SURFACE ENTRAPMENT OF CHITOSAN ON 3D PRINTED POLYLACTIC ACID SCAFFOLD

NOR HIDAYAH BINTI ZAKARIA

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy

School of Chemical and Energy Engineering
Faculty of Engineering
Universiti Teknologi Malaysia

.

AUGUST 2018

To the persons that always there during those difficult and trying times, Zakaria Hj Mohd Saad, Radiah binti Mohd Som, Nadirul Hasraf Mat Nayan and Nadhif Hasraf Nadirul Hasraf

ACKNOWLEDGEMENT

In the name of Allah S.W.T, the most gracious and the most merciful. Alhamdulillah, with the utmost blessing from Allah and in the remembrance to our prophet Muhammad P.B.U.H His most beloved messenger of all time, the path of my master degree has come to a completion.

I would like to take this opportunity to show my deepest gratitude to Dr. Saiful Izwan bin Dato' Abd Razak who had stand in front of me to led my may in achieving my master degree. This thesis has become a reality due to his wholly support and knowledge, guidance and moral support a along with the will from Allah S.W.T. The appreciation also goes to my co-supervisor, Assoc. Prof. Dr. Abdul Razak Rahmat for his provision on supervising my research.

Not to be forgotten my beloved parents, Zakaria Bin Mohd Saad and Radiah Binti Mohmad Som, my lovely husband and son, Nadirul Hasraf Bin Mat Nayan and Nadhif Hasraf Bin Nadirul Hasraf for placing their highest belief in me to complete this thesis. Same thanks to my family members and friends who are willing to bear with me through thick and torn together in making my dream come true. Finally, tremendous assistance from all of my colleagues in Polymeric biomaterials lab would not be forgotten and always be in my thought and prayers.

ABSTRACT

This thesis reports a surface entrapment of chitosan on 3D printed PLA scaffold which has the potential use in promoting bone regeneration. The 3D scaffold was designed using SolidWorks and printed by Up Plus 3D printer and then incorporated with chitosan. The entrapped scaffold time was varied from 5 to 90 s. The scaffold was characterized in respect of its mechanical and surface properties. Compressive test showed a higher compressive modulus properties in neat 3D printed PLA scaffolds and an optimum value of 22248 MPa at 15 s of chitosan immersion. The Fourier-transform infrared spectroscopy peak revealed an existence of biomacromolecule and new absorption peaks at 3357 and 1618 cm^{-1} compared to neat PLA on the scaffold while water contact angle showed an increase in hydrophilicity as entrapment time increased. The confocal laser scanning microscopy revealed the existence of entrapment areas approximately 8μm in depth. The scanning electron microscopy showed clearly 3D scaffold with high porosity, uniform distribution chitosan and a controlled and repetitive architecture on entrapped 3D printed scaffold. Immersion of neat and entrapped 3D printed PLA scaffold in simulated body fluid for 14 days resulted the formation of fully covered apatite layers on the surface of entrapped PLA scaffold whereas no change was observed in neat PLA scaffold. Overall, the mechanical and surface properties results showed the suitability of the combination of method and materials to develop 3D porous scaffold and their initial biocompatibility, both being valuable characteristic for tissue engineering applications.

ABSTRAK

Tesis ini melaporkan tindakbalas pemerangkapan permukaan kitosan pada perancah PLA bercetak tiga dimensi (3D) yang mempunyai keupayaan untuk digunakan bagi menggalakkan pertumbuhan semula tulang. Perancah 3D direka menggunakan *SolidWorks* dan dicetak oleh pencetak *3D Up Plus* yang mengandungi kitosan. Masa pemerangkapan adalah dari 5 saat ke 90 saat. Perancah dicirikan terhadap sifat mekanik dan sifat permukaannya. Ujian mampatan menunjukkan sifat modulus mampatan yang lebih tinggi dalam perancah PLA dicetak 3D dengan nilai optima 2248 MPa pada 15 s rendaman kitosan. Puncak spektroskopi inframerah transformasi Fourier menunjukkan kewujudan puncak biomakromolekul dan puncak penyerapan baharu pada 3357 dan 1618 cm^{-1} berbanding dengan PLA tanpa pemerangkapan manakala sudut sentuhan air menunjukkan peningkatan hidrofilik bila meningkatnya masa pemerangkapan. Mikroskop imbasan laser konfokal menunjukkan kewujudan kawasan pemerangkapan sedalam kira-kira 8 μm. Mikroskop elektron imbasan bagi perancah 3D yang memerangkap kitosan jelas menunjukkan permukaan berliang yang saling berkait, penyebaran kitosan yang seragam dan seni bina yang terkawal dan berulang. Hasil rendaman kedua - dua perancah di dalam cecair badan simulasi selama 14 hari menghasilkan pembentukan lapisan mineral di permukaan perancah PLA yang melalui proses pemerangkapan manakala tiada pembentukan yang dapat dilihat berlaku pada perancah 3D biasa. Secara keseluruhan, hasil keputusan ujikaji sifat mekanik dan sifat permukaan menunjukkan kesesuaian di antara kombinasi kaedah dan bahan di dalam pembentukan perancah berpori 3D dan biokeserasian awal, di mana kedua-duanya menjadi ciri-ciri penting untuk aplikasi kejuruteraan tisu.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS AND SYMBOLS

PLA	-	Poly (lactic acid)
SEM	-	Scanning Electron Microscopy
3D	-	Three Dimensional
AM	-	Additive manufacturing
CAD	-	Computer Aided Design
FDA	-	Food and Drug Administration
HA	-	Hydroxyl apatite
SBF	-	Simulated Body Fluid
RP	-	Rapid Prototyping
OH	-	Hydroxide Group
NaOH	-	Sodium Hydroxide
stl	-	sterealitography
CLSM	-	Confocal Laser Scanning Microscopy
SPIN	-	Surface Interpreting Network
ECM	-	Extracellular matrix
Ca/P	-	Calcium/Phosphate
FTIR	-	Fourier Transform Infrared Spectroscopy
WCA	-	Water contact angle

CHAPTER 1

INTRODUCTION

1.1 Overview

Rapid prototyping (RP), is an attractive tool in fabrication of scaffold in tissue engineering (Shirazi *et al.,* 2015). This technique can produce a complicated design with well-defined structures and reproducible architectures (Gross *et al*. 2014). It has open the possibility in making scaffold considering biomedical diagnostic from individual patient and needs (Ventola, 2014).

Modern 3D printing with the aid of computer design and automatic printing technology can tailor made the fabrication of scaffold (Guvendiren *et al.,* 2016). Most other techniques fails to produce this desired properties due to lack the capability of computer design. This includes the effects of geometry/architecture on cell response, and for computer modeling of the scaffold's behavior (Sears *et al.,* 2016). By using 3D printer, an improved mechanical performance of three-dimensional (3D) structures also can be obtained (Serra *et al*., 2013).

Polylactic acid (PLA) are one of the popular biomaterial reported to be used in 3D printing specified in Fused Deposition Modeling (FDM) technologies due to its low cost, nontoxicity and ease of processability (Guvendiren *et al.,* 2016) . Though there are few reports on the use of PLA biomaterial for production of 3D printed scaffolds, serious concerns are on PLA long-term biocompitability due to production of acidic by product, its hydrophilicity (Dong *et al*., 2010) and lack of functional group for covalent cell-recognition signal molecules in the PLA to promote cell adhesion (Zhu, 2002). Those drawbacks can lead to tissue inflammation and cell death. To remedy this, PLA often combined with other bioactive fillers such as calcium phosphate glass (Serra *et al.,* 2013), 45S5 bioactive glass (Estrada *et al.*, 2017), nanocellulose (Wang *et al.,* 2017) and hydroapatite (Corcione *et al.,* 2017). However, since both material have a distinct physical, chemical and biological properties (Barbosa *et al*., 2010), and the 3D fabrications used are costly since it requires preliminary processing compatibility with the 3D printer setup.

Other method to combat this drawback are by modifying PLA scaffold using surface modification technique either by physio sorption, covalent bonding (Serra *et al.,* 2013) or by creating surface interpenetrating networks (Quirk *et al*., 2001). Chitosan are one of the biomacromolecules that have been successfully modifies the surface of PLA (Cui *et al.,* 2003). Modifying PLA with chitosan can improve its osteoconductivity, biocompatibility and its suitable degradation rate (Dutta *et al*., 2004). To date, this method still lack of reports especially on the mechanical integrity and surface properties of the modified scaffold.

With the aid of this feasible method, degradable porous material scaffold were designed to integrate the cell proliferate and tissue regeneration. This is one way to deal with repair and recovery of cell and tissue by establish upon the utilization of polymer scaffold which serve to bolster, strengthen and at times sort out the removing tissue (Madihally, 1999). The mechanical expect for scaffold is also important as a mass transport biological delivery and tissue regeneration (Hollister, 2005).

Researchers had studied the experimental and clinical studies of 3D printed scaffold for biomimetic application in scaffold bioresorbable resource as well as its design. This work describe the fabrication of PLA-based scaffold by 3D printer. Chitosan molecules were entrapped in PLA to obtain 3D scaffold with high mechanical and high bioactive properties. The structures obtained are characterized in terms of mechanical behavior, surface properties as well as in vitro biomineralization studies.

1.2 Problem Statement

PLA is widely used in biomedical application and as biodegradable polymer that been approved by FDA (Almeida, 2013). Most studies focus on PLA mechanical and morphological improvement. Even PLA have been extensively studied especially in scaffold fabrication, the fabrication of PLA scaffold specifically through nozzle-based system are scarcely reported. Plus the resulting printed PLA scaffold usually produced lack in biological moieties which require additional process to activate the biological sites (Zhu *et al*., 2002). Thus making the study of the production of PLA scaffold via commercial 3D were limited to some extent.

PLA is problematic for tissue and implant engineering application due to its absence of biologically active site. Production of a stable and biocompatible PLA scaffold are limited since it may modifies main polymer structure or may require a subsequent process solvent removal from its final structure (Serra *et al.,* 2013). According to Zhu (2002), one of the strategies to render this properties is to design back the polymer backbone that have function monomer unit by introducing functional group on the surface or the polymer backbones.

It is reported, the most straight forward method is coating the surface of the biomaterial with bioactive molecules (Li *et al.,* 2009). This method however it is problematic due to it instability of the layers (coat) thus restrict it further application

in biomedical field (Shen, 2007). Since the PLA printed scaffold are limited in its biocompatibility, this limitation can be overcome with incorporation of chitosan *(*Cui *et al*., 2003). Chitosan are one of bioactive molecules that have been proved to have great osteoconductivity, biocompatibility, suitable degradation rate and minimal foreign body reaction (Cui *et al.*, 2003; Dutta *et al.,* 2004; Collection *et al.,* 2000; Shen *et al.,* 2000). Incorporation of chitosan in PLA structure will lead a more biocompatible scaffold structure (Rogina *et al.,* 2016).

Porous structure of 3D printed can promote faster healing. Though the printed scaffold method can produce a uniform and repetitive porosity, various cumbersome factors should be taken into consideration to design a porous and stable structure such as pore size and the exposition of elevated temperature of the polymer which may lead to denaturation and toxic production of PLA scaffold (Pfister *et al.,* 2003). Therefore, it is important have suitable intrinsic material properties but also geometry of the 3D scaffolds to design the new surface and tailor macrophage activation toward regenerative pathway (Zhu, 2002).

Being relatively new in the tissue engineering field, 3D printed PLA scaffold with incorporation of biomacromolecules has many unexplored features and characteristics. By combining 3D printing method together with suitable biodegradable polymers, fabrication of 3D scaffold it is possible with well-distinguished geometric, different characteristics and allowing the study of the effect of surface entrapment to those cell responses.

1.3 Objectives of the Study

There are three objectives to be achieved in this study. There are as following:

a) To design and prepare 3D printed PLA scaffold with surface entrapment of chitosan.

b) To study the effects of chitosan entrapment on the compressive strength and surface properties of the PLA modified scaffold
c) To investigate the preliminary *in vitro* biomineralization of the scaffold.

1.4 Scope of Study

In order to satisfy all the outlined objectives, the scopes of this research are undertaken according to the following.

Initially, PLA scaffold were designed and fabricated using 3D printer. The scaffold were first design using SolidWork drawing before converted to suitable format for the 3D printer system. The produced scaffold are characterized for its morphology and appearance.

Next step is to produce a bioactive scaffold by entrapment of chitosan in the surface of the 3D printed scaffold. The scaffolds were immersed in chitosan solution for period of 5, 10, 15, 30, 60 and 90s. The resulting entrapped scaffold were evaluated in terms of its mechanical and morphological properties. Mechanical test conducted were compressive strength of the scaffold. Other than that, surface properties such as FTIR, WCA and SEM were also being evaluated.

Consequently, the scaffold were tested for *in-vitro* bio mineralization to test its bioactivity. This was done through immersion in simulated body fluid solution (SBF) and followed by evaluation of hydroxyl apatite growth on the sample.

REFERENCES

Abd Razak,S. I Dahli, F. N.,Wahab, I.F., Abdul Kadir, M.R., Muhamad I.I., Mohd M.I., Yusof and Hassan, A. (2016). Improving viability of stem cells during syringe needle flow through the design of hydrogel cell carriers. *Tissue Engineering Part A, 18*(7-8), 806-815.

Almeida C. R., Serra T., Oliveira MI., Josep A. Planell, Barbosa M, Navarro M., (2014). *Impact of 3-D printed PLA- and chitosan-based scaffolds on human monocyte/macrophage responses: Unraveling the effect of 3-D structures on inflammation*, Elsevier Ltd.

Atala, A., & Yoo, J. J. (2015). *Essentials of 3D biofabrication and translation*. Academic Press, 1.

Austin P. R. (1984). Chitin solvents and solubility parameters. chitin, chitosan, and related enzymes. *Orlando: Academic Press, Inc.;. p. 227‑37*

Bandyopadhyay, A.; Bernard, S.; Xue, W.; Bose, S. (2006). Calcium phosphate-based resorbable Ceramics: Influence of MgO, ZnO, and SiO_2 dopants. *Journal American Ceramic . Soc. 89, 2675-2688.*

Barbosa JN, Amaral IF, Aguas AP, Barbosa MA. (2010). Evaluation of the effect of the degree of acetylation on the inflammatory response to 3D porous chitosan scaffolds. *Journal Biomedical*.

Benton, J. A., DeForest, C. A., Vivekanandan, V., & Anseth, K. S. (2009). Photocrosslinking of gelatin macromers to synthesize porous hydrogels that promote valvular interstitial cell function. *Tissue Engineering Part A*, 15(11), 3221-3230.

Cai, K.Y., Yao, K. D., Cui, Y.L., Yang, Z. M.,, Li, X.Q., Xie, H. Q., ,Qing T. W,, Gao LB. (2002). Influence of different surface modification treatments on poly(D, L-

lactic acid) with silk fibroin and their effects on the culture of osteoblast in vitro. *Biomaterials; 23:* 1603–1611

Cho, C. S. (2003). A novel degradable polycaprolactone networks for tissue engineering. *Biomaterials*, 24, 801–808.

Chua K.C., (2015). Bioprinting: principles and applications. *World scientific,Singapore*

Cima, L. G., Vacanti, J. P., Vacanti, C., Ingber, D., Mooney, D., Langer, R. (1991). Tissue engineering by cell transplantation using degradable polymer substrates. *Journal of Biomechanical Engineering.* 113, 143−151.

Collection, S. (2000). Scaffold development using 3D printing with a starch-based polymer. *Progress in Polymer Science (Oxford),* 10(3), pp.49–56.

Corcione, C.E., Gervaso, F., Scalera, F., Montagna, F., Maiullaro, T., Sannino, A., Maffezzoli, A. (2017). 3D printing of hydroxyapatite polymer-based composites for bone tissue engineering. *Journal of Polymer. Eng.* 37, 741-746.

Cui, Y. L., Hou, X., Qi, A. D., Wang, X. H., Wang, H., Cai, K. Y., Yin, Y. J., Yao, K. D. (2003). Biomimetic surface modification of poly (L-lactic acid) with gelatin and its effects on articular chondrocytes in vitro. *Journal Biomedical Material Resources*; 66: 770–778.

Cui, Y. L., Qi, A. D., Liu, W. G., Wang, X. H., Wang, H., Ma, D. M., Yao, K. D. (2003). Biomimetic surface modification of poly (L-lactic acid) with chitosan and its effects on articular chondrocytes in vitro. *Biomaterials*; 24: 3859–3868.

Davies, M. C., Tendler, S. J. B., Chan, W. C., Shakesheff, K. M., (2001). Controlling biological interactions with poly(lactic acid) by surface entrapment modification. *Langmuir*; 17: 2817–2820.

Dhandayuthapani, B., Yoshida, Y., Maekawa, T., and Kumar, D. S. (2011). Polymeric scaffold in tissue engineering application: a review. *International journal of polymer science* ; 28(28740-812)

Di Martino, A, Sittinger, M., Risbud, M. V. (2005). Chitosan: A versatile biopolymer for orthopaedic tissue-engineering. *Biomaterial*; 26(30):5983–90.

Dong, Y., Li, P., Chen, C.B., Wang, Z.H., Ma, P., Chen, G.Q. (2010). The improvement of fibroblast growth on hydrophobic biopolyesters by coating

with polyhydroxyalkanoate granule binding protein PhaP fused with cell adhesion motif RGD. *Biomaterial, 31,* 8921-8930.

Dutta, P.K., Duta, J. & Tripathi, V.S., (2004). Chitin and Chitosan: Chemistry, properties and applications. *Journal of Scientific and Industrial Research*, 63(1), pp.20–31.

Edith, D., Six, J.L. (2006). Surface characteristics of PLA and PLGA on the surface films. *Application Surface Science 253,* 2758-2764.

Estrada, S.A.M., Armendáriz, I.O., García, A.T., Paz, J.F.H., González, C.A.R. (2017). Evaluation of in vitro bioactivity of 45s5 bioactive glass/poly lactic acid scaffolds produced by 3D printing. *International Journal of Composite Materials. 7,* 144-149

Gross, B.C., (2014). Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences. *Analytical Chemistry*, 86(7), pp.3240–3253.

Gruber, P., O'brien, M. (2003). Polylactides "NatureWorksTM PLA. *Biopolymers*, 4, 235-252

Gualandi, C., (2011). Porous polymeric bioresorbable scaffold for tissue engineering, *Springer,* New York.

Guvendiren, M. (2016). Designing Biomaterials for 3D Printing. ACS Biomaterials Science and Engineering, 2(10), pp.1679–1693.

Hoc K., (2007). Modification of surface engineering using 3D printing material, *Europe Cell Materials*, 14, p. 1

Hollister, S. J. (2005). Porous scaffold design for tissue engineering. *Nature materials,* 4, 518-524

Hubbell, J. A. (1991). Solution technique to incorporate polyethylene oxide and other water-soluble polymers into surfaces of polymeric biomaterials. *Biomaterials*; 12: 144–153.

Hubbell, J.A. (1992). Surface physical interpenetrating networks of poly (ethylene terephthalate) and poly (ethylene oxide) with biomedical application. *Macromolecules*; 25: 226–232.

Hutmacher, D. W. (2001). Scaffold design and fabrication technologies for engineering tissues- state of the art and future perspectives. *Journal of Biomaterial Science. Polym. Ed.* 12, 107-124.

Ito, A., Mase, A., Takizawa, Y., Shinkai, M., Honda, H., Hata, K. I., Ueda, M., Kobayashi, T. (2003). Transglutaminase-mediated gelatin matrices incorporating cell adhesion factors as a biomaterial for tissue engineering, *Journal of Orthophedics*, 95, 196–199.

Kai, H., Wang, X., Madhukar, K. S., Qin, L., Yan, Y., Zhang, R., Wang, X. (2009). Fabrication of two-level tumor bone repair biomaterial based on rapid prototyping technique. *Biofabrication*, 83, 193-196

Karageorgiou, V., Kaplan, D. L. (2005). Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials*, 26(27), 5474-91.

Karande, S. T., Agrawal, M. C. (2008). Functions and requirement of synthetic scaffolds in tissue engineering. In: Nanotechnology and Tissue Engineering: The scaffolds. Laurencin, C. T. & Nair, L. S. (Ed.), CRC Group, *Taylor and Francis group*, 53.

Khang, G. (2012) Handbook of intelligent scaffold for tissue engineering and regenerative medicine, Pan Stanford publishing, *Taylor and Francis Group*, 546, 809-810

Kim, B. S., Baez, C. E., Atala, A. (2000). Biomaterials for tissue engineering, *World Journal Urology*. 1, (18), 2–9.

Kokubo, T., Takadama, H. (2006) How useful is SBF in predicting in vivo bone bioactivity?, *Biomaterials*, 27(15):2907–15.

Kong, L., Gao, Y., Cao, W., Gong, Y., Zhao, N., Zhang, X. (2005). Preparation and characterization of nano-hydroxyapatite/ chitosan composite scaffolds. *Journal Biomedical Material Resources*, A;75(2):275–82.

Kurita, K., Tomita, K., Ishi, S., Nishimura, S. I., Shimoda, K. (1993) b-chitin as a convenient starting material for acetolysis for efficient preparation of N-acetylchitooligosaccharide. *Journal of Polymer Science: A Polymer Chemistry* ; 31:2393‑5.

Laurencin, C. T., Ambrosio, A. M. A., Borden, M. D., Cooper, J. A., (2008) Nanotechnology and tissue engineering (the scaffold), *Tylor and Francis group*. 33:373‑9.

Laurencin, C. T., Ambrosio, A. M. A., Borden, M. D., Cooper, J. A., (1999). Tissue engineering: orthopedic applications, *Annual Revision Biomedical Fiber Engineering* 1, 19–46.

Lavik, E., Langer, R. (2004). Tissue engineering: current state and perspective, *Appl. Microbiol. Biotechnol.* 65 (1), 1–8.

Li, J., Shi, R. (2007). Fabrication of patterned multi-walled poly-l-lactic acid conduits for nerve regeneration. *Journal of Neuroscience Methods*, 165, 257-264.

Li, J., Shi, R. (2009). Surface characterization and biocompatibility of micro- and nano-hydroxyapatite / chitosan-gelatin network films. *Materials Science & Engineering* 29(4), pp.1207–1215.

Lin, H. R., Yeh, Y. J., (2004). Porous alginate/hydroxyapatite composite scaffolds for bone tissue engineering: preparation, characterization, and in vitro studies. *Journal of Biomedical Material Resources: Application Biomaterials*, 71(1):52–65.

Lin, L., Ju, S., Cen, L., Zhang, H., Hu, Q. (2008). Fabrication of porous, TCP scaffolds by combination of rapid prototyping and freeze drying technology. *Yi Peng, Xiaohong Weng (Eds.)*, pp. 88–91APCMBE 2008, IFMBE Proceedings 19, Springer-Verlag, Berlin Heidelberg.

Lu, L., Mikos, A. G., (1996). The importance of new processing techniques in tissue engineering. *Material Science Bulletin*; 11:28 32.

Lutolf, M., (2005). Synthetic miomaterials as instructive extracellular microenviroments for morpholgenisis in tissue engineering, *nature biotechnology*; 67: 175-179

Ma, P. X., Langer, R. (1999). Fabrication of biodegradable polymer foams for cell transplantation and tissue engineering. In Tissue Engineering Methods and Protocols, *Morgan, J., and Yarmush, M. (eds.)* Humana Press, NJ, 47

Mandal, B. B., Kundu, S. C. (2008). Non-bioengineered silk gland fibroin protein: characterization and evaluation of matrices for potential tissue engineering applications. *Biotechnology and Bioengineering*, 100 (6) 1237 – 50.

Nishikawa, H., Ueno, A., Nishikawa, S., Kido J., Ohishi, M., Inoue, H., Nagata, T. (2000). Sulfated glycosaminoglycan synthesis and its regulation by transforming growth factor-β in rat clonal dental pulp cells. *J Endodental*; 26: 169–171.

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 scaffold. *Biomaterials. 26 (4),* 433-441.

Pfister, A. (2003). Biofunctional rapid prototyping for tissue engineering applications: 3D bioplotting versus 3D printing. *Journal of Polymer Science Part A: Polymer Chemistry*, 42(3), pp.624–638.

Phelps, E.A. (2009). Characteristics of modification of PLA on chitosan: A medical discovery, *Regen Medicines*, (4) 65

Quirk, R.A., Davies, M.C., Tendler, S.J., Chan, W. C.; Shakesheff, K.M (2001). Controlling biological interactions with poly (lactic acid) by surface entrapment modification. *Langmuir*, *17*, 2817-2820.

Quirk, R.A.; Davies, M.C.; Tendler, S.J., Shakesheff, K.M. (2000) Surface engineering of poly (lactic acid) by entrapment of modifying species. *Macromolecules, 33*(2), 258-260.

Rasso, F., Marino, A., Giordano, M.Barbarisi, (2005). Smart materials for scaffolds for tissue engineering, *Journal of cellular physiology*, 48, 3982.

Rinaudo, D. (2006). Chitin and chitosan: properties and appliacation, *Elsevier*, 646-49, 270(2)

Rizzi, S.C., Heath, D. J., Coombes, A. G., Bock, N., Textor, M., Downes, S. (2001). Biodegradable polymer/hydroxyapatite composites: surface analysis and initial attachment of human osteoblasts. *Journal of Biomedical Material Resources* 255(4):475–86.

Rogina, A. et al., 2016. Macroporous poly (lactic acid) construct supporting the osteoinductive porous chitosan-based hydrogel for bone tissue engineering, *macromolecules*, (98) 172–181.

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

Sauced, S., Watanabe, K., Yamaguchi, Y., (2000). Differentiation/regeneration of oligodendrocytes entails the assembly of a cell-associated matrix. *International Journal of Devices Neuroscience*; 705–720.

Scheper, T. (2006). Advances in biochemical engineering/biotechnology- tissue engineering| Scaffold system for tissue engineering, *Springer*, 17 (2), 543-552

Sears, N. A. (2016). A Review of Three-Dimensional Printing in Tissue Engineering. Tissue Engineering, *Int. polymer application*, Part B: Reviews, 22(4), pp.298–310.

Serra, T., Mateos-Timoneda, M.A., Planell, J.A., Navarro, M. (2013) 3D printed PLA-based scaffolds: a versatile tool in regenerative medicine. *Organogenesis* 2013, *9*, 239-244.

Serra, T., Planell, J.A., Navarro, M., (2013). High-resolution PLA-based composite scaffolds via 3-D printing technology, *Elsevier Ltd.*, 117(3), 276-282

Shen, F. (2000). A study on the fabrication of porous chitosan / gelatin network scaffold for tissue engineering. *Polymer International*, 1599, 1596–1599.

Shen, F. (2007). Surface modification of polyester biomaterials for tissue engineering. *Biomedical materials*, 2, 24-37.

Shen, F., Jia, W. Y., Cui, Y. L., Shi, H. D., Yang, L. F., Yao, K. D., Dong, X. H., (2000). A study on the fabrication of porous chitosan/gelatin network scaffold for tissue engineering, *Society of Chemical Industry*.4, 235-253

Shinha, V. R., Singla, A. K., Wadhawan, S., Kaushiik, R., Kumria, R., Bansal, K., Dhawan, S. (2004). Chitosan microspheres as a potential carrier drugs, *International Journal of Pharmacy*, 274: 1-33

Shirazi, S. F .S. (2015). A review on powder-based additive manufacturing for tissue engineering: selective laser sintering and inkjet 3D printing. *Science and Technology of Advanced Materials*, 16(3), p.33502.

Smith, L. A., Beck, J. A., Ma, P. X. (2006). Nano fibrous scaffolds and their biological effects. In: Tissue Cell and Organ Engineering, *Kumar, C. (Ed.)*, pp 195, Wiley-VCH.

Song, W. H., Jun, Y. K., Han, Y., Hong, S.H. (2004). Biomimetic apatite coatings on micro-arc oxidized titania. *Biomaterials* , 25(17):3341–9

Sultana, N. *Biodegradable polymer-based scaffold for bone tissue engineering*. Malaysia ; Springer, 2013.

Thevenot, W. Hu, L. Tang, P. (2008). Current topics in medicinal chemistry, *Soft Material*, 8, 270.

Thomson, R. C., Yaszemski, M. J., Mikos, A. G., (1997). Polymer scaffold processing. Principles of tissue engineering, *Landes Co*., 263 (72).

Ventola, C. L. (2014). Medical Applications for 3D Printing: Current and Projected Uses. *P & T : a peer-reviewed journal for formulary management*, 39(10), pp.704–711.

Wang, J, W., Hon, M. H. (2003) Sugar-mediated chitosan/poly (ethyleneglycol)-β-dicalcium pyrophosphate composite: Mechanical and microstructural properties. *Journal of Biomedical Material Resources;* 64*:* 262–272.

Wang, Z., Xu, J., Lu, Y., Hu, L., Fan, Y., Ma, J., Zhou, X. (2017) Preparation of 3D printable micro/nanocellulose-polylactic acid (MNC/PLA) composite wire rods with high MNC constitution. *Indian Crop Production, 109,* 889-896.

Wedler, V. (2009). Rapid prototyping of anatomically shaped, tissue engineered implants for restoring congruent articulating surfaces in small joints. *Cell Proliferation* (4), 485-97

Widmer, M. S., (1998) Manufacture of porous biodegradable polymer conduits by an extrusion process for guided tissue regeneration. *Biomaterials,* 105 (3)

Widmer, M. S., Mikos, A. G., (1998) Fabrication of biodegradable polymer sca!olds for tissue engineering. In: Patrick Jr CW, Mikos AG, McIntire LV, editors. Frontiers in tissue engineering. *Elsevier Science*, p. 107(20).

Williams, D. F. (2008). Surface modification of poly (L-lactic acid) with chitosan Biomaterials, *Langmuir,* 29, 2941.

Y. Ikada, *Biomaterials*. Landon: Springer. 1994.

Yuan, X., Mak, A.F., Li, J. (2001). Formation of bone-like apatite on poly (L-lactic acid) fibers by a biomimetic process. *Journal of Biomedical Material Resources A, 57,* 140-150.

Zhu, H., Ji, A., Shen, J., Surface engineering of poly (DL-lactic acid) by entrapment of biomacromolecules. *Macromolecular Rapid Communications*. 2002. 23(14); 819 – 823.

LIST OF PUBLICATIONS

1. **Zakaria, N.H**., Mat Nayan, N.H., Razak, S. I. A. (2017). Effect of Biomacromolecules on the surface porosity and hydrophilicity of Polylactic Acid Scaffold, Asia international Multidisplinary conference, AIMC-2017-STE-971.