
*Faculty of Dentistry*

# "Odontogenic Differentiation of Human Dental Pulp Stem Cells using Mineral Trioxide Aggregate and Nanohydroxy Apatite"

## (In Vitro Study)

## Thesis

Submitted in partial fulfillment for Master Degree in Oral Biology

By

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# *Dedication*

To my beloved family

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# List of Abbreviations

| | |
|---|---|
| ABMSCs | Alveolar bone-derived mesenchymal stem cells |
| ALP | Alkaline Phosphatase |
| BMP 2 | Bone morphogenetic protein 2 |
| BMSCs | Bone marrow stem cells |
| CS | Chitosan |
| CS/nHAC | Chitosan nano-hydroxyapatite composite |
| COL1 | Collagen 1 |
| DFPCs | Dental follicle progenitor cells |
| DMEM | Dulbecco modified Eagle's medium |
| DPSCs | Dental pulp stem cells |
| DSPP | Dentin sialophosphoprotein |
| EMD | Enamel matrix derivative |
| FBS | Fetal bovine serum |
| GelMA | Gelatin methacrylate |
| GelMA/nHA | Gelatin methacrylate nano-hydroxyapatite microgels |
| GIC | Glass ionomer cement |
| GMSCs | Gingival mesenchymal stem cells |
| GMTA | Grey Mineral Trioxide Aggregate |
| HA | Hydroxyapatite |
| HA/TCP | Hydroxyapatite/Tricalcium phosphate |
| hBMSCs | Human bone marrow stem cells |
| hDPSCs | Human dental pulp stem cells |
| hPDLSCs | Human periodontal ligament stem cells |
| hTGSCs | Human tooth germ stem cells |
| MCGS | Mesenchymal cell growth supplement |
| MSCs | Mesenchymal stem cells |
| MTA | Mineral Trioxide Aggregate |
| nHA | Nano-hydroxyapatite |

| | |
|---|---|
| nHAC/PLA | Nano-hydroxyapatite/collagen/poly(L-lactide) |
| OCN | Osteocalcin |
| OPN | Osteopontin |
| PBS | Phosphate buffer solution |
| PCL | Polycaprolactone |
| PDLSCs | Periodontal ligament stem cells |
| PDGF | Platelet derived growth factor |
| $PGE_2$ | Prostaglandin $E_2$ |
| Pgt | Prostaglandin transporter |
| $PP_i$ | Pyrophosphate |
| PRP | Platelet rich plasma |
| q RT-PCR | Real-Time Quantitative Polymerase Chain Reaction |
| rhBMP 2 | recombinant human bone morphogenetic protein 2 |
| RQ | Relative quantification |
| SCAP | Stem cells from apical papilla |
| SCs | Stem cells |
| SDF-1 | Stromal cell-derived factor-1 |
| SEM | Scanning electron microscopy |
| SHEDs | Stem cells from human exfoliated deciduous teeth |
| TCP | Tricalcium phosphate |
| TGPCs | Tooth germ progenitor cells |
| WMTA | White Mineral Trioxide Aggregate |
| $\Delta C_T$ | Change in cycle threshold |

# Abstract

There has been an urge to shift from conventional therapies to the more promising regenerative strategy, since conventional treatment relies on synthetic materials to fill defects and replace missing tissues, lacking the ability to restore the tissues' physiological architecture and function. **Aim of the study:** The present study focused on the assessment of the role of two commonly used biomaterials namely; mineral trioxide aggregate (MTA) and nano hydroxy-apatite (nHA) as promoters of odontogenic differentiation of dental pulp stem cells (DPSCs). **Materials and Methods:** DPSCs were isolated, cultured in odontogenic media and divided into three groups; control group, MTA group and nHA group. Odontogenic differentiation was assessed by tracing genes characteristic of different stages of odontoblasts via qRT-PCR. Calcific nodules formation was evaluated by Alizarin red staining. **Results:** In both MTA and nHA groups, odontogenic differentiation of DPSCs was promoted in comparison to control group. **Conclusion**: MTA and nHA were capable of enhancing odontogenic differentiation of DPSCs. nHA was found to have a higher promoting effect. However, in the absence of odontogenic medium, MTA and nHA could not enhance odontogenic differentiation of DPSCs.

# Introduction

It is difficult to repair defects in hard tissues especially dental structures. Tooth enamel don't have the capacity of self-repairing, meanwhile cementum and dentin can regenerate in a limited capacity. Dental hard tissues are susceptible to caries invasion in a constant basis, furthermore, the pervasive nature of caries can destroy enamel and dentin leading to dental pulp inflammation, infection, and high probability of tooth loss. Hence, Dentin regeneration is paramount to prevent caries progression towards pulp tissues, thus maintaining tooth vitality and integrity.

There has been an urge to shift from conventional therapies to the more promising regenerative strategy, since conventional treatment relies on synthetic materials to fill defects and replace missing tissues, lacking the ability to restore the tissues' physiological architecture and function. Conventional therapies associated with treating pulp exposure has high morbidity rate, often requiring either root canal therapy or extraction. Tooth restoration, endodontic treatment, tooth extraction and its replacement involve multiple appointments and inconvenience. An alternative procedure to extraction or endodontic therapy is regenerative strategies, including cell-based therapies, in an attempt to maintain pulp vitality and avoid the more extensive treatment dictated by extraction or endodontic therapy.

Stem cells are unique type of cells that have the capacity for self-renewal where cell undergoes numerous cycles of cell division maintaining the undifferentiated state, and potency; cell can give rise to different cell phenotypes.

Stem cells can be broadly divided into embryonic stem cells, adult stem cells and induced pluripotent stem cells that are derived through genetic manipulation of somatic cells (Yu et al., 2007). Adult stem cells can be further subdivided into hematopoietic and mesenchymal stem cells. Dental stem cells are considered one of the promising sources of mesenchymal stem cells.

As for the materials used in regenerative medicine, Mineral Trioxide Aggregate (MTA) and nano hydroxyapatite (nHA) are promising materials which have wide clinical and regenerative uses.

Different studies performed on MTA, showed that it plays an important role in tissue regeneration through different mechanisms including activation of cementoblasts resulting in cementum formation which is of prime importance in regenerative endodontics (Baek et al., 2005), stimulation of the odontogenic differentiation capacity of stem cells (Seo et al., 2013), induction of stem cell proliferation with excellent biocompatibility (Amit et al., 2014).

Another material is nHA, due to its particle size which is close to the apatite crystals present naturally in human mineralized tissue having a wide range of use as a scaffold material in tissue engineering (He et al., 2011). It also has superior properties such as enhancing stem cells proliferation and differentiation into osteoblasts resulting in bone formation (Lee et al., 2012) in addition to its great role in guided tissue regeneration for enhancing bone regeneration in the field of periodontology (Vitti et al., 2013).

# Review of literature

## *Stem cells*

In recent years, stem cell research has grown exponentially owing to the recognition that stem cell-based therapies have the potential to improve the life of patients with conditions that range from Alzheimer's disease to cardiac ischemia and regenerative medicine, like bone or tooth loss.

Based on their ability to rescue and/or repair injured tissue and partially restore organ function, different types of stem/progenitor cells have been speculated. Growing evidence demonstrates that stem cells are primarily found in niches and that certain tissues contain more stem cells than others (Li et al., 2005).

The discovery of stem cells and recent advances in cellular and molecular biology has led to the development of novel therapeutic strategies that aim at the regeneration of many tissues that were injured by disease. Generally, stem cells have two major properties: they are capable of self-renewal and, upon division, they can give rise to cells that have the potential to differentiate (Bianco et al., 2008).

Over the last few years, medicine has begun to explore the possible applications of stem cells and tissue engineering towards the repair and regeneration body structures. It has been shown that stem cells can play an important role in future medical treatments since they can be readily grown and induced to differentiate into any cell types in culture (Meirelles et al., 2009).

Stem cells can be classified according to their potency and sources from which they are derived. According to potency, they can be divided into totipotent, pluripotent and multipotent cells. Totipotent cells which have the capability to give rise to all human

cells, such as brain, blood or heart cells. Pluripotent cells are embryonic stem cells that can give rise to all types of tissues, however they cannot bring about an entire organism. Multipotent cells are progenitor cell, such as hematopoietic stem cell and mesenchyme stem cell that have the capability to differentiate into a limited cell range within a tissue type (Hui et al., 2011).

Stem cells can also be classified into embryonic stem cells, adult stem cells, induced pluripotent stem cells and cancer stem cells. Embryonic stem cells are pluripotent stem cells that originated from the blastocyst inner cell mass, in embryo early-stage. They have two distinctive characteristics; they are capable of differentiation into all derivatives of three primary germ layers (pluripotency), and they are able to propagate themselves under defined conditions indefinitely (Young, 2011).

On the other hand, adult stem cells are multipotent stem cells, derived from mature tissues. They have limited potential compared to embryonic stem cells owing to the development stage of these cells. They can be classified according to their origin into endodermal, mesodermal, and ectodermal in origin. Endodermal adult stem cells types are pulmonary SCs, pancreatic SCs, gastrointestinal tract SCs, hepatic oval cells, mammary and prostatic gland SCs, ovarian and testicular SCs. Mesodermal adult stem cells types are mesenchymal stromal SCs, hematopoietic SCs, mesenchymal SCs, mesenchymal precursor SCs, multipotent adult progenitor cells, bone marrow SCs (BMSCs) and cardiac stem cells. Ectodermal adult stem cells types are neural SCs, skin SCs and ocular SCs (Barrilleaux et al., 2006).

The adult stem cells application in research and therapy is not controversial like embryonic stem cells application as the adult stem cells production does not require the embryonic destruction. Because adult stem cells can be obtained as autograft, the risk of tissue rejection is eliminated (Hui et al., 2011).