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Investigating bone mineral formed by different cell types in bone tissue engineering

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"*After climbing a great hill, one only finds that there are many more hills to climb*."
Nelson Mandela

ABSTRACT

Combining the suitable cells with three-dimensional (3D) biomaterial scaffolds provides a solution for replacing diseased and damaged tissue. The key requirement for bone tissue engineering scaffolds to be successful is their ability to mimic the physiological function, chemical and mechanical properties of the natural extra-cellular matrix (ECM), which is characterised by collagen nanofibers embedded with nano-hydroxyapatite crystals. This study investigates the proper media condition and the best electrospun scaffold materials for supporting osteogenic differentiation and matrix production. To achieve these objectives, both low and high cells density were cultured in the present and absent of β-glycerophosphate (BGP), ascorbic acid 2-phosphate (AA). Cells viability was tested by alamrBlue assay and ECM production of different cell lines was measured by using Alizarin red (Ar) and Picrosirius red (Sr) stains. For electrospinning, random and aligned nanofbrous scaffolds were electrospun from polycaprolactone (PCL) by incorporating different concentrations of hydroxyapatite (HA) nanoparticles. The morphology of the nanofibers was evaluated using scanning electron microscopy and *in vitro* biocompatibility of the scaffolds was assessed by culturing human embryonic stem cell-derived mesenchymal progenitor cells (hES-MP cells). Cells proliferation, alkaline phosphatase activity (ALP), minerals deposition and collagen production were investigated on different types of scaffolds. Results revealed that in order to enhance ECM minerals and collagen production both BGP and AA are required in the culture media. However, the presence of low concentration of nHA particles in random PCL scaffolds could be a potential substrate for osteoblasts proliferation and mineralisation in enhancing bone tissue regeneration.

LIST OF ABBREVIATIONS

%	percentage
°C	degree Celsius
I	one in Roman Numeral
α	alpha
β	beta
2D	two dimensional
3D	three dimensional
AA	ascorbic acid-2-phosphate
AB	alamarBlue
ALP	alkaline phosphatase
ANOVA	analysis of variance
ASC	adult stem cell
AR	Alizarin red
βGP	β-glycerophosphate
Ba	Barium
Ca	Calcium
cm	centimetre
cm^2	square centimetre
CO2	carbon dioxide
CO3	carbon trioxide
DCM	dichloromethane
Dex	dexamethasone
dH2O	distilled water
DMEM	Dulbecco's Modified Eagle's Medium
DNA	deoxyribonucleic acid
dsDNA	double strand deoxyribonucleic acid
ECM	extracellular matrix
ESC	embryonic stem cell
F	Iron
FBS	fetal bovine serum
FTIR	fourier transform infrared spectroscopy
g	gram
G	group/ condition
h	hour
HA	hydroxyapatite
HDF	human dermal fibroblast
HDMS	hexamethyldisilazane
hESC	human embryonic stem cell
hESMP	human embryonic stem cell-derived mesenchymal progenitor
HGF	human gingival fibroblasts
hMSC	human mesenchymal stem cell
kV	kilovolts
L	litre
M	molar

MEM	minimum essential medium
mg	milligram
µg	microgram
Mg	Magnesium
min	minute
mL	millilitre
µL	microliter
MLO-A5	murine long bone osteocyte A5
mm	millimetre
mM	millimolar
MSC	mesenchymal stem cells
n	number
NaOH	Sodium hydroxide
nHA	nano hydroxyapatite
nm/nM	nanometre
nmol	nanomole
O2	oxygen
OH	hydroxide
P/S	penicillin/streptomycin
P	Phosphorus
Pb	Lead
PBS	phosphate-buffered saline
PCL	polycaprolcatone
pNPP	p-nitrophenol phosphate substrate
Po	Polonium
PTH	Parathyroid hormone
Rpm	revolutions per minute
SD	standard deviation
SEM	scanning electron microscopy
SR	Sirius red
T75	tissue culture 75 cm^2
TE	tissue engineering
Zn	Zinc

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CHAPTER 1

Introduction

Tissue engineering bone grafts have great potential to be used in treating a number of bone abnormalities which are caused by congenital defects, tumour formation or traumatic injury or accidents of the bone [1]. The concepts of tissue engineering were first applied in the 1980s. Since then, the number of studies in this field has grown rapidly [2]. Much effort has been made to cultivate cells into functional bone grafts with suitable shapes and sizes to be used later as an alternative to the currently highly expensive bone grafts [3, 4]. Over half a million bone graft surgeries, which cost about $2.5 billion, are performed annually in the United States [5]. Tissue engineering has the potential to replace current therapeutic and surgical procedures that repair bone fractures and replace missing bone. These procedures are usually complex, fail to heal perfectly, are painful and may pose a significant health risk to the host. However, *in vitro* engineered bone grafts have the ability to be reabsorbed, replaced and healed as natural bone does; this will have a great impact on the field of science and medicine in the future. In fact, researches over the past two decades have demonstrated that the widespread application of engineered bone grafts is strongly dependent on the type of cells and scaffold that are selected to regenerate and repair the bone [6, 7].

In this paper, I will provide a brief review and a general introduction to bone structure and the modelling and remodelling mechanisms of bone, the methods of controlling the secretion of bone minerals and the most common bone diseases. In addition, I will report the role of scaffolds and hydroxyapatite nanoparticles in the field of bone tissue engineering and will focus on one method in fabricating these scaffolds.

The main objectives of my thesis were to compare the effect of different media supplemented conditions on the amount of calcium and collagen in both animal and human cell lines that have been selected to be differentiated into osteogenic cells. Also, to investigate mineral production by evaluating different concentrations of hydroxyapatite

nanoparticles presence in aligned and random PCL scaffolds that have been prepared by an electrospinning technique.

It is important to produce strong dense bone tissue by stimulating the osteoblasts cells to produce the right amount and type of minerals in the newly formed bone tissue that is similar to true human bone.