Expression of mineralization markers in isolated postnatal human dental pulp stem cells in diabetic patients.
(An in vitro study)

Thesis

Submitted to the faculty of oral and dental medicine
Cairo University
In partial fulfillment of the requirements for
Master's degree in Oral Biology

BY
Mohamed Shamel Mohamed
B.D.S.

Faculty of Oral and Dental Medecine
Cairo University
2010

Supervisors

Dr. Nahed Sedky Korany

Assistant professor of Oral biology

Department of Oral biology

Faculty of Oral and dental medicine

Cairo University

Dr. Heba Mohammad Hakam

Lecturer of Oral biology

Department of Oral biology

Faculty of Oral and dental medicine

Cairo University

I`m very grateful to **Prof. Dr. Hala Zaatar,** professor of oral biology and head of oral biology department, Faculty of dentisry, MSA University, for her great and continuous efforts and support. I`ve learned alot from you and words cannot explain my deep gratitude and respect to you.

I am greatly thankful to my friend Tarek Tantawy who aided me in the most difficult times through helping this work to be done in *Chuong Ting Biomedical Engineering cente , China.*

Special thanks to all my friends who tried to help and support me including all the staff members and my colleagues of oral biology department, Faculty of dentistry, MSA university.

And finally, to my family, I could have never done anything without you. Thank you for the continuous encouragement, support and love you always gave me.

Acknowledgement

I would like to express my deepest appreciation and gratitude to **Dr. Nahed Sedky Korany**, Assistant professor of Oral biology, Faculty of Oral and dental medicine, Cairo University, for allowing this work to be done under her own supervision and for the guidance, support and encouragement. Thank you for always being there when I needed you. You are a real example of how a researcher and more importantly a mentor should be.

My deepest thanks and appreciation to **Dr. Heba Mohammad Hakam**, Lecturer of Oral biology, Faculty of Oral and dental medicine, Cairo University, thank you for having patience with me, thank you for the wonderful remarks that made this thesis better.

A special gratitude is directed to **Prof. Dr. Ahmed Helmy Salama**, Professor of oral biology and head of oral biology department, Faculty of Oral and dental medicine, Cairo University, for his great and continuous effort in supporting, helping and encouraging me.

My great thanks to **Prof. Dr. Eman Abou El Ezz**, Professor and head of Oral and dental research division, National research center, for her detailed and constructive comments and helpful advices.

List of abbreviations

ALP: Alkaline phosphatase

BMMSC: Bone marrow mesenchymal stem cells

BMP: Bone morphogenic protein

BSP: Bone sialoprotein

DM: Diabetes Mellitus

DMEM: Dulbecco modified Eagle`s medium

DPP: Dentin phosphoprotein

DPSC: Dental pulp stem cells

DSPP: Dentin sialophosphoprotein

HSC: Hematopoietic stem cells

MC: Mesenchymal cells

MSC: Mesenchymal stem cells

OCN: Osteocalcin

PBS: Phosphate buffered solution

PDL: Periodontal ligament

PDLSC: Periodontal ligament stem cells

RT-PCR: Real time polymerase chain reaction

SCAP: Stem cells from apical papilla

SHED: Stem cells from human exfoliated deciduous teeth

UCB: Umbilical cord blood

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Abstract

Stem cells are characterized by the ability to form clones, self renew and differentiate into different types of cells. Stem cells derived from human dental pulp (DPSCs) have been shown to differentiate into odontoblasts in vitro after using BMP-2. The aim of the present study is to explore the variations in the differentiation potential of the DPSC into odontoblasts in diabetic adults, it also aims at investigating the possible influence of the origin of stem cells (coronal and radicular pulp) on their differentiation potential. Pulp tissue was extirpated from healthy individuals as well as from controlled and uncontrolled diabetic patients. Pulp tissue was divided into coronal and radicular parts and each were cultured for 30 days and then BMP-2 was added. Alizarin red staining was performed to confirm mineralization. RT-PCR was used to analyze expression of mineralization markers DSPP & Enamelysin. The results of this study showed that DPSCs of both healthy and diabetic groups had stem cell properties. DPSCs of the coronal pulp have a more proliferative capacity than that of the radicular pulp. Controlled diabetes mellitus provides relatively unfavorable conditions for the DPSCs to proliferate and differentiate while uncontrolled diabetes produces more deleterious effect on the DPSCs capacity for proliferation and differentiation. Expression of DSPP and enamelysin was positive in healthy and controlled diabetics groups, while in the uncontrolled diabetic group it was negative. It is concluded that DPSCs of the coronal pulp have a more proliferative capacity than that of the radicular pulp, controlled diabetes mellitus provides relatively unfavorable conditions for the DPSCs to proliferate and differentiate and uncontrolled diabetes produces more deleterious effect on the DPSCs capacity for proliferation and differentiation.

Key words: Pulp stem cells, Diabetes mellitus, Mineralization markers, odontoblasts.

Introduction and review of literature

Craniofacial tissue Engineering and stem cells

Tissue engineering is a novel and exciting field that aims to re-create functional, healthy tissues and organs in order to replace diseased, dying, or dead tissues. The field has developed due to the inadequate supply of organs and tissues for patients requiring organ and tissue replacement **(Darnell and Mooney, 2001).**

The restoration of craniofacial constituents, complicated by post-cancer surgeries, trauma and congenital malformations, and to obtain a reconstructed facial support, a seeing eye, a functioning tooth, or rehabilitated facial expressions remains a dream for the individuals affected **(Miura et al., 2006). Zaky and Cancedda, 2009** stated that "It is in the light of improvements in our understanding of wound repair, together with the recent advances in materials science and stem-cell and developmental biology, helping to target molecules and pathways to restore our regenerative capacity, that the "engineering" of our irreversibly affected tissues is progressing toward reality.

Craniofacial tissue engineering promises the regeneration or de novo formation of dental, oral, and craniofacial structures lost due to congenital anomalies, trauma, or diseases **(Mao et al., 2006)**. Structures of the craniofacial complex for which engineering has been attempted include facial skin, gingival tissue, mandibular condyles, salivary glands, muscles,

nerves, blood vessels, dental and periodontal tissues **(Zaky and Cancedda, 2009).**

For successful tissue engineering attempts, three main elements must be fulfilled, **progenitor/stem cells**, **inductive morphogenetic signals (growth factors)** and the **extracellular matrix scaffold (Nakashima , Iohara and Zheng, 2006) (Figure 1).**

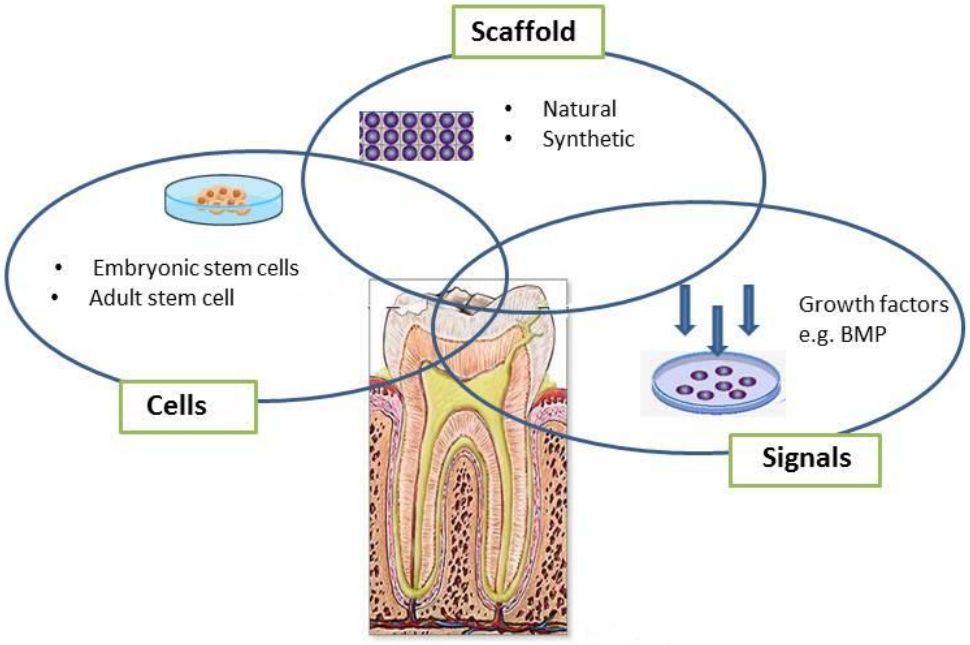

Figure (1): Triad of tissue engineering.

Virtually most of the craniofacial structures are derivatives of mesenchymal cells (MCs). During development MCs originating from the neural crest are known to migrate into the mesenchyme thus named ectomesenchymal cells and subsequently participate in the morphogenesis of virtually most of craniofacial structures such as cartilage, bone, ligaments, cranial sutures, musculature, tendons, the periodontium, and the teeth. These cells undergo asymmetric division, with one offspring cell differentiating toward an end-stage cell, while the other replicates into an offspring mesenchymal cell which, upon the completion of morphogenesis, continue to reside in various craniofacial tissues, and retain their status as mesenchymal stem cells which is an important type of stem cells **(Mao et al., 2006).**

Stem cells

Stem cells are defined as clonogenic, self-renewing cells which are capable of generating one or more specialized cell types **(Gronthos et al., 2002).**

Types of stem cells:

1- According to development:

Stem cells are categorized as either embryonic or as postnatal (they are also called organ-specific, tissue-specific, or adult stem cells) **(Leung and Verfaillie, 2005).**

Embryonic stem cells are derived from the inner cell mass of a developing blastocyst . They are considered as pluripotent cells as for being able to give rise to all the body's cell lineages (endoderm, mesoderm, and ectoderm) **(Smith, 2006)**. Postnatal stem cells are found in many tissues such as the umbilical cord, amniotic fluid, bone marrow, brain and teeth **(Gronthos et al., 2000)**. Postnatal stem cells are also derived from adipose tissue by suction-assisted lipectomy (liposuction) **(Mizuno and Hyakusoku, 2003)**.

Post natal stem cells have two distinct populations; the **hematopoietic stem cells (HSCs)** and the **mesenchymal stem cells (MSCs)**. Hematopoiesis is the production and maintenance of blood stem cells and their proliferation and differentiation into the cells of peripheral blood. HSCs have the ability to reconstitute the hematopoiesis as it gives rise to all blood cell lineages. The hematopoietic stem cells develope early in embryogenesis from mesoderm and becomes deposited in specific sites within the embryo. These sites include the bone marrow, liver, and yolk sac. Bone marrow stem cells may be more plastic and versatile than expected because they are multipotent and can be differentiated into many cell types both in vitro and in vivo **(Maria et al., 2007).**

MSCs originate from the mesodermal layer of the fetus **(Maria et al., 2007)** and postnatally they are found in the bone marrow as well as in a variety of tissues such as the periosteum, fat and skin. MSCs are multipotent cells that are capable of differentiating into cartilage, bone, muscle, tendon, ligament and fat **(Pittenger et al., 1999).** In a study conducted by **Jiang et al., 2002** a new kind of cells was discovered and was proved to obtain a pluripotent character as well as an ability to give rise to endodermal tissues

in addition to mesodermal tissues, the authors described that kind of cells as a multipotent adult progenitor cell**.**

2- <u>According to potency (plasticity):</u>

Potency refers to the differentiation potential of the stem cell into variable cell types **(Schöler, 2007).**

Accordingly, Stem cells can be divided into:

- **<u>Totipotent</u>**: stem cells that can differentiate into embryonic and extraembryonic (outside the embryonic body and discarded at birth) cell types. Such cells can construct a complete, viable, organism. They are produced from the fertilization process, in the inner cell mass of the blastocyst. Cells produced by the first few divisions of the fertilized egg are also totipotent (**Schöler, 2007).**
- **<u>Pluripotent</u>**: These stem cells are the descendants of totipotent cells and can differentiate into nearly all kinds of cells i.e. cells derived from any of the three germ layers **(Montoya, Verfaillie and Hu, 2005) (Figure 2).**
- **<u>Multipotent</u>** stem cells can give rise to a number of cells that belong to a closely related family of cells (e.g. Hematopoietic stem cell give rise to blood cells) **(Schöler, 2007) (Figure 3).**
- **<u>Oligopotent</u>** stem cells can differentiate into only limited kinds of cells, such as lymphoid or myeloid tissue stem cells **(Schöler, 2007).**

- **Unipotent** cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells) **(Schöler, 2007).**

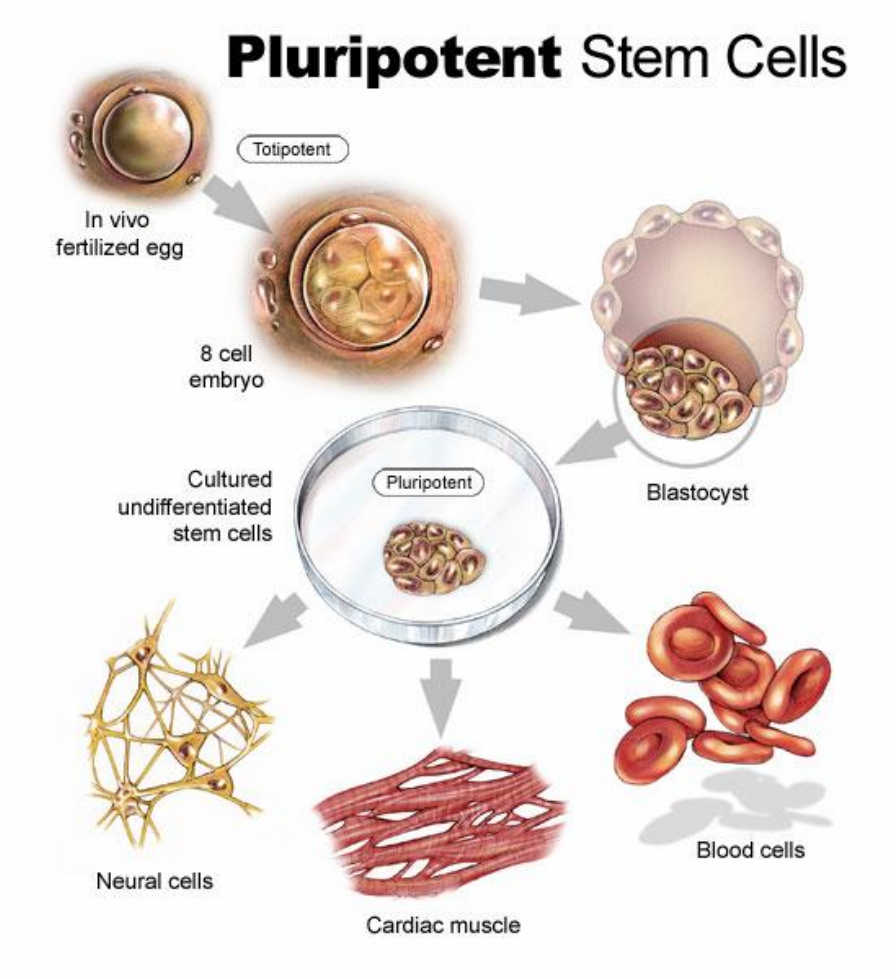

Figure (2): Diagram representing totipotent and pluripotent cells and their differentiation ability.

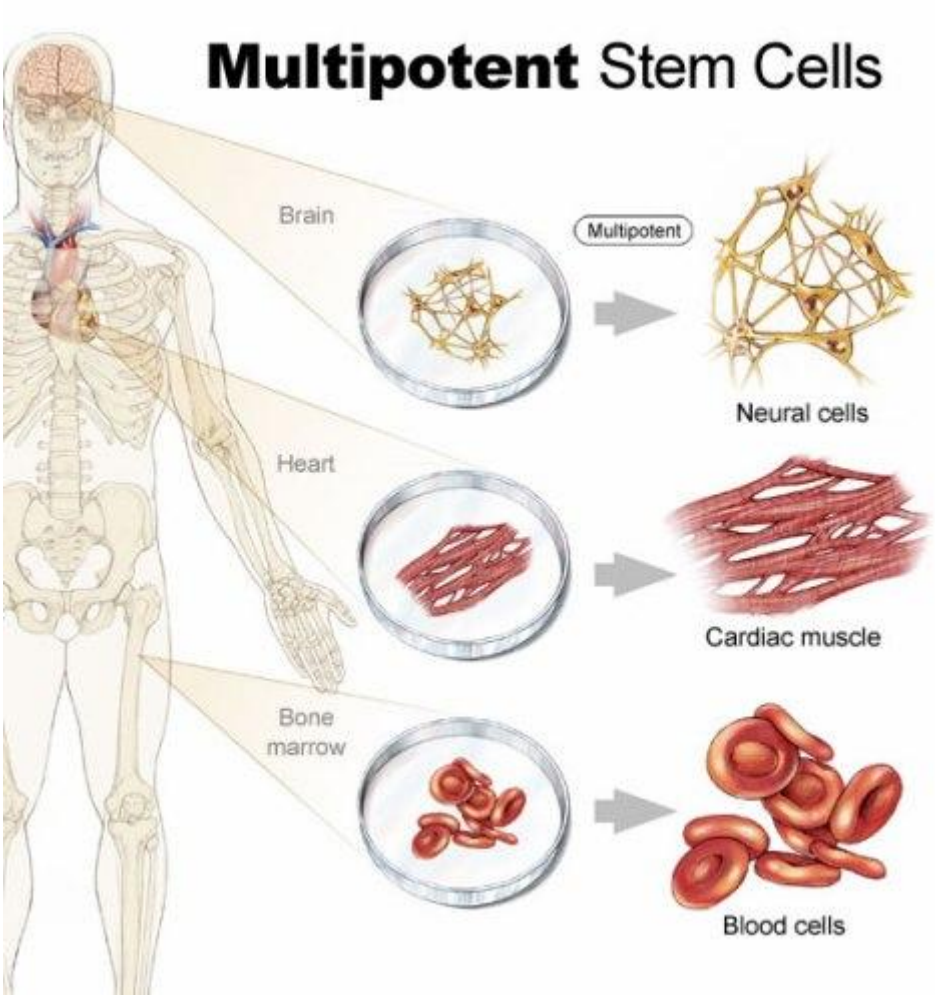

Figure (3): Diagram representing tissues containing multipotent cells