SYNTHESIS AND CHARACTERIZATION OF BIODEGRADABLE POLY(XYLITOL SEBACATE DODECANOATE)/ NANO-HYDROXYAPATITE COMPOSITES FOR POTENTIAL USED IN BIOMEDICAL APPLICATIONS

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To my beloved parents and family

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ABSTRACT

Xylitol-based polyesters such as poly(xylitol sebacate) (PXS) are said to be the best candidates for tissue engineering due to its tunable mechanical and degradation properties. In this study, dodecanedioic acid (DDA) was added into PXS as the additional monomer to increase the strength. Novel poly(xylitol sebacate dodecanoate) (PXSD) polymers was synthesized by using polycondensation method for potential used in tissue engineering. The starting materials for synthesizing PXSD are xylitol, sebacic acid (SA) and DDA and the ratio was varied (xylitol: SA: DDA), PXSD 1 (1:0.25:0.75) PXSD 2 (1:0.5:0.5) and PXSD 3 (1:0.75:0.25). The mixture synthesized at 120 °C for 24 hours under the present of nitrogen gas. After that it was further cured in an oven at 100 °C for 4 days. From the tensile test data, PXSD 1 was selected as the matrix to prepare PXSD/n-HA composite. Thermal stability and melting temperature slightly increased with the increasing in DDA ratio. Polymer crystallinity, mechanical properties such as tensile strength, percentage strain at break, Young's modulus and degradation rate of the polymer was also controlled by the monomer ratio between SA and DDA. Increasing the DDA content resulted in highly ordered structure, improved mechanical properties and reduced erosion time. Much faster degradation rate was found in PXSD 3 compared to the more crystalline polymer of higher DDA content. Another objective was to investigate the effect of n-HA amount to the polymer properties and it was varied to 5, 10, 15 and 20 wt.%. By increasing the n-HA within the matrix, tensile strength and Young's modulus were increased, but the percentage strain at break was reduced. The thermal properties showed no significant changes and distribution of the filler particles become well as the n-HA loadings were increased.

ABSTRAK

Poliester yang berasaskan xylitol seperti poly(xylitol sebakat) (PXS) dikatakan bersesuaian diaplikasikan sebagai bahan untuk kegunaan kejuruteraan tisu kerana sifat mekanik dan kadar degradasi yang boleh dimanipulasi. Di dalam penyelidikan ini, asid dodekanedioik (DDA) telah dicampurkan ke dalam PXS sebagai monomer tambahan untuk meningkatkan kekuatan bahan. Polimer PXSD disintesis menggunakan teknik polikondensasi. Bahan pemula yang digunakan untuk menghasilkan PXSD ialah xylitol, asid sebakat (SA) dan DDA dengan nisbah berlainan seperti (xylitol: SA: DDA); PXSD 1 (1: 0.25: 0.75), PXSD 2 (1: 0.5: 0.5) dan PXSD 3 (1: 0.75: 0.25). Campuran ini disintesis pada 120 °C selama 24 jam dengan kehadiran gas nitrogen, diikuti dengan pengawetan di dalam ketuhar selama 4 hari pada 100 °C. Kestabilan haba dan suhu lebur menunjukkan sedikit peningkatan apabila nisbah DDA ditingkatkan. Kehabluran, sifat mekanik dan degradasi polimer dikawal oleh nisbah monomer antara SA dan DDA. Peningkatan nisbah DDA menghasilkan menambahkan struktur hablur polimer, meningkatkan sifat mekanik dan melambatkan masa degradasi. PXSD 3 yang mempunyai nisbah DDA paling sedikit didapati mempunyai kadar degradasi yang paling cepat jika dibandingkan dengan polimer yang mempunyai struktur hablur yang tinggi. Objektif lain di dalam penyelidikan ini adalah untuk mengkaji kesan jumlah nano-hidroksiapatite (n-HA) terhadap sifat polimer yang terhasil. Jumlah n-HA di dalam sistem komposit divariasikan kepada 5, 10, 15 dan 20 wt%. Daripada data tegangan yang diperolehi, PXSD 1 telah dipilih sebagai matrik untuk menghasilkan PXSD/HA komposit nano. Peningkatan jumlah n-HA di dalam matrik menambahkan kekuatan tegangan dan modulus, tetapi peratus untuk terikan telah menurun. Sifat termal menunjukkan perubahan yang tidak begitu ketara dan taburan partikel pengiso bertambah baik apabila jumlah n-HA telah ditingkatkan.

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LIST OF ABBREVIATIONS

C	-	Carbon
CA	-	Citric acid
COOH	-	Carboxylic acid group
DDA	-	Dodecanedioic acid
DSC	-	Differential scanning calorimetry
ECM	-	Extracellular matrix
FDA	-	Food and Drug Administration
FESEM	-	Field emission scanning electron microscopy
FTIR	-	Fourier transform infrared spectroscopy
HA	-	Hydroxyapatite
NaOH	-	Sodium hydroxide
n-HA	-	Nano-hydroxyapatite
OH	-	Hydroxyl group
PBS	-	Phosphate buffer saline
PCL	-	Poly(caprolactone)
PGA	-	Poly(glycerol acid)
PGD	-	Poly(glycerol dodecanoate)
PGS	-	Poly(glycerol sebacate)
PLA	-	Poly(lactic acid)
PLGA	-	Poly(lactide-co- glycolide)
PPS	-	Poly(polyol sebacate)
PPSC	-	Poly(1,2- propanediol- sebacate- citrate)
PTFE	-	Polytetrafluoroethylene
PXS	-	Poly(xylitol sebacate)
PXSD	-	Poly(xylitol sebacate dodecanoate)

PXSD/n-HA	-	Poly(xylitol sebacate dodecanoate)/ nano-hydroxyapatite
SA	-	Sebacic acid
TE	-	Tissue engineering
T_g	-	Glass transition temperature
TGA	-	Thermogravimetric analysis
T_m	-	Melting temperature
XRD	-	X- ray diffraction

LIST OF SYMBOLS

Aa	-	Area of amorphous region
Ac	-	Area of crystalline region
E	-	Young's modulus
Eb	-	Elongation at break
N_2	-	Nitrogen
$T_{10\%}$	-	Degradation temperature at 10% mass loss.
Wo	-	Initial mass
W_D	-	Mass of dried sample
W_T	-	Mass at certain time point
Wt%	-	Weight percent
Xc	-	Percentage of crystallinity

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Methods to regenerate and restore bone tissue to its functional state have became a clinical necessity as the increase of aging population. This is because, with an advancing age, the imbalance of bone resorption and formation can lead to bone disease such as osteoporosis (Green *et al.,* 2002). Currently, the surgeon use donor tissue for bone repair which are known as allogenic and autologous. Allogenic is the process of cells transplant from different individual of the same species, while in autologous technique the cell donor and recipient is the same person. However, both techniques suffer from limitations. The current clinical "gold standard", autografting, requires a second surgery site, which is expensive and often associated with donor site morbidity, pain, infection, and hematoma formation. Allografting carries the potentials risk of immune rejection, as well as a lessening or even complete loss of bone inductive factor (Petricca *et al.,* 2002).

Construction of scaffolds for tissue engineering is considered as the best solution of the various current treatment drawbacks. Through tissue engineering,

researchers seek to regenerate human tissue, such as bone and cartilage that has been damaged by injury or disease. Over the time, the artificial scaffold which is lattice-like structure will resorbs into the body and leaving behind only the natural tissue (Pallua *et al.,* 2011).

Biodegradable elastomers are said to be the best candidates for tissue engineering applications since many tissues in the body have elastomeric properties, and these kind of material can mimic the structure of bone. Polyester based biodegradable polymer has been synthesized in this research by using xylitol, sebacic acid (SA), and dodecanedioic acid (DDA). According to the previous literature reported by Francesco *et al.,* (2009) three features of polyester elastomers including stable three dimensional network structure, certain elasticity which can provide mechanical stimulation for tissue engineering constructs, and appropriate mechanical strength especially matching with soft tissues and organ bodies have make it very suitable to be developed. Xylitol is a very versatile polymer platform use as the central monomer because it is composed of metabolites endogenous to the mammalian organism, yielding hydrogels with tunable mechanical properties and in vivo degradation (Bruggeman *et al.,* 2008). In addition, the mechanical properties of xylitol based elastomers are correspond to biologically relevant values that fall close to or are equal to those of various tissues such as acellular peripheral nerves, small diameter arteries, cornea and intervertebral discs (Bruggeman *et al.,* 2008).

Currently, the most widely used implantable degradable polymer are the poly (α-esters), in particular poly(glycolic acid) (PGA) and poly(lactic acid) (PLA) and their copolymers poly(lactic/glycolic) acid (PLGA). These FDA approved polymers are used in resorbable sutures and injectable drug delivery system. However these polymers display bulk erosion characteristics (Gopferich., 1996). Xylitol based polyester are said to be the best candidates since the mechanical and degradation rate can be tuned by simply adjust the monomer ratio. Poly(xylitol sebacate) (PXS) was previously synthesized by Bruggeman *et al.,* (2008) by reacting xylitol and SA via polycondensation reaction without the use of catalyst. However, PXS is very soft with a range of Young's modulus of 0.82 ± 0.15 to 5.33 ± 0.40 MPa. Therefore, an

additional monomer, DDA which has 12 carbon (12 C) atoms and two carboxylic groups (COOH) will be added to the reaction. It is anticipated that, it will improve the mechanical properties. Previously, DDA had been used as acid monomer to develop poly(glycerol-dodecanoate) (PGD), high modulus and high elongation at break were achived (Migneco *et al.,* 2009). Besides that, Djordjevic *et al.,* 2010, had reported that the combination of multifunctional acids, citric acid (CA) and SA yield hydrophilic polyesters with different properties by varying its mole ratio.

Nano-hydroxyapatite (n-HA), a type of inorganic bone mineral was used as filler in this research to further improve the mechanical and biological properties of the scaffold such as to impart osteoconductivity to the polymers (Qiu *et al.,* 2006). In addition to increase the modulus, HA has biocompatibility with several types of cells such as osteoblast, osteoclast, fibroblast and periodontal ligaments (Correlo *et al.,* 2005). Elastomer composite with HA may be a competent candidate for application in tissue engineering scaffold because its certain elastic properties can provide mechanical stimulation for tissue engineering construct, improved mechanical strength, adjustable biodegradation and comparably stable shape (Liu *et al.,* 2009). In this project, poly(xylitol sebacate dodecanedioate) (PXSD) with three different ratios were synthesized. n-HA was incorporated to further improve the mechanical properties and reduce the degradation rate. To our knowledge, the research and synthesis of PXSD polymer and PXSD/n-HA composites, have not been reported in the present day.

1.2 Problems Statement

In previous literature, PXS was synthesized by using xylitol and sebacic acid as starting monomer (Bruggeman *et al.*,2010). Xylitol-based polymers have been preferably employed by Bruggemann and coworkers since it has tunable mechanical and degradation properties by simply adjusting the monomer ratio. However, PXS is

very soft with a Young's modulus of only 0.82 ± 0.15 MPa. Due to this inadequate strength property DDA will be added as the third monomer to increase the strength and perhaps reduce the degradation rate by reducing the amount of degrading agent to permit into the polymer which can be achieved by the formation of crystalline segments. According to Djordjevic *et al., (*2010), the use of additional acid reacts with multifunctional alcohols in catalyst free polycondensation reactions, will yield hydrophilic polyesters with high degree of elasticity and therefore produce polymer with high menchanical properties. In addition to mechanical properties and degradation time, it is also an advantage for the polymer to have "dual behavior" (plastic-elastomeric transition) to enhance surgical implantation since it is much easier to position correctly a stiff device than a soft one, and this can be achieved by using a bulky polymer backbone (12 C dicarboxylic acid) (Migneco *et al.,* 2009).

In order to lower the degradation rate, n-HA was added to counteract with the degradation residue of the polymer and also to make it to more compatible for bone cells (Liang *et al.,* 2010). The effect of loading n-HA with different amount into PXSD has not yet been reported. It is expected that mechanical and degradation properties will be improved since it can affect the composites' morphology. Similar to other study, this research is performed in comparison to PLGA as control since PLGA has been used clinically over a very long period providing substantial data regarding their performances as biomaterials.

Several questions need to be answered are listed below:

1. What is the effect of different mole ratio of xylitol: SA: DDA on the mechanical properties and rate of degradation of PXSD elastomers.
2. What is the effect of different amount of n-HA on the mechanical properties and rate of degradation of composites.

1.3 Objectives of Research

The main objective of this research is to synthesis PXSD polyester and PXSD composite reinforced with n-HA. The PXSD/n-HA composites have the potential application in tissue engineering as a scaffold material. Other objectives are listed below:

1. To determine the best monomer ratio of SA and DDA (xylitol: SA: DDA) (1:0.25:0.75, 1:0.5:0.5, and 1:0.75:0.25) on the mechanical properties and degradation rate of the elastomers.
2. To investigate the effect of increasing weight percent of n-HA (5, 10, 15, and 20 wt%) on the mechanical properties and degradation rate of composites.

1.4 Scopes of the Study

The scopes of this research will cover the following:

1. Polycondensation reaction will be used to produce PXSD elastomers.
   Fourier transform infra-red (FTIR) spectroscopy will be used to identify the chemical bonds of the elastomers and composites.
2. Differential scanning spectroscopy (DSC) and Thermogravimetric analysis (TGA) will be used to study the thermal properties of elastomers and composites.

3. Field Emission Scanning electron microscope (FESEM) and x-ray diffraction (XRD) will be used to study the morphology of the elastomers and composites.
4. Tensile test will be carried out to evaluate the mechanical properties of elastomers and composites.
5. In vitro degradation test will be carried out to determine the rate of degradation of the elastomers and composites.

REFERENCES

Ahmad.R, R., Adnan, R., Abu.B, M., and Masudi, S.M. (2011). Synthesis and characterization of pure nanoporous hydroxyapatite. *Chemical sciences*. 22, 25-37.

Bruggeman, J.P., Bettinger, C.J., L.E. Nijst, C.L.E., Daniel, S., Kohane, and Langer, R. (2008). Biodegradable Xylitol-based polymers. *Advanced materials*. 20, 1922-1927.

Bruggeman, J.P., Bruin, B.J., Bettinger, C.J., Langer, R. (2008). Biodegradable poly(polyol sebacate) polymers. *Biomaterials*. 29, 4726-4735.

Bruggeman, J.P., Bettinger, C.J., and Langer,R. (2010). Biodegradable xylitol- based elastomers: In vivo behavior and biocompatibility. *Journal of Biomedical Materials Research.* 95, 92-103.

Calandrelli, L., Immirzi, B., Malinconico, M., Volpe, M.G., Oliva, A., and Ragione, F.D. (2000). Preparation and characterization of composites based on biodegradable polymers for "in vivo" application. *Polymer*. 41, 8027-8033.

Cho,J., Boccaccini,A.R., and Shaffer, M.S.P. (2009). Ceramic matrix composites containing carbon nanotubes. *Material Science*. 44, 1934-1951.

Chung, D.D.L. (2000). Thermal analysis of carbon fiber polymer-matrix composites by electrical resistance measurement. *Themochimica Acta*. 364, 121-132.

Chung, E.J., Sugimoto, M.J., and Ameer, G.A. (2011). The role of hydroxyapatite in citric acid- based nanocomposites: Surface characteristics, degradation, and osteogenicity in vitro. *Biomaterials*.

Correlo, V.M., Boesel, L.L., Bhattacharya, M., Mano.F, J., Neves.M, N., and Reis, R.L. (2005). Hydroxyapatite reinforced chitosan and polyester blends for biomedical applications. *Macromolecular Materials and Engineering*. 290, 1157-1165.

Correlo, V.M., Pinho, E.D., Pashkuleva, I., Bhattacharya, M., Neves, N.M., and Reis, L.R. (2007). Water absorption and degradation characteristics of chitosan-

based polyesters and hydroxyapatite composites. *Macromolecular Bioscience.* 7, 354–363.

Djordjevic, I., Choudhury, N.R., K.D, Naba., Kumar, S. (2010). Poly(octanediol- co- (citric acid)- co- (sebacic acid) elastomers: novel bio- elastomers for tissue engineering. *Society of Chemical Industry*. 60, 333-343.

Elkayar, A., Elshazly, Y., and Asaad, M. (2009). Properties of hydroxyapatite from bovine teeth. *Bone and Tissue Regeneration Insight.* 2, 31-36.

Hasegawa, S., Ishii., Tamura, J., Furukawa, T., Neo, M., Matsusue, Y., Shikinami, Y., Okuno, M., and Nakamura, T. (2006). A 5- 7 year in vivo study of high-strength hydroxyapatite/ poly(L- lactide) composite rods for the internal fixation of bone fractures. *Biomaterials*. 27, 1327-1332.

Kharkwal.H., Bala,K., and Katare, D.P. (2011). Biodegradable polymers, role in enhancing bioavailability of drug. *Biomedical and Pharmaceutical Sciences*. 5, 01-11.

Kordestani, S.S., Hanna, S., and Windle, A.H. (2000). The role of crystallinity in the deformation of main-chain liquid-crystalline copolyesters. *Polymer.* 41, 6616-6626.

Lee, L.Y., Wu, S.C., Fu, S.S., Zeng, S.Y., Leong, W.S., and Tan, L.P. (2009). Biodegradable elastomer for soft tissue engineering. *European Polymer Journal.* 45, 3249–3256.

Lei, L., Li, L., Zhang, L., Chen, C., and Tian, W. (2009). Structure and performance of nano- hydroxyapatite filled biodegradable poly(1,2- propanediol- sebacate-citrate) elastomers. *Polymer degradation and stability*. 94, 1494-1502.

Liang, S.L., Cook, W.D., Thouas, G.A., and Chen, Q.Z. (2010). The mechanical characteristics and in vitro biocompatibility of poly(glycerol sebacate)-bioglass elastomeric composites. *Biomaterials.* 31, 8516-8529.

Liu, Q., Jiang, L., Shi, R., and Zhang, L. (2012). Synthesis, preparation, in vitro degradation, and application of novel degradable bioelastomers. *Polymer sciences*. 37, 715-765.

Liu, Q., Wu, J., Tan, T., Zhang, L., Chen, D., and Tian, W. (2009). Preparation, properties and cytotoxicity evaluation of a biodegradable polyester elastomer composite. *Polymer degradation and stability*. 94, 1427-1435

Migneco, F., Huang, Y.C., Birla, R.K., and Hollister, S.J. (2009). Poly(glycerol-dodecanoate), a biodegradable polyester for medical devices and tissue engineering scaffolds. *Biomaterials.* 30, 6479-6484.

Mukhodpadhyay, S.K., Basu, D., and Banerjee, A. (1997). DSC, FTIR and TGA studies of vinyl acetate-2-ethylhexyl acrylate copolymer. *Journal of Applied Polymer Science*. 65, 2075-2080.

Pang, Y.X., and Bao, X. (2003). Influence of temperature, ripening time and calcinations on the morphology and crystallinity of hydroxyapatite nanoparticles. *Journal of European Ceramic Society*. 23, 1697-1704.

Pasupuleti, S., and Madras, G. (2011). Synthesis and degradation of sorbitol-based polymers. *Journal of Applied Polymer Science*. 121, 2861-2869.

Petricca, S.E., Marra, K.G., Kumta, P.N. (2006). Chemical synthesis of poly(lactic-co-glycolic acid)/ hydroxyapatite composites for orthopaedic applications. *Biomaterials.* 2, 277-286.

Qiu, H., Yang, J., Kodali, P., Koh, J., A.A, G. (2006). A citric acid- based hydroxyapatite composite for orthopedic implants. *Biomaterials.* 27, 5845-5854.

Serrano, M.C., Chung, E.J., and Ameer, G.A. (2010). Advances and applications of biodegradable elastomers in regenerative medicine. *Advance Functional Materials.* 20, 192-208.

Vaidya, U.R., and Nadkarni, M. (1987). Unsaturated polyester from PET waste: Kinetics of polycondensation. *Journal of Applied Polymer Science.* 34, 235-245.

Velazco.C, L.1., Nonell, J.M., Valdes, S.S., and Valle, F.R. (2009). Morphology and osteogenetic characteristics of polyamide/ nanohydroxyapatite biocomposites. *Polymer Bulletin*. 62, 99-110.

Wang, Y., A. A, G., Sheppard, B.J., and Langer, R. (2002). A tough biodegradable elastomers. *Biomaterials.* 20, 602-606.

Weng.H, T., Wahit, M.U., Kadir.A, M.R., and Wong, T.W. (2013). Mechanical and thermal properties of biodegradable hydroxyapatite/ poly(sorbitol malate) composites. *Journal of Science and Technology*. 35 (1), 57-61.

Wu, Y., Shi, R., Chen, D., Zhang, L., and Tian, W. (2010). Nanosilica filled poly(glycerol-sebacate-citrate) elastomers with improved mechanical properties, adjustable degradability, and better biocompatibility. *Applied polymer science*. 123, 1612-1620.

Xie, R., Fu, H., Ji, X., Yao, J. (2002). Characterization of the size- dependent optical properties of p- terphenyl nanocrystals. *Photochemistry and Photobiology.* 147,31-37

Xu, W., Liang, G., Wang, W., Tang, S., He, P., and Pan, W.P. (2003). PP-PP-g-MAH Nanocomposites. I. Intercalation. *Behavior and Microstructure.* 88, 3225-3231.