

Evaluation of the Cytotoxicity, Biocompatibility and Physical Properties of Two Bioceramic Sealers

Thesis

Submitted to the Faculty of Dentistry

Ain Shams University

For

Partial Fulfillment of Requirements of

The Doctor Degree in Endodontics

By

Soha Mohamed Ahmed Ismail

B.D.S(October 6 University) 2007

M.Sc in Endodontics, Cairo University (2013)

Faculty of Dentistry

Ain Shams University

(2018)

Supervisors

Prof. Dr. Ihab Elsayed Hassanein

Chairman and Professor of Endodontics

Faculty of Dentistry, Ain Shams University

Prof. Dr. Kariem Mostafa EL Batouty

Vice dean and Professor of Endodontics

Faculty of Dentistry, Ain Shams University

Dr. Dalia Yehia Ebrahim

Associate Professor and Head of Restorative and
Dental Materials Department

National Research Center

Cairo, Egypt

ACKNOWLEDGEMENTS

*First and foremost thanks are due to **Allah** the most beneficent, unlimited and continuous blessing on me.*

*My deepest gratitude, thanks, appreciation and respect goes to **Prof. Dr. Ihab Elsayed Hassanein**, Professor of Endodontics, Faculty of Dentistry, Ain Shams University, for his unsurpassed kindness, thoughtful guidance, extraordinary decency, unlimited help, care and support.*

*Countless thanks to **Prof. Dr. Kariem M. El Batouty** Professor of Endodontics , Faculty of Dentistry, Ain Shams University, for his unlimited kindness, care, concern , his valuable cooperation and helpful remarks.*

*Many thanks to **Dr. Dalia Yehia Ebrahim**, Associate Professor, Restorative and Dental Materials Department, National Research Center, for the facilities she offered to me through this work, and for her unlimited support.*

*Deep thanks and greatest appreciation to **Dr Engy Medhat Kataia**, Associate Professor, Restorative and Dental Materials Department, National Research Center, for her valuable guidance, great help and care.*

*My sincere gratitude to **Dr.Nermine R. Amin** , Assistant professor of Oral Pathology Department, Faculty of Oral and Dental Medicine, Cairo University for her sincere help and cooperation.*

DEDICATION

*This work is dedicated to the dearest person to my heart my **mum** whom I wish she had witnessed this moment with me. I missed you a lot.*

*I give my deepest expression of love and appreciation to my husband **Ahmed** who encouraged me, and put his academic profession on hold so I could achieve my dream. Thank you for the support and company during late nights of typing. I am truly thankful for having you in my life.*

*I also dedicate my success to my precious **dad** for his unlimited love, support and patience; no words can describe my gratitude to him. Your prayers have been answered.*

LIST OF CONTENTS

Title	Page
Introduction	1
Review of Literature	
○ Cytotoxicity	4
○ Biocompatability	10
○ Physical Properties	12
Aim of the study	22
Materials and Methods	23
Results	
○ Cytotoxicity	54
○ Biocompatability	61
○ Physical Properties	72
Discussion	83
Summary and Conclusions	101
References	103
Arabic Summary	120

LIST OF ABBREVIATIONS

Abbreviation	Detailed name
BC	Bioceramic
MTA	Mineral trioxide aggregate
DMEM	Dulbecco's modified Eagle Medium
EBSS	Earl's balanced salt solution
FBS	Fetal bovine serum
hTGSCs	Human tooth germ stem cells
MTT	3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide dye solution
PBS	Phosphate buffered saline
DMSO	Dimethyl sulphoxide
ELISA	Enzyme-linked immunosorbent assay
ISO	International organization for standardization
ADA	American dental association
ROI	Region of interest
PI	Pixel intensity
SEM	Scanning electron microscope
NaOCl	Sodium hypochlorite
EDTA	Ethylene diamine tetra acetic acid
IRM	Intermediate restorative material
HCl	Hydrochloric acid
hPDLCs	Human periodontal ligament cells

LIST OF TABLES

Tab. No	Subjects	Page
1	Tested sealers, their composition and manufacturer.	23
2	Cytotoxicity was rated based on cell viability relative to control as given by Dahl et al.	27
3	Mean and standard deviation (SD) values and results for comparison between cell viability percentage with different dilutions after 24 hours.	55
4	Mean and standard deviation (SD) values and for comparison between cell viability percentage with different dilutions after 72 hours.	56
5	Mean and standard deviation (SD) values and results for comparison between cell viability percentage with different dilutions in MTA-Fillapex group.	59
6	Mean and standard deviation (SD) values and results for comparison between cell viability percentage with different dilutions in Endosequence group.	60
7	Mean and standard deviation (SD) for inflammatory cell count for different tested sealers.	62
8	Overall comparison of inflammatory cell count between different tested sealers.	64
9	Mean and standard deviation (SD) for fibrous capsule thickness (um) for different tested sealers.	66
10	Overall comparison of fibrous capsule thickness (µm) between different tested sealers.	68
11	Mean and standard deviation (SD) for initial setting time for different tested sealer.	72
12	Mean and standard deviation (SD) for solubility for different tested sealers at all observation periods.	74

13	Overall comparison of solubility (%) between different tested sealers.	75
14	Mean and standard deviation (SD) for radio-opacity for different tested sealers in mm Al.	76
15	Mean and standard deviation (SD) for film thickness (um) for different tested sealers.	77
16	Mean percentage surface area of gaps for different tested sealers at all root levels.	78
17	Overall comparison of percentage of gaps between different tested sealers.	80

LIST OF FIGURES

Fig. No	Subjects	Page
1	Samples from each sealer immersed in extraction media.	27
2	Extracts filtered using Millex-GS sterile filter under strict sterile condition.	27
3	Cell diluted in fresh medium and seeded into 96-well plates.	28
4	Optical densities measured at 570nm, using an enzyme-linked immunosorbent assay (ELISA) plate reader. (A) plate introduced into ELISA reader, (B) cytotoxicity results giving on computer.	28
5	Insertion of polyethylene tube containing the sealer into the incision	33
6	Sutured surgical sites.	33
7	Cell count: inflammatory cells (red arrows), spindle shaped cells (black arrows).	33
8	Leica Q-win plus software.	34
9	Separate readings for capsule thickness were measured.	34
10	Broken solubility specimens.	35
11	Stainless steel ring molds.	37
12	Plaster of paris mold steps.	39
13	The Gilmore needle was carefully lowered vertically onto the flat surface of the sealer.	40
14	The operation was repeated until no indentation was seen.	40
15	Analytical balance.	42
16	Suspended sample in a container.	42

17	Sealer samples were positioned on image plate along with aluminum step-wedge.	44
18	Digora software.	44
19	The rim of each glass plate was marked.	45
20	Micrometer caliper.	45
21	Plastic syringe tubes with 0.1 ml graduations was sectioned into 0.1 ml divisions and used as molds to get the proper volume.	46
22	Load of 150 ± 2 N.	46
23	The combined thickness of the two glass plates and the specimen film was recorded. This was reading B.	46
24	Longitudinally sectioned roots.	51
25	K550X sputter coater.	51
26	Quanta 250 FEG SEM.	52
27	Image J 1.41a image analyzer software.	52
28	Bar chart showing mean cell viability percent for different dilutions of tested sealers after 24 hours.	56
29	Bar chart showing mean cell viability percent for different dilutions of tested sealers sealers after 72 hours.	57
30	Line chart showing mean cell viability percent with different concentrations in MTA-Fillapex group.	59
31	Line chart showing mean cell viability percent with different concentrations in Endosequence group.	60
32	Bar chart showing the mean inflammatory cell count for different tested sealers.	63

33	Line chart showing the mean inflammatory cell count for different tested sealers.	63
34	Bar chart showing the overall comparison of the mean inflammatory cell count between different tested sealers.	64
35	Bar chart showing the mean fibrous capsule thickness (um) for different tested groups.	67
36	Line chart showing the mean fibrous capsule thickness (um) for different tested groups.	67
37	Bar chart showing overall comparison of fibrous capsule thickness (um) between different tested groups.	68
38	Histological evaluation showing connective tissue reaction after one week observation period.	69
39	Histological evaluation showing connective tissue reaction after two weeks observation period.	70
40	Histological evaluation showing connective tissue reaction after three weeks observation period.	71
41	Bar chart showing the mean setting time (Hrs.) for different tested sealers.	72
42	Bar chart showing mean solubility for different tested sealers.	74
43	Line chart showing the mean solubility for different evaluation periods.	75
44	Bar chart showing the overall comparison of solubility (%) between different tested sealers.	75
45	Bar chart showing the mean radio-opacity (mm Al) for different tested sealers.	76
46	Bar chart showing the mean film thickness (um) for different tested sealers.	77
47	Bar chart showing the mean percentage of gaps distance for different tested sealers.	79

48	Bar chart showing the mean percentage of gaps distance for different root section.	79
49	Bar chart showing the overall comparison of percentage of gaps between different sealers.	80
50	Photomicrograph of MTA-fillapex (A), Endosequence (B) sealer /dentin interface at the coronal third (score 1).	81
51	Photomicrograph of MTA-Fillapex (A), Endosequence (B) sealer /dentin interface at the middle third (score 2) .	82
52	Photomicrograph of MTA-Fillapex (A), Endosequence (B) sealer /dentin interface at the apical third (score 3) .	82

Introduction

The properties of an ideal root canal sealer include creating a bacteria-resistant seal, adequate working time, low solubility, dimensional stability, adequate radiopacity, possessing antimicrobial activities, being tissue tolerant, and providing good adhesion between itself and the intraradicular dentin after setting ¹. New sealers are constantly being developed in attempts to provide all of these favorable properties.

Every year, new endodontic materials are developed to fulfill the objective of 3-dimensional sealing of root canal system with hopes of revolutionizing the endodontic obturation technique, but none of these materials have presented better results than the association of gutta-percha with conventional sealers.

The introduction of a bioceramic sealer allows us, for the first time, to take advantage of all the benefits associated with bioceramics and to not limit its use to merely root repairs and apical retrofills. This is only possible because of recent nanotechnology developments (the particle size of BC sealer is so fine, it can actually be used with a .012 capillary tips).

This material has been specifically designed as nontoxic calcium silicate cement that is easy to use as an endodontic sealer. In addition to its excellent physical properties, the purpose of BC sealer is to improve the convenience and delivery method of an excellent root canal sealer while simultaneously taking advantage of its bioactive characterization (it utilizes the water inherent in the dentinal tubules to drive the hydration reaction of the material, thereby shortening the setting time).

Bioceramics offer a variety of new treatment options with the potential for improving treatment prognosis in many endodontic

procedures. These materials appear to demonstrate biocompatibility and antimicrobial properties similar to that of MTA. Bioceramics are promising and may surpass traditionally used materials such as Ca(OH)_2 , Glass ionomer, composite and amalgam due to their seemingly superior biocompatibility and improved handling characteristics. With the majority of research on these new bioceramic products being benchtop studies, clinical efficacy cannot be determined. With further in vivo clinical research, these bioceramic products have the potential to become the preferred materials in endodontics for sealers, root repair materials, and pulp capping materials.

Endosequence BC Sealer (Brasseler USA, Savannah, GA) is a premixed bioceramic endodontic sealer. It contains water-free thickening vehicles to enable the sealer to be delivered in the form of a premixed paste.

Unlike calcium silicate and calcium phosphate cements, monobasic calcium phosphates are included in the sealer to facilitate reaction with calcium hydroxide to produce water and hydroxyapatite upon activation of the sealer by water.

Both the sorbed water derived from the external environment and that produced by the reaction between calcium phosphates and calcium hydroxide participate in the hydration of calcium silicate particles to generate a calcium silicate hydrate phase.

Hydroxyapatite is coprecipitated within the calcium silicate hydrate phase to produce a composite-like structure, reinforcing the set cement².

The introduction of a premixed calcium phosphate silicate-based sealer eliminates the potential of heterogeneous consistency during on-site mixing. Because the sealer is premixed with nonaqueous but water-