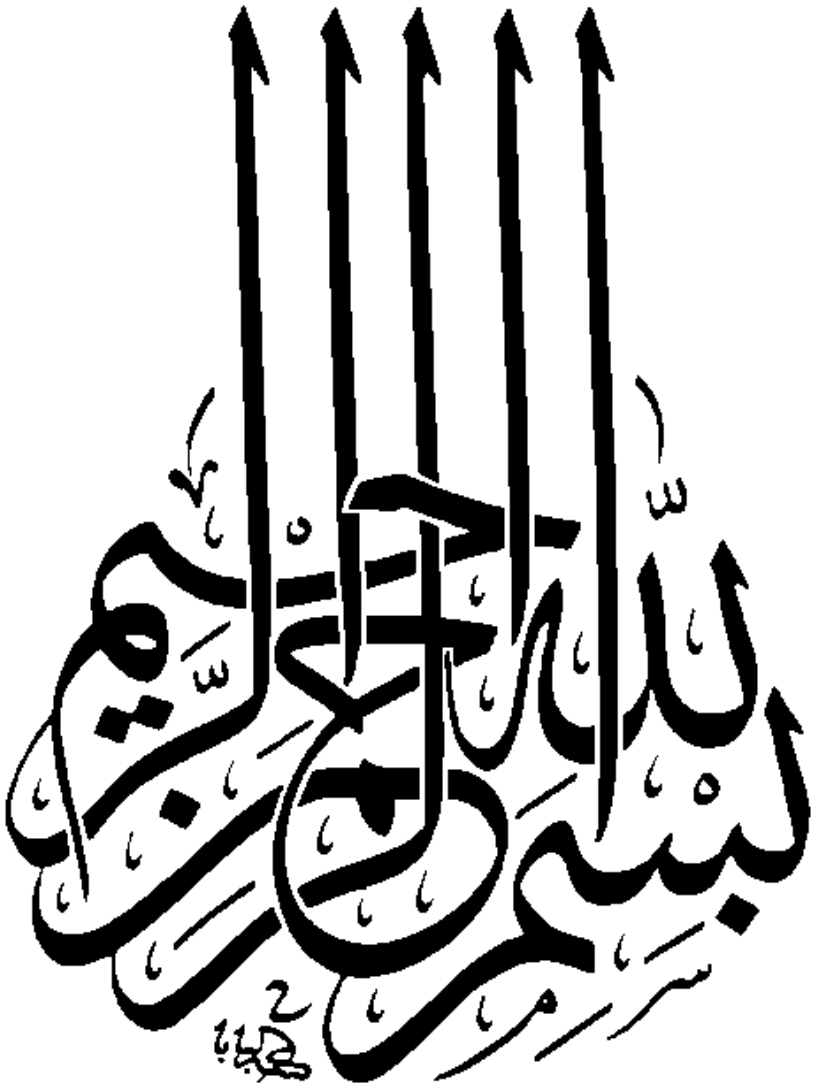

سبحانك

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صدق الله العظيم

Assessment of Adult Jaw Osteoblasts As a Source for Bone Tissue Engineering

Thesis

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By

Yasmine Ahmed Nassar
B.D.S.

Faculty of Oral and Dental Medicine,
Cairo University

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SUPERVISORS

Dr. Maha Mohamed Hakam
B.Ch.D., M.Sc., Ph.D. (Cairo)
Professor of Oral and Maxillofacial Surgery
Faculty of Oral and Dental Medicine,
Cairo University.

Dr. Nader Nabil ElBokle
B.Ch.D., M.Sc. (Cairo), Ph.D. (USA)
Lecturer of Oral and Maxillofacial Surgery
Faculty of Oral and Dental Medicine,
Cairo University.

Dr. Mervat M.F. ElDeftar
M.B.Ch., M.Sc., M.D (Cairo)
Lecturer of Pathology,
Tissue Culture and Cytogenetics Unit,
National Cancer Institute,
Cairo University.

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List of Abbreviations

ALP	alkaline phosphatase
BMP-2	bone morphogenetic protein-2
BMSCs	bone marrow stromal cells
BSP	bone sialoprotein
BSS	balanced Salt Solution
DMEM	Duelbecco's modification of Eagle's medium
D-PBSA	Duelbecco's phosphate-buffered saline lacking calcium and magnesium
ECM	extracellular matrix
FBS	fetal bovine serum
GA	general anaesthesia
HA	hydroxyapatite
IM	intramuscular
KCl	poassium chloride
M	molar
mM	millimolar
MOs	microorganisms
mPCL-TCP	medical grade polycaprolactone-tricalcium phosphate
mRNA	messenger ribonucleic acid
MSCs	mesenchymal stem cells
NaCl	sodium chloride
NCC	neural crest cells
OC	osteocalcin

OP	osteopontin
PGA	poly-glycolic acid
PLA	poly-lactic acid
PLGA	poly lactic-co-glycolic acid
RER	rough endoplasmic reticulum
RGD	Arginine-Glycine-Apartic acid
RT-PCR	reverse transcriptase-polymerase chain reaction
SCID	severe combined immunodeficient
SEM	scanning electron microscope
SOP	standard operating room procedures
TGF- β	transforming growth factor- β
UMCs	undifferentiated mesenchymal cells
UPW	ultra pure water
α MEM	alpha minimum essential medium
β-TCP	β-tricalcium phosphate

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Introduction

The current standard for reconstruction of large bone defects is the use autogenous bone grafts with its osteogenic, osteoinductive, and osteoconductive capabilities. However, the use of autogenous bone grafts is limited by morbidity of the donor site, limitation in the amount of available bone. On the other hand, alloplastic grafting materials lack osteogenicity and most of them lack osteoinductivity, while xenografts and allografts carry hazards of viral transmission [(1)]. Therefore, tissue engineering could be a promising tool for classic bone grafts and substitutes.

Tissue engineering is a field which combines the principles of engineering and life sciences to develop biological substitutes to restore lost tissue. Three strategies for engineering tissues exist. The first comprises the use of cells alone, the second holds implanting signalling molecules such as growth factors with their carrier, while the third approach is the use of cells carried on a scaffold [(2)].

Cells for tissue engineering could be obtained by a process called "Tissue Culture". The tissue culture technique consists of many steps to obtain a sufficient number of cells. In brief, a small tissue sample is obtained, then cells are placed in the conditions needed for their survival and proliferation and perhaps their differentiation into the targeted type of cells [(3)]. Then, the obtained cells are carried onto a scaffold and then implanted in vivo [(2)].

For bone tissue engineering, many types of cells have been investigated for the ability of forming bone. For instance, osteoblast like cells derived directly from bone samples [4, 5, 6, 7], bone marrow stromal cells (BMSCs) obtained from bone marrow [8, 9, 10, 11] [also called mesenchymal stem cells (MSCs)] and cells obtained from the periosteal layer [12, 13, 14, 15].

The jaw bone is an accessible source of bone derived osteoblasts. The present study was designed to test the feasibility of culturing osteoblasts from the adult canine mandibular bone and the bone forming ability of such cells in vitro.

Aim of Study

- To test the feasibility of isolating and culturing osteoblasts from adult canine jaw bone.
- Seeding of collagen sscaffolds.

Review of Literature

Bone is a specialized type of connective tissue. Owing to its mineralized extra cellular matrix, the bone tissue is a rigid tissue combined with a degree of elasticity due to the organic component of the extra cellular matrix (ECM) [16].

The inorganic component of bone consists mainly of calcium phosphates, in the form of hydroxyapatite (HA) crystals carrying a chemical formula of Ca_{10} $(PO_4)_6$ $(OH)_2$ [17]. Traces of other inorganic ions, for example, sodium, potassium, magnesium, and carbonates, are also present [18].

The organic matrix of bone consists of collagen type I and other proteins such as, osteonectin, osteopontin (OP), bone sialoprotein (BSP), osteocalcin (OC), fibronectin and others [17]. Each organic component has a role in the process of bone formation.

The principal component of the organic matrix of bone is collagen type I. It provides the backbone for bone mineral deposition, where hydroxyapatite crystals are deposited [17]. In addition to acting as a scaffold for mineral deposition, collagen type I binds other matrix proteins that aid in hydroxyapatite (HA) deposition. Collagen type I contains RGD (Arginine-Glycine-Apartic acid) sequences, which is an amino acid sequence that binds to the integrin family of receptors present on the cell surface. Therefore the

presence of such sequence on collagen and other bone proteins plays a role in attaching osteoblasts to the extra cellular matrix [19].

Osteonectin [20] and bone sialoprotein [21] play a role in the mineralization of the extracellular matrix of bone, while osteopontin and bone sialoprotein are involved in the attachment of osteoblasts to the ECM [19]. Osteocalcin [22] and osteopontin [23] mediate the role of osteoclasts in osteogenesis. Osteopontin is also involved in mediating the response of bone cells to mechanical stresses [24].

The cellular component of bone is represented mainly by osteoblasts, osteocytes and osteoclasts. Other cells are, osteoprogenitors, preosteoblasts, bone lining cells [25] and osteoclast precursors [26].

Osteoblasts are fully differentiated cells responsible for the production of the bone matrix. They are protein forming cells, as they form collagen and non collagenous bone proteins. Therefore, their cytoplasm is characterized by a well developed rough endoplasmic reticulum (RER), Golgi apparatus and mitochondria [27]. Osteoblasts also regulate mineralization of bone [16].

Osteocytes are mature osteoblasts. They occupy lacunae, within the mineralized bone matrix, and extend processes within canaliculi, to connect them with adjacent osteocytes, with the bone surfaces and with blood vessels nearby [28]. Gap junctions are present within these processes to allow for nutrient exchange [29]. Unlike osteoblasts, they contain little RER and mitochondria [27].

The function of osteocytes is not limited to nutrient transport across bone. Being mechanosensors they play an important role in functional adaptation [29].

Osteoclasts are multinucleated giant cells originating from the monocyte-macrophage lineage of hemopoietic cells. They are bone resorbing cells. In addition, they are involved in the process of calcium homeostasis [30].

Osteoclasts together with osteoblasts, work on the process of bone growth and remodelling throughout life, by simultaneous bone resorption and deposition [28].

The bone structure could be either in the form of loosely organized trabeculae, forming a network (cancellous or spongy bone), or densely packed lamellae (compact or lamellar bone) [16]. The unit structure of compact bone is the osteon or the Haversian system, while the cancellous bone is formed of struts and plates called trabeculae [18].

The outer surface of bone is covered by the periosteum which consists of two layers. The inner layer contacting bone (the osteogenic layer) consists of osteoprogenitor cells, and the outer layer which is the fibrous layer [27]. The endosteum which covers the inner surfaces of bone is formed of a single layer of fibroblasts and osteoprogenitor cells [18].

Cells used for Bone Tissue Engineering

The fact that bone forming cells, to be used in the field of bone tissue engineering, could be obtained from different types of tissues has been proved by many authors [5, 10, 14]. In addition bone cells could be obtained from donors of any age range [4].

Osteoblasts obtained from adult donors

The earliest report describing isolation of cells, that exhibited osteoblast-like characteristics, from adult human bone, was published by Mills et al. in 1979 [31]. Trabecular bone samples were obtained from patients with Paget's disease. Cultures contained osteoblast-like and osteoclast-like cells.

Attempts of characterizing cells derived from human bone specimens have been then reported. In 1985, Auf'mkolk et al. [4], established a successful culture model for extracting human osteoblast-like cells from trabecular bone specimens obtained during surgeries or during obtaining biopsies from donors ranging from 1 to 90 years of age, and they characterized such cells in culture in terms of morphology, osteocalcin production and synthesis of type I collagen.

Another study was conducted by Marie et al. (1989) [32] to characterize cells obtained from human trabecular bone samples. The cultured cells exhibited characteristics of osteoblasts. Cells produced collagen type I, showed a high alkaline phosphatase (ALP) activity and secreted osteocalcin bone protein.