UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF NANOCOMPOSITE 3D-SCAFFOLDS FOR BONE REPAIR

SAFFANAH KHUDER MAHMOOD

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DEVELOPMENT OF NANOCOMPOSITE 3D-SCAFFOLDS FOR BONE REPAIR

By

SAFFANAH KHUDER MAHMOOD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

أَوَلَمْ يَرَ الأنسان أَنَّا خَلَقْنَاهُ مِنْ نُطْفَةٍ فَإِذَا هُوَ خَصِيمٌ مُبِينٌ (77) وَضَرَبَ لَنَا مَثَلاً وَنَسِيَ خَلْقَهُ قَالَ مَنْ يُحْيِي الْعِظَامَ وَهِيَ رَمِيمٌ (78) قُلْ يُحْيِيهَا الَّذِي أَنْشَأَهَا أَوَّلَ مَرَّةٍ وَهُوَ بِكُلِّ خَلْقٍ عَلِيمٌ (79) **سورة يس**

DEDICATION

I stand in the mihrab of giving between desire and awe, the sincere desire to express what is mental and futile, and the dread of failing to fulfill part of my religion. I have given little to those who have given so much.

And To

My "eyelashes", my dear parents, may this be a step in the realization of their dreams.

My loyal brothers and sisters.

My greater family, members of the Faculty of Veterinary Medicine in general and the branch of Anatomy in particular, especially at the University of Mosul - Iraq and Malaysia, as a group and as individuals, and every one of those who have taught me character which I have found useful in my life in interaction with my teachers and my colleagues.

Finally to

All Aziz and Gal, to all those who are happy for my success; I feel my affection for all of them, and I give them my fruit as well as the essence of my thoughts.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree Doctor of Philosophy

DEVELOPMENT OF NANOCOMPOSITE 3D-SCAFFOLDS FOR BONE REPAIR

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October 2017

Chairman : Professor Md Zuki Abu Bakar @ Zakaria, PhD
Faculty : Veterinary Medicine

The demands for applicable tissue-engineered scaffolds that can be used to repair load-bearing segmental bone defects (SBDs) are vital and increasing. Significant bone problems named trauma, deformity and tumors leave the patients under the pressure of surgical complications, high cost, risk of infection, donor shortage and slow healing process. The main objective of this study is to develop porous nanocomposite scaffold from cockle shell nanopowder for SBD repair. In this study, 9 different combinations of nanocomposite porous scaffolds were fabricated using various proportion of cockle shell-derived $CaCO_3$ aragonite nanoparticles, gelatin, dextran and dextrin. The scaffold then used for repairing critical-size bone defect (2 cm) that made on the shaft of radial bone of 16 adult, male New Zealand White rabbits which divided into four groups (n=4): Group A (control), Group B (scaffold 5211), Group C ($5211_{GTA+Alginate}$) and Group D (5211_{PLA}). The defect site implanted with scaffold was assessed for 8 weeks by means of radiography, hematology, biochemistry, grossly and histology. The micron sized cockle shell-derived $CaCO_3$ powder obtained (75 µm) was transformed into nanoparticles using mechano-chemical and ball mill (top-down) methods of nanoparticle synthesis with the presence of surfactant BS-12 (dodecyl dimethyl bataine). The phase purity and crystallographic structures, the chemical functionality and the thermal characterization of the scaffolds' powder were analyzed using Fourier Transform InfraRed (FTIR) spectrophotometer, Powder X-Ray Diffractometer (PXRD) and Differential Scanning Calorimetry (DSC), respectively. Characterizations of the scaffolds were assessed by Scanning Electron Microscopy (SEM), porosity test, swelling test, water absorption test, degradation manner and mechanical test. The cytocompatibility of the scaffolds was assessed in terms of cell attachment, alkaline phosphatase (ALP) concentration, cell proliferation and capability to form mineralized bone nodules. The tests were conducted throughout *In vitro* cell culture using human Fetal OsteoBlast cells line (hFOB). Top-down methods produced cockle shell-derived $CaCO_3$ aragonite nanoparticles having size range of 15.94-55.21±6 nm which were determined using Field Emission Scanning Electron Microscopy (FESEM) and

Transmission Electron Microscopy (TEM). The aragonite form of calcium carbonate was identified in both PXRD and FTIR for all scaffolds, while the melting (T_m) and transition temperatures (T_g) were identified using DSC with the range of T_m 62.41-75.51°C and T_g 229.38-232.58°C. Engineering analyses showed that scaffolds possessed a 3D interconnected homogenous porous structure with pore sizes 8-526 µm, porosity 6-97%, mechanical strength 4-65 MPa, Young's Modulus104-296 MPa and enzymatic degradation rate 16-67% within 2, 4 and 10 weeks. The biological evaluation also showed that all scaffolds did enhance the osteoblast proliferation rate and improved the osteoblast function as demonstrated by the significant increase in ALP concentration. Radiographic examination showed new trabecular bone formation that signifies the bone healing/regeneration. This occurred in the defects edge as well as in the middle within one month which involved osteogenesis that moved within the central region and margin of the scaffold implant. This was attained with negligible tissue responses to a foreign body which was seen through hematology, biochemistry and histopathological analyses results. Grossly and histologically, after 8 weeks post-implantation the quantity of mature bone increased forming whole bone. The new bone tissue that was produced was successively matured within time as anticipated with increased mature cortical bone development and regeneration. Animal experiment revealed that the material used was able to resist load-bearing situations in extended usage without material breaking or generating stress protective effects to the bone of the host. This work signifies a key development in the healing of artificial bone grafts and suggests that the biomaterial of the grafted scaffold could possess great potential in prospective clinical uses where regeneration of bone is necessary.

Key words: 3D-porous scaffolds, bionanocomposite, tissue engineering, non-seeded, rabbits.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBUATAN PERANCAH-3D NONOKOMPOSIT BAGI MEMPERBAIKI TULANG

Oleh

SAFFANAH KHUDER MAHMOOD

Oktober 2017

Pengerusi : Profesor Md Zuki Abu Bakar @ Zakaria, PhD
Fakulti : Perubatan Veterinar

Permintaan bagi perancah tisu yang direka untuk membaiki kecacatan tulang segmental yang menampung beban (SBDs) adalah penting dan semakin bertambah. Masalah tulang yang signifikan terutama trauma, kecacatan dan tumor meninggalkan pesakit di bawah tekanan komplikasi pembedahan, kos yang tinggi, risiko jangkitan, kekurangan penderma dan proses penyembuhan yang perlahan. Objektif utama kajian ini adalah untuk mencipta perancah nanokomposit berpori dari serbuk nano yang didapati daripada cengkerang kerang untuk membaiki SBD. Dalam kajian ini, 9 kombinasi perancah berpori nanokomposit dibuat dengan menggunakan pelbagai komposisi nanopartikel aragonit $CaCO_3$ yang diperolehi daripada cengkerang kerang, gelatin, dektsran dan dekstrin. Perancah kemudian digunakan untuk membaiki kecacatan tulang ukuran kritikal (2 cm) yang dibuat pada batang tulang radial 16 ekor arnab putih New Zealand jantan yang dibahagikan kepada empat kumpulan (n = 4): Kumpulan A (kawalan), Kumpulan B (perancah 5211), Kumpulan C ($5211_{GTA+Alginat}$) dan Kumpulan D (5211_{PLA}). Tempat kecacatan yang ditanam dengan perancah dinilai selama 8 minggu dengan cara radiografi, hematologi, biokimia, pandangan kasar dan histologi. Serbuk $CaCO_3$ (75µm) bersaiz mikron yang diperolehi dari cengkerang kerang diubah menjadi nanopartikel menggunakan kaedah mekano-kimia dan pengisar bebola (atas bawah) sintesis nanopartikel dengan menggunakan surfaktan BS-12 (dodecyl dimethyl bataine). Struktur kristal dan fasa purifikasi, fungsi kimia dan ciri-ciri termal serbuk perancah dianalisis menggunakan spektrofotometer jelmaan Fourier inframerah (FTIR), Difensometer Sinar-X Serbuk (PXRD) dan Kalorimetri Pengimbasan Perbezaan (DSC). Ciri-ciri perancah dinilai oleh Pengimbasan Mikroskop Elektron (SEM), ujian-ujian keliangan, penggelembungan, penyerapan air, cara penurunan dan ujian mekanikal. Ketaksempurnaan sitokompatibiliti ditaksir dari segi penampanan sel, kepekatan fosfatase alkali (ALP), proliferasi sel dan keupayaan untuk membentuk nodul tulang mineral. Ujian telah dijalankan ke atas kultur sel *In vitro* dengan menggunakan garis sel-sel OsteoBlast janin manusia (hFOB). Nanopartikel aragonit $CaCO_3$ yang diperolehi dari cengkerang kerang menggunakan kaedah atas-bawah menmpunyai julat saiz 15.94-

55.21±6 nm yang ditentukan menggunakan Mikroskop Elektron Pengimbasan Pelepasan Medan (FESEM) dan Mikroskop Elektron Transmisi (TEM). Bentuk kalsium karbonat aragonit telah dikenal pasti oleh kedua-dua PXRD dan FTIR dalam semua perancah, manakala suhu lebur (T_m) dan suhu peralihan (T_g) telah dikenalpasti menggunakan DSC dengan julat T_m 62.41-75.51°C dan T_g 229.38-232.58°C. Analisis kejuruteraan menunjukkan bahawa perancah mempunyai struktur poros dalaman homogen 3D dengan saiz liang 8-526 μm, porositi 6-97%, kekuatan mekanikal 4-65 MPa, Young Modulus 104-296 MPa dan kadar degradasi enzimatik 16-67% dalam tempoh 2, 4 dan 10 minggu. Penilaian biologi juga menunjukkan bahawa semua perancah telah meningkatkan kadar percambahan osteoblast dan meningkatkan fungsi osteoblas seperti yang ditunjukkan oleh peningkatan ketara dalam kepekatan ALP. Pemeriksaan radiografi menunjukkan pembentukan tulang trabekular baru yang menandakan penyembuhan tulang / regenerasi. Ini berlaku di bahagian tepi kecacatan dan juga di bahagian pertengahan dalam masa satu bulan yang melibatkan osteogenesis yang bergerak di dalam kawasan tengah dan margin implan perancah. Ini dicapai dengan tindak balas tisu kepada badan asing yang boleh diabaikan seperti yang dilihat melalui hasil analisis hematologi, biokimia dan histopatologi. Secara kasar dan histologi, selepas 8 minggu pos-implantasi jumlah tulang matang telah meningkat dan membentuk seluruh tulang. Tisu tulang baru yang dihasilkan telah berturut-turut matang dalam masa seperti yang dijangkakan dengan perkembangan tulang kortikal dan regenerasi yang meningkat. Ujikaji ke atas haiwan mendedahkan bahawa bahan yang digunakan mampu menahan beban dalam penggunaan lanjutan tanpa memecahkan atau menghasilkan kesan perlindungan stres ke tulang perumah. Kerja-kerja ini menandakan perkembangan penting dalam penyembuhan graf tulang tiruan dan menunjukkan bahawa biobahan yang digunakan berpotensi besar dalam penggunaan klinikal yang memerlukan pertumbuhan semula tulang.

Kata kunci: perancah 3D-berliang, bionanokomposit, kejuruteraan tisu, tidak bertunas, arnab.

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I certify that a Thesis Examination Committee has met on 13 October 2017 to conduct the final examination of Saffanah Khuder Mahmood on his thesis entitled "Development of Nanocomposite 3D-Scaffolds for Bone Repair" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohamad Aris bin Mohd Moklas, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Jalila binti Abu, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Dewi Apri Astuti, PhD
Professor
Bogor Agricultural University
Indonesia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 November 2017

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.The members of the Supervisory Committee were as follows:

Md Zuki Abu Bakar @ Zakaria, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Intan Shameha Binti Abdul Razak, PhD
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Loqman Mohamad Yusof, PhD
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

_____

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Name of Chairman of Supervisory Committee: Professor Dr. Md Zuki Abu Bakar @ Zakaria

Signature: _____
Name of Member of Supervisory Committee: Dr. Intan Shameha Binti Abdul Razak

Signature: _____
Name of Member of Supervisory Committee: Dr. Loqman Mohamad Yusof

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µl	Microlitter
µm	Micrometer
2D	2 Dimensional
2θ	Two Theata
3D	3 Dimensional
5211	Cockle Shells Nanoparticles 50%, Gelatin 25%, Dextran 10% and Dextrin 15%
$5211_{GTA+Alginate}$	Cockle Shells Nanoparticles 50%, Gelatin 25%, Dextran 10% and Dextrin 15%, soaked in Crosslinking GTA and coated using Alginate
5211_{PLA}	Cockle Shells Nanoparticles 50%, Gelatin 25%, Dextran 10% and Dextrin 15%, coated using PLA
5400	Cockle Shells Nanoparticles 50%, Gelatin 40%, Dextran 5% and Dextrin 5%
6211	Cockle Shells Nanoparticles 60%, Gelatin 20%, Dextran 10% and Dextrin 10%
6300	Cockle Shells Nanoparticles 60%, Gelatin 30%, Dextran 5% and Dextrin 5%
7101	Cockle Shells Nanoparticles 70%, Gelatin 15%, Dextran 5% and Dextrin 10%
7200	Cockle Shells Nanoparticles 70%, Gelatin 20%, Dextran 5% and Dextrin 5%
8100	Cockle Shells Nanoparticles 80%, Gelatin 10%, Dextran 5% and Dextrin 5%
ACC	Amorphous Calcium Carbonates
ALP	Alkaline Phosphatase

ALT	Alanine Transaminase
ANOVA	One-Way Analysis Of Variance
AO	Acridine Orange
AST	Aspartate Aminotransferase
ATCC	American Type Culture Collection
B.W	Body Weight
BCA	Bicinchoninic Acid
BCP	Biphasic Calcium Phosphate
BMPs	Bone Morphogenic Proteins'
BMU	Basic Multicellular Unit
BS-12	Dodecyl Dimethyl Bataine
b-TCP	b-Tri Calcium Phosphate
Ca^{+2}	Calcium ion
$Ca_{10}(PO4)_6(OH)_2$	Chemical Structure of Hydroxyapatite
$CaCO_3$	Calcium Carbonate
C-C	Carbon-Carbon
CCAN	Cockle shell-derived $CaCO_3$ Aragonite Calcium Nanocrystals
CCN	Calcium Carbonate Nanoparticles
C-H	Carbon-Hydrogen group
CH_2C_{l2}	Dichloromethane
cm	Centimeter
C-O	Carbon-Oxygen group
CO_2	Carbon Dioxide
CP	Calcium Phosphate

CSD	Critical-Size Defect
CT	Computed Tomography
d (nm)	crystallite size (nm)
DBM	Demineralized Bone Matrix
DMEM	Dulbecco's Modified Eagle;S Medium
DMSO	DiMethyl SulfOxide
DSC	Differential Scanning Calorimetry
D_{TEM}	crystallite size (nm) using TEM
DW	Deionized Water
D_{XRD}	crystallite size (nm) using XRD
ECM	ExtraCellular Matrix
EDAC	1-Ethyl-3-3-DimethylAminopropyl Carbodiimide
EDS	Element Detection System
EDX	Energy Dispersive X-ray
EG	Ethylene Glycol
ELISA	Enzyme-Linked ImmunoSorbent Assay
ESEM	Environmental Scanning Electron Microscopy
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FDM	Freeze Drying Method
FESEM	Field Emission Scanning Electron Microscopy
FOS	Faculty Of Science
FTIR	Fourier Transform InfraRed
FWHM	Full Width at Half Maximum
g	Gram

G	L-Guluronic Acid
G418	Geneticin solution
GAG	GlycosAminoGlycan
GBR	Guided Bone Regeneration
GCE	Glassy Carbon Electrode
GTA	GluTarAldehyde
h	Hour
H&E	Haematoxylin and Eosin
HA	HydroxyApatite
HA	Hyaluronic Acid
ha	Hectare
Hb	Haemoglobin
HCL	Hydrocloric Acid
H_f	Heat of fusion
hFOB	human Fetal OsteoBlast Cells
HIV	Human Immune-deficiency Virus
HOB	Human OsteoBlast Cells
I/M	IntraMuscular
I/V	IntraVenous
IACUC	Institute of Animal Care and Use Committee
IU	International Unit
JCPDS	Joint Committee of Powder Diffraction Society
Kg	Kilogram
L	Litter
lit	Litter

M	1(4)-Linked D-Mannuronic Acid Monomer
MCHC	Mean Corpuscular Hemoglobin Concentration
M-CSF	Macrophage Colony-Stimulating Factor
MCV	Mean Corpuscular Volume
mg	Milligram
min	Minute
mins	Minutes
mL	MilliLiter
mm	Millimeter
MPa	Megapascals (Mpa Or N/Mm^2) Pascal (Pa) Uint = One Newton Per Square Meter
MRI	Magnetic Resonance Imaging
MSCs	MeSenchymal Cells
MTT	3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphynyltetrazolium Bromide
MV	Matrix Vesicles
NC	Normal Coral
NC	Natural Coral
nm	Nanometer
NPs	Nanoparticles
NZW	New Zealand White
O_2	Oxygen
O-H	Oxygen-Hydrogen group
OPG	OsteoProteGerin
P3HB	Poly-3-HydroxyButyrate
PBS	Phosphate Buffer Solution

PBS	PolyButylene Succinate
PCC	Precipitated Calcium Carbonate
PCL	PolyCaproLactone
PCL	Poly (E-CaproLactone)
PCV	Packed Cell Volume
P_{et}	Density of Ethanol
PET	Poly Ethylene Terephthalate
PG	Plane Geometry
PGA	Poly Glycolic Acid
pH	Power of Hydrogen
PHA	PolyHydroxyAlkanoates
PHB	PolyHydroxyButyrate
PHV	Hydroxyl-Valerate
PI	Propidium Iodide
PLA	PolyLactic Acid
PLGA	Poly-DL-Lactic-Co-Glycolic Acid
PLLA	Poly(L-Lactide) Acid
PMMA	Poly (Methyl MethAcrylate)
pNPP	P-NitroPhenyl Phosphate
PPO	PolyPhenol Oxidase
PSS−ACC	Poly (4-Sodium Styrene Sulfonate)-Stabilized Amorphous Calcium Carbonate
PTH	ParaThyroid Hormone
PXRD	Powder X-Ray Diffraction
R	Radius
RANK-L	Receptor for Activation of Nuclear Factor Kappa B Ligand

RBCs	Red Blood Cells
Reagent A	Sodium Carbonate, Sodium Bicarbonate, Bicinchoninic Acid and Sodium Tartrate In 0.1M Sodium Hydroxide
Reagent B	4% Cupric Sulphate
RM	Ringgit Malaysian
ROD	Renal OsteoDystrophy
rpm	round per minute
SBDs	Segmental Bone Defects
SE	Standard Error
SEM	Scanning Electron Microscopy
SG	Solid Geometry
solution A	Bicinchoninic Acid Solution
solution B	Copper Sulfate Solution
T	Thickness
t	Ton
TCP	TriCalcium Phosphate ($Ca_3 (PO_4)_2$)
TEM	Transmission Electron Microscopy
T_f	Temperature of freezing
Tg	glass Transition Temperature
TGFs	Transforming Growth Factors
Tm	melting Temperature
U/ml	Unit per milliliter
UPM	Universiti Putra Malaysia
UV	UltraViolet light
VPSEM	Variable Pressure Scanning Electron Microscopy
W_0	Dry Weight (Initial Weight)

W_1	Dry Weight
W_2	Wet Weight
W_d	Dry Weight
W_w	Wet Weight
β-TCP	β-TriCalcium Phosphate
μCT	micro-Computed Tomography
μg	Microgram

CHAPTER 1

GENERAL INTRODUCTION

The thoughts of restoring a damaged body have been in existence since the start of humankind with early history manifesting them as myths and magic. New understanding of the natural world, disease, trauma and the introduction of scientific methods enabled the production of an artificial prosthetic materials to restore the lost functions of organs and tissues. With the unfolding of the 20th century, the concept of substituting one tissue with another was developed. This has laid the foundation for the emergence of the field of tissue engineering which formally begun in 1987 (vacanti and vacanti, 2007). The science of designing and fabricating new tissues or materials for impairment repairs has since been widely studied and is constantly expanding. The bone possessing the highest regeneration potentials provides a classic example of a clear principle of a tissue engineering model (fisher and reddi, 2003).

Currently, novel nanotechnology approaches are being engaged in the tissue engineering. The human bone represents one of the most important organs of the human body. These rigid organs play an essential role in providing the needed support, protection and movement. These unique features of the bones are well manipulated in the field of tissue engineering in a constant search for an ideal bone replacement material. A major problem for bone surgery frequently presents secondary bone tumour, trauma or deformity (Buckwalter, 2004; Nihorbd, 2004; Brydone *et al*., 2010).

Bone injury, mainly is as a result of aged, deteriorating diseases or accidents. Many repair techniques have been suggested over the past decades. However almost all of them failed to produce long-lasting tissue repair (Salgado *et al*., 2002; van Gaalen *et al*., 2008). Bone replacement or transplantation involves the grafting of a new bone or a suitable replacement material between the spaces of a fractured bone or a defected bone in order to aid the healing process. Transplantation of bone is a fast growing field, which has a considerable influence on patients that suffer from bone tissue injury and infection (Sagar *et al*., 2013). Over a century, the process of bone grafting has been utilized by orthopedic surgeons due to the constant need for bone replacement. In medical procedures, grafting is commonly used to substitute damaged tissue. Presently the alternatives to treat these injuries are inadequate as they depend on allografts, autografts, and biomimetic or variety of synthetic materials and strategies (Da Silva, 2009). Autografts are osteoconductive, osteoinductive, with osteogenic characteristics (Cypher and Grossman, 1996; Ilan and Ladd, 2002). Even though, autografts are consider to be the standard for bone transplant, they likewise possess some restrictions because of probable donor morbidity, establishment of other medical complications and low tissue accessibility (Moore *et al*., 2001; Ilan and Ladd, 2002; Jakoi *et al*., 2015). The expectation of a graft substitute is highly dependent on the nature of the fracture or defect of the bone. This determines the use of the graft whether as simple void filler or as larger gap filler that acts like a scaffolding material to facilitate formation of new bone. In both

cases, the graft material acts as a structural support and strength provider (Ilan and Ladd, 2002).

To date, the choice of graft substitute marketed fulfills these criteria and one or more of the key principals of bone healing (osteoconduction, osteoinduction and osteogenesis) but not all. At the very least a grafting material designed should be osteoconductive in nature to be used as simple void fillers facilitating the formation of new bone cells. With the incorporation of growth factors such as Bone Morphogenic Proteins' (BMPs) that promotes cell growth, an osteoinductive nature could be conferred to a grafting material to promote an even faster rate of healing. The constant emergence of newly innovated or improved grafting materials keeps the field of bone tissue engineering an exciting avenue for future studies in order to fulfill these empty voids in producing a grafting material that fulfills the principals of a successful bone substitute material. Prosthetics from metals and bone cement fillers, polymers and ceramics are additional treatment options in addition to bone defect renovation or changing broken bone tissue. The entire predictable approaches to renovate and replace bone may be painful, taking longer time and may be discarded by the body (Nandi *et al*., 2010; Bose *et al*., 2012; Santos Jr. and de Carvalho Zavaglia, 2016). It is in this context that in the last decades tissue engineering arose as a substitute method to restore and redevelop injured tissues to avoide the prerequisite for everlasting implant (Mistry and Mikos, 2005; Nesic *et al*., 2006; Chung and Burdick, 2008).

Tissue engineering may be divided into diverse approaches, the best approach for the creation of strong tissue (such as, bone and cartilage) substitutes is by the combination of living cells, biologically dynamic molecules and temporary three-D (3-D) permeable scaffolds (Hutmacher *et al*., 2007). Substitute strategies have been intensely explored and scrutinized based on a tissue engineering approach, attempting to rise above the innate restrictions of the presently obtainable solutions to bone defects. Using this strategy, forming of bone by tissue engineering is through seeding cells which can develop into osteoblasts on greatly permeable biomaterials (Brydone *et al*., 2010; Bose *et al*., 2012). Base on Williams (1987), tissue engineering is definite as an multidisciplinary field that uses the values of engineering and the life sciences in the direction of the improvement of biological alternatives that maintain, reestablish or progress function of tissue. These replacements are commonly branded as "scaffolds".

In the last few decades, tissue engineering has arose as a hopeful substitute to treat or substitute loss function of tissues and organs that result from infection or distress (Scheller *et al.*, 2009; Torroni, 2009). The most studied approach includes the usage of artificial extracellular matrix (the scaffold) normally planned to be provisional and therefore made from bioresorbable or biodegradable polymers. Tissue engineering in recent time has boost up the awareness in producing permeable configurations for scaffolding in regeneration of tissue. The fundamental standard in tissue engineering is culturing of cells isolates from a patient, expanded, and even prompted to segregate *In vitro* in culturing of cell. Within *In vitro*, the cells seeded onto a scaffold further developed *In vitro*, ultimately in vibrant culture settings, after which is implanted into

the receipient deficiency which will act as an inductor for tissue redevelopment (Langer and Vacanti, 1993). Tissue engineering gives a prospective method to form tissues, organs and artificial graft products under laboratory circumstances in defeating the troubles of implantation refusal, diseases related with xenografts transmission and allografts, with deficiency in donation of organ (Blom *et al*., 2005; Lee *et al*., 2008; Navarro *et al*., 2008).

Bone tissue engineering is a multidisciplinary research area in which new approaches are developed to treat human patients suffering from bone loss or disease. The same as in tissue engineering, synthetic bone is formed by seeding cells that can grow to be osteoblasts on three dimensional porous scaffolds for incubation either *In vitro* or *in vivo* to motivate bone matrix production (Navarro *et al*., 2008; Brydone *et al*., 2010; Bose *et al*., 2012). The biological artificial bone is predictable to substitute the autogenous bone graft by providing parallel essential apparatus. Bone tissue engineering can be addressed to resolve a lot of troubles such as possibility of bacterial infection, donor shortage, high cost and slow vascularization (Navarro *et al*., 2004, 2008; Sagar *et al*., 2013). Bone repair is the normal objective for bone tissue engineering, it may be useful in healing or fixing broad variety of bone defects (Blom *et al*., 2005; Navarro *et al*., 2008; Brydone *et al*., 2010). As explained above, tissue engineering of bone needs three significant fundamentals: these are cellular components, extracellular matrix (ECM) and growth factors (Søballe , 1993; Nandi *et al*., 2010). There are a lot of different approaches which could be used in building bone tissue engineering. Among the approach is a seeding autologous osteogenic cell *In vitro* beside a biodegradable scaffold forming a scaffold–cell hybrid which can be called a tissue-engineered constructs. Chondrocytes and Mesenchymal stem cells osteoblasts, from rigid and soft tissues of the patient could be extended in culture and seeded onto a scaffold that would in a few manner die permitting fully normal bone tissue substitution (Czekanska *et al*., 2012; Biomed Central, 2015; Chen *et al*., 2015). A current report on the world marketplace of orthopedic implants and products industry indicated that the total drug orthopedic implant and device market to grow at a CAGR of approximatly 8.8% over the next decade to reach around $91.42 billion by 2025 (Glover, 2016).

Orthopedic implants develop with a growth rate of 7% to 10% over the last decade and this trend is predictable to carry on in the years to come (FDA, 2015). The global dental implants and prosthetics market is predictable to grow at a CAGR of 7.2% during the forecast period, to influence USD 12.32 Billion by 2021 (Sunita, 2010).

The major part of this market was thoracolumbar fixation, followed by inter body devices and cervical fixation, which together compose the whole market for spinal fusion (Mis and Vcf, 2009). The global foot and ankle devices market is composed to grow at a CAGR of about 7.9% over the next decade to reach about $7.82 billion by 2025 (ZMR, 2017).

The achievement of tissue engineering scaffold will appear into play to find out if it will sustain attachment of cell, growth and finally cell distinction into the proper tissue. Because of these, the bioresorbable scaffold should be biocompatible and having permeable related linkage to make easy vascularization and quick growing of a new produced tissue (Lee *et al*., 2008; Navarro *et al*., 2004, 2008). Consequently, numerous requirements were recognized as essential for the manufacturing of scaffolds in tissue engineering: the scaffold should have (1) connecting pores of a scale suitable to support incorporation and vascularization of tissues through allowing cell migration, conveying of gases, metabolites, nutrients and signal molecules both inside the scaffold and amongst the scaffold and the local environment, (2) substances that restricted the biodegradability or bioresorbability in order for the host tissue to finally substitute the scaffold through allowing to be break down by biological procedures at a rate compatible to the rate of tissue growth while supporting mechanical reliability at a giving time which vary from weeks to many months, (3) suitable surface chemistry to support cell connection, distinction and growing, (4) satisfactory mechanical properties, (5) not stimulate an adverse reaction, and (6) simple range of forms and dimensions (Li and Li, 2005; Lee *et al*., 2008; Navarro *et al*., 2004, 2008). Having these necessities in mind, numerous substances have been accepted or produced and made-up into scaffolds (Harrison, 2007).

A number of polymers are normally used in bone scaffolds, including collagen, hydroxyapatite, polylactic acid (PLA), polyglycolic acid (PGA) and polycaprolactone (PCL). Once artificial, scaffolding may sustain other surface modifications to improve their interactions with cells (Duan and Wang, 2010; Chang and Wang, 2011; Saber-Samandari *et al*., 2016a).

The fundamental principle of the present study was to use tissue engineering approach for restoration of critical size bone defect. The novel porous bioceramic scaffold has been developed using combination of cockle shell-drived $CaCO_3$ aragonite nanoparticles powder, gelatin, dextran, dextrin and deionized water. The cockle shell-drived $CaCO_3$ aragonite nanopowder (CCAN) was used for this study. The cockle (*Anadara granosa*) is certainly, the majority plentiful species that is cultured in Malaysia. A probable benefit of using cockle shells as a biomineral is that they could work as equivalents of calcium carbonate existing *in vivo*. CCAN is an inorganic nanocrystal synthesized using the top down approach of nanoparticle preparation. Cockles are dominant faunal bivalves present, sometimes comprising the entire bivalve fauna in deep shells beds on sandy mud flats in the upper parts of estuaries and harbors. They live in super abundance in the low tidal and shallow subtidal zones of most of the present-day estuaries and enclosed bays and harbours (Hayward, 1990). In Malaysia, the cockles (*Anadara granosa*) are cultivated in a large scale in the area of intertidal coastal bordering mudfield regions and in many part of South East Asian countries, mainly Thailand and Indonesia. They are by far, the most vital species cultured in Malaysia (Ibrahim, 1995). The cockle shells contain more than 98% $CaCO_3$ and thus, has the potential for the development of biomaterials for orthopedic applications (Awang-Hazmi *et al*., 2007).

Aragonite $CaCO_3$ polymorph is a thermodynamically less stable and less available form of crystalline $CaCO_3$ synthesized in laboratory. The size and shape of aragonite is strongly dependent on the preparation methods and conditions (Wang *et al*., 1999). Due to the huge striking properties of aragonite nanoparticles as a material of biomedical importance, researchers have paid huge attention on invention of methods for its controlled and facile synthesis at appropriate sizes and shapes using bottom up methods (Wang *et al*., 2006 a,b; Guo *et al*., 2007). Yet, none of these methods can promise production of pure aragonite nanoparticles of suitable sizes and shapes. Aragonites resulting from this production are often mixed with calcite (Guo *et al*., 2007) or calcite and vaterite (Chen and Xiang, 2009). Therefore, these methods may not be appropriate for specific biomedical applications. Though carbonation methods are found to be useful in industries and environmentally friendly, they are associated with the need for strict control of temperature, purified raw materials, and strenuous gas (CO_2 or combination of CO_2 and N_2) bubbling phases which are complicated, expensive and time consuming (Wang *et al*., 2007a). Other impurities such as BS-12 are also added to the final products (Wang *et al*., 2007a). Therefore, the top down approach of CCAN synthesis from its natural sources, for example cockle shells or sea shells is greatly promising (Islam *et al*., 2011).

The present study is undertaken to fabricate, characterize and biologically quantify these natural origin materials for potential tissue engineering applications in the form of a bone scaffold. The abundant availability of these materials and mainly their biocompatibility nature with significant similarities to the organic and mineral phases of the bone structure makes them an interesting candidate for the study. The use of cockle shells that are mainly considered as a waster product which are easily obtained with no cost and gelatin powder that are relatively cheap, coupled with simple laboratory techniques makes the production of the scaffold material to be extremely cost effective in regards to future commercialization if intended. Although drawbacks such as batch variations and limited mechanical stability may cause an issue, the advantages of using these materials for biomedical engineering clearly outweighs its limitations as justified through the findings of these study.

The constant demands for bone grafting materials, the drawbacks of the current grafting materials and techniques as well as the ever expending field of tissue engineering lays the foundation to embark on the current study in order to contribute to the development of the next generation of biomaterial based bone grafts.

Significant bone problems named trauma, deformity and tumors leave the patients under the pressure of surgical complications, high cost, risk of infection, donor shortage and slow healing process.

The hypothesis of the current study is that the fabricated porous nanocomposite bone scaffold is able to display the desired characteristics of an ideal bone grafting material and produce sufficient osteoconductive response in order to promote better bone healing.

Objectives of the study

The main objective of the study was to develop porous nanocomposite scaffold for critical size defect bone repair.

The specific objectives of this study were:

i. To synthesize and characterize calcium carbonate $CaCO_3$ nanoparticles in the aragonite phase from cockle shells.
ii. To develop porous nanocomposite scaffolds and determine their physical, chemical and biomechanical properties.
iii. To evaluate the porous nanocomposite scaffolds *In vitro* using cell line.
iv. To evaluate the porous nanocomposite scaffolds *in vivo* in a rabbitmodel.

REFERENCES

Abdal-hay, A., Khalil, K.A., Hamdy, A.S. and Al-Jassir, F.F. (2017). Fabrication of highly porous biodegradable biomimetic nanocomposite as advanced bone tissue scaffold. *Arabian Journal of Chemistry* 10 (2): 240–252.

Abu Bakar, M. S., Cheng, M. H., Tang, S. M., Yu, S. C., Liao, K., Tan, C. T., Khor, K.A. and Cheang, P. (2003). Tensile properties, tension-tension fatigue and biological response of polyetheretherketone hydroxyapatite composites for load-bearing orthopedic implants. *Biomaterials* 24 (13): 2245-2250.

Addadi, L., Raz, S. and Weiner, S. (2003). Taking advantage of disorder: Amorphous calcium carbonate and its roles in biomineralization. *Advanced Materials* 15 (12): 959-970.

Aguilar, F., Dusemund, B., Galtier, P., Gilbert, J., Gott, D.M., Grilli, S., Gürtler, R., König, J., Lambré, C., Larsen, J-C., Leblanc, J-C., Mortensen, A., Parent-Massin, D., Pratt, I., Rietjens, I.M.C.M., Stankovic, I., Tobback, P., Verguieva, T. and Woutersen, R.A. (2011). Scientific opinion on re-evaluation of calcium carbonate (E170) as a food additive. *European Food Safety Authority (EFSA) Journal* 9 (7): 2318-2354.

Aho, A. J., Eskola, J., Ekfors, T., Manner, I., Kouri, T. and Hollmen, T. (1998). Immune responses and clinical outcome of massive human osteoarticular allografts. *Clinical Orthopaedics* 346: 196-206.

Aizenberg, J., Lambert, G., Weiner, S. and Addadi, L. (2002). Factors involved in the formation of amorphous and crystalline calcium carbonate: A study of an ascidian skeleton. *Journal of the American Chemical Society* 124 (1): 32-39.

Ajikumar, P.K., Lakshminarayanan, R. and Valiyaveettil, S. (2004). Controlled deposition of thin films of calcium carbonate on natural and synthetic templates. *Crystal Growth and Design* 4 (2): 331-335.

Al-Hosney, H.A. and Grassian, V.H. (2004). Carbonic acid: An important intermediate in the surface chemistry of calcium carbonate. *Journal of the American Chemical Society* 126 (26): 8068-8069.

Altman, G. H., Lu, H. H., Horan, R. L., Calabro, T., Ryder, D., Kaplan, D.L., Stark, P. M., Richmond, J. C. and Vunjak-Novakovic, G. (2002). Advanced bioreactor with controlled application of multi-dimensional strain for tissue engineering. *Journal of Biomechanical Engineering* 124: 742–749.

Andrievski, R.A. (2003). Modern nanoparticle research in Russia. *Journal of Nanoparticle Research* 5 (5): 415-418.

Annabi, N., Nichol, J.W., Zhong, X., Ji, C., Koshy, S., Khademhosseini, A. and Dehghani, F. (2010). Controlling the porosity and microarchitecture of hydrogels for tissue engineering. *Tissue Engineering B* 16 (4): 371–383.

Annual fisheries statistics. (2003). Fisheries – Malaysia - Statistics. Kuala Lumpur. www.fao.org/docrep/008/ae934e/ae934e08.htm. *Annual Review of Physiology* 59: 575-599.

Antonio, C., Brunella, S., Francesca, R., Filippo, M.M., Paolo, B. and Carlo, G. (2006). The critical sized bone defect: Morphological study of bone healing. Annali della Facolta di Medicina Veterinaria, Universita di Parma, Vol. XXVI, 97-110.

Anvari-Yazdi, A., Yazdani, A., Talaei-Khozani, T. and Kalantar, M. (2013). Extraction and viability checking of various carbonated hydroxyapatite by Whartons' Jelly Mesenchymal stem cell. *Science International* 1 (5): 1-6.

Ardakani, M. H., Kavian, F., Moztarzadeh, F., Eslaminejad, M., Zamanian, A. and Bagheri, F. (2012). Poly (Lactic-co-glycolic) / nanostructured marinate porous composites for bone tissue engineering. I. Preparation and Morphology. *Key Engineering Materials* 493-494: 718-722.

Argintar, E., Edwards, S. and Delahay, J. (2011). Bone morphogenetic proteins in orthopaedic trauma surgery. *Injury* 42 (8): 730-734.

Armentano, I., Dottori, M., Fortunati, E., Mattioli, S. and Kenny, J.M. (2010). Biodegradable polymer matrix nanocomposites for tissue engineering: A review. *Polymer Degradation and Stability* 95 (11): 2126-2146.

Arnett, T.R. (2008). Extracellular pH regulates bone cell function. *The Journal of Nutrition* 138 (2): 415S-418S.

Arosa, F.A., Pereira, C.F. and Fonseca, A.M. (2004). Red blood cells as modulators of T cell growth and survival. *Current Pharmaceutical Design* 10 (2): 191-201.

Ashish, A. J. and Sergio, N. (2006). New dextrin supplementing fiber with innovation. *Pharmaceutical Technology* www.pharmtech.com.

Askarzadeh, K., Orang, F. and Moztarzadeh, F. (2005). Fabrication and characterization of a porous composite scaffold based on gelatin and hydroxyapatite for bone tissue engineering. *Iranian Polymer Journal* 14 (6): 511-520.

Atala, A. (2009). Engineering organs. *Biotechnology* 20: 575–592.

Athanasiou, K.A., Niederauer, G.G. and Agrawal, C.M. (1996). Sterilization, toxicity, biocompatibility and clinical applications of poly lactic acid/poly glycolic acid copolymers. *Biomaterials* 17 (2): 93-102.

Avella, M., Cosco, S., Di Lorenzo, M.L., Di Pace, E., Errico, M.E. and Gentile, G. (2006). Nucleation activity of nanosized $CaCO_3$ on crystallization of isotactic polypropylene in dependence on crystal modification, particle shape and coating. *European Polymer Journal* 42 (7): 1548-1557.

Avérous, L. (2008). Poly Lactic Acid: Synthesis, Properties and Applications. Chapter 021, pp. 433-435.

Avila, G., Misch, K., Galindo-Moreno, P. and Wang, H.L. (2009). Implant surface treatment using biomimetic agents. *Implant Dentistry* 18 (1): 17.

Avolio, R., Gentile, G., Avella, M., Carfagna, C. and Errico, M.E. (2013). Polymer–filler interactions in PET/$CaCO_3$ nanocomposites: Chain ordering at the interface and physical properties. *European Polymer Journal* 49 (2): 419-427.

Awang-Hazmi, A.J., Zuki, A.B.Z., Noordin, M.M., Jalila, A. and Norimah, Y. (2007). Mineral composition of the cockle (*Anadara granosa*) shells of west coast of Peninsular Malaysia and its potential as biomaterial for use in bone repair. *Journal of Animal and Veterinary Advances* 6 (5): 591-594.

Azami, M., Mohammad, R. and Fathollah, M. (2010a). Gelatin/hydroxyapatite nanocomposite scaffolds for bone repair. *Society of Plastic Engineers (SPE)*: 1-3.

Azami, M., Samadikuchaksaraei, A. and Poursamar, S.A. (2010b). Synthesis and characterization of a laminated hudroxyapatite/gelatin nanocomposite scaffold with controlled pore structure for bone tissue engineering. *International Journal of Artificial Organs* 33 (2): 86-95.

Azami, M., Tavakol, S., Samadikuchaksaraei, A., Hashjin, M.S., Baheiraei, N., Kamali, M. and Nourani, M.R. (2012). A porous hydroxyapatite/gelatin nanocomposite scaffold for bone tissue repair: *In vitro* and *in vivo* evaluation. *Journal of Biomaterials Science* 23: 2353-2368.

Badar, M., Lünsdorf, H., Evertz, F., Rahim, M.I., Glasmacher, B., Hauser, H. and Mueller, P.P. (2013). The formation of an organic coat and the release of corrosion microparticles from metallic magnesium implants. *Acta Biomaterialia* 9: 7580–7589.

Bailey, A. J., Light, N. D. and Atkins, E. D. (1980). Chemical cross-linking restrictions on models for the molecular organization of the collagen fiber. *Nature* 288: 408-410.

Bakar, Z.A., Hussein, B.F. and Mustapha, N.M. (2011). Cockle shell-based biocomposite scaffolds for bone tissue engineering. *Engineering, Regenerative Medicine and Tissue Engineering: Cells and Biomaterials*, Daniel Eberli (Ed.),

ISBN: 978-953-307-663-8, InTech, Available from: http://www.intechopen.com/books/regenerative-medicine-and-tissue-engineering-cells-and-biomaterials/cockle-shell-based-biocomposite-scaffold-for-bone-tissue-engineering.

Bancroft, G. N., Sikavitsas, V. I., van den Dolder, J., Sheffield, T. L., Ambrose, C. G., Jansen, J. A. and Mikos, A. G. (2002). Fluid flow increases mineralized matri deposition in 3D perfusion culture of marrow stromal osteoblasts in a dose-dependent manner. *Proceedings of the National Academy of Sciences of United States of America (PNAS)* 99: 12600-12605.

Bartolo, L. D., Rende, M., Morelli, S., Salerno, S.A.P. and Gordano, A. (2008). Influence of membrane surface properties on the growth of neuronal cells isolated form hippocampus. *Journal of Membrane Science* 325: 139-149.

Bauer, T. and Muschler, G. (2000). Bone graft materials: An overview of the basic science. *Clinical Orthopaedics* 371: 10-27.

Behnamghader, A., Bagheri, N., Raissi, B. and Moztarzadeh, F. (2008). Phase development and behavior of biphasic HA-TCP calcium phosphate materials prepared from hydroxyapatite and bioactive glass. *Journal of Materials Science: Materials in Medicine* 9 (1): 197-201.

Bernhardt, A., Despang, F., Lode, A., Demmler, A., Hanke, T. and Gelinsky, M. (2009). Proliferation and osteogenic differentiation of human bone marrow stromal cells on alginate-gelatin-hydroxyapatite scaffolds with anisotropic pore structure. *Journal of Tissue Engineering and Regenerative Medicine* 3 (1): 54-62.

Bernhardt, A., Lode, A., Peters, F. and Gelinsky, M. (2011). Novel ceramic bone replacement material osbone® in a comparative *In vitro* study with osteoblasts. *Clinical Oral Implants Research* 22 (6): 651-657.

Bernstein, A. (2011). Materials for orthopedic applications. SoMas, www.softmattergraduate.uni-freiburg.de.

Beu, A.G., Maxwell, P.A. and Brazier, R. (1990). Cenozoic Mollusca of New Zealand: New Zealand Geological Survey.

Bharatham, H., Zuki, B.Z., Perimal, E.K., Loqman, M.Y. and Hamid, M. (2014a). Development and characterization of novel porous 3D alginate-cockle shell powder nanobiocomposite bone scaffold (Research article). *BioMed Research International* 2014 Article ID 146723: 1-12.

Bharatham, H., Zuki, B.Z., Perimal, E.K., Loqman, M.Y. and Hamid, M. (2014b). Mineral and physiochemical evaluation of cockle shell (*Anadara granosa*) and

other selected molluscan shell as potential biomaterials. *Sains Malaysiana* 43 (7): 1023–1029.

Bianco, P., Riminucci, M., Gronthos, S. and Robey, P. G. (2001). Bone marrow stromal stem cells: Nature, Biology and potential applications. *Stem Cells* 19: 180-192.

Biomed Central. (2015). The role of Mesenchymal stem cells in bone fracture healing. Research, comment and community news in Biology and Medicine. *BMC Medicine*: 1-3. http://biome.biomedcentral.com.

Biopharma (2005). Course Notes in Pharmaceutical Freeze-Drying.

Birchall, J.D. (1971). Precipitated calcium carbonate: Google Patents.

Blackwood, K.A., Bock, N., Dargaville, T.R. and Woodruff, M.A. (2012). Scaffolds for growth factor delivery as applied to bone tissue engineering: Review article. *International Journal of Polymer Science* 2012: 1-25.

Blom, A. W., Cunningham, J.L., Hughes, G., Lawes, T. J., Smith, N., Blunn, G., Learmonth, I.D. and Goodship, A. E. (2005). The compatibility of ceramic bone graft substitutes as allograft extenders for use in impaction grafting of the femur. *Journal of Bone Joint Surgery* 87-B (3): 421-425.

Bobyn, J. D., Stackpool, G. J., Hacking, S. A., Tanzer, M. and Krygier, J. J. (1999). Characteristics of bone ingrowth and interface mechanics of a new porous tantalum biomaterial. *The Journal of Bone and Joint Surgery* 81 B (5): 907-914.

Boey, P.L., Maniam, G.P., Hamid, S.A. and Ali, M.H. (2011). Utilization of waste cockle shell (*Anadara granosa*) in biodiesel production from palm olein: Optimization using response surface methodology. *Elsevier Science* 90 (7): 2353-2358.

Borsari, V., Giavaresi, G., Fini, M., Torricelli, P., Salito, A., Chiesa, R., Chiusoli, L., Volpert, A., Rimondini, L. and Giardino, R. (2005). Physical characterization of different roughness titanium surfaces with and without hydroxyapatite coating and their effect on human osteoblast-like cells. *Journal of Biomedical Materials Research B: Applied Biomaterials* 75 (2): 359-368.

Bose, S., Roy, M. and Bandyopadhyay, A. (2012). Recent advances in bone tissue engineering scaffolds. *Trends in Biotechnology* 30 (10): 546–554.

Botchwey, E. A., Pollack, S. R., Levine, E. M. and Laurencin, C. T. (2001). Bone tissue engineering in a rotating bioreactor using a microcarrier matrix system. *Journal Biomedical Materials Research* 55: 242-253.

Boyne, N. P., Lambrianides, A. L. and Pollard, C. (2002). Incisional hernia through iliac crest bone graft donor sites. *ANZ Journal of Surgery* 72: 156-157.

Brydone, A. S., Meek, D. and Maclaine, S. (2010). Bone grafting, orthopaedic biomaterials and the clinical need for bone engineering. *Journal of Engineering in Medicine* 224: 1329.

Bucholz, R. W., Carlton, A. and Holmes, R. (1989). Interporous hydroxyapatite as a bone graft substitute in tibial plateau fractures. *Clinical Journal in Orthopaedics* 240: 53-62.

Buckwalter, J. A. (2004). Can tissue engineering help orthopaedic patients? Clinical needs and criteria for success. In: Tissue Engineering in Musculoskeletal Clinical Practice, edited by L. J. Sandell and A. J. Grodzinsky. *American Academy of Orthopaedic Surgeons*: 3-16.

Buczynski, C. and Chafetz, H.S. (1991). Habit of bacterially induced precipitates of calcium carbonate and the influence of medium viscosity on Mineralogy.

Burg, K. J. L., Porter, S. and Kellam, J. F. (2000). Biomaterial developments for bone tissue engineering. *Biomaterials* 21: 2347-2359.

Byrne, E.M., Farrell, E., McMahon, L.A., Haugh, M.G., O'Brien, F. J., Campbell, V.A., Prendergast, P.J. and O'Connell, B.C. (2008). Gene expression by marrow stromal cells in a porous collagen-glycosaminoglycan scaffold is affected by pore size and mechanical stimulation. *Journal of Materials Science: Materials in Medicine* 19: 3455-3463.

Cai, A., Xu, X., Pan, H., Tao, J., Liu, R., Tang, R. and Cho, K. (2008). Direct synthesis of hollow vaterite nanospheres from amorphous calcium carbonate nanoparticles via phase transformation. *Journal of Physical Chemistry C* 112 (30): 11324-11330.

Cai, K., Yao, K., Yang, Z., Qu, Y. and Li, X. (2007). Histological study of surface modified three dimensional poly (D, L-lactic acid) scaffolds with chitosan *in vivo*. *Journal of Materials Science: Materials in Medicine* 18: 2017-2024.

Calasans-Maia, M.D., Monteiro, M. L., Áscoli, F.O. and Granjeiro, J. M. (2009). The rabbit as an animal model for experimental surgery. *Acta Cirúrgica Brasileira* 24 (4): 325-328.

Caligur, V. (2008). Dextran and related polysaccharides. *BioFiles* 3:10-17.

Cao, W. and Hench, L. L. (1996). Bioactive materials. *Ceramics International* 22 (6): 493-507.

Carson, J.L., Terrin, M.L. and Jay, M. (2003). Anemia and postoperative Rehabilitation. *Canadian Journal of Anesthesia* 50 (6): 60-64.

Cartmell, S. H., Porter, B. D., Garcia, A. J. and Guldberg, R. E. (2003). Effects of medium perfusion rate on cell-seeded three-dimensional bone constructs *In vitro*. *Tissue Engineering* 6: 1197-1203.

Cavalu, S., Simon, V., Ratiu, C., Oswald, I., Vlad, S. and Ponta, O. (2014). Alternative approaches using animal model for implant biomaterials: Advantages and disadvantages. *Key Engineering Materials* 583: 101-106.

Chamberlain, L. J., Yannas, I. V., Hsu, H.P., Strichartz, G. and Spector, M. (1998a). Collagen-GAG substrate enhances the quality of nerve regeneration through collagen tubes up to level of autograft. *Experimental Neurology* 154: 315–329.

Chamberlain, L.J., Yannas, I.V., Arrizabalaga, A., Hsu, H.P., Norregaard, T.V. and Spector, M. (1998b). Early peripheral nerve healing in collagen and silicone tube implants: Myofibroblasts and the cellular response. *Biomaterials* 19: 1393-1403.

Champion, J.A., Katare, Y.K. and Mitragotri, S. (2007). Particle shape: A new design parameter for micro and nanoscale drug delivery carriers (a review). *Journal of Controlled Release* 121: 3-9.

Chan, C-M., Wu, J., Li, J-X. and Cheung, Y-K. (2002). Polypropylene/calcium carbonate nanocomposites. *Polymer* 43 (10): 2981-2992.

Chang, H.I. and Wang, Y. (2011). Cell responses to surface and architecture of tissue engineering scaffolds. *Regenerative Medicine and Tissue Engineering: Cells and Biomaterials*: 569-588.

Chang, S. C., Chuang, H., Chen, Y. R., Yang, L. C., Chen, J. K., Mardini, S., Chung, H. Y., Lu, Y. L., Ma, W. C. and Lou, J. (2004). Cranial repair using BMP-2 gene engineered bone marrow stromal cells. *Journal of Surgical Research* 119: 85-91.

Chantal, E. H., Molly S. S. and John, E. D. (2000). Engineering three dimensional bone tissue *In vitro* using biodegradable scaffolds: Investigating initial cell-seeding density and culture period. *Journal Biomedical Material Research* 51: 376-382.

Chen, C. S., Milan, M., Sui, H., Whitesides G. M. and Ingber, D. (1997). Geometric control of cell life and death. *Science* 276: 1425-1428.

Chen, C.Y., Ke, C.J., Yen, K.C., Hsieh, H.C., Sun, J.S. and Lin, F.H. (2015). 3D porous calcium-alginate scaffolds cell culture system improved human osteoblast cell clusters for cell therapy. *Theranostics* 5 (6): 643-655.

Chen, J. and Xiang, L. (2009). Controllable synthesis of calcium carbonate polymorphs at different temperatures. *Powder Technology* 189: 64-69.

Chen, J.H., Liu, Q.L., Zhang, X.H. and Zhang, Q.G. (2007a). Pervaporation and characterization of chitosan membranes cross-linked by 3-aminopropyltriethoxysilane. *Journal of Membrane Science* 292: 125–132.

Chen, Q. Z. and Boccaccini, A.R. (2006). Poly (D,L-lactic acid) coated 45S5 Bioglass®-based scaffolds: Processing and characterization. *Journal of Biomedical Materials Research A* 77 (3): 445–457.

Chen, Z., Li, C., Yang, Q. and Nan, Z. (2010). Transformation of novel morphologies and polymorphs of $CaCO_3$ crystals induced by the anionic surfactant SDS. *Materials Chemistry and Physics* 123: 534-539.

Cheng-Yu, W., Jing-Zhe, Z., Yan-Hua, L., Yu-Peng, G., Xu, Z., Yan-Hui, Y.D. and Zi-Chen, H.W. (2005). Synthesis in situ of active calcium carbonate nanoparticles via simulating biomineralization. *Chemical Journal* 26 (1): 13-15. http://www.cjcu.jlu.edu.cn/CN/Y2005/V26/11/13.

Cheung, H., Lau, K., Lu, T. and Hui, D. (2007). A critical review on polymer-based bioengineered materials for scaffold development. *Composites B: Engineering* 38 (3): 291-300.

Choi, S.J., Oh, J.M. and Choy, J.H. (2009). Toxicological effects of inorganic nanoparticles on human lung cancer A549 cells. *Journal of Inorganic Biochemistry* 103 (3): 463-471.

Christenson, E.M., Anseth, K.S., van den Beucken, J.J.J.P., Chan, C.K., Ercan, B., Jansen, J.A. and Lurecin, C.T. (2007). Nanobiomaterial applications in orthopedics. *Journal of Orthopaedic Research* 25 (1): 11-22.

Chu, T. M., Halloran, J. W., Hollister, S. J. and Feinberg, S. E. (2001). Hydroxyapatite implants with designed internal architecture. *Journal of Materials Science: Materials in Medicine* 12: 471-478.

Chu, T. M., Orton, D. G., Hollister, S. J., Feinberg, S. E. and Halloran, J. W. (2002). Mechanical and *in vivo* performance of hydroxyapatite implants with controlled architectures. *Biomaterials* 23: 1283-1293.

Chua, K.N., Chai, C., Lee, P.C., Ramakrishna, S., Leong, K.W. and Mao, H.Q. (2007). Functional nanofiber scaffolds with different spacers modulate adhesion and expansion of cryopreserved umbilical cord blood hematopoietic stem/progenitor cells. *Experimental Hematology* 35 (5): 771–781.

Chung, C. and Burdick, J.A. (2008). Engineering cartilage tissue. *Advanced Drug Delivery Reviews* 60 (2): 243-262.

Ciapetti, G., Ambrosio, L., Savarino, L., Granchi, D., Cenni, E., Baldini, N., Pagani, S., Guizzardi, S., Causa, F. and Giunti, A. (2003). Osteoblast's growth and function in porous poly ε-caprolactone matrices for bone repair: A preliminary study. *Biomaterials* 24 (21): 3815-3824.

Clarinval, A.M. and Halleux, J. (2005). Classification of biodegradable polymers. In: Smith R, editor. *Biodegradable Polymers for Industrial Applications*. 1st ed. Boca Raton, FL, USA: CRC Press. pp. 3–31.

Constantz, B., Ison, I., Fulmer, M., Poser, R., Smith, S. and VanWagoner, M. (1995). Skeletal repair by in situ formation of the mineral phase of bone. *Science* 267 (5205): 1796-1799.

Coradin, T., Bah, S. and Livage, J. (2004). Gelatin/silicate interactions: From nanoparticles to composite gels. *Colloids and Surfaces B: Biointerfaces* 35 (1): 53-58.

Cypher, T. J. and Grossman, J. P. (1996). Biological principles of bone graft healing. *Journal of Foot Ankle Surgery* 35: 413–417.

Czekanska, E.M., Stoddart, M.J., Richards, R.G. and Hayes, J.S. (2012). In search of an osteoblast cell model for *In vitro* research. *European Cells and Materials* 24: 1-17.

Da Silva, V.M. (2009). Development of New Chitosan Based Biodegradable Blends for Bone and Cartilage Tissue Engineering. PhD Thesis, Universidade do Minho.

Dahham, Kh. M. and Nainar, M.A.M. (2013). Mechanical properties and morphological studies on Pu-Ha biocomposite. *International Journal of Science and Research, India Online* 2 (8): 2319-7064.

Dalby, M.J., Gadegaard, N., Curtis, A.S. and Oreffo, R.O. (2007). Nanotopographical control of human osteoprogenitor differentiation. *Current Stem Cell Research and Therapy* 2 (2): 29e138.

Dangtungee, R., Yun, J. and Supaphol, P. (2005). Melt Rheology and extrudate swell of calcium carbonate nanoparticle-filled isotactic polypropylene. *Polygraph Tests* 24 (1): 2-11.

Datta, N., Holterf, H. L., Sikuritsas, V. I., Jansen, J. A. and Mikos, A. G. (2005). Effect of bone extracellular matrix synthesized *In vitro* on the osteoblastic differentiation of marrow stromal cells. *Biomaterials* 26: 971-977.

Dawson, J. I., Wahl, D. A., Lanham, S. A., Kanczler, J. M., Czernuszka, J. T. and Oreffo, R. O. (2008). Development of specific collagen scaffolds to support the osteogenic and chondrogenic differentiation of human bone marrow stromal cells. *Biomaterials* 29: 3105-3116.

Day, R.M., Boccaccini, A.R., Shurey, S., Roether, J.A., Forbes, A. and Hench, L.L. (2004). Assessment of poly glycolic acid mesh and bioactive glass for soft-tissue engineering scaffolds. *Biomaterials* 25: 5857–5866.

de Groot, K., de Putter, C., Smitt, P. and Driessen, A. (1981). Mechanical failure of artificial teeth made of dense calcium hydroxyapatite. *Science of Ceramics* 11: 433–437.

De Leeuw, N.H. and Parker, S.C. (1998). Surface structure and morphology of calcium carbonate polymorphs calcite, aragonite and vaterite: An atomistic approach. *The Journal of Physical Chemistry B* 102 (16): 2914-2922.

Dean, D. P. D., Topham, N. S. M. D., Rimnac, C. P.D., Mikos, A. G. P. D., Goldberg, D. P. M. D., Jepsen, K. P. D., Redtfeldt, R., Liu, Q., Pennington, D. and Ratcheson, R. (1999). Osseointegration of preformed poly methylmethacrylate craniofacial prostheses coated with bone marrow impregnated poly (DL-lactic-co-glycolic acid) foam. *Plastic and Reconstructive Surgery* 104 (3): 705-712.

Delabarde, C., Manson, J.A.E. and Plummer, J.C. (2011). Bioresorbable Nanocomposite Foams for Bone Tissue Engineering. PhD Thesis, Ecole Polytechnique Federale De Lausanne (EPFL). http://dx.doi.org/10.5075/epfl-thesis-5111.

D'Haese, P.C., Spasovski, G.B., Sikole, A., Hutchison, A., Freemont, T.J., Sulkova, S. and Balducci, A. (2003). A multicenter study on the effects of lanthanum carbonate (Fosrenol™) and calcium carbonate on renal bone disease in dialysis patients. *Kidney International* 63: S73-S78.

Di Lorenzo, M.L.M., Errico, E. and Avell, M. (2002). Thermal and morphological characterization of poly (ethylene terephthalate)/calcium carbonate nanocomposites. *Journal of Material Science* 37: 2351-2358.

Díaz-Rodríguez, P., González, P., Serra, J. and Landin, M. (2014). Key parameters in blood-surface interactions of 3D bioinspired ceramic materials. *Materials Science and Engineering C* 41: 232–239.

Dinopoulos, H., Dimitriou, R. and Giannoudis, P.V. (2012). Bone graft substitutes: What are the options? *The surgeon* 10: 230-239.

Dittrich, R., Tomandi, G., Despang, F., Bernhardt, A., Hanke, Th., Pompe, W. and Gelinsky, M. (2007). Scaffolds for hard tissue engineering by ionotropic gelatin

of alginate-influence of selected preparation parameters. *Journal of the American Ceramic Society* 90 (6): 1703-1708.

Domaschke, H., Gelinsky, M., Burmeister, B., Fleig, R., Hanke, T., Reinstorf, A., Pompe, W. and Rösen-Wolff, A. (2006). *In vitro* ossification and remodeling of mineralized collagen I scaffold. *Tissue Engineering* 12 (4): 949-958.

Donath, K., Rohrer, M.D. and Beck-Mannagetta, J. (1987). A histologic evaluation of a mandibular cross section one year after augmentation with hydroxyapatite particles. *Oral Surgery, Oral Medicine and Oral Pathology* 63 (6): 651-655.

Draget, K.I., Skjak-Braek, G. and Smidsrod, O. (1997). Alginate based new materials. *International Journal of Biological Macromolecules* 21 (1-2): 47-55.

Duan, B. and Wang, M. (2010). Customized Ca-P/PHBV nanocomposite scaffolds for bone tissue engineering: Design, fabrication, surface modification and sustained release of growth factor. *Journal of the Royal Society Interface*: 1-15.

Ducheyne, P., Radin, S., Heughebaert, M. and Heughebaert, J. C. (1990). Calcium phosphate ceramic coatings on porous titanium: Effect of structure and composition on electrophoretic deposition, vacuum sintering and *In vitro* dissolution. *Biomaterials* 11(4): 244-254.

Duelen, R. and Sampaolesi, M. (2017). Stem cell technology in cardiac regeneration: A pluripotent stem cell promise. *EBioMedicine* 16: 30–40.

Ehara, A., Ogata, K., Imazato, S., Ebisu, S., Nakano, T. and Umakoshi, Y. (2003). Effects of a-TCP and TetCP on MC3T3-E1 proliferation, differentiation and mineralization. *Biomaterials* 24 (5): 831-836.

El Ghannam, A., Amin, H., Nasr, T. and Shama, A. (2004). Enhancement of bone regeneration and graft material resorption using surface-modified bioactive glass in cortical and human maxillary cystic bone defects. *International Journal of Oral Maxillofacial Implants* 19 (2): 184-191.

Elgendy, H.M., Norman, M.E., Keaton, A.R. and Laurencin, C.T. (1993). Osteoblast-Like cell (MC3T3-E1) proliferation on bioerodible polymers: An approach towards the development of a bone-bioerodible polymer composite material. *Biomaterials* 14 (4): 263-269.

Fadhlia, Z.Z., Fauziah, A.A. and Nahulan, T. (2007). Spectroscopic studies of cow femurs and porites species coral from Sabah. *Journal of Nuclear and Related Technology (Special Edition)* 4: 181-184.

Fathi, M.H. and Mortazavi, V. (2007). Tantalum, niobium and titanium coatings for biocompa improvement of dental implants. *Dental Research Journal* 4 (2): 74-82.

FDA. 2015. www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/Implants.../June 2015.

Fellows, C.R., Matta, C. and Mobasheri, A. (2016). Applying proteomics to study crosstalk at the cartilage-subchondral bone interface in osteoarthritis: Current status and future directions. *EBioMedicine* 11: 2–4.

Ferrer, M.C.H. (2007). Development and Characterization of Completely Degradable Composite Tissue Engineering Scaffolds. PhD Thesis, University of Barcelona.

Fisher, J.P. and Reddi, A.H. (2003). Functional Tissue Engineering of Bone: Signals and Scaffolds. Chapter 5. II Bone: 1-29.

Fleming, J., Cornell, C. and Muschler, G. (2000). Bone cells and matrices in orthopaedic tissue engineering. *Orthopaedic Clinics North America* 31: 357-374.

Fouad, H., Elsarnagawy, T., Almajhadi, F.N. and Khalil, K.A. (2013). Preparation and *In vitro* thermo-mechanical characterization of electrospun PLGA nanofibers for soft and hard tissue replacement. *International Journal of Electrochemical Science* 8: 2293-2304.

Freed, L. E. and Vunjak-Novakovic, G. (1995). Cultivation of cell-polymer tissue constructs in simulated microgravity. *Biotechnology Progress* 46: 306-313.

Freed, L. E., Vunjak-Novakovic, G., Biron, R. J., Eagles, D. B., Lesnoy, D. C., Barlow, S. K. and Langer, R. (1994a). Biodegradable polymer scaffolds for tissue engineering. *Biotechnology* 12: 689-693.

Friedenstein, A. J., Chailakhyan, R. K. and Gerasimov, U. V. (1987). Bone marrow osteogenic stem cells: *In vitro* cultivation and transplantation in diffusion chambers. *Cell and Tissue Kinetics* 20: 263-272.

Fujihara, K., Kotaki, M. and Ramakrishna, S. (2005). Guided bone regeneration membrane made of poly caprolactone/calcium carbonate composite nanofibers. *Biomaterials* 26: 4139-4147.

Furukawa, T., Sato, H., Murakami, R., Zhang, J., Duan, Y.X., Noda, I., Ochiai, S. and Ozaki, Y. (2005). Structure, dispensability and crystallinity of poly (hydroxybutyrate)/poly (L-lactic acid) blends studied by FT-IR microspectroscopy and differential scanning calorimetry. *Macromoleculus* 38: 6445–6454.

Gasser, B. (2000). About composite materials and their use in bone surgery. *Injury* 31(Suppl 4): 48-53.

Gehrke, N., Colfen, H., Pinna, N., Antonietti, M. and Nassif, N. (2005). Superstructures of calcium carbonate crystals by oriented attachment. *Crystal Growth and Design* 5: 1317-1319.

Geiger, M., Li, R. H. and Friess, W. (2003). Collagen sponges for bone regeneration wit rhBMP-2. *Advanced Drug Delivery Reviews* 55: 1613-1629.

Gendler, E., Gendler, S. and Nimni, M. E. (1984). Toxic reactions evoked by glutaraldehyde-fixed pericardium and cardiac valve tissue bioprosthesis. *Journal of Biomedical Materials Research* 18: 727-736.

Genge, B. R., Sauer, G. R., Wu L. N., Mc Lean, F. M. and Wuthier, R. E. (1988). Correlation between loss of alkaline phosphatase activity and accumulation of calcium during matrix vesicle-mediated mineralization. *Journal Biological Chemistry* 263: 18513-18519.

Gettens, R.J., FitzHugh, E.W. and Feller, R.L. (1974). Calcium carbonate whites. *Studies in Conservation* 19 (3): 157-184.

Ghanaati, S., Unger, R.E., Webber, M.J., Barbeck, M., Orth, C., Kirkpatrick, J.A., Booms, P., Motta, A., Migliaresi, C., Sader, R.A. and Kirkpatrick C.J. (2011). Scaffold vascularization *in vivo* driven by primary human osteoblasts in concert with host inflammatory cells. *Biomaterials* 32 (32): 8150-8160.

Glover. 2016. www.marketsandmarkets.com/Market-Reports/dental implants.../Septembr2016.

Goldstein, A. S. (2001). Effect of seeding osteoprogenitor cells as dense clusters on cell growth and differentiation. *Tissue Engineering* 7 (6): 817-827.

Gomes, M. E. and Reis, R. L. (2004). Biodegradable polymers and composites in biomedical applications from catgut to tissue engineering. Part I: Available systems and their properties. *International Materials Reviews* 49 (55): 261-273.

Gomes, M. E., Azevedo, H. S., Moreira, A. R., Ellä, V., kellomäki, M. and Reis, R. L. (2008). Strach-poly (episilon-caprolactone) and starch-poly (Lactic-acid) fiber-mesh scaffolds for bone tissue engineering applications: Structure, mechanical properties and degeneration behaviour. *Journal of Tissue Engineering and Regenerative Medicine* 2 (5): 243-252.

Gomes, M. E., Sikavitsa, V. I., Behravesh, E., Reis, R. L. and Mikos, A. G. (2003). Effect of flow perfusion on the osteogenic differentiation of bone marrow

stromal cells cultured on starch-based three-dimensional scaffolds. *Journal of Biomedical Materials Research* 67A: 87-95.

Gomes, M.E., Holtorf, H.L., Reis, R.L. and Mikos, A.G. (2006). Influence of the porosity of starch-based fiber mesh scaffolds on the proliferation and osteogenic differentiation of bone marrow stromal cells cultured in a flow perfusion bioreactor. *Tissue Engineering* 12 (4): 801-809.

Gonzalez, C., Mariam, A.S., Manuela, D.L.T., Basilio, J.C., Francisco, D.P., Jose, A.V., Torre, B.J., Collia, F., Pedro, J.A., Vazquez, B. and Roman, J.S. (2006). Injectable and self-curing composites of acrylic/bioactive glass and drug systems. A histomorphometric analysis of the behavior in rabbits. *Biomaterials* 27 (9): 1778-1787.

González-Toro, D.C. and Thayumanavan, S. (2013). Advances in polymer and polymeric nanostructures for protein conjugation. *European Polymer Journal* 49: 2906–2918.

Gottrup, F. (2004a). A specialized wound-healing center concept: Importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. *The American Journal of Surgery* 187: 38S–43S.

Gottrup, F. (2004b). Oxygen in wound healing and infection. *World Journal of Surgery* 28 (3): 312-315.

Gough, J.E., Jones, J.R. and Hench, L.L. (2004). Nodule formation and mineralization of human primary osteoblasts cultured on a porous bioactive glass scaffold. *Biomaterials* 25 (11): 2039-2046.

Govindan, R., Kumar, G.S. and Girija, E.K. (2015). Polymer coated phosphate glass/hydroxyapatite composite scaffolds for bone tissue engineering applications. *RSC Advances* 5: 60188-60198.

Gower, L. and Tirrell, D. (1998). Calcium carbonate films and helices grown in solutions of poly (aspartate). *Journal of Crystal Growth* 191 (1-2): 153-160.

Gross-Aviv, T. and Vago, R. (2009). The role of aragonite matrix surface chemistry on the chondrogenic differentiation of Mesenchymal stem cells. *Biomaterials* 30 (5): 770-779.

Gu, W., Wu, C., Chen, J. and Xiao, Y. (2013). Nanotechnology in the targeted drug delivery for bone diseases and bone regeneration. *International Journal of Nanomedicine* 8: 2305-2317.

Guo, F., Li, Y., Xu, H., Zhao, G. and He, X. (2007). Size-controllable synthesis of calcium carbonate nanoparticles using aqueous foam film as templates. *Materials Letters* 61 (27): 4937-4939.

Guo, X., Yan, S., Shi, B. and Feng, Y. (2011). Effect of excessive vitamin A on alkaline phosphatase activity and concentrations of calcium-binding protein and bone Gla-protein in culture medium and CaBP mRNA expression in osteoblasts of Broiler Chickens. *Asian Australian Journal of Animal Science* 24 (2): 239.

Habibovic, P. and De Groot, K. (2007). Osteoinductive biomaterials properties and relevance in bone repair. *Journal of Tissue Engineering and Regenerative Medicine* 1 (1): 25-32.

Habibovic, P., Barrere, F., van Blitterswijk, C.A., de Groot, K. and Layrolle, P. (2002). Biomimetic hydroxyapatite coating on metal implants. *Journal of American Ceramic Society* 85 (3): 517-522.

Hacking, S. A., Harvey, E. J., Tanzer, M., Krygier, J. J. and Bobyn, J. D. (2003). Acid-etched microtexture for enhancement of bone growth into porous-coated implants. *The Journal of Bone and Joint Surgery* 85 B (8): 1182-1189.

Hager, E. A. (2004). Composite gelatin delivery system for bone regeneration. DBS. Massachusetts Institute of Technology.

Hak, D.J. (2007). The use of osteoconductive bone graft substitutes in orthopaedic trauma. *Journal of the American Academy of Orthopaedic Surgeons* 15 (9): 525-536.

Hallman, M. and Thor, A. (2008). Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry. *Periodontology 2000* 47 (1): 172-192.

Hamilton, D.W., Riehle, M.O., Monaghan, W. and Curtis, A.S. (2005). Articular chondrocytes passage number: Influence on adhesion, migration, cytoskeletal organization and phenotype in response to nano- and micro-metric topography. *Cell Biology International* 29: 408e21.

Hammadi, N.I., Abba, Y., Hezmee, M.N.M., Razak, I.S.A., Jaji, A.Z., Isa, T., Mahmood, S. Kh. and Zakaria, Z.A.B. (2017). Formulation of a sustained release Docetaxel loaded cockle shell-derived calcium carbonate nanoparticles against breast cancer. *Pharmaceutical Research* 34 (6): 1193–1203.

Han, J., Zhou, Z., Yin, R., Yang, D. and Nie, J. (2010). Alginate chitosan/hydroxyapatite polyelectrolyte complex porous scaffolds: Preparation and characterization. *International Journal of Biological Macromolecules* 46 (2): 199–205.

Hannouche, D., Petite, H. and Sedel, L. (2001). Current trends in the enhancement of fracture healing. *Journal of Bone and Joint Surgery* 83-B: 2.

Harada, S. and Rodan, G. A. (2003). Control of osteoblast function and regulation of bone mass. *Nature* 423: 349-355.

Harlan Laboratories. (2010). Calcium carbonate (nano): Oral gavage combined repeat dose toxicity study with reproduction/developmental toxicity screening test in the rat. Project number: 2974/0010. Unpublished study report provided by CCA Europe. May 2011.

Harley, B. A. C. and Gibson, L. J. (2008). *In vivo* and *In vitro* applications of collagen-GAG scaffolds. *Chemical Engineering Journal* 137: 102-121.

Harrison, K. (2007). Introduction to polymeric scaffolds for tissue engineering. In: *Biomedical Polymers*, ed. M. Jenkins, Wood head publishing Lts, Cambridge, pp.1-32.

Hatcher, B. M. (2004). Bioactive Organic-Inorganic Hybrids for Tissue Engineering Applications. PhD Thesis, University of Florida.

Haugh, M.G. (2009). The Development of Novel Scaffolds for Tissue Engineering with A Range of Structural and Mechanical Properties. PhD Thesis, University of Dublin, Trinity College Dublin.

Hautamäki, M. (2012). Repair of Segmental Bone Defects with Fiber-Reinforced Composite: A Study of Material Development and an Animal Model on Rabbits. PhD Thesis, Annales Universitas Turkuensis, Turku, Finland.

Hayward, B.W. (1990). *Kawerua Mollusca*. *Tane* 32: 1-9.

Hayward, B.W., Blom, W., Morley, M., Stephenson, A.B. and Hollis, C.J. (1994). Benthic Ecology of Whangape Harbour. *Northland Records of the Auckland Institute and Museum* 31: 219-230.

He, X-w., Liu, T., Chen, Y-x., Cheng, lD-j.,Li, X-r., Xiao, Y. and Feng, Y-l. (2008). Calcium carbonate nanoparticle delivering vascular endothelial growth factor-C siRNA effectively inhibits lymph angiogenesis and growth of gastric cancer *in vivo*. *Cancer Gene Therapy* 15: 193-202.

Hench, L. L. (1998a). Bioceramics. *Journal of American Ceramic Society* 81: 1705-1728.

Hench, L. L. (1998b). Biomaterials: A forecast for the future. *Biomaterials* 19 (16): 1419-1423.

Hench, L.L. and Polak, J.M. (2002). Third-generation biomedical materials. *Science* 295 (5557): 1014-1017.

Hendrich, C., Noth, U., Stahl, U., Merklein, F., Rader, C.P., Schutze, N., Thull, R., Tuan, R.S. and Eulert, J. (2002). Testing of skeletal implant surfaces with human fetal osteoblasts. *Clinical Orthopaedics and Related Research*: 278-394.

Henton, D.E., Gruber, P., Lunt, J. and Randall, J. (2005). Poly Lactic Acid Technology. 1741, Chapter 16: 527-578.

Herold, G. H., Hurvitz, A. and Tadmor, A. (1971). The effect of growth hormone on the healing of experimental bone defects. *Acta Orthopaedica Scandinavica Journal* 42 (5): 377-384.

Hill, J. (2008). *In vitro* study investigation the mechanical properties of acrylic bone cement containing calcium carbonate nanoparticles. *Journal of Materials Science: Materials in Medicine* 19 (11): 3327-3333.

Hillsley, M.V. and Frangos, J. A. (1994). A review: Bone tissue engineering: The role of interstitial fluid flow. *Biotechnology and Bioengineering* 43: 573-581.

Hing, K.A., Wilson, L.F. and Buckland, T. (2007). Comparative performance of three ceramic bone graft substitutes. *The Spine Journal* 7 (4): 475-490.

Ho, E. Y. (2005). Engineering Bioactive Polymer for the Next Generation of Bone Repair. PhD Thesis, Drexel University.

Hofmann, S., Hagenmuller, H., Koch, A.M., Muller, R., Vunjak-Novakovic, G., Kaplan, D.L., Merkle, H.P. and Meinel, L. (2007). Control of *In vitro* tissue-engineered bonelike structures using human Mesenchymal stem cells and porous silk scaffolds. *Biomaterials* 28 (6): 1152-1162.

Hoi-Yan, C., Kin-Tak, L., Tung-Po, L. and David, H. (2007). A critical review on polymer based bioengineered materials for scaffold development. *Composites B: Engineering* 38: 291-300.

Hollinger, J. O. and Chaudhari, A. (1992). Bone regeneration materials for the mandibular and craniofacial complex. *Journal Cells and Materials* 2: 143-151.

Holmes, R. E. (1979). Bone regeneration within a coralline hydroxyapatite implant. *Plastic Reconstructive Surgery* 63: 626-633.

Holmes, R., Bucholz, R. and Mooney, V. (1986). Porous hydroxyapatite as a bone graft substitute in metaphyseal defects: A histometric study. *Journal of Bone and Joint Surgery* 68A: 904.

Holsworth, R.E., Cho, Y.I. and Weidman, J. (2013). Effect of hydration on whole blood viscosity in firefighters. *Alternative Therapies in Health and Medicine* 19 (4): 44-49.

Holtorf, H. L., Jansen, J. A. and Mikos, A. G. (2006). Modulation of cell differentiation in bone tissue engineering constructs cultured in a bioreactor. *Advances in Experimental Medicine and Biology* 585: 225-241.

Holtorf, H. L., Sheffield, T. L., Ambrose, C. G., Jansen, J. A. and Mikos, A. G. (2005). Flow perfusion culture of marrow stromal cells seeded on porous biphasic calcium phosphate ceramics. *Annals of Biomedical Engineering* 33: 1238-1248.

Hoque, M.E., Shehryar, M. and Islam, K.M.N. (2013). Processing and characterization of cockle shell calcium carbonate ($CaCO_3$) bioceramic for potential application in bone tissue engineering. *Journal of Materials Science and Engineering* 2 (4): 1-5.

Hornberger, H., Virtanen, S. and Boccaccini, A.R. (2012). Biomedical coatings on magnesium alloys: A review. *Acta Biomaterialia* 8: 2442-2455.

Hu, R., Lin, C. and Shi, H. (2007). A novel ordered nanohydroxyapatite coating electrochemically deposited on titanium substrate. *Journal of Biomedical Materials Research A* 80 (3): 687–692.

Huang, R.L., Kobayashi, E., Liu, K. and Li, O. (2016). Bone graft prefabrication following the *in vivo* bioreactor principle. *EBioMedicine* 12: 43–54.

Huang, Y., Onyeri, S., Siewe, M., Moshfeghian, A. and Madihally, S. (2005). *In vitro* characterization of chitosan-gelatin scaffold for tissue engineering. *Biomaterials* 26: 7616-7627.

Hum, J., Luczynski, K.W., Nooeaid, P., Newby, P., Lahayne, O., Hellmich, C. and Boccaccini, A.R. (2013). Stiffness improvement of 45S5 bioglass ® -based scaffolds through natural and synthetic biopolymer coating: An Ultrasonic study. *Strain* 49: 431–439.

Hunt, T. K. and Hopf, H.W. (1997). Wound healing and wound infection. What Surgeons and anesthesiologists can do? *Surgical Clinics of North America* 77 (3): 587-606.

Hutmacher, D.W. and Cool, S. (2007). Concepts of scaffold-based tissue engineering: The rationale to use solid free-form fabrication techniques. *Journal of Cellular and Molecular Medicine* 11(4): 654-669.

Hutmacher, D.W., Schantz, J.T., Lam, G. X. F., Tan, K. C. and Lim, T. C. (2007). State of the art and future directions of scaffold-based bone engineering from a biomaterial perspective. *Journal of Tissue Engineering and Regenerative Medicine* 1 (4): 245-260.

Ibrahim, N. (1995). Trace element content of Malaysian cockles (*Anadara granosa*). *Food Chemistry* 54 (2): 1333-1335.

Ignatius, A. A., Betz, O., Augat, P. and Claes, L. E. (2001). *In vivo* investigations on composites made of resorbable ceramics and poly (lactide) used as bone graft substitutes. *Journal of Biomedical Materials Research* 58 (6): 701-709.

Ilan, D.I. and Ladd, A.L. (2002). Bone graft substitutes. *Operative Techniques in Plastic and Reconstructive Surgery* 9 (4): 151–160.

Ingber, D. E. (1997). Tensegrity: The architectural basis of cellular mechanotransduction. *Annual Review of Physiology* 59: 575-599. https://doi.org/10.1146/annurev.physiol.59.1.575.

Isa, T., Zakaria, Z.A.B., Rukayadi, Y., Hezmee, M.N.M., Jaji, A.Z., Imam, M.U., Hammadi, N.I. and Mahmood, S. Kh. (2016). Antibacterial activity of ciprofloxacin-encapsulated cockle shells calcium carbonate (aragonite) nanoparticles and its biocompatibility in macrophage J774A.1. *International Journal of Molecular Sciences* 17: 713.

Ishaug-Riley, S. L., Crane, G. M., Miller, M. J., Yasko, A. W., Yaszemski, M. J. and Mikos, A. (1997b). Ectopic bone formation by marrow stromal osteoblast transplantation using poly (D,L-lactic-co-glycolic acid) foams implanted into the rat mesentery. *Journal of Biomedical Material Research* 36: 1-8.

Islam, K.H., Zuki, A.B.Z., Ali, M.E., Zobir, M.H., Noordin, M.M., Loqman, M.Y., Miah, G., Wahid, H. and Hashim, U. (2013). A novel method for the synthesis of calcium (aragonite) nanoparticles from cockle shells. *Journal of Powder Technology*: 70-75.

Islam, K.H., Zuki, A.B.Z., Noordin, M.M., Zobir, M.H., Abd Rahman, N.S. and Ali, M.E. (2011). Characterization of calcium carbonate and its polymorphs from cockle shells (*Anadara granosa*). *Journal of Powder Technology* 213 (1-3): 188-191.

Islam, K.N., Zuki, A.b.Z., Ali, M.E., Hussein, M.Z.B., Noordin, M.M., Loqman, M.Y., Wahid, H., Hakim, M.A. and Hamid, S.B.A. (2012). Facile synthesis of calcium carbonate nanoparticles from cockle shells: Research article. *Journal of Nanomaterials* 2012: 1-5.

ISO (International Organization for Standardization). (2010). I.I.O.f. in Nanotechnologies Vocabulary Part 1: Core Terms, TS 80004-1.

Jäger, M., Feser, T., Denck, H. and Krauspe, R. (2005). Proliferation and osteogenic differentiation of Mesenchymal stem cells cultured onto three different polymers *In vitro*. *Annals of Biomedical Engineering* 33: 1319-1332.

Jaiswal, N., Haynesworth, S., Caplan, A. and Bruder, S. (1997). Osteogenic differentiation of purified culture expanded human Mesenchymal stem cells *In vitro*. *Journal of Cellular Biochemistry* 64: 295-312.

Jaji, A.Z., Zakaria, Z.A.B., Mahmud, R., Loqman, M.Y., Hezmee, M.N.M., Abba, Y., Isa, T. and Mahmood, S. Kh. (2017). Safety assessments of subcutaneous doses of aragonite calcium carbonate nanocrystals in rats. *Journal of Nanoparticle Research* 19:175.

Jakoi, A.M., Iorio, J.A. and Cahill, P.J. (2015). Autologous bone graft harvesting: A review of grafts and surgical techniques. *Musculoskeletal Surgery* 99 (3): 171-178.

Jamshidian, M., Tehrany, E.A., Imran, M., Jacquot, M. and Desobry, S. (2010). Poly Lactic Acid: Production, applications, nanocomposites and release studies. *Comprehensive Reviews in Food Science and Food Safety* 9: 552-571. Doi 10.1111/j.1541-4337.2010.00126.x.

Janicki, P. and Schmidmaier, G. (2011). What should be the characteristics of the ideal bone graft substitute? Combining scaffolds with growth factors and/or stem cells. *Injury* 42: S77-S81.

Jiang, T., Abdel-Fattah, W. I. and Laurencin, C. T. (2006). *In vitro* evaluation of chitosan/poly (Lactic acid-glycolic acid) sintered microsphere scaffolds for bone tissue engineering. *Biomaterials* 27 (28): 4894-4903.

Jin, H.H., Kim, D.H., Kim, T.W., Shin, K.K., Jung, J.S., Park, H.C. and Yoon, S.Y. (2012). *In vivo* evaluation of porous hydroxyapatite/chitosan-alginate composite scaffolds for bone tissue engineering. *International Journal of Biological Macromolecules* 51(5): 1079-1085.

Johnson, A.L., Stein, L.E. and Roe, S.C. (2014). Evaluation of collagen as a retainer for autogenous cancellous bone used in repair of full thickness cortical bone defects. *Researchgate.net* 16 (2): 146-150.

Jonsson, K. B., Frost, A., Nilsson, O., Ljunghall, S. and Ljunggren, Öten. (1999). Three isolation techniques for primary culture of human osteoblast-like cells. *Acta Orthopaedica Scandinavica* 70 (4): 365-373.

Jorge-Herrero, E., Fernandez, P., Turnay, J., Olmo, N., Calero, P., Garcõca, R., Freile, I. and Castillo-Olivares, J.L. (1999). Influence of different chemical cross-linking treatments on the properties of bovine pericardium and collagen. *Biomaterials* 20: 539-545.

Kamba, A.S., Ismail, M., Ibrahim, T.A.T., Zakaria, Z.A.B. and Gusau, L.H. (2014). *In vitro* ultrastructural changes of MCF-7 for Metastasise bone cancer and induction of apoptosis via mitochondrial cytochrome C released by $CaCO_3$/ Dox nanocrystals: Research article. *BioMed Research International* 2014: 1-14.

Kamba, S.A. and Zakaria, Z.A.B. (2014). Osteoblasts growth behavior on bio-based calcium carbonate aragonite nanocrystal. *BioMed Research International* 2014, Article ID 215097, 9 pages.

Kang, H. G., Kim, S. Y. and Lee, Y. M. (2006). Novel porous gelatin scaffolds by overrun/particle leaching process for tissue engineering applications. *Journal of Biomedical Material Research B: Applied Biomaterials* 79B: 388–397.

Kanitkar, M., Tailor, H.D. and Khan, W.S. (2011). The use of growth factors and Mesenchymal stem cells in orthopaedics. *The Open Orthopaedics Journal* 5: 271-275.

Katti, K. S. (2004). Biomaterials in total joint replacement. *Colloids and Surfaces B: Biointerfaces* 39 (3): 133-142.

Katz, J. (1981). Composite Materials Models for Cortical Bone. In: Cowin S, editor. *Mechanical Properties of Bone*. NY: Ed. A.S.M.E. pp.171-184.

Kawaguchi, H.H., Sakai, K., Sera, S., Nakajima, T., Ebisawa, Y. and Koyama, K. (1992). Synthesis of nanosized calcium carbonate (aragonite). *Colloid Polymer Science*: 270.

Kenley, R. A., Yim, K., Abrams, J., Ron, E., Turek, T., Marden, L. J. and Hollinger, J. O. (1993). Biotechnology and bone graft substitutes. *Pharmaceutical Research* 10 (10): 1393-1401.

Keränen, P., Itälä, A., Kooet, J., Kohonen, I., Dalstra, M., Kommonen, B. and Aro, H.T. (2007). Bioactive glass granules as extender of atuogenous bone grafting in cement less intercalary implant of the canine femur. *Scandinavian Journal of Surgery* 96: 243-251.

Kessel, R. G. (1989). Basic Medical Histology: The Biology of Cells, Tissue and Organs. pp. 144-145.

Khor, E. (1997). Methods for the treatment of collagenous tissues for bioprostheses. *Biomaterials* 18: 95-105.

Kikuchi, M., Matsumoto, H.N., Yamada, T., Koyama, Y., Takakuda, K. and Tanaka, J. (2004). Glutaraldehyde cross-linked hydroxyapatite/collagen self-organized nanocomposites. *Biomaterials* 25: 63–69.

Kim, H. W., Kim, H. E. and Salih, V. (2005). Stimulation of osteoblast responses to biomimetic nanocomposites of gelatin-hydroxyapatite for tissue engineering scaffolds. *Biomaterials* 26 (25): 5221-5230.

Kim, H. W., Knowles, J. C. and Kim, H. E. (2004). Development of hydroxyapatite bone scaffold for controlled drug release via poly (epsilon-caprolactone) and hydroxyapatite hybrid coatings. *Journal of Biomedical Materials Research* 70B: 240-249.

Kim, H., kim, H.M., Jang, J.E., Kim, C.M., Kim, E.Y., Lee, D. and Khang, G. (2013). Osteogenic differentiation of bone marrow stem cell in poly (Lactic-co-glycolic acid) scaffold loaded various ratio of hydroxyapatite. *International Journal of Stem Cells* 6 (1): 67–74.

Kim, H., Lee, J. H. and Suh, H. (2003b). Interaction of Mesenchymal stem cells and osteoblasts for *In vitro* osteogenesis. *Yonsei Medical Journal* 44: 187-197.

Kim, H.M., Miyaji, F., Kokubo, T., Nishiguchi, S. and Nakamura, T. (1999). Graded surface structure of bioactive titanium metal prepared by chemical treatment. *Journal of Biomedical Materials Research* 45: 100–107.

Kim, H.W., Lee, S.Y., Bae, C.J., Noh, Y.J., Kim, H.E., Kim, H.M. and Ko, J.S. (2003a). Porous ZrO_2 bone scaffold coated with hydroxyapatite with fluorapatite intermediate layer. *Biomaterials* 24: 3277–3284.

Kim, H.W., Noh, Y.J., Koh, Y.H., Kim, H.E. and Kim, H.M. (2002). Effect of CaF_2 on densification and properties of hydroxyapatite–zirconia composites for biomedical applications. *Biomaterials* 23: 4113–4121.

Kirkland, N.T., Birbilis, N. and Staiger, M.P. (2012). Assessing the corrosion of biodegradable magnesium implants: A critical review of current methodologies and their limitations. *Acta Biomaterialia* 8: 925–936.

Klemm, D. (2006). Advances in polymer science. *Polysaccharides II* 205: 199.

Kneser, U., Schaefer, D.J., Polykandriotis, E. and Horch, R.E. (2006). Tissue engineering of bone: The reconstructive surgeon's point of view. *Journal of Cellular and Molecular Medicine* 10 (1): 7-19.

Koegler, W.S. and Griffith, L.G. (2004). Osteoblast response to PLGA tissue engineering scaffolds with PEO modified surface chemistries and demonstration of patterned cell response. *Biomaterials* 25 (14): 2819-2830.

Kohn, D.H., Sarmadi, M., Helman, J.I. and Krebsbach, P.H. (2002). Effects of pH on human bone marrow stromal cells *In vitro*: Implications for tissue engineering of bone. *Journal of Biomedical Materials Research* 60 (2): 292-299.

Kolan, K.C.R., Leu, M.C., Hilmas, G.E. and Comte, T. (2013). Effect of architecture and porosity on mechanical properties of borate glass scaffolds made by selective laser sintering. *Rapid Prototyping Journal* 65: 816-826.

Kose, G. T., Korkusuz, F., Korkusuz, P., Purali, N., Özkul, A. and Hasirci, V. (2003). Bone generation on PHBV matrices: An *In vitro* study. *Biomaterials* 24: 4999-5007.

Krajewski, A., Ravaglioli, A., Roncari, E., Pinsco, P. and Montanari, L. (2000). Porous ceramic bodies for drug delivery. *Journal of Materials Science: Materials in Medicine* 11: 763–772.

Kreuter, J. (1983). Evaluation of nanoparticles as drug-delivery systems. III. Materials, stability, toxicity, possibilities of targeting and use. *Pharmaceutica Acta Helvetiae* 58: 242–250.

Kumbar, S.G., Laurencin, C.T. and Deng, M. (2014). Natural and Synthetic polymers. Elsevier, San Diego, USA.

Kuriyan, M. and Carson, J.L. (2005). Anemia and clinical outcomes. *Anesthesiology Clinics of North America* 23 (2): 315-325.

Küther, J., Seshadri, R., Knoll, W., Tremel, W. and Mater, J. (1998a). Templated crystallization of calcium and strontium carbonates on centered rectangular self-assembled monolayer substrates. *Chemistry* 8: 641.

Lam, C. X. F., Mo, X. M., Teoh, S. H. and Hutmacher, D. W. (2002). Scaffold development using 3D printing with a starch-based polymer. *Materials Science and Engineering* 20: 49–56.

Langer, R. and Vacanti, J.P. (1993). Tissue engineering. *Science* 260: 920-926.

Lanza, R.P. and Chick, W.L. (1997). Transplantation of encapsulated cells and tissues. *Surgery* 121 (1): 1-9.

Le´vesque, S. G., Lim, R. M. and Shoichet, M. S. (2005). Macroporous interconnected dextran scaffolds of controlled porosity for tissue engineering applications. *Biomaterials* 26: 7436–7446.

Leach, J.K., Kaigler, D., Wang, Z., Krebsbach, P.H. and Mooney, D.J. (2006). Coating of VEGF-releasing scaffolds with bioactive glass for angiogenesis and bone regeneration. *Biomaterials* 27: 3249–3255.

Lee, C. R., Grodzinsky, A. J. and Spector, M. (2001a). The effects of cross-linking of collagen-glycosaminoglycan scaffolds on compressive stiffness, chondrocyte-mediated contraction, proliferation and biosynthesis. *Biomaterials* 22: 3145-3154.

Lee, I., Han, S.W., Choi, H.J. and Kim, K. (2001b). Nanoparticle-directed crystallization of calcium carbonate. *Advanced Materials* 13 (21): 1617.

Lee, J., Cuddihy, M.J. and Kotov, N.A. (2008). Three-dimensional cell culture matrices: State of the art. *Tissue Engineering B* 14 (1): 61-86.

Lee, J.K., Lee, K.H. and Jin, B.S. (2001c). Structure development and biodegradability of uniaxially stretched poly (L-lactide). *European Polymer Journal* 37: 907.

Lee, K. Y., Shim, J. and Lee, H. G. (2004). Mechanical properties of collagen and gelatin composite films. *Carbohydrate Polymers* 56 (2): 251-254.

Lee, K., Silva, E. and Mooney, D. (2011). Growth factor delivery-based tissue engineering: General approaches and a review of recent developments. *Journal of the Royal Society Interface* 8 (55): 153-170.

Lee, K.Y. and Mooney, D.J. (2012). Alginate: Properties and biomedical applications. *Progress in Polymer Science* 37: 106-126.

Lee, S. J., Choi, J. S., Park, K. S., Khang, G., Lee, Y. M. and Lee, H. B. (2004). Response of MG63 osteoblast-like cells onto polycarbonate membrane surfaces with different micropore sizes. *Biomaterials* 25: 4699-4707.

Lee, S. M., Cho, D. H., Park, W. H., Lee, S. G., Han, S. O. and Drzal, L. T. (2005). Novel silk/ poly (butylenes succinate) biocomposites: The effect of short fiber content on their mechanical and thermal properties. *Composites Science and Technology* 65: 647–657.

Lee, S., Kim, S.H., Han, Y. and Kim, Y. (2001d). Synthesis and degradation of end-group functionalized poly lactide. *Journal of Polymer Science A: Polymer Chemistry* 39: 973.

Lee, S., Porter, M., Wasko, S., Lau, G., Chen, P.Y., Novitskay, E.E., Tomsia, A.P., Almutairi, A., Meyers, M.A. and McKittrick, J. (2012). Potential bone replacement materials prepared by two methods. *MRS proceeding*: 1418.

Leila, B., Hamid, R.R., Seyed, M.H. and Mohamad, A.S. (2010). Investigation of biphasic calcium phosphate/gelatin nanocomposite scaffolds as a bone tissue engineering. *Ceramics international* 8: 2421-2426.

Lemos, A.F., Rocha, J.H.G., Quaresma, S.S.F., Kannan, S., Oktar, F.N., Agathopoulos, S. and Ferreira, J.M.F. (2006). Hydroxyapatite nano-powders produced hydrothermally from nacreous material. *Journal of the European Ceramic Society* 26 (16): 3639-3646.

Li, M. and Mann, S. (2002). Emergent nanostructures: Water-induced mesoscale transformation of surfactant-stabilized amorphous calcium carbonate nanoparticles in reverse microemulsions. *Advanced Functional Materials* 12: 773-779.

Li, Q., Ding, Y., Li, F., Xie, B. and Qian, Y. (2002). Solvothermal growth of vaterite in the presence of ethylene glycol,1,2-propanediol and glycerin. *Journal of Crystal Growth* 236 (1-3): 357-362. Doi: 10.1016/s0022-0248(01)02130-3.

Li, S., de Wijn, J. R., Li, J., Layrolle, P. and de Groot, K. (2003). Macroporous biphasic calcium phosphate scaffold with high permeability/porosity ratio. *Tissue Engineering* 9 (3): 535-548.

Li, Z. and Li, Z. B. (2005). Repair of mandible defect with tissue engineering bone in rabbits. *ANZ Journal of Surgery* 75:1017–1021.

Li, Z., Ramay, H.R., Hauch, K.D., Xia, D. and Zhang, M. (2005). Chitosan–alginate hybrid scaffolds for bone tissue engineering. *Biomaterials* 26: 3919-3928.

Lian, J. B. and Stein, G. S. (1992). Concepts of osteoblast growth and differentiation: Basis for modulation of bone cell development and tissue formation. *Critical Reviews in Oral Biology and Medicine* 3: 269-305.

Lim, J.Y., Kim, S.H., Lim, S. and Kim, Y.H. (2003). Improvement of flexural strengths of poly (L-lactic acid) by solid-state extrusion: Extrusion through rectangular die. *Macromolecular Materials and Engineering* 288: 50–57.

Lin, A. S., Barrows, T. H., Cartmell, S. H. and Guldberg, R. E. (2003). Microarchitectural and mechanical characterization of oriented porous polymer scaffolds. *Biomaterials* 24: 481-489.

Lin, H.R. and Yeh, Y.J. (2004). Porous alginate/hydroxyapatite composite scaffolds for bone tissue engineering: Preparation, characterization and *In vitro* studies. *Journal of Biomedical Materials Research B: Applied Biomaterials* 71 (1): 52-65.

Lind, M. and Bunger, C. (2001). Factors stimulating bone formation. *European Spine Journal* 10: S102-S109.

Lindholm, P.F., Annen, K. and Ramsey, G. (2011). Approaches to minimize infection risk in blood banking and transfusion practice. *Infectious Disorders Drug Targets* 11 (1): 45-56.

Liu, H.C., Lee, I.C., Wang, J.H., Yang, S.H. and Young, T.H. (2004). Preparation of PLLA membranes with different morphologies for culture of MG-63 Cells. *Biomaterials* 25 (18): 4047-4056.

Liu, S., Jin, F., Lin, K., Lu, J., Sun, J., Chang, J., Dai, K. and Fan, C. (2013). The effect of calcium silicate on *In vitro* physiochemical properties and *in vivo* osteogenesis, degradability and bioactivity of porous β-tricalcium phosphate bioceramics. *Biomedical Materials* 8 (2): 1-2.

Liuyun, J., Yubao, L. and Chengdong, X. (2009). Preparation and biological properties of a novel composite scaffold of nano-hydroxyapatite/chitosan/carboxymethyl cellulose for bone tissue engineering. *Journal of Biomedical Science* 16 (1): 65.

Lode, A., Bernhardt, A., Kroonen, K., Springer, M., Briest, A. and Gelinsky, M. (2009). Development of a mechanically stable support for the osteoinductive biomaterial COLLOSS®E. *Journal of Tissue Engineering and Regenerative Medicine* 3 (2): 149-152.

Loh, Q. and Choong, C. (2013). Three-dimensional scaffolds for tissue engineering applications: Role of porosity and pore size. *Tissue Engineering B* 19 (6): 485–502.

Long, M. W. (2001). Osteogenesis and bone-marrow-derived cells. *Blood Cells, Molecules and Diseases* 27: 677- 690.

Loy, J.E., Guo, J.H. and Severtson, S.J. (2004). Role of adsorption fractionation in determining the $CaCO_3$ scale inhibition performance of poly disperse sodium poly acrylate. *Industrial and Engineering Chemistry Research* 43 (8): 1882-1887.

Lu, H.H., El-Amin, S.F., Scott, K.D. and Laurencin, C.T. (2003). Three-dimensional, bioactive, biodegradable, polymer-bioactive glass composite scaffolds with improved mechanical properties support collagen synthesis and mineralization of human osteoblast-like cells *In vitro*. *Journal Biomedical Materials* 64 (A): 465-474.

Lucas, A., Gaud, J., Carel, C., Michel, J.F. and Cathelineau, G. (2001). A synthetic aragonite-based ceramic as a bone graft substitute and substrate for antibiotics. *International Journal of Inorganic Materials* 3 (1): 87-94.

Ma, T., Yang, S. T. and Kniss, D. (2001). Oxygen tension influences proliferation and differentiation in a tissue-engineered model of placental trophoblast like cells. *Tissue Engineering* 7: 495–505.

Maeno, S., Niki,Y., Matsumoto, H., Morioka, H., Yatabe, T., Funayama, A., Toyama, Y., Taguchi, T. and Tanaka, J. (2005). The effect of calcium ion concentration on osteoblast viability, proliferation and differentiation in monolayer and 3D culture. *Biomaterials* 26: 4847–4855.

Majumdar, M. K., Thiede, M. A., Mosca, J. D., Moorman, M. and Gerson, S. L. (1998). Phenotypic and functional comparison of cultures of marrow-derived Mesenchymal Stem Cells (MSCs) and stromal cells. *Journal Cell Physiology* 176: 57-66.

Malafaya, P.B., Santos, T.C., van Griensven, M. and Reis, R.L. (2008). Morphology, mechanical characterization and *in vivo* neo-vascularization of chitosan particle aggregated scaffolds architectures. *Biomaterials* 29 (29): 3914-3926.

Malafaya, P.B., Silva, G.A. and Reis, R.L. (2007). Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Advanced Drug Delivery Reviews* 59 (4-5): 207-233.

Mann, F.A., Constantinescu, C.M. and Yoon, H.Y. (2011). Fundamentals of small animals' surgery. Wiley-Blackwell, A John Wiley and Sons, Ltd., pp. 8-16.

Manoli, F. and Dalas, E. (2000). Spontaneous precipitation of calcium carbonate in the presence of ethanol, isopropanol and diethylene glycol. *Journal of Crystal Growth* 218 (2): 359-364.

Mapara, M., Thomas, B.S. and Bhal, K.M. (2012). Rabbit as an animal model for experimental research. *Dental Research Journal (Isfahan)* 9 (1): 111-118.

Maria, M. and E-G. A. (2004). Bone Tissue Engineering Strategy Basal on Starch Scaffolds and Bone Marrow Cells Cultured in a Flow Perfusion Bioreactor. PhD Thesis, Universidade Do Minho Escola De Engenharia.

Martel, J. and Young, J.D. (2008). Purported nanobacteria in human blood as calcium carbonate nanoparticles. *Proceeding of the National Academy of Sciences of the United States of America* 105 (14): 5549-5554.

Martin, R. B., Chapman, M. W., Sharkey, N. A., Zissimos, S. L., Bay, B. and Shors, E. C. (1993). Bone ingrowth and mechanical properties of coralline hydroxyapatite after implantation. *Biomaterials* 14 (5): 341-348.

Mass, T., Drake, J.L., Haramaty, L., Rosenthal, Y., Schofield, O.M.E., Sherrell, R.M. and Falkowski, P.G. (2012). Aragonite precipitation by ''Proto-Polyps '' in coral cell cultures. *PLoS ONE* | www.plosone.org 7 (4): e35049.

Mastrogiacomo, M., Scaglione, S., Martinetti, R., Dolcini, L., Beltrame, F., Cancedda, R. and Quarto, R. (2006). Role of scaffold internal structure on *in vivo* bone formation in macroporous calcium phosphate bioceramics. *Journal of Biomaterials* 27: 3230–3237.

Mathew, B.M. and Mikos, A.G. (2007). Polymer scaffold fabrication. *Principals in Tissue Engineering: Academic Press*: 309-320.

Matta, C., Khademhosseinic, A. and Mobasheri, A. (2015). Mesenchymal stem cells and their potential for microengineering the chondrocyte niche. *EBioMedicine* 2: 1560–1561.

Mattila, R. H., Laurila, P., Rekola, J., Gunn, J., Lassila, L. V. J., Mäntylä, T., Aho, A.J. and Vallittu, P.K. (2009). Bone attachment to glass-fibre-reinforced composite implant with porous surface. *Acta Biomaterialia* 5 (5): 1639-1646.

McMahon, R.E., Wang, L., Skoracki, R. and Mathur, A.B. (2013). Development of nanomaterials for bone repair and regeneration. *Journal of Biomedical Materials Research B: Applied Biomaterials* 101(2): 387-397.

Mehta, R., Kumar, V., Bhunia, H. and Upadhyay, S.N. (2005). Synthesis of poly (Lactic acid): A review. *Journal of Macromolecular Science C: Polymer Reviews* 45: 325–349.

Melvik, J.E. and Dornish, M. (2004). Alginate as a carrier for cell immobilization. *Focus on Biotechnology* 8: 33-51.

Meng, D., Francis, L., Thompson, I.D., Mierke, C., Huebner, H., Amtmann, A., Roy, I. and Boccaccini, A.R. (2013a). Tetracycline-encapsulated P(3HB) microsphere-coated 45S5 Bioglass®-based scaffolds for bone tissue engineering. *Journal of Materials Science: Materials in Medicine* 24 (12): 2809–2817.

Meng, Z.X., Li, H.F., Sun, Z.Z., Zheng, W. and Zheng, Y.F. (2013b). Fabrication of mineralized electrospun PLGA and PLGA/gelatin nanofibers and their potential in bone tissue engineering. *Materials Science and Engineering C* 33 (2): 699–706.

Merriam-Webster online Medical Dictionary. An Encyclopedia Britannica Company. Retrieved online on 21/07/2015 from: http://www.merriam-webster.com/dictionary/calcium%20carbonate.

Metze, A.L., Grimm, A., Nooeaid, P., Roether, J.A., Hum, J., Newby, P.J., Schubert, D.W. and Boccaccini, A.R. (2013). Gelatin coated 45S5 bioglass® derived scaffolds for bone tissue engineering. *Key Engineering Materials* 541: 31–39.

Meyer, U., Joos, U. and Wiesmann, H. P. (2004a). Biological and biophysical principles in extracorporeal bone tissue engineering. Part I. *International Journal of Oral and Maxillofacial Surgery* 33: 325-332.

Mills, C.A., Navarro, M., Engel, E., Martinez, E., Ginebra, M.P., Planell, J., Errachid, A. and Samitier, J. (2006). Transparent micro- and nanopatterned poly (Lactic acid) for biomedical applications. *Journal of Biomedical Materials Research* 76: 781–787.

Mis and Vcf. (2009). U.S. Market for Spinal Implants. Medical Devices. *www.reportlinker.com*

Mistry, A. S. and Mikos, A. G. (2005). Tissue engineering strategies for bone regeneration. *Advances in Biochemical Engineering/Biotechnology* 94: 1–22.

Mittal, A., Negi, P., Garkhal, K., Vermas, S. and Kumar, N. (2010). Integration of porosity and bio-functionalization to form a 3D scaffold: Cell culture studies and *In vitro* degradation. *Biomedical Materials* 5 (4): 045001.

Mobini, S., Hoyer, B., Solati-Hashjin, M., Lode, A., Nosoudi, N., Samadikuchaksaraei, A. and Gelinsky, M. (2013). Fabrication and characterization of regenerated silk scaffolds reinforced with natural silk fibers for bone tissue engineering. *Journal of Biomedical Materials Research A* 101 (8): 2392-2404.

Mohamed, M., Yusup, S. and Maitra, S. (2012). Decomposition study of calcium carbonate in cockle shell. *Journal of Engineering Science and Technology* 7 (1): 1-10.

Mondrinos, M.J., Dembzynski, R., Lu, L., Byrapogu, V.K.C., Wootton, D.M., Lelkes, P.I. and Zhou, J. (2006). Porogen-based solid freeform fabrication of poly caprolactone–calcium phosphate scaffolds for tissue engineering. *Biomaterials* 27 (25): 4399-4408.

Moore, W. R., Graves, S. E. and Bain, G. I. (2001). Synthetic bone graft substitutes. *ANZ Journal of Surgery* 71(6): 354-361.

Moseke, C., Gelinsky, M., Groll, J. and Gbureck, U. (2013). Chemical characterization of hydroxyapatite obtained by wet chemistry in the presence of V, Co and Cu ions. *Journal of Materials Science and Engineering C* 33 (3): 1654-1661.

Munirah, S., Kim, S.H., Ruszymah, B.H. and Khang, G. (2008). The use of fibrin and poly (Lactic-co-glycolic acid) hybrid scaffold for articular cartilage tissue engineering: An *in vivo* analysis. *European Cells and Materials* 15: 41-52.

Murphy, C. M., Haugh, M. G. and O'brien, F. J. (2008). The Effect of Pore Size on Osteoblast Activity in Collagen-Glycosaminoglycan Scaffolds. TERMIS-EU. Porto.

Murugan, R. and Ramakrishna, S. (2004). Coupling of therapeutic molecules onto surface modified coralline hydroxyapatite. *Biomaterials* 25 (15): 3073-3080.

Mwangi, J. W. and Ofnfier, C. M. (2004). Cross-linked gelatin matrices: Release of a random coil macromolecular solute. *International Journal of Pharmaceutics* 278 (2): 319-327.

Nagai, Y., Yamazaki, C., Ma, K., Nozaki, T., Toyama, K. and Yamashita, T. (2012). Response of osteoblast-like MG63 cells to TiO_2 layer prepared by micro-arc oxidation and electric polarization. *Journal of the European Ceramic Society* 32 (11): 2647-2652.

Nagata, J. and Saito, M. (2006). Effects of simultaneous intakes of indigestible dextrin and diacylglycerol on lipid profiles in rats fed cholesterol diets. *Nutrition* 22: 395-400.

Naka, K., Huang, S.C. and Chujo, Y. (2006). *Langmuir* 22: 7760.

Nakagawa, H., Kamimura, M., Takahara, K., Hashidate, H., Kawaguchi, A., Uchiyama, S. and Miyasaka, T. (2006). Changes in total alkaline phosphatase level after hip fracture: Comparison between femoral neck and trochanter fractures. *Journal of Orthopaedic Science* 11: 135-139.

Nandi, S.K., Mukherjee, R.P., Kundu, B., De, D.K. and Basu, D. (2010). Orthopaedic applications of bone graft and graft substitutes: A review. *Indian Journal of Medical Research* 132: 15-30.

Narayan, R. (2009). Specialized fabrication processes: Rapid prototyping. *Biomedical materials*, Springer, Boston, US: 493-523.

Navarro, M., del Valle, S., Martinez, S., Zeppetelli, S., Ambrosio, L., Planell, J. A. and Ginebra, M.P. (2004). New macroporous calcium phosphate glass ceramic for guided bone regeneration. *Biomaterials* 25 (18): 4233–4241.

Navarro, M., Michiardi, A., Castaño, O. and Planell, J.A. (2008). Biomaterials in orthopaedics. *Journal of the Royal Society Interface* 5 (27): 1137-1158.

Nesic, D., Whiteside, R., Brittberg, M., Wendt, D., Martin, I. and Pierre Mainil-Varlet, P. (2006). Cartilage tissue engineering for degenerative joint disease. *Advanced Drug Delivery Reviews* 58 (2): 300-322.

Nihorbd. (2004). National institutes of health osteoporosis and related bone disease. National Resource Center. Accessed online on the 19th November. 2012 from http://www.osteo.org2004.

Nishida, J. and Shimamura, T. (2008). Methods of reconstruction for bone defect after tumor excision: A review of alternatives. *Medical Science Monitor* 14 (8): RA107-113.

Nkenke, E. and Stelzle, F. (2009). Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: A systematic review. *Clinical Oral Implants Research* 20 (S4): 124-133.

Novak, B.M. (1993). Hybrid nanocomposites materials between inorganic glasses and organic polymers. *Advanced Materials* 5: 422-433.

O'brien, F. J., Harley, B. A., Yannas, I. V. and Gibson, L. J. (2005). The effect of pore size on cell adhesion in collagen-GAG scaffolds. *Biomaterials* 26: 433- 441.

Oest, M.E., Dupont, K.M., Kong, H.J., Mooney, D.J. and Guldberg, R.E. (2007). Quantitative assessment of scaffold and growth factor-mediated repair of critically sized bone defects. *Journal of Orthopaedic Research*: 941-950.

Ohgushi, H., Okumura, M., Yoshikawa, T., Inboue, K., Senpuku, N., Tamai, S. and Shors, E.C. (1992). Bone formation processing porous calcium carbonate and hydroxyapatite. *Journal of Biomedical Materials Research* 26 (7): 885-895.

Okada, A., Uno, Y., McGeough, J.A., Fujiwara, K., Doi, K., Uemura, K. and Sano, S. (2008). Surface finishing of stainless steels for orthopedic surgical tools by large area electron beam irradiation. *CIRP Annals Manufacturing Technology* 57 (1): 223-226.

Olde Damink, L. H., Dijkstra, P. J., Van Luyn, M. J., Van Wachem, P.B., Nieuwenhuis, P. and Feijen, J. (1996). Cross-linking of dermal sheep collagen using a water-soluble carbodiimide. *Biomaterials* 17: 765-773.

Oliverira, A.L. and Reis, R. L. (2004). Pre-mineralization of starch/ polycrapolactone bone tissue engineering scaffolds by a calcium silicate-based process. *Journal of Materials Science: Materials in Medicine* 15: 533-540.

Olszta, M. J., Douglas, E. P. and Gower, L. B. (2003). Scanning Electron Microscopic on and bonding to calcium phosphate ceramic implants. *Materials Research Society Symposium* 14: 147-156.

Oryan, A., Alidadi, S., Moshiri, A. and Maffulli, N. (2014). Bone regenerative medicine: Classic options, novel strategies and future directions. *Journal of Orthopaedic Surgery and Research* 9: 18.

Owen, T.A., Aronow, M., Shalhoub, V., Barone, L.M., Wilming, L., Tassinari, M.S., Kennedy, M.B., Pockwinse, S., Lian, J.B. and Stein, G.S. (1990). Progressive development of the rat osteoblast phenotype *In vitro*: Reciprocal relationships in expression of genes associated with osteoblast proliferation and differentiation during formation of the bone extracellular matrix. *Journal of Cellular Physiology* 143 (3): 420-430.

Ozkan, M. (2004). Quantum dots and other nanoparticles: What they can offer to drug discovery. *Drug Discovery Today* 9 (24): 1065-1071.

Pabbruwe, M.B., Standard, O.C., Sorrell, C.C. and Howlett, C.R. (2004). Bone formation within alumina tubes: Effect of calcium, manganese and chromium dopants. *Biomaterials* 25 (20): 4901-4910.

Parch, I. P.D., Vittorio, O., Andreani, L., Piolanti, N., Cirillo, G., Iemma, F., Hampel, S. and Lisanti, M. (2013). How nanotechnology can really improve the future of orthopedic implants and scaffolds for bone and cartilage defects. *Journal of Nanomedine and Biotherapeutic Discovery* 3: 2.

Park, E. K., Lee, Y. E., Je-Yong, C., Sun-Ho, O., Hong-In, S., Kyo-Han, K., Kim, K. and Shin-Yoon, K. S. (2004). Cellular biocompatibility and stimulatory effects of calcium metaphosphate on osteoblastic differentiation of human bone marrow-derived stromal cells. *Journal Biomaterials* 25: 3403-3411.

Park, S. N., Park, J. C., Kim, H. O., Song, M. J. and Suh, H. (2002). Characterization of porous collagen/hyaluronic acid scaffold modified by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide cross-linking. *Biomaterials* 23: 1205-1212.

Parsons, A.J., Ahmed, I., Han, N., Felfel, R. and Rudd, C.D. (2010). Mimicking bone structure and function with structural composite materials. *Journal of Bionic Engineering* 7: S1-S10.

Pathak, R., Amarpal, A.H.P., Kinjavdekar, P., Pawde, A.M., Rashmi, M.T., Sharma, N. and Dhama, K. (2015). Bone Tissue Engineering: Latest trends and future perspectives. *Advances in Animal and Veterinary Sciences* 3 (4): 9-22.

Pearce, A.I., Richards, R.G., Milz, S., Schneider, E. and Pearce, S.G. (2007). Animal models for implant biomaterial research in bone: A review. *European Cells and Materials* 13: 1-10.

Pekkarinen, P. (2005). Effect of sterilization and delivery systems on the osteoinductivity of reindeer bone morphogenetic protein extract. *Dissertation*: 30-31.

Perez-Sanchez, M.J., Ramirez-Glindon, E., Lledo-Gil, M. and Calvo-Guirado, J.L. (2010). Biomaterials for bone regeneration. *Medicina Oral Patologia Oral Cirugia Bucal* 15: 517-522.

Perka, C., Schultz, O., Spitzer, R.S., Lindenhayn, K., Burmester, G.R. and Sittinger, M. (2000). Segmental bone repair by tissue-engineered periosteal cell transplants with bioresorbable fleece and fibrin scaffolds in rabbits. *Biomaterials* 21: 1145-1153.

Peter, M., Binulal, N. S. and Soumya, S. (2010). Nanocomposite scaffolds of bioactive glass ceramic nanoparticles disseminated chitosan matrix for tissue engineering applications. *Carbohydrate Polymers* 79 (2): 284–289.

Petite, H., Viateau, V., Bensaid, W., Meunier, A., de Pollak, C., Bourguignon, M., Oudina, K., Sedel, L. and Guillemin, G. (2000). Tissue engineering bone regeneration. *Nature Biotechnology* 18 (9): 959-963.

Petrochenko, P. and Narayan, R. (2010). Novel approaches to bone grafting: Porosity, Bone Morphogenetic Proteins, stem cells and the periosteum. *Journal of Long-Term Effects of Medical Implants* 20 (4): 303-315.

Philippart, A., Boccaccini, A.R., Fleck, C., Schubert, D.W. and Roether, J.A. (2015). Toughening and functionalization of bioactive ceramic and glaa bone scaffolds by biopolymer coating and infiltration: A review of the last 5 years. *Expert Review of Medical Devices* 12: 93–111.

Pilia, M., Guda, T. and Appleford, M. (2013). Development of composite scaffolds for load-bearing segmental bone defects. *BioMed Research International* 2013: 1-15.

Porter, A. E., Patel, N., Skepper, J. N., Best, S. M. and Bonfield, W. (2004). Effect of sintered silicate-substituted hydroxyapatite on remodeling processes at the bone-implant interface. *Biomaterials* 25: 3303-3314.

Porter, J.R., Ruckh, T.T. and Popat, K.C. (2009). Bone tissue engineering: A review in bone biomimetics and drug delivery strategies. *Biotechnology Progress* 25 (6): 1539-1560.

Porter, N. L., Pilliar, R. M. and Grynpas, M. D. (2001). Fabrication of porous calcium polyphosphate implants by solid freeform fabrication: A study of processing parameters and *In vitro* degradation characteristics. *Journal of Biomedical Material* 4 (56): 504-515.

Powell, H. M. and Boyce, S. T. (2006). EDC cross-linking improves skin substitute strength and stability. *Biomaterials* 27: 5821-5827.

Premnath, P., Tan, B. and Venkatakrishnan, K. (2012). Bioactive interlinked extracellular matrix–like silicon nano-network fabricated by femtosecond laser synthesis. *Bioresearch Open Access* 1 (5): 231–238.

Puelacher, W. C., Vacanti, J. P., Ferraro, N. F. S. and Vacanti, C. A. (1996). Femoral shaft reconstruction using tissue engineered growth of bone. *International Journal of Oral and Maxillofacial Surgery* 25: 223-228.

Puppi, D., Chiellini, F., Piras, A.M. and Chiellini, E. (2010). Polymeric materials for bone and cartilage repair. *Progress in Polymer Science* 35 (4): 403-440.

Purohit, R. and Venugopalan, P. (2009). Polymorphism. RESONANCE, September 2009, pp.882-893. http://www.ias.ac.in/resonance/Volumes/14/09/0882-0893.pdf.

Putnam, A.J. and Mooney, D.J. (1996). Tissue engineering using synthetic extracellular matrices. *Nature Medicine* 2 (7): 824-826.

Qi, R-J. and Zhu, Y-J. (2006). Microwave assisted synthesis of calcium carbonate (vaterite) of various morphologies in water-Ethylene glycol mixed solvents. *The journal of Physical Chemistry B* 110 (16): 8302-8306. Doi: 10.1021/jp060939s.

Quarles, L.D., Yohay, D.A., Lever, L.W., Caton, R. and Wenstrup, R.J. (1992). Distinct proliferative and differentiated stages of murine MC3T3-E1 cells in culture: An *In vitro* model of osteoblast development. *Journal of Bone and Mineral Research* 7 (6): 683-692.

Rainer, J.E. and Reto, L. (2012). Tissue engineering-nanomaterials in the musculoskeletal system. *Swiss Medical Weekly*: Review article 142: w13647.

Rajkumar, M., Meenakshisundaram, N. and Rajendran, V. (2011). Development of nanocomposites based on hydroxyapatite/sodium alginate: Synthesis and characterization. *Materials Characterization* 62 (5): 469–479.

Rani, V.V.D., Ramachandran, R., Chennazhi, K.P., Tamura, H., Nair, S.V. and Jayakumar, R. (2011). Fabrication of alginate/nanoTiO_2 needle composite scaffolds for tissue engineering applications. *Carbohydrate Polymers* 83 (2): 858-864.

Rashidi, N.A., Mohamed, M. and Yusup, S. (2012). The kinetic model of calcinations and carbonation of *Anadara Granosa*. *International Journal of Renewable Energy Research* 2 (3): 497-503.

Rath, S.N., Strobel, L.A., Arkudas, A., Beier, J.P., Maier, A.K., Greil, P., Horch, R.E. and Kneser, U. (2012). Osteoinduction and survival of osteoblasts and bone marrow stromal cells in 3D biphasic calcium phosphate scaffolds under static and dynamic culture conditions. *Journal of Cellular and Molecular Medicine* 16 (10): 2350-2361.

Ravi Kumar, M.N. (2000). Nano and microparticles as controlled drug delivery devices. *Journal of Pharmaceutical Sciences* 3: 234–258.

Razak, S.I.A., Sharif, N.F.A. and Rahman, W.A.W.A. (2012). Biodegradable polymers and their bone applications: A review. *International Journal of Basic and Applied Sciences* 12 (1): 31-49.

Reichert, J. C., Saifzadeh, S., Wullschleger, M. E., Epari, D. R., Schutz, M. A., Duda, G. N., Schell, H., van Griensven, M., Redl, H. and Hutmacher, D.W. (2009). The challenge of establishing preclinical models for segmental bone defect research. *Biomaterials* 30 (12): 2149-2163.

Reis, R.L. and San Roman, J. (2005). Components in Biodegradable Systems in Tissue Engineering and Regenerative Medicine. CRC Press: Boca Raton.

Ren, J., Zhao, P., Ren, T., Gu, S. and Pan, K. (2008). Poly (D, L-lactide)/nano-hydroxyapatite composite scaffolds for bone tissue engineering and biocompatibility evaluation. *Journal of Materials Science: Materials in Medicine* 19 (3): 1075-1082.

Rezania, A. and Healy, K. E. (2000). The effect of peptide surface density on mineralization of a matrix deposited by osteogenic cells. *Journal of Biomedical Materials Research* 52: 595-600.

Rose, F.R. and Oreffo, R. O. (2002). Bone tissue engineering: Hope vs hype. *Biochemical and Biophysical Research Communications* 292: 1-7.

Rosen, H. M. and McFarland, M. M. (1990). The biologic behavior of hydroxyapatite implanted into the maxillofacial skeleton. *Plastic Reconstructive Surgery* 85 (5): 718-723.

Ross, M.H. and Pawlina, W.A. (2006). Text and Atlas with Correlated Cell and Molecular Biology. 5th Edition, pp. 224-225.

Roszek, B., Jong, W. H. and Geertsma, R. (2005). Nanotechnology in medical applications: State-of-the-art in materials and devices. https://www.researchgate.net/publication/27451595_Nanotechnology...

Rucker, M., Laschke, M.W., Junker, D., Carvalho, C., Schramm, A., Mulhaupt, R., Gellrich, N-C. and Menger, M.D. (2006). Angiogenic and inflammatory

response to biodegradable scaffolds in dorsal skinfold chambers of mice. *Biomaterials* 27: 5027–5038.

Saber-Samandari, S. and Saber-Samandari, S. (2017). Biocompatible nanocomposite scaffolds based on copolymer-grafted chitosan for bone tissue engineering with drug delivery capability. *Materials Science and Engineering C* 75: 721-732.

Saber-Samandari, S., Saber-Samandari, S., Ghonjizade-Samani, F., Aghazadeh, J. and Sadeghi, A. (2016a). Bioactivity evaluation of novel nanocomposite scaffolds for bone tissue engineering: The impact of hydroxyapatite. *Ceramics International* 42 (9): 11055-11062.

Saber-Samandari, S., Saber-Samandari, S., Kiyazar, S., Aghazadeh, J. and Sadeghi, A. (2016b). *In vitro* evaluation for apatite-forming ability of cellulose-based nanocomposite scaffolds for bone tissue engineering. *International Journal of Biological Macromolecules* 86: 434-442.

Sachlos, E. and Czernuszka, J. T. (2003). Making tissue engineering scaffolds work review on the application of solid freeform fabrication technology to the production of tissue engineering scaffolds. *European Cell and Materials* 5: 29-40.

Sagar, N., Pandey, A.K., Gurbani, D., Khan, K., Singh, D., Chaudhari, B.P., Soni, V.P., Chattopadyay, N., Dhawan, A. and Bellare, J.R. (2013). *In vivo* efficacy of compliant 3D nano-composite in critical-size bone defect repair: A six month preclinical study in rabbit. *Plos One*, www.plosone.org, 8 (10): e77578.

Sahagian, M. E. and Goff, H. D. (1996). Fundamental Aspects of the Freezing Process, CRC Press.

Saini, S. and Wick, T. M. (2003). Concentric cylinder bioreactor for production of tissue engineered cartilage: Effect of seeding density and hydrodynamic loading on construct development. *Biotechnology Progress* 19: 510-521.

Sakata, S., Kei, T., Uchida, K. and Kaetsu, I. (2006). Nano-particle of hydrophobic poly lactic acid for DDS. *Polymer Preprints Japan* 55: 2074.

Sakolish, C.M., Esch, M.B., Hickman, J.J., Shuler, M.L. and Mahler, G.J. (2016). Modelling barrier tissues *In vitro*: Methods, achievements and challenges. *EBioMedicine* 5: 30–39.

Salerno, A., Zeppetelli, S., Di Maio, E., Iannace, S. and Netti, P. (2012). Architecture and properties of bi-model porous scaffolds for bone regeneration prepared via supercritical CO_2 foaming and porogen leaching combined process. *The Journal of Supercritical Fluids* 67: 114-122.

Salgado, A.J., Gomes, M.E., Chou, A., Coutinho, O.P., Reis, R.L. and Hutmacher, D.W. (2002). Preliminary study on the adhesion and proliferation of human osteoblasts on starch-based scaffolds. *Materials Science and Engineering C* 20 (1–2): 27-33.

Samartzis, D., Shen, F.H., Goldberg, E.J. and An, H.S. (2005). Is autograft the gold standard in achieving radiographic fusion in one-level anterior cervical discectomy and fusion with rigid anterior plate fixation? *Spine* 30 (15): 1756.

Santos Jr., A.R. and de Carvalho Zavaglia, C.A. (2016). Tissue Engineering Concepts. Reference Module in Materials Science and Materials Engineering.

Saraf, S. and Kumaraswamy, V. (2013). Basic research: Issues with animal experimentations. *Indian Journal of Orthopaedics* 47 (1): 6-9.

Sautier, J. M., Nefussi, J. R., Boulekbache, H. and Forest, N. (1990). *In vitro* bone formation on coral granules. *In vitro Cellular and Developmental Biology* 26: 1079-1085.

Sawada, K. (1997). The mechanisms of crystallization and transformation of calcium carbonates. *Pure and Applied Chemistry* 69 (5): 921-928.

Sawyer-Glover, A. M. and Shellock, F. G. (2000). Pre- MRI procedure screening: Recommendations and safety considerations for biomedical implants and devices. *Journal of Magnetic Resonance Imaging* 12 (1): 92-106.

Scheller, E.L., Krebsbach, P.H. and Kohn, D.H. (2009). Tissue engineering: State of the art in oral Rehabilitation. *Journal of Oral Rehabilitation* 36 (5): 368-389.

Schmelzer, E., Finoli, A., Nettleship, I. and Gerlach, J.C. (2015). Long- term three-dimensional perfusion culture of human adult bone marrow mononuclear cells in bioreactors. *Biotechnology and Bioengineering* 112 (4): 801-809.

Schnettler, R., Pfefferle, H-J., Kilian, O., Heiss, C., Kreuter, J., Lommel, D., Pavlidis, T., Stahl, J-P., Meyer, C., Wenisch, S. and Alt, V. (2005). Glycerol-l-lactide coating polymer leads to delay in bone ingrowth in hydroxyapatite implants. *Journal of Controlled Release* 106 (1-2): 154-161.

Searles, J. A. (2004). Freezing and Annealing Phenomena in Lyophilization. In RAY, L. and MAY, J. (Eds.) *Freeze-Drying/Lyophilization of Pharmaceutical and Biological Products*. 2nd ed. New York, Marcel Dekker.

Searles, J. A., Carpenter, J. F. and Randolph, T. W. (2001). Annealing to optimize the primary drying rate, reduce freezing-induced drying rate heterogeneity and determine T(g)' in pharmaceutical lyophilization. *Journal of Pharmaceutical Sciences* 90: 872-887.

Seo, Y.K., Yoon, H.H., Park, Y.S., Song, K.Y., Lee, W.S. and Park, J.K. (2008). Correlation between scaffold *in vivo* biocompatibility and *In vitro* cell compatibility using Mesenchymal and mononuclear cell cultures. *Cell Biology and Toxicology* 25 (5): 513-522.

Shahini, A., Yazdimamaghani, M., Walker, K.J., Eastman, M.A., Hatami-Marbini, H., Smith, B.J., Ricci, J.L., Madihally, S.V., Vashaee, D. and Tayebi, L. (2014). 3D conductive nanocomposite scaffold for bone tissue engineering. *International Journal of Nanomedicine* 9: 167-181.

Shan, D., Zhu, M., Xue, H. and Cosnier, S. (2007). Development of amperometric biosensor for glucose based on a novel attractive enzyme immobilization matrix: Calcium carbonate nanoparticles. *Biosensors and Bioelectronics* 22 (8): 1612-1617.

Shellock, F. G. (2002). Biomedical implants and devices: Assessment of magnetic field interactions with a 3.0-tesla MR system. *Journal of Magnetic Resonance Imaging* 16 (6): 721-732.

Shin, D. S., Choong, P. F., Chao, E. Y. and Sim, F. H. (2000). Large tumor endoprostheses and extracortical bone-bridging: 28 patients followed 10-20 years. *Acta Orthopaedica Scandinavica* 71(3): 305-311.

Shinoka, T. (2014). Development of a tissue-engineering vascular graft for use in congenital heart surgery. *EBioMedicine* 1: 12–13.

Sikavitasa, V. I., Bancroft, G. N., Lemoine, J. J., Liebschner, M. A. K., Dauner, M. and Mikos, A. G. (2005). Flow perfusion enhances the calcified matrix deposition of marrow stromal cells in biodegradable nonwoven fiber mesh scaffolds. *Annals of Biomedical Engineering* 33 (1): 63-70.

Sikavitsas, V. I., Bancroft, G.N. and Mikos, A. G. (2002). Formation of three-dimensional cell/polymer constructs for bone tissue engineering in a spinner flask and a rotating wall vessel bioreactor. *Journal of Biomedical Materials Research* 62: 136-148.

Sikavitsas, V. I., Temenoff, J. S. and Mikos, A. G. (2001). Biomaterials and bone mechanotransduction. *Biomaterials* 22 (19): 2581-2593.

Slivka, M. A., Leatherbury, N. C., Kieswertter, K. and Niederauer, G. G. (2001). Porous resorbable fiber-reinforced scaffolds tailored for articular cartilage repair. *Tissue Engineering* 7: 767–880.

SMI: PCC Morphology Comparison. (2012). Precipitated Calcium Carbonate (PCC). Chemical and physical properties. Accessed online: 18th November, 2012 from Specialty minerals. http://www.specialtyminerals.com/paper/pcc-

pigments/features-of-pcc/pcc-morphology-comparison/Stereochemical Requirements in Biomineralization. *Proceeding of the National Academy of Sciences of the United States of America* 82 (12): 4110-4114.

Smith, C.A., Richardson, S.M., Eagle, M.J., Rooney, P., Board, T. and Hoyland, J.A. (2014). The use of a novel bone allograft wash process to generate a biocompatible, mechanically stable and osteoinductive biological scaffold for use in bone tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine*, published by John Wiley and Sons Ltd.

Søballe, K. (1993). Hydroxyapatite ceramic coating for bone implant fixation: Mechanical and histological studies in dogs. *Acta Orthopaedica Scandinavica Supplementum* 255: 1-58.

Song, N., Zhang, H-b., Liu, H. and Fang, J-z. (2017). Effects of SiC whiskers on the mechanical properties and microstructure of SiC ceramics by reactive sintering. *Ceramics International* 43 (9): 6786–6790.

Sophia, C-N. C., Huoli, C., Yu-Ray, C., Lin-Cheng, Y., Jan-Kan, C., Samir, M., Hui-Ying, C., Yi-Lung, L., Wei-Chun, M. and Jueren, L. (2004). Cranial repair using BMP-2 gene engineered bone marrow stromal cells. *Journal of Surgical Research* 119: 85–91.

Sorkin, A. M., Dee, K. C. and Knothe Tate, M. L. (2004). "Culture shock" from the bone cell's perspective: Emulating physiological conditions for mechanobiological investigations. *American Journal of Physiology Cell* 287(6): C1527-C1536.

Sosnowski, S., Wozniak, P. and Lewandowska-Szumiel, M. (2006). Polyester scaffolds with bimodal pore size distribution for tissue engineering. *Macromolecular Bioscience* 6 (6): 425-434.

Soumya, S., Sajesh, K. M., Jayakumar, R., Nair, S. V. and Chennazhi, K. P. (2012). Development of a phytochemical scaffold for bone tissue engineering using Cissus quadrangularis extract. *Carbohydrate Polymers* 87 (2): 1787–1795.

Sousa, R.A., Correlo, V.M., Chung, S., Neves, N.M. and Reis, R.L. (2008). Processing of thermoplastic natural-based polymers: An overview of starch based blends. *In Handbook of Natural-based Polymers for Biomedical Applications*, N.M. Neves *et al*., Editors. CRC Press: Cambridge.

Stechschulte, L.A., Czernika, P.J., Rotter, Z.C., Tausif, F.N., Corzo, C.A., Marciano, D.P., Asteianc, A., Zheng, J., Bruning, J.B., Kamenecka, T.M., Rosen, C.J., Griffin, P.R. and Lecka-Czernik, B. (2016). PPARG post-translational modifications regulate bone formation and bone resorption. *EBioMedicine* 10: 174–184.

Stein, G. S. and Lian, J. B. (1993). Molecular mechanisms mediating proliferation/ differentiation interrelationships during progressive development of the osteoblastic phenotype. *Endocrine Reviews* 14: 424-442.

St-Pierre, J.P., Gauthier, M., Lefebvre, L.P. and Tabrizian, M. (2005). Three-dimensional growth of differentiating MC3T3-E1 pre-osteoblasts on porous titanium scaffolds. *Biomaterials* 26 (35): 7319-7328.

Stupp, S.I. and Braun, P.V. (1997). Molecular manipulation of microstructures: Biomaterials, ceramics and semiconductors. *Science* 277 (5330): 1242-1248.

Sugawara, A., Ishii, T. and Kato, T. (2003). Self-organized calcium carbonate with regular surface-relief structures. *Angewandte Chemie* 115 (43): 5457-5461.

Sunita. 2010. www.k5thehometeam.com/story/34361755/global-foot-and-ankle-devices.../ 27 January 2017.

Suryanarayana, C. and Norton, M.G. (1998). X-ray diffraction: A practical approach, New York: Plenum Press Publishing.

Taboas, J. M., Maddox, R. D., Krebsbach, P. H. and Hollister, S. J. (2003) Indirect solid free form fabrication of local and global porous, biomimetic and composite 3D polymer-ceramic scaffolds. *Biomaterials* 24: 181-194.

Takagishi, Y., Kawakami, T., Hara, Y., Shinkai, M., Takezawa, T. and Nagamune, T. (2006). Bone-like tissue formation by three-dimensional culture of MG63 osteosarcoma cells in gelatin hydrogels using calcium-enriched medium. *Tissue Engineering* 12 (4): 927-937.

Takayama, Y. and Mizumachi, K. (2009). Effect of lactoferrin-embedded collagen membrane on osteogenic differentiation of human osteoblast-like cells. *Journal of Bioscience and Bioengineering* 107: 191–195.

Tampieri, A., Sandr, M., Landi, E., Celotti, G., Roveri, N., Mattioli-Belmonte, M., Virgili, L., Gabnana, F. and Biaaini, G. (2005). HA/alginate hybrid composites prepared through bio-inspired nucleation. *Acta Biomaterialia* 1: 343-351.

Taniyama, K., Shirakata, Y., Yoshimoto, T., Takeuchi, N., Yoshihara, Y. and Noguchi, K. (2012). Bone formation using β-tricalcium phosphate/carboxymethyl-chitin composite scaffold in rat calvarial defects. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology* 116 (6): e450-e456.

Tanzer, M., Turcotte, R., Harvey, E. and Bobyn, J. D. (2003). Extracortical bone bridging in tumor endoprostheses: Radiographic and histologic analysis. *Journal of Bone and Joint Surgery* 85 A (12): 2365-2370.

Tautzenberger, A., Kovtun, A. and Ignatius, A. (2012). Nanoparticles and their potential for application in bone. *International Journal of Nanomedicine* 7: 4545.

Tavangarian, F. and Emadi, R. (2011). Preparation of bioactive nanostructure scaffold with improved compressive strength. *Ceramics-Silikáty* 55 (1): 49-53.

Teixeira, S., Fernandes, H., Leusink, A., Van Blitterswijk, C., Ferraz, M.P., Monteiro, F.D. and de Boer, J. (2010). *In vivo* evaluation of highly macroporous ceramic scaffolds for bone tissue engineering. *Journal of Biomedicine Materials Research A* 93 (2): 567-575.

Thompson, R. C., Jr Garg, A., Clohisy, D. R. and Cheng, E. Y. (2000). Fractures in large segment allografts. *Clinical Orthopaedics and Related Research* (370): 227-235.

Thrall, M.A., Weiser, G., Allison, R. and Campbell, T.W. (2012). Veterinary Hematology and Clinical Chemistry. John Wiley and Sons, USA.

Thumsorn, S., Yamada, K., Leong, Y.W. and Hamada, H. (2011). Development of cockle shell-derived $CaCO_3$ for flame retardancy of recycled PET/recycled PP blend. *Materials Sciences and Applications* 2: 59-69.

Tielinen, L.M. (2003). Bioabsorbable polymer and bone growth factor composites. Topics in Tissue Engineering, University of Oulu, Chapter 9: 1-11.

Torroni, A. (2009). Engineered bone grafts and bone flaps for maxillofacial defects: State of the art. *Journal of Oral and Maxillofacial Surgery* 67 (5): 1121-1127.

Tran, N. and Webster, T.J. (2011). Increased osteoblast functions in the presence of hydroxyapatite-coated iron oxide nanoparticles. *Acta Biomaterialia* 7 (3): 1298-1306.

Trentz, O.A., Hoerstrup, S.P., Sun, L.K., Bestmann, L., Platz, A. and Trentz, O.L. (2003). Osteoblasts response to allogenic and xenogenic solvent dehydrated cancellous bone *In vitro*. *Biomaterials* 24 (20): 3417-3426.

Truscello, S., Kerckhofs, G., Van Bael, S., Pyka, G., Schrooten, J. and Van Oosterwyck, H. (2011). Prediction of permeability of regular scaffolds for skeletal tissue engineering: A combined computational and experimental study. *Acta Biomaterialia* 8 (4): 1648-1658.

Tsai, W.B., Chen, C.H., Chen, J.F. and Chang, K.Y. (2006). The effects of types of degradable polymers on porcine chondrocyte adhesion, proliferation and gene expression. *Journal of Materials Science: Materials in Medicine* 17: 337e43.

Tuusa, S. M., Peltola, M. J., Tirri, T., Lassila, L. V. and Vallittu, P. K. (2007). Frontal bone defect repair with experimental glass-fiber-reinforced composite with bioactive glass granule coating. *Journal of Biomedical Materials Research B: Applied Biomaterials* 82 (1): 149-155.

Ueno, Y., Futagawa, H., Takagi, Y., Ueno, A. and Mizushima, Y. (2005). Drug-incorporating calcium carbonate nanoparticles for a new delivery system. *Journal of Control Release* 103: 93-98.

Urist, M. R. (1965). Bone formation by autoinduction. *Science* 150: 893.

Vacanti, J.P. and Vacanti, C.A. (2007). The history and scope of tissue engineering. Chapter 1. Principles of Tissue Engineering. 3th ed.: 3-6.

van Gaalen, S., Kruyt, M., Meijer, G., Mistry, A., Mikos, A., van den Beucken, J., Jansen, J., de Groot, K., Cancedda, R., Olivo, C., Yaszemski, M. and Dhert, W. (2008). Tissue Engineering of Bone. *Textbook on Tissue Engineering*. Chapter 19th. Elsevier, Amsterdam. pp. 559-610.

Varghese, S. and Elisseeff, J. (2006). Hydrogels for musculoskeletal tissue engineering. *Polymers for Regenerative Medicine*: 95-144.

Vasconcellos, L.M.R., Leite, D.O., Oliveira, F.N., Carvalho, Y.R. and Cairo, C.A.A. (2010). Evaluation of bone ingrowth into porous titanium implant: Histomorphometric analysis in rabbits. *Brazilain Oral Research* 24 (4): 399-405.

Vater, C., Lode, A., Bernhardt, A., Reinstorf, A., Heinemann, C. and Gelinsky, M. (2010). Influence of different modifications of a calcium phosphate bone cement on adhesion, proliferation and osteogenic differentiation of human bone marrow stromal cells. *Journal of Biomedical Materials Research A* 92 (4): 1452-1460.

Vecchio, K.S., Zhang, X., Massie, J.B., Wang, M. and Kim, C.W. (2007). Conversion of bulk seashells to biocompatible hydroxyapatite for bone implants. *Acta biomaterialia* 3 (6): 910-918.

Vega, M., G´omez-Quintero, T., Nu˜nez-Anita, R., Acosta-Torres, L. and Casta˜no, V. (2012). Polymeric and ceramic nanoparticles in biomedical applications. *Journal of Nanotechnology* 2012: Article ID 936041, 10 pages.

Venkateswarlu, K., Sreekanth, D., Sandhyarani, M., Muthupandi, V., Bose, A.C. and Rameshbabu, N. (2012). X-ray peak profile analysis of nanostructured hydroxyapatite and fluorapatite. *International Journal of Bioscience, Biochemistry and Bioinformatics* 2 (6): 389-393.

Verket, A., Tiainen, H., Haugen, H. J., Lyngstadaas, S.P., Nilsen, O. and Reseland, J.E. (2012). Enhanced osteoblast differentiation on scaffolds coated with TIO_2 compared to SIO_2 and cap coatings. *Biointerphases* 7: 36.

Verma, S. and Kumar, N. (2010). Effect of biomimetic 3D environment of an injectable polymeric scaffold on MG-63 osteoblastic-cell response. *Materials Science and Engineering* C3: 1118-1128.

Viateau, V. and Guillemin, G. (2004). Experimental Animal Models for Tissue-Engineered Bone Regeneration. *Engineered Bone*, edited by H. Petite and R. Quartro. Eurckah.com. Chapter 8: 1-17.

Virtanen, S. (2011). Biodegradable Mg and Mg alloys: Corrosion and biocompatibility. *Materials Science and Engineering B* 176: 1600–1608.

Vitale-Brovarone, C., Verne, E., Robiglio, L., Appendino, P., Bassi, F., Martinasso, G., Muzio, G. and Canuto, R. (2007). Development of glass ceramic scaffolds for bone tissue engineering: Characterization, proliferation of human osteoblasts and nodule formation. *Acta Biomaterialia* 3 (2): 199-208.

Vitte, J., Benoliel, A.M., Pierres, A. and Bongrand, P. (2004). Is there a predictable relationship between surface physical-chemical properties and cell behaviour at the interface? *European Cells and Materials* 7: 52e63.

Vuola, J. (2001). Natural coral and hydroxyapatite as bone substitutes. Academic dissertation.

Vuola, J., Göransson, H., Böhling, T. and Seljavaara, A. (1995). Bone marrows induce osteogenesis in hydroxyapatite and calcium carbonate implants. *Journal of Biomaterials* 17: 1761-1766.

Walsh, D., Lebeau, B. and Mann, S. (1999). Morphosynthesis of calcium carbonate (vaterite) microsponges. *Advanced Materials* 11 (4): 324-328.

Wang, C., Liu, Y., Bala, H., Pan, Y., Zhao, J., Zhao, X. and Wang, Z. (2007a). Facile preparation of $CaCo_3$ nanoparticles with self-dispersing properties in the presence of dodecyl dimethyl betaine. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 297 (1): 179-182.

Wang, C., Zhao, J., Zhao, X., Bala, H. and Wang, Z. (2006a). Synthesis of nanosized calcium carbonate (aragonite) via a polyacrylamide inducing process. *Journal of Powder Technology* 163 (3): 134-138.

Wang, K., Zhou, C., Hong, Y. and Zhang, X. (2012). A review of protein adsorption on bioceramics. *Journal of Interface Focus* 2 (3): 259-277.

Wang, L., Sondi, I. and Matijević, E. (1999). Preparation of uniform needle-like aragonite particles by homogeneous precipitation. *Journal of Colloid Interface Science* 218: 545-553.

Wang, M. (2003). Developing bioactive composite materials for tissue replacement. *Biomaterials* 24: 2133-2151.

Wang, X., Mabrey, J.D. and Agrawal, C.M. (1998). An interspecies comparison of bone fracture properties. *Biomedical Materials and Engineering* 8 (1): 1-9.

Wang, Y., Uemura, T., Dong, J., Kojima, H., Tanaka, J. and Tateishi, T. (2003). Application of perfusion culture system improves *In vitro* and *in vivo* osteogenesis of bone marrow derived osteoblastic cells in porous ceramic materials. *Tissue Engineering* 9: 1205-1214.

Wang, Y., Zhang, S., Zeng, X., Ma, L. L., Wang, W., Yan, W. and Qian, M. (2007b). Osteoblastic cell response on fluoridated hydroxyapatite coatings. *Acta Biomaterialia* 3: 191–197.

Wang, Y.W., Wu, Q., Chen, J. and Chen, G.Q. (2005). Evaluation of three-dimensional scaffolds made of blends of hydroxyapatite and poly (3-hydroxybutyrate-co-3hydroxyhexanoate) for bone reconstruction. *Biomaterials* 26 (8): 899-904.

Wang, Z., Xie, G., Wang, X., Li, G. and Zhang, Z. (2006b). Rheology enhancement of polycarbonate/calcium carbonate nanocomposites prepared by melt-compounding. *Materials Letters* 60 (8): 1035-1038.

Weadock, K., Olson, R. M. and Silver, F. H. (1983) Evaluation of collagen cross-linking techniques. *Biomaterials, Medical Devices and Artificial Organs* 11: 293-318.

Wen-De, X., Zhao-Ming, Z., Yong-Zhi, T., Zi-Xing, X., Zhun, X. and Jian-Ting, C. (2012). Repair of critical size bone defects with porous poly (D, L-lactide)/ nacre nanocomposite hollow scaffold. *Saudi Medical Journal* 33 (6): 601-607.

Wheeler, D. L., Eschbach, E. J., Hoellrich, R. G., Montfort, M. J. and Chamberland, D. L. (2000). Assessment of resorbable bioactive material for grafting of critical-size cancellous defects. *Journal of Orthopaedic Research* 18 (1): 140-148.

Whited, B.M., Whitney, J.R., Hofmann, M.C., Xu, Y. and Rylander, M.N. (2011). Pre-osteoblast infiltration and differentiation in highly porous apatite-coated PLLA electrospun scaffolds. *Biomaterials* 32 (9): 2294-2304.

Widmaier, E.P., Raff, H. and Strang, K.T. (2008). America: Vander's human physiology, 1-769. W.B. Saunders Company.

Wiebe, D., Megerman, J., L'italien, G. J. and Abbott, W. M. (1988). Glutaraldehyde release from vascular prostheses of biologic origin. *Surgery* 104: 26-33.

Wiesmann, H., Joos, U. and Meyer, U. (2004). Biological and biophysical principles in extracorporal bone tissue engineering. Part II. *International Journal of Oral and Maxillofacial Surgery* 33: 523-530.

William, R.W., Frank, V., Dean, M., Jason, A., Andy, L., Rema, O., Yan, Y., Hiroyuki, I. and Warwick, B. (2008). β-TCP bone graft substitutes in a bilateral rabbit tibial defect model. *Biomaterials* 29: 266-271.

Williams, D. F. (1987). Definitions in biomaterials. Proceedings of a consensus conference of the European Society for biomaterials, Vol. 4. Chester, England, March 3-5.New York: Elsevier.

Williams, D.F. (1987). Tissue-Biomaterial Interactions. *Journal of Materials Science* 22: 3421–3445.

Williams, J. and Lewis, J. (1982). Properties and an anisotropic model of cancellous bone from the proximal tibial epiphysis. *Journal of Biomechanics* 104: 50-56.

Williams, K. A., Saini, S. and Wick, T. M. (2002). Computational fluid dynamics modeling of steady-state momentum and mass transport in a bioreactor for cartilage tissue engineered. *Biotechnology Progress* 18: 951-963.

Winn, S. R., Uludag, H. and Hollinger, J. O. (1998). Sustained release emphasizing recombinant human Bone Morphogenetic Protein-2. *Advanced Drug Delivery Reviews* 31: 303-318.

Wollensak, G. and Spoerl, E. (2004). Collagen cross-linking of human and porcine sclera. *Journal of Cataract and Refractive Surgery* 30: 689-695.

Wollensak, G., Spoerl, E. and Seiler, T. (2003). Riboflavin/ultraviolet-induced collagen cross-linking for the treatment of keratoconus. *American Journal of Ophthalmology* 135: 620-627.

Wu, L. N., Ishikawa, Y., Sauer, G. R., Genge, B. R., Mwale, F., Mishima, H. and Wuthier, R.E. (1995). Morphological and biochemical characterization of mineralizing primary cultures of avian growth plate chondrocytes: Evidence for cellular processing of Ca_2 and Pi prior to matrix mineralization. *Journal of Cellular Biochemistry* 57: 218-237.

Xiang, H., Mu, Y., Hu, C. and Luo, X. (2017). Biocompatibility and toxicity of poly lactic acid/ferrosoferric oxide nanomagnetic microsphere. *Journal of Nanomaterials* 2017: Article ID 5429063, 8 pages.

Xiang, L., Xiang, Y., Wen, Y. and Wei, F. (2004). Formation of $CaCo_3$ nanoparticles in the presence of terpineol. *Materials Letters* 58 (6): 959-965. Doi: 10.1016/j.matlet.2003.07.034.

Xiao, W.D., Zhong, Z.M., Tang, Y.Z., Xu, Z.X. and Xu, Z. (2012). Repair of critical size bone defects with porous poly (D,L-Iactide)/ nacre nanocomposite hollow scaffold. *Saudi Medical Journal* 33 (6): 601-607.

Xu, A.W., Antonietti, M., Colfen, H. and Fang, Y.P. (2006). Uniform hexagonal plates of vaterite $CaCO_3$ mesocrystals formed by biomimetic mineralization. *Advanced Functional Materials* 16 (7): 903-908.

Xu, G., Yao, N., Aksay, I.A. and Groves, J.T. (1998). Biomimetic synthesis of macroscopic-scale calcium carbonate thin films: Evidence for a multistep assembly process. *Journal of the American Chemical Society* 120 (46): 11977-11985.

Xu, X., Han, J.T. and Cho, K. (2004b). Formation of amorphous calcium carbonate thin films and their role in biomineralization. *Chemistry of Materials* 16 (9): 1740-1746.

Yang, Y-F., Gai, G-S. and Fan, S-M. (2006). Surface nano-structured particles and characterization. *International Journal of Mineral Processing* 78 (2): 78–84.

Yannas, I. V., Lee, E., Orgill, D. P., Skrabut, E. M. and Murphy G. F. (1989). Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. *Proceeding of the National Academy of Sciences: USA* 86: 933-937.

Yannas, I.V. (2001). Tissue and Organ Regeneration in Adults. New York: Springer.

Yao, J., Radin, S., Leboy, S. and Ducheyne, P. (2005a). The effect of bioactive glass content on synthesis and bioactivity of composite poly (Lactic-co-glycolic acid)/bioactive glass substrate for tissue engineering. *Biomaterials* 26 (14): 1935-1943.

Yao, J., Radin, S., Reilly, G., Leboy, P.S. and Ducheyne, P. (2005b). Solution-mediated effect of bioactive glass in poly (Lactic-co-glycolic acid)-bioactive glass composites on osteogenesis of marrow stromal cells. *Journal of Biomedical Materials Research A* 75 (4): 794801.

Yazdimamaghani, M., Razavi, M., Vashaee, D. and Tayebi, L. (2014). Development and degradation behavior of magnesium scaffolds coated with poly caprolactone for bone tissue engineering. *Materials Letters* 132: 106-110.

Yefang, Z., Hutmacher, D.W., Varawan, S.L. and Meng, L.T. (2007). Comparison of human alveolar osteoblasts cultured on polymer-ceramic composite scaffolds and tissue culture plates. *International Journal of Oral and Maxillofacial Surgery* 36 (2): 137-145.

Yoshikawa, T., Davies, J. E., Ichijima, K., Ohgushi, H. and Tamai, S. (1998). Morphological study on mineralization of collagen fibers in *In vitro* bone formation. *Journal of Japanese Society Biomaterials* 16: 72-77.

Yoshikawa, T., Nakajima, H., Yamada, E., Akahane, M., Dohi, Y., Ohgushi, H., Tamai, S. and Ichijima, K. (2000). *In vivo* osteogenic capability of cultured allogeneic bone in porous hydroxyapatite: Immunosuppressive and osteogenic potential of FK506 *in vivo*. *Journal of Bone Minerals Research* 15: 1147-1157.

Yoshikawa, T., Peel, S.A.F., Gladstone, J.R. and Davies, J.E. (1997). Biochemical analysis of the response in rat bone marrow cell cultures to mechanical stimulation. *Biomedical Materials and Engineering* 7: 369-377.

Young, S., Wong, M., Tabata, Y. and Mikos, A. G. (2005). Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *Journal of the Controlled Release* 109: 256-274.

Yu, J., Guo, H., davis, S.A. and Mann, S. (2006). Fabrication of hollow inorganic microspheres by chemically induced self-transformation. *Advanced functional Materials* 16 (15): 2035-2041.

Yuehuei, H. An. and Friedman, R.J. (1999). Animal Models in Orthopedic Research. CRC Press, Inc., Boca Raton, Florida, USA. Chapter 29, pp. 659-674.

Zakaria, Z.A.B., Zakaria, N. and Kasimb, Z. (2004). Mineral composition of the cockle shell (*Anadora granosa*) shells, Hard Clamp (*Meretrix meretrix*) shells and corals (*Porites spp*): Comparative study. *Journal of Animal and Veterinary Advances* 3: 445-447.

Zeeman, R., Dijkstra, P. J., Van Wachem, P. B., Van Luyn, M. J., Hendriks, M., Cahalan, P. T. and Feijen, J. (1999) Successive epoxy and carbodiimide cross-linking of dermal sheep collagen. *Biomaterials* 20: 921-931.

Zeltinger, J., Sherwood, J.K., Graham, D.A., Mueller, R. and Griffith, L.G. (2001). Effect of pore size and void fraction on cellular adhesion, proliferation and matrix deposition. *Tissue Engineering* 7: 557–572.

Zhang, C.X., Zhang, J.L., Feng, X.Y., Li, Y.W., Zhao, J. and Han, B.X. (2008). Influence of surfactants on the morphologies of $CaCO_3$ by carbonation route with compressed CO_2. *Colloids Surfaces A* 324 (1-3): 167-170.

Zhang, M., Powers, R. M. and Wolfinbarger, L. (1997). Effect(s) of the demineralization process on the osteoinductivity of demineralized bone matrix. *Journal of Periodontology* 68 (11): 1085-1092.

Zhang, Y., Wang, J., Wang, J., Niu, X., Liu, J., Gao, L., Zhai, X. and Chu, K. (2015). Preparation of porous PLA/DBM composite biomaterials and experimental research of repair rabbit radius segmental bone defect. *Cell and Tissue Banking* 16 (4): 615-622.

Zhanga, Q., Rena, L., Sheng, Y., Ji, Y. and Fu, J. (2010). Control of morphologies and polymorphs of $CaCo_3$ via multi-additives system. *Materials Chemistry and Physics* 122: 156-163.

Zheng, H., Wu, J.J. and Wang, J. (2013). Evaluation of effectiveness and analysis of goal-directed blood transfusion in peri-operation of major orthopedic surgery in elderly patients. *Experimental and Therapeutic Medicine* 5 (2): 511-516.

Zimmermann, G. and Moghaddam, A. (2011). Allograft bone matrix versus synthetic bone graft substitutes. *Injury* 42 (Suppl 2): S16-S21.

Zuki, A.B., Bahaa, F.H. and Noordin, M.M. (2011). Cockle shell-based biocomposite scaffold for bone tissue engineering. *Regenerative Medicine and Tissue Engineering: Cells and Biomaterials*: 365-390.