شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

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جامعة عين شمس

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قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

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تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

HANAA ALY

Effect of Nano Hydroxyapatite Coated by Chitosan and Bioactive Glass Nanoparticles on Osteogenic Differentiation and Proliferation of Stem Cells of Apical Papilla

Thesis

Submitted to the Faculty of Dentistry - Ain Shams University
for Partial Fulfillment of the Requirements for Master's Degree in
Endodontics

By

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Dedication

This work is dedicated to

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My husband

who has always been there to support and encourage me throughout my journey, it wouldn't have been the same without him.

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LIST OF ABBREVIATIONS

Abbreviation	Full term
ALP	Alkaline Phosphatase
CD44	Family of cell surface glycoproteins with isoforms generated by alternate splicing of mRNA. Important in epithelial cell adhesion to hyaluronate in basement membranes and maintaining polar orientation of cells, also binds laminin, collagen and fibronectin
CD45	Leukocyte Common Antigen (LCA)
CD73	Aka ecto-5′-nucleotidase
DMEM	Dulbecco's Modified Eagle Medium
DPSC	Dental Pulp Stem Cell
FBS	Fetal Bovine Serum
FTIR	Fourier Transform Infrared spectra
MSCs	Mesenchymal Stem Cells
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide)
nBG	Nano Bioactive Glass
nHAP	Nano Hydroxyapatite
nHAP/CH	Nano Hydroxyapatite Coated by Chitosan
OM	Osteogenic Differentiation Medium
PSA	Penicillin G sodium, Streptomycin and Amphotericin B
RANKL	Receptor Activator of Nuclear factor Kappa-B Ligand
SCAP	Stem Cell of Apical Papilla
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscopy
XRD	X-Ray Diffraction

INTRODUCTION

Endodontic regenerative procedure (ERP) is a biological procedure to replace diseased, damaged, or missing structures including dentin, cells of the pulp-dentin complex and root structures to restore the normal physiologic functions of the pulp-dentin complex. Delivering and stimulation of the mesenchymal stem cells into root canal systems via different approaches during the regenerative procedures are necessary to attain a successful treatment. Because of their anti-inflammatory ability, these stem cells may survive in inflammatory situations and continue to play a critical role during the re-apexogenesis of developing roots arrested by periapical diseases[(1)].

Stem cell's origin is the mesenchyme, in vitro, they undergo multiline age differentiation and have magnificent clonal expansion capacity[(2)]. Different stem cells' sources have been identified including stem cells from human exfoliating deciduous teeth (SHED), dental pulp stem cells (DSPSCs), and stem cells from the apical papilla (SCAP). In 2006, SCAP were first discovered and isolated from the apical papilla tissue of incompletely developed tooth [(3)].

Evidence is supporting the hypothesis that SCAP appear to be the source of primary odontoblasts that are responsible for the formation of root dentin, whereas DPSCs are likely the source of replacement odontoblasts. Conservation of these stem cells when treating immature teeth may allow continuous root development[(4)]. SCAP have the characteristics of expression of MSCs markers, self-renewal, proliferation, migration, differentiation, and immunosuppression. Moreover, SCAP are capable of differentiating to various lineages of cells, such as osteogenic, odontogenic, neurogenic,

adipogenic, chondrogenic, and hepatogenic cells, which a promising source for stem cell-based therapy [5].

Biomaterials used in endodontic always come in direct contact with the pulp and periapical tissues. Therefore, they should be biocompatible and have no adverse effect on differentiation and proliferation of stem cells present in the area. Among the various bioactive materials developed, Bioactive Glass (BG) that the ability to bond to hard and soft tissue. In recent years, nano-sized Bioactive Glass (nBG) particles were presented and possess a higher specific surface area, a more regular size, than the traditional micron-sized BG particles [6].

Nano hydroxyapatite (nHAP) is another material that is widely used, because the particle size of this material is close to the size of natural apatite crystals present in human mineralized tissue which can used as a scaffold material in tissue engineering. Chitosan, a chitin's deacetylated derivative, is biodegradable, biocompatible, bio adhesion and lack of toxicity. It is a cationic biopolymer that has strong antibacterial properties [7].

Therefore, in this study, the Nanohydroxyapatite coated by chitosan and Nano bioactive glass were evaluated regarding their ability to trigger the osteogenic differentiation and proliferation of SCAP in vitro.

REVIEW OF LITERATURE

I. Use of Nanomaterials in regenerative endodontics:

Nano-BIOACTIVE GLASS:

Bioactive glass (BG) is highly biocompatible, osteoinductive, and osteoconductive calcium silicate–based biomaterial. It has been applied in periodontitis treatment, maxillary cystic bone defects treatment and implantation. Studies[8][9] have proven that BG regulates osteoblast behavior by altering the expression of several relative genes. These genes have an important function in cell proliferation, differentiation, and bone matrix formation. Moreover, the ionic products of BG dissolution have an indispensable function. Nanoscaled 45S5 bioactive glass particles can be considered a promising material for bone tissue engineering, providing very fast kinetics for bone-like hydroxyapatite mineralization without any toxic effects on osteoblast cells.[6]

Surface reactive Bioceramics were first developed by Hench and colleagues in the early 1970s. Since then, numerous studies have focused on bioactive glasses for bone tissue repair due to their ability to bond to bone. Glasses of various compositions such as melt-derived 45S5 Bioglasss and the sol–gel derived glasses of 58S composition have been investigated. It has been previously found that 45S5 Bioglasss causes osteoblast gene up regulation in response to extensive research due to its excellent potential to repair diseased or damaged tissue via regeneration rather than replacement. Third generation biomaterials which are bioactive, resorbable and which stimulate specific and controlled cell responses at the molecular level are now being investigated[10].

A melt-derived glass, 45S5 bioactive glass, with composition in weight percent**:** 45% SiO_2, 24.5% CaO, 24.5% Na_2O and 6% P_2O_5, was first developed in the early 1970s and in multiple studies has shown to have good