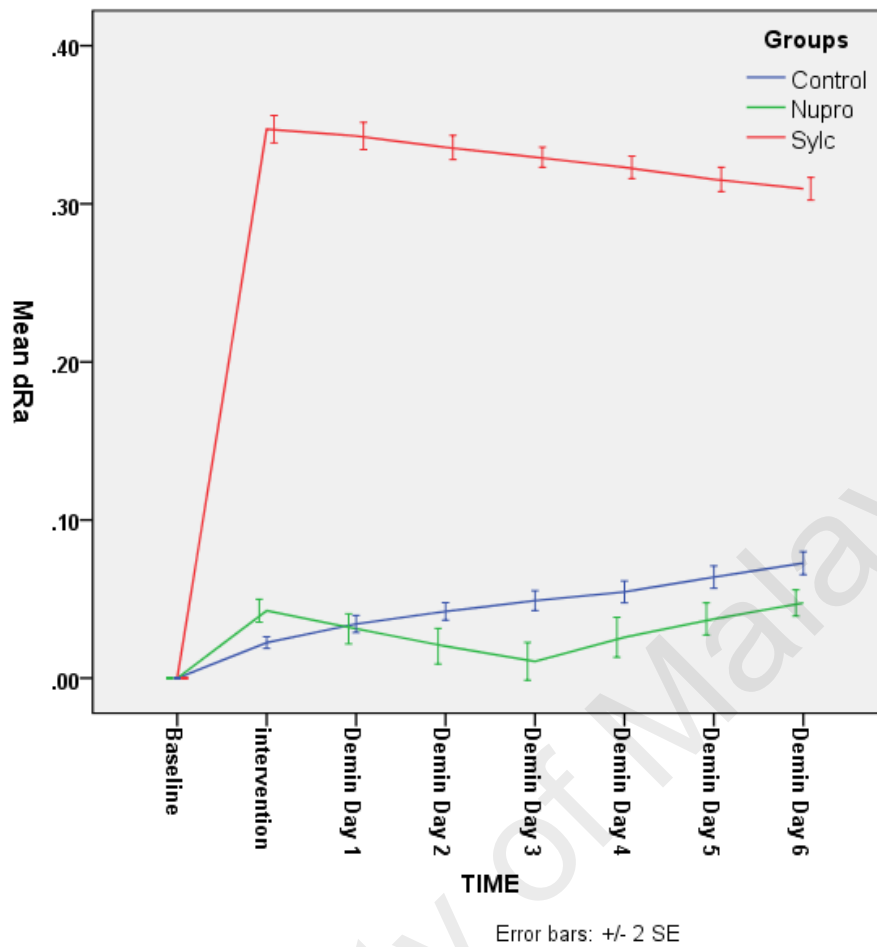


Figure 4.12 Change in ΔR_a from Baseline to Demineralisation Day 6 (Dentine).

4.4 Qualitative analysis of SEM images:

4.4.1 Enamel

4.4.1.1 Control Group

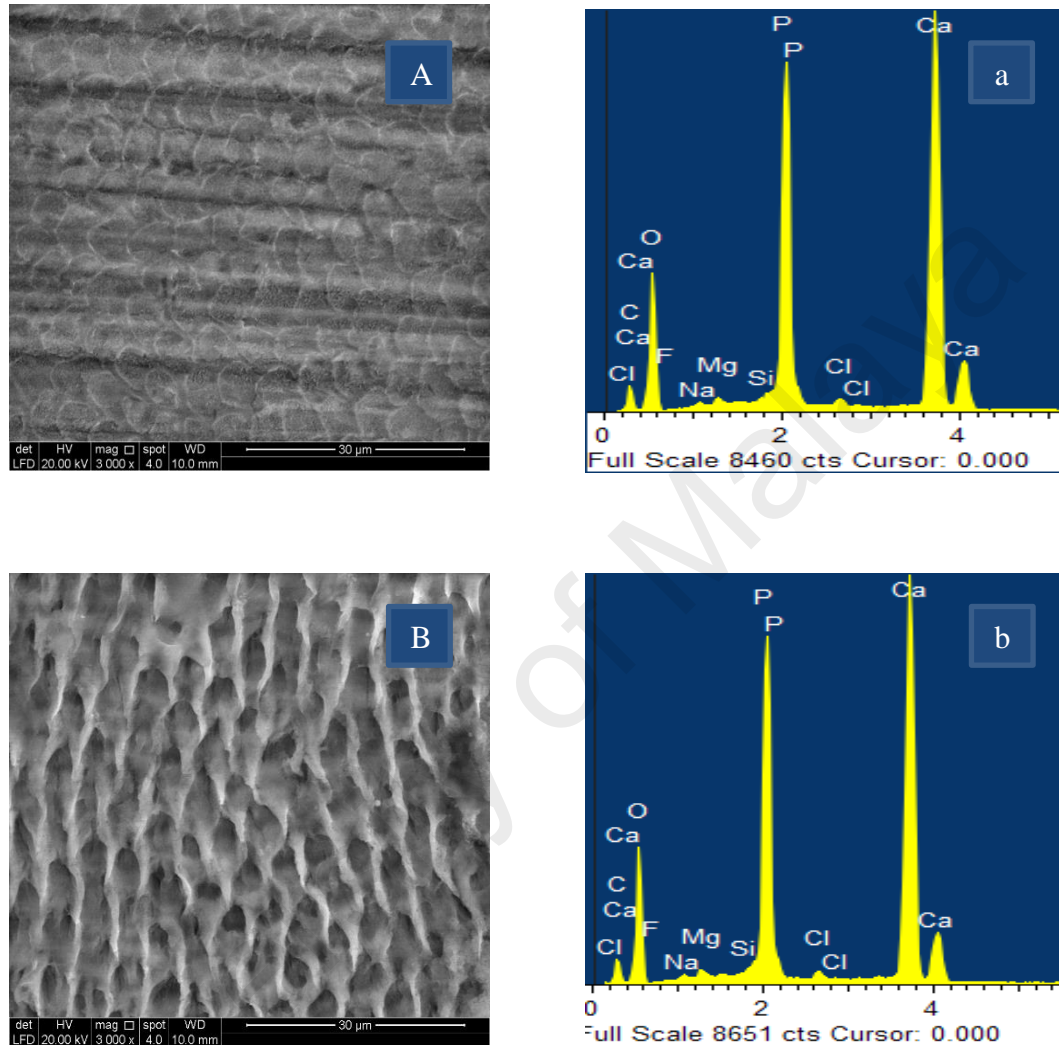


Figure: 4.13 SEM image and EDX element spectra of the surface of an enamel specimen in the Control group: after being subjected to one acidic challenge (A) and (a); and after the 6 days of demineralisation – remineralisation cycle (B) and (b).

Figure 4.13 (A) shows the enamel surface which has been subjected to 1 Day of acid challenge. The SEM image shows signs of early enamel erosion highlighting the prismatic structure of the enamel surface.

Figure 4.13 (B) shows a similar image of the enamel specimen after 6 Days of acidic challenge with accentuated demineralisation resulting in enamel surface with peak appearances and clearly defined prism boundaries.

4.4.1.2 Nupro Group

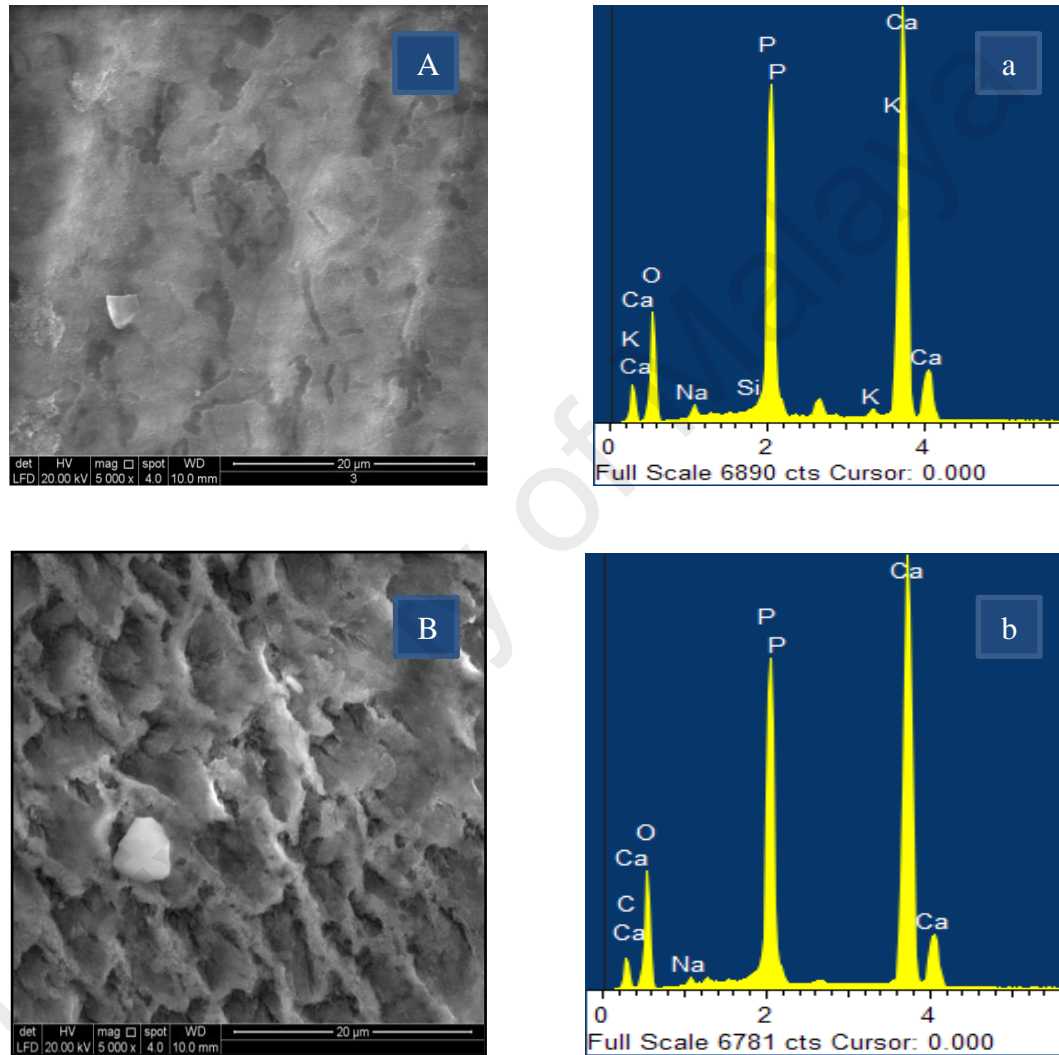


Figure: 4.14 SEM image and EDX element spectra of the surface of an enamel specimen in the Nupro group: after being subjected to one acidic challenge (A) and (a); and after the 6 days of demineralisation – remineralisation cycle (B) and (b).

For the first test group, the enamel specimens were treated with Nupro prophylactic paste and Figure 4.14. (A) Shows a homogenous layer of the material, Nupro on the surface of the enamel samples at 5000x magnification.

At the bottom right corner of Figure 4.14 (A), an angular shaped particle (about 3-4 μm) could be seen embedded within the homogenous layer.

After 6 days of Demineralisation-Remineralisation cycle, the large amount of the bioactive glass material had been removed from the surface of the enamel specimen, eroded enamel surface is very detectible with the presence of deep crypts as shown in Figure 4.14(B). However, some of the Nupro material can be seen within the eroded enamel prisms, at the top left corner of Figure 4.14 (B). This appears as a homogenous layer within the enamel prisms. An angular shaped particle could also be seen lodged within the eroded enamel prisms. This particle could be between 5-6 μm .

4.4.1.3 Sylc group

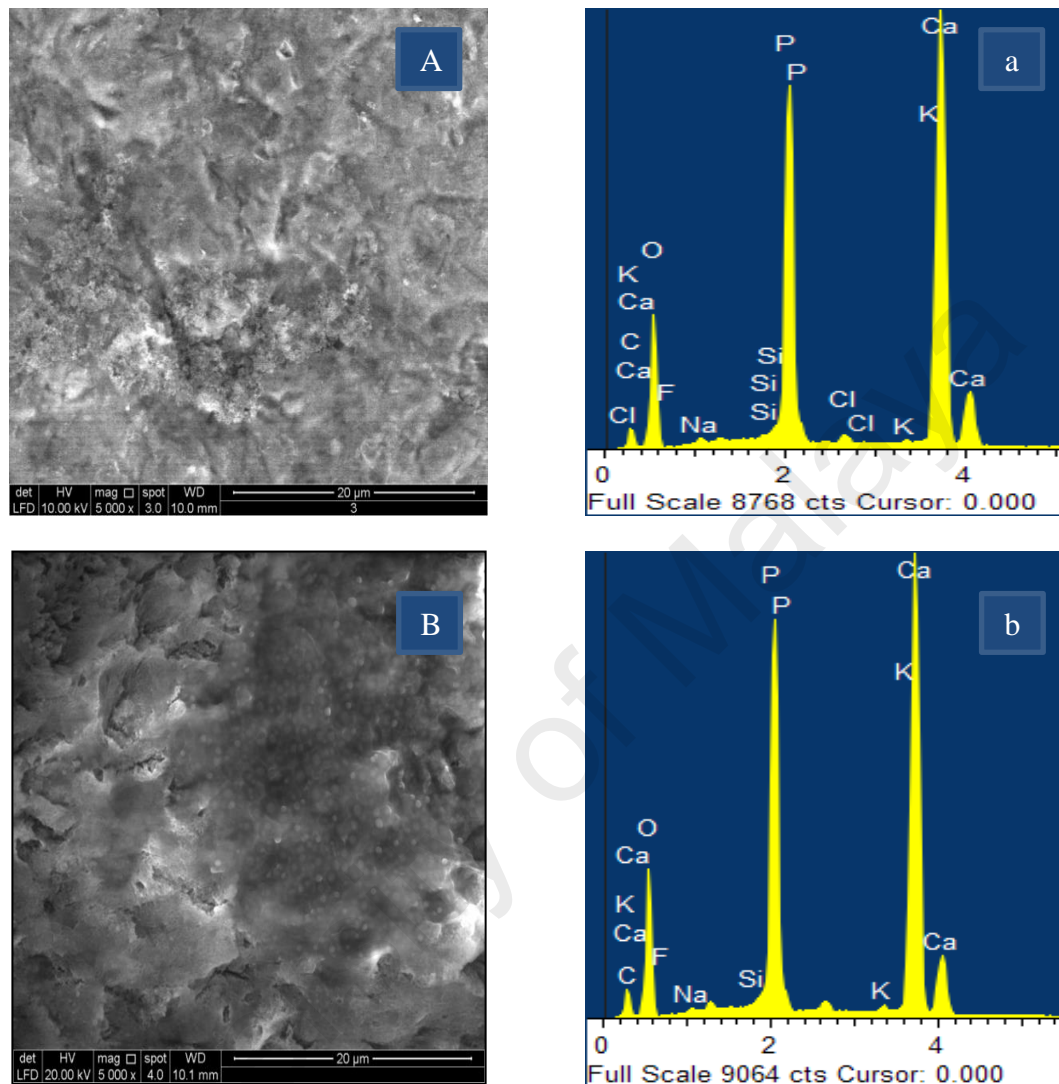


Figure: 4.15 SEM image and EDX element spectra of the surface of an enamel specimen in the Sylc group: after being subjected to one acidic challenge (A) and (a); and after the 6 days of demineralisation – remineralisation cycle (B) and (b)

Figure 4.15 shows the enamel sample in the second test group, Sylc. In Figure 4.15 (A), a uniform homogenous disorderly packed layer of the Sylc material can be seen deposited on the surface of the enamel samples at the Intervention stage.

Figure 4.15 (B) is an image of enamel specimen at the end of the 6 Days of Demineralisation-Remineralisation cycle giving an impression of condensed cloudy surface.

The outline of the enamel prisms could be seen especially at the top right corner. It appears that a large quantity of the bioactive glass material is still adhered on the surface of the enamel after 6 Days of acidic challenge although the homogeneity of the layer is not very constant.

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4.4.2 Dentine

4.4.2.1 Control Group

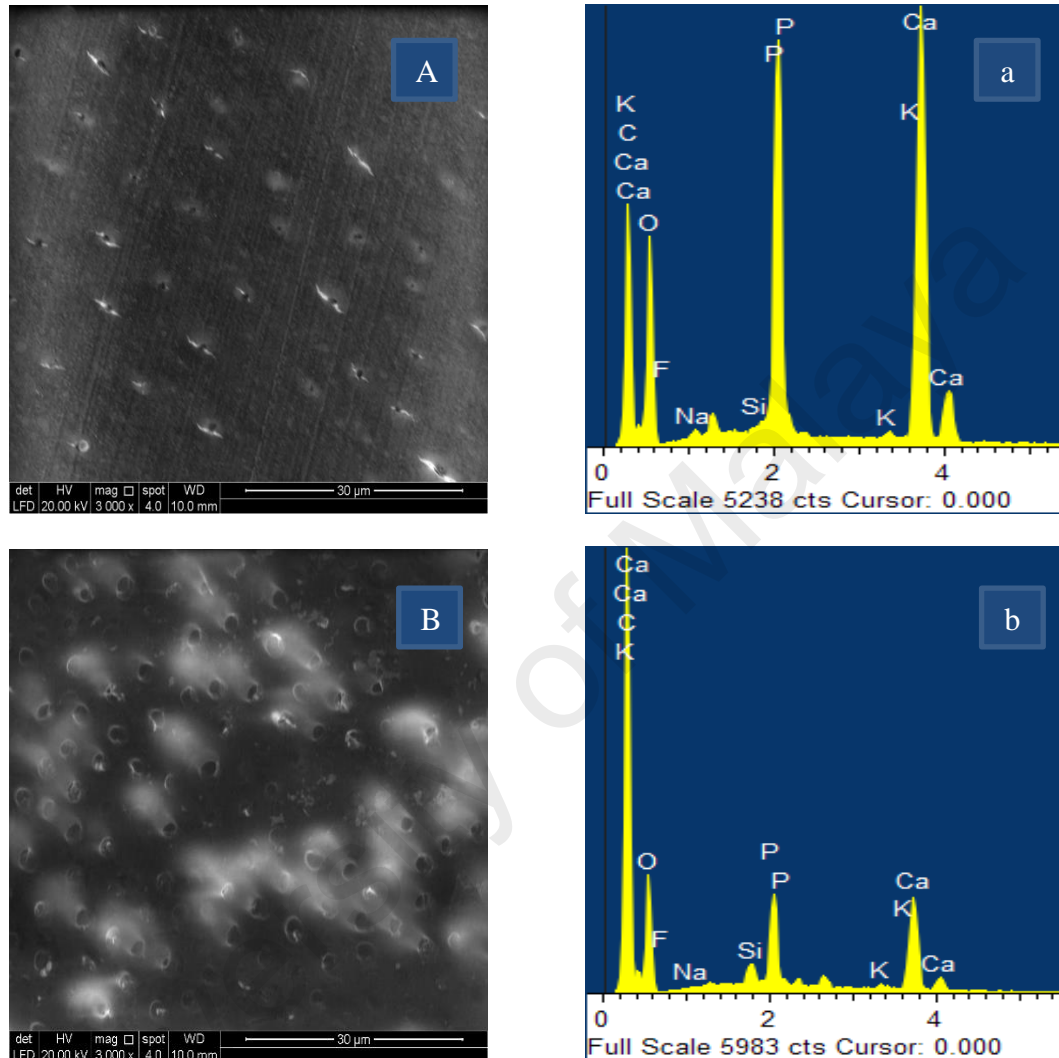


Figure: 4.16 SEM image and EDX element spectra of the surface of an dentine specimen in the Control group: after being subjected to one acidic challenge (A) and (a); and after the 6 days of demineralisation – remineralisation cycle (B) and (b)

Figure 4.16 (A) shows the dentine surface with one day post acid challenge. The SEM view demonstrates a spotted demineralised dentinal surface with few enlarged dentinal tubules.

Figure 4.16 (B) shows an analogous image of the dentine specimen 6 Days of post acid encounter. Wide open dentine tubules are obvious due to demineralisation.

4.4.2.2 Nupro Group

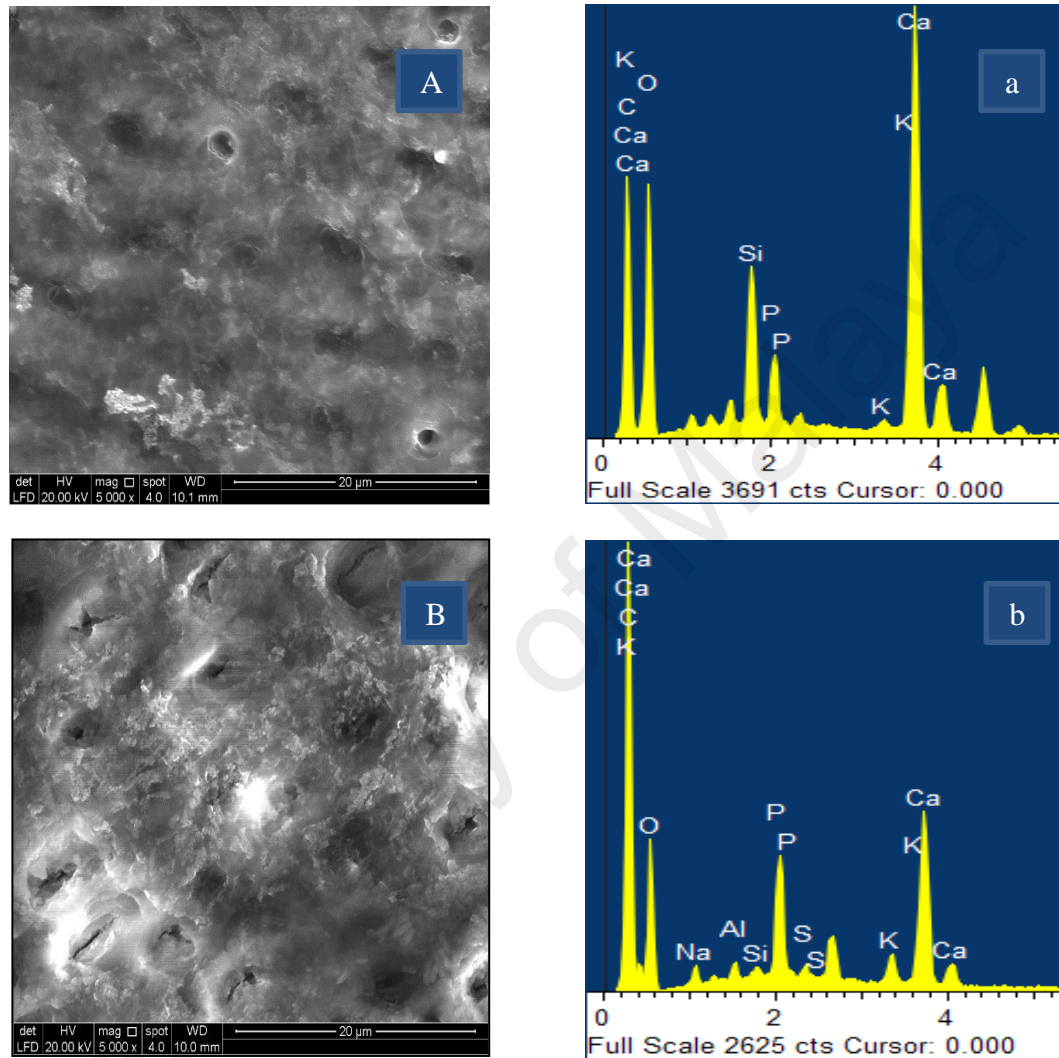


Figure: 4.17 SEM image and EDX element spectra of the surface of an dentine specimen in the Nupro group: after being subjected to one acidic challenge (A) and (a); and after the 6 days of demineralisation – remineralisation cycle (B) and (b).

For the first test group, the dentine specimens were treated with Nupro prophylactic paste and Figure 4.17(A) Scanning electron micrograph image at 5000x magnification showing the dentinal tubules covered by a homogenous layer of Nupro

material. A small rounded particle (about 2-3 μm in diameter) could be seen adjacent to a dentinal tubule (arrowhead).

After 6 Days of Demineralisation-Remineralisation cycle, most of the bioactive glass material had been removed from the surface of the dentine specimen, leaving an eroded dentine surface, as shown in Figure 4.17 (B). The SEM image shows patent dentinal tubules with some residues of bioactive glass particles plugged in some dentinal tubules.

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4.4.2.3 Sylc Group

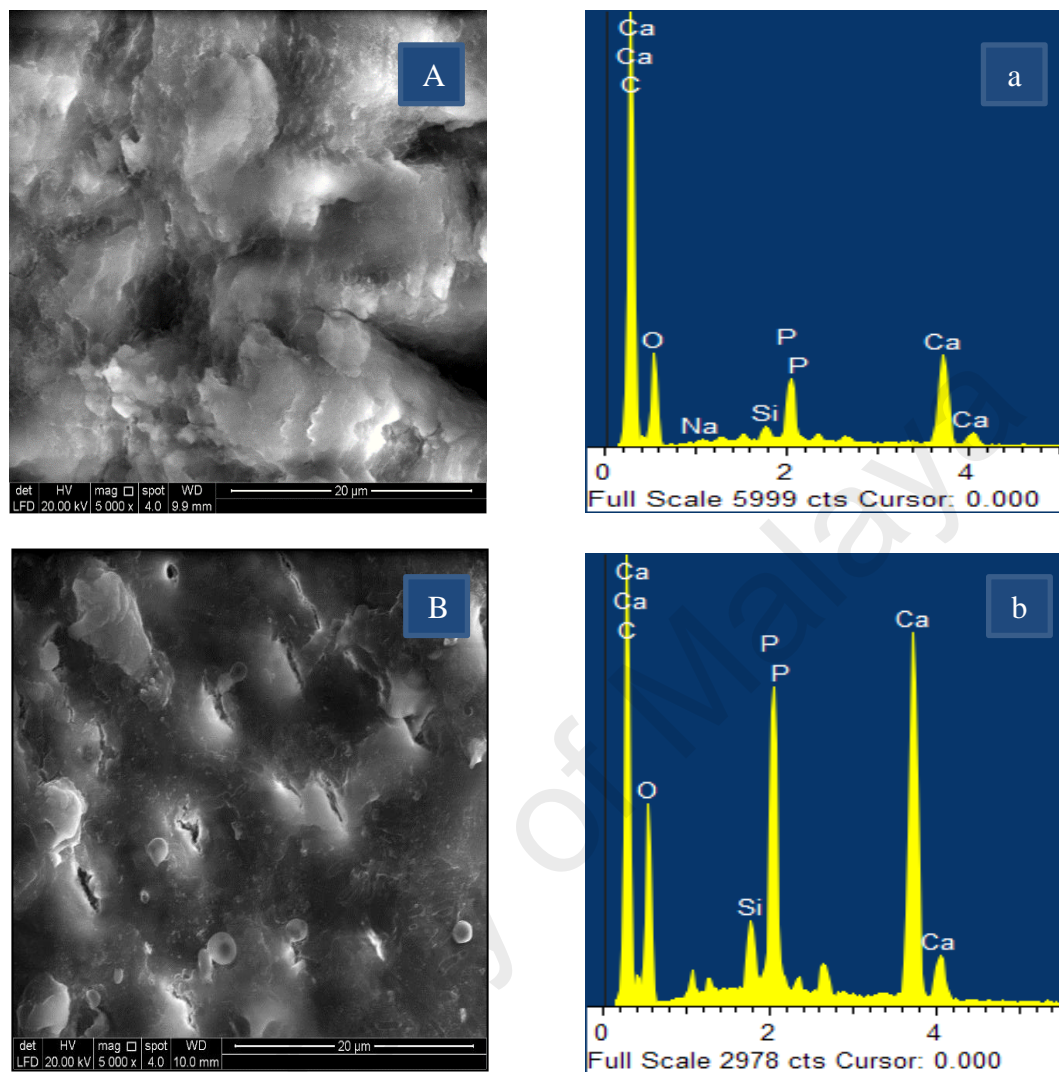


Figure: 4.18 SEM image and EDX element spectra of the surface of an dentine specimen in the Sylc group: after being subjected to one acidic challenge (A) and (a); and after the 6 days of demineralisation – remineralisation cycle (B) and (b)

Figure 4.18 (A) is the SEM micrograph of the dentine specimen treated with Sylc, showing a heterogeneous layer of the Sylc material deposit on the surface of the dentine specimen at the Intervention stage. At the end of the 6 Days of Demineralisation-remineralisation cycle, SEM microradiograph shows traces of the bioactive glass on the surface of dentine specimens (Figure B). Crystal-like deposits

were still adhered to the surface of dentine, albeit the heterogeneous layer of Sylc was less continuous after the 6-Day acid challenge.

4.5 Elemental analysis by Energy dispersive X-ray Spectroscopy (EDX)

The quantitative elementary analysis in the present study focused mainly on Calcium (Ca), Phosphorus (P), Oxygen (O) and Silicone (Si) elements in both enamel and dentine specimens. When compared within each group, the EDX spectrum showed a general trend of decrease in Ca, P and O on Day 6 of the demineralisation and remineralisation cycle when compared to Intervention day, in all three groups. This trend is observed on both enamel and dentine surfaces (Figures 4.19 and 4.20) and is indicative of the occurrence of demineralisation.

The weight percentage of Ca and P of each time point, when compared across groups, were higher in the two test groups, when compared to the Control group, with those in the Sylc group being the highest. Similar trends were again observed in both enamel and dentine specimens. However there were no obvious differences of the weight percentage of O, at each time point, when compared across groups in the enamel specimens.

Silicon (Si) was only observed in the two test groups. When compared within each test group, there was a decrease of Si on Day 6 of experimental cycle when compared to the Intervention day, to the extent that, Si was found to be absent on Day 6 in the Enamel Nupro group. The weight percentage of Si was greater in the Sylc group as compared to the Nupro in both enamel and dentine specimens.

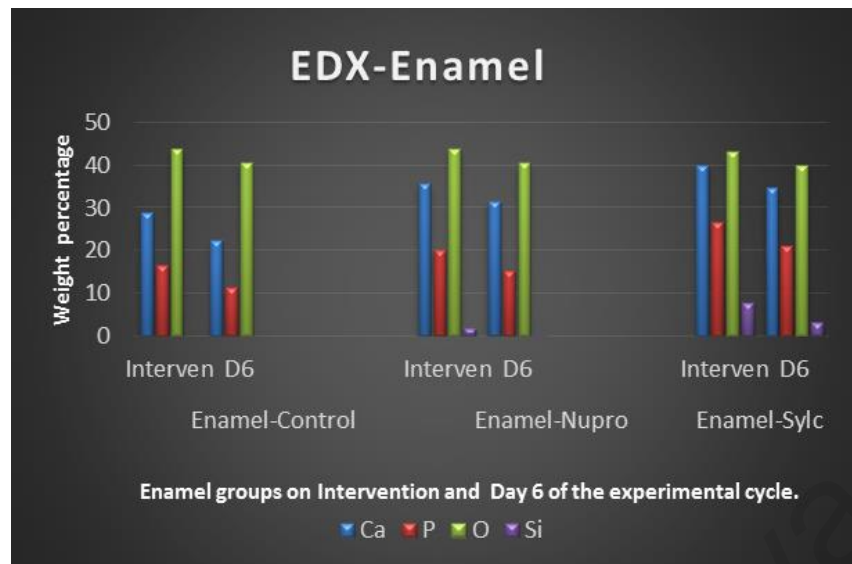


Figure 4.19: Elemental weight percentage in enamel groups at Intervention and Day 6 of experimental cycle.

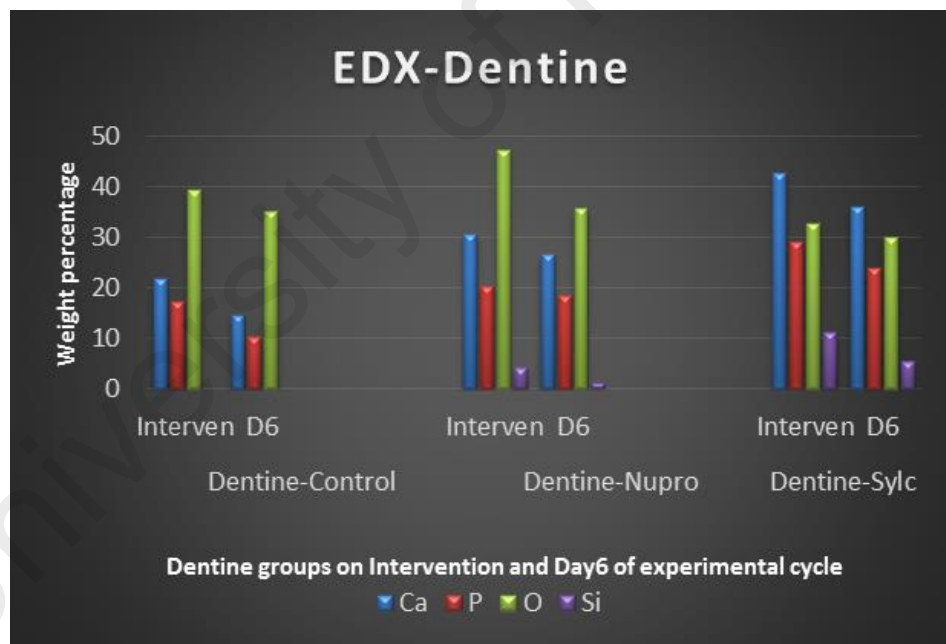


Figure.4.20: Elemental weight percentage in dentine groups at Intervention and Day 6 of experimental cycle.

4.6 Cross Sectional SEM

4.6.1 Enamel Nupro

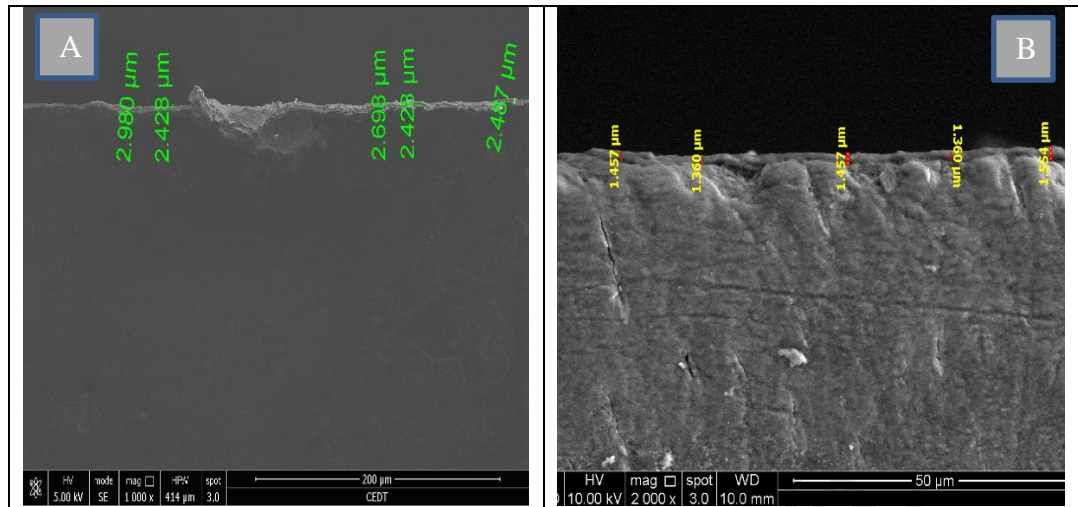


Figure 4.21(A): Cross-section SEM image of enamel Nupro specimen at Intervention (B) Cross-section SEM image of enamel Nupro specimen after six days of experimental cycle.

Figure 4.21(A) shows an SEM image of a longitudinally cross sectioned enamel specimen after application of Nupro. A continuous uneven layer of $2.64 \pm 0.24 \mu\text{m}$ (Mean \pm SD) can be noticed at this stage. In Figure 4.21(B), the image shows cross section view of enamel Nupro specimen on Day 6 of the Demineralisation-Remineralisation cycle. An uneven layer of $1.40 \pm 0.05 \mu\text{m}$ (Mean \pm SD) can be observed clearly. The thickness of the layer decreased greatly during the study cycle in this group.

4.6.2 Enamel_Sylc

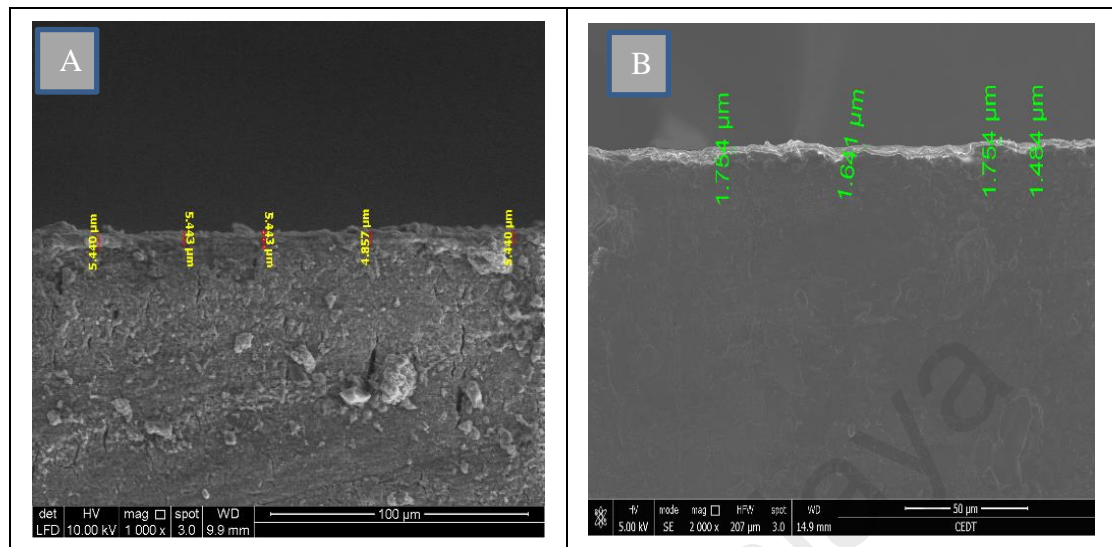


Figure 4.22(A) Cross-section SEM image of enamel Sylc specimen at Intervention (B) Cross-section SEM image of enamel Sylc specimen after six days of experimental cycle.

Figure 4.22(A) SEM image is showing picture of longitudinally cross sectioned enamel Sylc specimen after application of Sylc. A continuous irregular layer of Sylc, with an average thickness $5.19 \pm 0.50 \mu\text{m}$ (Mean \pm SD) can be noticed on Intervention. In Figure 4.22(B), the image shows cross section view of enamel Sylc specimen on Day 6 of the experimental cycle. In this image, a more regular layer of Sylc is observed with the average thickness of the material measuring at $1.70 \pm 0.08 \mu\text{m}$ (Mean \pm SD). It should be noted that the layer of the Bioactive glass (Sylc) was still present on the surface of the enamel specimen after 6 days of erosive challenge albeit demonstrating a reduced thickness.

4.6.3 Dentine Nupro

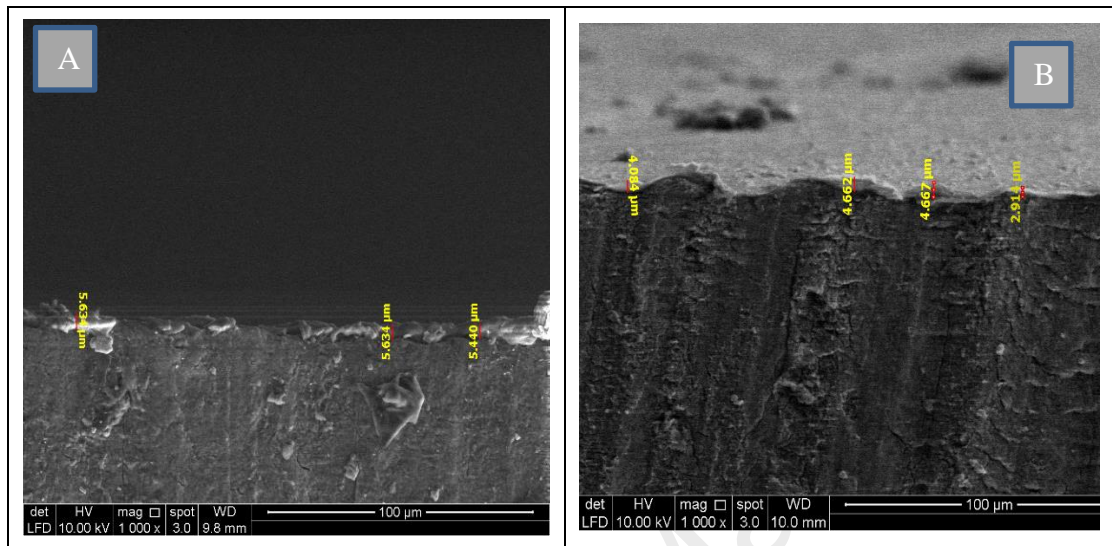


Figure 4.23(A) Cross-section SEM image of dentine Nupro specimen at Intervention (B) Cross-section SEM image of dentine Nupro specimen after six days of experimental cycle.

Figure 4.23(A) SEM image presenting longitudinally cross sectioned dentine Nupro specimen after application of Nupro at Intervention. The image shows an uneven layer of Nupro, with the thickness of the material averaging $5.57 \pm 0.09\mu\text{m}$ (Mean \pm SD) on the surface of the dentine specimen. In Figure 4.23(B) the image shows a similar cross section of dentine specimen after 6 days of remineralisation-demineralisation cycle, with the presence of a thin and wavy layer of Nupro measuring at $3.67 \pm 0.61\mu\text{m}$ (Mean \pm SD). Similar to enamel specimens, the bioactive glass material is still present on the surface of the specimen although with a reduced thickness, indicating the ability to resist the 6 days of acidic challenge. The layer seems wavy possibly due to lack of fine sectioning of the specimen.

4.6.4 Dentine_Sylc

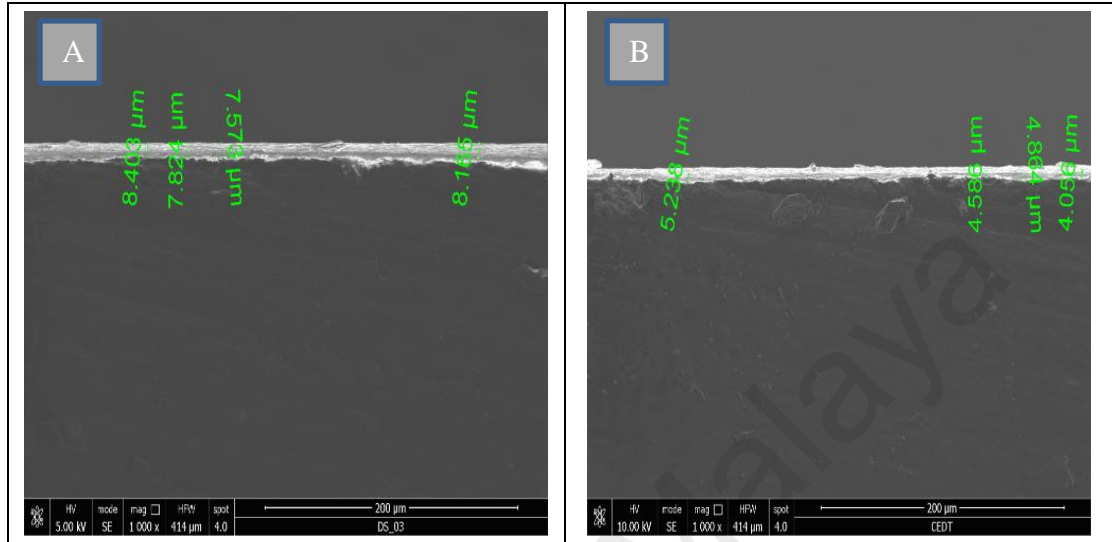


Figure 4.24(A) Cross-section SEM image of dentine Sylc specimen at Intervention (B) Cross-section SEM image of dentine Sylc specimen after six days of experimental cycle.

Figure 4.24 (A) and (B) shows the cross sectional view of dentine Sylc specimens at Intervention day and Day 6 respectively. The average thickness of Sylc deposited on the surface of the specimen at Intervention is $7.99 \pm 0.36 \mu\text{m}$ (Mean \pm SD) whilst on Day 6 of the experimental cycle, the thickness reduced, with an average thickness of $5.602 \pm 0.18 \mu\text{m}$ (Mean \pm SD). Although there is a decrease in the thickness of the Sylc layer at the end of Day 6, it should be noted that the remaining layer displays a similar continuous, smooth layer, when compared to the appearance of bioactive glass layer at Intervention.

CHAPTER 5

DISCUSSION

5.1 Discussion of Methods:

Research in the field of dentistry suggests that the media in which teeth are stored following harvesting influence bond strength and, consequently, microhardness of teeth (231). Moreover, one report (232) showed that although post-extraction time did not significantly affect dentine bond strength, the depth of preparation and storage media affect the adhesion properties of dentine. Mitchem et al. (233) further demonstrated that adhesion decreased significantly as the pulp was approached. In another study, Aulino et al. (234) found that no difference existed between the adhesive bond strengths of teeth stored in 0.9% thymol and distilled water and those stored in distilled water alone. It is for these reasons that all the teeth used in this study were stored in distilled water. Similarly, other authors (117, 152, 235) used distilled water to store tooth specimens although the temperature at which the specimens were stored varied between the studies. In a study that assessed fluoride treatment and resistance of dental enamel to soft drink erosion *in vitro*, Ren et al. (152) used freshly extracted human third molars, which they initially stored in normal saline at 4°C. Enamel slabs of 5 mm x 5 mm x 1.5 mm were then cut from the buccal and lingual surfaces of the third molars and moulded to form a 10 mm x 10 mm round disc. The enamel surfaces were ground under water coolant to achieve a flat surface and kept in distilled water at room temperature, prior to testing. Conversely, besides using an identical grinding and polishing protocol for preparing enamel and dentine samples as Ren et al. (235), we also stored our samples in distilled water at a temperature of 4 °C.

Many beverages, including fruit juices and carbonated drinks contain citric acid (236), and are implicated in the rising incidence of dental erosion (237). There is evidence that dental erosion caused by citric acids is different from that due to other weak or strong acids owing to the fact that citric acids have a more complex interaction

(55). Citrate ions can remove calcium from the crystal surface of teeth, and each acid anion has a different calcium complexation strength, which depends on the molecule structure and how readily it can form complexes with the calcium ion. As a result, citric acid has a double action, and it is very damaging to tooth surface (55). This property of citric acid was used in the current study and in several researches to investigate enamel and dental erosion in both human (238-240) and bovine teeth (241). While we used a pH of 3.2, similar to that used in the study conducted by Ranjitkar et al. (240), the pH used in the other studies varied, ranging from 2.2 in the study by Attin et al. (241) to greater than 2.3 but less than 6.3 in the study by Barbour et al. (238). According to a previous study (242), the critical pH below which dental enamel dissolves is considered to be 5.5, which is above the value used in our study; however, a more recent study (243) reported that the critical pH for dental enamel is not constant, but it is rather inversely proportional to calcium and phosphate concentrations in saliva.

In this study, the samples were immersed in 200 mL of citric acid and stirred at a constant speed of 400 rpm for 10 minutes at room temperature (24 °C). On the other hand, the testing materials Nupro Prophylaxis Paste and Sylc Prophy Powder were applied for comparatively shorter periods. This model was designed as such in order to simulate a probable clinical context in which oral hygiene products are usually applied for one minute and soft drinks—which are typically consumed cold or at room temperature (24 °C)—are sipped over ten minutes (244). The effect of temperature on dentine and enamel erosion by dietary acids has been described by West et al. (237). In their study, enamel and dentine samples from unerupted human third molars were separated into groups of five specimens and placed in citric acid over a temperature range of 5 to 60 °C for 10 minutes. Results demonstrated that at greater temperatures,

dentine and enamel erosion were increased. Similarly, increasing concentration of immersion medium and exposure time increased dental erosion (237).

As alluded to in the previous chapter, saliva is considered to be an important biological factor against dental erosion (88, 143-145). Previous research tested several therapies using *in vitro* protocols and saliva was, in most cases, used as a control to mimic the oral environment. Unfortunately, it is practically impossible to duplicate the properties of human saliva owing to its specific characteristics. Another setback is the lack of standardization on the formulation of saliva. Furthermore, various types of saliva can cause remineralisation of varying degrees.

The remineralisation solution used in this study is similar to that used by Amaechi and Higham (245), who demonstrated that the observed remineralisation of early enamel erosion could be achieved by natural saliva as well as by a solution at a neutral pH containing calcium, phosphate and fluoride at concentrations that are sufficient to cause remineralisation. Previous studies have confirmed the potential of artificial saliva (246) and calcifying solutions (247, 248) to remineralises softened or etched enamel. However, Amaechi and Higham (245) found that remineralisation in artificial saliva might have been limited by the presence of carboxyl methylcellulose, which was reported in previous studies (249, 250) to reduce the re-hardening potential of artificial saliva. It is believed that this effect of carboxyl methylcellulose is due to its property of forming complexes with calcium and/or phosphate ions or increasing the viscosity of the artificial saliva (245). The effect of carboxyl methylcellulose was established by the degree of remineralisation observed in the remineralising solution that contained the exact same constituents as the artificial saliva devoid of the carboxyl methylcellulose, but demonstrated a greater remineralising potential than the artificial saliva (245).

It is important to determine how much material tooth specimens have lost or gained during *in vitro* experiments. This can usually be achieved by techniques suitable for direct or indirect mineral quantification, such as microhardness tests, which we used in our study. The Knoop or Vickers hardness tests have been traditionally utilized to detect changes brought about by erosive substances on the surface of enamel. According to some reports (145, 204), these tests can detect surface changes shortly after dental tissue is exposed to an erosive substance. While the depth of penetration by Knoop and Vickers diamond indenters vary, it has been deduced that the Knoop indenter—used in the present study—has the capacity to detect changes that involve the most superficial layers of eroded enamel (208).

Surface roughness indicates the area between the roughness profile and the mean line or the integral of the absolute value of the roughness profile height over the evaluation length. The arithmetic average of deviations of the surface profile from an imaginary centreline, (R_a), is the most common surface roughness parameter used for reporting roughness in dentistry(214). Hence it was utilized in the current study.

Surface roughness is good for characterizing random type surfaces, such as grinding. However, R_a is limited in that it only gives information of the amplitudes but the value contains no information about the textural characteristics of a profile, for example, whether the profile contains more of peaks or troughs (255). In practice, the Bearing Area Curve (BAC) or Abbott-Firestone Curve has been used to assess surfaces since its creation in 1933 by Abbott and Firestone (251). The BAC provides the ratio of material length at any level, beginning from the highest point, also known as the bearing ratio, as a function of level. The BAC is produced from a surface profile by drawing a parallel or bearing linear distance from a reference or mean line (251). The sum of the

length of each material intercept along the line is computed and the proportion of this sum to the total length is calculated as the bearing ratio (t_p). Beginning from the highest peak to the lowest valley, this process is repeated along several bearing lines, and the fractional land length is plotted as a function of the height of each slice from the highest peak (251).

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5.2 Discussion of Results:

This study investigated the anti-erosive potential of Sylc® Prophy Powder and NUPRO® prophylaxis paste on dental hard tissue. Our analysis showed that Sylc® Prophy Powder and NUPRO® prophylaxis paste had high anti-erosive potentials, with Sylc® Prophy Powder showing a greater potential.

Parkinson & Willson (2010) suggested that significant increase in Surface microhardness from Baseline is indicative of remineralisation and intimate incorporation of the particles into the enamel surface and possible recrystallization of the hydroxyapatite crystals (252).

Statistically significant increase was noticed in Sylc enamel group for SMH compared to Baseline (Δ SMH (t_0)) up till Day 6 demineralisation. This suggests the 'remineralisation' effect of Sylc was still in place after 6 days of progressive erosion.

On the other hand, a net decrease in the mean SMH was observed for enamel specimens treated with Nupro. Statistically significant increase in SMH (t_0) was only detected on Intervention Day in Nupro group. The SMH (t_0) were not statistically different from the other days of demineralisation. This could potentially mean the protective effect was not sustained during further demineralisation. These findings are in line with the findings of Banarjee et al. (25) who reported that Sylc bioactive glass demonstrated a significantly higher remineralisation potential. Similar to our findings, other authors reported that Novamin-containing pastes and Nupro® Prophy Paste increased the SMH of dental tissue (38, 252).

A significant decrease in surface microhardness of enamel specimens from Baseline is indicative of progressive demineralisation. The progressive decrease in

SMH in both test groups is possibly due to gradual loss of Sylc and Nupro from the surface of enamel specimens as a result of continuous demineralisation from Day 1 to 6. A total of three SMH readings per specimen every day during the six days remineralisation-demineralisation cycle were recorded, due to this large standard error has been noticed in the results of SMH. This standard error could have been reduced by increasing number of readings at each time point during the experimental cycle.

The results of SMH of enamel test group specimens were further supported by elemental analysis of enamel Nupro and enamel Sylc group specimens after Intervention day and at the end of the study cycle. An increase in Ca, P, O and Si was observed in both test groups after application of test materials which conferred to higher surface Microhardness of enamel specimens due to remineralisation. The weight percentage of Ca, P, O and Si were higher in Sylc group as compared to Nupro. The evidence of remineralisation by application of bioactive glasses on enamel surfaces through elemental gain of Ca, P, O and Si has been reported in several studies ((12, 20). Similarly increase in surface Microhardness on enamel surface after application of BAGs on demineralised surface and remineralisation by elemental gain of Ca, P, O and Si has also been reported by Deng et al,(39).

A small quantity of Sylc material on the surface of the specimens at the end of Demineralisation Day 6, as seen on scanning electron micrographs (Figure 4.15 B & C), conferred a higher degree of hardness to enamel. Novamin, which is one of the constituents of Nupro® Prophy Paste, has been reported to deposit fine particles onto the surface of dentine and enamel, resulting in a series of reactions that cause the formation of a crystalline, hydroxycarbonate apatite layer (253). This hydroxyl

carbonate apatite layer is chemically and structurally similar to natural enamel and dentine, and it is resistant to acid challenges.

The presence of BAG layer on the test group specimens of both enamel and dentine was further confirmed by cross sectional SEM images taken after Intervention day and on Day 6 of the experimental cycle. The results of thickness of BAG layer by cross section SEM images were similar to another lab study conducted by Z.Dong et al,(20) in which different compositions of bioactive glasses were evaluated for teeth remineralisation effect on etched human enamel in simulated oral environment. The results indicated that the BAG deposits on enamel surface formed a homogenous and dense mineralized layer which was confirmed by SEM images and elemental analysis by EDX (20).The average thickness of the layer of BAG formed on the specimens treated with 45S bioactive glass (20) was recorded as 4 μ m which is almost similar to our results of enamel Sylc group. Gjorgievska E, Nicholson J.(12) also confirmed presence of BAG layer on cross sectional SEM image of demineralized enamel surface. The author reported treatment with any toothpaste containing bioactive glass Novamin® resulted in formation of a protective layer on enamel surface.

The thickness of BAG layer on the enamel specimens was compared to the penetration depth, h , of the indenter of surface Microhardness. This was to check that whether the indenter was on the BAGs layer or it has penetrated onto enamel surface. It confirmed that the indenter was not on the bioactive glass layer but rather it was on the enamel surface in Nupro group (Figure.4.21A) at Intervention day. However, in the Sylc group, (Figure.4.22A) the thickness of BAG layer at Intervention day when compared to the penetration depth of the indenter indicates that the indenter was on the BAG layer and not onto the enamel surface. Hence it was noted that Sylc® Prophy

Powder which has a 100% bioactive glass ingredient formed a thicker layer of BAG on the specimens which was more resistant to acidic challenges till the end of the experimental cycle. Comparing the thickness of BAGs layer on cross section SEM images of enamel test groups specimens with the penetration depth of indenter of SMH it was obvious that in both test groups specimens, the indenter was on the enamel surface and not on the BAG layer at the end of the experimental cycle (Table 4.4).

In measuring the mean surface roughness of enamel specimens, we observed a substantial increase in the Sylc group, followed by Nupro, and the least increase in surface roughness was recorded in the control group.

A significant increase in surface roughness in enamel specimens in the form of R_a from Baseline could be indicative of the following

1. Progressive demineralisation

The pattern of the roughness profile observed is of short wavelengths and big amplitudes as seen in Figure 4.5B

2. The presence or retention of bioactive glass particles on the surface

The roughness profile pattern for this situation is observed to be of big wavelengths and shorter amplitudes (Figure 4.6A and Figure 4.7A)

3. Or a combination of both

On the other hand, a significant decrease in surface roughness as measured with R_a from Baseline could be indicative of loss of the bioactive glass particles.

There was a statistically significant increase in R_a in enamel Sylc group from Baseline up till Day 6, where the pattern of roughness profile is represented in Figure 4.7B. In this image of enamel Sylc specimen at Day 6 of demineralisation, surface roughness profile graph shows high amplitudes of both the peaks and troughs but the

peaks appear to be higher in number compared to the troughs. The wavelengths do not defer from that of Intervention (Figure 4.7A), i.e. it is still bigger than that of Baseline. This is most likely due to the fact that there is still retention of the particles on the enamel surface at the end of 6 days of progressive erosion.

Enamel specimens treated with Nupro showed statistically significant differences in $\Delta R_a(t_0)$ up till Day 1 demineralisation and it could be postulated that there was significant retention of the particles only in one day of further demineralisation. Subsequent demineralisation had significantly reduced the presence of the particles.

We believe that the increase in surface roughness in the test groups was due to application of the test materials on the specimens, while in the control group, the increase was due to progressive demineralisation of the surface of the enamel specimens.

While we observed that enamel specimens in the Sylc group showed a decrease in the mean surface roughness through Days 1 to 6, those in the Nupro group showed a decrease through days 1 to 3 and a sudden increase on Day 4. The sudden increase in the Nupro group was probably because the material was gradually washed off the surface of the specimens. Furthermore, Yurdagüven et al. (254) showed that enamel had fine irregularities after treatment with a prophylaxis paste, which the authors suggested was probably due to the inorganic composition of enamel, while dentine samples had coarse regular scratches after treatment.

Similar to the enamel specimens, a significant increase in surface roughness in dentine in the form of R_a from Baseline could be indicative of:

1. Progressive demineralisation.

The pattern of the roughness profile observed is showing relatively regular dentine surface with graph showing peaks and troughs of similar amplitudes (Figure 4.9B).

2. The presence or retention of bioactive glass particles on the surface.

The roughness profile pattern for this situation is observed to be showing a relatively irregular dentine surface and the graph shows equal number of peaks and troughs. Both the peaks and troughs also exhibited relatively similar amplitude dimensions as seen in Figure 4.10A and Figure 4.11A.

3. Or combination of both.

Dentine specimens treated with Sylc showed statistically significant increase in R_a from Baseline up till Day 6 where the pattern of roughness profile is seen Figure 4.11B. This picture shows obvious homogenous irregular surface of dentine and the surface roughness profile shows peaks and troughs with similar amplitude as that at Intervention (Figure 4.11A). However, the wavelengths are slightly wider than at Intervention. This means that there is still retention of the particles on the dentine surface at the end of 6 days of progressive erosion and there is no sign of progressive demineralisation.

A similar increase in surface roughness values of dentine specimens was observed by Wang et al.(255) in control and treatment groups (bioactive glass 45S5 and modified bioactive glass). However, surface roughness values were highest for dentine specimens in the control group, followed by those treated with bioactive glass 45S5, and least for those treated with modified bioactive glass particles.

In the dentine Nupro group, statistically significant differences in $\Delta R_a(t_0)$ were found up till Day 1 demineralisation and it could be postulated that there was significant retention of the particles only in one day of further demineralisation. Subsequent demineralisation had significantly reduced the presence of the particles.

One report (252) compared the level of dentine tubule occlusion and dentine mineralization achieved with Novamin-containing toothpaste (Sensodyne® Repair & Protect) to that of other commercial toothpastes reported to occlude dentine tubules. In their study, dentine disc samples were randomly assigned to eight treatment groups and brushed twice-daily for four days with one of the seven tested toothpastes or deionized water. All the samples were subjected to two five-minute acid challenges and tested for SMH each day after treatment. It was found that a positive change from baseline was indicative of the surface becoming harder after treatment. Sensodyne® Repair & Protect caused a significantly higher increase in SMH than the other toothpastes. The increase in SMH after Sensodyne® Repair & Protect treatment was consistent with mineralization of the dentine surface and the formation of mineral deposits that were harder than dentine. In another study that tested the ability of bioactive-containing prophylactic pastes or air polishing/cutting powders in encouraging the remineralisation and occlusion of dentinal tubules, Sauro et al.(38) found that Sylc caused significant dentine remineralisation when compared to all the alternate test materials (3 wt.% mono potassium-mono hydrogen oxalate in water, sodium bicarbonate NaHCO_3 Cavitron®Prophy Powder, amino-acid-glycine $\text{NH}_2\text{CH}_2\text{COOH}$ EMSPerio, casein and phospho peptide-amorphous calcium phosphate § GC Tooth Mousse, 8% calcium carbonate-arginine § Colgate Sensitive Pro-Relief, and 5% calcium sodium phosphosilicate NovaMin® § NUPRO Solution Prophy Paste).

Ian Thompson in 2010 presented a report(256) which states that Sylc underwent a rapid surface reaction that allowed it to physically adhere to tooth structure, and microscopic and spectroscopic analysis of teeth that were treated with Sylc showed that the material penetrated into the tubules and formed mineral hydroxycarbonate apatite to protect the tooth surface.

Clinically significant results were obtained by Du Min et al. (257) in a study that assessed the efficacy of NovaMin for the treatment of dentin hypersensitivity. These results prompted researchers to hypothesize that NovaMin could be beneficial in remineralising and preventing demineralisation of tooth structures, especially dentine. The change in the mean surface roughness in the dentine specimens mirrored that of the enamel specimens. The differences in the dentine surface roughness data indicates the two test materials behave differently in their interaction with the exposed dentine surface.

In our study, the mean surface roughness values were higher for dentin samples than for enamel. A similar finding was reported by Yurdaguvan et al. (254) who found that dentine surfaces were more affected by the application of prophylaxis pastes than enamel surfaces. In their study, Yurdaguvan et al.(254) observed that the initial and final surface roughness readings were 0.024 ± 0.006 and 0.071 ± 0.015 for enamel and 0.030 ± 0.007 and 0.143 ± 0.029 for dentine.

In the current study, the degree of separation between Sylc and Nupro in dentine specimens was greater compared with the degree of separation in enamel, which is because Sylc adheres better to exposed dentine compared to enamel. Therefore, a smaller quantity of Sylc was dislodged after the demineralisation-remineralisation cycle. As in the case of the enamel specimens, the increase in surface roughness in the

dentine group was probably due to application of the test materials on the specimens. However, the increase in surface roughness of specimens in the control group was due to demineralisation as there was no treatment given to these specimens during the 6 Days of experimental cycle, but instead, the specimens were subjected to daily acidic challenge.

SEM images of dentine test group specimens showed deposition of Nupro and Sylc material heterogeneously on the dentine surface. The cross sectional dentine SEM images of Nupro (Figure.4.23A) and Sylc (Figure 4.24A) also confirmed the presence of bioactive glass layer after application of test materials on Intervention day. Further the remineralisation of dentine test groups specimens was confirmed by increased Ca,P,O and Si levels on Intervention day. At the end of the experimental cycle on Day 6, the cross sectional SEM images of dentine test group specimens showed uneven and thinner BAG layer in Nupro group (Figure.4.23B) while the dentine Sylc group (Figure.4.24B) still showed a continuous BAG layer but of reduced thickness when compared to Intervention day. This would be possibly due to continuous wash off of the layer during daily acidic challenges. The EDX spectra showed reduced levels of Ca, P, O and Si on Day 6 of experimental cycle in dentine test groups. Comparing the weight percentage values of Ca, P, O and Si between enamel and dentine test group specimens on Intervention day and at the end of the study cycle, dentine specimens showed higher values than enamel which indicates both tendencies; remineralisation and acid resistance were greater in dentine rather than on enamel.(Figure. 4.19-4.20)

The occlusion of demineralised open dentinal tubules by bioactive glass has also been reported by Sauro,(38) by SEM images and the mineral gain of Ca, P,O and Si has been proven through elemental analysis EDX. In this study both Sylc® Prophy Powder

and NUPRO® prophylaxis paste along with other in-office remineralising materials has been used for remineralisation of demineralised dentine surface. Sylc® Prophy Powder being a full concentration bioactive glass product showed best remineralising property when compared to all other test materials. Remineralisation by application of bioactive glasses by increased Ca, P,O and Si on demineralised dentine surfaces has also been reported in several studies (40, 42, 43), where the elemental analysis was done through EDX and the morphological changes were studied through SEM.

The reasons of the differences observed between Sylc® Prophy Powder and NUPRO® prophylaxis paste could be due to following reasons;

- I. Different methods of application.
- II. Different concentration of active ingredient; Sylc® Prophy Powder contains a 100% concentration of Bioactive glass while NUPRO® prophylaxis paste has lesser concentration of Bioactive glass along with fluoride and other ingredients.(Table 3.1).
- III. Different forms i.e. (paste vs powder).

The null hypothesis of this study which states that there is no anti erosive potential of Sylc® Prophy Powder and NUPRO® prophylaxis paste on demineralised enamel and dentine and there is no significant difference in the anti-erosive effect between Sylc® Prophy Powder and NUPRO® prophylaxis paste on demineralised enamel and dentine surfaces has been rejected.

CHAPTER 6

CONCLUSIONS

&

RECOMMENDATIONS

6.1 Conclusions for Enamel :

1. From this in-vitro study, it can be concluded that both Nupro and Sylc have exhibited anti-erosive potentials when subjected to multiple acidic exposures.
2. The anti-erosive effect of Sylc on enamel is better than Nupro on enamel.
3. Sylc showed anti-erosive potentials till the end of the 6 day Remineralisation-Demineralisation cycle and demonstrate remineralising potentials.
4. Nupro exhibited anti-erosive potential only during Intervention and did not appear to demonstrate remineralising potentials.

6.2 Conclusions for Dentine:

1. From this in-vitro study, it can be concluded that the anti-erosive effect of Sylc on dentine is greater than Nupro on dentine.
2. Sylc showed anti-erosive potentials till the end of the 6 day Remineralisation-Demineralisation cycle.
3. Nupro exhibited anti-erosive potentials up until Day 1 of the Remineralisation-Demineralisation cycle.

6.3 Recommendations:

1. To better assess and monitor the remineralising effects of Sylc and Nupro, cross sectional micro computerized tomography (Micro CT) could be used.
2. This *in-vitro* study was conducted as a preliminary study and the study design incorporated a six-day demineralisation-remineralisation cycle. However, further studies could include longer demineralisation-remineralisation cycles such as a 14-day cycle, 1 month or more.

3. In order to replicate oral cavity environment, the specimens should have been processed through thermo cycling between the Demineralisation-Remineralisation cycles.

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