

Evaluation of Osteoconductivity and Bioactivity of a Bioceramic Sealer

Thesis submitted to Endodontic Department, Faculty of Dentistry, Ain Shams University.

For

Partial fulfillment of requirements of the Doctorate Degree in Endodontics.

By

Mohamed Ahmed Elsayed Mousa
Assistant lecturer of Endodontics,
Assiut University
(B.D.S, 2003) (M.Sc, 2012)

Endodontic Department
Faculty of Dentistry
Ain Shams University
2017

Under Supervision of

Prof.Dr. Ihab Elsayed Hassanien
Chairman of Endodontic Department
Professor of Endodontics
Faculty of Dentistry
Ain Shams University

**Prof. Dr. Mohamed Mohamed Abdelrahman
Semieka**
Professor and head of veterinary surgery department
Faculty of veterinary medicine
Assiut University

Dr. Abeer Abd Elhakim Elgendy
Associated Professor of Endodontics
Faculty of Dentistry
Ain Shams University

List of content

List of content	i
List of tables.....	v
List of figures	vii
List of abbreviations.....	xviii
Introduction	1
Review of literature	3
I.A. Bioceramic sealers.....	3
I.B. Osteoconductivity of Calcium Silicate based bioceramic materials.....	4
I.C. Bioactivity of Calcium Silicate based bioceramic materials.....	22
Aim of the study.....	35
Materials and Methods.....	36
Tested materials.....	36
Methods:	37
I. First part: (Animal study)	37
I.A. Animal selection.....	37
I.B. Procedure	37
I.C. Post-operative care	45
I.D. Sacrificing animals.....	45
I.E. Sample preparation for histological analysis	45
I.F. Evaluation of osteoconductivity	48
II. - Second Part: Bioactivity (<i>in vitro</i>).....	63

II.A.	Sample preparation.....	63
II.B.	Sample classification (Figure 27)	63
II.C.	Sealer Insertion:	64
II.D.	Samples preservation.....	64
II.E.	Evaluation of bioactivity	67
Results	73
I. First Part (Osteoconductivity)	73
I.A.	Tissue reaction to tested materials:	73
I.B.	Immunohistochemical results (osteopontin expression)	92
II. Second Part (Bioactivity)	101
II.A.	Surface characterization and globular precipitations surface area (SEM)	101
II.B.	Results of elemental microanalysis (EDX)	117
Discussion	132
Summary	153
Conclusion	156
References	157

List of tables

Table 1: tested materials and their composition.	36
Table 2: The mean \pm SD values and results of comparison between inflammatory cells count in the three groups at two time intervals.	75
Table 3: The mean \pm SD values of the overall comparison in the inflammatory cell count among the three groups.	77
Table 4: The mean \pm SD values, and results of the comparison among the percentages of new bone formation in the three groups at two time intervals.	85
Table 5: The mean \pm SD values, and results of overall comparison among the percentages of bone formation in the three groups.	87
Table 6: The mean \pm SD values, and results of comparison between percentages of osteopontin in the three groups at two time points.	94
Table 7: The mean \pm SD values, and results of overall comparison between percentages of osteopontin in the three groups.	96
Table 8: The mean \pm SD values and results of comparison between percentage of globular precipitation surface area in the two groups at three time intervals.	104
Table 9: The mean values \pm SD values and results of overall comparison between percentage of surface area covered by globular precipitation in the two groups.	106

Table 10: The mean \pm SD values and results of comparison between ca content in surface apatite precipitations in the two groups at three time points.....	120
Table 11: The mean \pm SD values and results of overall comparison between Ca content in surface apatite precipitations of the study groups.	122
Table 12: The mean \pm SD values and results of comparison between Ca/P ratio in surface apatite precipitations in the two groups at three time intervals.	125
Table 13: The mean \pm SD values and results of overall comparison between Ca/P ratios in surface apatite precipitations of the two groups.	127

List of figures

Figure 1: Diagram of dog mandible, showing second, third, and fourth premolars included in this study	40
Figure 2: Preoperative x ray examination, access cavity preparation, and induction of periapical pathosis.....	41
Figure 3: Working length determination, apical perforation and root canal preparation.....	42
Figure 4: Schematic diagram showing classification of experimental groups.....	43
Figure 5: Obturation material was applied according to compared study groups.....	44
Figure 6: Postoperative radiograph showing apical sealer buff.	44
Figure 7: Samples preparation.....	47
Figure 8: High-resolution digital camera, mounted on a light microscope connected to computer supplied with image analysis software.	50
Figure 9: Inflammatory cells were measured digitally by the Image Analysis software Image J.....	50
Figure 10: An inflammatory cell was selected using polygon selections tool.	51

Figure 11: Measuring step was repeated for five cells with different sizes to identify the average size.....	51
Figure 12: Images were transformed in image J into 8-bit type.....	52
Figure 13: The Adjust/Threshold function was used to identify the area that was to be measured.	52
Figure 14: The Analyze Particles function.....	53
Figure 15: Dialog box was used to configure the circularity value and particle size range.	53
Figure 16: Threshold / Apply function to convert the image to black and white.....	54
Figure 17: Process/Binary/Watershed function.....	54
Figure 18: The Show/Outlines and Summarize functions was used to obtain the count and area fraction of the inflammatory cells. Note the original image on the right without optimization, and on the left after optimization for more accuracy.	55
Figure 19: A rectangular field within the section measuring 1360800 pixel	57
Figure 20: The region of interest (<i>ROI</i>) manager was used to save field dimensions.....	57
Figure 21: Using duplicate option, ROI was processed to a new separate image.....	58

Figure 22: Wand tracing tool was used to automated selection of new bone and bone matrix,The percentage area was calculated.	58
Figure 23: Positive osteopontin immunolabeling appears in red brownish color.	61
Figure 24: A diagram showing stained cell-collagen-osteopontin bond. .	61
Figure 25: ImmunohistochemiacI stained tissue with osteopontin appears at bone border in the periapical defect.....	62
Figure 26: Freehand selection tool was used to select positively stained cells and collagen matrix area.	62
Figure 27: Study design and classification of study groups.....	65
Figure 28: Sealer after injection into the prepared root canal space.	66
Figure 29: Specimens were hold in a tightly sealed labeled polyethylene tubes and incubated at 37 °C.	66
Figure 30: Specimens during sputter-coated with gold.	69
Figure 31: Specimens after gold sputtered.....	69
Figure 32: Images were transferred by ImageJ into 8-bit type.	70
Figure 33: Adjust/Threshold function was used to identify areas to be measured.....	70
Figure 34: Create Selection function from the Edit/Selection menus was then used to delineate the areas identified in the previous step.	71
Figure 35: Elemental atomic weight and ratio obtained by EDX.	71

Figure 36: Bar chart representing the effect of time on inflammatory cell count in the three study groups.....	76
Figure 37: Bar chart representing the effect of material on inflammatory cell count after the two time intervals.....	76
Figure 38: Bar chart representing over all inflammatory cell count in the study groups.....	78
Figure 39: Photomicrograph (H&E staining) of a section in Total Fill BC; one month samples, showing fibroblasts (black arrow) and inflammatory cell count (blue arrow) (x40).....	78
Figure 40: Photomicrograph (H&E staining) of a section in Total Fill BC; four months samples, showing root apex (blue arrow),osteocytes in a newly formed osteoid tissue (black arrow) and almost disappear of inflammatory cells (x20).	79
Figure 41: Photomicrograph (H&E staining) of a section in Total Fill BC ;four months samples, showing root apex (blue arrow) and spindle shaped undifferentiated mesenchymal cells in newly formed tissue (black arrow) (x40).	79
Figure 42: : Photomicrograph (H&E staining) of a section in Total Fill BC ;four months samples, showing traces of material (black arrow) with no signs of inflammatory tissue reaction (blue arrow) (x20).	80
Figure 43: Photomicrograph (H&E staining) of a section in MTA Fillapex ; one month samples, showing high inflammatory cell count (x40).	80

Figure 44: Photomicrograph (H&E staining) of a section in MTA Fillapex ;four months samples, showing osteocytes in a newly formed bone surrounded by chronic inflammatory cell (blue arrow) (x40).....	81
Figure 45: Photomicrograph (H&E staining) of a section in MTA Fillapex ; four months samples, showing perforated apex with some resorption and remodeling (black arrow) and chronic inflammatory cells (blue arrow). (x20).....	81
Figure 46: Photomicrograph (H&E staining) of a section in Positive control group ;one month samples, showing highly inflammatory reaction (blue arrow) related to root apex with active resorption areas (black arrow) (x10).....	82
Figure 47: Photomicrograph (H&E staining) of a section in positive control group; four months samples, showing abundant chronic inflammatory cells (x40).	82
Figure 48: Bar chart representing effect of time on percentage of new bone formation at the three groups.	85
Figure 49: Bar chart representing effect of material on percentage of new bone formation at two time intervals.	86
Figure 50: Bar chart representing over all percentage of bone formation in the study groups.	87
Figure 51: Photomicrograph (Goldner's trichrome staining) of a section in Total Fill BC; one month samples, showing the newly formed bone in the apical defect (blue arrow) surrounded by the old bone (black arrow) (x20).....	88

Figure 52: Photomicrograph (Goldner's trichrome staining) of a section in Total Fill BC;one month samples, showing island of newly formed bone in green color (black arrow)(x40).	88
Figure 53: Photomicrograph (Goldner's trichrome staining) of a section in Total Fill BC; four months samples, showing that the newly formed mineralized tissue was noted in close contact with the apex, surrounded by old bone (x20).....	89
Figure 54: Photomicrograph (Goldner's trichrome staining) of a section in MTA Fillapex; one month samples, showing the newly formed bone (black arrow) in the apical defect surrounded by old bone(blue arrow) (x20).....	89
Figure 55: Photomicrograph (Goldner's trichrome staining) of a section in MTA Fillapex; one month samples, showing the newly formed bone (black arrow) (x40).	90
Figure 56: Photomicrograph (Goldner's trichrome staining) of a section in MTA Fillapex; four months samples, showing the newly formed bone (blue arrow) surrounded by mineralized collagen matrix (black arrow) (x40).....	90
Figure 57: Photomicrograph (Goldner's trichrome staining) of a section in positive control group; one month samples, showing root apex (blue arrow) and remodeled old bone trabeculae (black arrow) (x40).	91
Figure 58: Photomicrograph (Goldner's trichrome staining) of a section in positive control group; four months samples, showing the apical defect (blue arrow) surrounded by newly mineralized tissue (black arrow) (x20).....	91

Figure 59: Bar chart representing effect of time on percentage of osteopontin expression in the three groups.	95
Figure 60: Bar chart representing effect of material on percentage of osteopontin expression at the two time intervals.....	95
Figure 61: Bar chart representing over all percentage of osteopontin in the study groups.....	97
Figure 62: Photomicrograph (antiosteopontin staining) of a section in Total Fill BC; one month samples, showing extracellular osteopontin expression (black arrow) in localized areas around the newly formed bone (blue arrow) (x40).	97
Figure 63: Photomicrograph (antiosteopontin staining) of a section in Total Fill BC; four months samples, showing intense osteopontin expression (black arrow) (x40).	98
Figure 64: Photomicrograph (antiosteopontin staining) of a section in MTA Fillapex; one month samples, showing brownish stained osteopontin (black arrow) around the newly formed bone (blue arrow) (x40).	98
Figure 65: Photomicrograph (antiosteopontin staining) of a section in MTA Fillapex; four month samples, showing mild extracellular osteopontin expression (black arrows) (x40).	99
Figure 66: Photomicrograph (antiosteopontin staining) of a section in positive control group; one month samples, showing resorption of root (black arrow) and bone tissue with few osteopontin stained areas was observed. (x10)	99

Figure 67: Photomicrograph (antiosteopontin staining) of a section in positive control group; one month samples, showing low extracellular osteopontin expression (black arrows) (x40).	100
Figure 68 : Photomicrograph (antiosteopontin staining) of a section in positive control group; one month samples, showing moderate extracellular osteopontin expression (black arrows) (x40).	100
Figure 69: Bar chart representing the effect of time on percentage of globular precipitation surface area in the study groups.	105
Figure 70: Bar chart representing the effect of material on percentage of globular precipitation surface area in the three time intervals.	105
Figure 71: Bar chart representing overall percentage of surface area covered by globular precipitation in study groups.	107
Figure 72: Total Fill BC Sealer SEM image (x120 magnification) after one day of soaking showing surface precipitates.	107
Figure 73: Total Fill BC Sealer SEM image (x1000 magnification) after one day of soaking showing surface precipitates.	108
Figure 74: Total Fill BC Sealer SEM image (x1000 magnification) after one day of soaking showing surface precipitates at dentin sealer interface.	108
Figure 75: SEM image of Total Fill BC Sealer (x160 magnification) after one week of soaking showing heavy precipitation and post setting sealer expansion.....	109

Figure 76: SEM image of Total Fill BC Sealer (x1000 magnification) after one week of soaking showing high bioactivity and deposition of circular precipitates.....	109
Figure 77: SEM image of Total Fill BC Sealer (x120 magnification) after two month of soaking, showing a thick apatite coating.....	110
Figure 78: SEM image of Total Fill BC Sealer (x1000 magnification) after two month of soaking showing surface completely covered by aggregated of microspherulites (scale bar 100 μ m).....	110
Figure 79: SEM image (x100 magnification) of MTA Fillapex sample after one day of soaking showing surface nearly free of deposits.....	111
Figure 80: SEM image (x1000 magnification) of MTA Fillapex sample after one day of soaking showing surface nearly free of deposits.....	111
Figure 81: SEM image (x1000 magnification) of MTA Fillapex sample after one day of soaking showing high Titanium oxide ingredients.....	112
Figure 82: SEM image (x1000 magnification) of MTA Fiillapex sample after one day of soaking showing no precipitates at the dentine–sealer interface.....	112
Figure 83: SEM image (x120 magnification) of MTA Fillapex sample after one week of soaking showing minimal amount of surface precipitation.....	113
Figure 84: SEM image (x1000 magnification) of MTA Fillapex sample after one week of soaking showing isolated islands of globular precipitation.....	113