

## Regenerative potentials of post natal stem cells in non vital immature teeth (An Animal study)

Thesis
Submitted to the Faculty of Dentistry,
AinShamsUniversity

For

Partial Fulfillment of Requirements of the DoctorateDegree in Endodontics

By

#### Adel Abdelwahed Mahmoud

Assistant Lecturer of Endodontics Future University (B.D.S, Ain Shams University-2006, M.D.S, Ain Shams University-2012)

2017

## **Supervisors**

### Prof. Ehab Elsayed Hassanien

Professor of Endodontics Head of Endodontic Department Faculty of Dentistry, Ain Shams University

### Prof. Alaa El Din Ismail

**Professor of General Surgery Faculty of Medicine, Ain Shams University** 

### Prof. Ahmed A .El Rahman Hashem

Professor of Endodontics Faculty of Dentistry, Ain Shams University

#### Dr. Maram Farouk Obeid

Lecturer of Endodontics Faculty of Dentistry, Ain Shams University

# Dedication

To the soul of my Great Father

To my Dearest Mother

To my Sweet wife

To my Lovely brothers and sister

# Acknowledgement

I would like to express my deep gratitude to **Professor Dr. Ehab Hassanien**, Professor of Endodontics, Head of Endodontic department, Faculty of Dentistry, Ain Shams University for his kind guidance, sincerity, extraordinary supervision and unlimited support and help throughout my academic and clinical work.

I would like to thank *Professor Dr. Alaa El Din Ismail* Professor of General Surgery Faculty of Medicine, Ain Shams Universityfor his excellent advice, valuable stimulating guidance and help during this study.

My sincere appreciation to **Professor Dr. Ahmed A .El Rahman Hashem,** Professor of Endodontics Faculty of Dentistry, Ain Shams University for his meticulous care, encouragement, insightful comments, unsurpassed support and patience throughout the course of this research.

My deepappreciation to **Dr. Maram Farouk Obeid,** Lecturer of Endodontics, Faculty of Dentistry, Ain Shams University for her sincere help, cooperation and encouragement during this study.

My sincere due respect, appreciation and gratitude to *Professor Or. Medhat Abdelrahman Kataya*, Professor of Endodontics, Faculty of Dentistry, Cairo University, Head of Endodontic department, Faculty of Oral and Dental Medicine, Future University for his valuable guidance and support over years of work. He has always guided me through the way providing all the help and care and kept me on course the entire way. For him, I will remain eternally indebted.

Special thanks to *DR*, *Ahmed Mohamed Abdellah*, Vet surgeon at experimental surgery unit, Medical Research Center, Ain Shams University for his sincere help during the animal work.

Special thanks to *Or. Amany Ahmed Rabea*, Lecturer, Oral biology department, Faculty of Oral and Dental Medicine, Future University for her sincere help.

#### **List of Contents:**

List of Figures	ii
List of Tables	vii
Introduction	1
Review of literature	4
- Management of open apex	4
I. Apexification	4
A. Calcium hydroxide	5
B. MTA apical plug	6
II. Regenerative endodontics	6
A. Revascularization	7
B. Tissue engineering protocol	20
Aim of the study	47
Materials and methods	
Results	72
Discussion	114
Summary and Conclusion	
References	134

#### List of Figures

Figure 1: Schematic diagram for samples classification
Figure 2:showing administration of general anesthesia
Figure 3:showing a sterile file size #25 was used to disrupt the remnant pulp tissue in the canals
Figure 4:showing injection of triple antibiotic paste
Figure 5:A: Showing induction of bleeding using hand file size #35.  B: Showing blood clots inside the root canals
Figure 6:showing INFUSE® Bone Graft
Figure 7:showing INFUSE® Bone Graft kit components62
Figure 8: showing rhBMP-2 mixed with 0.9 ml sterilized water63
Figure 9: showing the prepared collagen scaffold (DPSCs impregnated with rhBMP-2)placed inside the canal space
Figure 10: Showing sealing of access cavity using glass ionomer
restoration64
Figure 11:Histogram representing mean percent increase in root length between different subgroups and time periods
Figure 12:.Histogram representing mean percent increase in root thickness between different subgroups and time periods
Figure 13:Histogram representing mean apical closure percent between different subgroups and time periods
Figure 14: showing a representative sample of group I subgroup (A); Revascularization (one month) A: preoperative radiograph. B: radiograph one month following treatment
Figure 15: showing a representative sample of group I subgroup (B); [Scaffold impregnated with BMP-2(Infuse bone graft) and DPSCs] (one month). A: preoperative radiograph. B: radiograph one month following treatment

Figure 16:showing a representative sample of group II subgroup (A); Revascularization (three month). A: preoperative radiograph. B: radiograph three months following treatment
Figure 17:showing a representative sample of group II subgroup (B); [Scaffold impregnated with BMP-2(Infuse bone graft) and DPSCs] (three month). As preoperative radiograph. B: radiograph three months following treatment
Figure 18:showing a representative sample of group II subgroup (C); positive control A: preoperative radiograph. B: one month radiograph C: three-month radiograph.
Figure 19:showing a representative sample of group II subgroup (D); negative control A: preoperative radiograph. B: one month radiograph C: three-month radiograph.
Figure 20:Histogram representing mean inflammatory cell count between different subgroups and time periods
Figure 21:Photomicrograph showing moderate inflammatory cell infiltration in group I subgroup A (x400)89
Figure 22:Photomicrograph showing mild inflammatory cell infiltration in group I subgroup B (x400)
Figure 23:.Photomicrograph showing mild inflammatory cell infiltration in group II subgroup A (x400)
Figure 24:.Photomicrograph few inflammatory cells infiltration in group II subgroup B (x400)
Figure 25:Histogram representing prevalence of bone resorption between different subgroups and time periods
Figure 26:Photomicrograph showing active apical root resorption in group l subgroup A (x40)93

Figure 27:Photomicrograph showing no signs of apical root resorption of bone resorption in group I subgroup B(x40)93
Figure 28:Photomicrograph showing active bone resorption in some areas in group II subgroup A(X40)
Figure 29:Photomicrograph showing showed no signs of apical root resorption or bone resorption in group II subgroup E (x40)
Figure 30:Histogram representing mean vital tissues scores between differen subgroups and time periods
Figure 31:Photomicrograph showing connective tissue (C.T) in growth inside the pulp cavity reaching the apical third of the root canal in group I subgroup A (x40)
Figure 32:Photomicrograph showing C.T inside the pulp cavity reaching the middle third of the root canal in group I subgroup B (x40)98
Figure 33:Photomicrograph At a higher magnification showing tissue resembles the pulp tissue with variable amounts of inflammatory cells infiltration (black arrow), noticeable angiogenic activity (green arrows) and a layer of odontoblast-like cells undergo differentiation opposite to a predenting layer (blue arrow) in group I subgroup B (x200)
Figure 34:Photomicrograph showing C.T inside the pulp cavity reaching the middle third of the root canal in group II subgroup A(x40)
Figure 35:Photomicrograph at a higher magnification showing tissue resembles periodontal connective tissue (black arrow) with variable amounts of inflammatory cells infiltration (green arrows) and noticeable angiogenic activity (red arrow) in group II subgroup A (x400)
Figure 36:Photomicrograph showing C.T inside the pulp cavity reaching the coronal third of the root canal (blue arrow). In some specimens areas of a mineralized tissue resembling osteodentin with a layer of odontoblast-like cells (black arrow) could be detected in group II subgroup E (x40)

Figure 37:Photomicrograph at a higher magnification showing tissue resembles the pulp tissue with variable amounts of inflammatory cells infiltration. A definite layer of odontoblast-like cells could be observed opposite to a predentin layer (black arrow) in group II subgroup B (x400)
Figure 38:Histogram representing mean hard tissue formation scores between different subgroups and time periods
Figure 39:Photomicrograph showing apical hard tissue formation that resembles cementum—like tissue (black arrow) in group I subgroup A (x40)
Figure 40:Photomicrograph showing large areas of mineralized tissue that resembles osteodentin in group I subgroup B (X40),,,,106
Figure 41:Photomicrograph at a higher magnification showing large areas of mineralized tissue that resembles osteodentin covered with a layer of predentin (red arrow). Odontoblast-like cells could be observed entrapped inside the mineralized tissue (black arrow). Areas of tubular dentin (green arrow), and odontoblast-like cells opposite to the predentin layer (blue arrow) could be detected in group I subgroup B (x200)
Figure 42:Photomicrograph showingapical hard tissue formation in group II subgroup A (x40)
Figure 43:Photomicrograph at a higher magnificationshowing hard tissue that resembles cementum—like tissue covered by thin layer of cementoid (blue arrow). Cementocyte-like cells (black arrow) and empty lacunae with degenerated cementocyte-like cells (green arrow) in group II subgroup A (x200)
Figure 44:Photomicrograph showing odontoblast-like cells entrapped inside the mineralized tissue group II subgroup B (x40)
Figure 45:Photomicrograph at a higher magnification showing odontoblast-like cells entrapped inside the mineralized tissue (black arrow) opposite to predentin (red arrow) in group II subgroup B (x400)

Figure 47:Photomicrograph showing apical closure in group I subgroup A (x40)	Figure 46:Histogram representing prevalence of apical closure between different subgroups and time periods
Figure 49:Photomicrograph showing apical closure in group II subgroup A (x40)	
(x40)	

#### List of Tables

Table 1:The mean, standard deviation (SD) values for the percent changes in root length and statistical analysis among different subgroups and time periods
Table 2:The mean, standard deviation (SD) values for the percent changes in root thickness and statistical analysis among different subgroups and time periods
Table 3:The mean, standard deviation (SD) values for the apical closure percent changes and statistical analysis among different subgroups and time periods
Table 4:The mean, standard deviation (SD) values for the inflammatory cell count and statistical analysis among different subgroups and time periods 87
Table 5:Frequencies (n), percentages and results of for prevalence of bone resorption among different subgroups and time periods
Table 6:The mean, standard deviation (SD) values for the vital tissues scores and statistical analysis among different subgroups and time periods95
Table 7:The mean, standard deviation (SD) values for the hard tissue formation scores and statistical analysis among different subgroups and time periods
Table 8:Frequencies (n), percentages and results for prevalence of apical closure among different subgroups and time periods

Recent progress in tissue engineering technology has led to a growing interest in the development of regenerative endodontic procedures. Although current root canal treatment modalities offer high levels of success for many conditions, an ideal form of therapy might consist of regenerative approaches in which diseased or necrotic pulp tissues are removed and replaced with healthy pulp tissues to revitalize the teeth. The creation and delivery of new tissues to replace diseased, missing, or traumatized pulp is referred to as regenerative endodontics. This approach provides an innovative and novel range of biologically-based clinical treatments for endodontic disease.

Pulp necrosis of an immature permanent tooth from caries or trauma arrests further development and leaves the tooth with thin, weak walls that are prone to fracture. Endodontic treatment of such a tooth is difficult because the thin walls do not forgive much mechanical instrumentation, and the open apex is difficult or with conventional methods impossible to seal of lateral condensation or thermoplasticized techniques. The traditional treatment for these teeth is long-term calcium hydroxide application to induce apexification (an apical hard tissue barrier). More recent treatments have used an artificial barrier of mineral trioxide aggregate (MTA). Both of these techniques are followed by a traditional root filling, but they do not increase the fracture resistance of the walls. In fact, the long-term calcium hydroxide

therapy for apexification may leave the thin walls even more prone to fracture. Root-wall-strengthening methods with composite resin have been advocated, but they may limit the possibility of root canal retreatment if the need arises in the future.

Revascularization is a regenerative treatment and a biologically based alternative approach to treat necrotic immature teeth that, unlike apexification and artificial apical barrier techniques, allows continuation of root development. In this situation, the necrotic uninfected pulp acts as a scaffold for the ingrowth of new tissue from the periapical area. The absence of bacteria is important for successful revascularization because the new tissue will stop at the level it meets bacteria in the canal space.

One potential use of regenerative endodontic therapy may be the treatment of immature teeth with necrotic pulp which are based on the basic tissue engineering principles that include a triad of stem cells, morphogens or growth factors, and an extracellular matrix scaffold. Dental Pulp Stem Cells (DPSCs) were isolated for the first time in 2000 by Gronthos et al<sup>(1)</sup>. DPSCs can develop odontogenic/osteogenic, chondrogenic, or adipogenic phenotypes, depending on their exposure to different cocktails of growth factors and morphogens.

The role played by BMP-2 is reportedly crucial as a biological tool for dentin regeneration. Recombinant human BMP-2 stimulates the differentiation of adult pulp stem cells into