



بسم الله الرحمن الرحيم

∞∞∞∞

تم رفع هذه الرسالة بواسطة / حسام الدين محمد مغربي

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى

مسئولية عن محتوى هذه الرسالة.

ملاحظات : لا يوجد



# **The Effect of Different Remineralizing Agents on Restored Demineralized Enamel: An In vitro Comparative Study**

A Thesis Submitted to the Operative Dentistry Department,  
Faculty of Dentistry, Ain Shams University  
in Partial Fulfillment of the Requirements of the  
Doctor Degree in Operative dentistry

***BY***

***Nahla Hamed Mohamed Hamed***

B.D.S. Faculty of Dentistry, Ain Shams University, 2007

M.Sc Faculty of Dentistry, Ain Shams University, 2014

Assistant Lecturer of Operative Dentistry, Faculty of Dentistry,  
Ain Shams University

**Supervisors**

***Dr. Omaila Hassan Ghallab***

Professor of operative dentistry,  
Faculty of Dentistry, Ain Shams University

***Dr. Mohammed Nasser Mohammed Anwar***

Lecturer of Operative Dentistry,  
Faculty of Dentistry, Ain Shams University

## *Acknowledgement*

I would like to express my most sincere gratitude and grateful appreciation to *professor Dr. Omaíma Hassan Ghallab*, professor of operative dentistry, faculty of dentistry, Ain shams university for her kind guidance, sincerity and unlimited support.

I deeply thank and faithfully express my gratitude and appreciation to *Dr. Mohammed Nasser Mohammed Anwar*, Lecturer of operative dentistry, faculty of dentistry, Ain shams university for his excellent advice, valuable stimulating guidance and help during this study and throughout my academic and clinical work.

## *Dedication*

*To my support system, my backbone, my family.*

*My dearest mom and dad*

*My loving and supporting husband*

*My little heroes Abd Al Rahman & Ammar*

*My mother and father-in law*

*My caring sisters & brother*

*Thank you for being there for me every step of the way.*

# *List of Contents*

<b>Introduction.....</b>	<b>1</b>
<b>Review of literature.....</b>	<b>3</b>
<b>Effect of dental restorations on the adjacent enamel margins:.....</b>	<b>4</b>
1-Glass ionomer .....	4
2-Resin composite .....	7
<b>I-Remineralization approach: .....</b>	<b>10</b>
1-Saliva:.....	10
2-Fluoride:.....	11
3-Casein Phosphopeptides: .....	12
4-Fluoride varnish: .....	18
<b>II-Regeneration Approach .....</b>	<b>20</b>
Self-assembling peptides .....	21
<b>Demineralization-remineralization models .....</b>	<b>25</b>
Chemical models:.....	26
PH cycling models: .....	27
<b>Bovine teeth .....</b>	<b>29</b>
<b>Microhardness and mineral content testing:.....</b>	<b>32</b>
<b>Microleakage .....</b>	<b>36</b>
<b>Aim of the study.....</b>	<b>40</b>
<b>Materials and Methods.....</b>	<b>41</b>
<b>I- Materials: .....</b>	<b>41</b>
Calculation of sample size:.....	46
Samples grouping: .....	46
<b>II- Methods: .....</b>	<b>49</b>
II.1. Collection of the bovine incisors:.....	49
Preparation of the samples: .....	49
II.2. Restoration of the bovine incisors: .....	51
II.3. Demarcation of the enamel window: .....	54
II.4. Preparation of the demineralization solution:.....	55
II.5. Artificial enamel caries formation: .....	56

II.6. Application of remineralizing agents .....	57
PH cycling.....	64
<b>III-Samples testing .....</b>	<b>67</b>
Surface and Cross-Sectional Microhardness testing.....	67
Mineral Content Examination (EDAX) of Samples: .....	71
Microleakage leakage examination: .....	72
<b>Results .....</b>	<b>75</b>
<b>I- Enamel surface micro-hardness .....</b>	<b>75</b>
<b>II- Enamel cross-sectional microhardness .....</b>	<b>81</b>
<b>III- Enamel mineral content (EDAX analysis) .....</b>	<b>88</b>
Calcium: .....	88
B- Phosphorus:.....	89
C- Ca/P: .....	91
D- Fluoride: .....	95
<b>IV- Micro-leakage score .....</b>	<b>99</b>
<b>Discussion.....</b>	<b>112</b>
Rationale of this study .....	112
Discussion of methodology .....	113
Discussion of results .....	119
- Microhardness and mineral content results.....	119
-Microleakage results: .....	138
<b>Conclusions and recommendations.....</b>	<b>142</b>
<b>Summary.....</b>	<b>144</b>
<b>References.....</b>	<b>146</b>
<b>Arabic summary.....</b>	<b>.....</b>

## List of Tables

TABLE 1: LIST OF MATERIALS USED, PRODUCTS NAME AND THEIR COMPOSITION, MANUFACTURER AND PATCH NUMBER. -----	41
TABLE 2: : LEVELS OF THE STUDY. -----	48
TABLE 3: INTERACTION BETWEEN THE LEVELS OF THE STUDY REGARDING THE REMINERALIZING AGENTS. -----	48
TABLE 4: CORRESPONDING WEIGHT OF CHEMICALS USED IN DEMINERALIZING SOLUTION PREPARATION. -----	55
TABLE 5: EFFECT OF DIFFERENT VARIABLES AND THEIR INTERACTIONS ON SURFACE MICRO- HARDNESS OF ENAMEL MARGINS. -----	75
TABLE 6: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL SURFACE MICRO- HARDNESS FOR DIFFERENT REMINERALIZING AGENTS. -----	76
TABLE 7: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL SURFACE MICRO- HARDNESS FOR DIFFERENT REMINERALIZING AGENTS WITHIN RESTORATIVE MATERIAL AND SURFACE DISTANCES. -----	79
TABLE 8: EFFECT OF DIFFERENT VARIABLES AND THEIR INTERACTIONS ON ENAMEL CROSS- SECTIONAL MICRO-HARDNESS. -----	81
TABLE 9: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL CROSS-SECTIONAL MICRO-HARDNESS FOR DIFFERENT DEPTH DISTANCES. -----	82
TABLE 10: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL CROSS-SECTIONAL MICRO-HARDNESS FOR DIFFERENT REMINERALIZING AGENTS. -----	83
TABLE 11: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL CROSS-SECTIONAL MICRO-HARDNESS FOR DIFFERENT REMINERALIZING AGENTS WITHIN RESTORATIVE MATERIAL AND DEPTH DISTANCES. -----	86
TABLE 12: EFFECT OF DIFFERENT VARIABLES AND THEIR INTERACTIONS ON CALCIUM WEIGHT PERCENTAGE (%) -----	88
TABLE 13: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL CALCIUM WEIGHT PERCENTAGE (%) FOR DIFFERENT REMINERALIZING AGENTS WITHIN THE RESTORATIVE MATERIAL. -----	89
TABLE 14: EFFECT OF DIFFERENT VARIABLES AND THEIR INTERACTIONS ON ENAMEL PHOSPHORUS WEIGHT PERCENTAGE (%) -----	90
TABLE 15: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL PHOSPHORUS WEIGHT PERCENTAGE (%) FOR DIFFERENT REMINERALIZING AGENTS WITHIN THE RESTORATIVE MATERIALS. -----	91
TABLE 16: EFFECT OF DIFFERENT VARIABLES AND THEIR INTERACTIONS ON ENAMEL Ca/P RATIO -----	92



<b>TABLE 17: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL Ca/P RATIO FOR DIFFERENT RESTORATIVE MATERIALS</b>	92
<b>TABLE 18: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL Ca/P RATIO FOR DIFFERENT REMINERALIZING AGENTS.</b>	93
<b>TABLE 19: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL Ca/P RATIO OF REMINERALIZING AGENTS AND RESTORATIVE MATERIAL,</b>	95
<b>TABLE 20: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL FLUORIDE WEIGHT PERCENTAGE (%) FOR DIFFERENT RESTORATIVE MATERIALS</b>	96
<b>TABLE 21: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL FLUORIDE WEIGHT PERCENTAGE (%) FOR DIFFERENT REMINERALIZING AGENTS.</b>	97
<b>TABLE 22: MEAN, STANDARD DEVIATION (SD) VALUES OF ENAMEL FLUORIDE WEIGHT PERCENTAGE (%) FOR TESTED RESTORATIVE MATERIALS AND REMINERALIZING AGENTS.</b>	98
<b>TABLE 23: MEAN, STANDARD DEVIATION (SD) VALUES OF MICRO-LEAKAGE SCORE OF NANOHYBRID RESIN COMPOSITE AND CONVENTIONAL GLASS IONOMER TESTED. --</b>	103
<b>TABLE 24: MEAN, STANDARD DEVIATION (SD) VALUES OF MICROLEAKAGE SCORE FOR TESTED REMINERALIZING AGENTS.</b>	104
<b>TABLE 25: MEAN, STANDARD DEVIATION (SD) VALUES OF MICROLEAKAGE SCORE OF TESTED RESTORATIVE MATERIALS WITHIN REMINERALIZING AGENTS.</b>	105
<b>TABLE 26: MEAN, STANDARD DEVIATION (SD) VALUES OF MICRO-LEAKAGE SCORE FOR REMINERALIZING AGENTS WITHIN THE TESTED RESTORATIVE MATERIALS</b>	107

## *List of Figures*

<b>FIGURE 1: A PROTONATION STATES AND THE AMINO ACID SEQUENCE OF P11-4 AT PH 7.4.</b>	
<b>THE SIDE CHAINS ARE LABELLED ACCORDING TO THE PEPTIDE BACK.</b>	23
<b>FIGURE 2: SCHEMATIC DIAGRAM SHOWING NUMBER OF SAMPLES, STUDY GROUPS AND TESTS APPLIED.</b>	47
<b>FIGURE 3: BOVINE INCISOR EMBEDDED IN ACRYLIC RING BLOCK WITH ENAMEL FACING UPWARD.</b>	49
<b>FIGURE 4: SMOOTHENED LABIAL SURFACE USING THREE GRITS SILICON OF CARBIDE PAPERS (A):320, (B): 600, (C): 1200</b>	50
<b>FIGURE 5: CYLINDRICAL CAVITY PREPARATION WITH DIMENSIONS 1.5DEPTH X 5MM DIAMETER</b>	50
<b>FIGURE 6: (A)LIGHT CURE (B)UNIVERSAL ADHESIVE (C)RESIN COMPOSITE (D)ACID ETCHANT (E) RESTORED CAVITY</b>	51
<b>FIGURE 7: FINISHING OF RESIN COMPOSITE RESTORATIONS.</b>	52
<b>FIGURE 8: CONVENTIONAL GLASS IONOMER RESTORATION OF THE PREPARED CAVITY</b>	53
<b>FIGURE 9: FINISHING TOOLS OF GLASS IONOMER RESTORATIONS.</b>	53
<b>FIGURE 10: FOR DEMARCATION OF ENAMEL WINDOW USING 10x10MM GRAPH PAPER OVERLYING RESTORATIONS</b>	54
<b>FIGURE 11: EXPOSURE OF DEMARCATED ENAMEL WINDOW</b>	54
<b>FIGURE 12: SCHEMATIC REPRESENTATION FOR THE SEQUENCE OF BOVINE TEETH PREPARATION.</b>	55
<b>FIGURE 13: PH OF DEMINERALIZING SOLUTION ADJUSTED TO 4.4 MONITORED BY PH METER.</b>	56
<b>FIGURE 14:ARTIFICIAL CARIOUS LESION FORMATION BY IMMERSION IN 20ML DEMINERALIZING SOLUTION</b>	57
<b>FIGURE 15: ARTIFICIAL SALIVA PREPARATION</b>	58
<b>FIGURE 16: APPLICATION OF SELF-ASSEMBLING PEPTIDE.</b>	59
<b>FIGURE 17: (A) SELF ASSEMBLING PEPTIDE VIAL. (B): MICROPIPETTE</b>	60
<b>FIGURE 18: (A)DISTILLED WATER DISPENSED (B) INJECTION OF DISTILLED WATER INTO THE VIAL USING INSULIN SYRINGE.</b>	60
<b>FIGURE 19: APPLICATION OF THE SELF-ASSEMBLING PEPTIDE USING MICROBRUSH.</b>	60
<b>FIGURE 20:(A)MI VARNISH® AND MICROBRUSH (B)APPLICATION OF MI VARNISH® ON ENAMEL SURFACE USING MICROBRUSH</b>	61
<b>FIGURE 21: APPLICATION OF CURODONT PROTECT™ ON EXPOSED ENAMEL WINDOW FOR TWO MINUTES.</b>	62
<b>FIGURE 22: APPLICATION OF MI PASTE PLUS® ON EXPOSED ENAMEL WINDOW FOR THREE MINUTES</b>	63
<b>FIGURE 23: DIAGRAMMATIC REPRESENTATION OF PH CYCLING SEQUENCE</b>	66

<b>FIGURE 24: MICROHARDNESS TESTING MACHINE.</b>	67
<b>FIGURE 25: BOVINE TEETH PREPARATION FOR MICROHARDNESS TESTING</b>	68
<b>FIGURE 26: SECTIONED SAMPLE MOUNTED IN ACRYLIC MOLD.</b>	69
<b>FIGURE 27:: DIAGRAMMATIC REPRESENTATION FOR MICROHARDNESS TESTING (A)TOOTH SECTIONING (B) INDENTATION SITES S: SURFACE INDENTATIONS, C: CROSS SECTION INDENTATIONS</b>	70
<b>FIGURE 28: REPRESENTATIVE IMAGE FOR MINERAL CONTENT QUANTIFICATION USING EDAX ANALYSIS FOR BASELINE GLASS IONOMER RESTORED SAMPLE.</b>	71
<b>FIGURE 29: STEREOMICROSCOPE FOR MICROLEAKAGE EVALUATION</b>	73
<b>FIGURE 30: REPRESENTATIVE IMAGE FOR DETECTION OF MICROLEAKAGE. (A): NANOHYBRID RESIN COMPOSITE: SCORE 3. (B)CONVENTIONAL GLASS IONOMER: SCORE 3</b>	73
<b>FIGURE 31: BAR CHART VALUES OF ENAMEL SURFACE MICRO-HARDNESS FOR DIFFERENT REMINERALIZING AGENTS.</b>	76
<b>FIGURE 32: BAR CHART VALUES OF ENAMEL SURFACE MICRO-HARDNESS FOR DIFFERENT REMINERALIZING AGENTS WITHIN RESTORATIVE MATERIAL AND SURFACE DISTANCES.</b>	80
<b>FIGURE 33: BAR CHART SHOWING VALUES OF ENAMEL CROSS-SECTIONAL MICRO-HARDNESS FOR DIFFERENT DEPTH DISTANCES.</b>	82
<b>FIGURE 34: BAR CHART SHOWING VALUES OF ENAMEL CROSS-SECTIONAL MICRO-HARDNESS FOR DIFFERENT REMINERALIZING AGENTS.</b>	83
<b>FIGURE 35: BAR CHART SHOWING VALUES OF ENAMEL CROSS-SECTIONAL MICRO-HARDNESS FOR DIFFERENT REMINERALIZING AGENTS WITHIN RESTORATIVE MATERIAL AND DEPTH DISTANCES.</b>	87
<b>FIGURE 36: BAR CHART VALUES OF ENAMEL CALCIUM WEIGHT PERCENTAGE (%) FOR DIFFERENT REMINERALIZING AGENTS WITHIN THE RESTORATIVE MATERIAL.</b>	89
<b>FIGURE 37: BAR CHART SHOWING AVERAGE ENAMEL PHOSPHORUS WEIGHT PERCENTAGE (%) FOR DIFFERENT REMINERALIZING AGENTS WITHIN THE RESTORATIVE MATERIALS.</b>	91
<b>FIGURE 38: BAR CHART SHOWING VALUES OF ENAMEL Ca/P RATIO FOR DIFFERENT RESTORATIVE MATERIALS.</b>	93
<b>FIGURE 39: BAR CHART SHOWING VALUES OF ENAMEL Ca/P RATIO FOR DIFFERENT REMINERALIZING AGENTS.</b>	94
<b>FIGURE 40: BAR CHART SHOWING AVERAGE ENAMEL Ca/P RATIO REMINERALIZING AGENTS AND RESTORATIVE MATERIAL.</b>	95
<b>FIGURE 41: BAR CHART SHOWING AVERAGE ENAMEL FLUORIDE WEIGHT PERCENTAGE (%) FOR DIFFERENT RESTORATIVE MATERIALS.</b>	96
<b>FIGURE 42: BAR CHART SHOWING ENAMEL FLUORIDE WEIGHT PERCENTAGE (%) FOR DIFFERENT REMINERALIZING AGENTS.</b>	97

<b>FIGURE 43: BAR CHART SHOWING VALUES OF ENAMEL FLUORIDE WEIGHT PERCENTAGE (%) FOR DIFFERENT RESTORATIVE MATERIALS AND REMINERALIZING AGENTS. -----</b>	<b>99</b>
<b>FIGURE 44: BASELINE ENAMEL MARGIN MINERAL CONTENT MEASUREMENT ADJACENT TO NANOHYBRID RESIN COMPOSITE RESTORATION.-----</b>	<b>99</b>
<b>FIGURE 45: DEMINERALIZED ENAMEL MARGIN MINERAL CONTENT MEASUREMENT ADJACENT TO CONVENTIONAL GLASS IONOMER RESTORATION -----</b>	<b>100</b>
<b>FIGURE 46: ARTIFICIAL SALIVA TREATED ENAMEL MARGIN MINERAL CONTENT MEASUREMENT ADJACENT TO NANOHYBRID RESIN COMPOSITE RESTORATION.-----</b>	<b>100</b>
<b>FIGURE 47: MI PASTE PLUS<sup>®</sup> TREATED ENAMEL MARGIN MINERAL CONTENT MEASUREMENT ADJACENT TO CONVENTIONAL GLASS IONOMER RESTORATION. -----</b>	<b>101</b>
<b>FIGURE 48: MI VARNISH<sup>®</sup> TREATED ENAMEL MARGIN MINERAL CONTENT MEASUREMENT ADJACENT TO NANOHYBRID RESIN COMPOSITE RESTORATION. -----</b>	<b>101</b>
<b>FIGURE 49: CURODONT REPAIR<sup>™</sup> TREATED ENAMEL MARGIN MINERAL CONTENT MEASUREMENT ADJACENT TO CONVENTIONAL GLASS IONOMER RESTORATION. ----</b>	<b>102</b>
<b>FIGURE 50: CURODONT PROTECT<sup>™</sup> TREATED ENAMEL MARGIN MINERAL CONTENT MEASUREMENT ADJACENT TO NANOHYBRID RESIN COMPOSITE RESTORATION.-----</b>	<b>102</b>
<b>FIGURE 51: BAR CHART SHOWING AVERAGE MICRO-LEAKAGE SCORE OF NANOHYBRID RESIN COMPOSITE AND CONVENTIONAL GLASS IONOMER TESTED. -----</b>	<b>103</b>
<b>FIGURE 52: BAR CHART SHOWING VALUES OF MICROLEAKAGE SCORE FOR TESTED REMINERALIZING AGENTS. -----</b>	<b>104</b>
<b>FIGURE 53: BAR CHART SHOWING AVERAGE MICRO-LEAKAGE SCORE VALUES OF MICROLEAKAGE SCORE FOR TESTED RESTORATIVE MATERIALS WITHIN REMINERALIZING AGENTS. -----</b>	<b>106</b>
<b>FIGURE 54: BAR CHART SHOWING VALUES OF MICRO-LEAKAGE SCORE FOR REMINERALIZING AGENTS WITHIN THE TESTED RESTORATIVE MATERIALS. -----</b>	<b>107</b>
<b>FIGURE 55: BASELINE NANOHYBRID RESIN COMPOSITE SAMPLE WITH MICROLEAKAGE SCORE 3.-----</b>	<b>108</b>
<b>FIGURE 56: DEMINERALIZED NANOHYBRID RESIN COMPOSITE SAMPLE WITH MICROLEAKAGE SCORE 3.-----</b>	<b>108</b>
<b>FIGURE 57: ARTIFICIAL SALIVA TREATED ENAMEL ADJACENT TO CONVENTIONAL GLASS IONOMER SAMPLE WITH MICROLEAKAGE SCORE 3. NOTE THE GAP BETWEEN CAVITY FLOOR AND THE RESTORATION AND THE PRESENCE OF METHYLENE BLUE DYE IN THE BULK OF THE RESTORATION. -----</b>	<b>109</b>
<b>FIGURE 58: CURODONT PROTECT<sup>™</sup> TREATED ENAMEL ADJACENT TO NANOHYBRID RESIN COMPOSITE SAMPLE WITH MICROLEAKAGE SCORE 1. -----</b>	<b>109</b>
<b>FIGURE 59: CURODONT REPAIR<sup>™</sup> TREATED ENAMEL ADJACENT TO NANOHYBRID RESIN COMPOSITE SAMPLE WITH MICROLEAKAGE SCORE 3. -----</b>	<b>110</b>
<b>FIGURE 60: MI PASTE PLUS<sup>®</sup> TREATED ENAMEL ADJACENT TO NANOHYBRID RESIN COMPOSITE SAMPLE WITH MICROLEAKAGE SCORE 1. -----</b>	<b>110</b>

**FIGURE 61: MI VARNISH<sup>®</sup> TREATED ENAMEL ADJACENT TO NANOHYBRID RESIN COMPOSITE  
SAMPLE WITH MICROLEAKAGE SCORE 2 . ----- 111**

Regardless of the restorative material used, demineralization around the margins of restorations has been regarded as the principal cause for restoration replacement. Reduced dental restoration durability necessitates several restorative therapies, including the placement of larger and larger restorations and the execution of complex therapeutic procedures. As a result, restoring the balance between demineralization and remineralization has a significant impact on preventing or minimizing caries formation around restorations<sup>1</sup>.

Remineralization is the body's natural repair mechanism for non-cavitated carious lesions, in which calcium and phosphate from saliva or other topical sources diffuses into the tooth and, with the help of fluoride, repairs existing crystal remnants rather than forming new ones. The repaired crystalline structure is significantly more resistant to acid attack than the original structure and this is accredited to the new mineralized structure which comprises the harder fluorapatite crystals. Demineralization and remineralization occurs simultaneously in the oral cavity. Whether dental caries occurrence is progressive, static or reversal is determined by the balance between demineralization and remineralization. As a result, any factor that can thrust this balance toward remineralization can be utilized as a weapon in the war against dental caries disease<sup>2</sup>.

The principles of minimally invasive dentistry clearly dictate the need for clinically effective measures to remineralize early enamel caries lesions. While fluoride-mediated remineralization is the cornerstone of current caries management philosophies, a number of new remineralization strategies have been commercialized or are under development that claim to promote deeper remineralization of lesions, reduce the potential risks associated with high-fluoride oral care products, and facilitate caries control over a lifetime<sup>3</sup>. These non-fluoride remineralizing systems can be broadly categorized into

biomimetic enamel regenerative technologies and the approaches that repair caries lesions by enhancing fluoride efficacy<sup>2</sup>.

Few researches have been conducted to investigate the effect of remineralizing agents on the demineralized lesions adjacent to restorations. Therefore, This research highlights the effect of different remineralizing protocols on the integrity of the tooth and tooth/restoration margins.