STRONTIUM-HYDROXYAPATITE INCORPORATED
POLY(HYDROXYBUTYRATE-CO-HYDROXYVALERATE)/
POLY(LACTIC-CO-GLYCOLIC ACID) ELECTROSPUN NANOFIBERS
FOR BONE TISSUE ENGINEERING SCAFFOLD

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Biomedical Engineering)

School of Biomedical Engineering and Health Sciences
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Universiti Teknologi Malaysia

FEBRUARY 2020

DEDICATION

This thesis is dedicated to my beloved family for their guidance and support.
I love you all very much.

ACKNOWLEDGEMENT

Alhamdulillah, all praises to Allah that I finally able to finish my PhD study.

First and foremost, I would like to thank my previously main supervisor, Dr. Naznin Sultana for the encouragement, support, and advices, ideas, and contributions to the research. I would also like to express my sincere gratitude to my currently main supervisor, Dr. Syafiqah Saidin, and my co-supervisor, Dr. Saiful Izwan Abd. Razak for all their guidance, assistant, and support throughout this research.

I would like to acknowledge the Faculty of Engineering, School of Biomedical Engineering and Health Sciences, Universiti Teknologi Malaysia for the lab facilities. I am very grateful for the provided lab facilities where I mostly work on my research in the laboratory such as Material Preparation Lab and Cell Culture Lab.

I am greatly thankful to MyPhD scholarship which has financially supported me, given by the Ministry of Higher Education (MOHE) under the program MyBrain15, and also support from the grants from Research Management Centre (RMC), Universiti Teknologi Malaysia.

Finally, I am very thankful to my friends and my beloved family especially my parents, Hassan bin Mohamad and Salbeh binti Mohamad for their constant support and encouragement over the years.

ABSTRACT

Hydroxyapatite polymer nanofibers composites offer many advantages such as good osteoconductivity, bone bonding ability, and also mimicking the bone extracellular matrix (ECM). In particular, strontium-hydroxyapatite (Sr-HA) has the ability to enhance osteogenesis as compare to neat hydroxyapatite (HA). Therefore, the Sr-HA has been incorporated within polymer nanofiber scaffolds to develop composite materials for bone tissue application. In this study, biodegradable composites scaffolds were fabricated by electrospinning technique. It was composed of poly (lactic-co-glycolic acid) (PLGA) and poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV), optimized at 50:50 weight ratio with a solution concentration of 26 % (w/v). The physicochemical properties of the HA and Sr-HA nanoparticles were then characterized by field emission scanning electron microscopy (FESEM), energy dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), selected area electron diffraction analysis (SAED), surface area analysis, attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) and X-ray diffraction (XRD). Meanwhile, the physicochemical properties of the scaffolds, known as PHBV/PLGA, PHBV/PLGA/HA and PHBV/PLGA/Sr-HA were characterized by scanning electron microscopy (SEM), EDX, pore size, porosity, atomic force microscopy (AFM), water contact angle, ATR-FTIR, and thermogravimetric (TGA) analyses. Mechanical properties were quantified by tensile test. Other characterizations include bioactivity and biodegradation test were also performed. According to the results, optimization of electrospinning parameters had produce homogenous and smooth nanofibers with an average diameter of 600 - 700 nm and porosity of ~78 %. The addition of either HA or Sr-HA nanoparticles has improved the surface roughness, bioactivity, and tensile strength of the composites as compare to PHBV/PLGA scaffold. The nanofiber scaffolds have suitable mechanical properties for bone tissue application with a tensile strength up to ~1.3 MPa and a Young's Modulus of ~ 45 MPa. The scaffolds have slow degradation rate with less than 10 % weight loss that is suitable for bone regeneration. Finally, the biocompatibility of the scaffolds was evaluated through in vitro cell culture with human skin fibroblast cells (HSF 1184) and human fetal osteoblast cells (hFOB 1.19). Cellular activities such as morphology, attachment and proliferation, were analyzed by SEM, cytoskeletal staining, MTT, and live/dead assay. The results showed that the scaffolds have promoted cellular adhesion and proliferation due to their nanoscale topography similar to the ECM, in addition to porous and high surface roughness. The biocompatibility and cell viability of osteoblast were enhanced with a demonstration of greater alkaline phosphatase activity by the PHBV/PLGA/Sr-HA scaffold. In conclusion, we proposed that PHBV/PLGA/Sr-HA nanofiber scaffold can be a potential material for bone tissue application.

ABSTRAK

Hydroksiapatit-polimer serat-nano komposit menawarkan banyak kelebihan seperti keupayaan osteokonduktiviti yang baik, kebolehan ikatan tulang dan juga menyerupai matriks ekstraselular tulang (ECM). Khususnya, strontium-hidroksiapatit (Sr-HA) mempunyai keupayaan untuk meningkatkan osteogenesis berbanding dengan hidroksiapatit (HA). Oleh itu, Sr-HA telah dimasukkan ke dalam perancah serat-nano untuk membangunkan bahan komposit untuk aplikasi tisu tulang. Dalam kajian ini, perancah komposit bolehurai telah difabrikasi melalui teknik putaran elektro. Ianya diperbuat daripada polimer poli(asid laktik-ko-glikolik) (PLGA) dan poli(hidroksibutirat-ko-hidroksivalerat) (PHBV), dioptimumkan pada nisbah berat 50:50 dengan kepekatan larutan 26 % (w/v). Sifat fizikokimia nanopartikel HA dan Sr-HA telah dikaji menggunakan mikroskopi pelepasan medan pengimbasan elektron (FESEM), spektroskopi penyebaran tenaga sinar-X (EDX), mikroskopi penghantaran elektron (TEM), analisis kawasan terpilih difraksi elektron (SAED), analisis kawasan permukaan, jumlah refleksi dilemahkan - spektroskopi pengubah inframerah Fourier (ATR-FTIR) dan difraksi X-ray (XRD). Manakala, sifat fizikokimia perancah, dikenali sebagai PHBV/PLGA, PHBV/PLGA/HA dan PHBV/PLGA/Sr-HA telah dikaji menggunakan analisis mikroskopi pengimbasan elektron (SEM), EDX, saiz liang, keliangan, mikroskopi kuasa atom (AFM), sudut sentuhan air, ATR-FTIR dan termogravimetrik (TGA). Sifat mekanikal perancah telah dikira menggunakan ujian tegangan. Ujikaji lain termasuk ujian bioaktiviti dan biodegradasi juga telah dijalankan. Mengikut keputusan kajian, pengoptimuman parameter putaran elektro telah menghasilkan serat-nano yang licin dan sekata dengan diameter purata 600 - 700 nm dan keliangan ~78 %. Penambahan nanopartikel samada HA atau Sr-HA telah menambah baik kekasaran permukaan, bioaktiviti dan kekuatan tegangan komposit berbanding perancah PHBV/PLGA. Perancah serat-nano mempunyai sifat mekanikal yang sesuai untuk aplikasi tisu tulang dengan kekuatan tegangan sehingga ~1.3 MPa dan Modulus Young ~ 45 MPa. Perancah mempunyai kadar degradasi perlahan dengan kehilangan berat kurang daripada 10 % yang sesuai untuk pertumbuhan semula tulang. Akhirnya, biokeserasian perancah telah dinilai melalui in vitro kultur sel dengan sel kulit manusia (HSF 1184) dan sel tulang manusia (hFOB 1.19). Aktiviti selular seperti morfologi, lekatan dan percambahan sel telah dianalisis oleh SEM, pewarnaan sitoskeletal, ujian MTT dan hidup/mati. Keputusan menunjukkan perancah menggalakkan lekatan dan percambahan selular kerana topografi skala nano perancah yang mirip dengan ECM, sebagai tambahan kepada kekasaran permukaan dan keliangan yang tinggi. Keupayaan bioserasi dan daya maju sel tulang dipertingkatkan dengan aktiviti fosfatase alkali yang lebih besar oleh perancah PHBV/PLGA/Sr-HA. Kesimpulannya, kami mencadangkan perancah serat-nano PHBV/PLGA/Sr-HA boleh menjadi bahan yang berpotensi untuk aplikasi kejuruteraan tisu tulang.

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LIST OF ABBREVIATIONS

2D	-	Two-Dimensional
3D	-	Three-Dimensional
3D-P	-	Three-Dimensional Printing
AFM	-	Atomic Force Microscopy
ALP	-	Alkaline Phosphatase activity
ATR	-	Attenuated Total Reflection
BCP	-	Biphasic Calcium Phosphate
BET	-	Brunauer–Emmett–Teller
BMPs	-	Bone Morphogenetic Proteins
Ca	-	Calcium
CAD	-	Computer-Aided Design
Calcein AM	-	Calcein Acetoxymethyl Ester
$CaSiO_3$	-	Wollastonite
Cl^-	-	Chlorine Ion
CO_2	-	Carbon Dioxide
CO^{3}_{2-}	-	Carbonate Ion
Co-Cr	-	Cobalt-Chromium
CT	-	Computed Tomography
Cu^{2+}	-	Copper Ion
DMEM	-	Dulbecco's Modified Eagle Medium
DTG	-	Derivative Thermogravimetric
ECM	-	Extracellular Matrix
EDX	-	Energy Dispersive X-ray Spectroscopy
EthD-1	-	Ethidium Homodimer-1
F^-	-	Fluoride Ion
F-actin	-	Actin Filaments
FBS	-	Fetal Bovine serum
FDM	-	Fused Deposition Modeling
Fe^{2+}	-	Iron Ion
FESEM	-	Field Emission Scanning Electron Microscopy

FTIR	-	Fourier Transform Infrared Spectroscopy
G418	-	Geneticin
HA	-	Hydroxyapatite
hFOB	-	Human Fetal Osteoblast
HSF	-	Human Skin Fibroblast
K^+	-	Potassium Ion
Mg^{2+}	-	Magnesium Ion
Mn^{2+}	-	Manganese Ion
MTT	-	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
O	-	Oxygen
P	-	Phosphorus
Pb^{2+}	-	Lead Ion
PBS	-	Phosphate Buffer Saline
PCL	-	Polycaprolactone
PDMS	-	Polydimethylsiloxane
PE	-	Polyethylene
PEEK	-	Polyether Ether Ketone
PGA	-	Polyglycolic Acid
PHA	-	Polyhydroxyalkanoates
PHB	-	Polyhydroxybutyrate
PHBV	-	Poly(hydroxybutyrate-*co*-hydroxyvalerate)
PHV	-	Polyhydroxyvalerate
PLA	-	Polylactic Acid
PLGA	-	Poly(lactic-*co*-glycolic Acid)
PMMA	-	Poly(methyl Methacrylate)
PP	-	Polypropylene
PPF	-	Poly(propylene Fumarate)
PS	-	Polystyrene
PTFE	-	Polytetrafluoroethylene
PTMC	-	Poly(trimethylene Carbonate)
RP	-	Rapid Prototyping
RPM	-	Revolutions Per Minute
SAED	-	Selected Area Electron Diffraction

SBF	-	Simulated Body Fluid
SEM	-	Scanning Electron Microscopy
Si^{2+}	-	Silicon Ion
SLS	-	Selective Laser Sintering
Sr	-	Strontium
Sr^{2+}	-	Strontium Ion
SrHA	-	Strontium Hydroxyapatite
Ta	-	Tantalum
TCP	-	Tricalcium Phosphate
TEM	-	Transmission Electron Microscopy
TGA	-	Thermogravimetric Analysis
TGF-β	-	Transforming Growth Factor Beta
Ti	-	Titanium
TIPS	-	Thermally Induced Phase Separation
UHMWPE	-	Ultra High Molecular Weight Polyethylene
UV	-	Ultraviolet
XRD	-	X-ray Diffraction
Zn^{2+}	-	Zinc Ion

LIST OF SYMBOLS

%	-	Percent
°C	-	Degree Celsius
µl	-	Microliter
µm	-	Micrometer
cm	-	Centimeter
cp	-	Centipoise
d	-	Diameter
g	-	Gram
kV	-	Kilovolt
mA	-	Miliampere
ml	-	Mililiter
ml/h	-	Mililiter/Hour
mm	-	Milimeter
MPa	-	Megapascal
nm	-	Nanometer
r	-	Density
Ra	-	Average Roughness
Sw	-	Specific Surface Area
V	-	Volume
w/v	-	Weight/Volume
w/w	-	Weight/Weight
ε	-	Total Porosity
θ	-	Theta

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CHAPTER 1

INTRODUCTION

1.1 Background of the Research

Bone defects because of the consequences from injury, infection, disease, trauma and degenerative bone loss remain a challenge in global healthcare. Bone tissue, when injured, leads to dramatic changes in the quality of patient's life. It can limit the ability to perform basic tasks, such as walking and frequently causes social and psychological problems. There have also been deaths from insufficient amounts of ideal bone grafts and implanted system failure for bone regeneration (Giannoudis *et al.* 2005). More than 2.2 million bone graft surgery take place worldwide and the case of bone injuries could be increased in the future which leads to high demand for bone replacement (Murugan *et al.* 2009).

Currently, clinical interventions available for the bone defects and diseases treatment are relying on bone grafting which involves harvesting replacement tissue own by the patient, known as autograft, or transplantation of tissue from a donor known as allograft. However, the scarcity of suitable donor organs, particularly due to aging population is depending on immunosuppression drugs and the risk of disease transmission poses serious health problems that need to be addressed. Further, surgical complication rates are high and patients suffer from severe pain, hematoma, non-union, infections, nerve damage, hernias and fractures at the donor site, in addition to the original defect (Babensee *et al.* 1998, Murugan and Ramakrishna 2005, William *et al.* 2007). Thus, alternative treatments for critical bone defects are urgently needed in the current clinical setting.

Tissue engineering seeks to create an alternative treatment to minimize these complications and provides improved patient outcomes. Interdisciplinary field tissue engineering involves the utilization of engineering and biological sciences to develop biological replacement material that can restore, preserve or enhance tissue or organ function. Bone tissue engineering has become viable approach for the replacement of damaged bone tissue through the development of biomaterial substitute or tissue engineered scaffold that can completely restore the bone tissue original condition and function. The key factors for the success of bone tissue engineering involves the fabrication of three-dimensional scaffold that have multiple properties such as biocompatible, bio-resorbable, optimum degradation rate, highly porous and interconnected pores porosity, good mechanical strength similar to bone, and non-immunogenic. Furthermore, scaffolds must be osteoconductive that helps bone formation by enhancing cell adhesion, proliferation and regulating osteogenic differentiation of bone cells (Amini *et al.* 2012, Henkel *et al.* 2013).

Nanofibers, from submicron to a few nanometers in diameter, have recently become center of attention in medical and tissue engineering due to their properties such as optimum porosity, variable of pore size, high surface area-to-volume ratio and can imitate extracellular matrix (ECM) structure of human. Electrospinning uses an electric field to convert a polymer solution into continuous polymer fibers and had been applied to fabricate fibrous scaffolds that mimic the structure of natural ECM. The resulting fibers with continuous morphology and ultrafine structure which mimicking ECM structure is an effective method to the development of suitable scaffolds for bone tissue engineering (Xie *et al.* 2008, Agarwal *et al.* 2009). Selection of materials to produce a scaffold for bone tissue engineering application plays a pivotal role. The natural bone matrix component is comprising of organic/inorganic, collagen, proteins and bone mineral apatite. To fulfil the needs, the potential of biopolymers and their composites materials has been studied by many researches that is appropriate for bone tissue regeneration (Murugan and Ramakrishna 2005).

Of the various synthetic polymers studied as scaffold material, one of the most popular biodegradable polymers, poly(lactic-*co*-glycolic acid) (PLGA), have been approved by the U.S. Food and Drug Administration as well as biocompatibility and biodegradability. PLGA has been widely utilized as drug carriers, implant, surgical sutures, and scaffolds (Haider *et al.* 2014). On the other hand, biodegradable polyester poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) can be produced by microorganisms via fermentation. Natural origin PHBV polymer has received much attention for various applications in medicine due to its favorable properties such as thermoplasticity, piezoelectricity, non-toxicity, and biocompatibility. The degradation of PHBV is longer than other biodegradable and biocompatible polymers, that results in good mechanical integrity of tissue or scaffold (Bai *et al.* 2015). For these reasons, blending these two materials may control and alter the degradation properties of the scaffold that suit better for long duration treatment strategies of tissues like bone.

Hydroxyapatite (HA) is one of the bioactive ceramics which has a structural and compositional resemblance to the main component of bone matrix. Hydroxyapatite has good compatibility with bone tissue and has osteoconductivity property for bone tissue engineering application. The mechanical properties of composites can be improved by HA nanoparticles addition and also provide a good environment for protein adhesion, osteoconduction and osteoblast proliferation (Webster *et al.* 2001). The biological performance of HA also can be improved by the incorporation of bioactive ions such as strontium (Sr^{2+}), magnesium (Mg^{2+}), and carbonate (CO^{3}_{2-}).

Among various substitute ions, Sr^{2+} is producing beneficial effects on bone and has been widely explored as bone regeneration materials, because due to its ability to increase new bone formation and inhibit bone resorption. Several studies have proved that Sr-containing calcium phosphate ceramics enhanced the proliferation and differentiation of osteoblast cells. Through *in vitro* and *in vivo* studies, strontium-containing HA promotes attachment and mineralization of osteoblast, osseointegration and bone formation (Gopi *et al.* 2014, Hao *et al.* 2015). Nevertheless, the studies were limited to the synthesis of strontium substituted or modified for bone cement, calcium phosphate and bioactive glass (Tan *et al.* 2014).

Natural bone is a hierarchically nanostructured composites with the dispersion of apatite nanocrystals along collagen fibers (Olszta *et al.* 2007). The chemical compositions and porous structures of natural bones inspire us to design novel organic-inorganic bone scaffolds (Zhang *et al.* 2007, Tampieri *et al.* 2011). Herein, electrospinning method was applied to fabricate nanofiber scaffolds to imitate the architecture of the natural bone matrix. The use of the electrospinning process has shown to be a good method to fabricate polymeric nanofibrous scaffolds with structure that could closely mimic the ECM (Zhang *et al.* 2007).

The novelty of the current work reside in the combinational use of materials of the electrospun composite nanofiber which mimic ECM as scaffold for bone tissue engineering application. The PHBV/PLGA polymer provide as temporary biodegradable template that could mimic the degradation rate of bone tissue. Subsequently, Sr-HA nanoparticles was incorporated into the scaffolds with random nano-morphology and high bonding strength, which allows osteoblast cells to live directly on the scaffolds, and further proliferate and also differentiate. As reported in literature, strontium-containing hydroxyapatite (Sr-HA) appears to be an interesting bone substitute material for its ability for improving bone regeneration (Šupová 2015). The advantageous combination of each component may produce synergistic effect on tissue regeneration for bone tissue engineering. The scaffold can be preferred for tissue engineering of non-load bearing bone defects such as small bone defects, where it originally act as supporting and guiding factor that promotes the growth of bone tissue, and lastly gradually absorbed in the body.

1.2 Problem Statement

The development of scaffolds for bone regeneration is becoming essential in bone and tissue engineering field. Methods to treat bone defects resulting from trauma, tumor resection, developmental anomalies or fracture non-unions include autografts, allografts, synthetics or natural material scaffolds. Autografts are considered as the gold standard; however, they have several drawbacks of donor site morbidity, increase risk of infection and geometry mismatch. There is a need to tissue engineer a patient-specific bone graft that is autologous in nature to reduce the drawbacks associated with autografts and alternative bone grafts (Young and Chapman 1989, Arrington *et al.* 1996, Webster and Ahn 2007).

Biodegradable polymer nanofibers formation from electrospinning have been widely investigated for tissue engineering application. Nanofiber scaffolds made from biodegradable polymer has desirable properties such as nanostrucutre, biodegradability, optimum porosity, high surface area to volume ratio, support cells attachment, migration and proliferation, as well as differentiation linked to nano-morhology. However most of the biopolymers still lack of bioactivity sites (Frenot and Chronakis 2003). On the other hand, stronger bioceramics compared to polymers provide a mechanical stability to material scaffold prior to the new bone matrix formation by cells. However, ceramics are poor in mechanical properties due to its brittleness and fragility. To overcome the shortcomings, ceramic can be combined with biodegradable polymers to construct biomaterial composite (Murugan and Ramakrishna 2005).

In conclusion, the possible way to overcome those shortcoming is to develop a composite tissue substitute or scaffold that is able to provide a functional repair effective in directing cell growth, migration and differentiation *in vitro* and thereby may improve bone regeneration *in vivo*.

1.3 Objectives

The overall aim of the work presented in this thesis was to investigate the feasibility of the production of bone nanofiber scaffold, through the addition of Sr-HA, which capable to enhance bone tissue regeneration. The study can be divided into several objectives as follows:

1) To synthesize HA and Sr-HA nanoparticles and characterize their morphological and physicochemical properties for bone tissue engineering application.
2) To fabricate and optimize composite nanofiber scaffold consisted of PHBV, PLGA and Sr-HA using an electrospinning technique.

3) To characterize morphological, physicochemical and mechanical properties of PHBV/PLGA, PHBV/PLGA/HA, and PHBV/PLGA/Sr-HA scaffolds for bone tissue engineering application.
4) To evaluate bioactivity, biodegradation and biocompatibility of PHBV/PLGA, PHBV/PLGA/HA and PHBV/PLGA/Sr-HA scaffolds for bone tissue engineering application.

1.4 Scope of the Research

The scope of this research is based on the fabrication of Sr-HA incorporated PHBV/PLGA nanofiber scaffold using an electrospinning technique. The scaffold was composed of a biocompatible PHBV/PLGA mixture to achieve appropriate biodegradation at a slow rate suitable for bone tissue application. The incorporation of Sr-HA nanoparticles into the scaffold for osteoconductive and bioactive properties is beneficial for bone regeneration to reinforce and enhance biological interaction for bone tissue engineering application. The successful formation of nanofibers similar to ECM was controlled by the optimization of electrospinning parameters such as solution concentratrion and blending ratio of blend polymers which were varied and also voltage, tip-collector distance and flow rate which were fixed.

The physicochemical properties of the Sr-HA nanoparticles were characterized using field emission electron microscopy (FESEM), energy dispersive X-ray spectroscopy (EDX), transmission electron microscopy-selected area diffraction (TEM-SAED), surface area analyzer, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and X-ray diffractometer (XRD). Meanwhile, the physicochemical characteristic of the Sr-HA incorporated PHBV/PLGA scaffolds were characterized using several methods such as SEM, EDX, pore size, porosity, atomic force microscopy (AFM), water contact angle, ATR-FTIR and thermogravimetric (TGA) analyses.

The mechanical properties of the scaffolds were characterized through a tensile test. Another analyses such as bioactivity was conducted by scaffold’s immersion in a simulated body fluid (SBF) and the biodegradation property was assessed by scaffold’s immersion in a phosphate buffer saline (PBS). Finally, a biocompatibility characterization was carried out to determine the efficacy of resultant scaffolds for bone tissue engineering by culturing the scaffolds with human skin fibroblast and human fetal osteoblast. Cell viability, proliferation and differentiation were evaluated using multiple assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT), live/dead, immunofluorescence staining and alkaline phosphatase activity (ALP).

1.5 Significance of the Research

Bone tissue has an intrinsic ability to regenerate, repair and remodel. However, the increasing prevalence of trauma and diseases related to bones has led to an increased demand for bone graft replacement. Besides, with critical-sized bone defects, the bone tissue cannot heal over natural lifetime resulting in a permanent defect. In addition an autogenous bone graft is associated with donor site morbidity while an allograft is associated with infection and disease transmission. Therefore, an alternative solution such as tissue engineering which aims at replacing tissue damaged is needed to minimize the aforementioned complications.

Bone tissue engineering provides an innovative platform in regenerative medicine by the development of novel biological scaffold. The production of nanofiber scaffold is seen as a way in providing this biological substitute. The scaffold is said to help scaffold materials delivery to the appropriate location in the human body, maintain its three-dimensional structure that aids growth of new tissue with suitable tissue function. Scaffolds with bioactive property provide better environment than current clinical treatments. Bioactive composite scaffolds as bone replacement can prevent problem such as donor site morbidity from autograft, comes from patient’s own tissue to repair bone site defect. Beside, sterility of bioactive composite scaffolds will efficiently reducing the infections risk from allograft, where donated tissue from another human donor to the patient to treat the defect.

The results of this study could help researchers to achieve more efficient bone regeneration and gain new knowledge on how cells behavior can be influenced with the development of biomaterials and the design of nanofiber scaffold, with the goal of creating new functional tissue and new treatment for patients with bone defect. The findings of this study will determine the suitability of the scaffold before being implanted into the bone loss. The improvement in bone regeneration also can reduce various costs associated with long-term rehabilitation after bone grafting surgery. In conclusion, this research is essential for patient with bone defect problem and may improve the quality lives of thousands people.

REFERENCES

Adzila, S., Murad, M.C., and Sopyan, I., 2012. Doping Metal into Calcium Phosphate Phase for Better Performance of Bone Doping Metal into Calcium Phosphate Phase for Better Performance of Bone Implant Materials. *Recent Patents on Materials Science*, 5 (1), 18–47.

Agarwal, S., Wendorff, J.H., and Greiner, A., 2009. Progress in the field of electrospinning for tissue engineering applications. *Advanced Materials*, 21 (32–33), 3343–3351.

Ai, J., Ghorbani, F., Heidari K, S., and Biazar, E., 2011. Fabrication of coated - collagen electro-spun PHBV nanofiber film by chemical method and its cellular study. *International Journal of Nano Dimension*, 1 (3), 241–251.

Aina, V., Lusvardi, G., Annaz, B., Gibson, I.R., Imrie, F.E., Malavasi, G., Menabue, L., Cerrato, G., and Martra, G., 2012. Magnesium- and strontium-co-substituted hydroxyapatite: the effects of doped-ions on the structure and chemico-physical properties. *Journal of Materials Science: Materials in Medicine*, 23 (12), 2867–2879.

Akbarzadeh, R. and Yousefi, A., 2014. Effects of processing parameters in thermally induced phase separation technique on porous architecture of scaffolds for bone tissue engineering. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 102 (6), 1304–1315.

Allen, M.R. and Burr, D.B., 2014. Bone modeling and remodeling. *In*: *Basic and applied bone biology*. Elsevier, 75–90.

Amini, A.R., Laurencin, C.T., and Nukavarapu, S.P., 2012. Bone tissue engineering: recent advances and challenges. *Critical reviews in biomedical engineering*, 40 (5), 363–408.

Andrady, A.L., 2007. Factors affecting nanofiber quality. *Science and technology of polymer nanofibers. Wiley*, 81–110.

Ann, R., Thi, F., Nguyen, H., and Lee, B., 2011. Preparation and characterization of electrospun PCL/PLGA membranes and chitosan/gelatin hydrogels for skin bioengineering applications. *Journal of Materials Science: Materials in Medicine*, 22 (10), 2207–2218.

Anton, F., 1938. Method and apparatus for the production of fibers.

Anton, F., 1939. Method and apparatus for the production of artificial fibers.

Arima, Y. and Iwata, H., 2007. Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. *Biomaterials*, 28 (20), 3074–3082.

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.

Arrington, E.D., Smith, W.J., Chambers, H.G., Bucknell, A.L., and Davino, N.A., 1996. Complications of iliac crest bone graft harvesting. *Clinical orthopaedics and related research*, 329, 300–309.

Azis, Y., Jamarun, N., Arief, S., and Nur, H., 2015. Facile synthesis of hydroxyapatite particles from cockle shells (Anadaragranosa) by hydrothermal method. *Oriental Journal of Chemistry*, 31 (2), 1099–1105.

Babensee, J.E., Anderson, J.M., McIntire, L. V, and Mikos, A.G., 1998. Host response to tissue engineered devices. *Advanced drug delivery reviews*, 33 (1–2), 111–139.

Baek, J.-Y., Xing, Z.-C., Kwak, G., Yoon, K.-B., Park, S.-Y., Park, L.S., and Kang, I.-K., 2012. Fabrication and characterization of collagen-immobilized porous PHBV/HA nanocomposite scaffolds for bone tissue engineering. *Journal of Nanomaterials*, 2012, 1.

Bai, J., Dai, J., and Li, G., 2015. Electrospun composites of PHBV/pearl powder for bone repairing. *Progress in Natural Science: Materials International*, 25 (4), 327–333.

Bakhtiari, L., Rezaie, H.R., Hosseinalipour, S.M., and Shokrgozar, M.A., 2010. Investigation of biphasic calcium phosphate/gelatin nanocomposite scaffolds as a bone tissue engineering. *Ceramics International*, 36 (8), 2421–2426.

Balu, R., Singaravelu, S., and Nagiah, N., 2014. Bioceramic Nanofibres by Electrospinning. *Fibers*, 2 (3), 221–239.

Barrere, F., Mahmood, T.A., De Groot, K., and Van Blitterswijk, C.A., 2008. Advanced biomaterials for skeletal tissue regeneration: Instructive and smart functions. *Materials Science and Engineering: R: Reports*, 59 (1–6), 38–71.

Baumgarten, P.K., 1971. Electrostatic spinning of acrylic microfibers. *Journal of* *colloid and interface science*, 36 (1), 71–79.

Bayani, M., Torabi, S., Shahnaz, A., and Pourali, M., 2017. Main properties of nanocrystalline hydroxyapatite as a bone graft material in treatment of periodontal defects. A review of literature. *Biotechnology & Biotechnological Equipment*, 31 (2), 215–220.

Bhardwaj, N. and Kundu, S.C., 2010. Electrospinning: a fascinating fiber fabrication technique. *Biotechnology advances*, 28 (3), 325–347.

Biazar, E., Ai, J., Heidari, S., and Asefnejad, A., 2011. Fabrication of Collagen-Coated Poly (beta-hydroxy by Chemical and Physical Methods. *Oriental Journal of Chemistry*, 27 (2), 385–395.

Bigi, A., Boanini, E., Capuccini, C., and Gazzano, M., 2007. Strontium-substituted hydroxyapatite nanocrystals. *Inorganica Chimica Acta*, 360 (3), 1009–1016.

Bostman, O., Hirvensalo, E., Makinen, J., and Rokkanen, P., 1990. Foreign-body reactions to fracture fixation implants of biodegradable synthetic polymers. *The Journal of bone and joint surgery. British volume*, 72 (4), 592–596.

Bottino, A., Camera-Roda, G., Capannelli, G., and Munari, S., 1991. The formation of microporous polyvinylidene difluoride membranes by phase separation. *Journal of membrane science*, 57 (1), 1–20.

Bretcanu, O., Misra, S.K., Yunos, D.M., Boccaccini, A.R., Roy, I., Kowalczyk, T., Blonski, S., and Kowalewski, T. a., 2009. Electrospun nanofibrous biodegradable polyester coatings on Bioglass®-based glass-ceramics for tissue engineering. *Materials Chemistry and Physics*, 118 (2–3), 420–426.

Brunauer, S., Emmett, P.H., and Teller, E., 1938. Adsorption of gases in multimolecular layers. *Journal of the American chemical society*, 60 (2), 309–319.

Buchko, C.J., Chen, L.C., Shen, Y., and Martin, D.C., 1999. Processing and microstructural characterization of porous biocompatible protein polymer thin films. *Polymer*, 40 (26), 7397–7407.

Buczynska, J., Pamula, E., and Blażewicz, S., 2006. Modification of resorbable fibres with hydroxyapatite. *Eng Biomater*, 58, 85–89.

Burr, D.B., 2019. Bone morphology and organization. *In*: *Basic and applied bone biology*. Elsevier, 3–26.

Canillas, M., Pena, P., Antonio, H., and Rodríguez, M.A., 2017. Calcium phosphates for biomedical applications. *Boletín de la Sociedad Española de Cerámica y*

Vidrio, 56 (3), 91–112.

Capuccini, C., Torricelli, P., Boanini, E., Gazzano, M., Giardino, R., and Bigi, A., 2009. Interaction of Sr-doped hydroxyapatite nanocrystals with osteoclast and osteoblast-like cells. *Journal of Biomedical Materials Research - Part A*, 89 (3), 594–600.

Casper, C.L., Stephens, J.S., Tassi, N.G., Chase, D.B., and Rabolt, J.F., 2004. Controlling surface morphology of electrospun polystyrene fibers: effect of humidity and molecular weight in the electrospinning process. *Macromolecules*, 37 (2), 573–578.

Chandran, S., Shenoy, S.J., Babu S., S., P. Nair, R., Varma, H.K., and John, A., 2018. Strontium Hydroxyapatite scaffolds engineered with stem cells aid osteointegration and osteogenesis in osteoporotic sheep model. *Colloids and Surfaces B: Biointerfaces*, 163, 346–354.

Chang, H.-I. and Wang, Y., 2011. Cell responses to surface and architecture of tissue engineering scaffolds. *In*: *Regenerative medicine and tissue engineering-cells and biomaterials*. InTechOpen.

Chen, G., Ushida, T., and Tateishi, T., 2001. Preparation of poly (L-lactic acid) and poly (DL-lactic-co-glycolic acid) foams by use of ice microparticulates. *Biomaterials*, 22 (18), 2563–2567.

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

Ciocca, L., De Crescenzio, F., Fantini, M., and Scotti, R., 2009. CAD/CAM and rapid prototyped scaffold construction for bone regenerative medicine and surgical transfer of virtual planning: a pilot study. *Computerized Medical Imaging and Graphics*, 33 (1), 58–62.

Cui, Y., Liu, Y., Cui, Y., Jing, X., Zhang, P., and Chen, X., 2009. The nanocomposite scaffold of poly (lactide-co-glycolide) and hydroxyapatite surface-grafted with L-lactic acid oligomer for bone repair. *Acta biomaterialia*, 5 (7), 2680–2692.

Custers, R.J.H., Saris, D.B.F., Dhert, W.J.A., Verbout, A.J., Van Rijen, M.H.P., Mastbergen, S.C., Lafeber, F., and Creemers, L.B., 2009. Articular cartilage degeneration following the treatment of focal cartilage defects with ceramic metal implants and compared with microfracture. *JBJS*, 91 (4), 900–910.

Dash, M., Chiellini, F., Ottenbrite, R.M., and Chiellini, E., 2011. Chitosan - A versatile semi-synthetic polymer in biomedical applications. *Progress in polymer science*, 36 (8), 981–1014.

Deitzel, J.., Kleinmeyer, J., Harris, D., and Beck Tan, N.., 2001. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*, 42 (1), 261–272.

Deitzel, J.M., Kleinmeyer, J., Harris, D.E.A., and Tan, N.C.B., 2001. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*, 42 (1), 261–272.

Deligianni, D.D., Katsala, N.D., Koutsoukos, P.G., and Missirlis, Y.F., 2001. Effect of surface roughness of hydroxyapatite on human bone marrow cell adhesion, proliferation, differentiation and detachment strength. *Biomaterials*, 22 (1), 87–96.

Demir, M.M., Yilgor, I., Yilgor, E., and Erman, B., 2002. Electrospinning of polyurethane fibers. *Polymer*, 43 (11), 3303–3309.

Deng, X., Hao, J., and Wang, C., 2001. Preparation and mechanical properties of nanocomposites of poly (D, L-lactide) with Ca-deficient hydroxyapatite nanocrystals. *Biomaterials*, 22 (21), 2867–2873.

Dhandayuthapani, B., Yoshida, Y., Maekawa, T., and Kumar, D.S., 2011. Polymeric scaffolds in tissue engineering application: A review. *International Journal of Polymer Science*, 2011 (ii).

Doblaré, M., Garcıa, J.M., and Gómez, M.J., 2004. Modelling bone tissue fracture and healing: a review. *Engineering Fracture Mechanics*, 71 (13–14), 1809–1840.

Dong, Y., Liao, S., Ngiam, M., Chan, C.K., and Ramakrishna, S., 2009. Degradation Behaviors of Electrospun Resorbable Polyester Nanofibers. *Tissue Engineering Part B: Reviews*, 15 (3), 333–351.

Donoso, M.G., Méndez-Vilas, A., Bruque, J.M., and González-Martin, M.L., 2007. On the relationship between common amplitude surface roughness parameters and surface area: Implications for the study of cell–material interactions. *International Biodeterioration & Biodegradation*, 59 (3), 245–251.

Dorozhkin, S. V, 2007. Bioceramics based on calcium orthophosphates. *Glass and Ceramics*, 64 (11–12), 442–447.

Dorozhkin, S. V, 2016. *Calcium orthophosphate-based bioceramics and biocomposites*. John Wiley & Sons.

Doshi, J. and Reneker, D.H., 1995. Electrospinning process and applications of electrospun fibers. *Journal of Electrostatics*, 35 (2–3), 151–160.

Dunn, A.S., Campbell, P.G., and Marra, K.G., 2001. The influence of polymer blend composition on the degradation of polymer/hydroxyapatite biomaterials. *Journal of Materials Science: Materials in Medicine*, 12 (8), 673–677.

Dvir, T., Timko, B.P., Kohane, D.S., and Langer, R., 2011. Nanotechnological strategies for engineering complex tissues. *Nature nanotechnology*, 6 (1), 13–22.

Eliaz, N. and Metoki, N., 2017. Calcium phosphate bioceramics: a review of their history, structure, properties, coating technologies and biomedical applications. *Materials*, 10 (4), 334.

Epari, D.R., Taylor, W.R., Heller, M.O., and Duda, G.N., 2006. Mechanical conditions in the initial phase of bone healing. *Clinical Biomechanics*, 21 (6), 646–655.

Feng, X., 2009. Chemical and biochemical basis of cell-bone matrix interaction in health and disease. *Current chemical biology*, 3 (2), 189–196.

Fernandes, M., Adelina, A., Hechenleitner, W., and Manuel, J., 2015. Study of Thermal Degradation of PLGA , PLGA Nanospheres and PLGA / Maghemite Superparamagnetic Nanospheres. *material*, 18 (6), 1400–1406.

Fong, H., Chun, I., and Reneker, D.H., 1999. Beaded nanofibers formed during electrospinning. *Polymer*, 40 (16), 4585–4592.

Frenot, A. and Chronakis, I.S., 2003. Polymer nanofibers assembled by electrospinning. *Current Opinion in Colloid & Interface Science*, 8 (1), 64–75.

Gartner, L.P. and Hiatt, J.L., 2006. *Color textbook of histology e-book*. Elsevier Health Sciences.

Ge, M., Ge, K., Gao, F., Yan, W., Liu, H., Xue, L., Jin, Y., and Zhang, J., 2018. Biomimetic mineralized strontium-doped hydroxyapatite on porous poly (l - lactic acid) scaffolds for bone defect repair. *International Journal of Nanomedicine*, 13, 1707–1721.

Ge, Z., Jin, Z., and Cao, T., 2008. Manufacture of degradable polymeric scaffolds for bone regeneration. *Biomedical Materials*, 3 (2), 022001.

Giannoudis, P. V, Dinopoulos, H., and Tsiridis, E., 2005. Bone substitutes: an update. *Injury*, 36 (3), S20–S27.

Goddard, J.M. and Hotchkiss, J.H., 2007. Polymer surface modification for the attachment of bioactive compounds. *Progress in polymer science*, 32 (7), 698–725.

Gokmen, M.T. and Du Prez, F.E., 2012. Porous polymer particles—A comprehensive guide to synthesis, characterization, functionalization and applications. *Progress in polymer science*, 37 (3), 365–405.

Golin, A.P., 2014. Humidity Effect on the Structure of Electrospun Core-Shell PCL-PEG Fibers for Tissue Regeneration Applications, (May).

Goonoo, N., Bhaw-Luximon, A., and Jhurry, D., 2014. Drug loading and release from electrospun biodegradable nanofibers. *Journal of Biomedical Nanotechnology*, 10 (9), 2173–2199.

Gopi, D., Ramya, S., Rajeswari, D., Surendiran, M., and Kavitha, L., 2014. Development of strontium and magnesium substituted porous hydroxyapatite/poly(3,4-ethylenedioxythiophene) coating on surgical grade stainless steel and its bioactivity on osteoblast cells. *Colloids and Surfaces B: Biointerfaces*, 114, 234–240.

Gruber, R., Pietschmann, P., and Peterlik, M., 2008. Introduction to bone development, remodelling and repair. *In*: *Radiology of osteoporosis*. Springer, 1–23.

Guo, X.E., 2001. Mechanical properties of cortical bone and cancellous bone tissue. *Bone mechanics handbook*, 10, 1–23.

Gupta, D., Venugopal, J., Mitra, S., Giri Dev, V.R., and Ramakrishna, S., 2009. Nanostructured biocomposite substrates by electrospinning and electrospraying for the mineralization of osteoblasts. *Biomaterials*, 30 (11), 2085–2094.

Ha, Y.-M., Amna, T., Kim, M.-H., Kim, H.-C., Hassan, M.S., and Khil, M.-S., 2013. Novel silicificated PVAc/POSS composite nanofibrous mat via facile electrospinning technique: potential scaffold for hard tissue engineering. *Colloids and surfaces. B, Biointerfaces*, 102, 795–802.

Habraken, W., Habibovic, P., Epple, M., and Bohner, M., 2016. Calcium phosphates in biomedical applications: materials for the future? *Materials Today*, 19 (2), 69–87.

Haider, A., Gupta, K.C., and Kang, I.-K., 2014. PLGA/nHA hybrid nanofiber scaffold as a nanocargo carrier of insulin for accelerating bone tissue regeneration. *Nanoscale research letters*, 9 (1), 314.

Haider, A., Haider, S., Han, S., and Kang, I., 2017. Recent advances in the synthesis, functionalization and biomedical applications of hydroxyapatite : a review. *RSC Advances*, 7 (13), 7442–7458.

Haider, A., Haider, S., and Kang, I.-K., 2018. A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. *Arabian Journal of Chemistry*, 11 (8), 1165–1188.

Hallab, N., Merritt, K., and Jacobs, J.J., 2001. Metal sensitivity in patients with orthopaedic implants. *JBJS*, 83 (3), 428–436.

Han, D., Dong, J., Cao, D.J., Yu, Z.-Y., Xu, H., Chai, G., Guo-Xiong, S., and Ai, S.-T., 2011. Usage of Rapid Prototyping Technique in Customized Craniomaxillofacial Bone Tissue Engineering Scaffold. *In*: *Advanced Applications of Rapid Prototyping Technology in Modern Engineering*. IntechOpen.

Hannink, G. and Arts, J.J.C., 2011. Bioresorbability, porosity and mechanical strength of bone substitutes: what is optimal for bone regeneration? *Injury*, 42, S22–S25.

Hao, J., Acharya, a, Chen, K., Chou, J., Kasugai, S., and Lang, N.P., 2015. Novel bioresorbable strontium hydroxyapatite membrane for guided bone regeneration. *Clinical oral implants research*, 26 (1), 1–7.

Hassan, M.I., Sultana, N., and Hamdan, S., 2014. Bioactivity Assessment of Poly(ε -caprolactone)/Hydroxyapatite Electrospun Fibers for Bone Tissue Engineering Application. *Journal of Nanomaterials*, 2014, 1–6.

Hatano, K., Inoue, H., Kojo, T., Matsunaga, T., Tsujisawa, T., Uchiyama, C., and Uchida, Y., 1999. Effect of surface roughness on proliferation and alkaline phosphatase expression of rat calvarial cells cultured on polystyrene. *Bone*, 25 (4), 439–445.

He, L.-H., Standard, O.C., Huang, T.T.Y., Latella, B.A., and Swain, M. V, 2008. Mechanical behaviour of porous hydroxyapatite. *Acta Biomaterialia*, 4 (3), 577–586.

Hench, L.L., 1998. Bioactive materials: the potential for tissue regeneration. *Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and the Australian Society for Biomaterials*, 41 (4), 511–518.

Henkel, J., Woodruff, M.A., Epari, D.R., Steck, R., Glatt, V., Dickinson, I.C., Choong, P.F., Schuetz, M.A., and Hutmache, D.W., 2013. Bone Regeneration Based on Tissue Engineering Conceptions – A 21st Century Perspective. *Bone research*, 3, 216–248.

Hernandez, C.J., Majeska, R.J., and Schaffler, M.B., 2004. Osteocyte density in woven bone. *Bone*, 35 (5), 1095–1099.

Hollander, W., Petr Patka, P.A.T.Klein, C., and A.K.Heidendal, G., 1991. Macroporous calcium phosphate ceramics for bone substitution: a tracer study on biodegradation with 45Ca tracer. *Biomaterials*, 12 (6), 569–573.

Hong, S. and Kim, G., 2011. Fabrication of electrospun polycaprolactone biocomposites reinforced with chitosan for the proliferation of mesenchymal stem cells. *Carbohydrate Polymers*, 83 (2), 940–946.

Huang, C., Chen, S., Lai, C., Reneker, D.H., Qiu, H., Ye, Y., and Hou, H., 2006. Electrospun polymer nanofibres with small diameters. *Nanotechnology*, 17 (6), 1558–1563.

Huang, J., 2017. Design and Development of Ceramics and Glasses. *In*: *Biology and Engineering of Stem Cell Niches*. Elsevier, 315–329.

Huang, Z.-M., Zhang, Y.-Z., Kotaki, M., and Ramakrishna, S., 2003. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Composites Science and Technology*, 63 (15), 2223–2253.

Hutmacher, D., Kirsch, A., Ackermann, K.L., and Huerzeler, M.B., 1998. Matrix and carrier materials for bone growth factors: State of the art and future perspectives. *In*: *Biological matrices and tissue reconstruction*. Springer, 197–206.

Hutmacher, D.W., 2000. Scaffolds in tissue engineering bone and cartilage. *In*: *The Biomaterials: Silver Jubilee Compendium*. Elsevier, 175–189.

Hutmacher, D.W., 2001. Scaffold design and fabrication technologies for engineering tissues—state of the art and future perspectives. *Journal of Biomaterials Science, Polymer Edition*, 12 (1), 107–124.

Jeong, S.I., Ko, E.K., Yum, J., Jung, C.H., Lee, Y.M., and Shin, H., 2008. Nanofibrous poly(lactic acid)/hydroxyapatite composite scaffolds for guided tissue regeneration. *Macromolecular bioscience*, 8 (4), 328–38.

Ji, Y., Liang, K., Shen, X., and Bowlin, G.L., 2014. Electrospinning and characterization of chitin nanofibril/polycaprolactone nanocomposite fiber mats. *Carbohydrate polymers*, 101, 68–74.

Jin, L., Jiang, G., and Liu, S., 2014. Preparation of ultra-porous UPM / PHBV nanofibres using solvent-etching technology and drug-loading efficiency. *Indian Journal of Fibre & Textile Research (IJFTR)*, 39 (4), 430–433.

Jin, R.M., Sultana, N., Baba, S., Hamdon, S., and Ismail, A.F., 2015. Porous PCL/Chitosan and nHA/PCL/Chitosan Scaffolds for Tissue Engineering

Applications : Fabrication and Evaluation. *Journal of Nanomaterials*, 16 (1), 1–8.

Jose, M. V, Thomas, V., Johnson, K.T., Dean, D.R., and Nyairo, E., 2009. Aligned PLGA/HA nanofibrous nanocomposite scaffolds for bone tissue engineering. *Acta biomaterialia*, 5 (1), 305–315.

Kalita, S.J., Bhardwaj, A., and Bhatt, H.A., 2007. Nanocrystalline calcium phosphate ceramics in biomedical engineering. *Materials Science and Engineering C*, 27 (3), 441–449.

Kang, I.K., Xing, Z.C., Han, S.J., and Shin, Y.S., 2011. Fabrication of biodegradable polyester nanocomposites by electrospinning for tissue engineering. *Journal of Nanomaterials*, 2011, 1–18.

Karageorgiou, V. and Kaplan, D., 2005. Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials*, 26 (27), 5474–5491.

Karak, N., 2012. *Vegetable oil-based polymers: properties, processing and applications*. Elsevier.

Khalil, K.A., Fouad, H., Elsarnagawy, T., and Almajhdi, F.N., 2013. Preparation and Characterization of Electrospun PLGA / silver Composite Nanofibers for Biomedical Applications. *International journal of Electrochemical Science*, 8 (3), 3483–3493.

Kharaziha, M., Fathi, M.H., and Edris, H., 2013. Effects of surface modification on the mechanical and structural properties of nanofibrous poly(ε-caprolactone)/forsterite scaffold for tissue engineering applications. *Materials science & engineering. C*, 33 (8), 4512–4519.

Ki, C.S., Baek, D.H., Gang, K.D., Lee, K.H., Um, I.C., and Park, Y.H., 2005. Characterization of gelatin nanofiber prepared from gelatin–formic acid solution. *Polymer*, 46 (14), 5094–5102.

Kim, H.-W., Knowles, J.C., and Kim, H.-E., 2004. Hydroxyapatite/poly (ε-caprolactone) composite coatings on hydroxyapatite porous bone scaffold for drug delivery. *Biomaterials*, 25 (7–8), 1279–1287.

Kim, H.M., Himeno, T., Kokubo, T., and Nakamura, T., 2005. Process and kinetics of bonelike apatite formation on sintered hydroxyapatite in a simulated body fluid. *Biomaterials*, 26 (21), 4366–4373.

Kim, H.W., Knowles, J.C., and Kim, H.E., 2005. Hydroxyapatite porous scaffold engineered with biological polymer hybrid coating for antibiotic Vancomycin release. *Journal of Materials Science: Materials in Medicine*, 16 (3), 189–195.

Kim, M.S., Son, J., Lee, H., Hwang, H., Choi, C.H., and Kim, G., 2014. Highly porous 3D nanofibrous scaffolds processed with an electrospinning/laser process. *Current Applied Physics*, 14 (1), 1–7.

Kim, T.W., Park, J., and Hyon, S., 2014. PLGA nanofiber membranes loaded with epigallocatechin-3- O -gallate are beneficial to prevention of postsurgical adhesions. *International Journal of Nanomedicine*, 9, 4067–4078.

Kini, U. and Nandeesh, B.N., 2012. Physiology of bone formation, remodeling, and metabolism. *In*: *Radionuclide and hybrid bone imaging*. Springer, 29–57.

Kokubo, T., Kim, H.-M., and Kawashita, M., 2003. Novel bioactive materials with different mechanical properties. *Biomaterials*, 24 (13), 2161–2175.

Koombhongse, S., Liu, W., and Reneker, D.H., 2001. Flat polymer ribbons and other shapes by electrospinning. *Journal of Polymer Science Part B: Polymer Physics*, 39 (21), 2598–2606.

Koski, A., Yim, K., and Shivkumar, S., 2004. Effect of molecular weight on fibrous PVA produced by electrospinning. *Materials Letters*, 58 (3–4), 493–497.

Kumta, P.N., Sfeir, C., Lee, D.-H., Olton, D., and Choi, D., 2005. Nanostructured calcium phosphates for biomedical applications: novel synthesis and characterization. *Acta Biomaterialia*, 1 (1), 65–83.

Kurtz, S.M., Muratoglu, O.K., Evans, M., and Edidin, A.A., 1999. Advances in the processing, sterilization, and crosslinking of ultra-high molecular weight polyethylene for total joint arthroplasty. *Biomaterials*, 20 (18), 1659–1688.

Lam, W.M., Pan, H.B., Li, Z.Y., Yang, C., Chan, W.K., Wong, C.T., Luk, K.D.K., and Lu, W.W., 2010. Strontium-substituted calcium phosphates prepared by hydrothermal method under linoleic acid-ethanol solution. *Ceramics International*, 36 (2), 683–688.

Langer, R. and Vacanti, J.P., 1993. Tissue engineering. *Science (New York, N.Y.)*, 260 (5110), 920–926.

Lao, L., Wang, Y., Zhu, Y., Zhang, Y., and Gao, C., 2011. Poly(lactide-co-glycolide)/hydroxyapatite nanofibrous scaffolds fabricated by electrospinning for bone tissue engineering. *Journal of materials science. Materials in medicine*, 22 (8), 1873–1884.

Lebourg, M., Suay Antón, J., and Gomez Ribelles, J.L., 2010. Characterization of calcium phosphate layers grown on polycaprolactone for tissue engineering purposes. *Composites Science and Technology*, 70 (13), 1796–1804.

Lee, K.H., Kim, H.Y., Bang, H.J., Jung, Y.H., and Lee, S.G., 2003. The change of bead morphology formed on electrospun polystyrene fibers. *Polymer*, 44 (14), 4029–4034.

Lee, M.J., Park, J.B., Kim, H.H., Ki, C.S., Park, S.Y., Kim, H.J., and Park, Y.H., 2014. Surface coating of hydroxyapatite on silk nanofiber through biomineralization using ten times concentrated simulated body fluid and the evaluation for bone regeneration. *Macromolecular Research*, 22 (7), 710–716.

Lee, S.J. and Atala, A., 2013. Scaffold technologies for controlling cell behavior in tissue engineering. *Biomedical materials (Bristol, England)*, 8 (1), 010201.

LeGeros, R.Z., 2002. Properties of osteoconductive biomaterials: calcium phosphates. *Clinical Orthopaedics and Related Research®*, 395, 81–98.

Lei, Y., Rai, B., Ho, K.H., and Teoh, S.H., 2007. In vitro degradation of novel bioactive polycaprolactone—20% tricalcium phosphate composite scaffolds for bone engineering. *Materials Science and Engineering: C*, 27 (2), 293–298.

Lei, Y., Xu, Z., Ke, Q., Yin, W., Chen, Y., Zhang, C., and Guo, Y., 2017. Strontium hydroxyapatite/chitosan nanohybrid scaffolds with enhanced osteoinductivity for bone tissue engineering. *Materials Science and Engineering C*, 72, 134–142.

Leung, V. and Ko, F., 2011. Biomedical applications of nanofibers. *Polymers for Advanced Technologies*, 22 (3), 350–365.

Li, D., Sun, H., Jiang, L., Zhang, K., Liu, W., Zhu, Y., Fangteng, J., Shi, C., Zhao, L., Sun, H., and Yang, B., 2014. Enhanced Biocompatibility of PLGA Nano fi bers with Gelatin / Nano- Hydroxyapatite Bone Biomimetics Incorporation. *Acs Applied Materials & Interfaces*, 6 (12), 9402–9410.

Li, J. and Stocum, D.L., 2014. *Fracture Healing*. Basic and Applied Bone Biology. Elsevier Inc.

Li, W.J., Laurencin, C.T., Caterson, E.J., Tuan, R.S., and Ko, F.K., 2002. Electrospun nanofibrous structure: A novel scaffold for tissue engineering. *Journal of Biomedical Materials Research*, 60 (4), 613–621.

Li, X., Wang, L., Fan, Y., Feng, Q., Cui, F.Z., and Watari, F., 2013. Nanostructured scaffolds for bone tissue engineering. *Journal of Biomedical Materials Research - Part A*, 101 A (8), 2424–2435.

Li, X., Xie, J., Yuan, X., and Xia, Y., 2008. Coating Electrospun Poly (ε -caprolactone) Fibers with Gelatin and Calcium Phosphate and Their Use as Biomimetic

Scaffolds for Bone Tissue Engineering. *Langmuir*, 24 (24), 14145–14150.

Li, Z. and Wang, C., 2013. Effects of working parameters on electrospinning. *In*: *One-dimensional nanostructures*. Springer, 15–28.

Li, Z.H., Wu, J.M., Huang, S.J., Guan, J., and Zhang, X.Z., 2010. Strontium Hydroxyapatite Synthesis, Characterization and Cell Cytotoxicity. *Advanced Materials Research*, 160–162, 117–122.

Lim, J.Y., Hansen, J.C., Siedlecki, C.A., Runt, J., and Donahue, H.J., 2005. Human foetal osteoblastic cell response to polymer-demixed nanotopographic interfaces. *Journal of the Royal Society Interface*, 2 (2), 97–108.

Liu, H. and Hsieh, Y., 2002. Ultrafine fibrous cellulose membranes from electrospinning of cellulose acetate. *Journal of Polymer Science Part B: Polymer Physics*, 40 (18), 2119–2129.

Liu, X. and Ma, P.X., 2004. Polymeric scaffolds for bone tissue engineering. *Annals of biomedical engineering*, 32 (3), 477–486.

Liu, Y., Luo, D., and Wang, T., 2016. Hierarchical structures of bone and bioinspired bone tissue engineering. *Small*, 12 (34), 4611–4632.

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

Lowry, N., Han, Y., Meenan, B.J., and Boyd, a. R., 2017. Strontium and zinc co-substituted nanophase hydroxyapatite. *Ceramics International*, 43 (15), 12070–12078.

Ma, Z., He, W., Yong, T., and Ramakrishna, S., 2005. Grafting of gelatin on electrospun poly (caprolactone) nanofibers to improve endothelial cell spreading and proliferation and to control cell orientation. *Tissue engineering*, 11 (7–8), 1149–1158.

Mahyudin, F., Widhiyanto, L., and Hermawan, H., 2016. Biomaterials in orthopaedics. *In*: *Biomaterials and Medical Devices*. Springer, 161–181.

Mallick, S., Tripathi, S., and Srivastava, P., 2015. Advancement in scaffolds for bone tissue engineering: a review. *IOSR J. Pharm. Biol. Sci*, 10, 37–54.

Mason, C., 2007. Regenerative Medicine 2.0.

Megelski, S., Stephens, J.S., Chase, D.B., and Rabolt, J.F., 2002. Micro-and nanostructured surface morphology on electrospun polymer fibers. *Macromolecules*, 35 (22), 8456–8466.

Mellon, S.J. and Tanner, K.E., 2012. Bone and its adaptation to mechanical loading: a review. *International Materials Reviews*, 57 (5), 235–255.

Meng, W., Kim, S.-Y., Yuan, J., Kim, J.C., Kwon, O.H., Kawazoe, N., Chen, G., Ito, Y., and Kang, I.-K., 2007. Electrospun PHBV/collagen composite nanofibrous scaffolds for tissue engineering. *Journal of biomaterials science. Polymer edition*, 18 (1), 81–94.

Meng, Z.X., Zheng, W., Li, L., and Zheng, Y.F., 2010. Fabrication and characterization of three-dimensional nanofiber membrance of PCL–MWCNTs by electrospinning. *Materials Science and Engineering: C*, 30 (7), 1014–1021.

Middleton, J.C. and Tipton, A.J., 2000. Synthetic biodegradable polymers as orthopedic devices. *Biomaterials*, 21 (23), 2335–2346.

Mistry, A.S. and Mikos, A.G., 2005. Tissue engineering strategies for bone regeneration. *In*: *Regenerative medicine II*. Springer, 1–22.

Mit-uppatham, C., Nithitanakul, M., and Supaphol, P., 2004. Ultrafine electrospun polyamide-6 fibers: effect of solution conditions on morphology and average fiber diameter. *Macromolecular Chemistry and Physics*, 205 (17), 2327–2338.

Mitra, J., Tripathi, G., Sharma, A., and Basu, B., 2013. Scaffolds for bone tissue engineering: Role of surface patterning on osteoblast response. *RSC Advances*, 3 (28), 11073–11094.

Mittal, K.L., 2009. *Contact angle, wettability and adhesion*. CRC Press.

Miyoshi, H. and Adachi, T., 2014. Topography Design Concept of a Tissue Engineering Scaffold for Controlling Cell Function and Fate Through. *Tissue Engineering Part B: Reviews*, 20 (6), 609–627.

Mofokeng, J.P. and Luyt, A.S., 2015. Morphology and thermal degradation studies of melt-mixed poly(hydroxybutyrate-co-valerate) (PHBV)/poly(e-caprolactone) (PCL) biodegradable polymer blend nanocomposites with TiO2 as filler. *Polymer Testing*, 50 (10), 3812–3824.

Morais, J.M., Papadimitrakopoulos, F., and Burgess, D.J., 2010. Biomaterials/tissue interactions: possible solutions to overcome foreign body response. *The AAPS journal*, 12 (2), 188–196.

Morgan, E.F., Barnes, G.L., and Einhorn, T.A., 2013. The bone organ system: form and function. *In*: *Osteoporosis*. Elsevier, 3–20.

Moroni, L., Licht, R., Boer, J. De, Wijn, J.R. De, and Blitterswijk, C.A. Van, 2006. Fiber diameter and texture of electrospun PEOT / PBT scaffolds influence human mesenchymal stem cell proliferation and morphology , and the release of incorporated compounds. *Biomaterials*, 27 (28), 4911–4922.

Moutos, F.T., Estes, B.T., and Guilak, F., 2010. Multifunctional hybrid three-dimensionally woven scaffolds for cartilage tissue engineering. *Macromolecular bioscience*, 10 (11), 1355–1364.

Murugan, R., Liao, S., and Molnar, P., 2009. Skeletal regenerative nanobiomaterials. *In*: *Durnten-Zurich: Trans Tech Publications*. 3–27.

Murugan, R. and Ramakrishna, S., 2005. Development of nanocomposites for bone grafting. *Composites Science and Technology*, 65 (15–16), 2385–2406.

Nair, L.S. and Laurencin, C.T., 2007. Biodegradable polymers as biomaterials. *Progress in polymer science*, 32 (8–9), 762–798.

Nakamura, H., 2007. Morphology, function, and differentiation of bone cells. *Journal of hard tissue biology*, 16 (1), 15–22.

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

Ni, G.-X., Shu, B., Huang, G., Lu, W.W., and Pan, H.-B., 2012. The effect of strontium incorporation into hydroxyapatites on their physical and biological properties. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 100 (2), 562–568.

Ni, G.X., Yao, Z.P., Huang, G.T., Liu, W.G., and Lu, W.W., 2011. The effect of strontium incorporation in hydroxyapatite on osteoblasts in vitro. *Journal of Materials Science: Materials in Medicine*, 22 (4), 961–967.

Ning, C., Cheng, H., Zhu, W., Yin, Z., Chen, H., Zheng, H., Lei, S., Yin, S., and Tan, G., 2008. In vitro mineralization of surface-modified porous polycaprolactone scaffolds in simulated body fluid. *Applied Surface Science*, 255 (2), 429–431.

Okamoto, M. and John, B., 2013. Synthetic biopolymer nanocomposites for tissue engineering scaffolds. *Progress in Polymer Science*, 38 (10–11), 1487–1503.

Oliveira, a. L., Malafaya, P.B., and Reis, R.L., 2003. Sodium silicate gel as a precursor for the in vitro nucleation and growth of a bone-like apatite coating in compact and porous polymeric structures. *Biomaterials*, 24 (15), 2575–2584.

Olszta, M.J., Cheng, X., Jee, S.S., Kumar, R., Kim, Y.-Y., Kaufman, M.J., Douglas, E.P., and Gower, L.B., 2007. Bone structure and formation: A new perspective. *Materials Science and Engineering: R: Reports*, 58 (3–5), 77–116.

Oranger, A., Colaianni, G., and Grano, M., 2014. Bone cells. *In*: *Imaging of Prosthetic*

Joints. Springer, 3–13.

Palmero, P., 2016. Ceramic–polymer nanocomposites for bone-tissue regeneration. *In*: *Nanocomposites for Musculoskeletal Tissue Regeneration*. Elsevier, 331–367.

Pan, H., Zhao, X., Darvell, B.W., and Lu, W.W., 2010. Apatite-formation ability-predictor of 'bioactivity'? *Acta biomaterialia*, 6 (11), 4181–4188.

Park, G.E. and Webster, T.J., 2005. A review of nanotechnology for the development of better orthopedic implants. *Journal of Biomedical Nanotechnology*, 1 (1), 18–29.

Park, J.B. and Lakes, R.S., 2007. Polymeric implant materials. *Biomaterials*, 173–205.

Park, K.E., Lee, K.Y., Lee, S.J., and Park, W.H., 2007. Surface characteristics of plasma-treated PLGA nanofibers. *Macromolecular Symposia*, 249–250, 103–108.

Paşcu, E.I., Stokes, J., and McGuinness, G.B., 2013. Electrospun composites of PHBV, silk fibroin and nano-hydroxyapatite for bone tissue engineering. *Materials science & engineering. C, Materials for biological applications*, 33 (8), 4905–16.

Patel, N.R. and Gohil, P.P., 2012. A review on biomaterials: scope, applications & human anatomy significance. *International Journal of Emerging Technology and Advanced Engineering*, 2 (4), 91–101.

Peng, F., Yu, X., and Wei, M., 2011. In vitro cell performance on hydroxyapatite particles/poly(L-lactic acid) nanofibrous scaffolds with an excellent particle along nanofiber orientation. *Acta biomaterialia*, 7 (6), 2585–92.

Pielichowska, K. and Blazewicz, S., 2010. Bioactive Polymer / Hydroxyapatite (Nano) composites for Bone Tissue Regeneration, 30–59.

Piskin, E., 1995. p. *Journal of Biomaterials Science, Polymer Edition*, 6 (9), 775–795.

Place, E.S., George, J.H., Williams, C.K., and Stevens, M.M., 2009. Synthetic polymer scaffolds for tissue engineering. *Chemical society reviews*, 38 (4), 1139–1151.

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.

Prabhakaran, M.P., Venugopal, J., and Ramakrishna, S., 2009. Electrospun nanostructured scaffolds for bone tissue engineering. *Acta biomaterialia*, 5 (8), 2884–2893.

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.

Purbrick, M., Ambrosio, L., VENTRE, M., and NETTI, P., 2010. Natural composites: structure–property relationships in bone, cartilage, ligament and tendons. *In*: *Biomedical Composites*. Elsevier, 3–24.

Qian, L. and Zhang, H., 2011. Controlled freezing and freeze drying: a versatile route for porous and micro-/nano-structured materials. *Journal of Chemical Technology & Biotechnology*, 86 (2), 172–184.

Ramakrishna, S., 2000. Biomedical applications of polymer composite materials. *In*: *Proc. of the second Asian-Australasian Conference on Composite Materials (ACCM-2000), Korea. Advanced Institute of Science and Technology: Korea*. A431-0A436.

Ramakrishna, S., 2005. *An introduction to electrospinning and nanofibers*. World Scientific.

Raucci, M.G., Giugliano, D., Alvarez-Perez, M. a., and Ambrosio, L., 2015. Effects on growth and osteogenic differentiation of mesenchymal stem cells by the strontium-added sol–gel hydroxyapatite gel materials. *Journal of Materials Science: Materials in Medicine*, 26 (2), 90.

Reneker, D.H. and Chun, I., 1996. Nanometre diameter fibres of polymer , produced by electrospinning. *Nanotechnology*, 216 (7), 216–223.

Rezwan, K., Chen, Q.Z., Blaker, J.J., and Boccaccini, A.R., 2006. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials*, 27 (18), 3413–3431.

Rho, J.-Y., Kuhn-Spearing, L., and Zioupos, P., 1998. Mechanical properties and the hierarchical structure of bone. *Medical engineering & physics*, 20 (2), 92–102.

Rieger, E., Dupret-Bories, A., Salou, L., Metz-Boutigue, M.-H., Layrolle, P., Debry, C., Lavalle, P., and Vrana, N.E., 2015. Controlled implant/soft tissue interaction by nanoscale surface modifications of 3D porous titanium implants. *Nanoscale*, 7 (21), 9908–9918.

Robb, B. and Lennox, B., 2011. The electrospinning process, conditions and control. *In*: *Electrospinning for Tissue Regeneration*. Elsevier, 51–66.

Rogel, M.R., Qiu, H., and Ameer, G.A., 2008. The role of nanocomposites in bone regeneration. *Journal of Materials Chemistry*, 18 (36), 4233–4241.

Rogina, A., 2014. Electrospinning process: Versatile preparation method for biodegradable and natural polymers and biocomposite systems applied in tissue engineering and drug delivery. *Applied Surface Science*, 296, 221–230.

Roseti, L., Parisi, V., Petretta, M., Cavallo, C., Desando, G., Bartolotti, I., and Grigolo, B., 2017. Scaffolds for bone tissue engineering: state of the art and new perspectives. *Materials Science and Engineering: C*, 78, 1246–1262.

Salgado, A.J., Coutinho, O.P., and Reis, R.L., 2004. Bone tissue engineering: state of the art and future trends. *Macromolecular bioscience*, 4 (8), 743–765.

Sampath, U., Ching, Y., Chuah, C., Sabariah, J., and Lin, P.-C., 2016. Fabrication of porous materials from natural/synthetic biopolymers and their composites. *Materials*, 9 (12), 991.

Sargeant, A. and Goswami, T., 2006. Hip implants: paper V. Physiological effects. *Materials & design*, 27 (4), 287–307.

Schumacher, M., Gelinsky, M., and Schumacher, M., 2015. Strontium modified calcium phosphate cements – approaches towards targeted stimulation of bone turnover. *Journal of Materials Chemistry B*, 3 (23), 4626–4640.

Sebastian, A. and Loots, G.G., 2018. Genetics of Sost/SOST in sclerosteosis and van Buchem disease animal models. *Metabolism*, 80, 38–47.

Seitz, H., Rieder, W., Irsen, S., Leukers, B., and Tille, C., 2005. Three-dimensional printing of porous ceramic scaffolds for bone tissue engineering. *Journal of Biomedical Materials Research Part B*, 74 (2), 782–788.

Shalumon, K.T., Anulekha, K.H., Chennazhi, K.P., Tamura, H., Nair, S. V, and Jayakumar, R., 2011. International Journal of Biological Macromolecules Fabrication of chitosan / poly (caprolactone) nanofibrous scaffold for bone and skin tissue engineering. *International Journal of Biological Macromolecules*, 48 (4), 571–576.

Shaw, D.J., 1980. *Introduction to colloid and surface chemistry*. Butterworths.

Sheikh, F. a, Ju, H.W., Moon, B.M., Park, H.J., Kim, J.H., Lee, O.J., and Park, C.H., 2013. A novel approach to fabricate silk nanofibers containing hydroxyapatite nanoparticles using a three-way stopcock connector. *Nanoscale Research Letters*, 8 (1), 303.

Shi, C., Yuan, Z., Han, F., Zhu, C., and Li, B., 2016. Polymeric biomaterials for bone regeneration. *Annals of Joint*, 1, 27.

Shuai, C., Sun, H., Wu, P., Gao, C., Yang, Y., and Guo, W., 2017. RSC Advances in fl uence of introducing SrO, 21749–21757.

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

Smith, B.D. and Grande, D.A., 2015. The current state of scaffolds for musculoskeletal regenerative applications. *Nature Reviews Rheumatology*, 11 (4), 213–222.

Song, W., Markel, D.C., Wang, S., Shi, T., Mao, G., and Ren, W., 2012. Electrospun polyvinyl alcohol-collagen-hydroxyapatite nanofibers: a biomimetic extracellular matrix for osteoblastic cells. *Nanotechnology*, 23 (11), 115101.

Srivastava, C.M. and Purwar, R., 2016. Fabrication of robust Antheraea assama fibroin nanofibrous mat using ionic liquid for skin tissue engineering. *Materials Science and Engineering C*, 68, 276–290.

Stanciu, G.A., Sandulescu, I., Savu, B., Stanciu, S.G., Paraskevopoulos, K.M., Chatzistavrou, X., Kontonasaki, E., and Koidis, P., 2007. Investigation of the hydroxyapatite growth on bioactive glass surface. *J. Biomed. Pharm. Eng*, 1 (1), 34–39.

Stevens, A. and Lowe, J.S., 2005. *Human histology*. Mosby.

Stevens, M.M., 2008. Biomaterials for bone tissue engineering. *Materials today*, 11 (5), 18–25.

Subia, B., Kundu, J., and Kundu, S., 2010. Biomaterial scaffold fabrication techniques for potential tissue engineering applications. *In*: *Tissue engineering*. IntechOpen, 141–159.

Subramaniam, A. and Sethuraman, S., 2014. Biomedical applications of nondegradable polymers. *In*: *Natural and Synthetic Biomedical Polymers*. Elsevier, 301–308.

Sui, G., Yang, X., Mei, F., Hu, X., Chen, G., Deng, X., and Ryu, S., 2007. Poly-L-lactic acid/hydroxyapatite hybrid membrane for bone tissue regeneration. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*, 82 (2), 445–454.

Sukigara, S., Gandhi, M., Ayutsede, J., Micklus, M., and Ko, F., 2003. Regeneration of Bombyx mori silk by electrospinning—part 1: processing parameters and geometric properties. *Polymer*, 44 (19), 5721–5727.

Šupová, M., 2015. Substituted hydroxyapatites for biomedical applications: A review. *Ceramics International*, 41 (8), 9203–9231.

Suslu, A., Albayrak, A.Z., Urkmez, A.S., Bayir, E., and Cocen, U., 2014. Effect of surfactant types on the biocompatibility of electrospun HAp/PHBV composite nanofibers. *Journal of Materials Science: Materials in Medicine*, 25 (12), 2677–2689.

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 (1), 181–194.

Tamada, Y. and Ikada, Y., 1993. Effect of preadsorbed proteins on cell adhesion to polymer surfaces. *Journal of colloid and interface science*, 155 (2), 334–339.

Tamayol, A., Akbari, M., Annabi, N., Paul, A., Khademhosseini, A., and Juncker, D., 2013. Fiber-based tissue engineering : Progress , challenges , and opportunities. *Biotechnology Advances*, 31 (5), 669–687.

Tampieri, A., Sprio, S., Sandri, M., and Valentini, F., 2011. Mimicking natural bio-mineralization processes: A new tool for osteochondral scaffold development. *Trends in Biotechnology*, 29 (10), 526–535.

Tan, S., Zhang, B., Zhu, X., Ao, P., Guo, H., Yi, W., and Zhou, G.-Q., 2014. Deregulation of Bone Forming Cells in Bone Diseases and Anabolic Effects of Strontium-Containing Agents and Biomaterials. *BioMed research international*, 2014, 814057.

Tang, X., Thankappan, S.K., Lee, P., Fard, S.E., Harmon, M.D., Tran, K., and Yu, X., 2014. Polymeric biomaterials in tissue engineering and regenerative medicine. *In*: *Natural and Synthetic Biomedical Polymers*. Elsevier, 351–371.

Tavares, D.D.S., Resende, C.X., Quitan, M.P., Castro, L.D.O., Granjeiro, J.M., and Soares, G.D.A., 2011. Incorporation of strontium up to 5 Mol. (%) to hydroxyapatite did not affect its cytocompatibility. *Materials Research*, 14 (4), 456–460.

Taylor, G., 1969. Electrically Driven Jets. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 313 (1515), 453–475.

Temenoff, J.S. and Mikos, A.G., 2000. Injectable biodegradable materials for orthopedic tissue engineering. *Biomaterials*, 21 (23), 2405–2412.

Teo, W. and Ramakrishna, S., 2009. Electrospun nanofibers as a platform for multifunctional , hierarchically organized nanocomposite. *Composites Science and Technology*, 69 (11–12), 1804–1817.

Thomas, S., Grohens, Y., and Ninan, N., 2015. *Nanotechnology Applications for Tissue Engineering*. William Andrew.

Thomas, V., Dean, D.R., Jose, M. V, Mathew, B., Chowdhury, S., and Vohra, Y.K.,

2007. Nanostructured biocomposite scaffolds based on collagen coelectrospun with nanohydroxyapatite. *Biomacromolecules*, 8 (2), 631–637.

Tong, H., Wang, M., Li, Z., and Lu, W.W., 2010. Electrospinning, characterization and in vitro biological evaluation of nanocomposite fibers containing carbonated hydroxyapatite nanoparticles. *Biomedical materials*, 5 (5), 054111.

Tong, H.W. and Wang, M., 2010. Forming Fibrous Nanocomposite Tissue Engineering Scaffolds Through Electrospinning: a Comparative Study of Three Fabrication Routes. *Bioceramics Development and Applications*, 1, 1–4.

Vagaská, B., Bačáková, L., Filovaá, E., and Balík, K., 2010. Osteogenic cells on bio-inspired materials for bone tissue engineering. *Physiological research*, 59 (3).

Vallet-Regí, M., 2001. Ceramics for medical applications. *Journal of the Chemical Society, Dalton Transactions*, 97–108.

Velard, F., Braux, J., Amedee, J., and Laquerriere, P., 2013. Inflammatory cell response to calcium phosphate biomaterial particles: an overview. *Acta biomaterialia*, 9 (2), 4956–4963.

Velasco, M.A., Narváez-Tovar, C.A., and Garzón-Alvarado, D.A., 2015. Design, materials, and mechanobiology of biodegradable scaffolds for bone tissue engineering. *BioMed research international*, 2015.

Wang, H.-S., Fu, G.-D., and Li, X.-S., 2009. Functional Polymeric Nanofibers from Electrospinning. *Recent Patents on Nanotechnology*, 3 (1), 21–31.

Wang, X. and Ye, J., 2008. Variation of crystal structure of hydroxyapatite in calcium phosphate cement by the substitution of strontium ions. *Journal of Materials Science: Materials in Medicine*, 19 (3), 1183–1186.

Webster, T., 2000. Enhanced functions of osteoblasts on nanophase ceramics. *Biomaterials*, 21 (17), 1803–1810.

Webster, T.J. and Ahn, E.S., 2007. *Nanostructured biomaterials for tissue engineering bone.* Tissue Engineering II. Springer Berlin Heidelberg.

Webster, T.J., Siegel, R.W., and Bizios, R., 2001. Nanoceramic surface roughness enhances osteoblast and osteoclast functions for improved orthopaedic/dental implant efficacy. *Scripta Materialia*, 44 (8–9), 1639–1642.

Wei, G. and Ma, P.X., 2004. Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. *Biomaterials*, 25 (19), 4749–4757.

William Jr, G., Einhorn, T.A., Koval, K., McKee, M., Smith, W., Sanders, R., and

Watson, T., 2007. Bone Grafts and Bone Graft Substitutes in Orthopaedic Trauma Surgery: A Critical Analysis. *JBJS*, 89 (3), 649–658.

Williams, D.F., 1999. *The Williams dictionary of biomaterials*. Liverpool University Press.

Williams, D.F., 2008. On the mechanisms of biocompatibility. *Biomaterials*, 29 (20), 2941–2953.

Wilson, E.E., Awonusi, A., Morris, M.D., Kohn, D.H., Tecklenburg, M.M.J., and Beck, L.W., 2006. Three structural roles for water in bone observed by solid-state NMR. *Biophysical journal*, 90 (10), 3722–3731.

Woodruff, M.A. and Hutmacher, D.W., 2010. The return of a forgotten polymer—Polycaprolactone in the 21st century. *Progress in polymer science*, 35 (10), 1217–1256.

Wraighte, P.J. and Scammell, B.E., 2006. Principles of fracture healing. *Surgery (Oxford)*, 24 (6), 198–207.

Wu, H., Fan, J., Chu, C.-C., and Wu, J., 2010. Electrospinning of small diameter 3-D nanofibrous tubular scaffolds with controllable nanofiber orientations for vascular grafts. *Journal of materials science. Materials in medicine*, 21 (12), 3207–3215.

Xiao-Yan, W., Yi, Z.U.O., Huang, D., Xian-Deng, H.O.U., and Yu-Bao, L.I., 2010. Comparative study on inorganic composition and crystallographic properties of cortical and cancellous bone. *Biomedical and Environmental Sciences*, 23 (6), 473–480.

Xie, J., Li, X., and Xia, Y., 2008. Putting Electrospun Nanofibers to Work for Biomedical Research. *Macromolecular rapid communications*, 29 (22), 1775–1792.

Xing, Z.C., Chae, W.P., Baek, J.Y., Choi, M.J., Jung, Y., and Kang, I.K., 2010. In vitro assessment of antibacterial activity and cytocompatibility of silver-containing phbv nanofibrous scaffolds for tissue engineering. *Biomacromolecules*, 11 (5), 1248–1253.

Yanagida, H., Okada, M., Masuda, M., Ueki, M., Narama, I., Kitao, S., Koyama, Y., Furuzono, T., and Takakuda, K., 2009. Cell adhesion and tissue response to hydroxyapatite nanocrystal-coated poly (L-lactic acid) fabric. *Journal of bioscience and bioengineering*, 108 (3), 235–243.

Yang, D., Zhang, J., Xue, J., Nie, J., and Zhang, Z., 2013. Electrospinning of Poly(3-

hydroxybutyrate-co-3-hydroxyvalerate) nanofibers with feature surface microstructure. *Journal of Applied Polymer Science*, 127 (4), 2867–2874.

Yang, F., Both, S.K., Yang, X., Walboomers, X.F., and Jansen, J. a, 2009. Development of an electrospun nano-apatite/PCL composite membrane for GTR/GBR application. *Acta biomaterialia*, 5 (9), 3295–3304.

Yang, F., Yang, D., Tu, J., Zheng, Q., Cai, L., and Wang, L., 2011. Strontium enhances osteogenic differentiation of mesenchymal stem cells and in vivo bone formation by activating Wnt/catenin signaling. *Stem cells*, 29 (6), 981–991.

Yaszemski, M.J., Payne, R.G., Hayes, W.C., Langer, R., and Mikos, A.G., 1996. Evolution of bone transplantation: molecular, cellular and tissue strategies to engineer human bone. *Biomaterials*, 17 (2), 175–185.

Ye, W.P., Du, F.S., Jin, W.H., Yang, J.Y., and Xu, Y., 1997. In vitro degradation of poly (caprolactone), poly (lactide) and their block copolymers: influence of composition, temperature and morphology. *Reactive and Functional Polymers*, 32 (2), 161–168.

Yildirim, E.D., Besunder, R., Pappas, D., Allen, F., Güçeri, S., and Sun, W., 2010. Accelerated differentiation of osteoblast cells on polycaprolactone scaffolds driven by a combined effect of protein coating and plasma modification. *Biofabrication*, 2 (1), 14109.

Yoon, J.J., Kim, J.H., and Park, T.G., 2003. Dexamethasone-releasing biodegradable polymer scaffolds fabricated by a gas-foaming/salt-leaching method. *Biomaterials*, 24 (13), 2323–2329.

Yoon, Y. Il, Moon, H.S., Lyoo, W.S., Lee, T.S., and Park, W.H., 2008. Superhydrophobicity of PHBV fibrous surface with bead-on-string structure. *Journal of Colloid and Interface Science*, 320 (1), 91–95.

Young, E.M. and Chapman, M.W., 1989. Morbidity at bone graft sites. *Journal of orthopaedic trauma*, 3 (3), 192–195.

Yu, W., Sun, T., Qi, C., Ding, Z., and Zhao, H., 2017. Strontium-Doped Amorphous Calcium Phosphate Porous Microspheres Synthesized through A Microwave-Hydrothermal Method Using Fructose 1 , 6-Bisphosphate as An Organic Phosphorus Source : Application in Drug Delivery and Enhanced Bone Regeneration. *ACS Applied Materials & Interfaces*, 9 (4), 3306–3317.

Yuan, H., Kurashina, K., de Bruijn, J.D., Li, Y., De Groot, K., and Zhang, X., 1999. A preliminary study on osteoinduction of two kinds of calcium phosphate ceramics. *Biomaterials*, 20 (19), 1799–1806.

Yuan, X., Zhang, Y., Dong, C., and Sheng, J., 2004. Morphology of ultrafine polysulfone fibers prepared by electrospinning. *Polymer International*, 53 (11), 1704–1710.

Zhang, C., Salick, M.R., Cordie, T.M., Ellingham, T., Dan, Y., and Turng, L.S., 2015. Incorporation of poly(ethylene glycol) grafted cellulose nanocrystals in poly(lactic acid) electrospun nanocomposite fibers as potential scaffolds for bone tissue engineering. *Materials Science and Engineering C*, 49, 463–471.

Zhang, Y., Ouyang, H., Lim, C.T., Ramakrishna, S., and Huang, Z.-M., 2005. Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds. *Journal of biomedical materials research. Part B, Applied biomaterials*, 72 (1), 156–65.

Zhang, Y., Su, B., Venugopal, J., Ramakrishna, S., and Lim, C., 2007. Biomimetic and bioactive nanofi brous scaffolds from electrospun composite nanofibers. *International Journal of Nanomedicine*, 1 (9), 623–638.

Zhao, S., Zhang, J., Zhu, M., Zhang, Y., Liu, Z., Tao, C., Zhu, Y., and Zhang, C., 2015. Acta Biomaterialia Three-dimensional printed strontium-containing mesoporous bioactive glass scaffolds for repairing rat critical-sized calvarial defects. *Acta Biomaterialia*, 12, 270–280.

Zhao, Y.Y., Yang, Q.B., Lu, X.-F., Wang, C., and Wei, Y., 2005. Study on correlation of morphology of electrospun products of polyacrylamide with ultrahigh molecular weight. *Journal of Polymer Science Part B: Polymer Physics*, 43 (16), 2190–2195.

Zhou, H. and Lee, J., 2011. Nanoscale hydroxyapatite particles for bone tissue engineering. *Acta Biomaterialia*, 7 (7), 2769–2781.

Zhou, W.Y., Wang, M., Cheung, W.L., Guo, B.C., and Jia, D.M., 2008. Synthesis of carbonated hydroxyapatite nanospheres through nanoemulsion. *Journal of materials science. Materials in medicine*, 19 (1), 103–110.

Zong, X., Kim, K., Fang, D., Ran, S., Hsiao, B.S., and Chu, B., 2002. Structure and process relationship of electrospun bioabsorbable nanofiber membranes. *Polymer*, 43 (16), 4403–4412.