T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PEDIATRIC DENTISTRY

EVALUATION OF THE EFFECT OF TOOTHPASTES WITH DIFFERENT INGREDIENTS ON THE SURFACE MICROHARDNESS AND SURFACE PROPERTIES OF ARTIFICIAL INCIPIENT CARIES, *IN VITRO*

DOCTOR OF PHILOSOPHY THESIS

NECMİYE BULAM, DENTIST

Istanbul-2021

T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PEDIATRIC DENTISTRY

EVALUATION OF THE EFFECT OF TOOTHPASTES WITH DIFFERENT INGREDIENTS ON THE SURFACE MICROHARDNESS AND SURFACE PROPERTIES OF ARTIFICIAL INCIPIENT CARIES, *IN VITRO*

DOCTOR OF PHILOSOPHY THESIS

NECMİYE BULAM, DENTIST

SUPERVISOR
PROF. DR. SENEM SELVİ KUVVETLİ

ASSISTANT SUPERVISOR
DR. ASSOC. PROF. KÜBRA TONGUÇ ALTIN

Istanbul-2021

THESIS APPROVAL FORM

Institute : Yeditepe University Institute of Health Sciences
Programme : Doctoral Program In Pediatric Dentistry
Title of the Thesis : Evaluation of the effect of toothpastes with different ingredients on the surface microhardness and surface properties of artificial incipient caries, *in vitro*.
Owner of the Thesis : Necmiye BULAM
Examination Date : 21.05.2021

This study have approved as a Doctorate Thesis in regard to content and quality by the Jury.

	Title, Name-Surname (Institution)
Chair of the Jury: Supervisor:	Prof. Dr. Senem SELVİ KUVVETLİ (Yeditepe University)
Member/Examiner:	Prof. Dr. Aslı TOPALOĞLU AK (Istanbul Aydın University)
Member/Examiner:	Prof. Dr. Elif Bahar TUNA İNCE (Istanbul University)
Member/Examiner:	Assoc. Prof. Dr. Elif SUNGURTEKİN EKÇİ (Yeditepe University)
Member/Examiner:	Dr. Assoc. Prof. Gülengül DUMAN (Yeditepe University)

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work, that I adhered to ethical behavior at all stages from planning to writing, that I obtained all the information in this thesis within academic and ethical rules, that I cited all the information and interpretations that were not obtained through the research and included these sources in the list of references, and that I have not violated patents or copyrights during the conduct and writing of my thesis.

NECMİYE BULAM

ACKNOWLEDGEMENTS

First of all, I would like to thank **Prof. Dr. Senem SELVİ KUVVETLİ**, Head of the Department of Pediatric Dentistry and my thesis advisor, whom I've seen as a role model since my first years in dentistry for her academic achievements, for guiding me into pediatric dentistry, making me proud to be instructed by her, never hesitating to share her knowledge and experience with me, and always supporting me,

I would also like to thank my dear teacher **Prof. Dr. Didem ÖZDEMİR ÖZENEN**, whom I've admired for her researcher spirit and vast knowledge, her invaluable support throughout my doctorate education, sharing with me her deep experience, and protecting and being there for me with great care,

To **Assoc. Prof. Dr. Elif SUNGURTEKİN EKÇİ** for supporting me throughout my education with her kind and motivating words,

To **Assoc. Dr. Kübra TONGUÇ ALTIN** for her great guidance both in the clinic and in social relations and helping me as a mentor at all stages of my thesis,

To my teachers **Assoc. Prof. Dr. Zerrin HATİPOĞLU**, **Assoc. Dr. G. Deniz BAYRAK, Assoc. Dr. Derya TABAKÇILAR**, and **Assoc. Dr. Elif YAMAN DOSDOĞRU**, for all their support,

To Hard Tissue Laboratory Technician at the Faculty of Dentistry at Y.U., **İsmethan DERELİ** for helping me with all the laboratory work and answering all my questions,

To my teammates at Yeditepe Pedodontics, **Dr. Seda EKER**, **Dt. M. Zeynep NİZAM**, **Dt. Naz YAZ ÇAKIR**, and **Dt. Mohanid ALMOZUGHİ** for their invaluable friendship,

To my dear friends **Dt. Ceren ÜSTÜNEL**, **Dt. Fulya BİNER**, **Dt. Gülce Ecem DOĞANCALI**, **Dt. Gizem CANBEK**, and **Dt. Duygucan PEÇENEK** for their continuous support and motivation since we've first began our training,

To **Ozan KARADUMAN** for his unrequited love and patience throughout my life, his devotion to my work, and for always believing in me,

And finally, to my dear mother **Gülenay BULAM**, my dear father **Şaban BULAM**, and my dear sister **Selinay BULAM** for their continuous love, supporting me throughout my education life, helping me stand on my own feet, and giving me hope despite all difficulties.

TABLES OF CONTENT

THESIS APPROVAL FORM ii

DECLARATION iii

ACKNOWLEDGEMENTS iv

LIST OF TABLES ix

LIST OF FIGURES xi

LIST OF GRAPHS xvi

SYMBOLS and ABBREVIATION xvii

1. INTRODUCTION and PURPOSE 3

2. GENERAL INFORMATION 7

2.1. Tooth Enamel 7

2.1.1. Physical Structure of Tooth Enamel 8

2.1.2. Chemical Structure of Tooth Enamel 11

2.2. Dental Caries 18

2.2.1. Factors in Formation of Tooth Caries 21

2.2.2. Demineralization-Remineralization Cycle 32

2.3. Incipient Caries Lesion 35

2.3.1. Clinical Appearance of Incipient Caries Lesions 36

2.3.2. Histological Structure of Incipient Caries Lesions 37

2.4. Demineralization and Remineralization Evaluation Methods 40

2.4.1. Microradiography 40

2.4.1.1. Transversal Microradioraphy (TMR) 41

2.4.1.2. Longitudinal Microradiography (LMR) 41

2.4.1.3. Wavelength-Independent Microradiography (WIM) 42

2.4.2. Energy-dispersive spectroscopy (EDS) 42

2.4.3. Atomic Froce Microscopy (AFM)……..42

2.4.4. Polarized Light Microscopy (PLM)..... 43

2.4.5. Quantitative Light Fluorescence (QLF)..... 43

2.4.6. Confocal Laser Scanning Microscopy (CLSM) 44

2.4.7. Scanning Electron Microscopy (SEM) 44

2.4.8. Surface Microhardness (SMH) 45

2.4.9. X-Ray Diffraction (XRD) 47

2.5. Agents Used in Remineralization of Incipient Caries Lesions 49

2.5.1. Topical Fluoride Applications 49

2.5.1.1. Topical Fluoride Agents 49

2.5.1.2. Topical Fluoride Application Methods..... 52

2.5.2. Calcium Phosphate Technology 58

2.5.2.1. Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP-ACP) 58

2.5.2.2. Sodium Trimetaphosphate (sTMP)..... 59

2.5.2.3. Dicalcium Phosphate Dihydrate (DCPD) 60

2.5.2.4. Tricalcium Phosphate (TCP) 61

2.5.2.5. Calcium Carbonate ($CaCO_3$)..... 62

2.5.3. Theobromine..... 62

2.5.4. Self-Assembling Peptides 63

2.5.5. Arginine Technology 64

2.5.6. Nano-hydroxyapatite (N-HAP)..... 65

2.5.7. Bioactive Glass 68

2.6. Toothpastes 74

2.6.1. History of Toothpastes..... 74

2.6.2. Structure of Toothpastes 75

2.6.2.1. Abrasives 76

2.6.2.2. Moisturizers 78

2.6.2.3. Soaps and Detergents 79

2.6.2.4. Binding and Thickening Agents 79

2.6.2.5. Sweeteners 80

2.6.2.6. Therapeutic Agents 80

3. MATERIALS and METHODS 84

3.1. Toothpaste Used in the Study 84

3.1.1. Dentiste Premium & Natural White Toothpaste 86

3.1.2. Biomin™ C Toothpaste 87

3.1.3. Colgate® Triple Action Toothpaste 88

3.1.4. Signal Kids Toothpaste 89

3.2. Preparation and Storage of Teeth 90

3.3. Preparation and Polishing of Samples 92

3.4. Baseline Surface Microhardness Measurement 94

3.5. Groups 96

3.6. Preparation of Demineralization Solution 99

3.7. Post-Demineralization (PD) Surface Microhardness Measurement 100

3.8. Preparation of Remineralization Solution 100

3.9. Treatment 100

3.10. Post-Treatment (PT) Surface Microhardness Measurement 104

3.11. Examining Scanning Electron Microscope (SEM) Images 104

3.12. X-Ray Diffraction (XRD) Analysis 106

3.13. Statistical Analysis 107

4. RESULTS 108

4.1. Surface Microhardness Test Results 108

4.2. SEM Images 115

4.3. XRD Analysis Results 130

4.3.1. XRD Analysis of Intact Enamel Samples 130

4.3.2. XRD Analysis of Demineralized Enamel Samples 131

4.3.3. XRD Analysis of Post-Treatment Samples 133

4.3.3.1. XRD analysis of N-HAP samples 133

4.3.3.2. XRD analysis BioC samples 134

4.3.3.3. XRD analysis of 1450 F samples…..……..136

4.3.3.4. XRD analysis of 1000 F samples 138

4.3..3.5. XRD analysis of negative control samples……..140

5. DISCUSSION 143

6. CONCLUSIONS 170

7. REFERENCES 174

8. ANNEXES 201

LIST OF TABLES

Table 1. Agents contained in toothpastes and percentages (26) 76

Table 2. RDA values (191) 78

Table 3. General properties of the toothpastes used in the research 84

Table 4. Coding experimental and control groups and toothpastes used in treatment 96

Table 5. Treatment cycle 101

Table 6. Mean baseline, post-demineralization, and post-treatment surface microhardness values according to groups 108

Table 7. Paired comparison of baseline, post-demineralization, and post-treatment surface microhardness values of all groups 110

Table 8. Paired comparison of baseline-PD, baseline-PT, and PD-PT surface microhardness values across all groups according to Newman-Keuls Multiple Comparison Test 112

Table 9. Reference codes, chemical formulas, and names of crystal phases dominant in XRD analysis of intact enamel samples 130

Table 10. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of demineralized enamel samples 132

Table 11. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of N-HAP samples 133

Table 12. Reference codes, chemical formulas and mineral names of crystal phases dominant in XRD analysis of BioC samples 135

Table 13. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of 1450 F samples 136

Table 14. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of 1000 F samples 138

Table 15. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of negative control samples 140

LIST OF FIGURES

Figure 1. Hydroxyapatite crystal (3) 7

Figure 2. SEM imageFigure of enamel prisms and matrix between prisms (3) 9

Figure 3. SEM imageFigure of enamel prisms formed by hydroxyapatite crystals (R) (3) ... 10

Figure 4. Crystal structure of hydroxyapatite (2) 12

Figure 5. Keyhole appearance of enamel prisms (1) 13

Figure 6. Keyhole appearance of enamel prisms in SEM (1) 13

Figure 7. Hunter-Schreger bands (20). 14

Figure 8. A- Dentin tissue and B-Striae of Retzius (14) 15

Figure 9. SEM imageFigure of perikymata (14) 15

Figure 10. Perikymata (14) 16

Figure 11. Enamel lamella (14) 17

Figure 12. Light microscope imageFigure of enamel brick (14) 17

Figure 13. Factor caries model of the 1960s (24) 19

Figure 14. Factors for the formation of tooth caries (26) 20

Figure 15. Complex dental caries model accepted today (26) 21

Figure 16. SEM imagefigure showing the diversity of microorganisms in MDP (13) 26

Figure 17. Corn cob appearance formed by microorganisms (23) 27

Figure 18. Stephan Curve (34) 30

Figure 19. Demineralization-remineralization balance (13) 32

Figure 20. Demineralization (42) 33

Figure 21. Clinical Appearance of Incipient Caries Lesions (55) 36

Figure 22. Histological appearance of incipient caries lesions (13) 39

Figure 23. Pore volumes of four layers forming incipient caries lesions (13) 40

Figure 24. Appearance of Vickers diamond tip (63) 46

Figure 25. Appearance of Knoop diamond tip (63) 46

Figure 26. X-ray diffraction on a crystal plane (79) 48

Figure 27. Dentiste' Premium & Natural White Toothpaste (N-HAP) 86

Figure 28. Biomin™ C Toothpaste (BioC) 87

Figure 29. Colgate®Triple Action Toothpaste (1450 F) 88

Figure 30. Signal Kids Toothpaste (1000 F) 89

Figure 31. All toothpastes used in the research 90

Figure 32. Optical microscope used in the research 91

Figure 33. Brushing teeth with a polishing brush and fluoride-free paste 92

Figure 34. Buehler Phoenix Beta Grinder Polishing Device 93

Figure 35. Attaching 6x6 mm adhesive tapes to polished enamel surfaces 93

Figure 36. Polishing samples with nail polish 94

Figure 37. Surface Microhardness Measurement Device 95

Figure 38. Dentiste Premium & Natural White group (N-HAP) 97

Figure 39. Biomin™ C group (BioC) 97

Figure 40. Colgate® Triple Action group (1450 F) 98

Figure 41. Signal Kids group (1000 F) 98

Figure 42. 37ºC oven where samples were stored 99

Figure 43. Brushing areas for negative control samples 101

Figure 44. Magnetic mixer 102

Figure 45. Electric toothbrush and sensitive brush head 103

Figure 46. Sensitive brush heads for each sample 103

Figure 47. Gold Coating Device 105

Figure 48. Scanning Electron Microscope (Carl ZEISS, EVO 40, Germany) 105

Figure 49. X-Ray Diffraction Device 106

Figure 50. Intact enamel surface figures at x200 magnification 115

Figure 51. Intact enamel surface image at x2000 magnification 116

Figure 52. Demineralized enamel surface figure at x500 magnification 117

Figure 53. Demineralized enamel surface figure at x3500 magnification 117

Figure 54. Demineralized enamel surface figure at x5000 magnification 118

Figure 55. Post-treatment enamel surface figure in the N-HAP group at x200 magnification 119

Figure 56. Post-treatment enamel surface figure in the N-HAP group at x3500 magnification 119

Figure 57. Post-treatment enamel surface figure in the N-HAP group at x5000 magnification 120

Figure 58. Post-treatment enamel surface figure in the BioC group at x200 magnification 121

Figure 59. Post-treatment enamel surface figure in the BioC group at x3500 magnification 121

Figure 60. Post-treatment enamel surface figure in the BioC group at x5000 magnification 122

Figure 61. Post-treatment enamel surface figure in the 1450 F group at x200 magnification 123

Figure 62. Post-treatment enamel surface figure in the 1450 F group at x3500 magnification 123

Figure 63. Post-treatment enamel surface figure in the 1450 F group at x5000 magnification 124

Figure 64. Post-treatment enamel surface figure in the 1000 F group at x200 magnification 125

Figure 65. Post-treatment enamel surface figure in the 1000 F group at x3500 magnification 125

Figure 66. Post-treatment enamel surface figure in the 1000 F group at x5000 magnification 126

Figure 67. Post-treatment SEM figure in the NC (+) group at x200 magnification..... 127

Figure 68. Post-treatment SEM figure in the NC (+) group at x3500 magnification..... 127

Figure 69. Post-treatment SEM figure in the NC (+) group at x5000 magnification..... 128

Figure 70. Post-treatment SEM figure in the NC (-) group at x200 magnification 129

Figure 71. Post-treatment SEM figure in the NC (-) group at x3500 magnification .. 129

LIST OF GRAPHS

Graph 1. Distribution of baseline, post-demineralization, and post-treatment surface microhardness values across all groups 113

Graph 2. Comparison of baseline, post-demineralization, and post-treatment surface microhardness values across all groups 114

Graph 3. XRD analysis of intact enamel samples 131

Graph 4. XRD analysis of demineralized enamel samples 132

Graph 5. XRD analysis of N-HAP samples 134

Graph 6. XRD analysis of BioC samples 135

Graph 7. XRD analysis of 1450 F samples 137

Graph 8. XRD analysis findings of 1000 F samples 139

Graph 9. XRD analysis of negative control samples 140

Graph 10. XRD analyses of all treatment groups 142

SYMBOLS and ABBREVIATION

Ca	: Calcium
PO_4	: Phosphate
MDP	: Microbial Dental Plaque
HA	: Hydroxyapatite
OH	: Hydroxyl
FAP	: Fluorapatite
N-HAP	: Nano-hydroxyapatite
Na	: Sodium
HCA	: Hydroxycarbonate Apatite
$Ca_{10}(PO_4)_6(OH)_2$	: Hydroxyapatite
pH	: Power of Hydrogen
nm	: Nanometer
µm	: Micrometer
µ	: Micron
SEM	: Scanning Electron Microscopy

S. mutans	: *Streptococcus mutans*
S. sobrinus	: *Streptococcus sobrinus*
K	: Potassium
S. sanguinis	: *Streptococcus sanguinis*
S. oralis	: *Streptococcus Oralis*
S. mitis	: *Streptococcus Mitis*
MS	: Mutans Streptococci
F-ATPase	: F-type Adenosine Triphosphatase
CaF_2	: Calcium Fluoride
HAP	: Hydroxyapatite
FAP	: Fluoroapatite
TMR	: Transverse Microradiography
L_d	: Lesion Depth
ΔZ	: Mineral Change Value
LMR	: Longitudinal Microradiography

WIM	: Wavelength-Independent Microradiography
EDS	: Energy-Dispersive Spectroscopy
AFM	: Atomic Force Microscopy
PLM	: Polarized Light Microscopy
QLF	: Quantitative Light-Induced Fluorescence
CLSM	: Confocal Laser Scanning Microscopy
LM	: Light Microscopy
Au	: Gold
Pt	: Platinum
Pd	: Palladium
C	: Carbon
SMH	: Surface Microhardness
PD	: Post-Demineralization
PT	: Post-Treatment
mm	: Millimeter
XRD	: X-Ray Diffraction

NaF	: Sodium Fluoride
SnF_2	: Stannous Fluoride
Sn_2PO_4OH	: Stannous Phosphate
$Sn_3F_3PO_4$	: Stannous Fluorophosphate
TiF_4	: Titanium Tetrafluoride
AmF	: Amine Fluoride
APF	: Acidulated Phosphate Fluoride
F^-	: Fluorine
mg	: Milligram
ISO	: International Organization for Standardization
SMFP	: Sodium Monofluorophosphate
AAPD	: American Academy of Pediatric Dentistry
EAPD	: European Academy of Pediatric Dentistry
CPP-ACP	: Casein Phosphopeptide-Amorphous Calcium Phosphate
CPP-ACFP	: Casein Phosphopeptide-Amorphous Calcium Fluoride Phosphate

sTMP	: Sodium Trimetaphosphate
TCP	: Tricalcium Phosphate
Ftcp	: Functionalized Tricalcium Phosphate
$CaCO_3$	: Calcium Carbonate
HCA	: Hydroxycarbonate Apatite
FDA	: Food and Drug Administration
ADA	: American Dental Association
RDA	: Relative Dentin Abrasivity
SLS	: Sodium Lauryl Sulfate
^{o}C	: Degrees Celsius
VHN	: Vickers Hardness Number
N	: Newton
ICDD	: Interface Control/Design Document
NCSS	: Number Cruncher Statistical System
CaP	: Calcium Phosphide

$Ca_{10}(PO_4)_6CO_3$	: Calcium Phosphate Carbonate
CPC	: Calcium Phosphate Carbonate
$Ca_5F(PO_4)_3$	: Calcium Fluoride Phosphate
$Ca_5(PO_4)_3(OH)$	: Calcium Hydroxide Phosphate
$Ca_2(SiO_4)$	: Calcium Silicate
CAS	: Calcium Silicate
$Ca_{10}(PO_4)_5CO_3F_{1.5}(OH)_{0.5}$	: Calcium Fluoride Carbonate Phosphate Hydroxide
CFA	: Carbonate Fluorapatite
$Ca_{10}(PO_4)_6CO_3$	: Calcium Phosphate Carbonate
$Ca_{5.061}(P_{2.87}O_{11.46})F_{0.89}$	: Calcium Phosphate Fluoride
$Ca_{10}(OH)_2(PO_4)_6$	: Calcium Hydroxyphosphate
CO_2	: Carbon Dioxide
CDC	: Centers for Disease Control and Prevention

ABSTRACT

Bulam N. 2020. Evaluation of the effect of toothpastes with different ingredients on the surface microhardness and surface properties of artificial incipient caries lesions, *in vitro*. Yeditepe University Institute of Health Sciences, PhD Thesis, Istanbul.

In the present study, the aim was to evaluate and compare the effect of toothpastes with different fluoride concentrations and toothpastes with alternative remineralization agents on the surface microhardness and surface properties of artificial incipient caries, *in vitro*. Enamel samples from bovine incisors were divided into 5 groups as 4 study groups (N-HAP, BioC, 1450F, and 1000F) and 1 control group (n=13). Baseline surface microhardness (SMH) values were measured for all samples and they were kept in a demineralization solution at 37°C for 72 hours, after which the artificial incipient caries lesions were created. Post-demineralization (PD) SMH values and post-treatment (PT) SMH values with toothpastes in the study groups were measured following a 14-day pH cycle. Baseline, PD, and PT surface properties of the samples were evaluated using a Scanning Electron Microscope (SEM) (n=3). The crystal phases that occurred on the enamel surfaces were determined by X-Ray Diffraction (XRD) (n=3). Statistical analyses were done using one-way analysis of variance, Newman Keuls, Tukey, Kruskal Wallis, and Dunn's tests. The findings were evaluated at a significance level of $p<0.05$. There were statistically significant differences between all groups in terms of mean PT SMH values ($p=0.0001$). The highest mean SMH among the study groups was observed with 1450 F, and the lowest was observed with N-HAP. PT SMH values were higher than PD SMH values, but lower than baseline across all study groups. SEM examinations revealed mineral gains on enamel surfaces after treatment across study groups. XRD analysis revealed calcium phosphide and calcium phosphate carbonate on enamel surfaces after demineralization and predominant presence of HAP and FAP in the 1000F and 1450F groups, HAP in the N-HAP group, and calcium silicate in the BioC group after treatment. In conclusion, it can be suggested that fluoride toothpaste remains the best choice for preventing dental caries and toothpastes with bioactive glass and N-HAP could be alternatives to fluoride toothpastes.

Keywords: Incipient caries lesion, demineralization, remineralization, fluoride, nano-hydroxyapatite, bioactive glass, toothpaste.

ABSTRACT (TURKISH)

Bulam N. 2021. Farklı içerikteki diş macunlarının yapay başlangıç çürük lezyonlarının yüzey mikrtosertliğine ve yüzey özelliklerine olan etkisinin in vitro koşullarda değerlendirilmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi, İstanbul.

Bu çalışmanın amacı, farklı konsantrasyonlarda fluorid ve fluoride alternative remineralizasyon ajanları içeren diş macunlarının yapay başlangıç çürüklerinin yüzey mikrosertliğine ve yüzey özelliklerine olan etkisinin in vitro koşullarda karşılaştırmalı olarak değerlendirilmesidir. Sığır kesici dişlerinden hazırlanan mine örnekleri 4 çalışma (N-HAP, BioC, 1450F, 1000F) ve bir kontrol olmak üzere 5 gruba ayrıldı (n=13). Başlangıç yüzey mikrosertliği (YMS) değerleri ölçülen örnekler, demineralizasyonu solüsyonunda 37 °C' de 72 saat boyunca bekletildi ve yapay başlangıç çürük lezyonları oluşturuldu. Demineralizasyon sonrasında (DS) ve 14 günlük pH döngüsü içerisinde çalışma gruplarındaki diş macunları ile tedavi sonrasında (TS) YMS değerleri ölçüldü. Başlangıç, DS ve TS'de örneklerin yüzey özellikleri Taramalı Elektron Mikroskop (SEM) ile değerlendirildi (n=3). Mine yüzeylerinde meydana gelen kristal fazları ise X Işını Kırınımı (XRD) yöntemi ile belirlendi (n=3). İstatistiksel analizler tek yönlü varyans analizi, Newman Keuls, Tukey, Kruskal Wallis, Dunn's testleri kullanılarak yapıldı. Sonuçlar, anlamlılık $p<0,05$ düzeyinde değerlendirildi. Tüm gruplar arasında, TS YMS ortalamaları açısından istatistiksel olarak anlamlı farklılık gözlemlendi (p=0,0001). Çalışma grupları arasında en yüksek YMS ortalaması 1450 F, en düşük N-HAP grubunda elde edildi. Tüm çalışma gruplarında TS YMS değerlerinin DS'den yüksek, ancak başlangıç değerlerinden düşük olduğu görüldü. SEM incelemelerinde, tüm çalışma gruplarında TS'de mine yüzeyinde mineral kazanımı izlendi. XRD analizinde, DS mine yüzeylerinde kalsiyumfosfit ve kalsiyumfosfatkarbonat, TS ise, ağırlıklı olarak, 1000F ve 1450F gruplarında HAP ve FAP, N-HAP grubunda HAP, BioC grubunda kalsiyum silikat izlendiği belirlendi. Sonuç olarak, diş çürüğünden korunmada halen en iyi seçimin fluoridli diş macunu olduğu, biyoaktif cam ve N-HAP içeren diş macunlarının ise fluoridli diş macunlarına alternatif olabileceği düşünülmektedir.

Anahtar Kelimeler: Başlangıç çürük lezyonu, demineralizasyon, remineralizasyon, fluorid, nanohidroksiapatit, biyokaktif cam, diş macunu.

1. INTRODUCTION and PURPOSE

Dental caries is a multifactorial, infectious, and chronic disease that involves an interaction of host-dependent factors like microbial dental plaque, tooth surface, and saliva. Dental caries occurs by the dissolution of calcium (Ca) and phosphate (P) ions from dental hard tissues due to acidic by-products resulting from the fermentation of dietary carbohydrates by cariogenic microorganisms in the microbial dental plaque (1).

Demineralization is the mineral loss on the dental hard tissues due to acids that are formed by the fermentation of dietary carbohydrates by cariogenic microorganisms in the microbial dental plaque (MDP). The reprecipitation of dissolved minerals on tooth enamel is named remineralization. There is a balance between demineralization and remineralization in the mouth (2).

An incipient caries lesion is the earliest stage of dental caries, and dental caries could still be prevented and treated at this stage. Incipient caries lesions are limited to the enamel. In these types of lesions, demineralization can continue under the intact enamel tissue. When demineralization continues, various levels of cavitation and loss of tooth hard tissues occur. Hence, early diagnosis and treatment of incipient caries lesions is crucial (1).

Today, fluoride applications are considered the gold standard for preventing dental caries. Fluoride compounds not only prevent demineralization and but also provide remineralization in a number of ways. Preventing the formation of MDP, inhibiting the acids produced by the microorganisms in MDP, ensuring the formation of fluorapatite, and ensuring the precipitation of calcium and phosphate ions on the body of the lesion are mechanisms of action of fluoride to prevent tooth decay and provide remineralization. The intake routes of fluoride for preventing dental caries are systemic and topical

applications. However, today, it is accepted that the presence of fluoride in the mouth at certain, even low concentrations, is more effective for preventing tooth decay and this fluoride is provided by topical fluoride applications after tooth eruption (3).

Topical fluoride applications are highly effective methods that have been preferred for many years for arresting incipient caries and providing remineralization. When high concentrations of fluoride compounds are used in topical fluoride applications, calcium fluoride globules are formed, which act as a kind of fluoride reservoir by dissolving when the pH in the oral cavity decreases during acid attacks, and this type of fluoride is called loosely bound fluoride. When low-concentration fluoride compounds are used regularly, fluor ions (F^-) replace hydroxyl (OH^-) ions in the hydroxyapatite cyrstals (HA) within the structure of tooth enamel, forming acid-resistant, less soluble fluorapatite (FAP) crystals; This type of fluoride is called firmly bound fluoride (3,4).

Fluoride applications, while the most effective method known for preventing dental caries, can exhibit adverse effects when not applied in appropriate doses and under ideal conditions. Researchers are searching for alternative agents to fluoride to prevent the toxic effects which can be caused by the ingestion of fluoride in young children with no extrusion reflex and also physically and mentally disabled individuals, and to minimize the risk of fluorosis in systemic fluoride intake (5).

With the advances in nanotechnology, in recent years, nano-hydroxyapatite (N-HAP) has been used frequently in biomedicine and dentistry as a bioactive and biocompatible material. The structure of nano-hydroxyapatite crystals has been examined, revealing that they have chemical and structural similarities with enamel crystals. Since it is a biomimetic, bioactive, and biocompatible material, it has been preferred often in dentistry in recent years. Nano-hydroxyapatite is used in dentistry to

eliminate dentin sensitivity, to prevent erosion, and to prevent dental caries (in remineralization studies). As research on the potential remineralization effect of N-HAP on tooth caries has increased, it has begun to be added to toothpastes and mouthwashes (6–8).

Bioactive glass, known with its chemical name calcium sodium phosphosilicate, was first developed in 1969 by Hench et al. as a bone regeneration material. The development and success associated with bioactive glass over the years is attributed to its high biocompatibility (9). As bioactive glass contacts body fluids like saliva, surface reactions that increase the pH begin to occur. As sodium (Na) ions in calcium sodium phosphosilicate particles are replaced with hydrogen ions in body fluids, the chain of reactions begins. The ion exchange that occurs leads to a rapid release of calcium and phosphate ions from the particle structure. With the loss of sodium ions, a gel layer rich in silica begins to form on the material surface. This layer facilitates the precipitation and accumulation of the incoming calcium and phosphate in materials and body fluids. Over time, the calcium phosphate layer begins to thicken, forming the hydroxycarbonate apatite (HCA) layer. This layer is chemically similar to the hydroxyapatite in tooth structure. With the ionic reaction of all ions in the bioactive glass and the combination of the HCA layer and bone tissue, surface reactions are completed (10–12).

With the increasing incidence of dental caries worldwide, preventive dentistry practices have gained significance. Although fluoride is the gold standard for preventing dental caries, research is carried out on different remineralization agents, like agents containing calcium phosphate, self-combining peptides, arginine bicarbonate, theobromine, hydroxyapatite, and bioactive glass, and these have begun to be added to oral and dental health products on the market.

The aim of the present study was to evaluate and compare the effect of toothpastes with different fluoride concentrations and toothpastes with alternative remineralization agents on the surface microhardness and surface properties of artificial incipient caries, *in vitro*.

2. GENERAL INFORMATION

2.1.Tooth Enamel

Tooth enamel is a cell-free hard tissue, of ectodermal origin, composed of 95% inorganic compounds, 4% water, and 1% organic compounds like protein and lipid, formed by ameloblastsvisible on the outer part of the tooth, and covering the anatomical crown (13).

Amelogenesis, that is, the formation of tooth enamel, occurs in 3 stages: cell differentiation, secretion of the matrix, and maturation of the matrix. Following amelogenesis, as the ameloblast cells disappear, the enamel tissue turns into a non-cellular and non-living structure. Tooth enamel is a highly mineralized, porous tissue in the matrix that consists of hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$], the main component of which is calcium phosphate, and it is the hardest tissue in the body (Figure 1) (14).

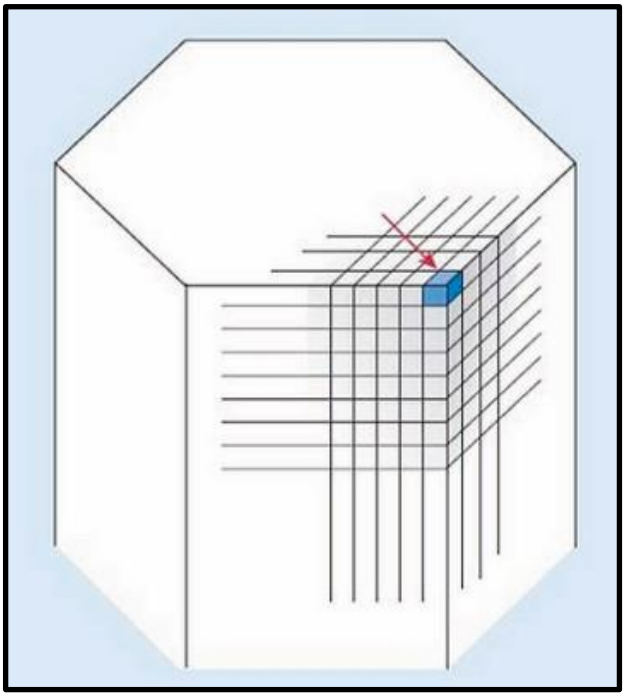


Figure 1. Hydroxyapatite crystal (3)

2.1.1. Physical Structure of Tooth Enamel

Tooth enamel is the hardest tissue in the human body because of its crystal structure and mineral salts. This ensures its durability against chewing forces. Also, enamel has a different thickness in various parts of the crown. . The enamel on the occlusal surface of posterior teeth and the incisal edges of anterior teeth is thicker than the others. The hardness of the tooth enamel decreases towards the inner layers of the tooth, reaching its lowest level at the dentin border (15).

Tooth enamel protects vital tissues like the pulp and dentin. The color of tooth enamel changes based on its thickness and the color of the underlying dentin tissue. The transparency of enamel is associated with its homogeneity and calcification (15).

The enamel consists of an enamel prism, a prism sheath, and an interprismatic matrix in between the two (Figure 2). 95-98% of it consists of hydroxyapatite crystals, and the remaining consists of an organic structure. Enamel crystals are arranged parallel to the long axis of themselves and of the enamel prisms (16).

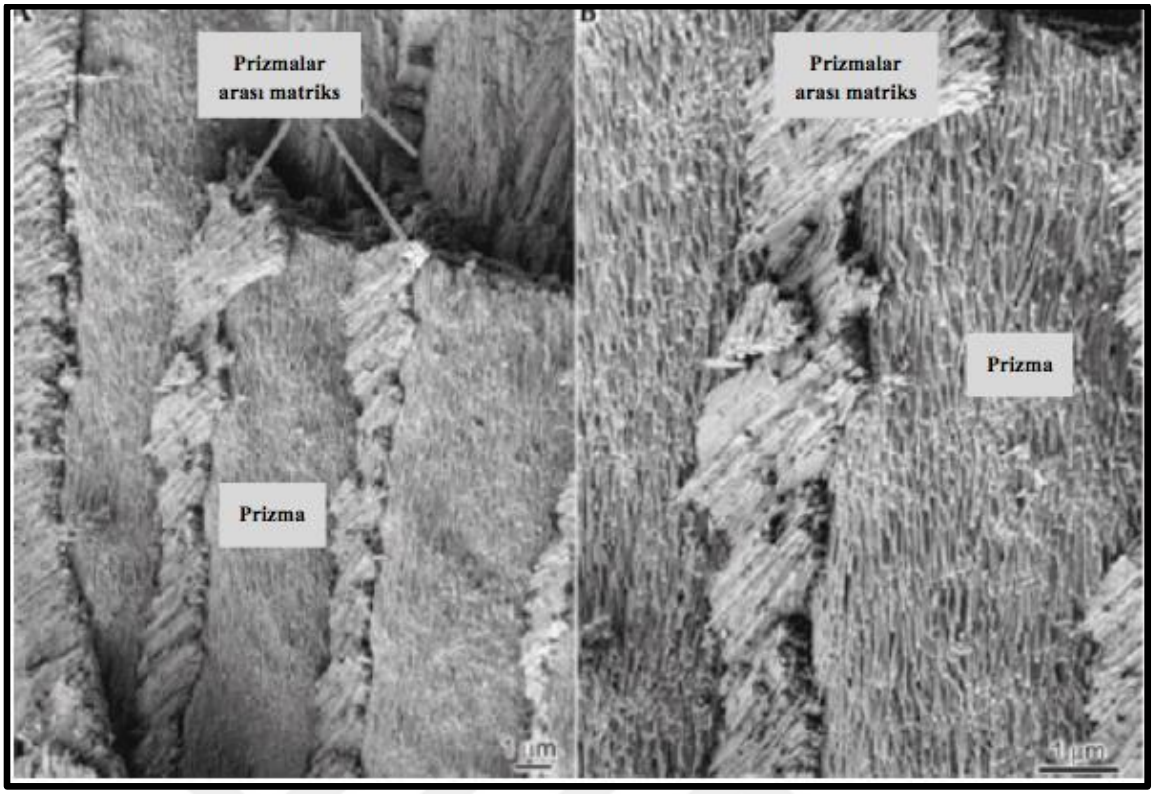


Figure 2. SEM imageFigure of enamel prisms and matrix between prisms (3)

The main components of enamel prisms are thin and elongated hydroxyapatite crystals in millions of different sizes and shapes (Figure 3). The different directions and firm attachment of enamel crystals make the enamel prisms durable. The long axis of the hydroxyapatite crystals in the center of the head of the enamel prisms is almost parallel to the long axis of the enamel prisms. The hydroxyapatite crystals in the tail are inclined to the axis of the enamel prism at an increasing angle of 65 degrees. The direction of enamel crystals is thought to have a role in the formation of tooth caries or acid etching. Also, the arrangement of the crystal structures within the enamel prisms can make the tooth enamel more sensitive or resistant to acid attacks. Dissolution due to acid attacks is observed in the head and central parts of the enamel prisms (13,14).

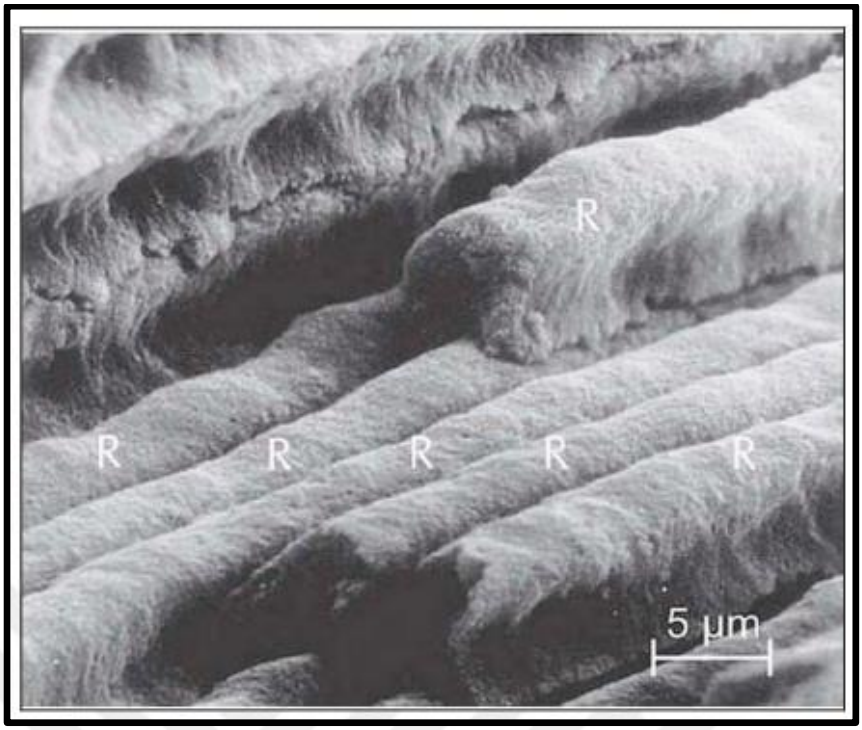


Figure 3. SEM imageFigure of enamel prisms formed by hydroxyapatite crystals (R) (3)

Enamel is a hard tissue with low tensile strength and high elastic modulus. These properties cause tooth enamel to be fragile. Fractures in the enamel tissue are often caused by traumatic injuries or caries in the dentin tissue (13).

With its semi-permeability, enamel allows the diffusion of ions, low-molecular-weight molecules and various liquids. It allows the passage of elements like calcium, while restricting some other molecules. Nevertheless, the permeability of tooth enamel may decrease over time due to changes in the structure of the enamel matrix. The ability of enamel to absorb some ions explains its potential for demineralization and remineralization (17).

2.1.2. Chemical Structure of Tooth Enamel

95-98% of tooth enamel consists of inorganic components. 1-2% of it consists of organic substances, while 2% consists of water. However, 1% of tooth enamel is known to contain sodium, magnesium, fluorine, iron, and manganese salts. Due to these minerals, it is a tissue which is highly mineralized (13).

The inorganic structure of tooth enamel tissue consists of calcium hydroxyapatite in hexagonal apatite structure, formed by the reaction of orthocalcium phosphate and water (Figure 4). Hydroxyapatite is the main component that forms the inorganic part of tooth enamel. It constitutes 90-92% of its volume. The water within tooth enamel is largely bound to hydroxyapatite crystals, while a small part is free within the organic components. The water in the remaining part envelops the apatite crystals like a shell (13).

The organic content of a matured enamel tissue contains short peptide fragments that are very rich in Pro, Glx, and Gly by-products, formed by the breakdown of amelogenin protein. Large protein compounds like siathelin, free amino acids, and lipids make up the other organic content of tooth enamel (18).

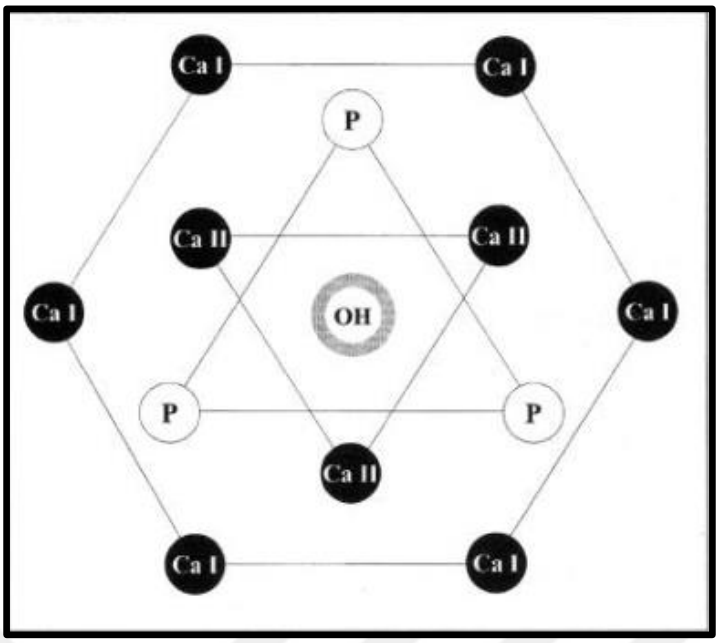


Figure 4. Crystal structure of hydroxyapatite (2)

During embryological development, tooth enamel is formed by ameloblasts. First, a prismless enamel dentin membrane is secreted over the predentin layer. Then, the ameloblasts begin to secrete 4 µm of enamel matrix daily (14). Enamel prisms consist of about 1000 hydroxyapatite crystals coming together. Hydroxyapatite crystals are hexagonal in structure. They have a hydration layer and are surrounded by a layer of protein and lipids. Hydroxyapatite crystals have an average diameter of 50 nanometers (nm) and an average length of over 100 nm (14).

Enamel prisms formed by hydroxyapatite crystals are observed horizontally and vertically extending from the enamel dentin border to the outer part of the enamel. Enamel prisms are shaped like hexagonal prisms and are 4 -6 micrometers (µm) in diameter. They consist of a head 5 microns (µ) in width and a tail 1µ in width (14). The round heads of enamel prisms are located between the tails of the two neighboring enamel prisms. The heads of enamel prisms extend towards the occlusal or incisal edge of the tooth, while

the tail extends cervically. Cross-sectional examination with a scanning electron microscopy (SEM) reveals that enamel prisms exhibit a characteristic appearance in the form of a 'keyhole' (Figures 5 and 6) (13).

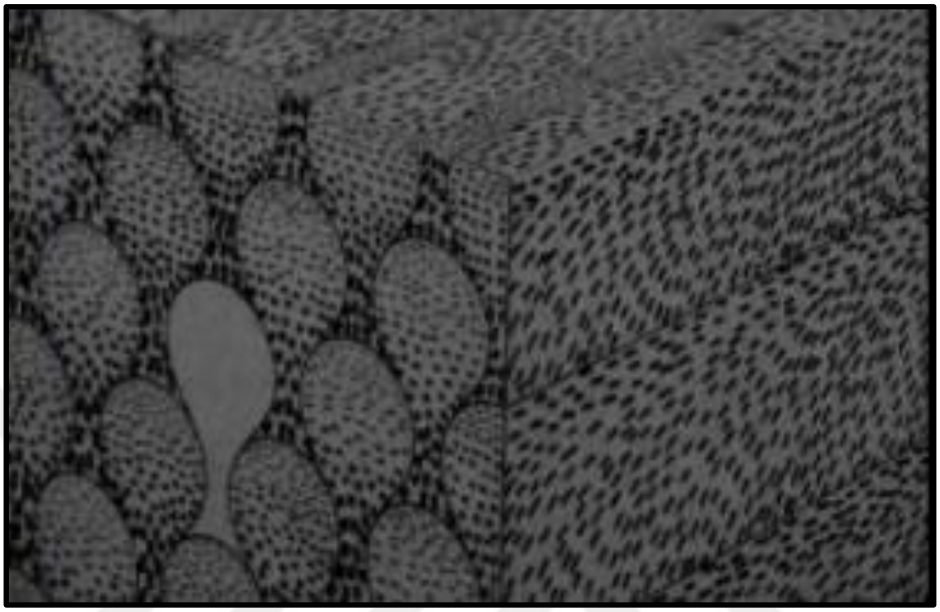


Figure 5. Keyhole appearance of enamel prisms (1)

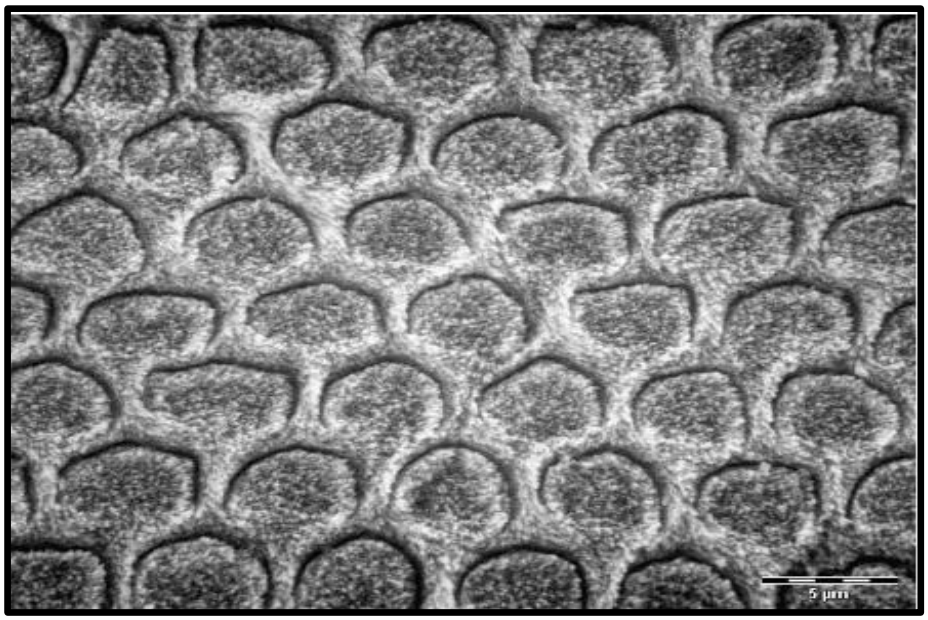


Figure 6. Keyhole appearance of enamel prisms in SEM (1)

There are 1 μm spaces between enamel prisms. This space between prisms contains a matrix consisting of organic substances and water. Enamel prisms are vertical at cusp crests and horizontal at the enamel dentin border. These slopes formed by prisms come together at different angles, getting arranged groups. This is reported to provide the durability of enamel against chewing and biting forces (19).

Examining enamel prisms under light reveals that light passes through some of the enamel prisms but not through other prism groups adjacent to these. This way, dark and light bands that continue along the long axis of enamel prisms are observed. These are called 'Hunter-Schreger bands' (20) (Figure 7).

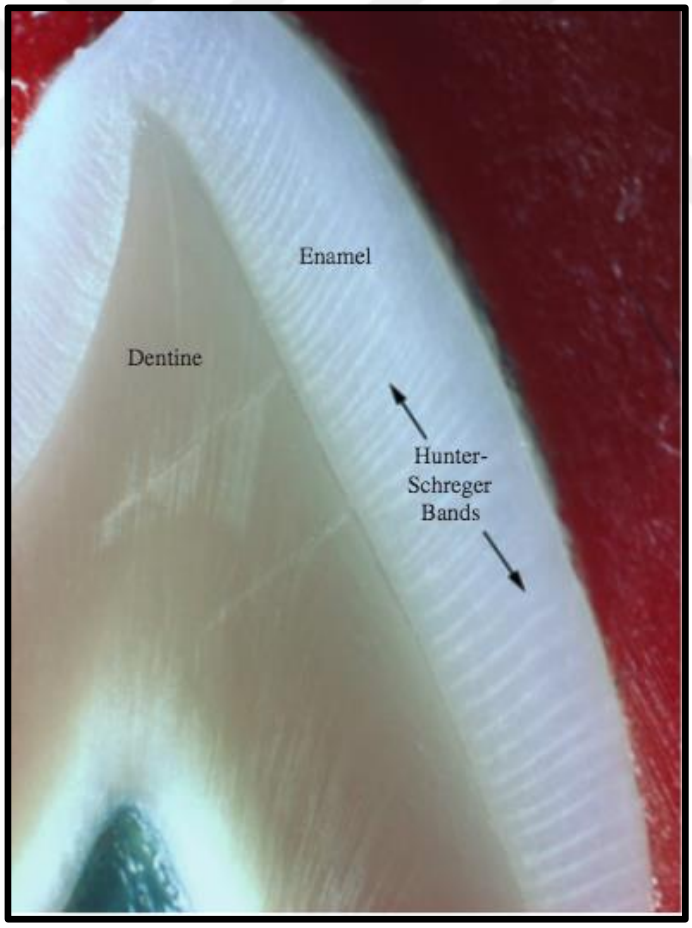


Figure 7. Hunter-Schreger bands (20).

At times of low activation during mineralization, lines where pauses are observed in tooth enamel are called 'Striae of Retzius' (Figure 8). The recessed parts formed by a stria of Retzius reaching the outer surface of the tooth enamel are called 'Perikymata'. These formations are horizontal and at regular intervals on the enamel surface (14) (Figures 9 and 10).

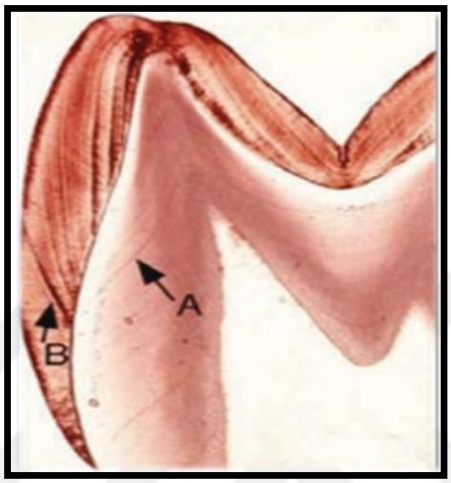


Figure 8. A- Dentin tissue and B-Striae of Retzius (14)

Figure 9. SEM imageFigure of perikymata (14)

Figure 10. Perikymata (14)

Formations that extend from the enamel dentin border toward the inner surface of the enamel are called enamel bricks. Enamel bricks have considerably more enamel proteins than normal enamel. Cracks that may be observed on the enamel surface are enamel lamellae. Enamel lamellae can extend to different depths from the enamel dentin border. Enamel lamellae can appear either during the formation of enamel or during tooth function. They often have a leaf-shaped appearance (Figures 11 and 12) (20).

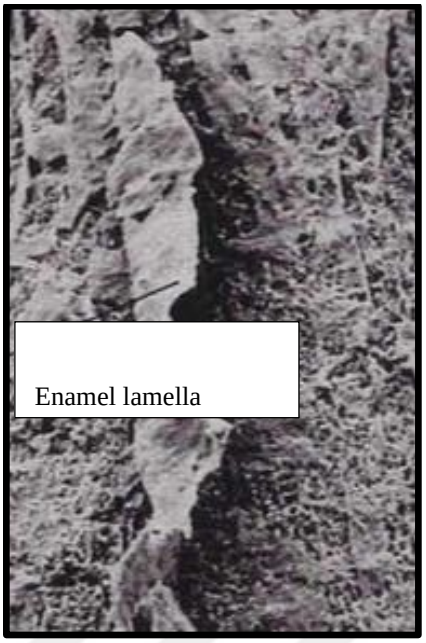


Figure 11. Enamel lamella (14)

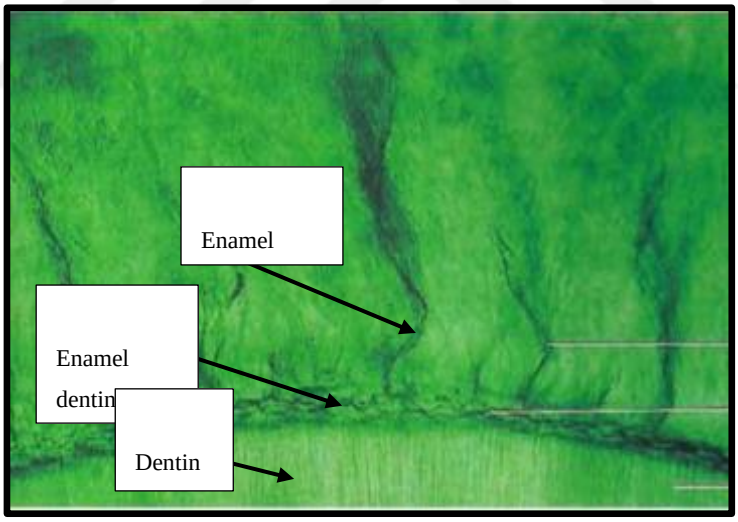


Figure 12. Light microscope imageFigure of enamel brick (14)

2.2.Dental Caries

Dental caries is a multifactorial, infectious, and chronic disease that involves the interaction of host-dependent factors like microbial dental plaque, dental hard tissues, and saliva. The process of dental caries begins by the dissolution of calcium and phosphate ions from the dental hard tissues due to the presence of acidic by-products resulting from the metabolization of dietary fermentable carbohydrates by the cariogenic microorganisms in the MDP (1).

There are many theories on the formation of dental caries. Among these, the oldest was the idea that a 'toothworm', believed to live in the center of the tooth, caused caries and toothache (21). Later, in late 1700s, the 'vital theory' emerged, claiming that inflammation within damaged teeth caused lesions in the teeth. In 1835, Robertson stated that dental caries was the result of the accumulation and fermentation of food residues in the teeth and surrounding tissues (22). In 1890, Miller put forth the 'chemoparasitic theory,' which has evolved to this day. The theory states that structures resembling lemon salt formed by bacteria are the first stage in the occurrence of caries. Yet, Miller's theory failed to explain that the source of bacteria and the acids produced by these bacteria is microbial dental plaque (23). The chemoparasitic theory has become more acceptable as numerous researchers, including GV Black (1898) who has made valuable contributions to dentistry, carried out studies stating that the source of acids causing dental caries is 'gelatinous microbial plaque'. (24)

There are 3 hypotheses in the etiology of dental caries. These are specific plaque hypothesis, nonspecific plaque hypothesis, and ecological plaque hypothesis (24).

Specific plaque hypothesis: According to this hypothesis *Streptococcus mutans (S. mutans)* and *Streptococcus sobrinus (S. sobrinus)* species have a role in the formation of dental caries. This hypothesis is established on a small number of specific bacteria (24).

Nonspecific plaque hypothesis**:** This hypothesis states that dental caries occurs by the activity of plaque microflora consisting of more than one bacterial species (24).

Ecological plaque hypothesis: This states that dental caries occurs by the changes in the balance of the local microflora (24).

The factors required for the formation of dental caries, which today is accepted as a multifactorial disease, have been modified and schematized in numerous ways over the years.

In the 1960s, it was believed that the combination of three factors was sufficient for dental caries to occur. These factors were host, nutrition, and microbial dental plaque (Figures 13) (1) (24).

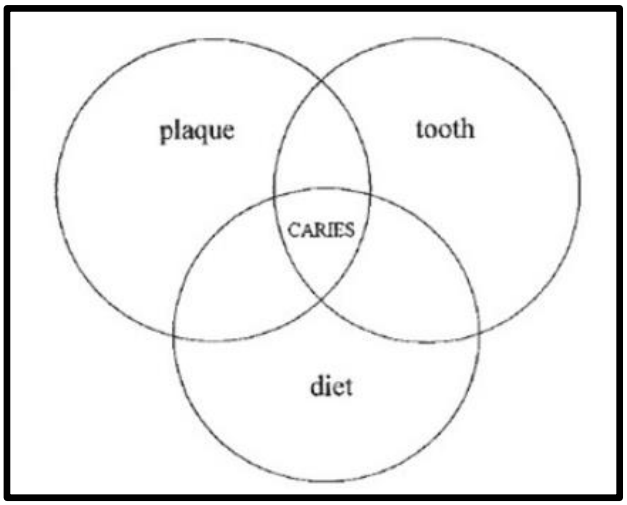


Figure 13. Factor caries model of the 1960s (24)

In the following years, it was reported that four main factors needed to coexist for the formation of dental caries. For dental caries to occur, all these factors must be found together. These are:

a) host (tooth hard tissue)
b) cariogenic microorganisms
c) nutrition
d) and time (Figures 14) (2,24,25).

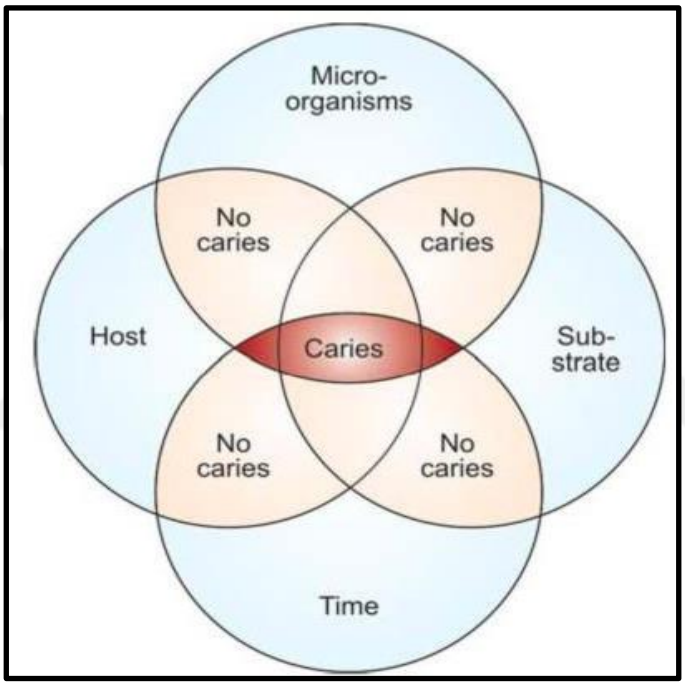


Figure 14. Factors for the formation of tooth caries (26)

In recent years, research has reported many factors to affect the formation of dental caries, such as socioeconomic status, education level, income level, behavioral status, culture, time, genetic factors, body defense system, saliva, nutrition, immunological factors, environmental factors, and fluoride intake. This model with many factors is called the complex dental caries model (Figure 15). The factors specified in the model help protect the integrity of dental hard tissues, while they can also cause damage in these tissues (27)

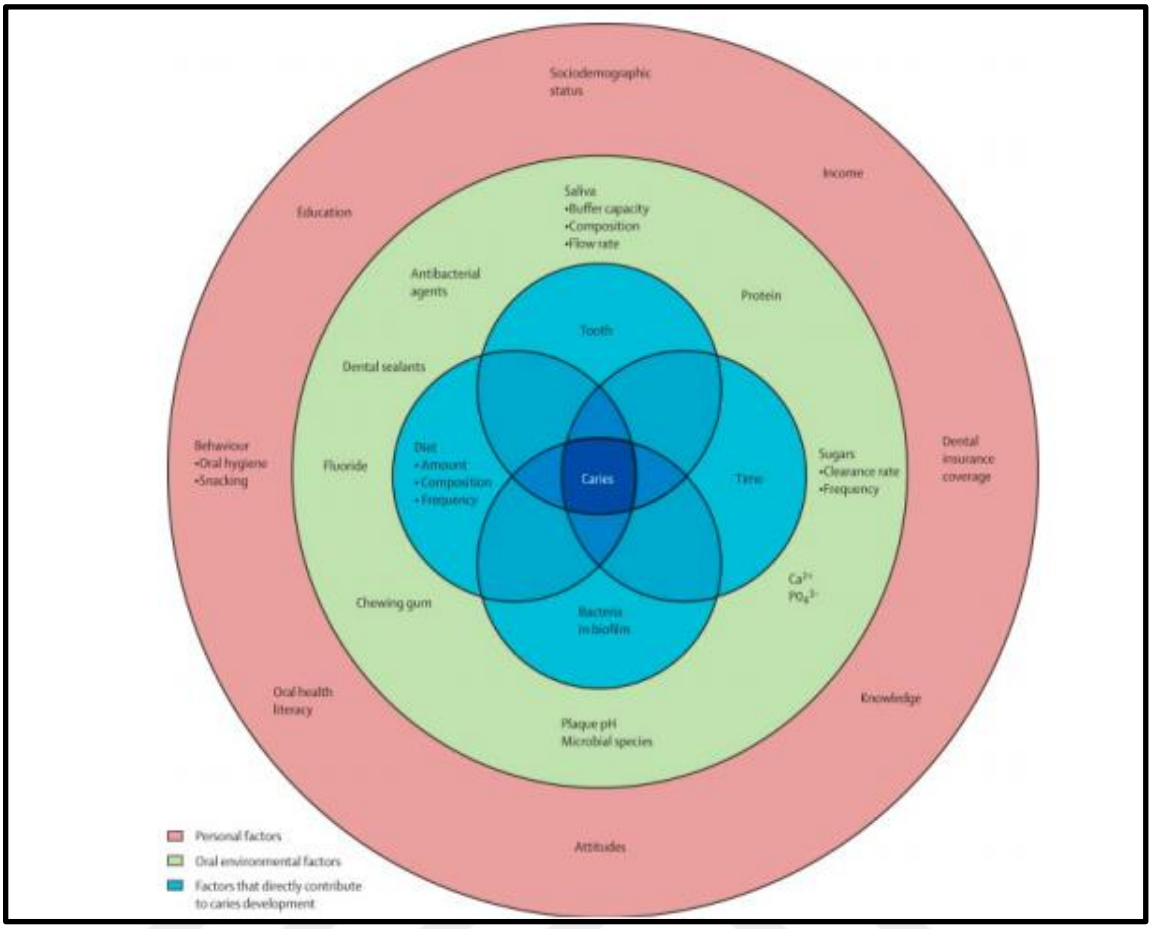


Figure 15. Complex dental caries model accepted today (26)

2.2.1. Factors in Formation of Tooth Caries

The factors that are influential in the formation of dental caries, which is a multifactorial disease, are defined as individual and environmental factors. Factors belonging to the host include those related to the teeth, saliva, microbial dental plaque, and microorganisms. Environmental factors include nutrition, time, socioeconomic status, and education level (27).

The current position of the teeth in the arch, the time after eruption, the surface morphology and structure of the teeth are key factors in the formation and progression of dental caries. The position of the teeth in the arch are crucial for ease of cleaning, plaque

accumulation, and exposure of teeth to acid attacks. Areas prone to excessive plaque accumulation have a high risk of dental tooth caries. The occlusal surfaces of posterior teeth are high-risk areas due to difficulty in cleaning and higher plaque accumulation. The lesser plaque accumulation on the lingual surfaces of the lower anterior teeth, easier cleaning, and benefiting more from the washing effect of saliva explain the lower incidence of caries in this region (27).

The structure of tooth enamel itself is another factor that affects tooth's resistance to caries. The size, type, and proximity of hydroxyapatite crystals affect the solubility of tooth enamel. After eruption, teeth are more susceptible to caries because tooth enamel has not yet fully maturated and is not easy to clean in the mouth. The alignment of the teeth on the arch, crowded teeth, orthodontic treatment, and restorations with poor margin alignment also lead to plaque accumulation in the mouth, preparing the ground for the formation of tooth caries (13).

Saliva is secreted from the major (submandibular, sublingual, parotid) and minor (buccal, lingual, palatine) salivary glands in the mouth. Saliva secreted from major salivary glands constitutes 95% of the saliva in the mouth. Saliva is 99% water and 1% organic and inorganic components. Daily saliva secretion varies from person to person, often in the range of 500-1500 ml (28). Amino acids, urea, uric acid, ammonia, glucose, lipid, cholesterol, and proteins constitute the organic part of saliva, and calcium, sodium, potassium (K), sulfate, chlorine, and bicarbonates constitute its inorganic part. The organic content of saliva often contains mucin, albumin, immunoglobulins, enzymes, hormones, DNA, polypeptide, oligopeptides, antibacterial proteins, and viruses. Glycopreteins in saliva prevent bacterial adhesion and pellicle formation. Mucins, the

mucous proteins in saliva, have two main types, namely MG-1 and MG-2. Mucins have certain functions like defending against microorganisms and preventing dehydration (29).

MG-1 has a high molecular weight. It binds to the tooth surface, acting as a barrier against acid attacks.

MG-2 has a low molecular weight. It acts by binding to microorganisms (29).

Saliva also contains IgG and IgM, but IgA is the most dominant and most important immunoglobin in the mouth. IgA prevents the aggregation of bacteria and their adhesion to tissues. With its antigen-antibody mechanism against bacteria, viruses, and their by-products, IgA provides the neutralization of these by-products. Contained in the organic content of saliva, lysozyme breaks down the cell wall of microorganisms. The ammonia and polyamines released by the metabolization of sialin increase pH. Histalin prevents pellicle formation and shows antimicrobial properties. Lactoferrin binds the iron necessary for the growth of microorganisms and removes them from the environment, prevents the colonization of microorganisms, and shows bacteriostatic properties. It also has bactericidal effects against Mutans Streptococci (MS)(30).

Sodium (Na) can be found in the inorganic part of saliva and is responsible for the regulation of the acid-base balance. Calcium (Ca) concentration increases based on the flow rate of saliva, playing a role in increasing bicarbonate and pH. The concentration of inorganic phosphate (PO_4) is inversely proportional to the flow rate of saliva. Bicarbonate is the key buffering component of saliva. Fluoride in the saliva is known to prevent demineralization and increase remineralization (29).

Saliva provides a media for ion exchange for hard dental tissues. Fluoride helps calcium, phosphate, and magnesium ions diffuse into the tooth enamel, facilitating the maturation of tooth enamel. Hence, the surface hardness of tooth enamel increases and it becomes more resistant to the formation of tooth caries. When saturated with Ca, Mg, P, and OH^- ions, saliva contributes to remineralization and prevents demineralization. Fluorine is known to be one of the most important ions in the demineralization-remineralization cycle (28).

Saliva also facilitates chewing, swallowing, and speaking functions by its lubrication property. The excess amount of water in its content helps remove food residues, particularly carbohydrates, from teeth and surrounding tissues. With the help of saliva, foods become softer and more thickened in the mouth, facilitating swallowing of foods (28).

Under normal conditions, saliva has a pH of 6.5-7.5. Organic acids that are released by the metabolization of fermentable carbohydrates by cariogenic bacteria can lower plaque pH below the critical value of 5.5. However, with the buffering capacity of saliva, plaque pH rises within 30 minutes after eating. With the buffering function of carbonic acid, bicarbonate, and phosphates in saliva, acids are neutralized. In stimulated saliva, the carbonic acid-bicarbonate system is active, but in unstimulated saliva, phosphates come in. The task of buffering in saliva mainly belongs to bicarbonate and phosphates. Although, if pH falls below 4-4.5, the proteins in the saliva begin to contribute to buffering. With stimulation, saliva increases in amount and its structure turns from mucous secretion to serous secretion (31).

Dental caries occurs when microbial dental plaque is allowed to organize and is not removed from the environment for a certain time. The community of microorganisms that accumulate in soft tissues in the mouth is called the oral biofilm. MDP is defined as a sticky, amorphous, gelatinous, organic accumulation with various types of microorganisms, formed by proteins and polysaccharides, that settle on tooth surfaces that cannot be mechanically cleaned with saliva, like the tongue, lips, and cheeks (31). MDP has been reported to contain many acidogenic and basic microorganisms and its composition differs during periods of milk dentition, mixed dentition, and permanent dentition (32).

There is a continuous ion exchange between the tooth surface and saliva. In the presence of MDP, the tooth surface cannot benefit from the washing effect of saliva and the buffering of saliva is prevented. This causes the acidogenic bacteria within MDP to contact the tooth surface for a long time, which is the main factor that leads to dental caries (Figure 16) (33).

Plaque formation is a dynamic process. The adhesion, growth, organization, separation, and readhesion of bacteria take place within the same process. The formation of MDP can be summarized as follows:

1. Pellicle formation
2. Adhesion of a single bacterial cell (0-4 hours)
3. Multiplication and forming small colonies of adhering bacteria (4-24 hours)
4. Variety of bacterial species increasing and colonies growing (1-14 days)
5. Mature plaque formation (2 weeks or more) (34).

Shortly after the mechanical cleaning of teeth, a transparent, colorless, thin, and flat layer called the pellicle begins to form on the tooth surface, containing saliva proteins,

phosphoproteins, glycoproteins, and lipids. At the end of 24 hours, its thickness reaches 0.01-1μm. This layer contains defense proteins like IgA, lysozyme, and albumin, but does not contain bacteria. With the formation of this layer, bacteria begin to adhere on the pellicle. The bacteria adhere to the pellicle due to hydrophobic ion, electrostatic, and Van der Waals forces. As bacteria become organized over time, pellicle formation begins to increase. As the thickness of the biofilm increases, the amount of oxygen diffused into the matrix decreases. As the bacteria settled on the surface consume oxygen, an anaerobic flora occurs in the deep layers of the biofilm (13,34) (Figure 13).

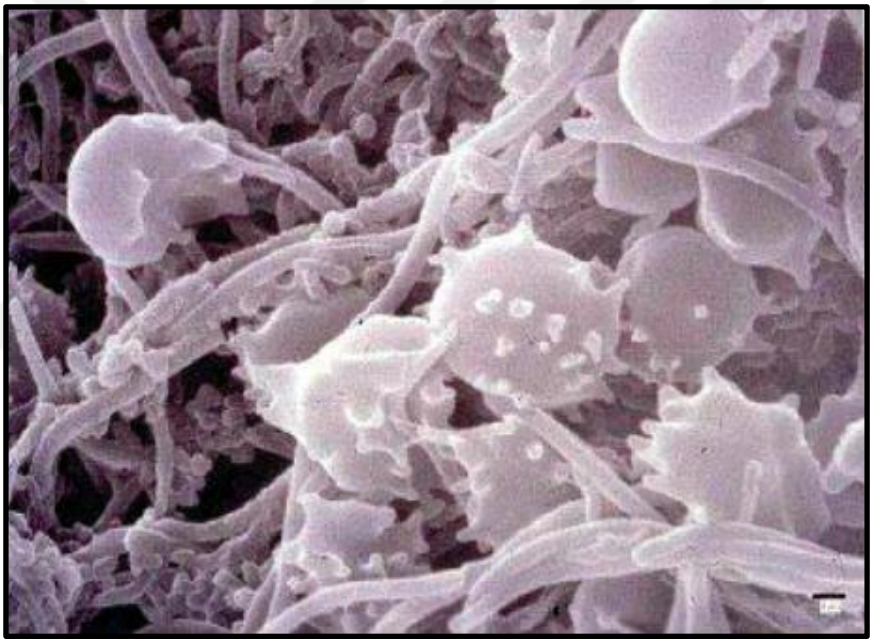


Figure 16. SEM imagefigure showing the diversity of microorganisms in MDP (13)

The most significant criterion for preventing the formation of MDP is to ensure effective oral and dental health. Besides, rough restorations in the mouth with poor margins and unpolished surfaces, high viscosity but low amount of saliva, nutrition rich in carbohydrates, and the alignment of teeth on the arch are some factors that increase the amount and speed of MDP formation (13).

First, *Streptococcus sanguis (S. sanguis)*, *Streptococcus oralis (S. oralis)*, and *Streptococcus mitis (S. mitis)* are colonized. The initial flora also includes Actinomyces species and gram-negative bacteria. The growth of the plaque occurs by the division of cells in the initial phase. The growth of microcolonies perpendicular to the surface of the tooth supports this. The plaque continues to grow with microorganisms coming from the saliva and the microorganisms in the upper layer form a structure in the shape of a 'corn cob' (Figure 17) (33).

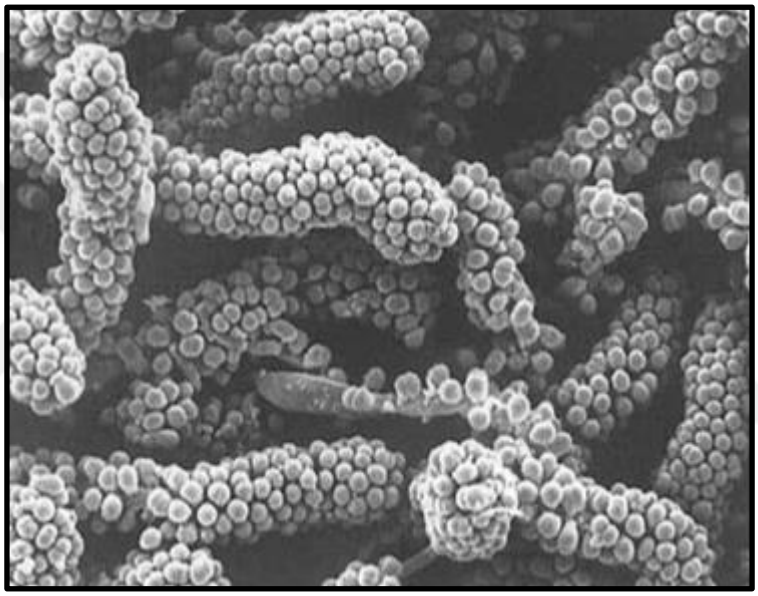


Figure 17. Corn cob appearance formed by microorganisms (23)

Research reports that there are various ecological areas in the tongue, cheek, gingival fluid, smooth surfaces of teeth, pits, and fissures in the mouth, and many different types of microorganisms in these areas. The bacteria groups Mutans Streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) and Lactobacilli are known to be closely associated with the formation of dental caries. Microorganisms that cause dental caries are distinguished from other microorganism types by rapidly metabolizing fermentable carbohydrates and producing acid. These microorganisms are capable of producing extracellular and intracellular polysaccharides. As extracellular

polysaccharides, they can synthesize glucan and fructan. Intracellular polysaccharides are known as stored polymers that are similar to glycogen (13).

Some types of Mutans Streptococci (MS), particularly *S. mutans and S. sobrinus* stand out as the most effective species known to be involved in the formation of dental caries by metabolizing fermentable carbohydrates, resulting in acid formation in the oral cavity (acidogenic), having the capacity to lower the pH even more in these acidic conditions (aciduric), and having the ability to survive and grow in this acidic environment (acidophilic). These virulence factors play a crucial role in the pathogenicity of dental caries (13).

The metabolization of dietary sucrose by MS has a key role in the formation of dental caries. Their accumulation on the pellicle and the extracellular polysaccharides that are necessary for their colonization are significant. The glycosyltransferase enzyme synthesized by MS allows the catabolization of glucan from sucrose. With the phosphotransferase enzyme system, carbohydrates can be taken into the bacterial cell. These properties make MS more effective than other types of bacteria (35). When they cannot find nutrients, they survive by using stored intracellular polysaccharides, and the acids released by metabolizing these cause a decrease in plaque pH. If this low pH persists, demineralization of hard dental tissues occurs (36).

MS is divided into 7 subgroups according to carbohydrate antigens and DNA hybridization. The most common types of MS that are isolated in humans are *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) (32,37).

S. mutans, has a key role in the formation of dental caries. In order of density, they are mostly found in occlusal fissures, proximal surfaces of molars, and vestibular surfaces

of anterior teeth. With the excellular polysaccharides they synthesize, they can easily colonize on tooth surfaces. Since they are acidogenic, when they metabolize fermentable dietary carbohydrates, the organic acids that are released as by-products cause a decrease in plaque pH. Since they are also aciduric and acidophilic, they can survive and reproduce in an acidic environment when the pH decreases (15).

It is known that the ability of *S. mutans* to survive in this acidic environment and to tolerate acids is due to the F-type adenylpyrophosphatase (F-ATPase) enzyme. The F-ATPase enzyme is accepted to drag protons out of the cell, preventing the decrease of intracellular pH, so acid-sensitive enzymes, DNA, and proteins are not damaged (37).

It has been reported that infants first encounter *S. mutans* during the eruption of the first primary tooth. This transition is believed to be caused by people in close contact with the infant, like the mother or the caregiver (38).

S. sobrinus is an acidogenic and aciduric microorganism with adhesion property, like *S. mutans*. It is the most isolated microorganism in dental caries following *S. mutans*. *It is reported that S. sobrinus is rarer in environments where S. mutans* is not present (39). Although *S. mutans* is often isolated in caries lesions, research reports that the cariogenicity of *S. sobrinus* is higher than *S. mutans* and that it is more effective in the formation of soft caries lesions (40).

Lactobacilli have a secondary effect in deep caries lesions. They have been reported to be frequently isolated in caries lesions with cavitation. In cases with no tooth caries, they have quite a low presence rate in MDP. Their presence in incipient caries lesions has also been found to be low. Like Mutans Streptococci, Lactobacilli are also

highly sensitive to dietary carbohydrates. Their acidogenic, aciduric, and adhesion abilities are significant for dental caries (32,41).

The role of nutrition is very important in the formation of dental caries. Cariogenic bacteria metabolize consumed fermentable carbohydrates, releasing organic acids. These released acids lead to a rapid decrease in plaque pH (in 1-3 minutes). Plaque pH remains acidic for a certain time and may then rise to a normal value (pH=7) within 30-60 minutes. The first to express this rise and fall in pH was Dr. Robert Stephan. These changes in pH are defined by the 'Stephan curve,' his own creation (Figure 18) (4). Stephan examines the changes in pH due to foods intake in this curve (42).

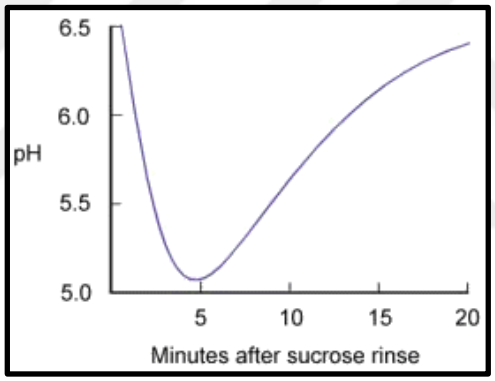


Figure 18. Stephan Curve (34)

Fermentable carbohydrates are known as the main cause of caries. Among sugars, sucrose has the highest potential to cause caries. Low-molecular-weight monosaccharides and disaccharides enter into the MDP and are used by cariogenic microorganisms; the released organic acids cause calcium and phosphate ions to dissolve from hard dental tissues, initiating tooth caries. These acids are neutralized by the buffering effect of saliva, increasing the pH (15).

Research has reported that the frequency of intake of sugary foods is more significant than their amount. The constant low plaque pH due to the frequent consumption of foods with high cariogenicity contributes to the formation of dental caries (43,44).

The duration for which sugary foods stay in the mouth also affects their cariogenicity. Easily soluble foods can be easily cleaned from the oral environment, while sticky ones can remain on teeth for a long time (44).

Another key factor in the consumption of cariogenic foods is at what meal they are consumed. Saliva secretion is higher during main meals. Hence, consuming cariogenic foods during meals rich in protein and fat reduces their carious activity. Individuals on a protein-rich diet have higher levels of urea in their saliva and consequently the saliva has an increased buffering capacity (15,44).

Research also reports that foods rich in calcium and phosphate reduce the cariogenicity of foods, decreasing the formation of dental caries (45).

It is well known that organic acids released in the presence of a suitable environment, nutrients, and microorganisms that can ferment these nutrients lead to the destruction of hard dental tissues, initiating dental caries. For this chain of reactions to begin, all factors that cause dental caries must coexist for a certain time (minimum of 30 minutes). This reflects that time is a crucial and effective factor in the formation of caries lesions (15).

2.2.2. Demineralization-Remineralization Cycle

Demineralization is the mineral loss on dental hard tissues due to acids that are formed by the fermentation of dietary carbohydrates by cariogenic microorganisms in the MDP. The reprecipitation of dissolved minerals into tooth enamel is named remineralization. There is a balance between demineralization and remineralization in the mouth (Figure 19) (2).

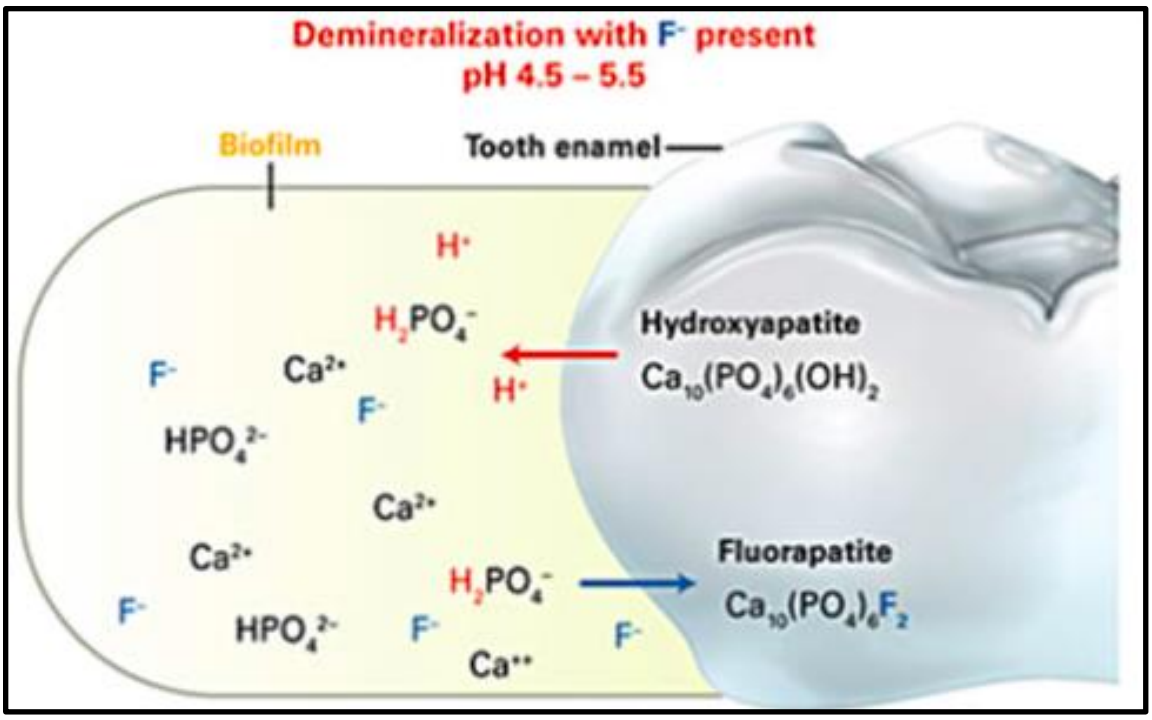


Figure 19. Demineralization-remineralization balance (13)

Intact enamel crystals are reported to be 40 nm thick and very small in size. The structure of enamel crystals is very similar to hydroxyapatite, although they do not have a pure mineral structure. Since they consist of more than one ion, they are much easier to dissolve than pure hydroxyapatite or fluorapatite. Hydroxyapatite has been shown to dissolve more easily than fluorapatite due to containing carbonate instead of fluorine in its crystal structure (46).

Demineralization in hard dental tissues takes place in two stages. First, by the metabolization of fermentable carbohydrates by cariogenic bacteria, acids like lactate that can diffuse with water are released between crystals. These acids diffuse from the structure of the enamel crystal from a suitable place, dissolving calcium and phosphate. These dissolved minerals join the liquid medium between the crystals (Figure 20) (47).

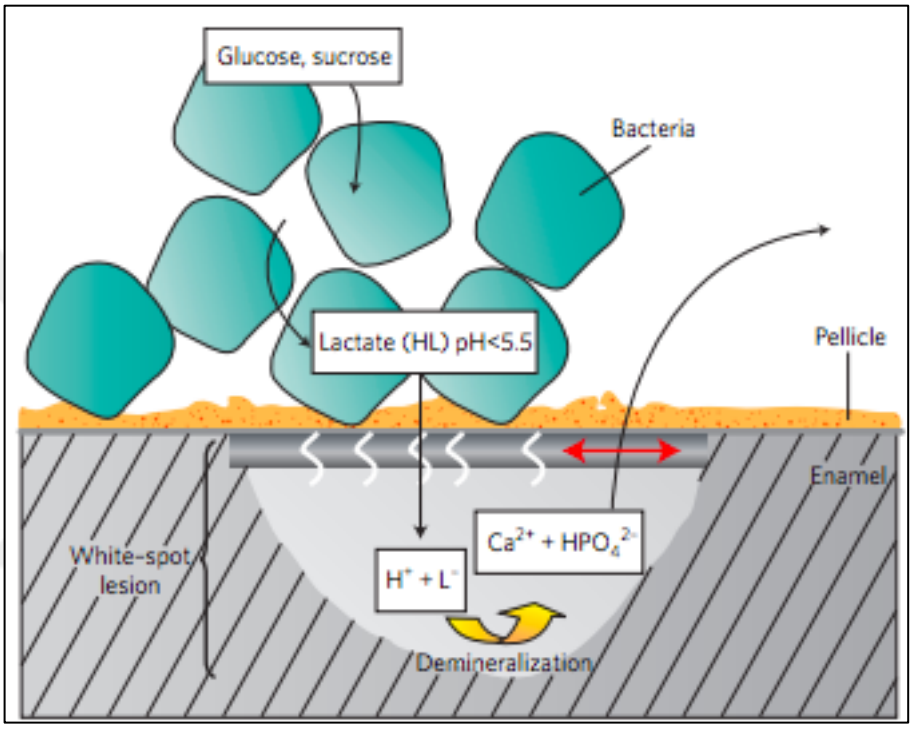


Figure 20. Demineralization (42)

With the acid attacks in the MDP, calcium fluoride (CaF_2), hydroxyapatite (HAP), and fluorapatite (FAP) dissolve. If these acid attacks persist, the dissolved minerals increase the saturation degree of the plaque, slowing down the dissolution of enamel crystals. When the pH reaches 7 again, the minerals and crystals that precipitate from within the dissolved minerals are reorganized. Of these minerals, some form hydroxyapatite and some fluorapatite (48).

If there is sufficient amount of fluorine ions on enamel crystal surface before or during demineralization, these fluorine ions penetrate the enamel crystals, preventing demineralization. For this to take place, fluoride must be applied topically and be present on the tooth surface and within the MDP (49).

The reprecipitation of dissolved calcium, phosphate, and other ions into demineralized areas at neutral pH is called remineralization. With the advances in minimally invasive treatments in today's dentistry, great importance is attached to remineralization studies and remineralization treatments (49,50).

Saliva plays a key role in remineralization. Beside the saturation of calcium and phosphate in saliva, it is also important for fluorine ion to be present in the medium to act as a catalyst for these ions. With the calcium and phosphate ions in its content, fluorine ion precipitates on demineralized surfaces, providing remineralization. This new structure is called fluoridated hydroxyapatite, or fluorapatite. It is known that this new structure dissolves at lower pH values than hydroxyapatite and is more resistant to acid attacks (51).

The lesion should not reach the cavitation stage for the remineralization of the enamel surface. The white, opaque, non-cavitated enamel lesions observed in enamel preserve their original crystal structures. Researchers report that the calcium and phosphate ions penetrating the demineralized enamel surface through the outer enamel membrane provide the remineralization of incipient caries lesions, and this new structure is more resistant to acid attacks than the original enamel structure (51).

The microbial dental plaque is also crucial in providing remineralization. In caries lesions covered with plaque, minerals like calcium, phosphate, and fluoride within the

plaque fluid diffuse to demineralized surfaces with their ability to move in liquid media, providing remineralization. If the concentration of these minerals in the mouth is low, acid attacks become long and frequent, this balance in the mouth cannot be maintained, and it progresses toward demineralization (52).

High flow rate and buffering capacity of saliva, high concentration of inorganic components in saliva, decreasing dietary carbohydrates, low number of cariogenic bacteria, shallow and inactive caries lesion, regular application or use of remineralizing agents, and mechanical control of the microbial dental plaque are crucial factors that accelerate this remineralization process (53).

2.3. Incipient Caries Lesion

An incipient caries lesion is the earliest stage of dental caries, where it is known to be possible to arrest and treat dental caries. Incipient caries lesions are limited to enamel (54). The surface structure of an incipient caries lesion is more porous than the surface of a healthy tooth enamel and this is a demineralized area where cavitation does not occur. Incipient caries lesions are also referred to as 'flat surface lesions' or 'white spot lesions' (1).

In these lesions, there is a demineralization process that continues under the intact enamel tissue. When demineralization continues, various levels of cavitation and loss of tooth hard tissues occur. Hence, early diagnosis and treatment of incipient caries lesions gains importance (1).

2.3.1. Clinical Appearance of Incipient Caries Lesions

One early symptom of dental caries occurring on smooth enamel surfaces is in the form of a white spot lesion (Figure 21). Since the mineral loss that occurs in incipient caries lesions changes the enamel's light reflectivity, they tend to have a chalky, opaque, and matte appearance. White spot lesions can be observed once the tooth surface is dried. (1,55)

Figure 21. Clinical Appearance of Incipient Caries Lesions (55)

Incipient caries lesions are often covered with plaque. These types of lesions can turn into cavitation based on the changes in the oral environment, or can be diagnosed at an early stage and stopped and treated (55).

Incipient enamel lesions may be confused with hypocalcified enamel surfaces. Air drying and examination with a blunt probe are crucial in the differential diagnosis of both lesions. Incipient caries lesions appear translucent when moist and opaque white when dried using air spray. The translucent appearance of the enamel in opaque enamel lesions is an optical phenomenon that is observed based on the width of the spaces between enamel crystals (56). However, hypocalcified enamel surfaces show an opaque

appearance despite the humidity or dryness of the medium. Often, no plaque accumulation is observed on surfaces with hypocalcified enamel defects. Cavitation is not observed in either lesion, although it is reported that incipient caries lesions have a softer and porous surface (57).

2.3.2. Histological Structure of Incipient Caries Lesions

Examining incipient enamel lesions histologically, it is observed that the early mineral loss first occur in the center of the enamel prisms. This can be explained by the fact that the low crystal density in the central region of the prisms allows acids from outside and proton diffusion (58).

According to Darling, incipient caries lesions consist of 4 layers. These layers, from the outer surface to the pulp, are the surface layer, lesion body, dark layer, and translucent layer (Figure 22) (59).

The surface layer is the outermost layer of enamel caries, a hard layer that is difficult to dissolve. The surface layer is less affected by caries compared to the other layers, with less mineral loss. The pores in the surface layer are larger than those in healthy enamel structure. This layer has a pore volume of 5% and a lesion width between 20-100 μm. Remineralization can be achieved since the surface layer allows ion exchange. Also, this layer can become more remineralized by the accumulation of dissolved minerals from the deeper caries layers. Besides, it contains more fluoride. By removing this hypermineralized area by processes like polishing, the enamel tissue loses resistance against caries. Arresting a caries lesion at this stage is crucial for caries prevention (13,60).

The lesion body is the largest part of enamel caries and the main layer where demineralization takes place. This layer is located 15-30 μm below the intact enamel surface. It has 24% less mineral content by volume compared to healthy enamel. The lesion body is highly porous and has the largest pore volume. Its pore volume varies within the lesion body and often tends to increase from the periphery toward the center. Pore volume can be around 25% in the center and 5% in the periphery. Since these pores can also facilitate the infiltration of bacteria into the lesion body, microorganisms can be found in this layer. Also, beacause there are more dissolved minerals in this layer, striae of Retzius can be observed more easily. (60,61)

The dark layer indicates that many demineralization and remineralization cycles take place within the lesion. In regions with less mineral loss, these pores are smaller in size. The quinoline solution that is used in polarized light microscopy examinations cannot pass through these micropores, and since polarized light is blocked, this layer is observed as dark under the microscope. The pore volume in this layer varies from 2 to 4%. The pores in the enamel structure get narrower as the caries lesion is remineralized and the permeability of the enamel tissue decreases. The width of the dark layer may vary based on the rate of progression of the caries. In cases of rapid progression, the dark layer is observed to be thinner, and during slow progression, it appears thicker. This layer involves higher demineralization than the surface layer and less demineralization than the lesion body. (60,62)

The translucent layer is the deepest layer of the lesion. When a quinoline solution with the same refractive index as tooth enamel is applied for polarized light microscope examination, the caries lesion gets filled with this solution, appearing like an intact enamel. Under light microscope, this region appears as translucent. Hence, this

layer is called the transparent or translucent layer. Here, the pore volume between enamel prisms is 1%. This is equivalent to nearly 10 times that of an intact enamel tissue (62). According to research, the translucent layer is found in 25% of primary teeth and 50% in permanent teeth (13).

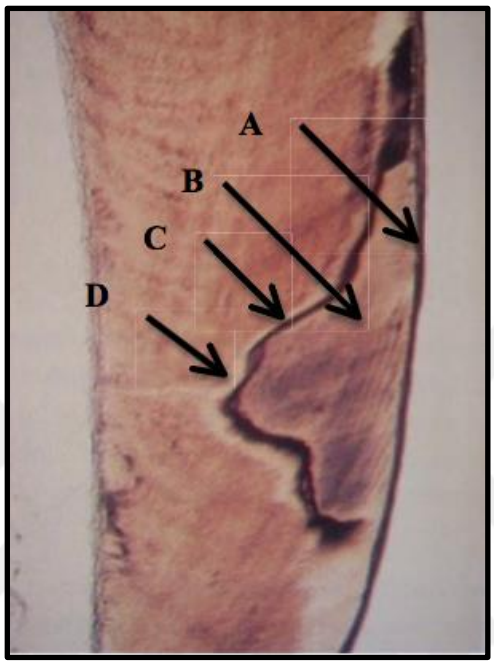


Figure 22. Histological appearance of incipient caries lesions (13)

A: Surface Layer **B:** Lesion Body **C:** Dark Layer **D:** Translucent Layer

Research reports that each of the four layers observed in histological examinations of incipient caries lesions have different pore volumes. Pore volume is less than 5% in the surface layer, 5-25% in the lesion body, 2-4% in the dark layer, and 1% in the translucent layer (Figure 23) (24).

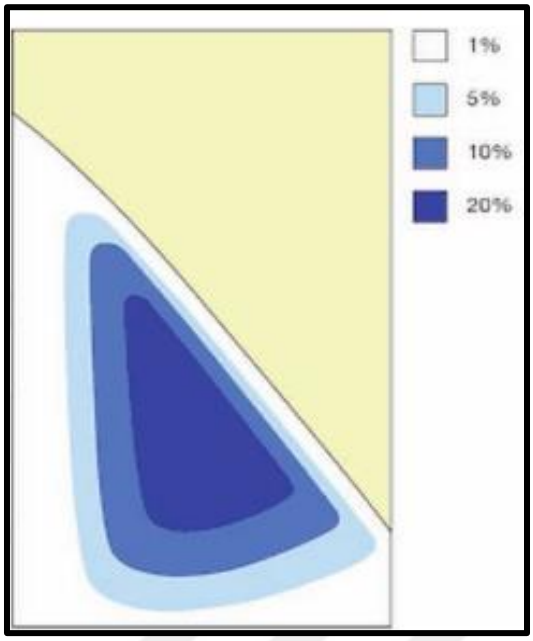


Figure 23. Pore volumes of four layers forming incipient caries lesions (13)

2.4.Demineralization and Remineralization Evaluation Methods

When determining demineralization and remineralization processes occurring in tissues, specifying which minerals are lost or gained and their amount is of great importance. Therefore, today, numerous methods are used to determine and compare demineralization and remineralization in dental hard tissues

2.4.1. Microradiography

Used since the 1940s, this method gives information about the mineral content of tissues by the absorption of X-rays. Tissues containing hydroxyapatite crystals that can absorb X-rays, like enamel, dentin, bone, and cartilage can be examined using this method (63). It is often preferred for determining demineralization and remineralization in both *in situ* and *in vitro* studies. As a very sensitive method, it is used to determine the morphology of enamel and dentin samples and their mineral content. When examining for remineralization, the researcher calculates how much of the X-ray sent from the source is absorbed by the minerals (63).

It has three types.

- Longitudinal microradiography examines samples in thin sections.
- Transverse microradiography examines them in thick sections.
- Wavelength-independent microradiography provides examination of the whole tooth (64).

2.4.1.1.Transversal Microradioraphy (TMR)

It is the most commonly used microradiography method. 50-200 μm thick samples obtained from enamel or dentin perpendicular to the tooth surface are placed in parallel on a radiographic film (63).

TMR gives two different parameters. These are lesion depth L_d (μm) and mineral change value (ΔZ, volume %μm or $kg.m^{-2}$). ΔZ helps determine the amount of mineral between the intact tissue and the lesion, and L_d is determined from the distribution of minerals. If a tissue is demineralized, L_d and ΔZ increase, and if it is remineralized, L_d and ΔZ decrease. Because very thin sections are obtained, it is a destructive and very sensitive technique. It can be used to examine enamel and dentin tissues in *in vitro* and *in situ* studies (64). Determining the distribution of minerals and numerical measurement of mineral loss or gain are among its advantages. Disadvantages include time-consuming examination and the samples being easily spoiled due to using thin sections (64).

2.4.1.2.Longitudinal Microradiography (LMR)

In this method, the amount of mineral in tissues can be measured more than once. It allows a chain of measurements as the samples are not damaged during measurement. X-rays are sent parallel to the longitudinal axis of the tooth. Sections are thicker compared

to TMR and samples are not damaged. Unlike TMR, LMR expresses the change in total mineral content. Although, it gives no information on the depth or mineral content of the lesion body (65).

2.4.1.3.Wavelength-Independent Microradiography (WIM)

This is a quite new method that uses high-energy polychromatic X-rays. The amount of mineral in hard dental tissues can be determined without damaging the surface of the sample. It allows measurements of 0.3-0.6 mm thick sections (64).

2.4.2. Energy-dispersive spectroscopy (EDS)

In EDS, electron beams are directed toward sample surfaces, the X rays reflected from the surface are analyzed, and the element content on the examined surface can be determined. Even 1µm wide phases can be examined using this method. The main use of this technique is numerical measurement of the amount of major and minor elements of minerals and solid materials (66).

2.4.3. Atomic Force Microscopy (AFM)

Atomic force microscopy, is a scanning force microscopy with high contrast and resolution. It is preferred in nano-level examinations due to its high resolution. This high resolution property leads to time-consuming examinations (67). AFM can be used for detailed examination of demineralization in enamel and dentin tissues. The single collagen fiber in demineralized dentin can be monitored this way. Since it can measure large differences at the atomic level, it is useful for determining early surface changes. Advantages include use in humid conditions, less artefacts in images, and performing series of examinations in succession (68).

2.4.4. Polarized Light Microscopy (PLM)

It is a sensitive and qualitative method used on hard dental tissues. This method allows the measurement of lesion depth and the size of caries layers, but gives no information on mineral density. Examinations are done on 80 μm thick sections. Mineral loss and gain can be observed when examining demineralization and remineralization. It gives information about the quantitative volumes of the pores in enamel (69). Polarized light microscopy is a laborious but cost-effective method. It helps determine lesion depth but cannot determine the inner or outer layers of the lesion. It allows qualitative assessment of changes in tissues (70).

2.4.5. Quantitative Light Fluorescence (QLF)

This is a diagnostic device that provides *in vivo* and *in vitro* assessment of parameters regarding dental caries, dental calculus, bacterial activity, microbial dental plaque, discoloration, and tooth whitening. It is also preferred in measurement of caries lesions. The mineral loss occurring in dental caries can be determined by the scattering of light (71).

Dental hard tissues have their own natural fluorescence. This property is called autofluorescence. Intact tissue appears green, and when the amount of fluorescence decreases in demineralized areas, the carious tissue appears as a dark area. When hard dental tissue is exposed to blue light, the fluorescence in its structure is stimulated and green fluorescence is released. The fluorescence in hard dental tissue decreases with demineralization. Hence, a caries lesion monitored by QLF has lower fluorescence than intact tooth tissue. Thus, demineralized areas appear as dark areas on QLF (71).

Repeated measurements can be made by recording the images on a computer, the changes in the mineral content of lesions can be measured at different times, and differences in lesion depth can be detected (71). In QLF examinations, fluorosis in enamel appears in a dark color due to the porous enamel structure. Porphyrins that are metabolized by bacteria in external dental calculus, microbial dental plaque, or infected caries lesions appear as red fluorescence in QLF examinations (71).

2.4.6. Confocal Laser Scanning Microscopy (CLSM)

Confocal Laser scanning microscope was developed in 1955 as a non-destructive microscopic tomography technique used particularly in cell biology. It has been used in dentistry studies since 1983. Light is obtained from a focused laser source and scans are made in lines on the sample. All reflections from outside the focused area are blocked so that only the focused area is monitored. This method allows examinations without taking sections from the teeth. Samples are not prepared in thin sections, they can be examined in humid conditions, and do not need to be dried, which prevents errors during examination. Besides, samples can be prepared quickly, there is no need for preparation before the examination, the researcher is not under the risk of X-ray radiation unlike microradiography, and surface properties, area, and volume can be determined and monitored in 3 dimensions (65,72).

2.4.7. Scanning Electron Microscopy (SEM)

Scanning electron microscopy was developed by Manfred von Ardenne in 1938 and introduced to the market by the Cambridge Instrument Company in 1965. It works by sending high-voltage electrons to the tooth surface, collecting the interaction between the electron and the surface while scanning the surface, and reflecting the signals on a screen

after they pass through amplifiers. It allows detailed examination of the topographic structure of enamel surface and changes that occur in it. It is often preferred in research due to its focal depth, discrimination power, and image analysis combination. Images obtained with SEM have very good depth of field. It is suitable for defining the morphology and surface properties of samples (73,74).

In SEM examinations, surface properties, morphology, and sequences of the elements or atoms in composition can be observed. Compared to Light Microscopy (IM), SEM has 300 times higher focal depth and 20-100,000 times clearer vision. It can obtain highly detailed images at various magnifications (x20 - x10,000 or higher) (75).

For the surface to be visual, the samples must be covered with a material that reflects electrons. Samples can be coated with a thin layer of gold (Au), platinum (Pt), gold-palladium (Au/Pd) alloy, or carbon (C). This is called 'sputtering' or 'sputter coating' (76).

2.4.8. Surface Microhardness (SMH)

This method was first used by Koulourides in *in situ* demineralization and remineralization studies (77). It is preferred in the analysis of mineral content of hard tissues like teeth. A surface microhardness device operates under IM, the diamond tip being perpendicular to the sample surface, by applying a certain weight for a certain time. Knoop or Vickers diamond tips are used for measurements. Knoop tip leaves a deeper mark on the surface than Vickers diamond tip using the same load. Both diamond tips leave the same diagonal depth, but with different diagonal widths. It is 1.4 µm in Vickers and 3.5 µm in Knoop. The surface images of these notches also differ. Vickers diamond tip leaves a square-shaped surface appearance, while Knoop diamond tip leaves a rhombic shape (Figures 24 and 25) (63,64).

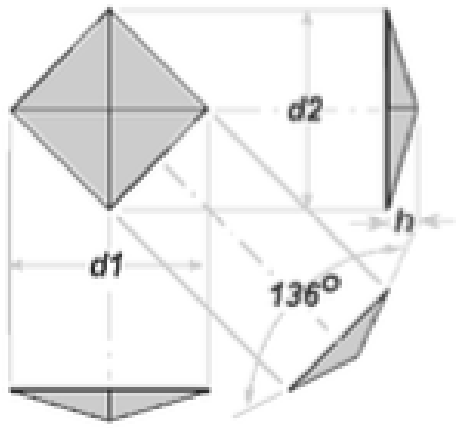


Figure 24. Appearance of Vickers diamond tip (63)

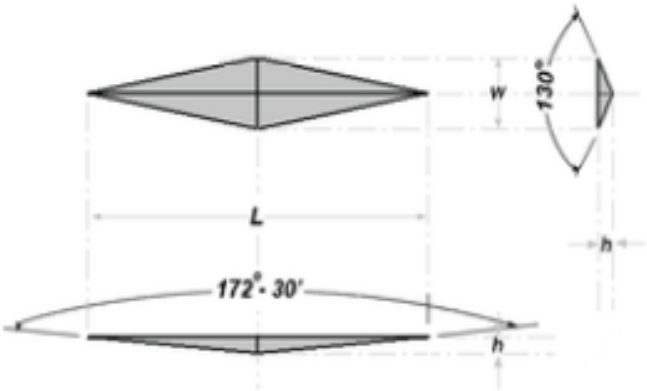


Figure 25. Appearance of Knoop diamond tip (63)

In Vickers surface hardness measurements, a certain force is applied on the sample from a peak angle of 136° with a square-based and pyramid-shaped diamond tip at a certain time, and a trace is created. The load applied to the sample surface should be such that no cracks occur on the surface. After the force is removed, the diagonals of the square-shaped trace are measured and this is transferred to a measurement screen with a microscope attached to the device. With the help of movable arms on the measurement

screen, the lengths of the diagonals are measured separately. The result is obtained by taking the average of the measurements (70).

Vickers hardness value or Vickers hardness number (VHN) is calculated by dividing the test load in kg by the area of the trace in mm^2 using the following equation:

VHN=1,854 x force / (base diagonal)^2 kgf/ mm^2 (77)

The smaller the trace area, the greater the Vickers surface microhardness value, indicating that the material is harder. The hardness of the material is inversely proportional to the size of the trace. The higher the surface microhardness of the material, the higher the VHN. Hence, surface microhardness and demineralization-remineralization studies give information about mineral gains. Nevertheless, it gives limited information on lesion depth. Since the measurements are made perpendicular to the outer surface of the tooth, the tooth surface must be polished before measurement. This method allows repeated measurements on the same sample (70,78).

2.4.9. X-Ray Diffraction (XRD)

This method gives information about the geometric arrangement of the atoms of the crystals and the distances between them. XRD is a technique that can determine the distance between the atomic planes of the crystal along with the unit cell size of a crystal plane (79).

X-ray diffraction is the reflection of X-rays from a crystal plane by hitting the crystalline atomic planes. During diffraction, incoming X-rays reach the atomic planes below the crystal surface. This shows that diffraction is not related to the surface. If the X-ray beam hits the crystal planes at any other angle, diffraction cannot occur. This is

because the X-rays reflected from the crystal planes have a phase difference due to the different lengths of their pathways. The rays cancel each other, and no diffraction peak can be observed (79).

When the X-ray beam hits the atomic planes at a certain angle known as the 'Bragg angle,' the path of the reflected rays will be equal to multiples of the wavelength (λ), so the rays will have the same phase. When the diffracted X-rays, meaning those that are reflected from the atomic planes, are in the same phase, a diffraction pattern occurs. To obtain a diffraction pattern, there must be a certain correlation between the angle that X-rays hit the atomic planes (θ), the distance between atomic planes (d), and the wavelength of incoming X-rays (λ). Diffraction occurs when an X-ray beam strikes parallel atom planes at a θ angle (Figure 26) (79).

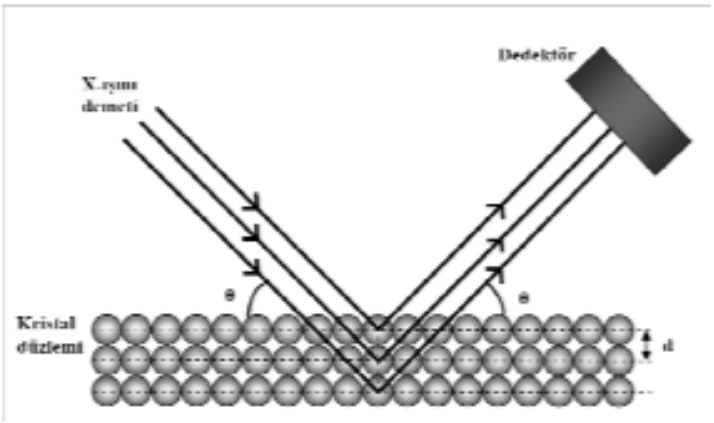


Figure 26. X-ray diffraction on a crystal plane (79)

2.5.Agents Used in Remineralization of Incipient Caries Lesions

2.5.1. Topical Fluoride Applications

Today, fluoride applications are considered the gold standard for preventing and managing tooth caries. Fluoride hardens demineralization in the mouth, while facilitating remineralization in many ways. Fluoride prevents dental caries by preventing microbial dental plaque formation, inhibiting acid production by microorganisms in MDP, providing fluorapatite formation, and facilitating the precipitation of calcium and phosphate ions to the lesion body. The methods of application of fluoride agents are classified as systemic and topical applications. So far, no research has proven how much of the preventive effect of fluoride on dental caries can be attributed to before or after eruption, although in light of data from clinical and laboratory studies, the main effect is accepted to take place after eruption of teeth. It is known that low levels of fluoride on the surface of hard dental tissues, microbial dental plaque, saliva, and soft tissues around the teeth can also maintain demineralization/remineralization balance, so research reports that this level of fluoride should be present in the oral environment throughout the day. This continuously low levels of fluoride can be provided by topical applications by professionals or individuals (3,80).

2.5.1.1. Topical Fluoride Agents

Topical fluoride agents can be applied professionally by dentists, taking into account the needs of the individuals, or individually at home.

Sodium fluoride (NaF) is a common fluoride agent that was the first to be used in topical applications and is very effective for preventing dental caries. It was first used in

1942. NaF is found in many forms including solution, gel, varnish, toothpaste, and mouthwash. Agents containing NaF do not irritate the gums and oral mucosa since they have neutral pH. Beside preventing dental caries, they can be used in patients with dry mouth (3).

Another fluoride agent, tin fluoride (SnF_2), was first used in preventive dentistry as a salt formed by fluorine with tin in the 1950s. It is also known as stannous fluoride**.** It can penetrate the enamel surface shortly after application and provides a very good bond. After application, calcium fluoride (CaF_2) is formed by the combination of the calcium in the structure of the tooth with the fluoride in tin fluoride, and here, the phosphates and the tin phosphate (Sn_2PO_4OH) surrounding the tooth form the tin fluorophosphate ($Sn_3F_3PO_4$). These compounds precipitate on the enamel, protecting it like an armor preventing it from dissolving (81,82). It has been shown by *in vitro* research that SnF_2 significantly reduces plaque formation and acidogenicity (83,84). SnF_2 has a quite high protective effect against dental caries, although it causes discoloration in the teeth and leaves a bad taste in the mouth, limiting its use. It is often found in toothpaste and mouthwashes (85).

Titanium tetrafluoride (TiF_4) has been used in dentistry since the 1980s. It has a role in preventing dental caries, sensitivity, and erosion . Agents containing TiF_4 are found in solutions, gels, and varnishes at concentrations ranging from 1 to 4%. Since TiF_4 is an acidic agent, research reports that it can affect demineralization on the enamel surface and increase the penetration depth of fluoride ions (86).

Reed and Bibby (1976) evaluated the effect of TiF_4 and acidulated phosphate fluoride (APF) in preventing tooth caries in a 3-year study *in vivo*, and reported that 1

minute of TiF_4 application once a year was statistically significant in reducing tooth caries compared to 4 minutes of APF application once a year (87).

Büyükyılmaz et al. (1994) assessed the effectiveness of 1% TiF_4 application around orthodontic brackets in protection from tooth caries for 4 weeks *in vivo*, concluding that 1% TiF_4 application significantly decreased lesion depth and mineral loss, proving its effectiveness in preventing tooth caries (88).

Amine fluoride (AmF) has been used in dentistry as a topical fluoride agent since 1957, particularly in European countries. With their surface-active properties, they bind tightly to tooth enamel, forming CaF_2. They are found in toothpastes, mouthwashes, and gels (89,90).

Acidulated phosphate fluoride (APF) has emerged in the 1960s by adding 10% orthophosphoric acid to fluoride preparations to prevent demineralization on the enamel surface and to increase fluoride uptake, supporting FAP formation. Professionally applied APF preparations have a pH of 3.0, containing 1.23% (12.300 ppm F^-) NaF. They are often available in gel form in the market (85). Clinical studies report that its effectiveness in preventing dental caries is on average 23-26% (91,92).

Research reports that applying preparations containing APF to the tooth surface causes formation of sufficient amounts of CaF_2, which makes it enough to apply it for longer periods compared to other fluoride agents, like 6 to 12 months (93,94).

Jiang et al. (2004) carried out a randomized controlled clinical study evaluating the protective effect of gels and foams containing APF against caries after 6 months of professional application, reporting no statistically significant difference between the

effectiveness of gels or foams containing APF in reducing flat surface caries in permanent first molars (95).

2.5.1.2. Topical Fluoride Application Methods

Topical fluoride applications are highly effective methods that have been preferred for many years for arresting incipient caries and providing remineralization. Topical fluoride applications can be applied individually or professionally by a dentist. In these applications, when fluoride is constantly present in the mouth and the pH of the mouth decreases against acid attacks, acting as a reservoir, CaF_2 is formed, called a loosely bound fluoride (85). Also, the replacement of hydroxyl ions (OH^-) with fluorine (F^-) ions in the hydroxyapatite in the tooth structure leads to fluorapatite, which is more resistant to acid and more difficult to dissolve, called a firmly bound fluoride (4).

2.5.1.2.1. Professional Topical Fluoride Application

Professional topical fluoride applications are performed by dentists using products with fluoride concentrations ranging from 5,000 to 22,600 ppm. In these applications, the fluoride compounds are given by solutions, gels, varnishes, or foams, or they are ensured to be constantly present in the mouth by dental materials and devices that release fluoride slowly. Professional topical fluoride applications are applied in 3, 6, or 12-month periods according to the needs of individuals and depending on their caries risk group (96).

Gels containing high concentrations of fluoride have been used in dental practice for many years. Fluoride gels are obtained by adding hydroxyethylcellulose into the solution to increase viscosity. In gel form, they may contain 2% NaF or 1.23% APF, and amorphous calcium phosphate may be added in some products (97). Fluoride gels

containing APF have been in use since 1968. It contains 1.23% fluoride ion (12300 ppm) and has a pH of 3-4. In clinical practice, the maximum recommended dose per tray is 5 milliliters. The application time of APF gels is recommended as 4 minutes. They should be applied by dentists in periods of 3, 6, or 12 months, according to the caries risk group (98).

Fluoride varnishes were first developed in Europe in 1964 under the trade name 'Duraphat.' Long-term research has reported that fluoride varnishes have quite high effectiveness for preventing tooth caries and are safe to use. Varnishes can stay on the tooth surface for longer times than other topical fluoride applications, acting as a reservoir by slowly releasing fluoride. It is considered the most appropriate professional local fluoride application method for being easy to apply in young children and being easy to tolerate. Fluoride varnishes also provide a recharging of fluoride in glass ionomer restorations in the mouth (99).

Numerous clinical studies, systematic reviews, and meta-analyses on the effect of fluoride varnishes in preventing dental caries during primary and permanent dentition have concluded that they reduce demineralization and increase remineralization (100).

The most commonly used fluoride varnishes are those containing 5% NaF. They are used for improving of incipient caries lesions, preventing caries in high risk patients, and eliminating excessive dentin sensitivity (101).

Before applying fluoride varnishes, the microbial dental plaque on the teeth should be cleaned and the teeth should be dried. It should be applied to the tooth surface in a thin layer. After application, teeth should not be brushed and food and drinks should be

avoided for 1 hour. The application should then be repeated in 3, 4, or 6 month periods based on the risk group of the individual (101).

2.5.1.2.2. Individual Topical Fluoride Applications

Individual topical fluoride applications include fluoride toothpastes, mouthwashes, and dental floss. These applications include products containing 0.2 to 1 mg of fluoride per milliliter and low concentrations of fluoride, ranging from 200 to 1450 ppm (102).

Fluoride toothpastes are the most ideal method of individual topical fluoride applications for preventing dental caries and protecting community oral and dental health. Fluoride toothpastes were first introduced in 1955. In 1995, the International Standardization Organization (ISO) has determined the maximum concentration of fluoride ions in fluoridated toothpastes as 1500 ppm (103).

The fluoride ions in fluoridated toothpastes act as a reservoir in the mouth. They prevent demineralization and ensure remineralization (104).

Systematic reviews have reported that using fluoridated toothpaste is effective in reducing dental caries (98,105–107). It is stated that widespread use of fluoride toothpastes in developed countries has been highly effective in reducing dental caries (108,109).

In their systematic review, ***Marinho et al. (2003)*** reported that using fluoride toothpaste in children and adolescents reduced dental caries by 37% during primary dentition and 24% during permanent dentition (98).

Walsh et al. (2010) conducted a systematic review on the effectiveness of toothpastes containing different concentrations of fluoride in protecting against dental caries in permanent teeth and found that fluoridated toothpastes containing 1000 ppm and above were effective in preventing dental caries, effectiveness against caries was associated with dose, and effectiveness increased with higher concentrations of fluoride (110).

Fluoridated toothpastes may contain fluoride compounds like NaF, sodium monofluorophosphate (NaMFP), SnF_2, AmF, or combinations thereof (111). The first formulations of fluoride toothpastes marked an incompatibility between abrasives and fluoride compounds. This problem was solved by adding sodium monofluorophosphate compatible with abrasives containing calcium or by adding calcium-ion-free abrasives into toothpastes. Today, abrasive systems containing silica are used rather than abrasives containing calcium (111).

NaMFP has been used as a source of fluoride in toothpastes for many years. NaMFP can release fluoride ions by hydrolysis with its reaction with saliva enzymes, while NaF can directly supply fluoride ions into saliva (111).

In their systematic review, ***Marinho et al. (2003)*** reported no statistically significant difference was found between the effectiveness of fluoride compounds with different ingredients, and found that NaMFP and NaF had a similar effect in preventing dental caries (98).

In another systematic review, ***Wright et al. (2014)***, reported that fluoride toothpastes were effective in preventing dental caries, and that care should be taken when using them in children under 6 years of age due to the dental fluorosis risk (106).

The American Academy of Pediatric Dentistry (AAPD) recommends using fluoride toothpastes no larger than a rice grain twice a day for children under the age of 3 years, and no larger than a pea twice a day for children aged 3-6 years. Although, they have no recommendation for the concentration of fluoride in toothpastes (112).

The European Academy of Pediatric Dentistry (EAPD) recommends toothpastes containing 1000 ppm fluoride no larger than a rice grain twice a day for children aged between 6 months and 2 years, toothpastes containing 1000 ppm fluoride no larger than a pea twice a day for children aged 2-6 years, and toothpastes containing 1450 ppm fluoride 1-2 cm long twice a day for children aged 6 years and over (113).

With the development of topical fluorides applications over time, easy-to-apply and time-efficient mouthwashes have also been developed (114). Research reports that the combination of mouthwashes with other topical fluoride applications is more effective in preventing dental caries (114–116).

In terms of fluoride concentrations, fluoride mouthwashes are divided into two as those containing 0.05 (230 ppm) NaF and 0.2 (900 ppm) NaF. Mouthwashes with low fluoride concentrations (200 ppm) are suitable for daily use, and those with high concentrations (900 ppm) are suitable for weekly use (117). The standard mouthwashes in the market are those with a low fluoride concentration and neutral pH, containing 0.05% NaF (118).

One study evaluated the effectiveness of using a high-concentration mouthwash (900 ppm) containing NaF once a week in children aged 5-13 years for preventing tooth caries in primary molars, and reported that they prevented dental caries to a great extent (115).

Fluoride mouthwash is not recommended for use in children younger than 6 years of age. It is rather recommended for individuals who cannot do mechanical cleaning, those in high risk of caries, patients receiving fixed orthodontic treatment, and those with decreased salivary flow rate (116,117,119).

There are no reported side effects to using mouthwashes containing fluoride. However, some mouthwashes (fluoride mouthwash with added hexidite) cause abrasion on the teeth, albeit at a low level, if used before brushing teeth. The disadvantage of fluoride mouthwash is acute or chronic fluoride poisoning. This is easy to prevent in adults and children using it under adult supervision (118).

The EAPD does not recommend using fluoride mouthwashes in children under the age of 6 years due to the risk of swallowing. It is reported to be effective in preventing dental caries in children older than 6 years of age and can be used in parental control (113).

The AAPD recommends using fluoride mouthwashes in children aged 6 years and over (112).

Using dental floss with fluoride is also recommended in addition to brushing to prevent or reduce the formation of aproximal caries, which is common in young adults and children. Dental floss containing fluoride provides recovery of fluoride in dissolved enamel, helping prevent dental caries by reducing the colonization of *S. mutans* (120).

2.5.2. Calcium Phosphate Technology

2.5.2.1. Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP-ACP)

The preventive properties of milk and other dairy products against caries have been demonstrated in numerous studies. However, they must be consumed in large amounts to have this effect. Hence, researchers have investigated separation of protective factors in milk and using them in oral care products (121).

Casein is a phosphoprotein that is predominantly found in milk. Casein phosphopeptides (CPP) stabilize amorphous calcium phosphate (ACP), forming the CPP-ACP complex and maintaining the mineral saturation of enamel. CPP-ACP prevents bacterial colonization, increases calcium (Ca) and phosphate (PO_4) levels in dental plaque, and binds free Ca and PO_4 to dental hard tissues. This way, it prevents demineralization and facilitates remineralization (122).

The CPP-ACP technology is present in many products in the market, including remineralizing preparations, toothpastes, chewing gums, mouthwashes, and fluoride varnishes and gels for professional use (123).

The synergistic CPP-ACP and fluoride are used together for preventing caries and providing remineralization, named as Casein Phosphopeptide-Amorphous Calcium Fluorophosphate (CPP-ACFP) (124).

De Souza et al. (2014) reported that enamel surfaces treated with CPP-ACFP had a rather homogeneous appearance (124).

Research shows that using toothpastes containing CPP-ACP together with fluoride provides more effective remineralization (123–125).

The direct correlation between nutrition and oral health has been known for many years. Foods consumed in daily nutrition increase the concentration of Ca and P ions in the mouth, showing anticariogenic properties, which is key for protecting oral and dental health. Milk and dairy products facilitate remineralization by preventing demineralization with their casein content, maintaining the balance in the mouth (122).

Grewal et al. (2017) conducted a study on the effectiveness of cheese consumption and using toothpastes containing CPP-ACP and fluoride on remineralization on the tooth enamel *in situ*, and found that cheese consumption and using products containing CPP-ACP increased the calcium and phosphate rates in enamel more than fluoride toothpastes, with no statistically significant difference between the two products. It has been suggested that, in addition to the use of fluoride toothpastes, adding dairy products like cheese to the diet of children with high caries risk can be effective in preventing dental caries (122).

Evaluating all research on CPP-ACP, we see that it is a remineralization agent that can be recommended by dentists for its high remineralization ability, accessibility, and ease of use.

2.5.2.2. Sodium Trimetaphosphate (sTMP)

Sodium trimetaphosphate is one of the most commonly preferred compounds to increase the effectiveness of fluoride toothpastes with its sodium and phosphate ion content. Adding sTMP to toothpastes has been reported to be effective in preventing demineralization (126).

One study assessed the significance of adding calcium to fluoride toothpastes in enamel remineralization, finding that adding calcium increased the remineralization of subsurface enamel lesions (125).

Danelon et al. (2013) reported that preparations containing low fluoride concentrations with added sTMP had similar success with preparations containing high fluoride concentrations in terms of their remineralization effect on incipient enamel lesions (126).

Favretto et al. (2018) investigated the effectiveness of toothpastes containing sTMP in occluding dentinal tubules, and found that the occlusion of micro and nano-sized sTMPs in toothpaste was higher than that in toothpaste containing 1100 ppm fluoride alone (127).

There is a limited number of studies on sTMP in the literature. Yet, the effectiveness of sTMP in preventing demineralization and increasing remineralization stands out.

2.5.2.3. Dicalcium Phosphate Dihydrate (DCPD)

DCPD base is used in fluoride toothpastes to increase the remineralization effect of fluoride (128).

Wefel et al. (1987) evaluated the combined use of DCPD and fluoride in toothpastes and found a positive interaction between DCPD and fluoride in their combined use and that there was higher fluorapatite formation, achieving faster remineralization (129).

Sullivan et al. (1997) assessed the effects of using toothpaste containing DCPD on

calcium (Ca) levels in the mouth and Ca activity in plaque fluid *in vivo*, finding that toothpastes containing DCPD released Ca ions in the mouth, which could provide remineralization for incipient enamel lesions (128).

2.5.2.4. Tricalcium Phosphate (TCP)

Tricalcium phosphate helps remineralization by increasing the decreased pH in the microbial dental plaque with its calcium and phosphate ion content. Tricalcium phosphate [$(Ca_3PO_4)_2$] has two forms as alpha and beta. The beta form is less soluble, making the alpha form more commonly preferred. Alpha tricalcium phosphate increases the free Ca and PO_4 levels in the mouth, suggesting that it provides remineralization in dental hard tissues (130).

Rirattanapong et al. (2017) compared the effectiveness of fluoride toothpastes with and without TCP on remineralization in enamel lesions in primary teeth, concluding that adding TCP to fluoride toothpastes increased the remineralization potential in enamel lesions, and that toothpastes with lower and higher concentrations had equal remineralization effect (130).

Functionalized tricalcium phosphate (fTCP) is a new formulation obtained by adding inorganic and organic parts of TCP like carboxylic acid and surfactant. Toothpastes containing fTCP combined with 5% fluoride are available in the market (Clinpro White Varnish, 3M ESPE) (131).

Elkasssas et al. (2014) evaluated the remineralization activity of fTCP in incipient caries lesions *in vitro* and reported that fTCP provided a significantly high rate of remineralization, increasing the resistance of tooth enamel against acid attacks (132).

2.5.2.5. Calcium Carbonate ($CaCO_3$)

Calcium Carbonate ($CaCO_3$) is an abrasive alkaline buffer that is commonly found in toothpastes. It is often used together with NaMFP (133). Toothpastes containing $CaCO_3$ have an alkaline pH value and buffering capacity, although their solubility is quite low at neutral pH. This property limits the effect of $CaCO_3$ on the acidogenicity of MDP. Even long after brushing, $CaCO_3$ particles are stored in MDP. When pH decreases, the solubility of $CaCO_3$ increases, showing anticaryogenic properties (53).

Hornby et al. (2014) reported that toothpastes containing $CaCO_3$ significantly increased calcium levels in MDP, increasing its remineralization potential on demineralized enamel surface (134).

2.5.3. Theobromine

Theobromine is an alkaloid from the methylxanthine family that contains theophylline and caffeine-like compounds, and its main source is the cocoa plant. It is obtained as a by-product from cocoa outer leaves in chocolate production (135).

Theobromine increases the size of hydroxyapatite crystals in the enamel, increasing the microhardness of tooth enamel. It provides resistance against acid changes in the mouth, affecting remineralization (136). Theobromine is reported to have a remineralization effect comparable to fluoride (137).

Amaechi et al. (2014) assessed the effectiveness of toothpastes containing theobromine in occluding dentinal tubules *in vitro* and reported equal effectiveness of toothpastes containing theobromine with or without fluoride in terms of occluding

dentinal tubules. This efficiency was reportedly higher than toothpastes containing Novamine (bioactive glass). They suggested that toothpastes containing theobromine could be recommended for eliminating dentin sensitivity (135).

Qasthari et al. (2018) examined the effect of toothpastes containing theobromine and NaMFP on enamel surface roughness after demineralization *in vitro* and reported that using theobromine toothpaste caused less roughness on demineralized enamel surface (138).

Ranju et al. (2018) assessed and compared the antimicrobial efficacy of toothpastes containing theobromine and low fluoride on *S.mutans* in MDP *in vitro*, and found a statistically significant decrease in the number *S.mutans* and plaque scores in both toothpaste groups, albeit with no significant difference between the groups. It has been reported that theobromine toothpastes could be used as a safe antibody agent for preventing dental caries (139).

2.5.4. Self-Assembling Peptides

One of the current remineralization agents, self-assembling peptides can form a tissue scaffold similar to enamel matrix proteins and crystals, providing mineralization. The peptides in the remineralization agent can penetrate the demineralized enamel, providing self-regeneration in line with the regeneration of the enamel in the lesion area (140).

The P11-4 peptide form is often used in remineralization studies on peptides. *In vitro* remineralization studies with artificial caries have shown that P11-4 diffuses into caries lesions, facilitating the formation of hydroxyapatite. It has also been reported that, by the remineralization of caries lesions, there is an increase in surface microhardness regardless

of lesion depth (140–142).

2.5.5. Arginine Technology

As an amino acid, arginine is found naturally in human saliva. Since its natural protective effect for oral health is well-known, arginine technology has been developed. The mechanism of toothpastes containing arginine technology is explained by the breakdown of amino acids in saliva by the arginine deaminase enzyme system and the release of highly alkaline ammonia. The release of ammonia increases the pH in the mouth, and therefore preparing an ideal environment for remineralization (143).

There have been a number of demineralization and remineralization studies on the anti-caries effectiveness of products with arginine technology, mainly produced to prevent dentin sensitivity. Positively charged arginine binds to negatively charged dentin surface. It also carries the calcium and phosphate ions in saliva, forming a plug in dentinal tubules. The reduction of fluid passage in the dentinal tubules prevents dentin sensitivity (144,145).

Research reports that the combination of 8% arginine, calcium carbonate, and 1450 ppm fluoride (sodium monofluorophosphate) in toothpastes is effective for providing remineralization in incipient caries lesions and preventing the formation of new caries (143).

Cheng et al. (2015) evaluated the remineralization effect of arginine and fluoride in artificial incipient caries lesions *in vitro* and concluded that combined use of arginine and fluoride increased fluoride uptake in enamel, providing statistically significantly more remineralization than arginine alone (144).

2.5.6. Nano-hydroxyapatite (N-HAP)

Hydroxyapatite is the main component of dental hard tissues like enamel and dentin. It makes up 95% of enamel and 75% of dentin in weight. Hydroxyapatite is responsible for the mechanical properties of dental hard tissues (6).

Emerging with the advances in nano technology, nano-hydroxyapatite has been often used in biomedicine and dentistry in recent years. The chemical structure of N-HAP is very similar to that of tooth enamel. It is frequently preferred in dentistry in recent years for being a biomimetic, bioactive, and biocompatible material (6).

Examining the structure of artificially produced nano-hydroxyapatite crystals has revealed that they had chemical and structural similarities with enamel crystals. Also, *in vitro* studies have shown N-HAP to have less solubility compared to other calcium phosphate compounds. Hence, nano-hydroxyapatite was developed to increase the solubility of hydroxyapatite and the rates of Ca and PO_4 ions, focusing remineralization studies in this area (7).

Nanohydroxyapatite is used in dentistry for eliminating dentin sensitivity, preventing erosion, and preventing and arresting tooth decay (in remineralization studies) (7,8).

Vano et al. (2018) conducted a randomized controlled double-blind clinical study on the effectiveness of toothpastes containing fluoride and N-HAP in reducing dentin sensitivity. They concluded that toothpaste containing N-HAP was effective in preventing sensitivity by occluding dentinal tubules, statistically significantly more so than toothpaste containing only fluoride in 2-4 weeks (8).

With increased research on the potential remineralization effect of nano-hydroxyapatite on dental caries, it has begun to be added to toothpastes and mouthwashes (146).

The pH of N-HAP in oral care products like toothpaste and mouthwash ranges from 5 to 9, but a pH of 6.8-7.5 is preferred to ensure similarity with the physiological conditions in the mouth. The pH changes that occur when N-HAP is present in the environment affect the physicochemical properties of N-HAP particles, changing their efficiency in providing remineralization (147).

There are different theories on the mechanism of action of nano-hydroxyapatite in the remineralization of enamel. Some researchers have argued that this remineralization ability takes place by nanoparticles precipitating in demineralized enamel gaps (148).

Another theory is that N-HAP acts as a calcium reservoir in the mouth, increasing calcium levels saliva and facilitating remineralization by preventing pH changes (149,150).

Another study reports that under neutral conditions, hydroxyapatite could not fully provide remineralization, but it dissolves more and increases ion release and penetration depth in acidic environments, accelerating remineralization (151).

Huang et al. (2010) evaluated the remineralization potential of Galla chinensis and N-HAP alone and in combination in incipient caries lesions *in vitro*, finding that combined use of Galla chinensis and N-HAP showed a synergistic effect on incipient caries lesions. They also stated that using N-HAP alone was insufficient to remineralize

all incipient caries lesions, limiting ion diffusion to the deep lesion area. However, when N-HAP was used in combination with Galla chinensis or fluoride, this could be overcome, achieving better success in preventing demineralization (146).

Some *in vitro* studies on the remineralization capacity of toothpastes containing nano-hydroxyapatite and fluoride have shown N-HAP to have higher remineralization capacity (149,151,152). Other studies have concluded that toothpastes containing N-HAP and fluoride had similar effects in arresting the progression of incipient caries lesions (153). Research has shown that combined use of N-HAP and fluoride in toothpastes had a synergistic effect, resulting in higher remineralization values (6,154–156).

Huang et al. (2009) evaluated the remineralization effect of agents containing different concentrations (1%, 5%, 10%, 15%) of nanohydroxyapatite on enamel with artificial incipient caries and reported that the concentration of 10% N-HAP was the most effective in terms of remineralization (157). Other studies have supported this finding, arguing that 10% N-HAP was the most appropriate concentration for the remineralization of incipient caries lesions (158–160).

Huang et al. (2011) also investigated the remineralization effect of nano and microstructured hydroxyapatite crystals *in vitro* and showed that nano-structured hydroxyapatite crystals had better remineralization effect than microstructure crystals. They reported that nano-structured hydroxyapatite crystals also provided remineralization on the outer surface of the lesion, having limited effect in deeper lesion areas. They highlighted that the remineralization area created by N-HAP on the outer surface of the caries lesion prevented ion transfer to the deep lesion area, failing to provide complete remineralization (161).

Mielczarek et al. (2014) applied fluoride toothpaste alone and in combination with N-HAP to teeth with artificial incipient caries and compared their effects on remineralization. They observed that, while remineralization was achieved in both groups, the surface microhardness values did not return to their baseline values (156).

Hegazy et al. (2016) assessed the effect of N-HAP, fluoride, and chlorhexidine mouthwashes in preventing MDP accumulation and gingivitis, along with their remineralization effect in incipient caries lesions on 81 children aged 7-12 years *in vivo*. Mouthwashes were used twice a day and measurements were made using Diagnodent. They reported that all three mouthwashes reduced the amount of MDP and gingivitis compared to baseline values, but those containing N-HAP and fluoride provided remineralization in incipient caries lesions (162).

Amechi et al. (2019) evaluated the remineralization potential of toothpastes containing hydroxyapatite and 500 ppm fluoride in incipient caries *in situ* and reported that toothpaste containing 10% hydroxyapatite had similar remineralization effect with that containing 500 ppm fluoride, with no statistically significant difference between the two (163).

2.5.7. Bioactive Glass

Calcium sodium phosphosilicate, in other words, bioactive glass, was first developed by Hench et al. as a bone regeneration material in 1969. The development and success associated with bioactive glass over the years is attributed to its high biocompatibility (9).

Bioactive glasses are known as biomaterials. Biomaterials are divided into four groups. These are composites, metals, polymers, and ceramics. The most distinctive propoerty of bioactive glasses, which are ceramics, is that they form the 'hydroxycarbonate apatite (HCA) layer, providing bonding with tissues (164).

The formula of the standard bioactive glass approved by the Food and Drug Administration (FDA) is known as 45S5 (Bioglass®). Bioactive glasses contain silicon, sodium, calcium, and phosphorus oxide. By weight, bioactive glass contains 45% SiO2, 24.5% Na2O, 24.5% CaO, and 6% P2O5. When the bioactive glass is modified, its SiO2 content changes, but P2O5 remains constant. During production, having a silica rate below 60% and a high CaO/P2O5 rate ensures that the bioactive glass has a highly reactive surface (9).

As bioactive glass contacts body fluids like saliva, surface reactions that increase the pH begin to occur. When sodium ions in calcium sodium phosphosilicate particles begin to replace hydrogen ions in body fluids, the chain of reactions begins. The ion exchange that occurs leads to a rapid release of calcium and phosphate ions from the particle structure. With the loss of sodium ions, a gel layer rich in silica begins to form on the material surface. This layer facilitates the precipitation and accumulation of calcium and phosphate coming from materials and body fluids. Over time, the calcium phosphate layer begins to thicken, and the HCA layer forms. This layer is chemically similar to the hydroxyapatite in tooth structure. When all ions in bioactive glass enter an ionic reaction and HCA layer and bone tissue are combined, the surface reactions end (10–12).

Due to their osteoconductive properties, they form the HCA layer by releasing calcium, sodium, and phosphate ions to the environment, providing chemical bonding with the bone tissue. With these properties, bioactive glasses can be used in the remineralization of dental hard tissues (9).

Key properties of bioactive glasses include their enzymatic activity, supporting the formation of a 3D vascular structure, helping differentiate mesenchymal cells in hard tissue, and forming organic bonds with hard tissue. Researchers have aimed to reduce the size of glass particles to nano levels, increasing their reactivity. A small particle size can allow the material to pass into smaller areas like dental hard tissues (165).

Bioactive glasses are used in dentistry as bone graft materials, for providing bone regeneration, in pulpotomy and pulp capping treatments, for removing dentin sensitivity, and providing remineralization in hard dental tissues (9).

Bioactive glasses that are used in dentistry are available in the market as S53P4 (Bonalive®), 45S5 (Bioglass®), Novamin®, Biogran®, Perioglass®, BioMin™ (10).

A new generation bioactive glass material, Biomin toothpaste is distinguished from other bioactive glasses with its high phosphate and less silica content. It was first produced as a bone graft material. It has two forms as Biomin C and Biomin F. Biomin C contains no fluoride, and its active ingredient is calcium chloro-phosphosilicate. Biomin F does contain fluoride, and its active ingredient is fluoro calcium phosphosilicate (166,167).

As another bioactive glass, Novamine was started to be used in protecting oral and dental health in 1996. It was patented and started to be used by the manufacturer

company in 2009. By the binding of its calcium, sodium, phosphate, and silica content to the tooth surface, hydroxycarbonate apatite is formed, which is similar to the structure of enamel and dentin. It occludes dentinal tubules and is often used to eliminate dentin sensitivity. It joins the structure of toothpastes, increasing the resistance of tooth enamel against acid attacks, and provides remineralization in dental hard tissues (168,169).

Numerous *in vitro* studies have reported that bioactive glass can provide remineralization on demineralized enamel surfaces and increase the surface microhardness value above the baseline value (5,165,170–176).

Some *in vitro* studies that evaluated bioactive glass in combination with fluoride have concluded that bioactive glass had a higher remineralization potential on demineralized surfaces than when used alone, creating a synergistic effect during combined use (169,176–178).

Burwell et al. (2009) examined and compared the remineralization potential of calcium sodium phosphosilicate (Novamin) toothpaste with fluoide toothpaste on bovine teeth with artificial incipient caries, *in vitro*. They reported that toothpastes containing bioactive glass had a remineralizing effect on enamel surface. They emphasized that it formed an HCA layer on the tooth surface and continued to release ions for up to 2 weeks, preventing demineralization against acid attacks and providing more effective remineralization when used in combination with fluoride (177).

Narayana et al. (2014) determined the remineralization effect of dental health products containing bioactive glass (Novamine), CPP-ACP, CPP-ACPF, and fluoride (1000 ppm AmF) in artificial incipient lesions in the enamel of large molar teeth and investigated mineral gain or loss with SEM-EDS using the pH cycle method for 10 days

in vitro. They reported that toothpaste containing bioactive glass provided statistically significant remineralization and could be used for treating incipient caries lesions by a minimally invasive approach (5).

Benjasuwantep et al. (2017) assessed the remineralization effect of bioactive glass, CPP-ACP, and fluoride toothpastes on artificial caries lesions in primary teeth by microtomography *in vitro*, and stated that toothpastes containing bioactive glass, 500 ppm fluoride, and CPP-ACP all had similar remineralization potential, with no statistically significant difference. Although, they reported that toothpaste containing 1000 ppm fluoride had a statistically significantly higher remineralization potential than all other products (179).

Güçlü et al. (2017) evaluated the remineralization effect of bioactive glass, hydroxyapatite (HAP) particles, and CPP-ACP complex in white spot lesions on the surface of permanent teeth with SEM-EDX *in vitro*. They found that under neutral pH conditions, bioactive glass and CPP-ACP could be effective on subsurface lesions by releasing calcium and phosphate ions, but HAP did not provide remineralization. They stated that with the dissolution of HAP in acidic environment, ion release occurred and remineralization on the lesion body was achieved. They also reported that bioactive glass and CPP-ACP covered the enamel surface in acidic environment, albeit with low remineralization potential in the lesion body (180).

Wang et al. (2011) evaluated the effectiveness of toothpastes containing Novamine (bioactive glass) in preventing dentin sensitivity using SEM-EDX *in vitro* and concluded that toothpastes containing bioactive glass significantly decreased dentin sensitivity (172).

Kulal et al. (2016) evaluated the effects of toothpastes containing N-HAP, Novamine (bioactive glass), and arginine on eliminating dentin sensitivity and occluding dentinal tubules using SEM *in vitro*. The authors concluded that all three toothpastes were effective in occluding dentinal tubules and that N-HAP toothpaste had a higher effect in eliminating dentin sensitivity compared to the others (181).

Cruz et al. (2018) evaluated the effect of toothpastes containing bioactive glass in eliminating dentin sensitivity *in vitro*, and reported that they were effective in eliminating dentin sensitivity and could be recommended for patients with sensitivity problems (182).

Pereira et al. (2018) assessed and compared the effect of toothpastes containing Biomin, Novamine, and fluoride in occluding dentinal tubules *in vitro* and demonstrated that toothpaste containing biomine could occlude dentinal tubules more than those with Novamine and fluoride. They also found that toothpastes containing Biomin and Novamine had a higher resistance against acid attacks than fluoride toothpaste, and Biomin was more resistant than Novamine (183).

Manchery et al. (2019) evaluated the remineralization potential of toothpastes containing bioactive glass (novamine), N-HAP (10%), and fluoride (1450 ppm AmF) on artificial incipient caries lesions in premolars using a polarized light microscope *in vitro*. They reported that after a 7-day pH cycle, all three toothpastes provided remineralization on incipient caries lesions, and N-HAP toothpaste had a statistically significantly higher remineralization effect than bioactive glass and fluoride toothpastes. Even though fluoride toothpaste provided more remineralization than bioactive glass toothpaste, the difference was not statistically significant (184).

2.6. Toothpastes

The most effective method for preventing dental caries and periodontal disease and for protecting oral and dental health is to remove microbial dental plaque from teeth and surrounding tissues by mechanical cleaning. In this method, toothbrushes and toothpastes have a crucial place. Toothpastes are available in many forms like powder, gel, or paste in the market (185).

2.6.1. History of Toothpastes

Ancient Egyptians had medical books like the Ebers Papyrus, compiled in the 1500s BC, containing data dating back to the 4000s BC. This book contains a recipe for how to clean teeth (186). Ancient Egyptians developed a tooth cream from ox hooves, myrrh, eggshells, and pumice to clean their teeth. They would add water to this cream before each use. Romans were the first to add aromas to this cream for better taste and more pleasant breath. Toothpastes were also used in China and India during the same time. The toothpaste used in China contained agents like ginseng, mint, and salt. The content of these toothpastes has been reported to be similar to toothpastes today (103).

The most common problems with toothpastes in ancient times were their high abrasiveness, bad taste, and high costs. With the industrialization in the 18th century, dental powders began to be developed by the cooperation of doctors, dentists, and chemists toward the end of the 18th century; sodium bicarbonate and sodium borate were added. In the early 19th century, glycerin was added to toothpaste to prevent it from drying out. Later, strontium was introduced to strengthen the teeth and to reduce sensitivity (103).

Peabody was the first to add soap to dental powders in 1824. Then, in the 1850s, John Harris added chalk to tooth powder. Toothpastes were first put in glass jars by Colgate&Co in 1873, and Dr. Washington Sheffield from Connecticut was the first to put toothpaste in collapsible tubes in 1892 (103).

One of the most important breakthroughs in the history of toothpaste was the introduction of fluoride compounds in 1914. British patent GB declared the formulations of toothpastes containing sodium fluoride in a file named 'advances in toothpastes.' Despite all this information, it remains unclear when fluoride toothpastes were first sold. In 1955, Procter & Gamble launched Crest in the USA, the first mass-market fluoride toothpaste (0.4% stannous fluoride) (103).

Dr. Joseph Muhler, after 10 years of research, developed a toothpaste containing 1000 ppm fluoride (tin fluoride) and calcium phosphate at the University of India. In 1954, after a clinical study on this toothpaste, it was reported that it significantly reduced dental caries in children (187).

The American Dental Association (ADA) first opposed the addition of fluoride in toothpastes due to its fluoride toxicity concerns,, but later approved it in 1960. In the 1980s, antiplaque agents were introduced to manage supragingival microbial dental plaque and tartar formation. Today, the ingredients of toothpastes continue to improve, and new agents are being added (103).

2.6.2. Structure of Toothpastes

Today, there are many types of toothpastes on the market. Most of these toothpastes contain similar agents. Toothpastes most often contain abrasives, water, moisturizers,

detergents, binding agents, sweeteners, therapeutic agents, colorants, and preservatives (Table 1) (26).

Table 1. Agents contained in toothpastes and percentages (26)

Agents contained in toothpastes	**%**
Abrasives	20-40
Water	20-40
Moisturizers	20-40
Detergents	1-2
Binding Agents	2
Sweeteners	2
Therapeutic Agents	5
Colorants and Preservatives	1

2.6.2.1. Abrasives

The key function of toothpastes is mechanically removing microbial dental plaque from tooth surface and eliminating external stain. With the abrasives in toothpastes, stains

on tooth surface can be physically eliminated. Abrasives are a requisite for eliminating external discoloration. An ideal toothpaste should have low abrasive property and an occlusive effect on dentinal tubules (188).

Abrasives that are commonly used in toothpastes are calcium carbonate, dicalcium phosphate dihydrate, alumina, silica, calcium pyrophosphate, and sodium bicarbonate (189).

$CaCO_3$ based abrasives are often found in toothpastes together with NaMFP. This formulation is commonly preferred in developing countries for its low cost. When $CaCO_3$-based abrasives are used together with NaF, the calcium ions in $CaCO_3$ react with free fluoride and render the toothpaste inactive, so inert abrasives like silica are preferred. Silica-based abrasives have higher costs than $CaCO_3$ based abrasives, but they are often preferred by manufacturers because they provide benefits like whitening teeth and protecting gingival health by additional ingredients (15).

The degree of abrasiveness of a toothpase depends on the hardness, particle size, and particle shape of the abrasives. Also, the pressure exerted by the toothbrush, the brushing technique, and the hardness of the bristles also affect the abrasion on the teeth (189).

Dyer et al. (2000) demonstrated that pellicle formed on tooth surface had a protective effect against abrasion caused by toothpaste *in vitro*. They reported that the amount of roughening in hard tissues by abrasives should not exceed the adhesion index (0.2 μm) of the bacterial plaque (190).

The abrasive properties of toothpastes are evaluated by a scale called 'Relative Dentin Abrasivity (RDA) Index' by the American Dental Association (ADA) (Table 2) (191).

Table 2. RDA values (191)

0-70	Low abrasive
70-100	Medium abrasive
100-150	Highly abrasive
150-250	Regarded as a harmful limit

2.6.2.2. Moisturizers

Until 1930, the water in toothpastes would evaporate over time and the solid content would remain on the outside, shortening the shelf life of the toothpaste. Later, moisturizers were added to toothpastes to solve this problem. Moisturizers prevent moisture loss in toothpastes and eliminate the risk of hardening. They also have sweetening effects. Besides, they strengthen the structure of the toothpaste, leaving a better taste in the mouth (192).

The most commonly preferred moisturizers are glycerin, sorbitol, propylene glycol, and mannitol. Of these, the most common are sorbitol and glycerin (192).

2.6.2.3. Soaps and Detergents

When toothpastes were first produced, soaps were used as cleaning agents. The aim was to remove debris and microbial dental plaque by the foaming effect of soaps. The disadvantages of soaps included nausea by mucous membrane irritation and bad taste (26).

When detergents were added to toothpastes, soaps were removed from their content. Today, the most widely used detergent in toothpastes is sodium lauryl sulphate (SLS) at a concentration of 1-2%. SLS functions as a foaming agent and provides antimicrobial activity (193).

SLS achieves this antiplaque effect in 3 ways:

- Killing microorganisms
- Lowering surface energy
- Denaturing protein

SLS can penetrate deep into tissues. Its commercially allowable concentration in toothpastes is 1-3% (193).

2.6.2.4. Binding and Thickening Agents

Binding and thickening agents stabilize the toothpaste, preventing the separation of solid and liquid phases. They also control the consistency of the toothpaste. Choosing the right binding agent with the right concentration ensures that the toothpaste can be easily squeezed out of the tube and look smooth on the toothbrush. Toothpastes contain these agents at a rate of 0.5-2.5% (194).

The most widely used binding agents are sodium carboxymethyl cellulose, carregeenates, sodium magnesium silicate, magnesium aluminum silicate, alginate, and colloidal silica (194).

2.6.2.5. Sweeteners

The taste of toothpastes is very important in their selection. Toothpastes that leave a pleasant taste and a fresh feeling in the mouth after use are preferred more by individuals. Soluble saccharin is often added to toothpastes. Anise, eucalyptus, mint, and cinnamon are also used. Besides, oils like thymol and menthol are added to toothpastes to provide antibacterial activity (195).

The first sweeteners added to toothpastes were sugar and honey. Due to their cariogenic effect, these agents were replaced by non-cariogenic sweeteners like saccharin, sorbitol, mannitol, and cyclamate. Glycerine, which is used to moisturize toothpastes, also has a sweetening effect. Xylitol, which cannot be metabolized by bacteria and does not cause acid formation, is a common sweetener (196).

2.6.2.6. Therapeutic Agents

2.6.2.6.1. Toothpastes That Prevent Caries

The most common therapeutic agents added to toothpastes are fluoride compounds. Today, fluoride is considered the gold standard for preventing dental caries. The presence of fluoride in numerous toothpastes on the market since the 1980s has made its use in toothpastes widespread. Fluoride is present in toothpastes as sodium fluoride, sodium monofluorophosphate, amine fluoride, or stannous fluoride or combinations thereof (116).

The efficacy of fluoride toothpastes varies based on the concentration of fluoride, frequency of use, amount of toothpaste used, and habit of washing mouth after brushing (116).

It is mandatory to indicate the percentage and concentration of fluoride content on the packaging of toothpastes (197). Beside fluoride, agents like xylitol, calcium, phosphate, and sodium bicarbonate are also used in toothpastes that prevent caries (196).

2.6.2.6.2. Toothpastes That Prevent Plaque Formation and Show Antibacterial Effect

While the main purpose of toothpastes is to remove microbial dental plaque from tooth surface by brushing, they also need to have a potential to slow the regeneration of microbial dental plaque. They should have a broad spectrum of actions against bacteria and yeasts. They should be chemically stable in the mouth. They should have no side effects like discoloration or inflammation, and should not cause taste disturbances (194).

Plaque-inhibiting agents can be divided into certain groups, like preventing bacterial involvement and proliferation, removing plaque, and changing plaque ecology. The effectiveness of plaque-inhibiting agents is evaluated based on their ability to stay in the mouth and having slow release. This property is often found in uncharged phenolic agents like fluoride, triclosan, and cationic molecules and metal salts like zinc. Zinc reduces bacterial proliferation in the microbial dental plaque. It is also used in combination with antimicrobial agents like chlorhexidine, citrate, hexetidine, and sanguinary for increased synergistic effect (194).

Triclosan has a broad spectrum of antibacterial activity. It was first added to toothpastes in the early 1990s to prevent gingivitis and plaque formation. It is often combined with a slow-release copolymer or an antimicrobial agent like zinc (194).

2.6.2.6.3. Toothpastes That Prevent Calculus Formation

Toothpastes that prevent the formation of calculus can prevent the mineralization of microbial dental plaque. They are effective against supragingival calculus formation. They do not remove calculus from the environment, but prevent the formation of new calculus. They contain mineralization inhibitors like zinc salts, diphosphonates, and pyrophosphates. Toothpastes containing 3-3.3% pyrophosphate prevent the formation of calculus (194).

2.6.2.6.4. Toothpastes That Prevent Dentin Sensitivity

Dentin sensitivity is a common disturbance in the society. Dentin sensitivity is often accompanied by gingival recession. When the dentinal tubules are exposed, the protoplasmic extensions of odontoblasts are also exposed into the mouth. Changes in the fluid within dentinal tubules stimulate odontoblasts and nerves become depolorized, causing pain. The first method to prevent dentin sensitivity is to occlude or cause sclerosis on the exposed dentinal tubules (198).

The most common desensitizing agents in toothpastes that prevent dentin sensitivity are potassium nitrate and stannous fluoride. The desensitizing effect of potassium nitrate takes place by the depolarization of nerve cells, while stannous fluoride prevents dentin sensitivity by occluding dentinal tubules (194).

Strontium ions occlude dentinal tubules by precipitating and prevent sensitivity. Hence, they were added to toothpaste in the form of strontium chloride for a time.

Fluoride, stannous fluoride, potassium, aluminum, and ferric oxalates are agents used in toothpastes for desensitization (194). .

2.6.2.6.5. Whitening Toothpastes

Whitening toothpastes remove discoloration on tooth surface by mechanical or chemical methods. Mechanical whitening is achieved with abrasives, while chemical whitening is done with surface-active agents or bleaching agents (199).

Whitening toothpastes are highly abrasive and contain bleaching agents like peroxides, sodium bicarbonate, or proteolytic enzymes that remove discoloration. Agents like pyrophosphate, hexametaphosphate, or citrate can be added to these toothpastes to increase their whitening effect. Their effect on removing discoloration on teeth changes based on the amount of abrasive they contain. The abrasive properties of whitening toothpastes are also affected by the shape, size, and hardness of their particles, and their pH (194).

3. MATERIALS and METHODS

3.1. Toothpaste Used in the Study

The active ingredients, manufacturers, and content of the toothpastes used in this study are presented in Table 3.

Table 3. General properties of the toothpastes used in the study

Brand	Manufacturer	Active Ingredient	Content
Dentiste' Premium & Natural White	Siam Cosmeceutical Co., Ltd. Lamlukka, Prathumthan, Thailand.	Nano-hydroxyapatite	Sorbitol, hydrated silica, aqua, xylitol, glycerin, sodium lauryl sulfate, PVP, nano-hydroxyapatite (N-HAP), green tea extract, sodium tricalcium phosphate, cellulose gum, methylparaben sodium, sodium saccharin, menthol, titanium dioxide, ascorbic acid, mica, peppermint oil, eucalyptus oil, clove oil, sage extract, chamomile extract, fennel extract, licorice extract, cinnamon bark extract, CI 42090
Biomin™ C	BiominTechnologies Limited, Fountain Street, Fenton, England	Chloro calcium phosphorus silicate	Glycerin, silica, PEG, sodium lauryl sulfate, **chloro calcium phosphorus silicate** titanium dioxide, sweetener, carbopol, and potassium acesulfame.

Signal Kids	Unilever, London, United Kingdom	Sodium Fluoride	Aqua, hydrogenated starch hydrolysate, hydrated silica, PEG-32, sodium lauryl sulfate, cellulose gum, sodium saccharin, sodium fluoride (1000 ppm F), benzyl alcohol, limonene, CI 73360, CI 77891
Colgate®Triple Action	Colgate Palmolive, New York, USA	Sodium monofluorophosphate	Calcium carbonate, aqua, sorbitol, sodium lauryl sulfate, hydrated silica, sodium monofluorophosphate (1450 ppm F), aroma, cellulose gum, magnesium aluminum silicate, sodium carbonate, benzyl alcohol, sodium saccharin, sodium bicarbonate, eugenol, CI74160, CI74260

3.1.1. Dentiste Premium & Natural White Toothpaste

The product contains sorbitol, hydrated silica, aqua, xylitol, glycerin, sodium lauryl sulfate, PVP, nano-hydroxyapatite, green tea extract, sodium tricalcium phosphate, cellulose gum, metal paraben sodium, sodium saccharin, menthol, titanium dioxide, ascorbic acid, mica, peppermint oil, eucalyptus oil, clove oil, sage extract, chamomile extract, fennel extract, licorice extract, cinnamon bark extract, CI 42090.

Figure 27. Dentiste' Premium & Natural White Toothpaste (N-HAP)

3.1.2. Biomin™ C Toothpaste

The product contains glycerin silica, PEG, sodium lauryl sulfate, chloro calcium phosphorus silicate, titanium dioxide, sweetener, carbopol, and potassium acesulfame.

Figure 28. Biomin™ C Toothpaste (BioC)

3.1.3. Colgate® Triple Action Toothpaste

The product contains calcium carbonate, aqua, sorbitol, sodium lauryl sulfate, hydrated silica, sodium monofluorophosphate (1450 ppm), flavor, cellulose gum, magnesium aluminum silicate, sodium carbonate, benzyl alcohol, sodium saccharine, sodium bicarbonate, augenol, CI74160, CI74260.

Figure 29. Colgate®Triple Action Toothpaste (1450 F)

3.1.4. Signal Kids Toothpaste

The product contains aqua, hydrogenated starch hydrolysate, hydrated silica, PEG-32, sodium lauryl sulfate, cellulose gum, sodium saccharine, sodium fluoride (1000 ppm F), benzyl alcohol, limonene, CI 73360, CI 77891.

Figure 30. Signal Kids Toothpaste (1000 F)

Figure 31. All toothpastes used in the research

3.2. Preparation and Storage of Teeth

65 bovine central incisors were used in this *in vitro* study. Bovine teeth were examined under an optical microscope (MM-6000B Inverted Metal Microscope, Yamer, Izmir). The teeth with no cracks, fractures, hypoplasia, or caries were included, and others were excluded (Figure 32).

Figure 32. Optical microscope used in the research

The teeth were washed with distilled water after polishing with a fluoride-free paste (Figure 33). They were kept in a saline solution containing 0.1% thymol in a refrigerator at +4°C until the experiments.

Figure 33. Brushing teeth with a polishing brush and fluoride-free paste

3.3.Preparation and Polishing of Samples

The crowns of the teeth were reduced to the cemento-enamel junction and cut longitudinally using a micromotor and a diamond separator attached to a dental handpiece (Acurata GmbH & Co. KG, Thurmansbag) under water cooling. Two samples were obtained from each tooth. Enamel samples were embedded in acrylic blocks (Imicryl Acrylic Repair Material, Konya, TR) prepared in standard metal molds with their labial surfaces exposed. All enamel surfaces were polished using 600, 800, 1200 grit silicon carbide abrasives (Atlas Brand English Abrasives, UK) in a Phoenix Beta Grinder-Polisher device (Buehler, Lake Bluff, IL, USA) to obtain a smooth surface (Figure 34). Standardised enamel surfaces were obtained by fixing an 6x6 mm adhesive tape to the labial surface of the teeth (Figure 35). All remaining surfaces were covered with 2 layers

of acid-resistant nail polish (Figure 36). The polished samples were washed with distilled water and dried. Samples were kept in a humid environment to prevent from losing water and drying.

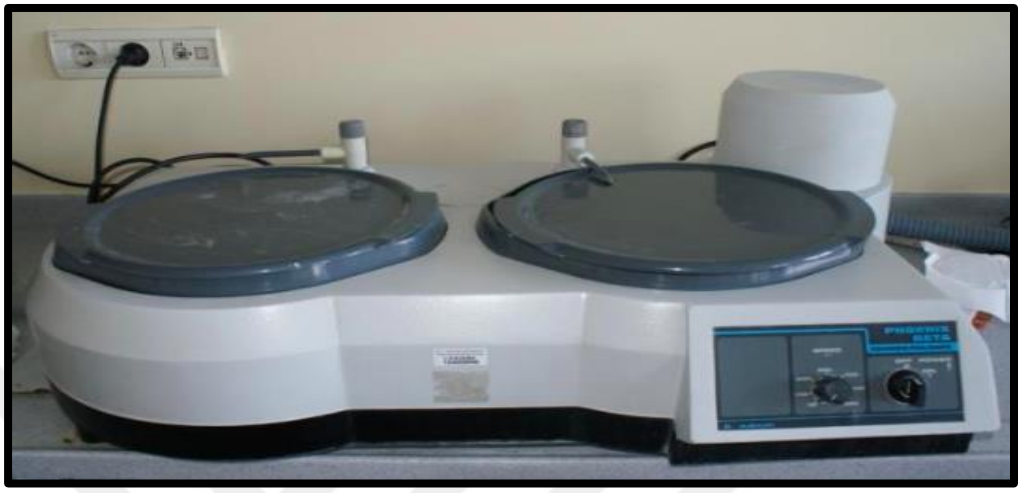


Figure 34. Buehler Phoenix Beta Grinder Polishing Device

Figure 35. Attaching 6x6 mm adhesive tapes to polished enamel surfaces

Figure 36. Polishing samples with nail polish

3.4.Baseline Surface Microhardness Measurement

Surface microhardness (SMH) measurements were carried out in the Hard Tissue Laboratory of the Faculty of Dentistry at Yeditepe University. 6x6 mm adhesive tapes were removed from the buccal surfaces of 65 randomly selected samples, and the baseline surface microhardness measurements of all samples were made using a Buehler® Micromet 5114 microhardness analyzer (Lake Bluff, Illiniois, USA) with a Vickers tip (Figure 37).

Prepared enamel samples were placed on the SMH measuring device in a way to keep their upper and lower surfaces parallel. By calculating the notches formed by the Vikers diamond tip on the enamel surface by the measuring system of the device, Vickers Hardness Numbers (VHN) were obtained. Measurements were carried out by applying a

force of 100 grams to tooth surface for 10 seconds. Surface microhardness values were obtained by measuring from five different points on each sample and recorded. The average of the surface microhardness values obtained from five different points were taken and recorded.

Figure 37. Surface Microhardness Measurement Device

3.5. Groups

65 samples with baseline surface microhardness values were divided into 5 groups with 13 samples in each group. The distribution of the experimental groups and their coding according to toothpaste and method are given in Table 4.

Table 4 Coding of the experimental and control groups and toothpastes used in treatment

Experimental Groups and Control Group (n=13)	**Toothpaste Used in Treatment**
N-HAP	Dentiste' Premium & Natural White
BioC	Biomin™ C
1450 F	Colgate® Triple Action (1450 ppm fluorid)
1000 F	Signal Kids (1000 ppm fluorid)
Negative control [NK(+)]	Brushing without toothpaste
Negative control [NK(-)]	No brushing

Figure 38. Dentiste Premium & Natural White group (N-HAP)

Figure 39. Biomin™ C group (BioC)

Figure 40. Colgate® Triple Action group (1450 F)

Figure 41. Signal Kids group (1000 F)

3.6. Preparation of Demineralization Solution

The demineralization solution was prepared in the Pharmaceutical Technology Laboratory of the Faculty of Pharmacy at Yeditepe University. The solution was prepared as 50mM acetic acid, 2,2 mM $Ca(NO_3)_2$ and 2.2 mM KH_2PO_4 according to the recommendations of Ten Cate et al. (200). Each sample (n=13) was kept in an 8 ml demineralization solution at 37°C for 72 hours according to the method of *Huang et al.* (2009) and artificial incipient caries lesions were formed on enamel surfaces (157).

Figure 42. 37ºC oven where samples were stored

3.7. Post-Demineralization (PD) Surface Microhardness Measurement

Enamel samples with artificial incipient caries lesions were removed from the demineralization solution and rinsed with distilled water and dried. Then, the post-demineralization surface microhardness values of 65 samples were measured using a Buehler® Micromet 5114 microhardness analyzer (Lake Bluff, Illiniois, USA) with a Vickers tip by applying 100 grams of force for 10 seconds. Surface microhardness values were obtained by measuring from five different points on each sample and recorded. The average of the surface microhardness values obtained from five different points were taken and recorded.

3.8. Preparation of Remineralization Solution

A solution with the same content of that used by *Ten Cate et al.* (1983) was prepared in the Faculty of Pharmacy at Yeditepe University. This remineralization solution contained 20 mmol l^{-1} HEPES, 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl, and 1 mM NaN_3 (200)**.**

3.9. Treatment

Enamel samples were subjected to a pH cycle, including exposure to demineralization-remineralization solutions for 14 days according to Featherstone et al. (1990) (200). All demineralized enamel samples were kept at 37°C in a demineralization solution with a pH of 4.5 for 6 hours and then in a remineralization solution with a pH of 7 for 18 hours. Throughout this cycle, enamel samples were brushed by the same researcher twice a day for 2 minutes with toothpastes in the study groups (Table 5), rinsed with distilled water, and immersed back into the solution (202).

Table 5. Treatment cycle scheme

6 HOURS OF DEMINERALIZATION
2 MINUTES OF BRUSHING
18 HOURS OF REMINERALIZATION
2 MINUTES OF BRUSHING

Lines were drawn on the middle of the buccal surfaces of negative control samples using a fine-tipped acetate pen before each brushing; one half was brushed with distilled water [NK(+)], and the other half was left in distilled water [NK(-)] (Figure 43).

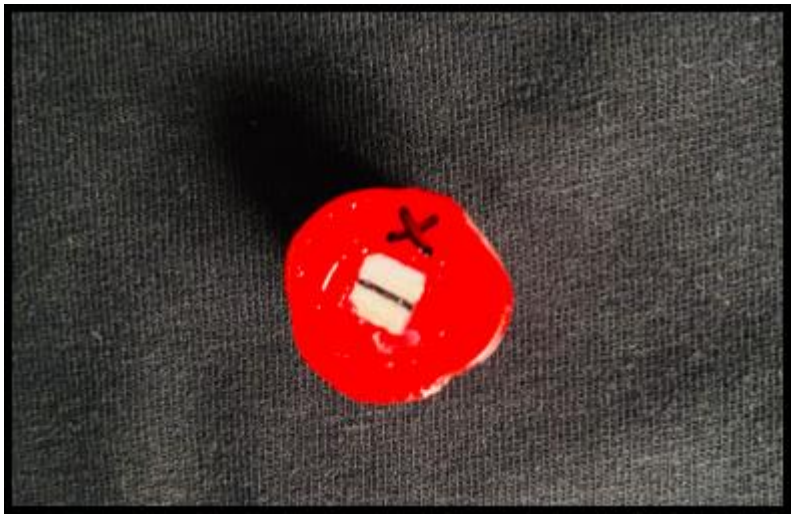


Figure 43. Brushing areas for negative control samples

The EN ISO 11609 (European standards for preparing artificial saliva/toothpaste mixture) were used for preparation as stated by *Tschopp et al.* (2011) (152,203). According to these standards, the toothpastes were diluted with distilled water at a rate of 1:3. Then, the mixture was mixed with a magnetic stirrer for 3 minutes to obtain a more homogeneous structure according to *Mehta et al.* (2014) (Figure 44) (165). Toothpaste mixtures were prepared daily during the study period. After each enamel sample was brushed, teeth were washed with distilled water and placed in solutions. Enamel samples were brushed using an Oral B electric toothbrush **(**Oral-B Genius 9000, Procter & Gamble, Germany) and a precision brush head (Oral B precision brush head, Procter & Gamble, Germany) (Figure 45 and Figure 46). The electric toothbrush was charged every 100 minutes. The bristles of the toothbrush were kept parallel to the enamel surfaces and 150 Newtons (N) were applied to enamel samples. Each enamel sample was brushed by the same researcher using separate brush heads.

Figure 44. Magnetic stirrer

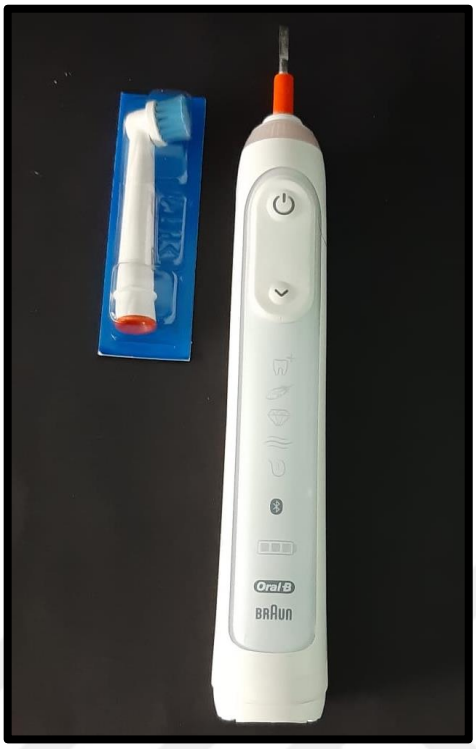


Figure 45. Electric toothbrush and sensitive brush head

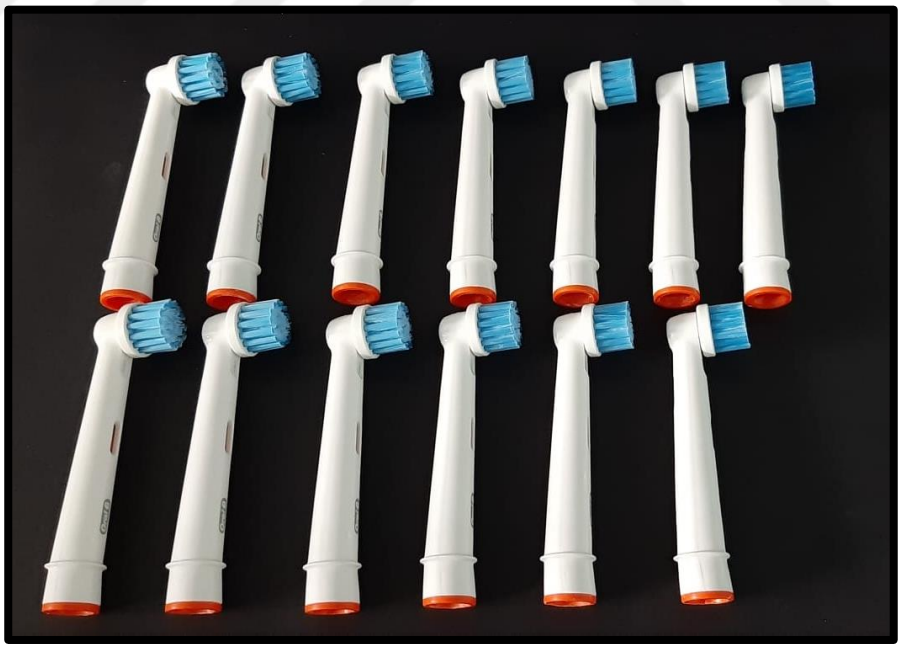


Figure 46. Sensitive brush heads for each sample

3.10. Post-Treatment (PT) Surface Microhardness Measurement

Post-treatment surface microhardness measurements were made using a Buehler® Micromet 5114 surface microhardness analyzer (Lake Bluff, Illiniois, USA) with a Vickers tip by applying 100 grams of force for 10 seconds. Surface microhardness values were obtained by measuring from five different points on each sample and recorded. The average of the surface microhardness values obtained from five different points were taken and recorded. All baseline, post-demineralization, and post-remineralization surface microhardness measurements were carried out by the same researcher.

3.11. Examining Scanning Electron Microscope (SEM) Images

The surface properties of the samples were examined with a Scanning Electron Microscope (Carl ZEISS, EVO 40, Germany) in the Nanobiotechnology Laboratory of the Genetics and Bioengineering Department at Yeditepe University.

21 samples from each group were examined on SEM as 3 samples each for baseline and post-demineralization imaging and 5 for post-treatment imaging. The surfaces of the samples embedded in acrylic resin were plated with gold in a gold coating device (Baltec, SCD 005 Sputter Coater) (Figure 47) and images were examined on SEM (Carl ZEISS, EVO 40, Germany) (Figure 48) at x500, x1000, x1500, and x35000 magnifications.

Figure 47. Gold Coating Device

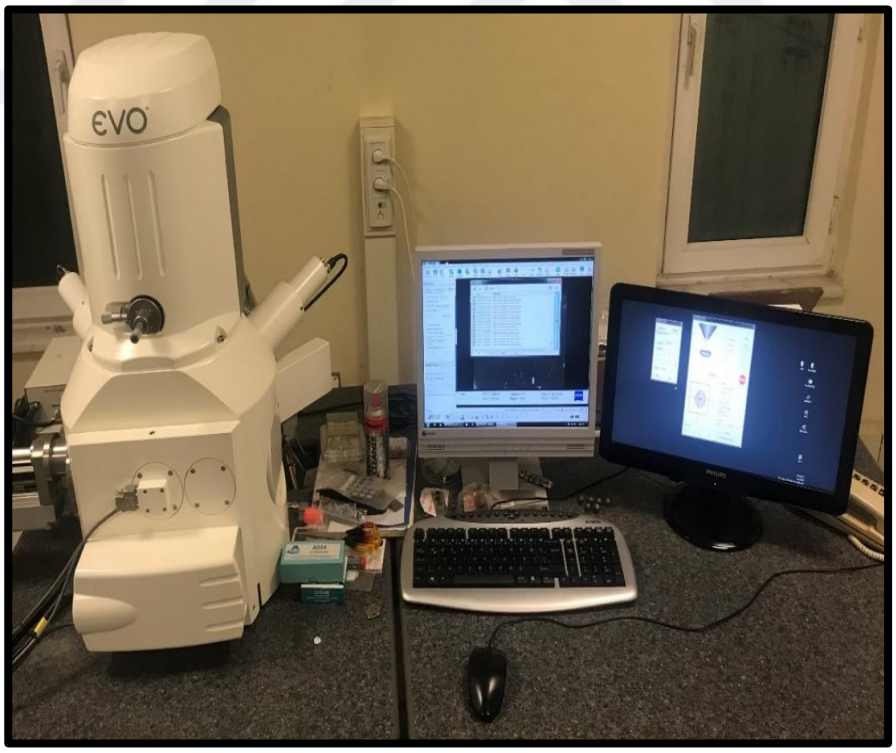


Figure 48. Scanning Electron Microscope (Carl ZEISS, EVO 40, Germany)

3.12. X-Ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analyses were carried out in the X-ray Laboratory of the Chemistry - Metallurgy Faculty, Metallurgy and Materials Engineering Department at Istanbul Technical University. Analyses were performed using an X-ray diffractometer (X'PERT^3 MRD, Malvern Panalytical, United Kingdom).

21 samples from each group were analyzed for XRD to examine the crystal phase on the enamel surfaces as 3 samples from each group at baseline and post-demineralization and 5 samples from each group at post-treatment.

The anode material was selected as copper, X-rays operating at a power of 60 kV-55 mA were used, and the scanning angle was set at as 0-80º. The analysis findings were obtained using X'Pert HighScore Plus 2.2b software and ICDD, ICSD (Interface Control/Design Document) databases.

Figure 49. X-Ray Diffraction Device

3.13. Statistical Analysis

Statistical analyses were carried out on NCSS (Number Cruncher Statistical System) 2007 Statistical Package Software (Utah, USA). Descriptive statistical methods were used for data evaluation (mean, standard deviation, median, interquartil range). The distribution of variables was analyzed using the Shapiro-Wilk normality test. For normally distributed variables, paired one-way analysis of variance was used for time comparisons, Newman–Keuls multiple comparison test for subgroup comparisons, one-way analysis of variance for group comparisons, and Tukey's multiple comparison test for subgroup comparisons. For non-normally distributed variables, Kruskal Wallis-H test was used for intergroup comparisons and Dunn's multiple comparison test for subgroup comparisons. The findings were evaluated at $p<0.05$ significance level.

4. RESULTS

4.1. Surface Microhardness Test Results

Table 6 presents the mean and standard deviation surface microhardness values of the experimental and control groups at baseline, post-demineralization, and post-treatment, comparison of baseline, post-demineralization (PD), and post-treatment (PT) values by One-Way Analysis of Variance and Paired One-Way Analysis of Variance. Table 7 shows paired comparisons of groups according to Tukey's Multiple Comparison Test, performed due to the statistical differences between the groups in the Paired One-Way Analysis of Variance.

Table 6. Baseline, post-demineralization, and post-treatment surface microhardness mean and standard deviation values and their comparison between applications and groups

	Baseline	**PD**	**PT**	**p***
N-HAP	339.03±8.46	253.44±2.96	296.98±7.40	**0.0001**
BioC	342.76±7.61	248.15±4.33	313.07±5.83	**0.0001**
1450 F	346.89±7.39	248.31±4.80	322.26±2.36	**0.0001**
1000 F	347.10±7.81	252.15±5.09	299.27±5.56	**0.0001**
NC(+)	342.14±6.37	251.53±5.83	209.31±5.20	**0.0001**
NC(-)	337.99±7.42	251.13±5.45	202.95±7.91	**0.0001**
p‡	**0.009**	**0.037**	**0.0001**	

* Paired One-Way Analysis of Variance ‡One-Way Analysis of Variance

Mean baseline surface microhardness values were 337.99±7.42 for the study group and 347.10±7.81 for the control group, with a statistically significant difference (p=0.009) (Table 6). The 1450 ppm fluoride group had a statistically significantly lower baseline surface microhardness than the N-HAP and negative control (unbrushed) groups (p=0.047, p=0.04), and the 1000 ppm fluoride group had a statistically significantly lower baseline surface microhardness than the N-HAP and negative control (unbrushed) groups (p=0.043, p=0.033); no other statistically significant difference was found between the other groups (p>0.05) (Table 7).

It was found that baseline surface microhardness values decreased significantly across all groups after demineralization and reached 248.15±4.33 and 253.44±2.96, with a statistically significant difference (p=0.037) (Table 6). The N-HAP group had a statistically significantly higher mean post-demineralization surface microhardness than the bioactive glass and 1450 ppm fluoride groups (p=0.041, p=0.046), but no statistically significant difference was observed with the other groups (p>0.05) (Table 7).

Mean post-treatment surface microhardness values were found to vary between 202.95±7.91 and 322.26±2.36 across all groups. There was a statistically significant difference between all groups (p=0.0001). Among the experimental groups, the highest mean surface microhardness was obtained in the 1450 ppm fluoride group and the lowest mean value was found in the N-HAP group. (Table 6). Mean post-treatment surface microhardness valuse were found to be statistically significantly lower in both negative control (brushed) and negative control (unbrushed) groups compared to all experimental groups (p=0.0001) (Table 7).

Table 7. Paired comparison of baseline, post-demineralization, and post-treatment surface microhardness values of all groups

Tukey's Multiple Comparison Test	Baseline	PD	PT
N-HAP / BioC	0.806	**0.041**	**0.0001**
N-HAP / 1450 F	**0.047**	**0.046**	**0.0001**
N-HAP / 1000 F	**0.042**	0.984	0.924
N-HAP / NC(+)	0.899	0.914	**0.0001**
N-HAP / NC(-)	0.999	0.828	**0.0001**
BioC / 1450 F	0.728	0.999	**0.003**
BioC / 1000 F	0.684	0.295	**0.0001**
BioC / NC(+)	0.998	0.484	**0.0001**
BioC / NC(-)	0.594	0.617	**0.0001**
1450 F /1000 F	0.998	0.339	**0.0001**
1450 F/ NC(+)	0.596	0.537	**0.0001**
1450 F/ NC(-)	**0.043**	0.671	**0.0001**
1000 F/ NC(+)	0.549	0.999	**0.0001**
1000 F / NC(-)	**0.033**	0.995	**0.0001**
NC(+) / NC(-)	0.726	0.999	0.086

The N-HAP group was found to have a statistically significantly lower mean post-treatment surface microhardness than the bioactive glass and 1450 ppm fluoride groups ($p=0.0001$), but had statistically significant difference with the other groups. The 1000 ppm fluoride group was found to have a statistically significantly lower mean post-treatment surface microhardness than the bioactive glass and 1450 ppm fluoride groups ($p=0.0001$). In brief, the bioactive glass and 1450 ppm fluoride groups had statistically significantly higher mean post-treatment surface microhardness values than the N-HAP and 1000 ppm fluoride groups (Table 7).

The bioactive glass group had a statistically significantly lower mean post-treatment surface microhardness than the 1450 ppm fluoride group ($p=0.003$), but no statistically significant difference with any of the other groups ($p>0.05$).

Table 8 shows paired comparisons of applications for each group according to Newman-Keuls Multiple Comparison Test, performed due to the statistical differences between the surface microhardness values found in One-Way Variance Analysis. Accordingly, there were statistically significant differences between baseline-PD, baseline-PT, and PD-PT surface microhardness values across all groups.

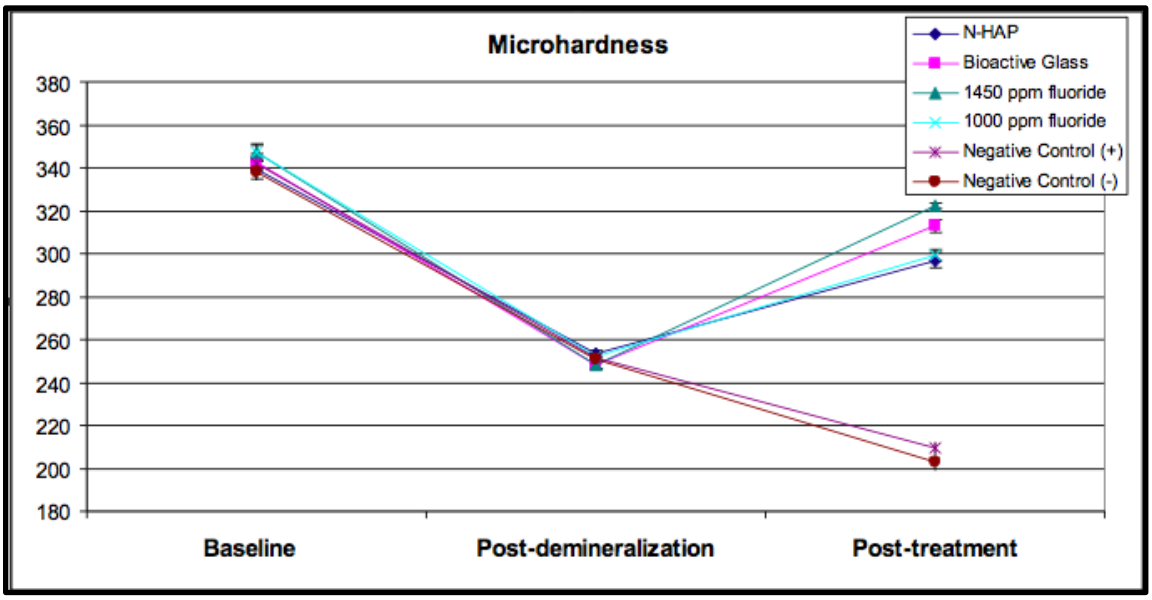
Table 8. Paired comparison of baseline-PD, baseline-PT, and PD-PT surface microhardness values across all groups according to Newman-Keuls Multiple Comparison Test

Newman-Keuls Multiple Comparison Test	N-HAP	BioC	1450 F	1000 F	NC(+)	NC(-)
Baseline / PD	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Baseline / PT	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
PD / PT	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

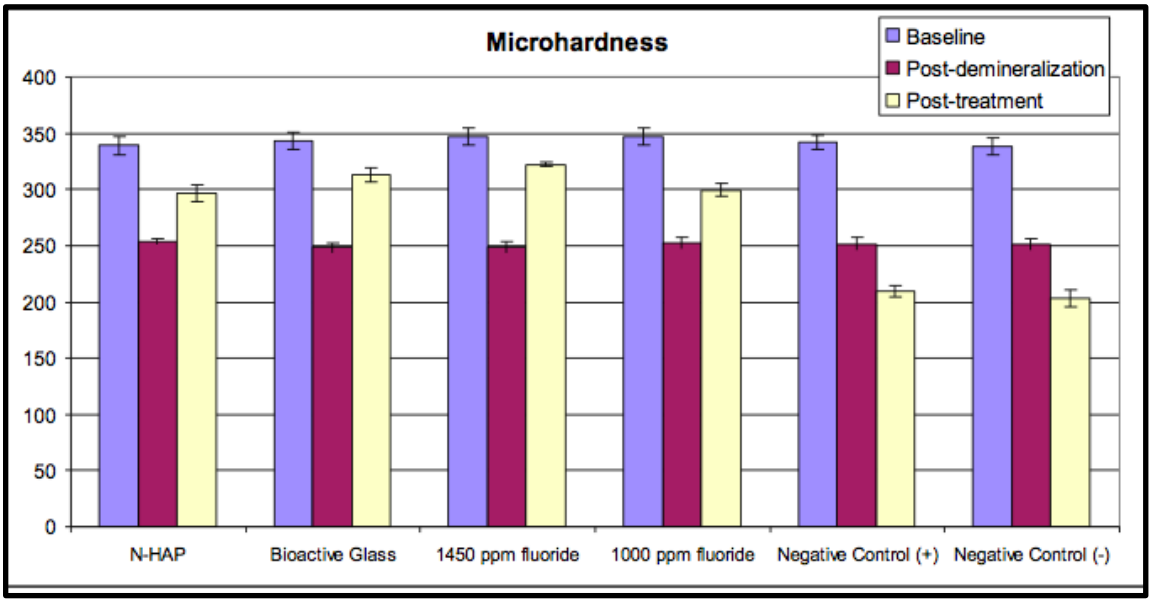
There were statistically significant differences between mean baseline, PD, and PT surface microhardness values across all groups (p=0.0001). Mean baseline surface microhardness was statistically significantly higher than mean PD and PT values (p=0.0001), and mean PT value was statistically significantly higher than mean PD value (p=0.0001) (Table 8).

There was a similar statistically significant difference between mean baseline, PD, and PT surface microhardness values in the negative control (unbrushed) and negative control (brushed) groups (p=0.0001). Mean baseline surface microhardness was statistically significantly higher than mean PD and PT values (p=0.0001), and mean PD value was statistically significantly higher than mean PT value (p=0.0001) (Table 8).

Graphs 1 and 2 show the distribution of the baseline, post-demineralization, and post-treatment surface microhardness values of all experimental and negative control groups. Mean PT surface microhardness values were higher than mean PD values across all experimental groups, but these values could not reach the baseline surface microhardness values.



Graph 1. Distribution of baseline, post-demineralization, and post-treatment surface microhardness values across all groups



Graph 2. Comparison of baseline, post-demineralization, and post-treatment surface microhardness values across all groups

4.2. SEM Images

SEM images of intact enamel at x200 and x2000 magnifications revealed that the surface followed a normal anatomical enamel structure, surface was smooth, and integrity was undisturbed. The enamel crystals on the enamel surface were observed to be in the shape of a keyhole **(**Figures 50 and 51).

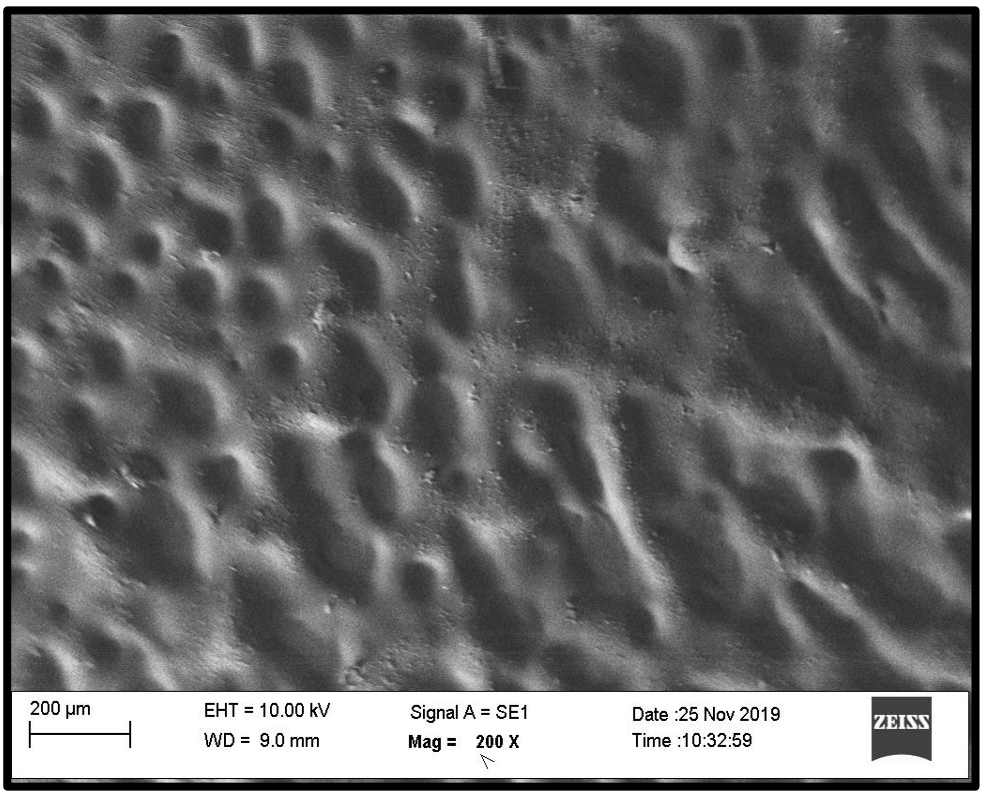


Figure 50. Intact enamel surface image at x200 magnification

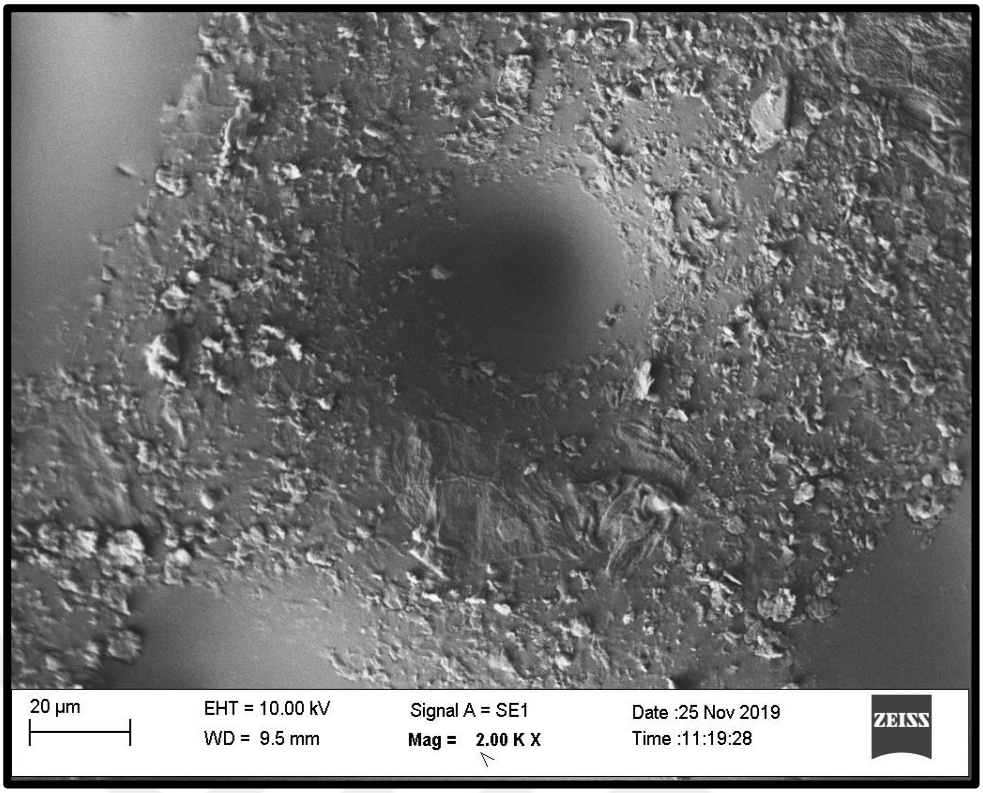


Figure 51. Intact enamel surface image at x2000 magnification

Images of demineralized enamel samples at x500, x3500, and x5000 magnifications revealed that the orientation of the hydroxyapatite crystals was disrupted, and the keyhole image of the enamel crystals disappeared. The structure on the enamel surface was observed to be rather scattered with increased porosity. Some areas on the enamel surface revealed deep prismatic holes. Because precipitation was higher in the core part of the enamel prisms than the periphery, a honeycomb appearance was observed in these areas **(**Figures 52-54).

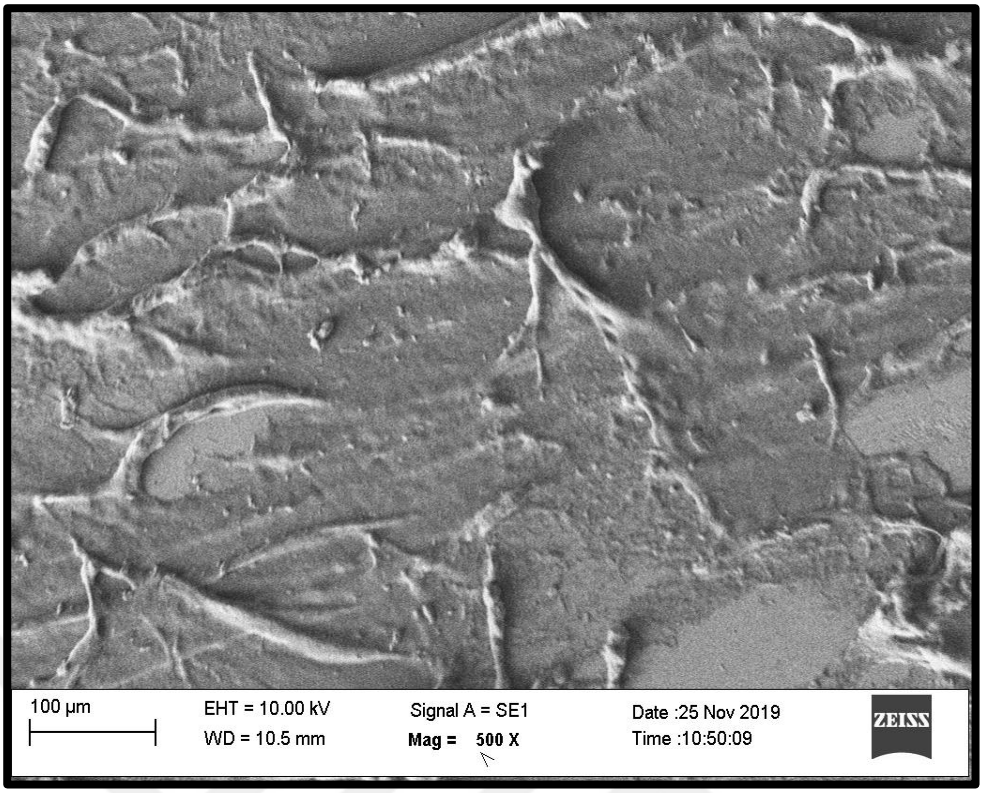


Figure 52. Demineralized enamel surface image at x500 magnification

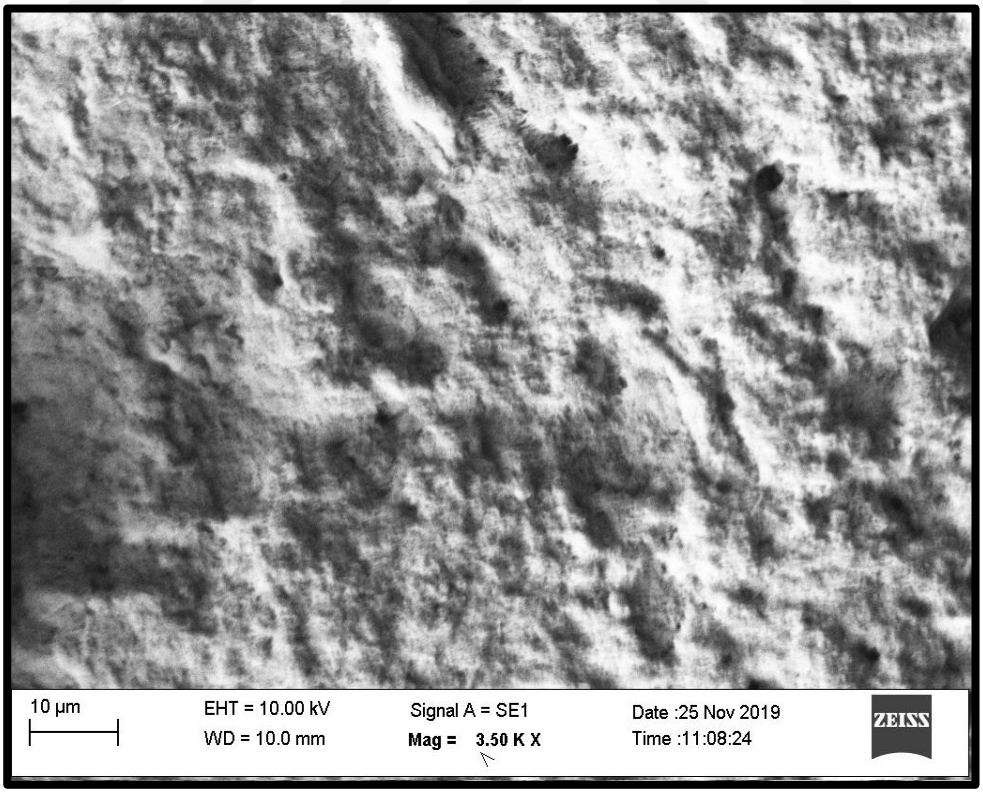


Figure 53. Demineralized enamel surface image at x3500 magnification

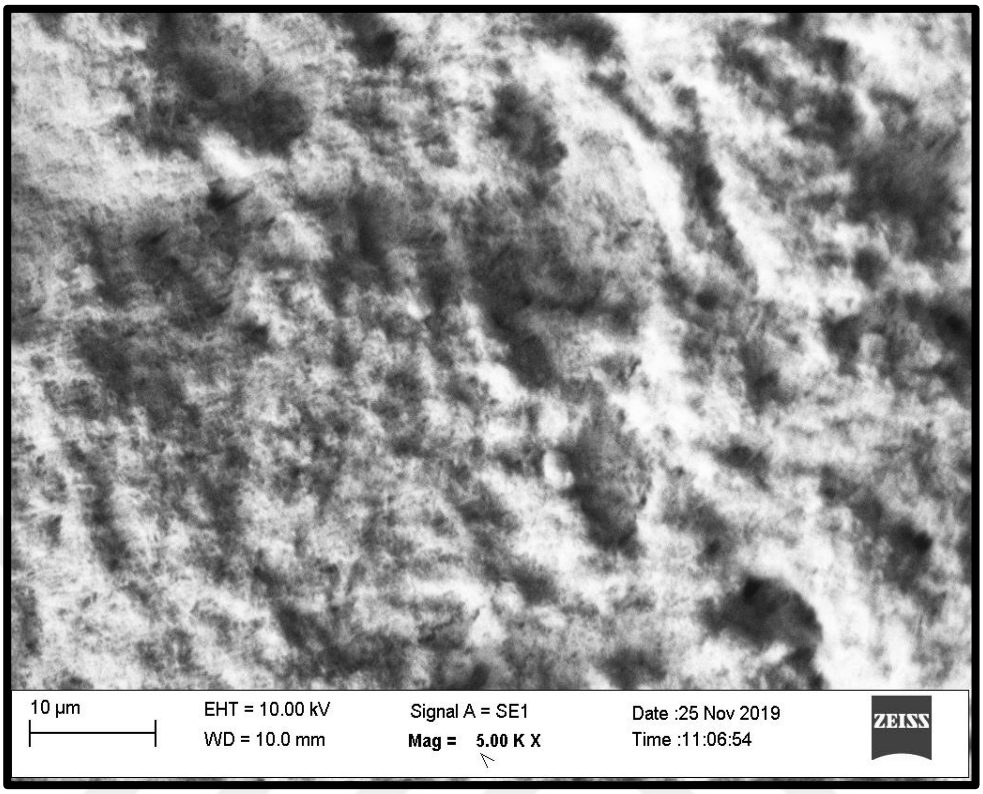


Figure 54. Demineralized enamel surface image at x5000 magnification

Post-treatment images of tooth surfaces in the N-HAP group at x200, x3500, and x5000 magnifications revealed that remineralization was achieved in some areas of the demineralized enamel surface and acicular crystals were precipitated. Uneven accumulation of crystals was observed on the enamel surface. The porous structure on the enamel surface was still present, and no homogeneous structure was observed. The honeycomb appearance that emerged by the precipitation of enamel crystals did not disappear (Figures 55-57).

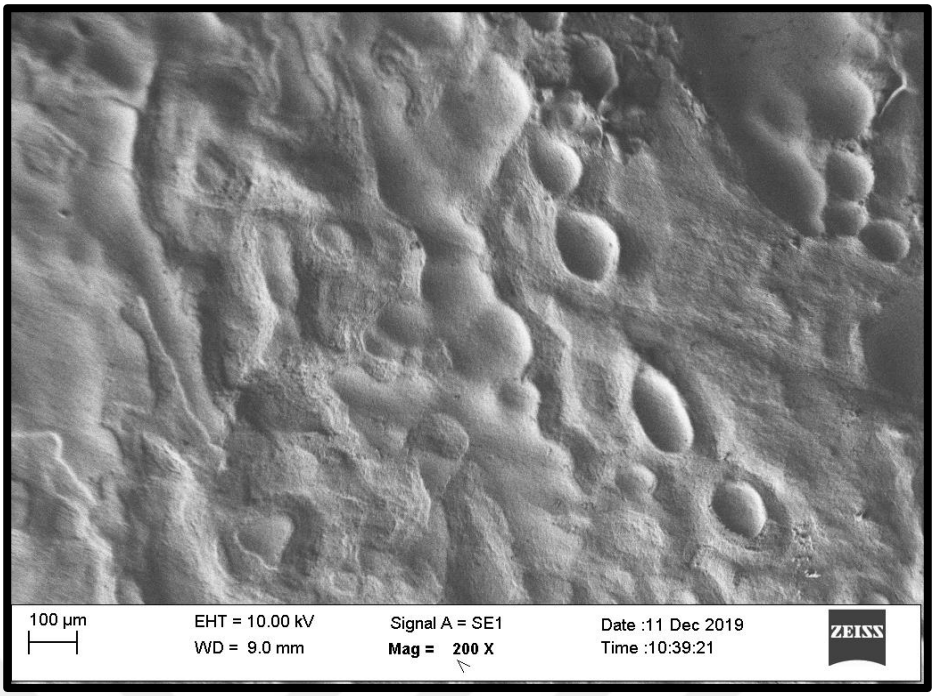


Figure 55. Post-treatment enamel surface image in the N-HAP group at x200 magnification

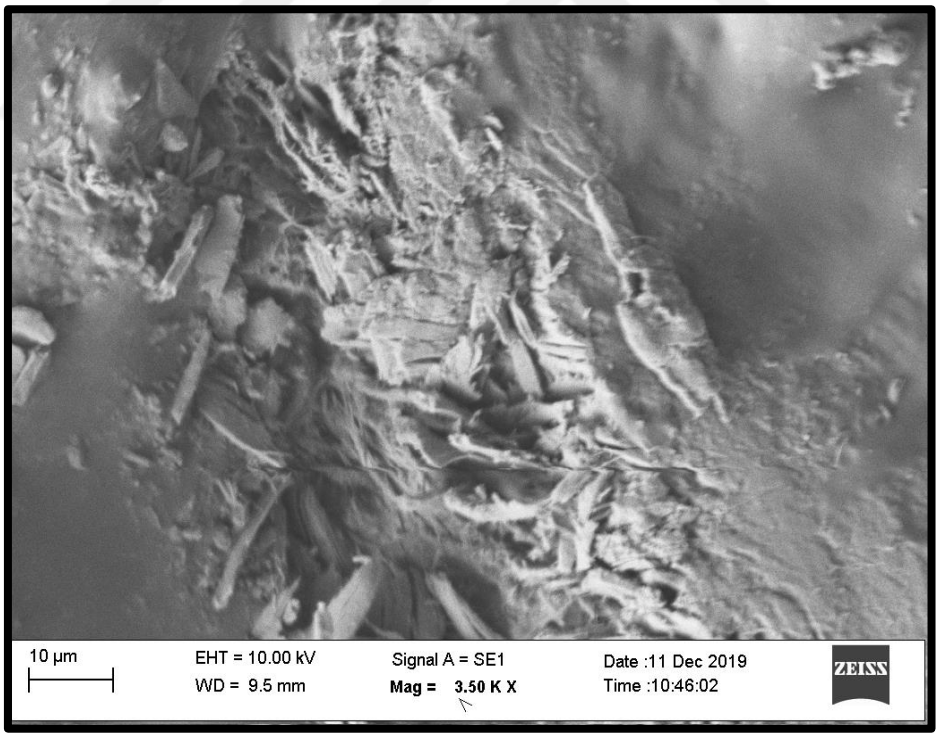


Figure 56. Post-treatment enamel surface image in the N-HAP group at x3500 magnification

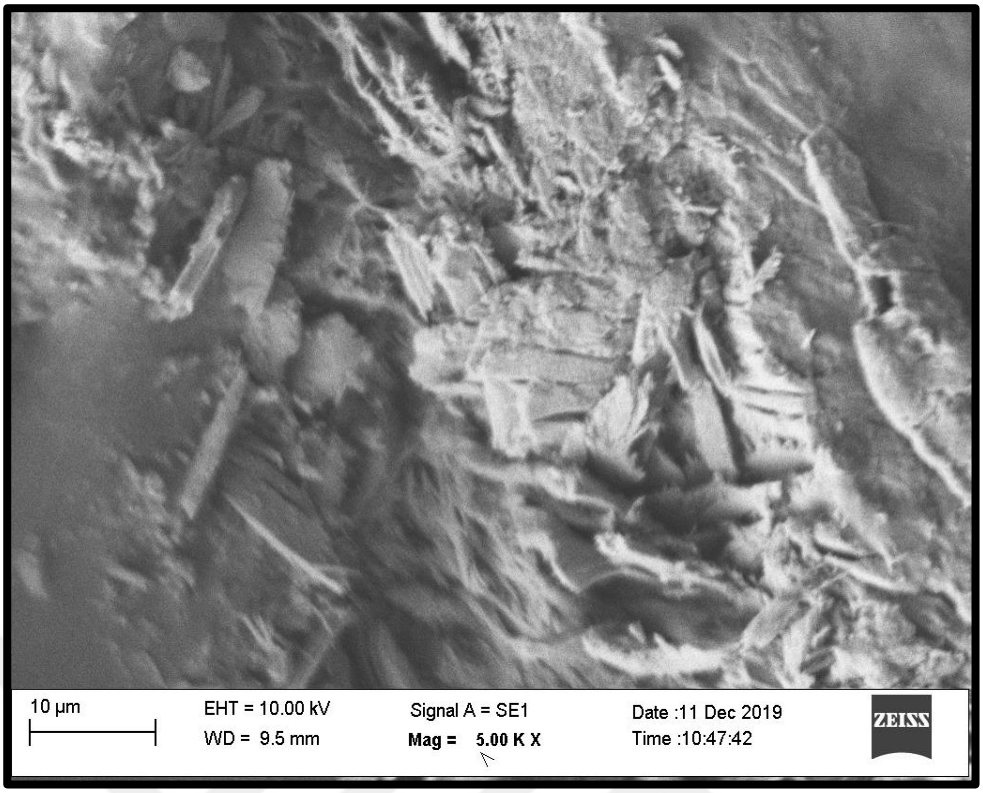


Figure 57. Post-treatment enamel surface image in the N-HAP group at x5000 magnification

Post-treatment surface images in the BioC group at x200, x3500, and x5000 magnifications revealed a flatter and uniform enamel surface with the accumulation of remineralization agent on the decalcified enamel surface. The porosity that was observed on the enamel surface after demineralization began to decrease, and crystals were found to precipitate in remineralized areas (Figures 58-60).

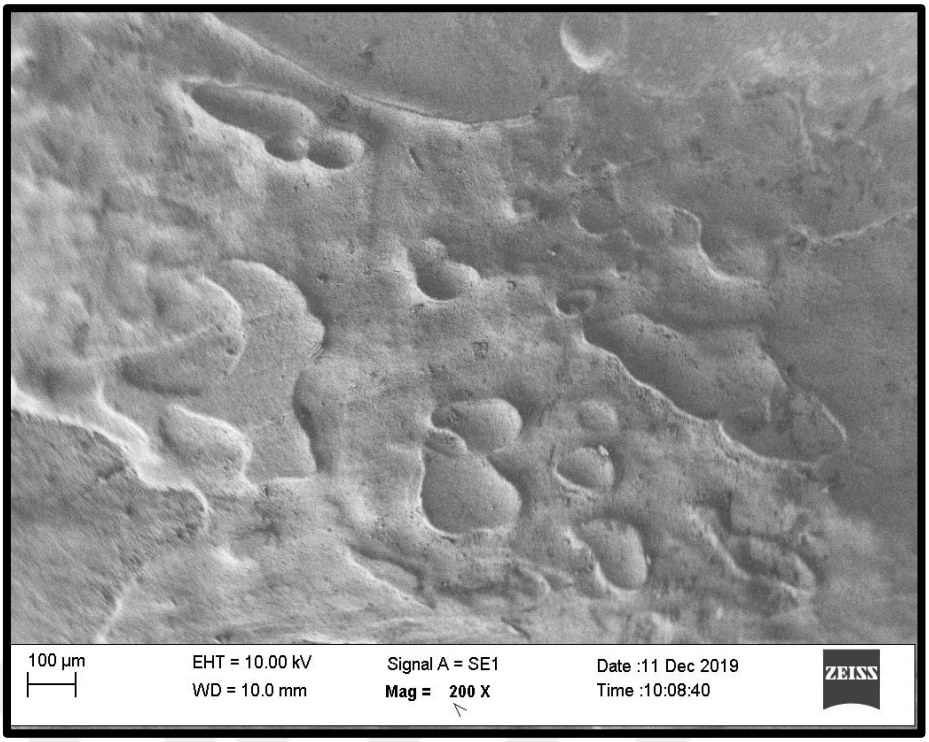


Figure 58. Post-treatment enamel surface image in the BioC group at x200 magnification

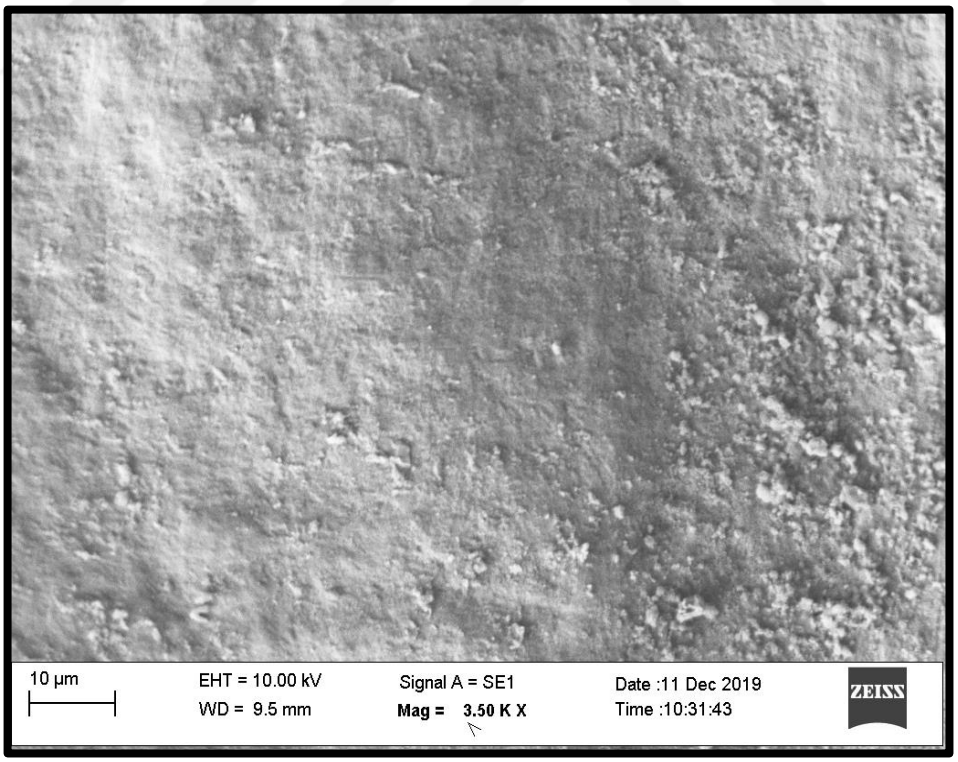


Figure 59. Post-treatment enamel surface iamge in the BioC group at x3500 magnification

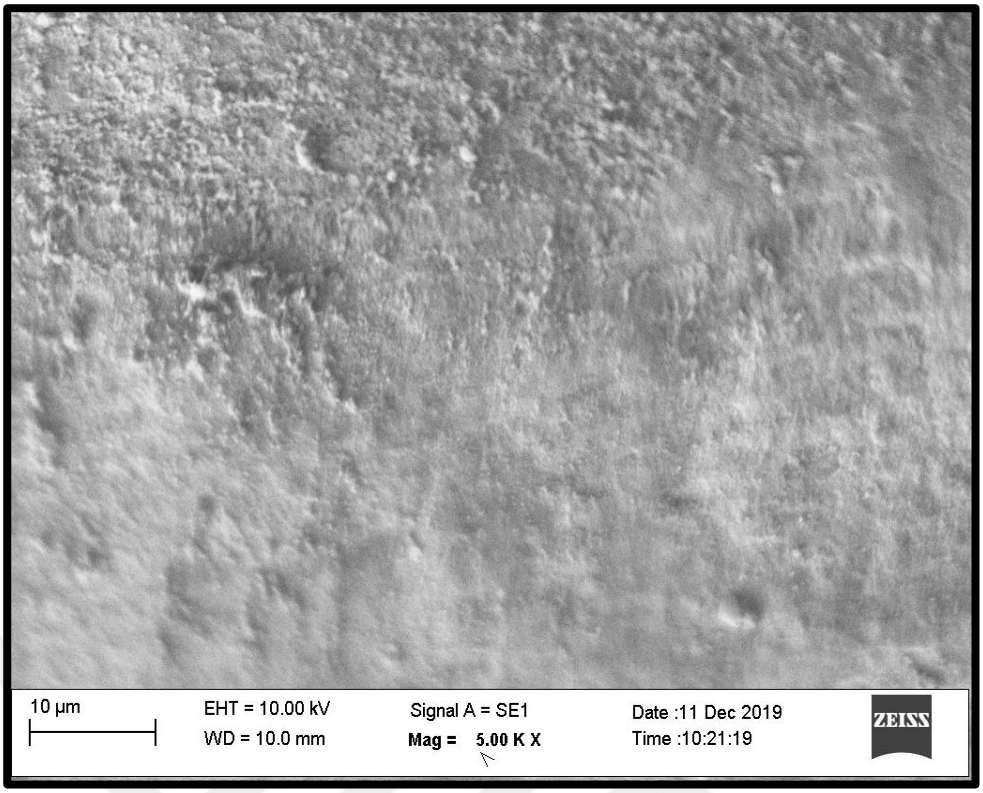


Figure 60. Post-treatment enamel surface iamge in the BioC group at x5000 magnification

Post-treatment enamel surface images of the 1450 F group at x200, x3500, and x5000 magnifications revealed mineral filling on the surface and flatter and uniform appearance. Filling of micropores was observed on the enamel. The honeycomb appearance after demineralization was found to disappear (Figures 61-63).

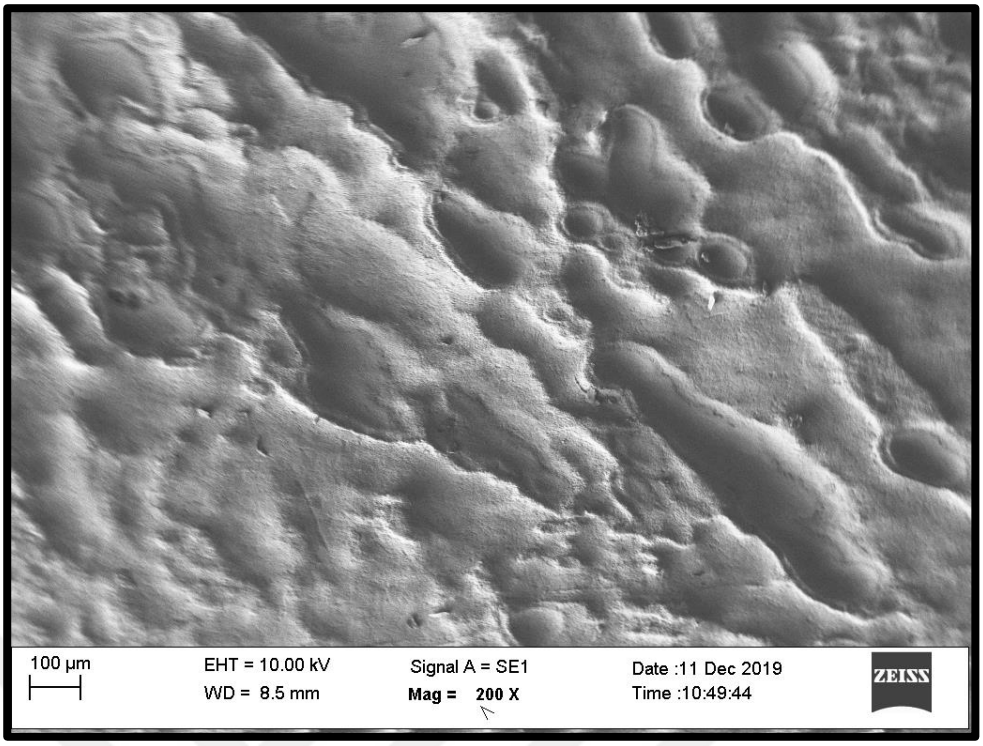


Figure 61. Post-treatment enamel surface image in the 1450 F group at x200 magnification

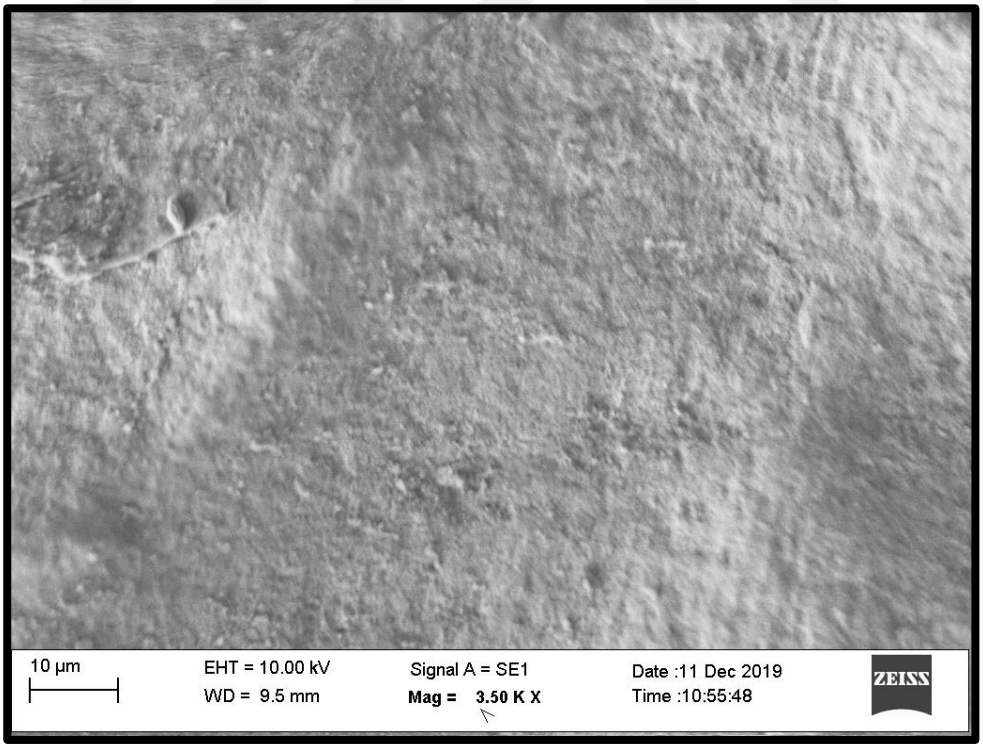


Figure 62. Post-treatment enamel surface image in the 1450 F group at x3500 magnification

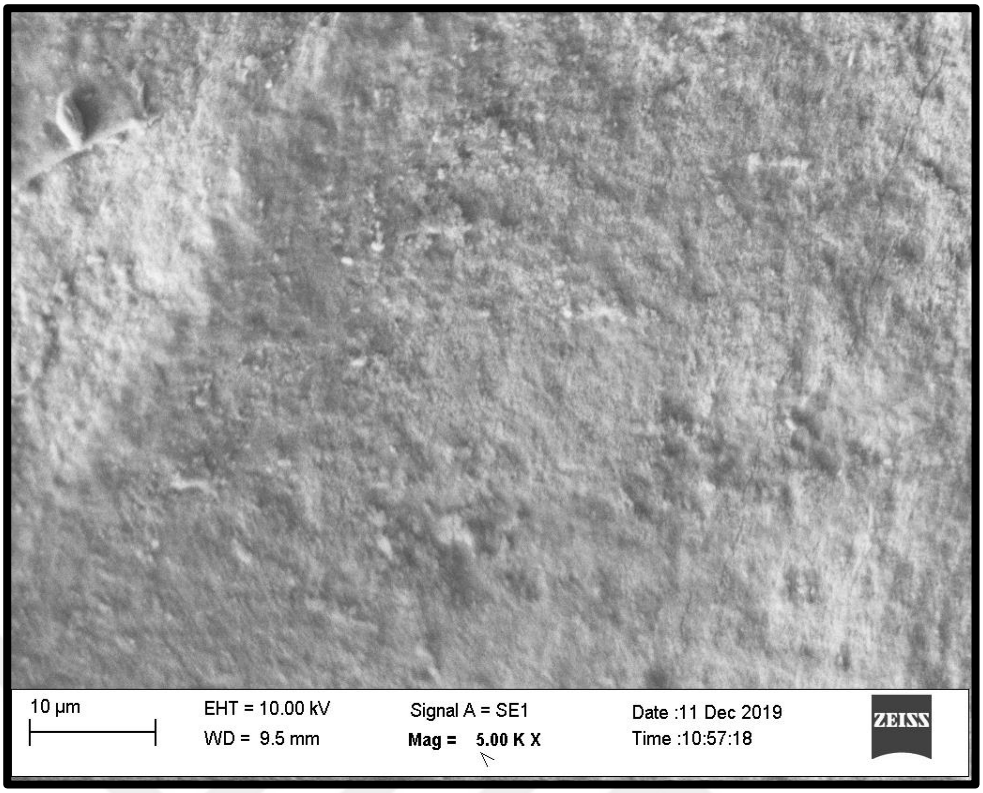


Figure 63. Post-treatment enamel surface image in the 1450 F group at x5000 magnification

Post-treatment enamel surface images of the 1000 F group at x200, x3500, and x5000 magnifications revealed decreased porosity, and the dissolved crystals began to precipitate. There were small globular precipitations in the area, and in some areas these combined to form larger ones. It was found complete remineralization was not achieved. Also, the micropores in some areas were not covered (Figures 64-66).

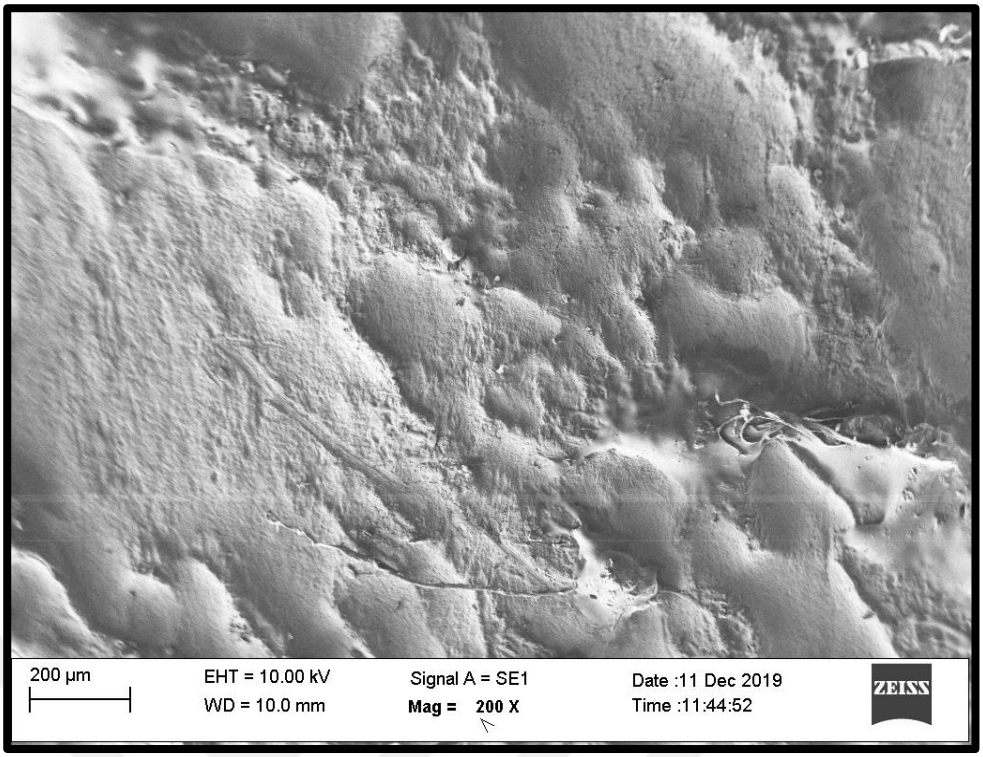


Figure 64. Post-treatment enamel surface image in the 1000 F group at x200 magnification

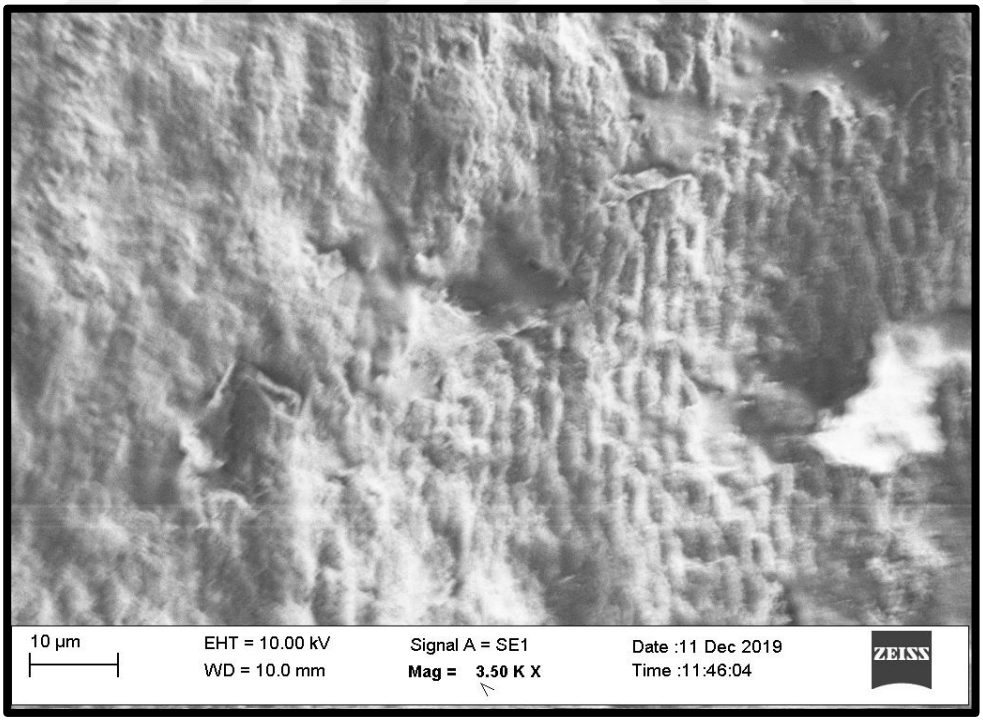


Figure 65. Post-treatment enamel surface image in the 1000 F group at x3500 magnification

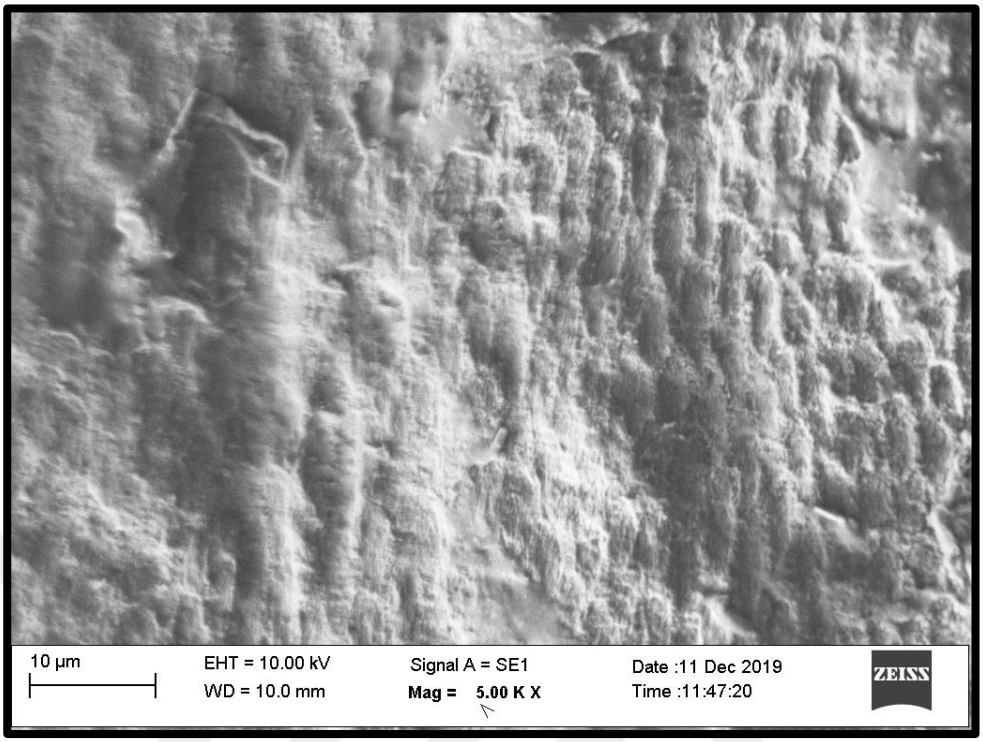


Figure 66. Post-treatment enamel surface image in the 1000 F group at x5000 magnification

In the NC (+) group that received no treatment agent, where enamel samples were kept in distilled water and brushed, surface porosity was higher than the group that was only kept in distilled water, and there was a highly dispersed appearance on the enamel surface. Honeycomb appearance was observed on the enamel surface (Figures 67-69).

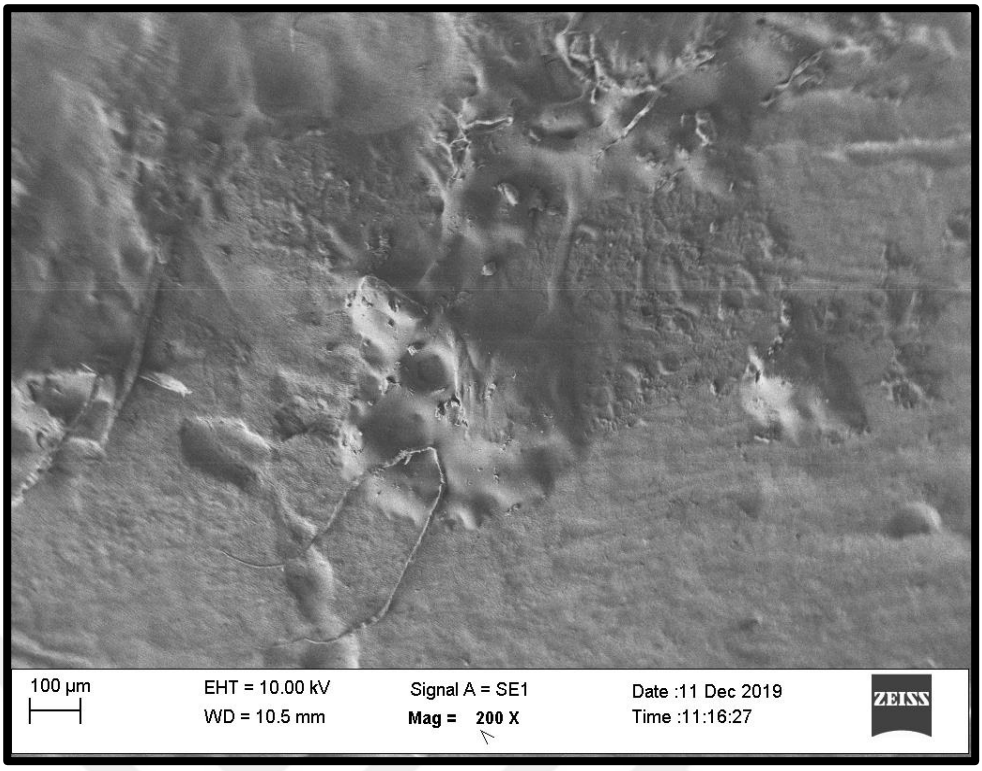


Figure 67. Post-treatment SEM iamge in the NC (+) group at x200 magnification

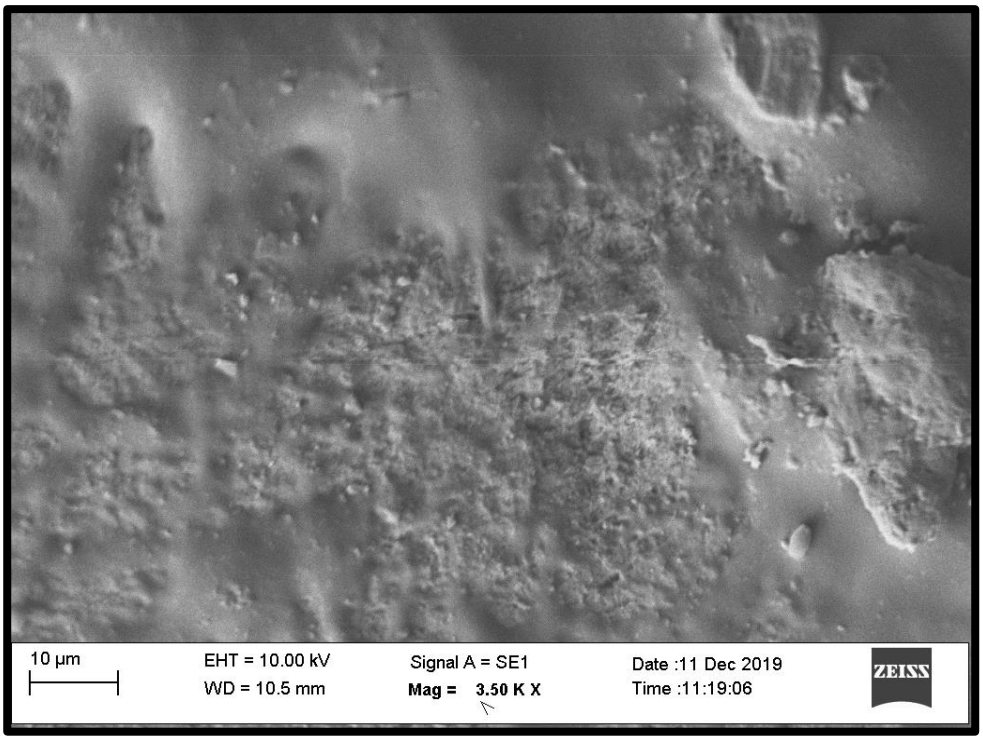


Figure 68. Post-treatment SEM image in the NC (+) group at x3500 magnification

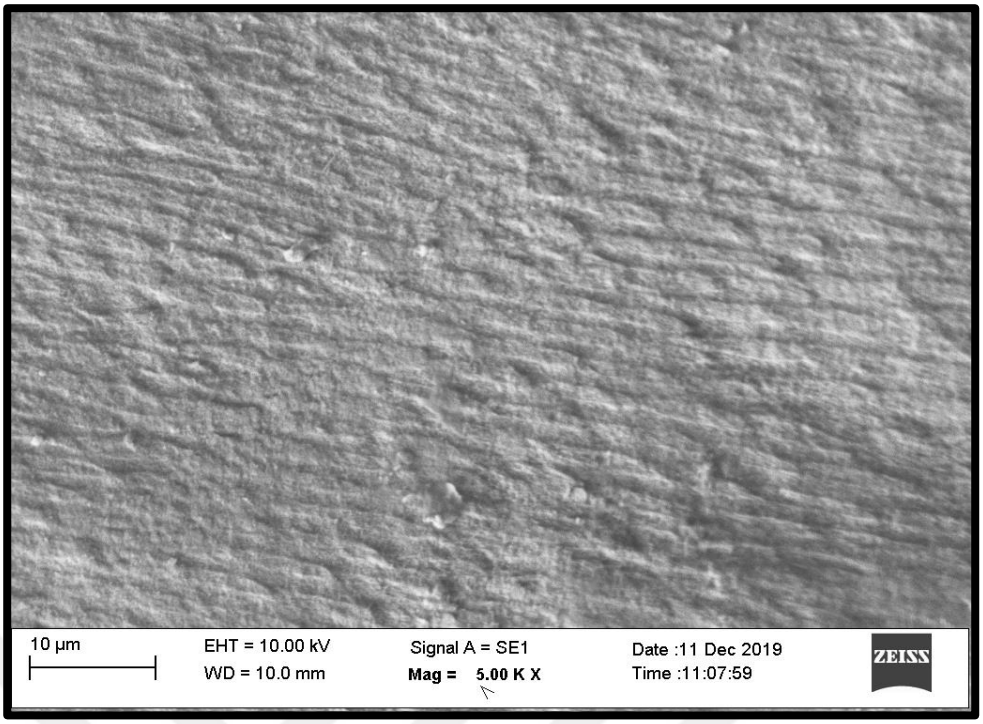


Figure 69. Post-treatment SEM image in the NC (+) group at x5000 magnification

In the NC (-) group that received no treatment agent, where enamel samples were only kept in distilled water, figures at x200 and x3500 magnifications revealed irregular and amorphous enamel surface and widespread porosities **(**Figures 70 and 71).

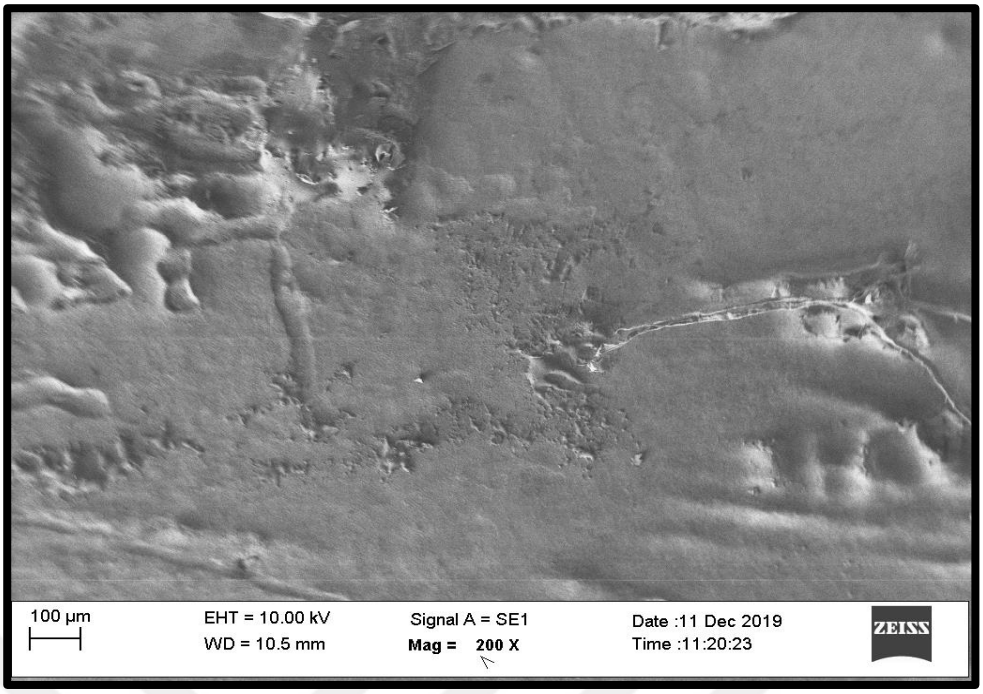


Figure 70. Post-treatment SEM image in the NC (-) group at x200 magnification

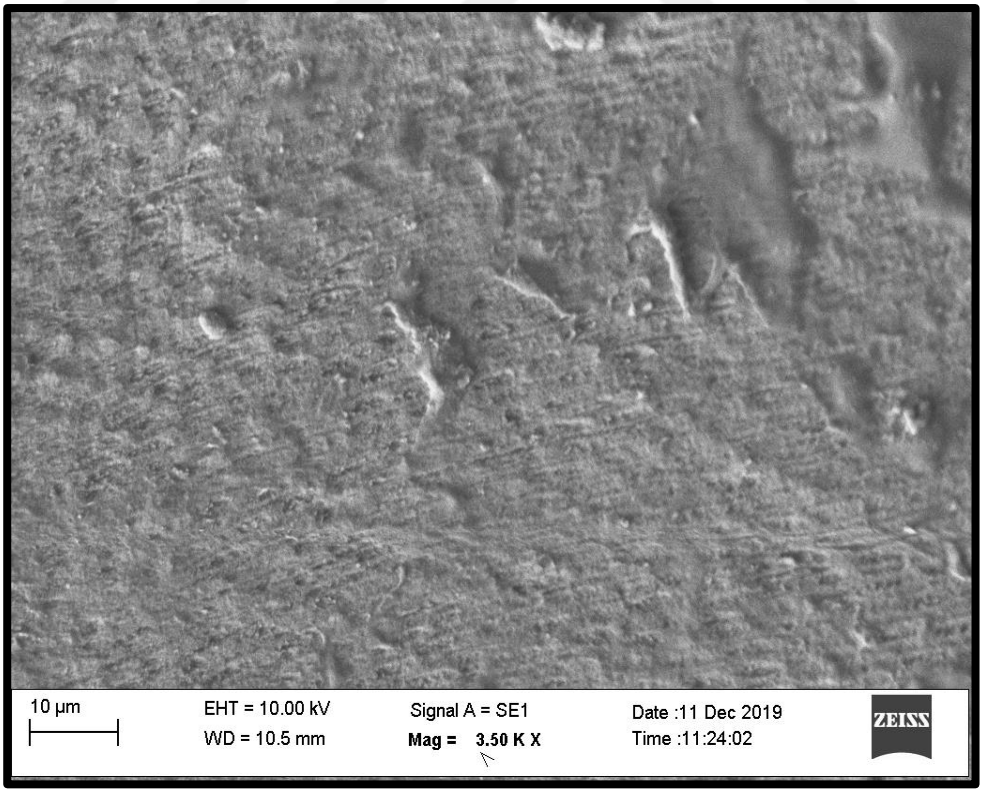


Figure 71. Post-treatment SEM image in the NC (-) group at x3500 magnification

4.3. XRD Analysis Results

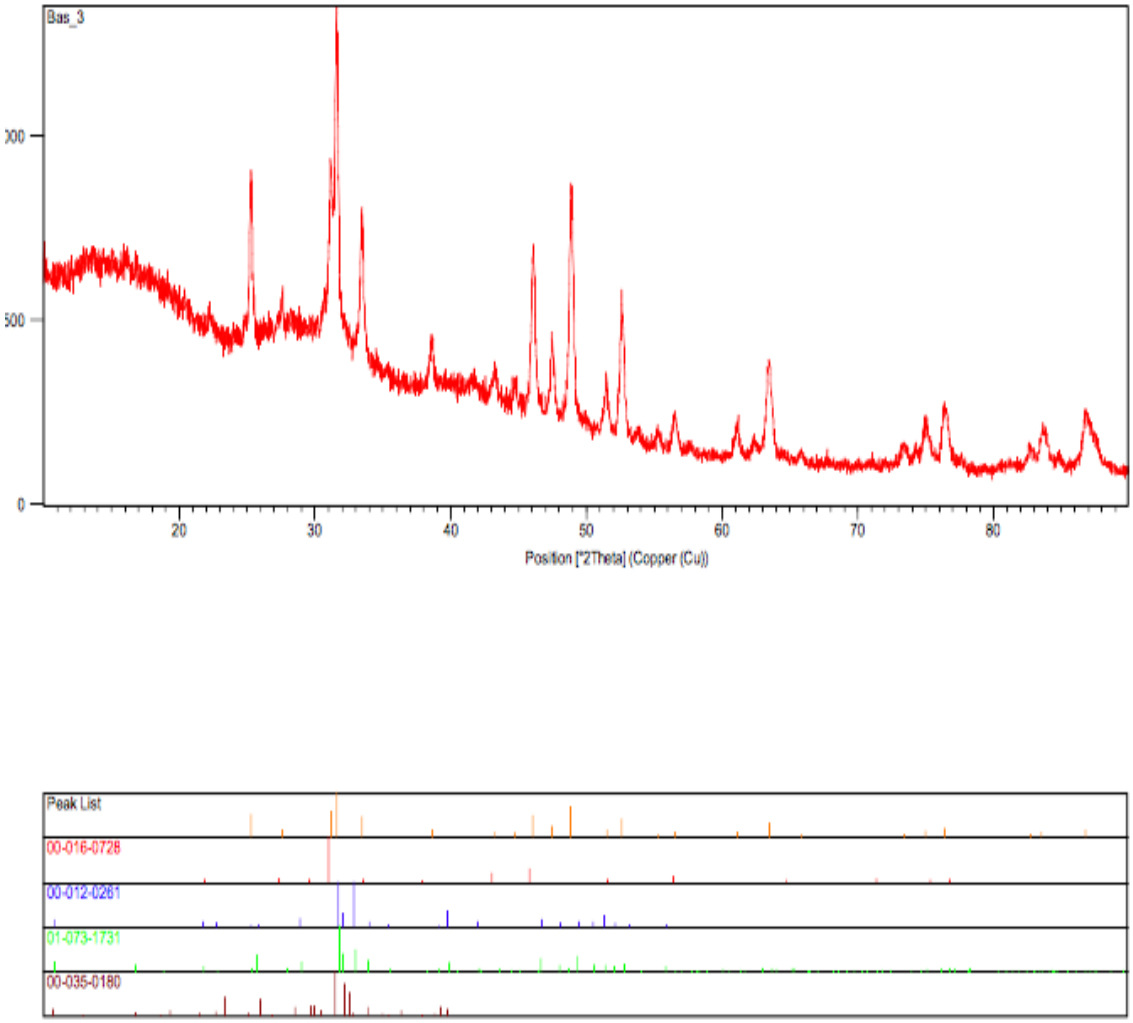
XRD peaks occurring in tooth tissue with crystal structure were compared with all crystal phases containing similar elements using X'pert Highscore RIR (reference intensity ratio), and which crystal structure the peaks belonged to were determined. All compounds containing the same element with analyzed dental tissues were obtained based on their percentages of proximity to each other, and compounds that intersected with each other were selected. After all peaks were matched, the reference catalogs of the phases were examined, the peaks revealed in dental tissue were re-evaluated, and cross-checked. The reference catalogs used in the evaluation are given in Annex 1 -9.

4.3.1. XRD Analysis of Intact Enamel Samples

XRD analysis of intact enamel samples revealed that crystal phases with reference codes 00-016-0728, 00-035-0180, 00-012-0261, and 01-073-1731 were dominant (peaked) (Graph 3). The reference codes, chemical formulas, and mineral names of these phases are given in Table 9.

Table 9. Reference codes, chemical formulas, and names of crystal phases dominant in XRD analysis of intact enamel samples

Reference Code	**Chemical Formula**	**Mineral Name**
00-016-0728	CaP	Calcium phosphide
00-035-0180	$Ca_{10}(PO_4)_6CO_3$	Calcium Phosphate Carbonate (CPC)
00-012-0261	$Ca_5F(PO_4)_3$	Fluorapatite
01-073-1731	$Ca_5(PO_4)_3(OH)$	Hydroxylapatite



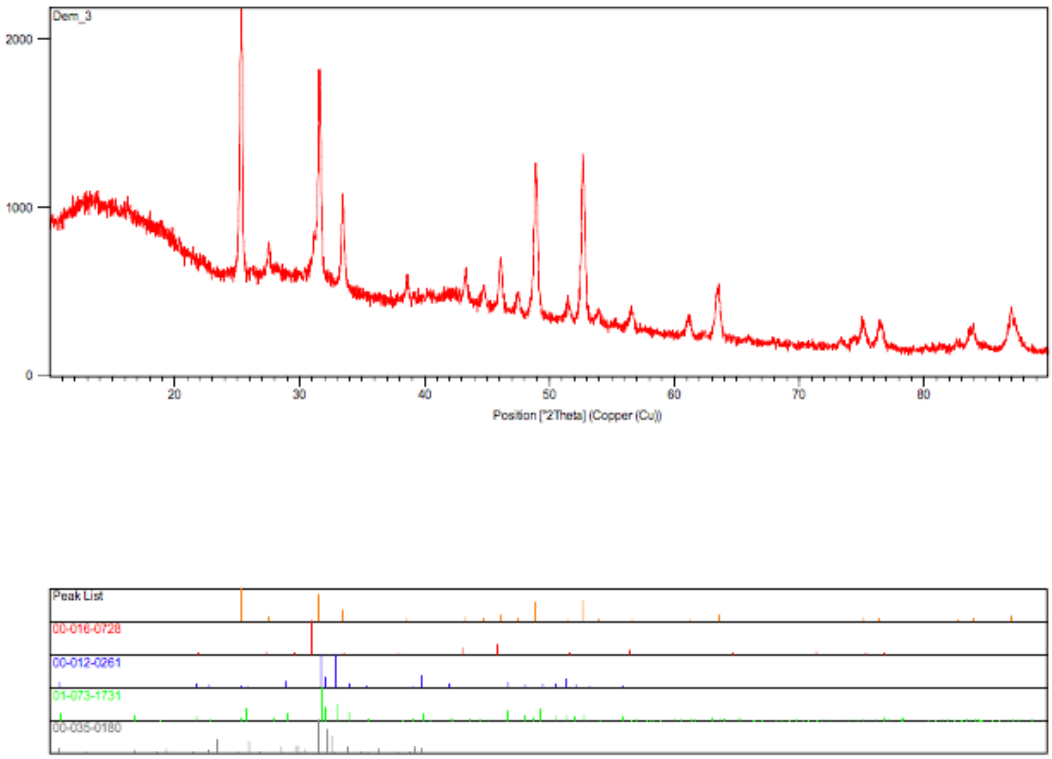
Graph 3. XRD analysis of intact enamel samples

4.3.2. XRD Analysis of Demineralized Enamel Samples

XRD analysis of demineralized enamel samples revealed that crystal phases with reference codes 00-016-0728, 00-035-0180, 00-012-0261, and 01-073-1731 were dominant (peaked) (Graph 4). The reference codes, chemical formulas, and mineral names of these phases are given in Table 10.

Table 10. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of demineralized enamel samples

Reference Code	**Chemical Formula**	**Mineral Name**
00-016-0728	CaP	Calcium phosphide
00-035-0180	$Ca_{10}(PO_4)_6CO_3$	Calcium Phosphate Carbonate
00-012-0261	$Ca_5F(PO_4)_3$	Fluorapatite
01-073-1731	$Ca_5(PO_4)_3(OH)$	Hydroxylapatite



Graph 4. XRD analysis of demineralized enamel samples

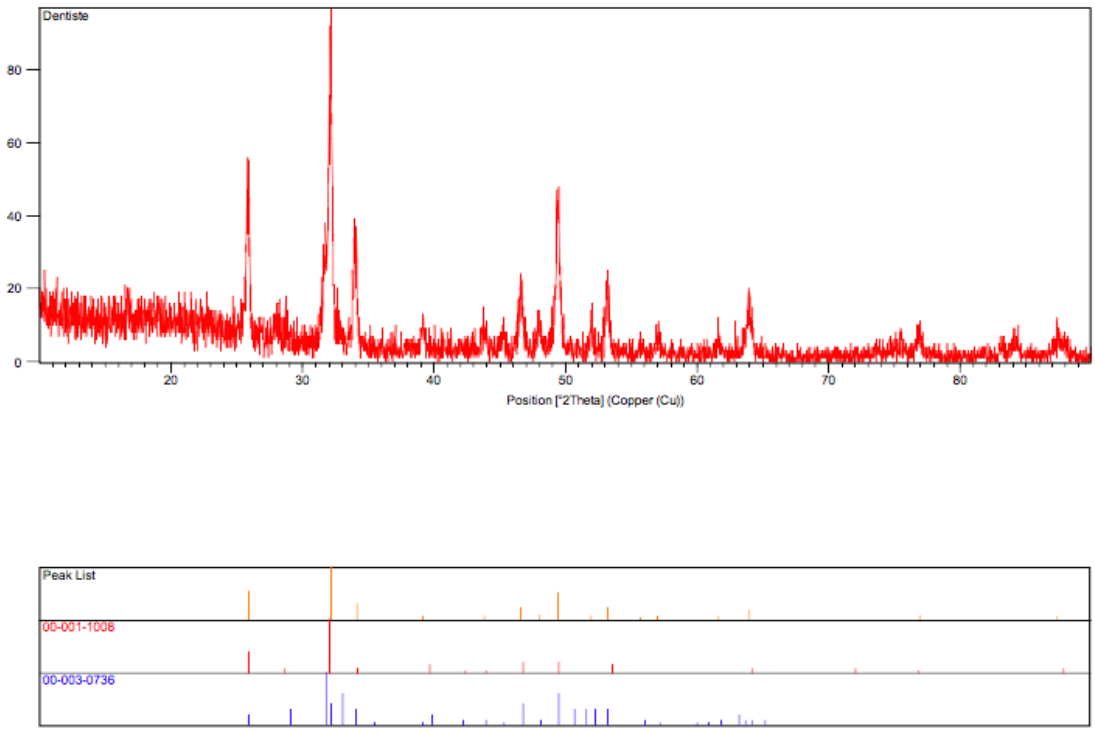
4.3.3. XRD Analysis of Post-Treatment Samples

4.3.3.1. XRD analysis of N-HAP samples

Post-treatment XRD analysis of demineralized enamel samples with Dentiste Premium & Natural White, the toothpaste containing nano-hydroxyapatite, revealed that crystal phases with reference codes 00-003-0736 and 00-035-0180 were dominant (peaked) (Graph 5). The reference codes, chemical formulas, and mineral names of these phases are given in Table 11.

Table 11. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of N-HAP samples

Reference Code	**Chemical Formula**	**Mineral Name**
00-003-0736	$Ca_5(PO_4)_3F$	Fluorapatite
00-001-1008	$Ca_{10}(OH)_2(PO_4)_6$	Hydroxyapatite



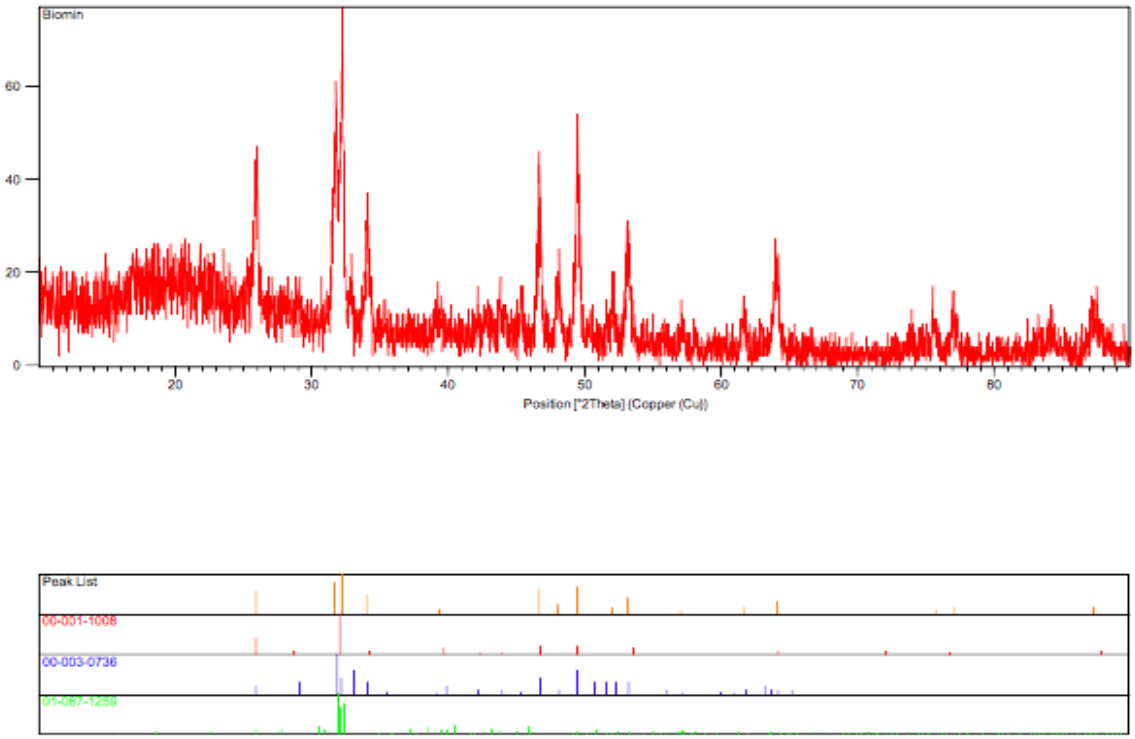
Graph 5. XRD analysis of N-HAP samples

4.3.3.2. XRD analysis BioC samples

Post-treatment XRD analysis of demineralized enamel samples with Biomin ™ C toothpaste containing bioactive glass revealed that crystal phases with reference codes 01-087-1259, 00-003-0736, and 00-001-1008 were dominant (peaked) (Graph 6). The reference codes, chemical formulas, and mineral names of these phases are given in Table 12.

Table 12. Reference codes, chemical formulas and mineral names of crystal phases dominant in XRD analysis of BioC samples

Reference Code	**Chemical Formula**	**Mineral Name**
01-087-1259	$Ca_2(SiO_4)$	Calcium silicate (CAS)
00-003-0736	$Ca_5(PO_4)_3F$	Fluorapatite
00-001-1008	$Ca_{10}(OH)_2(PO_4)_6$	Hydroxyapatite



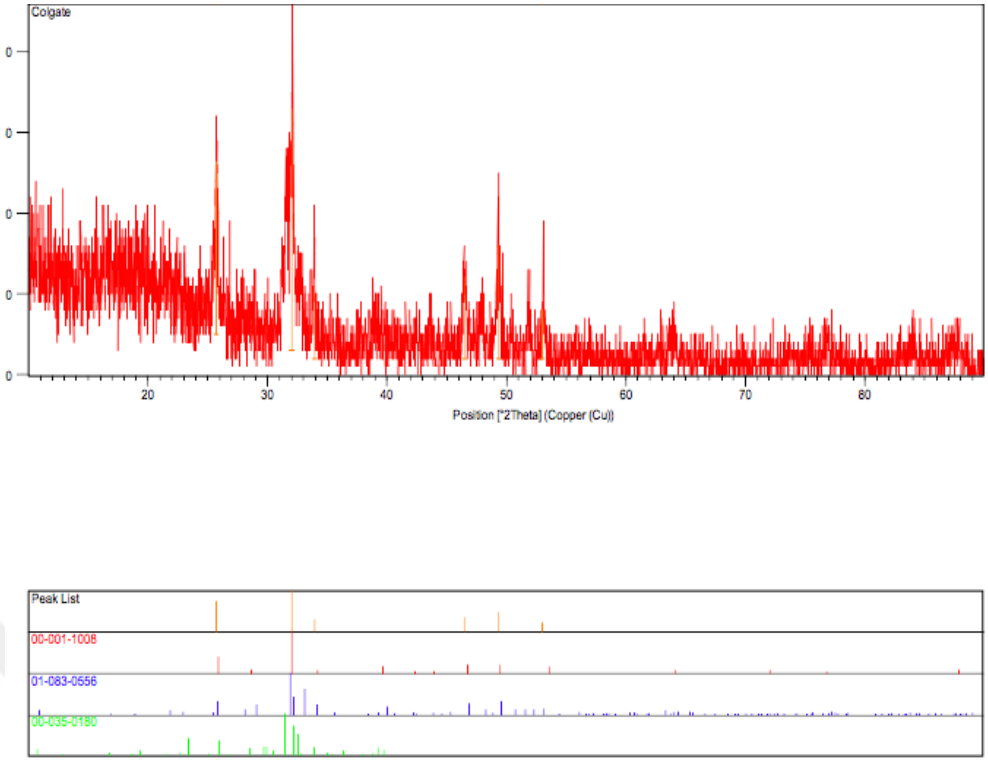
Graph 6. XRD analysis of BioC samples

4.3.3.3. XRD analysis of 1450 F samples

Post-treatment XRD analysis of demineralized enamel samples with Colgate® Triple Action toothpaste containing 1450 ppm fluoride revealed that crystal phases with reference codes 00-035-0180, 01-083-0556, and 00-001-1008 were dominant (peaked) (Graph 7). The reference codes, chemical formulas, and mineral names of these phases are given in Table 13.

Table 13. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of 1450 F samples

Reference Code	**Chemical Formula**	**Mineral Name**
00-035-0180	$Ca_{10}(PO_4)_6CO_3$	Calcium Phosphate Carbonate (CPC)
01-083-0556	$Ca_{5.061}(P_{2.87}O_{11.46})F_{0.89}$	Fluorapatite
00-001-1008	$Ca_{10}(OH)_2(PO_4)_6$	Hydroxyapatite



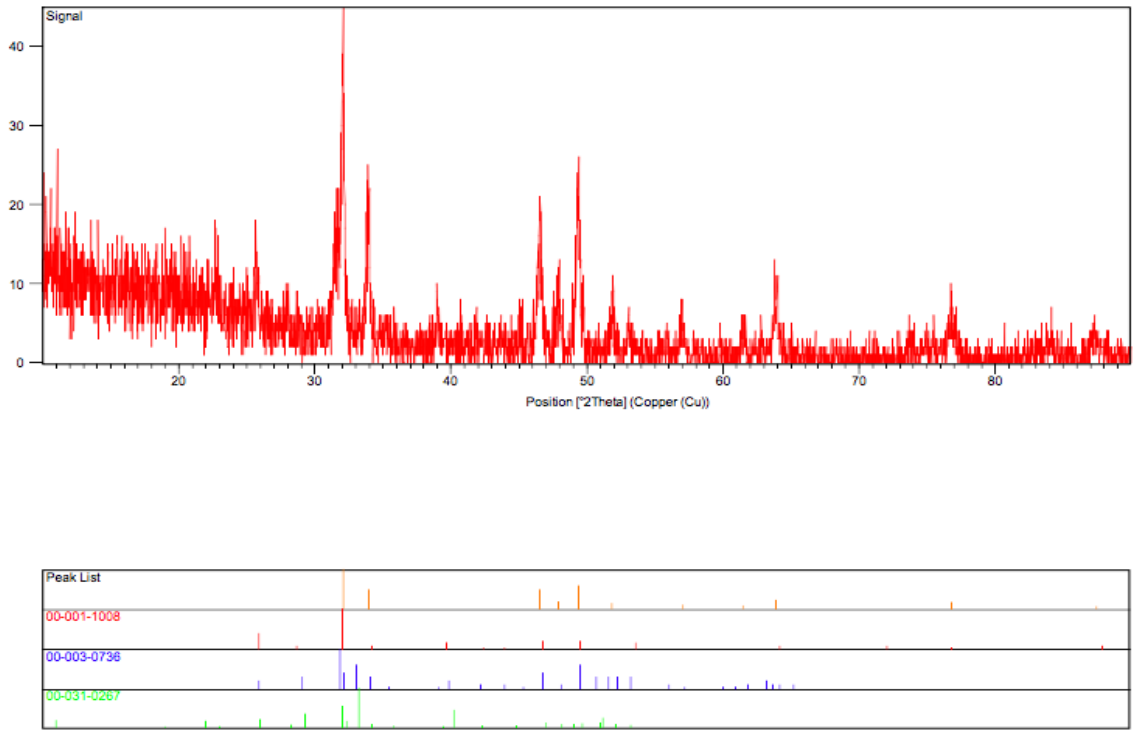
Graph 7. XRD analysis of 1450 F samples

4.3.3.4. XRD analysis of 1000 F samples

Post-treatment XRD analysis of demineralized enamel samples with Colgate® Triple Action toothpaste containing 1000 ppm fluoride revealed that crystal phases with reference codes 00-031-0267, 00-003-0736, and 00-001-1008 were dominant (peaked) (Graph 8). The reference codes, chemical formulas, and mineral names of these phases are given in Table 14.

Table 14. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of 1000 F samples

Reference Code	**Chemical Formula**	**Mineral Name**
00-031-0267	$Ca_{10}(PO_4)_5CO_3F_{1.5}(OH)_{0.5}$	Carbonate fluorapatite
00-003-0736	$Ca_5(PO_4)_3F$	Fluorapatite
00-001-1008	$Ca_{10}(OH)_2(PO_4)_6$	Hydroxyapatite



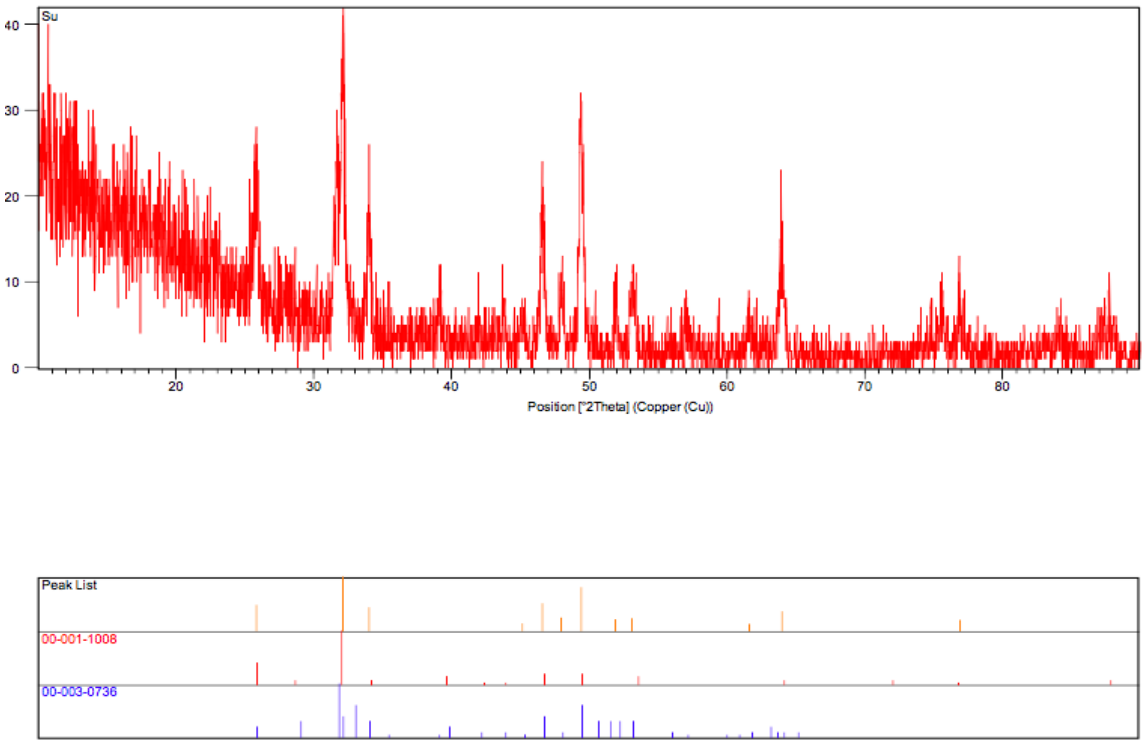
Graph 8. XRD analysis findings of 1000 F samples

4.3.3.5. XRD analysis of negative control samples

Post-treatment XRD analysis of demineralized enamel samples only treated with water revealed that crystal phases with reference codes 00-003-0736 and 00-001-1008 were dominant (peaked) (Graph 9). The reference codes, chemical formulas, and mineral names of these phases are given in Table 15.

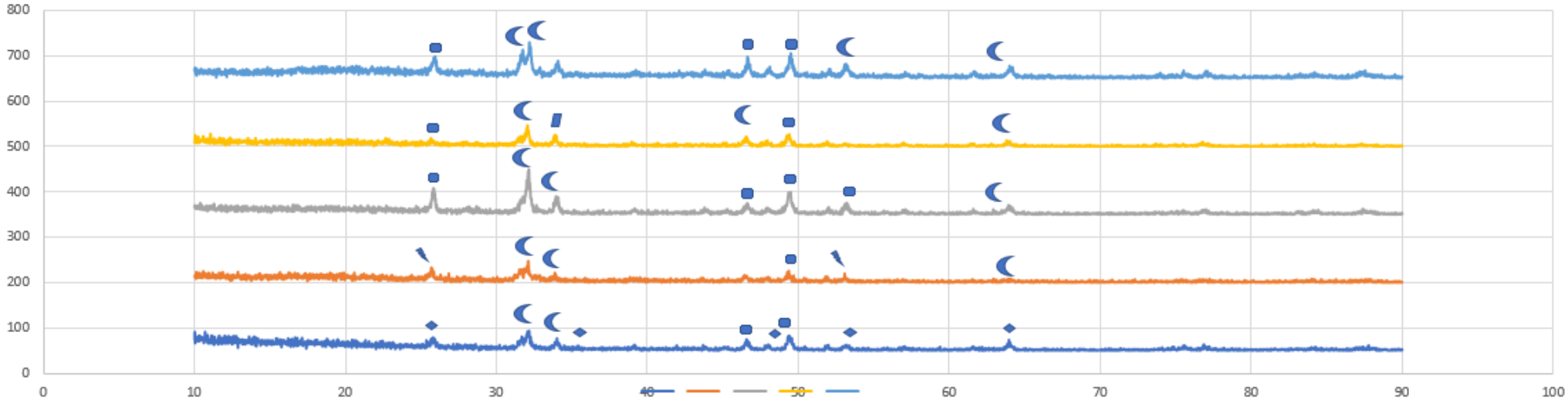
Table 15. Reference codes, chemical formulas, and mineral names of crystal phases dominant in XRD analysis of negative control samples

Reference Code	**Chemical Formula**	**Mineral Name**
00-003-0736	$Ca_5(PO_4)_3F$	Fluorapatite
00-001-1008	$Ca_{10}(OH)_2(PO_4)_6$	Hydroxyapatite



Graph 9. XRD analysis of negative control samples

HAP and FAP were predominantly observed on intact enamel surface, and inorganic structures like calcium phosphide and calcium phosphate carbonate with simpler and unstable structures formed on enamel surfaces with incipient caries lesions. Post-treatment XRD analyses revealed that HAP and FAP were predominantly observed in fluoride toothpaste groups, HAP was predominantly observed in the N-HAP toothpaste group, and CAS was predominantly observed in the bioactive glass toothpaste group **(**Graph 10).



Graph 10. XRD analyses of all treatment groups

HA =
FA =
CAS =
CFA =
CPC =

N-HAP =
BioC =
1450 F =
1000 F =
Water =

Hydroxyapatite = HAP
Fluorapatite = FAP
Calcium phosphate carbonate = CPC
Carbonated fluorapatite = CFA
Calcium silicate = CAS

5. DISCUSSION

This study aimed to measure the effects of toothpastes with different fluoride concentrations and toothpastes with different remineralization agents on artificial caries lesions and changes in surface microhardness, and to compare them by XRD analysis. Also, surface changes in enamel samples at baseline, post-demineralization, and post-remineralization were examined using SEM.

Demineralization is the loss of minerals in hard dental tissues that occurs by the plaque pH decreasing below the critical level, 5.5, due to acids produced by the fermentation of dietary carbohydrates by cariogenic microorganisms in MDP. Remineralization, on the other hand, is the re-precipitation of dissolved minerals with increased pH in the mouth. There is a balance between demineralization and remineralization in the mouth (2).

The increasing incidence of dental caries worldwide has given preventive dentistry practices further importance. Besides, *in vitro* and *in vivo* studies on using fluoride and other remineralization agents that can be alternatives to fluoride for preventing incipient caries lesions and their remineralization have gained momentum.

Emerging with advances in nano technology, nano-hydroxyapatite (N-HAP) is used in many areas in dentistry for being a biomimetic, bioactive, and biocompatible material (204). Examining the structure of artificially produced N-HAP crystals have revealed that they had chemical and structural similarities to enamel crystals. Also, *in vitro* research has shown N-HAP to have less solubility than other calcium phosphate compounds. Hence, nano-hydroxyapatites were developed to increase the solubility of hydroxyapatite and the levels of Ca and P ions released, which has been the main focus of remineralization studies (204).

Bordea et al. (2020) reported in their systematic review on using N-HAP in dentistry that its use was promising due to its physical, chemical, mechanical, and biological properties. N-HAP has already been used in many areas like coating titanium implants, eliminating dentin sensitivity, tissue engineering, bone regeneration, and preventing dental caries, and further *in vitro* and *in vivo* research is needed for its widespread use in dentistry (204). Thus, toothpaste containing N-HAP was selected for one of the treatment groups in this thesis to evaluate its remineralization effect on incipient caries lesions.

Bioactive glass is used in numerous areas of dentistry practices, including as bone graft material, for providing bone regeneration, pulpotomy and capping, eliminating dentin sensitivity, and providing remineralization in hard dental tissues (9). With their osteoconductive properties, they release the calcium, sodium, and phosphate ions within their structure and form the HCA layer, providing chemical bonding to the bone tissue. With these properties, bioactive glass can be used for the remineralization of hard dental tissues (9).

A number of *in vitro* studies have reported that bioactive glass can provide remineralization in demineralized enamel surfaces and increase surface microhardness compared to baseline (5,165,170–176).

As a new generation bioactive glass product, BioMin™ is distinguished from previous bioactive glass materials for containing higher phosphate and lower silica. It was first produced as a bone graft material. It has two forms as Biomin C and Biomin F. Biomin C does not contain fluoride and its active ingredient is calcium chloro-phosphosilicate. Biomin F does contain fluoride and its active ingredient is fluoro calcium phosphosilicate (166,167).

Our literature review revealed some studies on the new BioMin™ technology, mostly on eliminating dentin sensitivity (166,182,183,205,206). Research on providing remineralization in incipient caries lesions is limited, and those that exist are often on Biomin F (207,208). Therefore, in the present study, Biomin C toothpaste was chosen to investigate the effect of bioactive glass toothpaste on the remineralization of incipient caries lesions. .

Today, fluoride is considered the gold standard for preventing dental caries. It prevents demineralization and enhances remineralization. The continuous presence of fluoride in the mouth hampers MDP formation, inhibits microorganisms from metabolizing carbohydrates and producing acid, provides the formation of fluorapatite, and facilitates the precipitation of calcium and phosphate ions that are dissolved from tooth enamel during demineralization. Today, the consensus for preventing caries deems it more effective to have a certain concentration of fluoride in the mouth using topical applications (3). Fluoride toothpaste is the most widely used method for preventing dental caries and maintaining oral health (117).

Previous *in vitro*, *in situ*, and clinical studies have showed that toothpastes containing 1000 ppm fluoride are effective for preventing dental caries (209–211). ***Walsh et al. (2010)*** published a systematic review on Cochrane and reported that toothpastes containing high concentrations of fluoride (1000 ppm and above) should be preferred for preventing dental caries (212).

The Centers for Disease Control and Prevention (CDC) Guideline contains numerous studies on the effect of fluoride toothpaste for preventing dental caries in the United States and European countries, reporting that toothpastes containing 1500 ppm

fluoride are more effective in preventing dental caries and recommending using toothpastes with high-concentration fluoride based on individual needs in children and adolescents at high risk of caries (213).

The updated guideline by the European Academy of Pediatric Dentistry (EAPD) on the use of fluoride in children recommends the minimum fluoride concentration in toothpaste as 1000 ppm for children aged under 6 years and 1450 ppm for children aged 6 years and over (113).

In the light of this information, in this study, toothpastes containing 1000 ppm and 1450 ppm fluoride were preferred for the treatment groups.

Research on cariology in dentistry has often been carried out *in vitro*. Previous studies have reported numerous *in vitro* demineralization models with artificial caries lesions in enamel samples (214–218). Among these, the simplest method is an *in vitro* demineralization model created with acid buffers. In this model, buffer solutions contain acetic or lactic acid. While preparing the solutions, attention should be paid to the pH of the solution and its levels of calcium, phosphate, and fluoride ions. The prepared solution should have a pH of 4.5-5. Artificial caries lesions have a similar histological appearance with natural caries lesions. However, the lack of microorganisms and saliva and the inability to fully imitate the physiological demineralization-remineralization cycle raise concerns about the reliability of the findings of *in vitro* studies (214–216). Another disadvantage is that the excess fluoride released from the gel-like material accumulates on the tooth surface, inhibiting demineralization (217).

Another *in vitro* demineralization model uses acids produced by bacteria. This method involves keeping samples in a storage media and exposing them to organic acids

that are released by the fermentation of bacteria. Its disadvantages include the inability to monitor pH changes in the environment or the concentrations of calcium, phosphate, and fluoride ions (218).

The pH cycle *in vitro* demineralization model was first introduced by ***ten Cate et al.*** in 1982 (200). Today, it is well known that there is a demineralization and remineralization cycle in the mouth under physiological conditions. *In vitro* studies using this method expose samples to demineralization for 6 hours and to remineralization for 18 hours to mimic the physiological cycle in the mouth. This allows to create an environment that is close to the physiological conditions in the oral cavity, which is considered the most significant advantage of this method (219).

In studies using the *in vitro* demineralization model, factors like the levels of chemicals used in demineralization and remineralization solutions and the pH of the solutions, how much of the samples are exposed to the solution, the preparation process, the cycle regime, and the pH cycle can result in changes (219).

In vitro pH cycle models with longer demineralization than remineralization investigate the effect of fluoride on reducing demineralization; likewise, *in vitro* pH cycle models with longer remineralization than demineralization examine the effect of fluoride on increasing remineralization (220).

Based on this knowledge, for the pH cycle used here, the enamel samples were exposed to a demineralization solution with a pH of 4.5 for 6 hours and a remineralization solution with a pH of 7 for 18 hours. The solutions were renewed at regular intervals to prevent the content and the effect of the solutions from decreasing.

In vitro studies on incipient enamel lesions have often made use of extracted human teeth or bovine teeth (6,121,149,152,157,179,180,221,222). Bovine teeth are easier to obtain compared to human teeth and their inorganic content is straighter and more uniform. It is also more porous than human teeth, which allows the demineralization and remineralization cycle to occur faster. Considering this difference, here, bovine incisors were kept in the demineralization solution for 72 hours, as reported by ***Huang et al.*** (157). Bovine incisors are larger than human incisors in size, which facilitates the preparation of samples *in vitro*. Besides, artificial caries lesions in bovine teeth have a similar structure and mineral distribution to human teeth (219). Hence, bovine teeth are preferred over human teeth in many *in vitro* demineralization-remineralization studies (6,149,152,157,222). Thus, bovine teeth were preferred instead of extracted human teeth in the present study.

Saline solution containing 0.1% thymol is known to have an antimicrobial effect without any adverse effects on the histological structure of artificial demineralized surfaces (223). All the teeth used here were kept in a saline solution containing 0.1% thymol at +4°C in a refrigerator until the experiments, as reported by ***Huang et al. (2009)***, ***Mielczark et al. (2014)***, and ***Vyavhare et al. (2015)*** (121,156,157).

Clinically, toothpastes are diluted in the mouth, in proportion to secreted saliva. To mimic this under *in vitro* conditions, toothpastes are diluted in accordance with EN ISO 11609 (European standards for toothpaste mixture preparation) (203). In this study, the toothpastes were diluted with distilled water at a rate of 1:3, as reported by ***Itthagarun et al. (2010) and Mehta et al. (2011)***. The toothpastes were mixed using a magnetic stirrer for 3 minutes to obtain a homogeneous mixture (153,165).

An electric toothbrush was preferred so that brushing could mimic *in vivo* conditions. Previous *in vitro* studies have shown the ideal force for brushing to be 1.5 N, and forces between 1.5-3 N are acceptable without causing abrasion in hard dental tissues (138,222,224). The electric toothbrush used in this study could apply a force of 1.5-3 N and had a pressure warning feature for forces exceeding 3 N. All samples were brushed using a precision-headed toothbrush by the same applicator under *in vitro* conditions, keeping the bristles parallel to the sample surface and without applying any force. A separate brush head was used for each sample.

Phaneuf et al. were the first to report in **1962** that electric toothbrushes had a statistically significantly lower brushing force than manual brushes (225). ***Weijden et al (1996)*** further supported this in their research in the following years (226). In this thesis, an electric toothbrush was preferred to control the force on the enamel surfaces and to treat them without causing abrasion.

Yaacob et al. (2014) in a systematic review on the effects of electric and manual toothbrushes on oral health reported that electric toothbrushes were statistically significantly more effective in cleaning microbial dental plaque and maintaining oral and dental health in short-term use (227).

To prevent the electric toothbrush from losing power and to ensure equal performance across all samples, the toothbrush used here was charged every 100 minutes, as in ***Maden et al.*** (228).

Pamir et al. (2007) carried out an *in vitro* research where they reported that 10-minute brushing on a sample was equivalent to nearly 2 months of brushing twice a day for 2 minutes for an individual with 28 teeth (229). Accordingly, 14 days of brushing

twice a day for 2 minutes would correspond to about 12 months of brushing. Hence, the data obtained here can correspond to about 1 year of regular brushing.

There are numerous methods to examine post-demineralization and post-remineralization surface hardness and surface properties in tooth enamel *in vitro*. Research has shown that mineral gain or loss in hard dental tissues is directly associated with surface microhardness (77).

The surface microhardness (SMH) method was first used by ***Koulourides*** in demineralization and remineralization studies *in situ* (77). It is often preferred for analyzing the mineral content of teeth and similar hard tissues. It is done by measuring under a light microscope the notch on the sample surface after applying a certain force perpendicular to the surface for a certain period using a diamond tip. Knoop or Vickers diamond tips are used for these measurements. Knoop tips leave a deeper mark on the surface than Vickers tips on the same load. They both create the same diagonal depth, although different diagonal widths. The trace left by a Vickers diamond tip shows a square-shaped appearance while a Knoop diamond tip leaves a rhombic appearance. The surface microhardness of the material is inversely proportional to the size of the trace. A higher surface microhardness corresponds to a higher Vickers hardness number, meaning that the material is harder (63,64).

Featherstone et al. (1983) compared the methods of microradiography, which is accepted asthe gold standard today and surface microhardness for evaluating demineralization and remineralization *in vitro* and reported that both methods gave similar results for determining mineral changes in hard dental tissues, suggesting that either could be an alternative to the other (230).

Magalhaes et al. (2009) examined the correlation between surface microhardness and transversal microradiography (TMR) in artificial enamel lesions *in vitro* and found that SMH could be an alternative to TMR. They also reported that SMH could not give a clear evaluation about the mineral content of the lesion, but could give information about its mechanical properties. They suggested using different methods together to determine both the remineralization potential and mechanical properties of enamel lesions (231).

In the present study, surface microhardness of enamel samples from bovine incisors were measured at baseline, post-demineralization, and post-treatment between study and control groups using a surface microhardness device with a Vickers tip. The literature review revealed that measurements were made from five different points on each sample by applying 100 grams of force to the surface for 10 seconds in the previous studies (149,155,156,174). Therefore, in this thesis, all surface microhardness measurements were made from five different points on the tooth surface by applying 100 grams of force to the tooth surface for 10 seconds. Care was taken to place the enamel samples with their bottom and top surfaces parallel to each other on the SMH measurement device.

The measurements were made perpendicular to the outer surface of the tooth, so the surface had to be polished before measurements. Obtaining homogeneous and smooth surfaces has been reported to be significant in terms of the standardization of sample surfaces. Research also reports removing the fluoride-rich layer on the surface by polishing and allowing the sample to enter demineralization faster during the pH cycle (219). For this purpose, all samples examined here were polished using 600, 800, and 1200 grid silicon carbide abrasives and all samples were washed and dried to remove the residues on the surface after sanding, as in ***Comar et al. (2013)*** (6). ***Huang et al. (2009),***

Mielczark et al. (2014), and ***Oliveira et al. (2017)*** have reported that their samples were embedded in acrylic resin before sanding and polishing (156,157,222). Here, all enamel samples were embedded in acrylic resin placed in standard metal molds for processing at equal angles and amounts during grinding and polishing.

To obtain standardized enamel surfaces, 6x6 mm tapes were attached to the samples and all areas except the tape were covered with acid-resistant nail polish, as reported by ***Huang et al. (2011)*** (149).

SEM imaging is done by grinding high-voltage electrons to and scanning the tooth surface, collecting the interaction between the electron and the surface with the sensors, and reflecting them to a screen after passing them through signal amplifiers. SEM examinations can help observe the surface morphology and the changes on the surface, the elements in the composition of the surface, and even their atom arrangement (73,74).

Numerous studies on the effect of remineralization agents *in vitro* have observed demineralized areas, surface changes, and mineral deposits on sample surfaces using SEM (121,132,146,157,180,221,232). In the present study, SEM was preferred to visually assess the surface properties of enamel samples and surface changes occurring with mineral loss and regain after treatment with different toothpastes and in a control group.

In this thesis study, the post-demineralization SMH values of all enamel samples were found to be statistically significantly lower than baseline, as in a number of *in vitro* studies (121,146,149,152,153,155–157,233), which suggests that the content of the demineralization solution was effective in producing incipient carious lesions in bovine tooth enamel surfaces.

It was observed that the post-treatment surface microhardness values of all treatment groups failed to reach baseline values, as in ***Huang et al. (2009), Huang et al. (2010), Mielczarek et al. (2014)***, ***Swarup et al. (2012), Vyavhare et al. (2015), Ebadifer et al. (2017),*** and ***Alhussain et al. (2018)***. This indicates that complete remineralization could not be achieved in any of the groups (121,149,151,155–157,208). The treatment groups with the closest surface microhardness values to baseline were the 1450 ppm fluoride toothpaste and bioactive glass toothpaste groups, similar to ***Neto et al. (2009)***, ***Mony et al. (2015)***, and ***Wang et al. (2016)*** (174,234,235). The group with the highest difference between baseline and post-treatment surface microhardness was the negative control group that received no treatment, similar to ***Huang et al. (2009), Neto et al. (2009)***, ***Huang et al. (2010)***, and ***Vyavhare et al. (2015)*** (121,146,149,234).

The inability to obtain baseline SMH values after treatment in the treatment groups was thought to be due to not benefiting from the effects of saliva under physiological conditions. The buffering capacity of saliva increases the pH and the Ca and PO_4 ions dissolved from tooth enamel cause over-saturation, and remineralization occurs on demineralized crystal surfaces. Yet, it is known that stimulated saliva has a higher flow rate and is more effective in preventing dental caries (236).

Considering the SMH values of the negative control group (brushed) at baseline (342.14±6.37), post-demineralization (251.53±5.83), and post-treatment (209.31±5.20), post-treatment levels were found to be lower than post-demineralization values. It is believed that, despite keeping the negative control samples in the remineralization solution, demineralization continued, the ions in the solution diffused on sample surfaces, and no fluoride or similar remineralization agent was applied to these surfaces to prevent dissolution, which is why the post-treatment SMH values were the lowest. In this thesis

study, it was concluded that brushing with distilled water alone was insufficient in preventing demineralization, as reported by ***Stookey et al. (1993)*** (111).

The enamel samples prepared here were distributed randomly in the groups. There were significant differences between the groups in terms of baseline (p=0.009) and post-demineralization (p=0.037) SMH levels. This was associated with the fact that the bovine teeth were obtained from different animals and were of different ages and mineral density.

The SEM examinations revealed certain differences after demineralization compared to baseline surface morphology, like dissolution on the enamel surface, disoriented hydroxyapatite orientation, and presence of a porous structure. After applying the remineralization agents, it was seen that all groups except the negative control group had mineral gains, remineralization was achieved, but none of the surface morphologies were the same with the baseline, the closest being the 1450 ppm fluoride toothpaste group, as in ***Huang et al. (2009), Mony et al. (2015), Vyavhare (2015), Güçlü et al. (2017)***, and ***Chaudary et al. (2017)*** (157,174,180,237).

Examinations using surface microhardness device or SEM cannot yield information about the crystal structure at the mineral phase. Research reports that surface microhardness is sensitive for detecting early demineralization in hard dental tissues, while SEM helps examine the surface morphology (70,78).

The X-ray diffraction method, where X-rays sent to a crystal plane are reflected from the atomic planes, gives information about the geometric arrangement of the atoms of the crystals and their distances. This technique can help determine the distance between the atomic planes and the unit cell sizes of a crystal plane (79).

During diffraction, incoming X-rays reach the atomic planes below the crystal surface, which means that diffraction is not superficial. If an incoming X-ray beam meets an irregular crystal plane and hits it at any other angle, diffraction cannot occur. This is caused by the different path lengths of the reflected X-rays and the phase differences between then. This way, the rays coming to the surface interfere with each other, and no diffraction peak can be observed (79).

Remineralization studies pay great importance to the structure and properties of crystal phases formed by the ions that are deposited in hard dental tissues. Changes in a new crystal phase also affect the properties of that phase. Hence, research reports that an XRD analysis can help clarify the structural changes in the crystal phases and the properties of the crystal structure (79).

Research with XRD analysis of a new crystal phase formed on remineralized enamel surfaces is limited. Thus, in this study, an XRD analysis was done for a detailed examination of the new crystal phases formed by remineralization and use of toothpastes with different contents.

Different parts of enamel are known to have different content and phase structure. These can be easily analyzed by X-ray diffraction. Each element in the tooth structure and the phases formed by that element have different diffraction values. Accordingly, existing phases or those that occur later in the teeth can be detected *in vitro* and *in situ*. Here, no process was applied, and despite observing phases with different mineral content and structure in intact bovine incisors, the main phase structure was composed of hydroxyapatite. Some researchers have observed carbonated apatite or fluorapatite structures in natural tooth structure and suggested that this may be associated with the

amount of fluoride and carbon dioxide (CO_2) in water (238–240). Considering this information, the presence of fluorapatite structure in healthy bovine teeth used here was associated with the concentration of fluoride in the waters where the bovine lived.

According to the XRD analysis, FAP and HAP phases were predominant in intact enamel samples and calcium phosphate and calcium phosphate hydroxide phases were predominant in demineralized enamel samples. This indicates that most of the FAP and HAP compounds were dissolved within the demineralization solution, which they were kept in to form incipient caries. The decreased post-demineralization SMH values, the loss of orientation on demineralized enamel surfaces, and the presence of porosity on SEM support these findings.

When applied topically to tooth enamel, fluoride can be observed in either a loosely-bound structure like CaF_2 or in a firmly-bound structure like FAP. Some researchers have reported that fluoride varnishes with high fluoride concentrations primarily produce CaF_2 *in situ*, which may be a result of the protective effect of proteins in saliva, unlike under *in vitro* conditions (241).

Comar et al. (2017) evaluated the effect of titanium tetrafluoride (TiF_4) on intact and carious bovine teeth using XRD analysis *in vitro* and observed the predominance of HAP and CaF_2 in the NaF group. They reported that detecting CaF_2 in enamel samples treated with NaF was because of using artificial saliva and its rich Ca ion content. In this study, HAP, FAP, and similar structures were frequently observed on the XRD analysis of demineralized samples after treatment with fluoride toothpaste (1000 ppm NaF and 1450 ppm SMFP), but no CaF_2 was observed (242).

There was a statistically significant decrease from baseline SMH to post-treatment SMH in the demineralized enamel samples of the negative control group brushed with water alone (p=0.0001) and XRD analysis revealed mineral structures like calcium phosphate or calcium phosphate carbonate, which were less stable after demineralization. SMH were low and HAP and FAP-like structures were observed, which are more stable. It is believed that these phases (HAP and FAP) on the demineralized enamel surface are due to the precipitation of minerals during time in the remineralization solution within the pH cycle, although they did not increase the SMH value.

Huang et al. (2010) evaluated the effect of N-HAP and Galla Chinensis alone and in combination on incipient caries lesions in bovine tooth enamel using surface microhardness, polarized light microscope, XRD, and SEM *in vitro*. They reported that their combined use provided a statistically significant increase in SMH compared to their use alone, along with decreased surface porosity on SEM. Their XRD analyses revealed that HAP was predominantly present in the N-HAP groups, similar to our findings (146).

Cheng et al. (2010) examined the structures formed on demineralized bovine tooth enamel samples after remineralization with N-HAP using XRD and reported that HAP was predominant in the N-HAP group, as observed here (243).

Huang et al. (2011) evaluated the remineralization potential of N-HAP on incipient caries lesions in bovine teeth using surface microhardness, polarized light microscope, and XRD *in vitro* and observed that HAP was predominant after remineralization with N-HAP, as determined in the present study (149).

The Na and H ions in bioactive glass particles (sodium potassium silicate) change place and increase the pH of the environment. With increased pH, the Ca and P ions accumulate on the surface, forming a rich saturated layer of Ca ions on the surface (244).

In this study, calcium silicate was predominant on the XRD analysis of enamel samples with incipient caries lesions after 14 days of bioactive glass toothpaste treatment and HAP and FAP were also observed. Due to the lack of research on the chemical phases on enamel surfaces after remineralization with bioactive glass agents *in vitro*, no comparison could be made with our findings.

Kanwall et al. (2018) evaluated the dissolution of bioactive glass toothpastes and their effect in forming HAP-like structures in buffering solutions with different content *in vitro*. The authors reported that the presence of fluoride in bioactive glass toothpaste was effective in terms of forming FAP-like structures by remineralization (245). Unlike ***Kanwall et al.***, in this study, calcium silicate was observed to be predominant on XRD after treatment with toothpaste containing bioactive glass and no fluoride.

The literature review yielded a striking finding; studies often evaluate the remineralization potentials of toothpastes *in vitro*, but not the effect of brushing on demineralized enamel surfaces. Here, to assess the effect of brushing on demineralized enamel surfaces, half of the samples in the negative control group were brushed with distilled water alone using an electric toothbrush, while the other half was left untreated. After SMH measurements at the end of the 14-day pH cycle, there was no statistically significant difference between the SMH values of samples that were brushed with distilled water and those that were only kept in distilled water. SEM examinations revealed higher porosity on demineralized enamel surfaces in the brushed group, along

with a quite dispersed appearance on the enamel surface.

Addy et al. (2003) suggested that brushing would cause no abrasion on the tooth enamel, although some abrasion could be observed on the dentin surface. They also reported that this abrasion was mostly due to toothpastes and their abrasive content, and brushing teeth alone caused less abrasion. The authors highlighted that other factors affecting abrasion included the frequency and style of brushing, the structure of the bristles, and the use of electric or manual toothbrushes (246).

Lippert et al. (2004) reported that brushing leads to minor changes in the morphological and nanomechanical properties of softened enamel surfaces, also marking the significance of the lubricating effect of saliva for preventing abrasion on demineralized enamel surfaces (218).

Joiner et al. (2007) evaluated the natural protective effect of pellicle on enamel and dentin surfaces and found that pellicle formation significantly reduced abrasion (193).

Wiegand et al. (2007) examined the effect of brushing forces on abrasion on softened enamel surfaces and found that a brushing force of 4.5 N caused statistically significantly more abrasion than 1.5, 2, and 3.5 N (247).

This thesis study was carried out *in vitro*, hence we could not benefit from the protective effects of saliva and pellicle. Considering the SMH findings, there was no statistically significant difference between the brushed and non-brushed negative control subgroups in terms of mean SMH values. This can be explained by the brushing forces being controlled by an electric toothbrush and therefore remaining constant, and each sample being brushed by the same applicator with a separate brush head.

Emerging with the advances in nano technology, N-HAP is a biomimetic, bioactive, and biocompatible material that is used in dentistry to eliminate dentin sensitivity, prevent abrasion, and provide remineralization (204).

Regarding the remineralization effect of the N-HAP toothpaste used here, the post-demineralization SMH values of bovine teeth increased statistically significantly, although remaining below baseline, and complete remineralization could not be achieved.

Huang et al. (2009) evaluated the baseline remineralization effect of agents containing different concentrations of nano-hydroxyapatite (1%, 5%, 10%, 15%) on artificial incipient caries enamel samples and found that 10% nano-hydroxyapatite was the most effective concentration in terms of remineralization (157). Other studies after this research have also argued that a 10% is the most suitable concentration for nano-hydroxyapatite in terms of the remineralization of incipient caries lesions (158–160). The toothpaste used in this study also contained a 10% concentration of N-HAP.

In their study, ***Huang et al. (2009)*** reported that the solubility of N-HAP in the solution had a key role in the remineralization of demineralized surfaces over time. The authors remarked that by keeping N-HAP in the pH cycle for a shorter time, hydroxyapatite could not dissolve completely, failing to provide enough Ca^{2+} and $PO^{3-}{}_4$ ions to the environment and having a limited effect in providing remineralization (157). Based on this, the statistically significantly lesser increase in the SMH of N-HAP toothpaste compared to 1450 ppm fluoride toothpaste suggests that the increase observed with N-HAP after the 14-day pH cycle was insufficient in reaching the baseline values.

Huang et al. (2011) investigated the role of hydroxyapatite crystals in providing remineralization and their remineralization effect between micro or nano structures *in*

vitro and reported that nano-hydroxyapatite crystals provided better remineralization than micro-hydroxyapatite crystals, and that nano-hydroxyapatite crystals provided remineralization on the outer surface of the lesion, although with limited effect at the depths of the lesion. The authors found that the remineralization area of N-HAP on the outer surface of the lesion prevented ion transfer to deeper areas, failing to provide complete remineralization. They also suggested that micro-structured hydroxyapatite had limited effect in providing remineralization, resembling the apatite structure of tooth enamel, and a limited diffusion of Ca^{2+} and $PO^{3-}{}_4$ ions to the lesion (161). Similar to ***Huang et al.***, we found that N-HAP provided remineralization in the outer part of the lesion by increased post-treatment SMH and mineral filling on SEM.

The pH value of N-HAP in oral care products like toothpastes and mouthwashes varies between 5-9 and a pH of 6.8-7.5 is preferred to better mimic the physiological conditions of the mouth (248). The pH changes in an environment with N-HAP affect the physicochemical properties of N-HAP particles and their effect in providing remineralization (147).

Li et al. (2008) reported that N-HAP particles dissolved more under acidic conditions, increased ion release and penetration depth, showed better regain to demineralized surfaces, and had statistically significantly less mineral loss on demineralized surfaces (148).

Itthaguran et al. (2010) evaluated the effect of N-HAP (10%) and fluoride (950 ppm NaF) toothpastes on artificial caries lesions in human tooth enamel using microradiography *in vitro*. Both toothpastes were found to have a statistically significant effect in reducing lesion depth, although with no significant difference between them.

The authors concluded that toothpastes containing 10% of N-HAP can be recommended as an alternative to toothpastes containing 950 ppm fluoride (153). In parallel to their findings, in this study, the N-HAP (10%) toothpaste group and the 1000 ppm fluoride toothpaste group had a similar increase in post-treatment SMH values, with no statistically significant difference between the two groups.

Najibfard et al. (2011) examined the effect of toothpastes with different concentrations of N-HAP (5%, 10%) and a toothpaste with 1100 ppm of fluoride (NaF) on the remineralization of incipient caries lesions *in situ*. Lesion depth and mineral loss were measured by microradiography after 28 days of treatment. They found a statistically significant decrease in lesion depth and mineral loss across all groups, although no significant difference between the three toothpastes in terms of mineral gain (160). Unlike their research, an *in vitro* demineralization model was used in this thesis study. At the end of the 14-day pH cycle, the increase in SMH in the N-HAP toothpaste group was not statistically significant compared to the 1000 ppm fluoride group, and post-treatment mineral gain was observed in both groups on SEM.

Tschoppe et al. (2011) evaluated remineralization in bovine teeth with incipient caries lesions using toothpastes with 7%, 20%, and 24% N-HAP and 0.14% AmF by microradiography and found that N-HAP toothpastes provided statistically significantly more remineralization than the fluoride group, although with no significant difference among the N-HAP groups (152). Similar to their results, ***Swarup et al. (2012)*** reported that toothpaste containing 10% N-HAP provided statistically significantly more remineralization than fluoride toothpaste (2% NaF) (151).

Vyavhare et al. (2015) evaluated the effect of N-HAP (10%), CPP-ACP (10%), and NaF (1000 ppm) toothpastes on remineralization in artificial caries lesions in human tooth enamel after a 12-day pH cycle using SMH and SEM. The SMH values of the N-HAP and NaF groups on different days (days 3, 6, 9, and 12) were statistically significantly higher than the CPP-ACP and negative control groups (deionized water). Similar to the findings obtained here, there was no statistically significant difference between the N-HAP and fluoride groups in terms of SMH on days 3, 6, and 12. Also, post-treatment SEM images revealed mineral gain in the porosities on enamel and irregularities in surface morphology in the N-HAP (10%) and fluoride (1000 ppm NaF) groups after demineralization (121).

Ebadifar et al. (2017) evaluated the effect of N-HAP toothpaste on the SMH of artificial caries lesions in extracted human teeth *in vitro* and found that the combined use of N-HAP and fluoride (7% N-HAP, 1000 ppm NaF) provided statistically significantly higher remineralization than the group with fluoride alone (1000 ppm NaF) (155). Numerous other *in vitro* studies argue for the same finding, that the combined use of nano-hydroxyapatite and fluoride toothpastes create a synergistic effect, increasing remineralization potential on demineralized surfaces (6,154,156). This thesis, however, evaluated the remineralization effect of N-HAP alone.

Amechi et al. (2019) evaluated the remineralization potential of hydroxyapatite and 500 ppm fluoride (AmF) toothpastes on incipient caries *in situ* and found that 10% hydroxyapatite toothpaste had similar remineralization potential with 500 ppm fluoride toothpaste, with no statistically significant difference. They suggested that the 10% N-HAP toothpaste could be an alternative to 500 ppm fluoride (AmF) toothpaste (163). In the present study, the difference between the 10% N-HAP toothpaste and 1000 pmm

fluoride toothpaste after treatment was not statistically significant, again suggesting that 10% N-HAP toothpaste could be an alternative to 1000 ppm fluoride (NaF) toothpaste.

Burwell et al. (2009) compared the remineralization potentials of calcium sodium phosphosilicate toothpaste (Novamine) and fluoride toothpaste on bovine teeth with artificial incipient caries using SEM and SMH *in vitro*. SEM examinations revealed a relatively flat and uniform appearance on the enamel surface after treatment with bioactive glass toothpaste, indicating that bioactive glass could be a potential remineralization agent, with statistically significantly increased post-treatment SMH values after treatment. The authors also highlighted that the combined use of bioactive glass and fluoride had a synergistic effect, showing better effect on the remineralization of incipient caries lesions (177).

Gjorgievska et al. (2013) evaluated the effect of bioactive glass, hydroxyapatite, and fluoride (1040 ppm NaF) toothpastes on the remineralization of artificial incipient caries lesions in permanent molars using SEM-EDX *in vitro* and reported that bioactive glass and hydroxyapatite toothpastes increased Ca and PO_4 levels on the enamel surface and provided remineralization. They also found that the fluoride toothpaste groups provided higher remineralization and a higher Ca/PO_4 ratio (249). Similar to their findings, in this study, post-treatment SMH values were statistically significantly higher in the bioactive glass toothpaste group than the 1000 ppm fluoride toothpaste group, although with no statistically significant difference.

Narayana et al. (2014) examined the remineralization effect of oral dental health products containing bioactive glass (Novamine), CPP-ACP, CPP-ACPF, and fluoride (1000 ppm AmF) on artificial incipient lesions in molar teeth enamel and mineral gains

and losses after a 10-day pH cycle using SEM-EDS *in vitro* (5). They concluded that bioactive glass toothpaste provided statistically significantly higher remineralization and that it could be used in the treatment of incipient caries lesions by a minimally invasive approach, parallel to the findings obtained here.

Mony et al. (2015) also reported that 1450 ppm fluoride (SMFP) toothpaste had a statistically significantly higher remineralization potential than the bioactive glass toothpaste group *in vitro* (174). Their findings on SMFP and bioactive glass toothpastes are in parallel to the results reached in the present study.

Haghgo et al. (2016) compared the remineralization potentials of toothpastes containing bioactive glass (Novamine) and N-HAP on artificial incipient caries in primary incisors using Vickers SMH *in vitro* and reported that post-treatment SMH values were higher in the bioactive glass toothpaste (Novamine) group than the N-HAP toothpaste group, as determined here. Contrary to our findings, however, ***Haghgo et al.*** found the difference between the groups to be not significant (175).

Güçlü et al. (2017) evaluating the effects of bioactive glass, hydroxyapatite particles, and CPP-ACP complex on the remineralization of white spot lesions in permanent teeth using SEM-EDX *in vitro*. They concluded that bioactive glass and CPP-ACP could be effective on sub-surface lesions by releasing calcium and phosphate ions under neutral conditions, while HAP could not provide remineralization. They reported that, in acidic environment, dissolution of HAP led to ion release and remineralization in the lesion body. Bioactive glass and CPP-ACP covered the enamel surface, although with low remineralization potential on the lesion body (180).

Benjasuwantep et al. (2017) found that bioactive glass toothpaste had a similar remineralization potential with 500 ppm fluoride toothpaste *in vitro*, with no statistically significant difference between the two, unlike the findings obtained in the present study. Yet, they also reported that toothpaste containing bioactive glass had a statistically significantly lower remineralization effect compared to 1000 ppm fluoride toothpaste (179). In this study, the post-treatment SMH of the bioactive glass toothpaste was statistically significantly higher than the 1000 ppm fluoride toothpaste.

Gangwar et al. (2019) evaluated the remineralization potential of Novamine toothpaste on artificial incipient caries in primary teeth using Vickers SMH and SEM *in vitro* and reported that Novamine provided statistically significantly higher remineralization on the enamel surface following demineralization, with a very flat and uniform surface appearance on SEM (250). In this thesis, there was a statistically significant increase in post-treatment SMH compared to post-demineralization with the toothpaste containing bioactive glass. Still, these could not reach the baseline values, and the post-treatment porosity of the enamel surface was decreased on SEM, showing a flatter and relatively uniform appearance.

Manchery et al. (2019) evaluated the remineralization potential of bioactive glass (Novamine), N-HAP (10%), and fluoride (1450 ppm AmF) toothpastes on artificial incipient caries lesions in premolars after a 7-day pH cycle using polarized light microscopy *in vitro*. They observed that all three toothpastes provided remineralization in the lesions, with the N-HAP toothpaste providing statistically significantly higher remineralization compared to both. Besides, the fluoride toothpaste group provided more remineralization than the bioactive glass group, although with no statistically significant difference (184). However, in this study, the bioactive glass toothpaste group had a statistically significantly higher post-treatment SMH than the N-HAP group and a statistically significantly lower post-treatment

SMH than the 1450 ppm fluoride group. The difference between these findings is believed to stem from the use of different teeth, methods, and toothpaste brands.

Fluoride compounds in different formulations are known to have similar remineralization potentials; their effect on preventing tooth caries is associated with the concentration of fluoride in the content and increased concentrations are more effective in preventing tooth caries (251).

Twetman et al. (2003) conducted a systematic review where they reported fluoride toothpastes to be effective in preventing dental caries, remarking that 1500 ppm fluoride toothpaste was more effective than 1000 ppm fluoride toothpaste during primary dentition (105).

Hatipoğlu et al. (2019) assessed the effect of toothpastes with different fluoride concentrations on the surface microhardness of demineralized enamel surfaces *in vitro* and reported that the 1450 ppm fluoride toothpaste group provided a statistically significantly higher increase in SMH than the 1000 ppm fluoride toothpaste group (252).

Manosubsak et al. (2019) examined the effect of toothpastes with different fluoride concentrations (1000, 1450, and 5000 ppm) on SMH, fluoride content, and surface morphology changes in artificial incipient caries (SEM-EDS) *in vitro* and found that the 5000 ppm fluoride group provided statistically the highest increase in SMH, and unlike the findings reached here, no statistically significant difference between the 1000 and 1450 ppm fluoride toothpaste groups. Again, different from our findings, they observed quite similar changes in surface morphology between 1000 and 1450 ppm fluoride toothpaste groups on SEM after treatment (253).

The fluoride in fluoride toothpastes can be found in the form of chemical compounds like sodium fluoride (NaF), sodium mono fluorophosphate (SMFP), stannous fluoride (SnF_2), or combinations thereof. SMFP has been reported to have the ability to release fluoride ions by hydrolysis by its reaction with saliva enzymes. NaF can provide fluoride ions directly into saliva (111). NaF and SMFP reportedly have a similar effect on preventing dental caries, although some researchers highlight that SMFP has a limited remineralization effect *in vitro* (98,254,255).

In the present study, the 1450 ppm fluoride (SMFP) toothpaste group provided statistically significantly higher remineralization compared to 1000 ppm fluoride (NaF) after treatment, which indicates that SMFP can provide remineralization in demineralized enamel surfaces *in vitro*. Still, the higher remineralization potential of SMFP is believed to be related to the higher fluoride concentration in the SMFP toothpaste than the NaF toothpaste used in this research.

Accordingly, in this study, post-treatment SMH values were statistically the highest with the 1450 ppm fluoride toothpaste, and SEM examinations revealed a rather flat and uniform appearance on the enamel surface.

The present study aimed to evaluate and compare the effect of toothpastes with different concentrations of fluoride and alternative remineralization agents on the SMH and surface properties of artificial incipient caries *in vitro* and demonstrated statistically significantly higher post-demineralization SMH values across all treatment groups but the negative control group. The treatment group with the highest increase in post-treatment SMH was the 1450 ppm fluoride toothpaste group, followed by the bioactive glass toothpaste group, with statistically significant higher increase in SMH than the N-

HAP and 1000 ppm fluoride groups. The difference between the N-HAP and 1000 ppm fluoride toothpaste groups was not statistically significant. In the light of this information, we believe that fluoride toothpastes remain the best choice for preventing dental caries, with 1450 ppm fluoride toothpaste being more effective than 1000 ppm fluoride toothpaste, and bioactive glass and N-HAP toothpastes could be alternatives to fluoride toothpastes based on individual needs.

Limitations:

- The *in vitro* nature of the study and therefore not reflecting the physiological conditions of the mouth, particularly in terms of the cariogenic microorganisms in microbial dental plaque,
- Again, trying to imitate the saliva and plaque fluid in the mouth using demineralization and remineralization solutions, which only include inorganic components and not organic components like saliva proteins and enzymes that can perform enzymatic reactions,
- Also, the *in vitro* nature of the study did not allow to assess certain factors of dental caries, like individual variables, diet, and oral hygiene habits, as it is a multifactorial disease.

6. CONCLUSIONS

1. Surface microhardness values of incipient caries lesions obtained by keeping bovine teeth enamel samples in a demineralization solution for 72 hours were statistically significantly lower than baseline. It can be said that the content of the prepared demineralization solution and the time the samples were kept in the solution were effective in terms of forming incipient caries lesions in bovine teeth.

2. As a result of treating incipient caries lesions in enamel with toothpastes of different content for 14 days, statistically significant remineralization was achieved across all treatment groups, but post-treatment surface microhardness values failed to reach baseline values in all groups ($p=0.0001$). The groups closest to baseline surface microhardness values were the bioactive glass (Biomin C) and 1450 ppm fluoride (Colgate Triple Action) toothpaste groups, although with no statistically significant difference between them ($p>0.05$).

3. Remineralization could not be achieved in negative control groups, and post-treatment surface microhardness values obtained with distilled water treatment for 14 days were lower than post-demineralization surface microhardness values. Post-demineralization surface microhardness values were statistically significantly higher than post-remineralization surface microhardness values ($p=0.0001$)

4. This study also evaluated the effectiveness of brushing, and in this regard, no statistically significant difference was found between the samples of the two negative control groups ($p>0.05$). In light of this information, it was concluded that electric toothbrush, soft toothbrush bristles, and brushing with certain force made no difference on demineralized enamel surfaces.

5. After 14 days of pH cycle, the statistically significantly highest remineralization was found in the 1450 ppm fluoride group (Colgate Triple Action) across all experimental groups, and the statistically significantly least remineralization was observed in the negative control groups ($p=0.0001$) (brushed and unbrushed). There was no statistically significant difference between the negative control groups ($p>0.05$).

6. Mean post-treatment surface microhardness in the N-HAP group (Dentiste Premium & Natural White) was statistically significantly lower than the bioactive glass (Biomin C) and 1450 ppm fluoride groups (Colgate Triple Action) ($p=0.0001$).

7. Mean post-treatment surface microhardness in the 1000 ppm fluoride group (Signal kids toothpaste) was statistically significantly lower than the bioactive glass (Biomin C) and 1450 ppm fluoride groups (Colgate Triple Action) ($p=0.0001$).

8. Mean post-treatment surface microhardness in the bioactive glass group (Biomin C) was statistically significantly lower than the 1450 ppm fluoride group (Colgate Triple Action) ($p=0.003$), with no statistically significant difference between the other groups ($p>0.05$).

9. The fact that the bioactive glass group (Biomin C) had a higher mean post-treatment surface microhardness value than the 1000 ppm fluoride group suggests that toothpastes containing bioactive glass can be offered as an alternative to toothpastes containing 1000 ppm fluoride.

10. According to baseline SEM examinations, all enamel surfaces followed a normal anatomical enamel structure, with flat surface and undisturbed integrity. The enamel crystals on the enamel surface were observed to be in the shape of a keyhole.

11. Post-demineralization SEM examinations revealed that the orientation of the hydroxyapatite crystals was distorted, and the keyhole appearance of the enamel crystals

disappeared. The structure on the enamel surface was observed to be dispersed. Porosity on the enamel surface was increased, and due to the disturbance of the enamel prism, a honeycomb appearance was observed on the enamel surface.

12. Post-treatment SEM examinations revealed mineral filling and remineralization on enamel across all groups except the negative control group. Among the experimental groups, those with the highest remineralization and loss of porosity in the enamel and a rather uniform surface compared to the other groups were the bioactive glass (Biomin) and 1450 ppm fluoride (Colgate Triple Action) groups.

13. Post-treatment SEM examinations revealed that negative control enamel surfaces brushed with distilled water were slightly more dispersed than negative control surfaces that were only kept in distilled water. This suggests that brushing causes some disturbance on demineralized enamel surfaces.

14. Surface microhardness measurements and SEM examinations revealed that toothpastes with different active ingredients (N-HAP, bioactive glass, and fluoride) all provided remineralization.

15. Based on the XRD analysis findings, the presence of HA and FAP was observed predominantly on the intact enamel surfaces, and inorganic structures like calcium phosphide and calcium phosphate carbonate with simpler and unstable structure were formed on the enamel surfaces with incipient caries lesions.

16. According to post-treatment XRD analyses, HA and FAP were predominantly observed in the groups containing fluoride, HA was observed predominantly in the group containing N-HAP, and calcium silicate was observed predominantly in the group containing bioactive glass.

17. It was found that baseline, post-demineralization, and post-treatment measurements and analyses were correlated, and the images and findings obtained here supported each other.

18. Today, there is an increased momentum for *in vitro* and *in vivo* studies on fluoride agents and remineralization agents that could be alternatives to fluoride for preventing and remineralizing incipient caries lesions. Materials containing N-HAP and bioactive glass, although unable to provide remineralization as effective as the gold standard fluoride, are quite promising for the remineralization of incipient caries lesions. Further *in vitro* and *in vivo* research is needed to make a more precise determination on the effect of N-HAP and bioactive glass materials on incipient caries lesions.

7. REFERENCES

1. Featherstone JDB. The science and practice of caries prevention. J Am Dent Assoc. 2000 July;131(7):887–899.

2. Harris NO, García-Godoy F, Nathe CN. Primary preventive dentistry. 7th edition. NJ: Pearson; 2009. 24–72 p.

3. Groeneveld A, Van Eck AAMJ, Backer Dirks O. Fluoride in caries prevention: Is the effect pre- or post-eruptive? J Dent Res. 1990 Feb;69(2_suppl):751–755.

4. Featherstone JD, Doméjean S. The role of remineralizing and anticaries agents in caries management. Adv Dent Res. 2012 Aug;24(2):28–31.

5. Narayana SS, Deepa VK, Ahamed S, Sathish ES, Meyappan R, Satheesh Kumar K S. Remineralization efficiency of bioactive glass on artificially induced carious lesion an in-vitro study. J Indian Soc Pedod Prev Dent [serial online] 2014 Feb;32:19-25. Available from: https://www.jisppd.com/text.asp?2014/32/1/19/127047 DOI: 10.4103/0970-4388.127047

6. Comar LP, Souza BM, Gracindo LF, Buzalaf MAR, Magalhães AC. Impact of experimental nano-HAP pastes on bovine enamel and dentin submitted to a pH cycling model. Braz Dent J. 2013;24(3):273–278.

7. Enax J, Epple M. Synthetic hydroxyapatite as a biomimetic oral care agent. Oral Heal Prev Dent. 2018;16(1):7–19.

8. Vano M, Derchi G, Barone A, Pinna R, Usai P, Covani U. Reducing dentine hypersensitivity with nano-hydroxyapatite toothpaste: a double-blind randomized controlled trial. Clin Oral Investig. 2018 Jan;22(1):313–320.

9. Kulan M, Ulukapı İ. Diş Hekimliğinde Biyoaktif Camlar. Journal of İstanbul University Faculty of Dentistry. 2011;1(45):65–70.

10. Jones JR. Reprint of: Review of bioactive glass: From Hench to hybrids. Acta Biomater. 2015;23(S):S53–82. http://dx.doi.org/10.1016/j.actbio.2015.07.019

11. Hu YC, Zhong JP. Osteostimulation of bioglass. Chin Med J (Engl). 2009 Oct;122(19):2386–2389.

12. Kaur G, Pandey OP, Singh K, Homa D, Scott B, Pickrell G. A review of bioactive glasses: Their structure, properties, fabrication and apatite formation. J Biomed Mater Res - Part A. 2014 Jan;102(1):254–274.

13. Roberson TM, Heymann HO, Swift EJ. Sturdevant's Art and Science of Operative Dentistry: Cariology. 7th edition. Missouri; Elseiver Mosby: 2006. p. 96-98.

14. Nanci A. Ten Cate's Oral histology: Development, structure, and function. 9th ed. St Louis, Missouri: Elsevier; 2018. p. 1–11.

15. Fejerskov O, Nyvad O, Kidd EAM. Pathology of dental caries. In: Fejerskov O, Kidd EAM, editors. Dental caries: the disease and its clinical management 2nd ed. Oxford: Blackwell Munksgaard; 2008. p. 354–377.

16. Ameachi BT. Caries Managment by Modifying Diet. In: Meyer-Lückel H, Paris S, Ekstrand K. Caries Management–Science and Clinical Practice. New York: Thieme; 2013. p. 170.

17. Rozzi FR. Enamel structure and development and its application in hominid evolution and taxonomy. J Hum Evol. 1998; 35: 327-330.

18. A. Belcourt, S. Gillmeth. EDTA soluble protein of human mature normal enamel. Calcified tissue international. 1979 Dec;28(1):227-231.

19. Chiego DJ. Essentials of Oral Histology and Embryology: A Clinical Approach, 5th Edition. St Louis, Missouri: Elsevier; 2019. p. 178–195.

20. Lynch CD, O'Sullivan VR, Dockery P, McGillycuddy CT, Sloan AJ. Hunter-Schreger Band patterns in human tooth enamel. J Anat. 2010 July;217(2):106–115.

21. Ring ME. Dentistry: An illustrated history. New York: Harry N Abrams. Inc. & Mosby-Year Book, Inc; 1985. p. 100-105.

22. Robertson W. Diseases of the Teeth. Bost Med Surg J. 1835;12(6):91–94. DOI: 10.1056/NEJM183503180120602

23. Miller WD. The Micro-Organism of the Human Mouth. Basel: Karger Publishers; 1973. p. 68-101.

24. Fejerskov O, Nyvad O, Kidd EAM. Dental caries: what is it? In: Fejerskov O, Kidd EAM, editors. Dental caries: the disease and its clinical management 2nd ed. Oxford: Blackwell Munksgaard; 2008. p. 7-11.

25. Bowden GHW. The microbial ecology of dental caries. Microb Ecol Health Dis. 2000 July;12(3):138–148.

26. Harris NO, Segura A. The developing carious lesion. In: Harris NO, Garcia-Godoy F. Primary preventive dentistry 6th ed. New Jersey: Upper Saddle River; 2004. p. 45.

27. Selwitz RH, Ismail AI, Pitts NB. Dental caries. The Lancet. 2007 Jan;369(9555):51–59.

28. Carlson ER, Schlieve T. Salivary Gland Malignancies. Oral Maxillofac Surg Clin North Am. 2019 Feb;31(1):125-144.

29. Dodds MWJ, Johnson DA, Yeh CK. Health benefits of saliva: A review. J Dent. 2005 Mar;33(3):223–233.

30. Lagerlöf F, Oliveby A. Caries-protective factors in saliva. Adv Dent Res. 1994 July;8(2):229–38.

31. Rudney JD. Saliva and dental plaque. Adv Dent Res. 2000 Dec;14(1):29–39.

32. Van Houte J. Role of Micro-organisms in Caries Etiology "Use of Che-motherapeutic Agents for Caries. J Dent Res. 1994 Mar;73(3):672–681.

33. Forrest JL, Hujoel PP. Introduction to Evidence-Base Decision Making. In: Quirynen M, Teughels W, Haake SK NM. Carranza's Clinical Periodontology, 12th edition. St. Louis, Missiouri: Elsevier; 2015. p. 134–169.

34. Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. J Ind Microbiol. 1995 Sept;15(3):169–175.

35. Loesche WJ. Role of Streptococcus mutans in Human Dental Decay. Microbiol Rev. 1986 Dec;50(4):353–380.

36. Tenuta LMA, Cury JA. Fluoride: Its role in dentistry. Braz Oral Res. 2010;24(1):9–17.

37. Lembo FL, Longo PL, Ota-Tsuzuki C, Rodrigues CRMD, Mayer MPA. Genotypic and phenotypic analysis of Streptococcus mutans from different oral cavity sites of caries-free and caries-active children. Oral Microbiol Immunol. 2007 Aug;22(5):313–319.

38. De Abreu Da Silva Bastos VDA, Freitas-Fernandes LB, Da Silva Fidalgo TK, Martins C, Mattos CT, De Souza IPR. Mother-to-child transmission of Streptococcus mutans: A systematic review and meta-analysis. J Dent. 2015 Feb;43(2):181–191.

39. Martinez AR, Abranches J, Kajfasz JK, Lemos JA. Characterization of the Streptococcus sobrinus acid-stress response by interspecies microarrays and proteomics. Mol Oral Microbiol. 2010 Sept;25(5):331–342.

40. Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG, Galvin JL, et al. Molecular analysis of bacterial species associated with childhood caries. J Clin Microbiol. 2002;40(3):1001–1009.

41. Smiech-Slomkowska G, Jablonska-Zrobek J. The effect of oral health education on dental plaque development and the level of caries-related Streptococcus mutans and Lactobacillus spp. Eur J Orthod. 2007 Apr;29(2):157–160.

42. Kidd E, Fejerskov O. Esseentials of Dental Caries. 4th ed. New York: Oxford University Press; 2016. p. 7-10.

43. Burt BA, Eklund SA, Morgan KJ, Larkin FE, Guire KE, Brown LO, et al. The Effects of Sugars Intake and Frequency of Ingestion on Dental Caries Increment in a Three-year Longitudinal Study. J Dent Res. 1988 Nov;67(11):1422–1429.

44. Szpunar SM, Eklund SA, Burt BA. Sugar consumption and caries risk in schoolchildren with low caries experience. Community Dent Oral Epidemiol. 1995;June 23(3):142–146.

45. Krol DM. Dental caries, oral health, and pediatricians. Curr Probl Pediatr Adolesc Health Care. 2003 Sep;33(8):253–270.

46. Nelson DGA, Featherstone JDB, Duncan JF CT. Effect of carbonate and fluoride on the dissolution behaviour of synthetic apatites. Caries Res. 1983;17:200–211.

47. Hannig M, Hannig C. Nanomaterials in preventive dentistry. Nat Nanotechnol. 2010; 5(8):565–569.

48. Cury JA, Tenuta LMA. Enamel remineralization: Controlling the caries disease or treating early caries lesions? Braz Oral Res. 2009 Sept;23(1):23–30.

49. Ten Cate JM, Featherstone JDB. Mechanistic aspects of the interactions between fluoride and dental enamel. Crit Rev Oral Biol Med. 1991 July;2(3):283–296.

50. Ten Cate JM. Remineralization of caries lesions extending into dentin. J Dent Res. 2001 May;80(5):1407–1411.

51. Peters MC. Strategies for noninvasive demineralized tissue repair. Dent Clin 2010 July;54(3):507–525.

52. González-Cabezas C. The chemistry of caries: Remineralization and demineralization events with direct clinical relevance. Dent Clin North Am. 2010 July;54(3):469–478.

53. Li X, Wang J, Joiner A, Chang J. The remineralisation of enamel: A review of the literature. J Dent. 2014 Jun;42(1):12–20.

54. Çelik EU, Yazkan B, Katırcı G. Başlangiç Çürük Lezyonlarının Tedavisi. Atatürk Üniversitesi Diş Hekim Fakültesi Derg. 2011; 21(1):48–56.

55. Larsen MJ. The nature of early caries lesions in enamel. J Dent Res. 1986; July 65(7):1030–1031.

56. Holmen L, Thylstrup A, Øgaard B, Kragh F. A Scanning Electron Microscopic Study of Progressive Stages of Enamel Caries in vivo. Caries research. 1985;19(4):355–367.

57. Kudiyirickal MG, Ivancaková R. Early enamel lesion part I. Classification and detection. Acta Medica (Hradec Kralove). 2008;51(3):145–149.

58. Coolidge TB, Brightman V. Enamel caries-1957. J Am Dent Assoc. 1958 May; 56(5): 630-633.

59. Darling AI. Studies of the early caries lesion of enamel caries with transmitted light, polarized light and radiography. Br Dent J 1956; 101:289–297.

60. Mount GJ. Defining, classifying, and placing incipient caries lesions in perspective. Dent Clin North Am. 2005 Oct;49(4):701–723.

61. Pathak S, Roopa K, Poornima P, Neena I. White spot lesions: A literature review. J Pediatr Dent. 2015 Feb;3(1):1-7.

62. Pinkham JR. Pediatric Dentistry Infancy Through Adolescence, 4ed. Philadelphia: Elsevier Saunders ;2005. p. 199–203.

63. White DJ, Faller RV, Bowman WD. Demineralization and Remineralization Evaluation Techniques-Added Considerations. J Dent Res. 1992 Apr;71(3):929–933.

64. Arends J, ten Bosch JJ. Demineralization and remineralization evaluation techniques. J Dent Res. 1992 Apr;71:p 924-928.

65. Sønju Clasen AB, Øgaard B, Duschner H, Ruben J, Arends J, Sönju T. Caries development in fluoridated and non-fluoridated deciduous and permanent enamel in situ examined by microradiography and confocal laser scanning microscopy. Advances in dental research. 1997 Nov;11(4): 442-447.

66. Schlueter N, Hara A, Shellis RP, Ganss C. Supplementary Material for: Methods for the Measurement and Characterization of Erosion in Enamel and Dentine. Caries Res. 2011 May; 45(1):13-23.

67. Lombardini M, Ceci M, Colombo M, Bianchi S, Poggio C. Preventive effect of different toothpastes on enamel erosion: AFM and SEM studies. Scanning Microsc. 2014 Dec;36(4):401–410.

68. Zhang LL, Li JIY, Zhou XD, Cui FUZ, Li W. Effects of Galla chinensis on the surface topography of initial enamel carious lesion: An atomic force microscopy study. Scanning Microsc. 2009 Jan;31(5):195–203.

69. Manning RH, Edgar WM. Intra-oral models for studying de- and remineralization in man: methodology and measurement. J Dent Res. 1992 Apr;71(3):895–900.

70. Ten Bosch JJ, Angmar-Månsson B. Invited Review: A Review of Quantitative Methods for Studies of Mineral Content of Intra-oral Incipient Caries Lesions. J Dent Res. 1991 Jan;70(1):2–14.

71. Stookey GK. Optical Methods- quantitative light fluorescence. J Dent Res. 2004 July;83(1):84–88.

72. González-Cabezas C, Fontana M, Dunipace AJ, Li Y, Fischer GM, Proskin HM, et al. Measurement of Enamel Remineralization Using Microradiography and Confocal Microscopy: A Correlational Study. Caries Res. 1998 Sept;32(5):385–392.

73. McMullan D. Scanning electron microscopy 1928–1965. Scanning. 1995 May;17(3):175–185.

74. Yañez MJ, Barbosa SE. Changes in particle area measurements due to SEM accelerating voltage and magnification. Microsc Res Tech. 2003 July;61(5):463–468.

75. Saghiri MA, Asgar K, Lotfi M, Karamifar K, Saghiri AM, Neelakantan P, et al. Back-scattered and secondary electron images of scanning electron microscopy in dentistry: A new method for surface analysis. Acta Odontol Scand. 2012 Jan;70(6):603–609.

76. Paradella TC, Bottino MA. Scanning Electron Microscopy in modern dentistry research. Brazilian Dent Sci. 2012 Oct;15(2):43–48.

77. Wei SHY, Koulourides T. Electron Microprobe and Microhardness Studies of Enamel Remineralization. J Dent Res. 1972 Mar;51(2):648–651.

78. Şakar-Deliormanli A, Güden M. Microhardness and fracture toughness of dental materials by indentation method. Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 2006 Oct;76(2):257–264.

79. Xue J, Zhang L, Zou L, Liao Y, Li J, Xiao L, et al. High-resolution X-ray microdiffraction analysis of natural teeth. J Synchrotron Radiat. 2008;15(3):235–238.

80. Featherstone JDB. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol. 1999 Feb; 27(1):31-40.

81. Newbrun E. Topical fluorides in caries prevention and management: a North American perspective. J Dent Educ [Internet]. 2001 Oct;65(10):1078–1083. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11699980

82. Clark-Perry D, Levin L. Comparison of new formulas of stannous fluoride toothpastes with other commercially available fluoridated toothpastes: A systematic review and meta-analysis of randomised controlled trials. Int Dent J. 2020 Dec;70(6):418–426.

83. Svantun B, Gjermo P, Eriksen HM, Róslla G. A comparison of the plaque-inhibiting effect of stannous fluoride and chlorhexidine. Acta Odontol Scand. 1977 Jul;35(5):247–250.

84. Svatun B, Attramadal A. The effect of stannous fluoride on human plaque acidogenicity in situ (stephan curve). Acta Odontol Scand. 1978 Jun;36(4):211–218.

85. Ogard B, Seppä L, Rølla G. Professional topical fluoride applications-clinical efficacy and mechanism of action. Adv Dent Res. 1994 July;8(2):190–201.

86. Wiegand A, Magalhães AC, Attin T. Is titanium tetrafluoride (TiF_4) effective to prevent carious and erosive lesions? A review of the literature. Oral Heal Prev Dent. 2010 Apr;8(2):159-164.

87. Reed AJ, Bibby BG. Preliminary Report on Effect of Topical Applications of Titanium Tetrafluoride on Dental Caries. J Dent Res. 1976 May;55(3):357–358.

88. Büyükyilmaz T, Tangugsorn V, Øgaard B, Arends J, Ruben J, Rølla G. The effect of titanium tetrafluoride (TiF_4) application around orthodontic brackets. Am J Orthod Dentofac Orthop. 1994 Mar;105(3):293–296.

89. Naumova EA, Niemann N, Aretz L, Arnold WH. Effects of different amine fluoride concentrations on enamel remineralization. J Dent. 2012 Sept;40(9):750–755.

90. Arnold WH, Haase A, Hacklaender J, Gintner Z, Bánóczy J, Gaengler P. Effect of pH of amine fluoride containing toothpastes on enamel remineralization in vitro. BMC Oral Health. 2007 Oct;7(1):1-5.

91. Limeback H, Robinson C. Fluoride therapy Comprehensive preventive dentistry. New York: John Wiley & Sons; 2012. p. 251-282.

92. Johnston DW. Current status of professionally applied topical fluorides. Community Dent Oral Epidemiol. 1994 June;22(3):159–163.

93. Retief DH, Bradley EL, Holbrook MH, Switzer P. Enamel Fluoride Uptake, Distribution and Retention from Topical Fluoride Agents. Caries Res [Internet]. 1983;17(1):44–51. Available from: https://www.karger.com/Article/FullText/260647

94. Bruun C, Thylstrup A, Uribe E. Loosely bound fluoride extracted from natural carious lesions after topical application of APF in vitro: (Short communication). Caries Res. 1983;17(5):458–460.

95. Jiang H, Tai BJ, Du MQ, Peng B. Effect of professional application of APF foam on caries reduction in permanent first molars in 6-7-year-old children: 24-month clinical trial. J Dent. 2005 July;33(6):469–473.

96. Horst JA, Tanzer JM, Milgrom PM. Fluorides and Other Preventive Strategies for Tooth Decay. Dent Clin North Am. 2018 Apr;62(2):207–234.

97. Ripa LW. Review of the Anticaries Effectiveness of Professionally Applied and Self -applied Topical Fluoride Gels. J Public Health Dent. 1989 Dec;49(5):297–309.

98. Walsh T, Worthington HV, Glenny AM, Marinho VC, Jeroncic A. Fluoride toothpastes of different concentrations for preventing dental caries. Cochrane database of systematic reviews, 2019 Mar;2019(3). DOI: 10.1002/14651858.CD007868.pub3.

99. Petersson LG. fluoride mouthrinses and fluoride varnishes. Caries Res. 1993;27(1):35–42.

100. Marinho VCC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2013 July; 2013(7).

101. Donly KJ. Fluoride varnishes. J Calif Dent Assoc [Internet]. 2003 Mar;31(3):217–219. Available from: http://dx.doi.org/10.14219/jada.archive.2000.0232

102. Marinho VCC, Higgings JPT, Logan S, Sheiham A. Topical fluoride (toothpastes ,mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. Cochrane database of systematic reviews, 2003 Oct;2003(4).

103. Lippert F. An introduction to toothpaste - its purpose, history and ingredients. Toothpastes. 2013;23:1–14.

104. O'Mullane DM, Baez RJ, Jones S, Lennon MA, Petersen PE, Rugg-Gunn AJ, et al. Fluoride and oral health. Community Dent Health. 2016 June;33(2):69–99.

105. Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand. 2003 Jul;61(6):347–355.

106. Wright JT, Hanson N, Ristic H, Whall CW, Estrich CG, Zentz RR. Systematic review: Fluoride toothpaste efficacy and safety in children younger than 6 years: A systematic review. J Am Dent Assoc. 2014 Feb;145(2):182–189.

107. Twetman S. Caries prevention with fluoride toothpaste in children: an update. Eur Arch Paediatr Dent. 2009;10(3):162–167.

108. Haugejorden O, Nord A, Klock KS. Direct evidence concerning the "major role" of fluoride dentifrices in the caries decline: A 6-year analytical cohort study. Acta Odontol Scand. 1997 Jan;55(3):173–180.

109. Pollick H. The role of fluoride in the prevention of tooth decay. Pediatric Clinics. 2018 Oct;65(5):923-940.

110. Walsh T, Worthington HV, Glenny AM, Appelbe P, Marinho VC, Shi X. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2010 Jan 20;(1):CD007868.

111. Stookey GK, DePaola PF, Featherstone JBD, Fejerskov O, Möller IJ, Rotberg S, et al. A Critical Review of the Reltive Anticaries Efficacy of Sodium Fluoride and Sodium Monofluorophospate Dentifrices. Caries Res. 1993;27(4):337–360.

112. American Academy of Pediatric Dentistry. Policy on use of fluoride. The Reference Manual of Pediatric Dentistry. Pediatr Dent. 2018;40(6):49–50.

113. Toumba KJ, Twetman S, Splieth C, Parnell C, van Loveren C, Lygidakis N. Guidelines on the use of fluoride for caries prevention in children: an updated EAPD policy document. Eur Arch Paediatr Dent. 2019 Nov;20(6):507–516.

114. Brambilla E. Fluoride - Is It Capable of Fighting Old and New Dental Diseases? An Overview of Existing Fluoride Compounds and Their Clinical Applications. Caries Res. 2001;35(1):6–9.

115. Twetman S, Petersson LG, Axelsson S, Dahlgren H, Holm AK, Källestål C, et al. Caries-preventive effect of sodium fluoride mouthrinses: A systematic review of controlled clinical trials. Acta Odontol Scand. 2004 July;62(4):223–230.

116. Bidwell J. Fluoride mouthrinses for preventing dental caries in children and adolescents. Public Health Nurs. 2018 Jan;35(1):85–87.

117. Clarkson JJ, McLoughlin J. Role of fluoride in oral health promotion. Int Dent J. 2000 Jun;50(3):119–128.

118. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride, Community Dentistry and Oral Epidemiology. Community Dent Oral Epidemiol. 1999 Feb;27(1):31–40.

119. Chang HS, Walsh LJ, Freer TJ. Enamel demineralization during orthodontic treatment. Aetiology and prevention. Aust Dent J. 1997 Oct;42(5):322–327.

120. Sambunjak D, Nickerson JW, Pericic TP, Johnson TM, Imai P, Tugwell P, et al. Flossing for the management of periodontal diseases and dental caries in adults. Cochrane Database Syst Rev. 2011 Dec;12.

121. Vyavhare S, Sharma D, Kulkarni V. Effect of Three Different Pastes on Remineralization of Initial Enamel Lesion: An in Vitro Study . J Clin Pediatr Dent. 2015 Mar;39(2):149–160.

122. Grewal N, Gumber S, Kaur N. Comparative evaluation of enamel remineralization potential of processed cheese, calcium phosphate-based synthetic agent, and a fluoride-containing toothpaste: An in situ study. J Indian Soc Pedod Prev Dent. 2017 Jan;35(1):19–27.

123. Shetty S, Hegde MN, Bopanna TP. Enamel remineralization assessment after treatment with three different remineralizing agents using surface microhardness: An in vitro study. J Conserv Dent. 2014 Jan;17(1):49–52.

124. De Souza CC, Cury JLM, Coutinho TCL, Da Silva EM, Tostes MA. Effect of different application frequencies of CPP-ACP and fluoride dentifrice on Demineralized enamel: A laboratory study. Am J Dent. 2014 Aug;27(4):215–219.

125. Shen P, Walker GD, Yuan Y, Reynolds C, Stanton DP, Fernando JR, et al. Importance of bioavailable calcium in fluoride dentifrices for enamel remineralization. J Dent. 2018 Nov;78:59–64.

126. Danelon M, Takeshita EM, Sassaki KT, Carlos A, Delbem B. In situ evaluation of a low fluoride concentration gel with sodium trimetaphosphate in enamel remineralization. Am J Dent. 2013 Feb;26(1):15–20.

127. Favretto CO, Delbem ACB, Moraes JCS, Camargo ER, de Toledo PTA, Pedrini D. Dentinal tubule obliteration using toothpastes containing sodium trimetaphosphate microparticles or nanoparticles. Clin Oral Investig. 2018 Feb;22(9):3021–3029.

128. Sullivan RJ, Charig A, Blake-Haskins J, Zhang YP, Miller SM, Strannick M, et al. In Vivo Detection of Calcium From Dicalcium. Adv Dent Res. 1997 Nov;11(4):380–387.

129. Wefel JS, Harless JD. The Use of Saturated DCPD in Remineralization of Artificial Caries Lesions in vitro. J Dent Res. 1987 Nov;66(11):1640–1643.

130. Rirattanapong P, Vongsavan K, Saengsirinavin C, Waidee S. Enhancing remineralization of primary enamel lesions with fluoride dentifrice containing tricalcium phosphate. Southeast Asian J Trop Med Public Health. 2017 Mar;48(2):494–500.

131. Karlinsey RL, MacKey AC, Walker ER, Frederick KE. Surfactant-modified β-TCP: Structure, properties, and in vitro remineralization of subsurface enamel lesions. J Mater Sci Mater Med. 2010 Apr;21(7):2009–2020.

132. Elkassas D, Arafa A. Remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions. J Dent. 2014 Apr;42(4):466–474.

133. To-O K, Kamasaka H, Nishimura T, Kuriki T, Saeki S, Nakabou Y. Absorbability of calcium from calcium-bound phosphoryl oligosaccharides in comparison with that from various calcium compounds in the rat ligated jejunum loop. Biosci Biotechnol Biochem. 2003;67(8):1713–1718.

134. Hornby K, Ricketts SR, Philpotts CJ, Joiner A, Schemehorn B, Willson R. Enhanced enamel benefits from a novel toothpaste and dual phase gel containing calcium silicate and sodium phosphate salts. J Dent. 2014 June;42(1):39–45.

135. Amaechi BT, Mathews SM, Mensinkai PK. Effect of theobromine-containing toothpaste on dentin tubule occlusion in situ. Clin Oral Investig. 2014 Mar;19(1):109–116.

136. Kargul B, Özcan M, Peker S, Nakamoto T, Simmons WB, Falster AU. Evaluation of human enamel surfaces treated with theobromine: a pilot study. Oral Health Prev Dent. 2012;10(3):275–282.

137. Amaechi BT, Porteous N, Ramalingam K, Mensinkai PK, Ccahuana Vasquez RA, Sadeghpour A, et al. Remineralization of artificial enamel lesions by theobromine. Caries Res. 2013 Sept;47(5):399–405.

138. Qasthari AI, Irawan B, Herda E. The influence of brushing with theobromine and sodium monofluorophosphate toothpaste on enamel surface resistance to roughness after demineralization. J Phys Conf Ser. 2018;1073(3). doi :10.1088/1742-6596/1073/3/032005

139. Ranju MB, Girish MS, Prashanth S. Comparative evaluation of antimicrobial efficacy of theobromine toothpaste and a low-fluoridated toothpaste against plaque streptococcus mutans in children. World J Dent. 2018 July;9(3):215–219.

140. Kirkham J, Firth A, Vernals D, Boden N, Robinson C, Shore RC, et al. Self-assembling peptide scaffolds promote enamel remineralization. J Dent Res. 2007 May;86(5):426–430.

141. Deyhle H, Dziadowiec I, Kind L, Thalmann P, Schulz G, Müller B. Mineralization of Early Stage Carious Lesions In Vitro—A Quantitative Approach. Dent J. 2015 Oct;3(4):111–122.

142. Schmidlin P, Zobrist K, Attin T, Wegehaupt F. In vitro re-hardening of artificial enamel caries lesions using enamel matrix proteins or self-assembling peptides. J Appl Oral Sci. 2016 Feb;24(1):31–36.

143. Amaechi BT. Remineralization Therapies for Initial Caries Lesions. Curr Oral Heal Reports. 2015 Apr;2(2):95–101.

144. Cheng X, Xu P, Zhou X, Deng M, Cheng L, Li M, et al. Arginine promotes fluoride uptake into artificial carious lesions in vitro. Aust Dent J. 2015 Mar;60(1):104–111.

145. Bae JH, Kim YK, Myung SK. Desensitizing toothpaste versus placebo for dentin hypersensitivity: A systematic review and meta-analysis. J Clin Periodontol. 2015 Feb;42(2):131–141.

146. Huang S, Gao S, Cheng L, Yu H. Combined effects of nano-hydroxyapatite and Galla chinensis on remineralisation of initial enamel lesion in vitro. J Dent. 2010 Oct;38(10):811–819.

147. Harding IS, Rashid N, Hing KA. Surface charge and the effect of excess calcium ions on the hydroxyapatite surface. Biomaterials. 2005 Dec;26(34):6818–6826.

148. Li L, Pan H, Tao J, Xu X, Mao C, Gu X, et al. Repair of enamel by using hydroxyapatite nanoparticles as the building blocks. J Mater Chem. 2008 July;18(34):4079–4084.

149. Huang S, Gao S, Cheng L, Yu H. Remineralization potential of nano-hydroxyapatite on initial enamel lesions: An in vitro study. Caries Res. 2011 Nov;45(5):460–468.

150. Onuma K, Yamagishi K, Oyane A. Nucleation and growth of hydroxyapatite nanocrystals for nondestructive repair of early caries lesions. J Cryst Growth. 2005 Aug;282(1–2):199–207.

151. Rao A, Swarup Js. Enamel surface remineralization: Using synthetic nanohydroxyapatite. Contemp Clin Dent. 2012 Oct;3(4):433.

152. Tschoppe P, Zandim DL, Martus P, Kielbassa AM. Enamel and dentine remineralization by nano-hydroxyapatite toothpastes. J Dent. 2011 June;39(6):430–437.

153. Itthagarun A, Martyn King N, Cheung Y-M. The effect of nano - hydroxyapatite toothpaste on artificial enamel carious lesion progression : an in - vitro pH - cycling study. Hong Kong Dent J. 2010 June;7:61–66.

154. Mielczarek A, Gedrange T, Michalik J. An in vitro evaluation of the effect of fluoride products on white spot lesion remineralization. Am J Dent. 2015 Feb;28(1):51–56.

155. Ebadifar A, Nomani M, Fatemi SA. Effect of nano-hydroxyapatite toothpaste on microhardness of artificial carious lesions created on extracted teeth. J Dent Res Dent Clin Dent Prospects. 2017 Mar;11(1):14–17.

156. Mielczarek A, Michalik J. The effect of nano-hydroxyapatite toothpaste on enamel surface remineralization. An in vitro study. Am J Dent. 2014 Dec;27(6):287–290.

157. Huang SB, Gao SS, Yu HY. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. Biomed Mater. 2009 June;4(3):1–5.

158. King NM, Itthagarun A, Cheung M. Remineralization by Nanohydroxyapatite-Containing Dentifrice: a pH Cycling Study Using Slurry. J Dent Res. 2006;85(Special Issue B Brisbane Abstracts).

159. Itthagarun A, King NM, Cheung M. Remineralization Effects of Nanohydroxyapatite-Containing Dentifrice : a pH-Cycling Study Using Supernatant. J Dent Res, 2006;85:000-000.

160. Najibfard K, Chedjieu I, Ramalingam K, Amaechi BT. Remineralization of early caries by a nano-hydroxyapatite dentifrice. J Clin Dent. 2011;22(5):139–43.

161. Huang S, Gao S, Cheng L, Yu H. Remineralization potential of nano-hydroxyapatite on initial enamel lesions: An in vitro study. Caries Res. 2011 Nov;45(5):460–468.

162. Hegazy SA, Salama RI. Antiplaque and remineralizing effects of Biorepair mouthwash: A comparative clinical trial. Pediatr Dent J. 2016 Dec;26(3):89–94.

163. Amaechi BT, AbdulAzees PA, Alshareif DO, Shehata MA, Lima PP de CS, Abdollahi A, et al. Comparative efficacy of a hydroxyapatite and a fluoride toothpaste for prevention and remineralization of dental caries in children. BDJ Open. 2019 Dec 9;5(18):1-9.

164. Ceyhan T, Günay V, Capoğlu A, Sayrak H, Karaca C. Production and characterization of a glass-ceramic biomaterial and in vitro and in vivo evaluation of its biological effects. Acta Orthop Traumatol Turc. 2007;41(4):307–313.

165. Mehta AB, Kumari V, Jose R, Izadikhah V. Remineralization potential of bioactive glass and casein phosphopeptide-amorphous calcium phosphate on initial carious lesion: An in-vitro pH-cycling study. J Conserv Dent. 2014 Jan;17(1):3–7.

166. S. Ashwin, K. Swatika DNK. Comparative Evaluation of Desensitizing Efficacy of Dentifrice Containing 5% Fluoro Calcium Phosphosilicate versus 5% Calcium Sodium Phosphosilicate: A Randomized Controlled Clinical Trial. Contemp Clin Dent. 2018 July;9(3):330-336.

167. Reddy GV, Surakanti JR, Vemisetty H, Doranala S, Hanumanpally JR, Malgikar S. Comparative assessment of effectiveness of Biomin, NovaMin, herbal, and potassium nitrate desensitizing agents in the treatment of hypersensitive teeth: A clinical study. J NTR Univ Heal Sci. 2019 Apr;8(1):55–58.

168. Wefel JS. NovaMin®: likely clinical success. Adv Dent Res. 2009 July;21(1):40–43.

169. Alauddin SS. In vitro remineralization of human enamel with bioactive glass containing dentifrice using confocal microscopy and nanoindentation analysis for early caries defense [dissertation]. Gainesville, FL: University of Florida; 2004.

170. Milly H, Festy F, Andiappan M, Watson TF, Thompson I, Banerjee A. Surface pre-conditioning with bioactive glass air-abrasion can enhance enamel white spot lesion remineralization. Dent Mater. 2015 May;31(5):522–533.

171. Gjorgievska ES, Nicholson JW, Slipper IJ, Stevanovic MM. Remineralization of demineralized enamel by toothpastes: A scanning electron microscopy, energy dispersive X-ray analysis, and three-dimensional stereo-micrographic study. Microsc Microanal. 2013;19(3):587–595.

172. Wang Z, Jiang T, Sauro S, Pashley DH, Toledano M, Osorio R, et al. The dentine remineralization activity of a desensitizing bioactive glass-containing toothpaste: An in vitro study. Aust Dent J. 2011 Oct;56(4):372–381.

173. Alexander L, Kommi PB, Arani N, Hanumanth S, Kumar VV, Sabapathy RS. Evaluation of kinetic friction between regular and colored titanium molybdenum alloy archwires. Indian J Dent Res. 2018 Apr:29(2);212-216.

174. Mony S, Rao A, Shenoy R, Suprabha BS. Comparative evaluation of the remineralizing efficacy of calcium sodium phosphosilicate agent and fluoride based on quantitative and qualitative analysis. J Indian Soc Pedod Prev Dent. 2015 Sep;33(4):291–295.

175. Haghgoo R, Ahmadvand M, Moshaverinia S. Remineralizing effect of topical novamin and nanohydroxyapatite on caries-like lesions in primary teeth. J Contemp Dent Pract. 2016 Aug;17(8):645–649.

176. Vahid Golpayegani M, Sohrabi A, Biria M, Ansari G. Remineralization Effect of Topical NovaMin Versus Sodium Fluoride (1.1%) on Caries-Like Lesions in Permanent Teeth. J Dent (Tehran). 2012 Mar;9(1):68–75.

177. Burwell AK, Litkowski LJ, Greenspan DC. Calcium sodium phosphosilicate (NovaMin®): remineralization potential. Adv Dent Res. 2009 July;21(1):35–39.

178. Wang Y, Li X, Chang J, Wu C, Deng Y. Effect of tricalcium silicate (Ca_3SiO_5) bioactive material on reducing enamel demineralization: An in vitro pH-cycling study. J Dent. 2012 Dec;40(12):1119–1126.

179. Benjasuwantep P, Rirattanapong P, Vongsavan K. The remineralization effect of bioactive glass on enamel caries-like lesions in primary teeth. Southeast Asian J Trop Med Public Health. 2017 Sept;48(5):1127–1132.

180. Güçlü ZA, Gjorgievska ES, Coleman NJ. An in vitro comparison of the enamel remineralisation potential of bioactive glass, hydroxyapatite and CPP-ACP. Acta Phys Pol A. 2017 June;131(3):571–575.

181. Kulal R, Jayanti I, Sambashivaiah S, Bilchodmath S. An in-vitro comparison of nano hydroxyapatite, novamin and proargin desensitizing toothpastes -A SEM study. J Clin Diagnostic Res. 2016 Oct;10(10):ZC51–ZC544.

182. Da Cruz LPD, Hill RG, Chen X, Gillam DG. Dentine Tubule Occlusion by Novel Bioactive Glass-Based Toothpastes. Int J Dent. 2018 Apr;2018. https://doi.org/10.1155/2018/5701638

183. Pereira R, Dg G, Shaikh K, Phad S. Comparative Evaluation of Desensitizing Dentifrices containing BioMin® , Novamin® and Fluoride on Dentinal Tubule Occlusion before and after a Citric Acid Challenge – A scanning Electron Microscope in-vitro Study. J Odontol. 2018;2(1):1–6.

184. Manchery N, John J, Nagappan N, Subbiah GK, Premnath P. Remineralization potential of dentifrice containing nanohydroxyapatite on artificial carious lesions of enamel: A comparative in vitro study. Dent Res J (Isfahan). 2019 Sept;16(5):310–317.

185. Wülknitz P. Cleaning power and abrasitivity of European Toothpastes. Adv Dent Res. 1997 Nov;11(4):576–579.

186. Fischman SL. The history of oral hygiene products: How far have we come in 6000 years? Periodontol 2000. 1997 Nov;15(1):7–14.

187. Muhler JC, Radike AW, Nebergall WH, Day HG. Dentifrice on Caries Reduction in Children. J Dent Res. 1954 Oct;33(5);606–612.

188. Claydon NCA, Moran J, Bosma ML, Shirodaria S, Addy M, Newcombe R. Clinical study to compare the effectiveness of a test whitening toothpaste with a commercial whitening toothpaste at inhibiting dental stain. J Clin Periodontol. 2004 Nov;31(12):1088–1091.

189. Moore C, Addy M. Wear of dentine in vitro by toothpaste abrasives and detergents alone and combined. J Clin Periodontol. 2005 Nov;32(12):1242–1246.

190. Dyer D, Addy M, Newcombe RG: Studies in vitro of abrasion by different manual toothbrush heads and a standard toothpaste. J Clin Periodontol 2000 Dec; 27(2):99–103.

191. González-Cabezas C, Hara AT, Hefferren J, Lippert F. Abrasivity testing of dentifrices-Challenges and current state of the art. Monogr Oral Sci. 2013;23:100-107.

192. Forward GC, James AH, Barnett P, Jackson RJ. Gum health product formulations: What is in them and why? Periodontol 2000. 1997 Oct;15(1):32–39.

193. Joiner A, Schwarz A, Philpotts CJ, Cox TF, Huber K, Hannig M. The protective nature of pellicle towards toothpaste abrasion on enamel and dentine. J Dent. 2008 May;36(5):360–368.

194. Davies R, Scully C, Preston AJ. Dentifrices - An update. Med Oral Patol Oral Cir Bucal. 2010 Nov;15(6):976–982.

195. Takatsuka T, Exterkate RAM, Cate JM. Effects of Isomalt on enamel de- and remineralization, a combined in vitro pH-cycling model and in situ study. Clin Oral Investig. 2008 Dec;12(2):173–177.

196. Chunmuang S, Jitpukdeebodintra S, Chuenarrom C, Benjakul P. Effect of xylitol and fluoride on enamel erosion in vitro. J Oral Sci. 2007;49(4):293–297.

197. Davies RM, Ellwood RP, Davies GM. The rational use of fluoride toothpaste. Int J Dent Hyg. 2003 July;1(1):3–8.

198. Markowitz K, Pashley DH. Discovering new treatments for sensitive teeth: The long path from biology to therapy. J Oral Rehabil. 2008 Sept;35(4):300–315.

199. Altenburger MJ, Bernhart J, Schicha TD, Wrbas KT, Hellwig E. Comparison of in vitro fluoride uptake from whitening toothpastes and a conventional toothpaste in demineralised enamel. Schweiz Monatsschr Zahnmed. 2010 Jan;120(2):104–113.

200. ten Cate JM, Duijsters PPE. Influence of fluoride in solution on tooth demineralization. Caries Res. 1983;17(3):193-199.

201. Featherstone JDB, Glena R, Shariati M, Shields CP. Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. J Dent Res. 1990 Feb;69(2.):620–625.

202. Featherstone JDB, O'Reilly MM, Shariati M, Brugler S.Enhancement of remineralisation in vitro and in vivo. In: Leach SA, edıtor. Factors relating to demineralisation and remineralisation of the teeth. Oxford: IRL Press Ltd; 1986. p 23-34.

203. International Organization for Standardization. BS EN ISO 11609: BSI Standards Publication Dentistry-Dentifrices-Requirements, test methods and marking. Switzerland. 2017.

204. Bordea IR, Candrea S, Alexescu GT, Bran S, Băciuț M, Băciuț G, et al. Nano-hydroxyapatite use in dentistry: a systematic review. Drug Metab Rev. 2020 May;52(2):319–332.

205. Hussain KA, Kadiyala S, Lakshmi AY, Hindumathi M. Fetal transcerebellar diameter to abdominal circumference ratio (TCD/AC) and to femur length ratio (TCD/FL) in the assessment of normal fetal growth. J NTR Univ Heal Sci. 2019 Apr;8(1):5–10.

206. Gautam V, Halwai HK. Comparison of clinical efficacy of four dentifrices in the management of dentinal hypersensitivity. Int J Sci Res. 2017 Feb;6(2):239-240.

207. Bakry AS, Abbassy MA, Alharkan HF, Basuhail S, Al-Ghamdi K, Hill R. A novel fluoride containing bioactive glass paste is capable of re-mineralizing early caries lesions. Materials. 2018 Sept;11(9):1636.

208. Alhussain A, Alhaddad A, Ghazwi M, Farooq I. Remineralization of artificial carious lesions using a novel fluoride incorporated bioactive glass dentifrice. Dent Med Probl. 2018 Nov 23;55(4):379–382.

209. White DJ. Reactivity of Fluoride Dentifrices with Artificial Caries. Caries Res. 1987;21(2):126–140.

210. Paes Leme AF, Machado Tabchoury CP, Zero DT, Cury JA. Effect of fluoridated dentifrice and acidulated phosphate fluoride application on early artificial carious lesions. Am J Dent. 2003 Apr;16(2):91–95.

211. Mensinkai PK, Ccahuana-Vasquez RA, Chedjieu I, Amaechi BT, Mackey AC, Walker TJ, et al. In situ remineralization of white-spot enamel lesions by 500 and 1,100 ppm F dentifrices. Clin Oral Investig. 2012 July;16(4):1007–1014.

212. Walsh T, Worthington HV, Glenny AM, Appelbe P, Marinho VC, Shi X. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2010 Jan 20;(1):CD007868.

213. Kohn WG, Maas WR, Maltviz DM, Presson SM, Shaddix KK. Recommendations for using fluoride to prevent and control dental caries in the United States.MMWR Recommendations and Reports. 2001 Aug;50(RR-14).

214. Featherstone JDB, Mellberg JR. Relative Rates of Progress of Artificial Carious Lesions in Bovine, Ovine and Human Enamel. Caries Res. 1981;15(1):109–114.

215. Ten Cate JM. In Situ Models, Physico-Chemical Aspects. Adv Dent Res. 1994 Jul;8(2):125–133.

216. Featherstone JDB, Rodgers BE. Effect of Acetic, Lactic and other Organic Acids on the Formation of Artificial Carious Lesions. Caries Res. 1981;15(5):377–385.

217. Tsanidis V, Koulourides T. An in vitro Model for Assessment of Fluoride Uptake from Glass-ionomer Cements by Dentin and its Effect on Acid Resistance. J Dent Res. 1992 Jan;71(1):7–12.

218. Yu OY, Zhao IS, Mei ML, Lo EC-M, Chu C-H. A Review of the Common Models Used in Mechanistic Studies on Demineralization-Remineralization for Cariology Research. Dent J. 2017 Jun;5(2):20.

219. Buzalaf MAR, Hannas AR, Magalhães AC, Rios D, Honório HM, Delbem ACB. Ph-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: Strengths and limitations. J Appl Oral Sci. 2010 Aug;18(4):316–334.

220. Tenuta LMA, Cury JA. Laboratory and Human Studies to Estimate Anticaries Efficacy of Fluoride Toothpastes. Toothpastes. 2013: 23;108–124.

221. Wang Y, Mei L, Gong L, Li J, He S, Ji Y, et al. Remineralization of early enamel caries lesions using different bioactive elements containing toothpastes: An in vitro study. Technol Heal Care. 2016 Sept;24(5):701–711.

222. Esteves-Oliveira M, Santos NM, Meyer-Lueckel H, Wierichs RJ, Rodrigues JA. Caries-preventive effect of anti-erosive and nano-hydroxyapatite-containing toothpastes in vitro. Clin Oral Investig. 2017 Mar;21(1):291–300.

223. Marsh PD, Nyvad B. The oral microflora and biofilms on teeth. In: Fejerskov O, Kidd EAM, editors. The dental caries. The disease and its clinical managment. 2nd ed. Oxford: Blackwell; 2009. p. 163-168.

224. Suryana M, Irawan B, Soufyan A. The Effects of Toothpastes Containing Theobromine and Hydroxyapatite on Enamel Microhardness after Immersion in Carbonated Drink. J Phys Conf Ser. 2018 Aug;1073(3):032010.

225. Phaneuf EA, Harrıngton JH, Dale PP, Shklar G. Automatic toothbrush: a new reciprocating action. J Am Dent Assoc. 1962 Jul;65(1):12–25.

226. Van der Weijden GA, Timmerman MF, Reijerse E, Snoek CM, Van der Welden U. Toothbrushing force in relation to plaque removal. J Clin Periodontol. 1996 Aug;23(8):724–729.

227. Yaacob M, Worthington HV, Deacon SA, Deery C, Walmsley AD, Robinson PG, et al. Powered versus manual toothbrushing for oral health. Cochrane Database Syst Rev. 2014 June;(6).

228. Maden EA, Altun C, Polat GG, Basak F. The In vitro Evaluation of the effect of xyliwhite, probiotic, and the conventional toothpastes on the enamel roughness and microhardness. Niger J Clin Pract. 2018 Mar;21(3):306–311.

229. Pamir T, Korkut ZO, Tezel H, Timur K, Özata, F. Aşındırıcılık değerleri farklı beyazlatıcı diş macunlarının kompozit rezinlerin yüzey pürüzlülüğü ve mikrosertliğine etkilerinin incelenmesi. Acta Odontol Turc. 2007; 24(2):89-95.

230. Featherstone JDB, ten Cate JM, Shariati M, Arends J. Comparison of Artificial Caries-Like Lesions by Quantitative Microradiography and Microhardness Profiles. Caries Res [Internet]. 1983;17(5):385–391. Available from: https://www.karger.com/Article/FullText/260692

231. Magalhães AC, Moron BM, Comar LP, Wiegand A, Buchalla W, Buzalaf MAR. Comparison of cross-sectional hardness and transverse microradiography of artificial carious enamel lesions induced by different demineralising solutions and gels. Caries Res. 2009 Dec;43(6):474–483.

232. Amaechi BT, Porteous N, Ramalingam K, Mensinkai PK, Ccahuana Vasquez RA, Sadeghpour A, et al. Remineralization of artificial enamel lesions by theobromine. Caries Res. 2013 Sept;47(5):399–405.

233. Talaat DM, Abdelrahman AAM, Abdelaziz RH, Nagy D. Effect of two remineralizing agents on initial caries-like lesions in young permanent teeth: An in vitro study. J Contemp Dent Pract. 2018 Oct;19(10):1181–1188.

234. Neto FCR, Maeda FA, Turssi CP, Serra MC. Potential agents to control enamel caries-like lesions. J Dent. 2009 Oct;37(10):786–790.

235. Wang Y, Mei L, Gong L, Li J, He S, Ji Y, et al. Remineralization of early enamel caries lesions using different bioactive elements containing toothpastes: An in vitro study. Technol Heal Care. 2016 Sept;24(5):701–711.

236. Stookey GK. The effect of saliva on dental caries. J Am Dent Assoc. 2008 May;139(5):11S-17S.

237. Chaudhary I, Tripathi AM, Yadav G, Saha S. Effect of Casein Phosphopeptide–amorphous Calcium Phosphate and Calcium Sodium Phosphosilicate on Artificial Carious Lesions: An in vitro Study. Int J Clin Pediatr Dent. 2017 Feb;10(3):261–266.

238. LeGeros RZ, Sakae T, Bautista C, Retino M, LeGeros JP. Magnesium and carbonate in enamel and synthetic apatites. Adv Dent Res. 1996 Nov;10(2):225–231.

239. Mathew M, Takagi S. Structures of biological minerals in dental research. J Res Natl Inst Stand Technol. 2001 Nov;106(6):1035.

240. Ten Gate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. Eur J Oral Sci. 2007 Oct;105(5):461–465.

241. Lippert F, Hara AT, Martinez-Mier EA, Zero DT. In vitro caries lesion rehardening and enamel fluoride uptake from fluoride varnishes as a function of application mode. Am J Dent. 2013;26(2):81–85.

242. Comar LP, Souza BM, Al-Ahj LP, Martins J, Grizzo LT, Piasentim IS, et al. Mechanism of Action of TiF_4 on Dental Enamel Surface: SEM/EDX, KOH-Soluble F, and X-Ray Diffraction Analysis. Caries Res. 2018 Jan;51(6):554–567.

243. Cheng L, Li J, Hao Y, Zhou X. Effect of compounds of Galla chinensis on remineralization of enamel surface in vitro. Arch Oral Biol. 2010 June;55(6):435–440.

244. Khijmatgar S, Reddy U, John S, Badavannavar AN, D Souza T. Is there evidence for Novamin application in remineralization?: A Systematic review. J Oral Biol Craniofacial Res. 2020 Apr;10(2):87–92.

245. Kanwal N, Brauer DS, Earl J, Wilson RM, Karpukhina N, Hill RG. In-vitro apatite formation capacity of a bioactive glass - containing toothpaste. J Dent [Internet]. 2018 Jan;68:51–58.

246. Addy M, Hunter ML. Can tooth brushing damage your health? Effects on oral and dental tissues. Int Dent J. 2003 Sept;53(3):177–186.

247. Wiegand A, Köwing L, Attin T. Impact of brushing force on abrasion of acid-softened and sound enamel. Arch Oral Biol. 2007 Nov;52(11):1043–1047.

248. Olsson C, Emilson CG, Birkhed D. An in vitro study of fluoride release from a resin-modified glass ionomer cement after exposure to toothpaste slurries of different pH. Clin Oral Investig. 2000 Dec;4(4):233–237.

249. Gjorgievska ES, Nicholson JW, Slipper IJ, Stevanovic MM. Remineralization of demineralized enamel by toothpastes: A scanning electron microscopy, energy dispersive X-ray analysis, and three-dimensional stereo-micrographic study. Microsc Microanal. 2013;19(3):587–595.

250. Gangwar A, Jha K, Thakur J, Nath M. In Vitro evaluation of remineralization potential of novamin on artificially induced carious lesions in primary teeth using scanning electron microscope and vickers hardness. Indian J Dent Res [Internet]. 2019 Nov;30(4):590.

251. Sicca C, Bobbio E, Quartuccio N, Nicolò G, & Cistaro A. (2016). Prevention of dental caries: A review of effective treatments. Journal of clinical and experimental dentistry. 2016 Dec;8(5): e604-e610.

252. Hatipoğlu Z, Özbay Yavlal G, Kargül B. Effects of Different Fluoride-containing Toothpastes on In Vitro Enamel Remineralization. Bezmialem Science 2019;7(1):12-17.

253. Manosubsak N, Sukarawan W, Sriarj W. The remineralization quality by fluoridated dentifrice on artificial incipient caries lesion. Mahidol Dent J. 2019 Apr;39(1):53–62.

254. Toda S, Featherstone JD. Effects of fluoride dentifrices on enamel lesion formation. J Dent Res. 2008 Mar;87(3):224–227.

255. Mellberg JR, Chomicki WG, Mallon DE, Castrovince LA. Remineralization in vivo of artificial caries lesions by a monofluorophosphate dentifrice. Caries Res. 1985;19(2):126–135.

8. ANNEXES

ANNEX 1: Reference Catalog for Calcium Phosphide 00-016-0728

Name and formula

Reference code: 00-016-0728

PDF index name: Calcium Phosphide

Empirical formula: CaP
Chemical formula: CaP

Crystallographic parameters

Crystal system: Unknown

RIR: -

Subfiles and Quality

Subfiles: Inorganic
Alloy, metal or intermetalic
Quality: Doubtful (O)

Comments

Sample preparation: Prepared by heating Ca (PH_2)$_2$ at 600 C for 4 hours.
Additional pattern: To replace 3-682.
Reason "O" quality: O assigned because unindexed.

References

Primary reference: Pytlewski, Nixon., *Inorg. Chem.*, **2**, 763, (1963)

Peak list

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1				4,06000	21,874	4,0
2				3,26000	27,335	6,0
3				3,02000	29,555	4,0
4				2,88000	31,027	100,0
5				2,67000	33,537	4,0
6				2,37000	37,934	2,0
7				2,10000	43,038	20,0
8				1,98000	45,790	30,0
9				1,77000	51,596	4,0
10				1,63000	56,403	12,0
11				1,44000	64,678	4,0
12				1,32000	71,403	6,0
13				1,26000	75,374	4,0
14				1,24000	76,809	4,0

ANNEX 2: Reference Catalog for Calcium Phosphate 00-035-0180

Name and formula

Reference code:	00-035-0180
PDF index name:	Calcium Phosphate Carbonate
Empirical formula:	$CCa_{10}O_{27}P_6$
Chemical formula:	$Ca_{10}(PO_4)_6CO_3$

Crystallographic parameters

Crystal system:	Monoclinic
Space group:	Pa
Space group number:	7
a (Å):	9,5294
b (Å):	19,1000
c (Å):	6,8601
Alpha (°):	90,0000
Beta (°):	89,9700
Gamma (°):	90,0000
Volume of cell (10^6 pm^3):	1248,62
RIR:	-

Subfiles and Quality

Subfiles:	Inorganic
Quality:	Blank (B)

Comments

General comments:	A Guinier camera with CoKa radiation was also used. Cell parameters generated by least squares refinement.
Structure:	Structure similar to apatite.
Unit cell:	Reference reports: a=9.5571(3), b=19.114(2), c=6.8716, g=120.36.

References

Primary reference:	Elliott, J. et al., *J. Appl. Crystallogr.*, **13**, 618, (1980)

Peak list

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	1	1	0	8,24332	10,724	14,0
2	0	0	1	6,84714	12,919	1,0
3	1	3	0	5,27269	16,801	5,0
4	0	4	0	4,77086	18,583	1,0
5	2	1	0	4,58414	19,347	10,0
6	-1	3	1	4,12760	21,511	2,0
7	0	4	1	3,91861	22,673	7,0
8	2	-1	1	3,80537	23,358	40,0
9	1	5	0	3,54172	25,124	3,0
10	0	0	2	3,43056	25,952	35,0
11	-2	3	1	3,32074	26,826	1,0
12	3	1	0	3,12807	28,512	17,0
13	-3	2	0	3,00975	29,658	20,0
14	2	5	0	2,98165	29,944	20,0
15	0	6	1	2,92986	30,486	10,0
16	3	3	0	2,83746	31,504	100,0
17	-2	0	2	2,77871	32,188	70,0
18	-2	1	2	2,74698	32,570	50,0
19	0	7	0	2,72759	32,808	2,0
20	3	4	0	2,64294	33,890	19,0
21	0	5	2	2,56247	34,988	4,0
22	0	7	1	2,52805	35,480	1,0
23	2	6	1	2,47017	36,340	10,0
24	2	7	0	2,37007	37,933	1,0
25	-1	8	0	2,31592	38,855	2,0
26	0	0	3	2,29245	39,269	19,0
27	0	1	3	2,26521	39,761	13,0

ANNEX 3: Reference Catalog for Fluorapatite 00-012-0261

Name and formula

Reference code:	00-012-0261
Mineral name:	Fluorapatite
PDF index name:	Calcium Fluoride Phosphate
Empirical formula:	$Ca_5FO_{12}P_3$
Chemical formula:	$Ca_5F(PO_4)_3$

Crystallographic parameters

Crystal system:	Hexagonal
a (Å):	9,4240
b (Å):	9,4240
c (Å):	6,8880
Alpha (°):	90,0000
Beta (°):	90,0000
Gamma (°):	120,0000
Calculated density (g/cm^3):	3,16
Volume of cell (10^6 pm^3):	529,78
Z:	2,00
RIR:	-

Status, subfiles and quality

Status:	Marked as deleted by ICDD
Subfiles:	Inorganic Mineral
Quality:	Blank (B)

Comments

Deleted by:	Deleted by Post Parcel of May 10, 1963 (for 15-876).

References

Primary reference:	Carobbi, Mazzi., *Atti Accad. Naz. Lincei, Cl. Sci. Fis., Mat. Nat., Rend.*, **5**, 159, (1959)

Peak list

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	1	0	0	8,21000	10,767	16,0
2	2	0	0	4,09000	21,712	10,0
3	1	1	1	3,90000	22,783	8,0
4	2	0	1	3,52000	25,281	4,0
5	0	0	2	3,45000	25,803	4,0
6	2	1	0	3,09000	28,871	18,0
7	2	1	1	2,81600	31,751	100,0
8	1	1	2	2,78700	32,090	30,0
9	3	0	0	2,72200	32,878	100,0
10	2	0	2	2,63400	34,009	10,0
11	3	0	1	2,53300	35,409	4,0
12	2	1	2	2,30000	39,135	2,0
13	3	1	0	2,26400	39,783	35,0
14	3	1	1	2,15000	41,989	10,0
15	2	2	2	1,94300	46,713	16,0
16	3	1	2	1,89000	48,104	8,0
17	2	1	3	1,84000	49,498	10,0
18	3	2	1	1,80500	50,524	12,0
19	4	1	0	1,77900	51,316	25,0
20	3	0	3	1,75400	52,102	8,0
21	0	0	4	1,72100	53,178	4,0
22	2	2	3	1,64300	55,918	4,0

ANNEX 4: Reference Catalog for Hydroxylapatite 01-073-1731

Name and formula

Reference code:	01-073-1731
Mineral name:	Hydroxylapatite
ICSD name:	Calcium Phosphate Hydroxide
Empirical formula:	$Ca_5HO_{13}P_3$
Chemical formula:	$Ca_5 (PO_4)_3 (OH)$

Crystallographic parameters

Crystal system:	Hexagonal
Space group:	P63/m
Space group number:	176
a (Å):	9,4000
b (Å):	9,4000
c (Å):	6,9300
Alpha (°):	90,0000
Beta (°):	90,0000
Gamma (°):	120,0000
Calculated density (g/cm^3):	3,14
Measured density (g/cm^3):	3,08
Volume of cell (10^6 pm^3):	530,30
Z:	2,00
RIR:	1,09

Subfiles and Quality

Subfiles:	Inorganic Mineral ICSD Pattern
Quality:	Calculated (C)

Comments

Additional pattern:	See PDF 9-432 and PDF 84-1998.
ICSD collection code:	024240
Test from ICSD:	Calc. density unusual but tolerable. No R value given. At least one TF missing.

References

Primary reference:	*Calculated from ICSD using POWD-12++*, (1997)
Structure:	Hendricks, S.B., Jefferson, M.E., Mosley, V.M., *Z. Kristallogr., Kristallgeom., Kristallphys., Kristallchem.*, **81**, 352, (1932)

Peak list

Peak list

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	1	0	0	8,14064	10,859	21,0
2	1	0	1	5,27689	16,788	13,4
3	1	1	0	4,70000	18,866	0,3
4	2	0	0	4,07032	21,818	12,2
5	1	1	1	3,88979	22,844	1,6
6	2	0	1	3,50971	25,357	8,0
7	0	0	2	3,46500	25,689	37,2
8	1	0	2	3,18821	27,963	7,7
9	2	1	0	3,07687	28,997	21,2
10	2	1	1	2,81215	31,795	100,0
11	1	1	2	2,78900	32,066	38,4
12	3	0	0	2,71355	32,983	49,3
13	2	0	2	2,63845	33,950	23,5
14	3	0	1	2,52675	35,499	5,2
15	2	2	0	2,35000	38,269	0,2
16	2	1	2	2,30071	39,122	3,4
17	1	3	0	2,25781	39,897	20,2
18	2	2	1	2,22552	40,501	0,3
19	1	3	1	2,14674	42,056	7,4
20	3	0	2	2,13639	42,269	3,1
21	1	1	3	2,07314	43,624	5,7
22	4	0	0	2,03516	44,481	1,1
23	2	0	3	2,00901	45,092	1,6
24	4	0	1	1,95270	46,467	2,9
25	2	2	2	1,94489	46,665	30,2
26	3	1	2	1,89166	48,059	15,2
27	2	3	0	1,86759	48,719	7,1
28	2	1	3	1,84732	49,288	34,1
29	2	3	1	1,80326	50,576	14,5
30	1	4	0	1,77643	51,395	13,9
31	4	0	2	1,75485	52,074	10,7
32	0	0	4	1,73250	52,798	15,9
33	1	0	4	1,69455	54,075	0,6
34	3	2	2	1,64400	55,881	11,2
35	1	1	4	1,62558	56,571	0,6
36	1	3	3	1,61465	56,988	2,4
37	2	0	4	1,59410	57,792	0,2
38	5	0	1	1,58497	58,156	0,5
39	1	4	2	1,58079	58,325	0,3
40	3	3	0	1,56667	58,902	0,1
41	2	4	0	1,53844	60,093	3,5
42	3	3	1	1,52810	60,542	2,5
43	2	1	4	1,50964	61,362	2,7
44	2	4	1	1,50187	61,714	1,0
45	5	0	2	1,47356	63,033	5,8
46	3	0	4	1,46025	63,675	6,6
47	3	2	3	1,45232	64,064	5,6
48	5	1	1	1,43061	65,155	6,9
49	3	3	2	1,42753	65,313	5,8
50	1	4	3	1,40819	66,325	0,7
51	4	2	2	1,40608	66,437	0,8
52	2	2	4	1,39450	67,061	0,1

52	2	2	4	1,39450	67,061	0,1
53	1	3	4	1,37448	68,171	0,4
54	1	0	5	1,36634	68,634	0,2
55	6	0	0	1,35677	69,186	0,1
56	1	5	2	1,34709	69,755	2,5
57	3	4	0	1,33831	70,280	0,5
58	6	0	1	1,33080	70,736	0,7
59	4	0	4	1,31922	71,451	0,4
60	4	3	1	1,31403	71,777	2,4
61	5	2	0	1,30355	72,445	2,8
62	2	4	3	1,28046	73,966	2,5
63	2	3	4	1,27014	74,669	2,4
64	2	1	5	1,26371	75,115	4,7
65	3	4	2	1,24843	76,197	3,9
66	1	4	4	1,24030	76,787	6,8
67	5	1	3	1,23543	77,145	4,0
68	1	6	1	1,22198	78,155	2,9
69	2	5	2	1,22006	78,301	6,3
70	2	2	5	1,19383	80,366	0,1
71	5	0	4	1,18644	80,971	0,1
72	3	1	5	1,18120	81,405	0,9
73	4	4	0	1,17500	81,926	2,6
74	1	6	2	1,16869	82,465	0,8
75	7	0	0	1,16295	82,961	0,4
76	3	3	4	1,16202	83,042	0,3
77	4	3	3	1,15801	83,394	2,2
78	0	0	6	1,15500	83,661	2,3
79	2	4	4	1,15036	84,075	2,1
80	7	0	1	1,14691	84,386	1,3
81	4	0	5	1,14557	84,508	0,4
82	1	0	6	1,14355	84,692	0,5
83	5	2	3	1,13526	85,457	0,5
84	6	2	0	1,12890	86,054	0,2
85	1	1	6	1,12163	86,750	2,3
86	5	1	4	1,11738	87,162	1,7
87	6	2	1	1,11422	87,472	1,1
88	2	3	5	1,11299	87,593	3,1
89	7	0	2	1,10251	88,642	2,2
90	1	6	3	1,09352	89,566	1,4

ANNEX 5: Reference Catalog for Fluorapatite 00-003-0736

Name and formula

Reference code:	00-003-0736
Mineral name:	Fluorapatite
PDF index name:	Calcium Fluoride Phosphate
Empirical formula:	$Ca_5FO_{12}P_3$
Chemical formula:	$Ca_5(PO_4)_3F$

Crystallographic parameters

Crystal system:	Hexagonal
Space group:	P63/m
Space group number:	176
a (Å):	9,3800
b (Å):	9,3800
c (Å):	6,8900
Alpha (°):	90,0000
Beta (°):	90,0000
Gamma (°):	120,0000
Calculated density (g/cm^3):	3,19
Measured density (g/cm^3):	3,18
Volume of cell (10^6 pm^3):	525,00
Z:	1,00
RIR:	-

Status, subfiles and quality

Status:	Marked as deleted by ICDD
Subfiles:	Inorganic Mineral
Quality:	Blank (B)

Comments

Deleted by:	Berry parcel February 25, 1964, for 15-876.
Color:	Green
Sample source:	Specimen from Faraday Tw., Ontario, Canada.
Optical data:	A=1.630, B=1.634, Sign=-
Melting point:	1680

References

Primary reference:	McConnell, D., *Am. Mineral.*, **22**, 981, (1937)
Unit cell:	Bragg., *"Atomic Structure of Minerals"*

Peak list

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	0	0	2	3,44000	25,879	20,0
2	2	1	0	3,07000	29,063	30,0
3	2	1	1	2,81000	31,820	100,0
4	1	1	2	2,78000	32,173	40,0
5	3	0	0	2,71000	33,027	60,0
6	2	0	2	2,63000	34,062	30,0
7	3	0	1	2,53000	35,452	5,0
8	2	1	2	2,30000	39,135	5,0
9	3	1	0	2,26000	39,856	20,0
10	3	1	1	2,14000	42,195	10,0
11	1	1	3	2,06000	43,917	10,0
12	2	0	3	2,00000	45,306	5,0
13	2	2	2	1,94000	46,789	40,0
14	3	1	2	1,89000	48,104	10,0
15	2	1	3	1,84000	49,498	60,0
16	3	2	1	1,80000	50,674	30,0
17	4	1	0	1,77000	51,596	30,0
18	4	0	2	1,75000	52,230	30,0
19	0	0	4	1,72000	53,212	30,0
20	2	2	3	1,64000	56,029	10,0
21	3	1	3	1,61000	57,168	5,0
22	4	2	0	1,54000	60,026	5,0
23	4	0	3	1,52000	60,899	5,0
24	4	2	1	1,50000	61,799	10,0
25	5	0	2	1,47000	63,204	20,0
26	5	1	0	1,46000	63,687	10,0
27	3	2	3	1,45000	64,179	10,0
28	5	1	1	1,43000	65,186	10,0

ANNEX 6: Reference Catalog for Hydroxyapatite 00-001-1008

Name and formula

Reference code:	00-001-1008
Mineral name:	Hydroxyapatite
PDF index name:	Calcium Hydroxide Phosphate
Empirical formula:	$Ca_{10}H_2O_{26}P_6$
Chemical formula:	$Ca_{10}(OH)_2(PO_4)_6$
Second chemical formula:	$10CaO \cdot 3P_2O_3 \cdot H_2O$

Crystallographic parameters

Crystal system:	Unknown
Measured density (g/cm^3):	3,08
RIR:	-

Status, subfiles and quality

Status:	Marked as deleted by ICDD
Subfiles:	Inorganic Mineral Pharmaceutical
Quality:	Blank (B)

Comments

Deleted by:	Berry Parcel of July 31, 1958.
Color:	Colorless
General comments:	Reference reports hexagonal.
Optical data:	A=1.6413, B=1.6452, Sign=-
Melting point:	1400d

References

Primary reference:	Hanawalt. et al., *Anal. Chem.*, **10**, 475, (1938)

Peak list

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1				3,44000	25,879	40,0
2				3,11000	28,681	8,0
3				2,79000	32,054	100,0
4				2,62000	34,196	8,0
5				2,27000	39,673	16,0
6				2,13000	42,402	4,0
7				2,06000	43,917	4,0
8				1,94000	46,789	20,0
9				1,84000	49,498	20,0
10				1,71000	53,547	16,0
11				1,45000	64,179	8,0
12				1,31000	72,033	8,0
13				1,24000	76,809	4,0
14				1,11000	87,889	8,0

ANNEX 7: Reference Catalog for Calcium Silicate 01-087-1259

Name and formula

Reference code:	01-087-1259
ICSD name:	Calcium Silicate
Empirical formula:	Ca_2O_4Si
Chemical formula:	$Ca_2 (SiO_4)$

Crystallographic parameters

Crystal system:	Orthorhombic
Space group:	Pnma
Space group number:	62
a (Å):	6,8709
b (Å):	5,6010
c (Å):	9,5563
Alpha (°):	90,0000
Beta (°):	90,0000
Gamma (°):	90,0000
Calculated density (g/cm^3):	3,11
Volume of cell (10^6 pm^3):	367,76
Z:	4,00
RIR:	0,91

Status, subfiles and quality

Status:	Diffraction data collected at non ambient temperature
Subfiles:	Inorganic ICSD Pattern
Quality:	Calculated (C)

Comments

ICSD collection code:	082997

References

Primary reference:	*Calculated from ICSD using POWD-12++*, (1997)
Structure:	Mumme, W., Cranswick, L., Chakoumakos, B., *Neues Jahrb. Mineral., Abh.*, **170**, 171, (1996)

Peak list

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	1	0	1	5,57864	15,874	1,4
2	0	0	2	4,77815	18,555	3,7
3	1	1	1	3,95257	22,476	3,6
4	1	0	2	3,92284	22,649	3,9
5	2	0	0	3,43545	25,914	8,9
6	2	0	1	3,23289	27,569	2,8
7	1	1	2	3,21314	27,742	9,8
8	2	1	0	2,92847	30,501	15,9
9	1	0	3	2,88996	30,917	8,4
10	0	2	0	2,79995	31,937	100,0
11	2	0	2	2,78932	32,062	65,3
12	0	1	3	2,76895	32,305	74,7
13	1	1	3	2,56824	34,907	0,8
14	1	2	1	2,50283	35,850	0,8
15	2	1	2	2,49683	35,939	1,7
16	0	2	2	2,41609	37,183	10,6
17	0	0	4	2,38908	37,619	0,9
18	2	0	3	2,33583	38,510	17,1
19	1	2	2	2,27928	39,505	8,0

19	1	2	2	2,27928	39,505	8,0
20	1	0	4	2,25656	39,920	9,0
21	3	0	1	2,22723	40,468	19,7
22	2	2	0	2,17066	41,571	1,3
23	2	1	3	2,15587	41,869	0,2
24	2	2	1	2,11674	42,681	4,7
25	1	1	4	2,09307	43,188	11,1
26	3	1	1	2,06960	43,702	3,1
27	3	0	2	2,06530	43,798	7,3
28	1	2	3	2,01114	45,041	5,1
29	2	2	2	1,97629	45,880	17,3
30	2	0	4	1,96142	46,248	4,2
31	3	1	2	1,93776	46,847	0,2
32	3	0	3	1,85955	48,943	0,5
33	2	1	4	1,85119	49,179	1,2
34	1	0	5	1,84135	49,459	3,2
35	0	2	4	1,81756	50,151	0,2
36	0	1	5	1,80885	50,409	2,2
37	2	2	3	1,79378	50,863	9,2
38	1	3	1	1,77048	51,581	0,1
39	3	1	3	1,76482	51,758	0,1
40	1	2	4	1,75712	52,002	0,2
41	3	2	1	1,74317	52,450	4,1
42	4	0	0	1,71773	53,287	2,4
43	4	0	1	1,69063	54,211	1,1
44	1	3	2	1,68581	54,379	1,0
45	2	0	5	1,67019	54,930	2,5
46	3	2	2	1,66218	55,217	0,4
47	3	0	4	1,65330	55,539	0,1
48	4	1	0	1,64223	55,946	1,6
49	2	3	0	1,64041	56,014	1,8
50	2	3	1	1,61676	56,907	3,8
51	0	3	3	1,61073	57,140	6,4
52	2	2	4	1,60657	57,301	4,7
53	0	0	6	1,59272	57,847	1,5
54	3	1	4	1,58567	58,128	4,2
55	1	3	3	1,56821	58,839	0,1
56	4	1	2	1,55306	59,470	1,6
57	3	2	3	1,54914	59,636	1,0
58	1	2	5	1,53857	60,087	1,6
59	4	0	3	1,51191	61,259	2,2
60	1	1	6	1,49526	62,016	0,6
61	3	0	5	1,46743	63,327	0,4
62	4	2	0	1,46423	63,482	0,4
63	4	1	3	1,45967	63,703	1,9
64	4	2	1	1,44734	64,311	2,4
65	2	0	6	1,44498	64,429	1,6
66	1	3	4	1,43848	64,755	0,5
67	2	2	5	1,43446	64,959	0,9
68	3	3	1	1,43080	65,146	0,8
69	3	2	4	1,42371	65,510	1,0
70	3	1	5	1,41952	65,728	1,1
71	0	4	0	1,40025	66,750	2,3
72	3	3	2	1,38447	67,612	2,3
73	5	0	1	1,36019	68,988	0,1
74	1	4	1	1,35720	69,161	0,8
75	4	1	4	1,35334	69,387	0,9
76	0	4	2	1,34374	69,954	0,1
77	1	0	7	1,33901	70,238	0,2
78	0	3	5	1,33554	70,447	0,4
79	4	2	3	1,33041	70,760	2,1
80	0	1	7	1,32636	71,008	1,4
81	5	1	1	1,32177	71,293	0,9
82	5	0	2	1,32065	71,362	0,9
83	1	4	2	1,31876	71,480	0,9
84	1	3	5	1,31100	71,969	0,3
85	3	0	6	1,30761	72,185	0,2
86	1	1	7	1,30231	72,525	0,1
87	3	2	5	1,29980	72,688	0,2
88	2	4	0	1,29668	72,891	0,7
89	2	4	1	1,28412	73,721	1,9
90	4	0	5	1,27758	74,161	0,7

90	4	0	5	1,27758	74,161	0,7
91	3	1	6	1,27337	74,448	0,4
92	2	0	7	1,26869	74,769	0,1
93	4	3	0	1,26410	75,087	0,4
94	5	0	3	1,26178	75,249	0,2
95	4	3	1	1,25318	75,857	0,1
96	2	4	2	1,25142	75,982	0,2
97	4	2	4	1,24842	76,198	0,1
98	4	1	5	1,24479	76,460	0,1
99	3	3	4	1,23775	76,974	0,4
100	5	1	3	1,23093	77,480	0,1
101	5	2	1	1,22351	78,039	0,3
102	4	3	2	1,22206	78,149	0,3
103	0	4	4	1,20805	79,232	0,2
104	2	4	3	1,20099	79,791	0,4
105	0	0	8	1,19454	80,309	0,9
106	5	0	4	1,19119	80,581	0,8
107	3	4	1	1,18544	81,053	0,1
108	1	0	8	1,17688	81,768	0,1
109	4	3	3	1,17496	81,930	0,3
110	3	0	7	1,17266	82,125	0,5
111	5	1	4	1,16513	82,772	0,7
112	4	2	5	1,16234	83,015	0,6
113	3	4	2	1,15899	83,308	0,5
114	2	2	7	1,15563	83,605	0,2
115	3	3	5	1,15372	83,774	0,4
116	5	2	3	1,15040	84,071	0,5
117	3	1	7	1,14778	84,307	0,3
118	6	0	0	1,14515	84,546	0,3
119	4	1	6	1,14332	84,713	0,8
120	2	4	4	1,13964	85,051	0,5
121	6	0	1	1,13702	85,293	0,1
122	2	0	8	1,12828	86,113	0,1
123	6	1	0	1,12194	86,720	0,2
124	3	4	3	1,11858	87,045	0,1
125	4	3	4	1,11733	87,167	0,1
126	1	4	5	1,11429	87,465	0,2
127	0	5	1	1,11258	87,634	0,1
128	2	1	8	1,10606	88,284	0,1
129	0	3	7	1,10200	88,694	0,2
130	0	2	8	1,09876	89,025	0,6
131	5	2	4	1,09615	89,293	1,0

ANNEX 8: Reference Catalog for Fluorapatite 01-083-0556

Name and formula

Reference code:	01-083-0556
Mineral name:	Fluorapatite
ICSD name:	Calcium Phosphate Fluoride
Empirical formula:	$Ca_{5.061}F_{0.89}O_{11.46}P_{2.87}$
Chemical formula:	$Ca_{5.061}(P_{2.87}O_{11.46})F_{0.89}$

Crystallographic parameters

Crystal system:	Hexagonal
Space group:	P63/m
Space group number:	176
a (Å):	9,3713
b (Å):	9,3713
c (Å):	6,8859
Alpha (°):	90,0000
Beta (°):	90,0000
Gamma (°):	120,0000
Calculated density (g/cm^3):	3,12
Volume of cell (10^6 pm^3):	523,71
Z:	2,00
RIR:	1,14

Subfiles and Quality

Subfiles:	Inorganic Mineral ICSD Pattern
Quality:	Calculated (C)

Comments

Additional pattern:	See PDF 15-876.
ICSD collection code:	079652
Test from ICSD:	At least one SOF implausible.

References

Primary reference:	*Calculated from ICSD using POWD-12++*, (1997)
Structure:	Andreev, Yu.G., *J. Appl. Crystallogr.*, **27**, 288, (1994)

Peak list

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	1	0	0	8,11578	10,893	10,8
2	1	0	1	5,25063	16,872	1,1
3	1	1	0	4,68565	18,924	0,9
4	2	0	0	4,05789	21,885	10,3
5	1	1	1	3,87384	22,939	5,8
6	2	0	1	3,49600	25,458	4,3
7	0	0	2	3,44295	25,857	31,5
8	1	0	2	3,16953	28,131	12,4
9	2	1	0	3,06748	29,087	23,1
10	2	1	1	2,80203	31,913	100,0
11	1	1	2	2,77449	32,238	42,4
12	3	0	0	2,70526	33,087	60,8

12	3	0	0	2,70526	33,087	60,8
13	2	0	2	2,62532	34,125	24,0
14	3	0	1	2,51792	35,628	3,9
15	2	2	0	2,34283	38,391	0,4
16	2	1	2	2,29032	39,307	3,7
17	1	3	0	2,25091	40,024	18,9
18	2	2	1	2,21796	40,645	2,3
19	1	3	1	2,13951	42,205	3,6
20	3	0	2	2,12717	42,461	1,7
21	1	1	3	2,06127	43,888	4,5
22	4	0	0	2,02895	44,625	1,6
23	2	0	3	1,99784	45,358	5,8
24	2	2	2	1,93692	46,868	25,3
25	3	1	2	1,88401	48,267	12,0
26	2	3	0	1,86189	48,877	3,7
27	2	1	3	1,83777	49,562	31,2
28	2	3	1	1,79734	50,755	12,1
29	1	4	0	1,77101	51,564	11,2
30	4	0	2	1,74800	52,294	11,1
31	0	0	4	1,72148	53,162	13,7
32	1	0	4	1,68401	54,442	0,3
33	3	2	2	1,63775	56,113	6,5
34	5	0	0	1,62316	56,662	0,2
35	1	1	4	1,61587	56,941	0,3
36	1	3	3	1,60710	57,281	3,0
37	2	0	4	1,58477	58,164	0,2
38	5	0	1	1,57986	58,363	1,8
39	4	1	2	1,57487	58,565	1,2
40	3	3	0	1,56188	59,101	0,9
41	2	4	0	1,53374	60,296	4,3
42	3	3	1	1,52319	60,758	3,6
43	4	0	3	1,52017	60,891	2,2
44	1	2	4	1,50123	61,743	2,8
45	2	4	1	1,49705	61,934	2,7
46	5	0	2	1,46818	63,291	9,2
47	5	1	0	1,45764	63,803	1,3
48	3	0	4	1,45236	64,062	6,8
49	3	2	3	1,44598	64,379	6,9
50	5	1	1	1,42604	65,390	6,1
51	3	3	2	1,42237	65,580	4,3
52	4	1	3	1,40215	66,648	1,5
53	2	2	4	1,38724	67,459	0,1
54	1	3	4	1,36741	68,572	0,4
55	1	0	5	1,35777	69,128	0,1
56	6	0	0	1,35263	69,428	0,3
57	1	5	2	1,34230	70,040	1,7
58	4	3	0	1,33423	70,527	0,3
59	5	0	3	1,32527	71,076	0,5
60	1	1	5	1,32129	71,322	0,5
61	4	0	4	1,31266	71,864	0,6
62	4	3	1	1,30986	72,041	3,0
63	2	0	5	1,30412	72,409	1,0
64	5	2	0	1,29957	72,703	2,9
65	3	3	3	1,29128	73,245	0,3
66	5	2	1	1,27702	74,199	2,1
67	2	4	3	1,27524	74,320	2,3
68	2	3	4	1,26400	75,094	0,8
69	6	0	2	1,25896	75,447	1,1
70	2	1	5	1,25637	75,630	4,5
71	3	4	2	1,24408	76,511	1,6
72	6	1	0	1,23765	76,982	1,7

73	1	4	4	1,23441	77,221	5,4
74	5	1	3	1,23048	77,514	3,5
75	3	0	5	1,22730	77,752	1,5
76	1	6	1	1,21813	78,449	1,0
77	2	5	2	1,21584	78,626	4,8
78	2	2	5	1,18725	80,904	0,3
79	5	0	4	1,18097	81,424	0,7
80	1	3	5	1,17475	81,948	0,1
81	4	4	0	1,17141	82,232	1,6
82	1	6	2	1,16468	82,811	0,6
83	3	5	0	1,15940	83,272	0,6
84	3	3	4	1,15673	83,507	1,0
85	4	3	3	1,15350	83,794	3,5
86	0	0	6	1,14765	84,319	1,4
87	2	4	4	1,14516	84,545	2,7
88	1	0	6	1,13634	85,356	0,3
89	5	2	3	1,13088	85,867	1,2
90	2	6	0	1,12546	86,382	0,2
91	1	1	6	1,11470	87,425	2,0
92	5	1	4	1,11242	87,649	1,7
93	4	4	2	1,10898	87,991	0,8
94	2	3	5	1,10721	88,168	2,0
95	2	0	6	1,10433	88,458	1,1
96	7	0	2	1,09877	89,024	4,9
97	1	6	3	1,08937	90,000	0,2

ANNEX 9: Reference Catalog for Carbonate Fluorapatite 00-031-0267

Name and formula

Reference code: 00-031-0267

Mineral name: Carbonatefluorapatite
PDF index name: Calcium Fluoride Carbonate Phosphate Hydroxide

Empirical formula: $CCa_{10}F_{1.5}H_{0.5}O_{23.5}P_5$
Chemical formula: $Ca_{10}(PO_4)_5CO_3F_{1.5}(OH)_{0.5}$

Crystallographic parameters

Crystal system: Hexagonal
Space group: P63/m
Space group number: 176

a (Å): 9,3460
b (Å): 9,3460
c (Å): 6,8870
Alpha (°): 90,0000
Beta (°): 90,0000
Gamma (°): 120,0000

Measured density (g/cm^3): 3,12
Volume of cell (10^6 pm^3): 520,97
Z: 1,00

RIR: -

Subfiles and Quality

Subfiles: Inorganic
Mineral
Common Phase
Quality: Blank (B)

Comments

Color: Colorless
General comments: Optical data on specimen from Richtevsveld, South Africa.
Sample source: Specimen from Staffel, Germany.
Analysis: Analysis (wt.%): CaO 51.42, MgO 1.35, Na_2O 1.17, K_2O 0.38, P_2O_5 40.33, CO_2 2.70, F 3.89, H_2O 0.63.
Optical data: A=1.6155, B=1.627, Q=1.6785, Sign=-, 2V=0-36°
Additional pattern: To replace 21-141.

References

Primary reference: Brophy, Nash., *Am. Mineral.*, **53**, 445, (1968)
Optical data: de Villiers, J., *Am. J. Sci.*, **240**, 443, (1942)

Peak list

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	1	0	0	8,04000	10,996	18,0
2	1	1	0	4,67000	18,988	2,0
3	2	0	0	4,04000	21,984	16,0
4	1	1	1	3,86000	23,022	2,0
5	0	0	2	3,43000	25,956	20,0
6	1	0	2	3,16000	28,218	6,0
7	2	1	0	3,05000	29,258	35,0
8	2	1	1	2,79000	32,054	55,0
9	1	1	2	2,76900	32,304	16,0
10	3	0	0	2,69200	33,255	100,0
11	2	0	2	2,61900	34,210	8,0
12	3	0	1	2,50600	35,803	4,0
13	2	1	2	2,28400	39,420	2,0
14	3	1	0	2,24000	40,227	45,0
15	3	1	1	2,13400	42,319	4,0
16	4	0	0	2,02100	44,810	4,0
17	2	2	2	1,93100	47,020	12,0
18	3	1	2	1,88700	48,185	8,0
19	3	2	0	1,85500	49,071	8,0
20	2	1	3	1,83400	49,671	10,0
21	3	2	1	1,79000	50,978	12,0
22				1,78300	51,192	25,0
23	3	0	3	1,75400	52,102	8,0
24	0	0	4	1,72000	53,212	6,0