

**THE STUDY OF USING NATURAL HYDROXYAPATITE  
AS A FILLER FOR POLY(LACTIC ACID) COMPOSITES**

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**A Thesis Submitted in Partial Fulfillment of Requirements for the  
Degree of Master of Engineering in Polymer Engineering  
Suranaree University of Technology**

**Academic year 2009**

การศึกษาการใช้ไฮดรอกซีอะปาไทต์ธรรมชาติเพื่อเป็นสารตัวเติม  
ในพอลิแลกติกแอซิดคอมโพสิต

สุริยัน รักแม่

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต  
สาขาวิชาวิศวกรรมพอลิเมอร์  
มหาวิทยาลัยเทคโนโลยีสุรนารี  
ปีการศึกษา 2552

**THE STUDY OF USING NATURAL HYDROXYAPATITE AS  
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Suranaree University of Technology has approved this thesis submitted in partial fulfillments of the requirement for a Master's Degree.

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สุริยัน รักแม่ : การศึกษาการใช้ไฮดรอกซีอะปาไทด์ธรรมชาติเพื่อเป็นสารตัวเติมใน  
พอลิแลกติกแอซิดคอมโพสิต (THE STUDY OF USING NATURAL  
HYDROXYAPATITE AS A FILLER FOR POLY(LACTIC ACID) COMPOSITES)  
อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.นิธินาถ สุภกาญจน์, 139 หน้า.

วิทยานิพนธ์นี้ศึกษาความเป็นไปได้ในการนำไฮดรอกซีอะปาไทด์ธรรมชาติมาใช้เป็นสาร  
ตัวเติมในพอลิแลกติกแอซิดคอมโพสิต โดยไฮดรอกซีอะปาไทด์ธรรมชาติที่ใช้ในงานวิจัยนี้ได้มา  
จากกระดูกวัวที่ผ่านการเผาและบด เมื่อนำผงกระดูกที่ได้มาวิเคราะห์ทางสัณฐานวิทยาด้วยกล้อง  
จุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) วิเคราะห์สารประกอบและความเป็นผลึกด้วยเครื่องวัด  
การเลี้ยวเบนของรังสีเอกซ์ (XRD) และวิเคราะห์หมู่ฟังก์ชันเครื่องอินฟราเรดสเปกโตรสโคปี  
(FTIR) แล้วพบว่าผงกระดูกวัวที่ผ่านการเผาแล้วจะได้ผลึกของไฮดรอกซีอะปาไทด์ที่มีหมู่  
คาร์บอกเนตเป็นส่วนประกอบและเกาะกลุ่มกันแน่น

พอลิเมอร์คอมโพสิตระหว่างพอลิแลกติกแอซิดกับผงไฮดรอกซีอะปาไทด์ถูกเตรียมขึ้นโดย  
วิธีผสมที่ต่างกันสองวิธีเพื่อเลือกวิธีการผสมที่ดีที่สุดในการผลิตชิ้นงานพอลิแลกติกแอซิด  
คอมโพสิต ซึ่งวิธีแรกคือการใช้สารละลายคลอโรฟอร์มละลายพอลิแลกติกแอซิดก่อนที่จะผสมผง  
ไฮดรอกซีอะปาไทด์ลงไป (solution-mixing technique) และวิธีที่สองคือการใช้ความร้อนเพื่อหลอม  
พอลิแลกติกแอซิดก่อนที่จะผสมผงไฮดรอกซีอะปาไทด์ลงไป (melt-mixing technique) ซึ่งจากผล  
การตรวจสอบสมบัติทางกลและสมบัติทางความร้อนของพอลิแลกติกแอซิดคอมโพสิตที่เตรียมจาก  
กระบวนการผสมทั้งสองแบบ แสดงให้เห็นว่าคอมโพสิตที่ผ่านกระบวนการผสมแบบหลอมจะมี  
การกระจายตัวของผงไฮดรอกซีอะปาไทด์ สมบัติทางกล และอุณหภูมิการสลายตัวที่สูงกว่า  
กระบวนการผสมโดยใช้ตัวทำละลายคลอโรฟอร์ม ดังนั้นในการวิจัยนี้จึงเลือกวิธีการผสมแบบที่ใช้  
ความร้อนเพื่อหลอมพอลิแลกติกแอซิดก่อนที่จะผสมผงไฮดรอกซีอะปาไทด์ลงไปเพื่อใช้ในการ  
เตรียมคอมโพสิต แต่อย่างไรก็ตามคอมโพสิตที่ผ่านการผสมด้วยวิธีการผสมแบบนี้ยังคงมีสมบัติ  
บางประการที่จำเป็นต้องได้รับการปรับปรุงให้ดีขึ้นคือความเข้ากันได้ระหว่างไฮดรอกซีอะปาไทด์  
กับพอลิแลกติกแอซิดรวมถึงการสลายตัวของพอลิแลกติกแอซิดที่เกิดขึ้นระหว่างการเตรียม  
คอมโพสิต

การปรับปรุงพื้นผิวของไฮดรอกซีอะปาไทด์เพื่อเพิ่มความเข้ากันได้ระหว่างไฮดรอกซี  
อะปาไทด์กับพอลิแลกติกแอซิดทำได้โดยใช้สารคู่ควบไซเลน (silane coupling agents) 2 ชนิด คือ  
3-อะมิโนโพรพิลไตรเอทอกซีไซเลน (3-aminopropyltriethoxysilane (APES)) และ 3-เมทาครีลออกซี  
โพรพิล ไตรเมทอกซีไซเลน (3-methacryloxypropyltrimethoxysilane (MPTS)) หลังจากปรับปรุง

พื้นผิวของไฮดรอกซีอะปาไทต์ด้วยสารคู่ควบไซเลนและนำมาตรวจสอบด้วยเครื่อง FTIR และเครื่องเอกซเรย์ฟลูออเรสเซนซ์แบบกระจายพลังงาน (EDXRF) พบว่ามีสารคู่ควบไซเลนถูกดูดซับอยู่บนผิวของไฮดรอกซีอะปาไทต์ นอกจากนี้ยังพบว่ากลุ่มของไฮดรอกซีอะปาไทต์ที่ผ่านการปรับปรุงพื้นผิวแล้วมีขนาดลดลง ผลการศึกษาทางสัณฐานวิทยาหลังนำไฮดรอกซีอะปาไทต์ที่ผ่านการปรับปรุงพื้นผิวแล้วมาผสมกับพอลิแลคติกแอซิด พบว่าพื้นผิวของไฮดรอกซีอะปาไทต์สามารถเข้ากันได้ดีขึ้นกับเนื้อของพอลิแลคติกแอซิด รวมทั้งมีการกระจายตัวของไฮดรอกซีอะปาไทต์ในเนื้อของพอลิแลคติกแอซิดที่ดีขึ้น ด้วยเหตุนี้สมบัติทางกลและอุณหภูมิจึงมีการสลายตัวของคอมโพสิตจึงมีค่าสูงขึ้น นอกจากนี้เมื่อทดสอบด้วยเครื่องโครมาโทกราฟีแบบเจลเลือกผ่าน (GPC) ยังพบว่าการปรับปรุงพื้นผิวของไฮดรอกซีอะปาไทต์ด้วยสารคู่ควบไซเลนสามารถลดการสลายตัวของโมเลกุลพอลิแลคติกแอซิดระหว่างเตรียมคอมโพสิตได้ด้วย

การศึกษการสลายตัวของพอลิแลคติกแอซิดคอมโพสิตในสภาพแวดล้อมจำลอง โดยการแช่คอมโพสิตในสารละลายบัฟเฟอร์ของฟอสเฟต พบว่าคอมโพสิตที่ผสมด้วยไฮดรอกซีอะปาไทต์ที่ผ่านการปรับปรุงพื้นผิวมีการเปลี่ยนแปลงของความเป็นกรด-ด่างของสารละลายบัฟเฟอร์ของฟอสเฟตในระหว่างการแช่ ขนาดและสัณฐานวิทยาของชิ้นทดสอบ รวมทั้งโมเลกุลของพอลิแลคติกแอซิดน้อยกว่าคอมโพสิตที่ผสมด้วยไฮดรอกซีอะปาไทต์ที่ไม่ผ่านการปรับปรุงพื้นผิว โดยเป็นผลมาจากการยึดเหนี่ยวระหว่างไฮดรอกซีอะปาไทต์และเนื้อพอลิแลคติกแอซิดที่ดีขึ้นทำให้สารละลายบัฟเฟอร์ของฟอสเฟตมีโอกาสที่จะซึมเข้าไปในเนื้อของคอมโพสิตน้อยได้ลดลง ส่งผลให้สายโซ่ของพอลิแลคติกแอซิดเกิดการสลายตัวลดลง โดยสามารถทดสอบได้ด้วยเครื่อง GPC นอกจากการทดสอบการสลายตัวแล้วการศึกษาความไวทางชีวภาพ (Bioactivity) ของคอมโพสิตในสารละลายเทียมของร่างกายมนุษย์ (simulated body fluid (SBF)) ยังแสดงให้เห็นว่าการใช้ไฮดรอกซีอะปาไทต์จากกระดูกวัวสามารถเหนี่ยวนำให้เกิดการตกตะกอนของแคลเซียมฟอสเฟตบนคอมโพสิตได้และมีจำนวนมากขึ้นตามระยะเวลาในการแช่ อีกทั้งเมื่อทดสอบความเป็นพิษของสารละลายที่สกัดจากคอมโพสิตกับเซลล์กระดูกของมนุษย์ ผลที่ได้ชี้ให้เห็นว่าสารสกัดจากคอมโพสิตที่ได้ไม่มีความเป็นพิษต่อเซลล์กระดูกของมนุษย์ ณ สภาวะการทดลองที่กำหนด

สาขาวิชา วิศวกรรมพอลิเมอร์

ปีการศึกษา 2552

ลายมือชื่อนักศึกษา \_\_\_\_\_

ลายมือชื่ออาจารย์ที่ปรึกษา \_\_\_\_\_

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม \_\_\_\_\_

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม \_\_\_\_\_

SURIYAN RAKMAE : THE STUDY OF USING NATURAL  
HYDROXYAPATITE AS A FILLER FOR POLY(LACTIC ACID)  
COMPOSITES. THESIS ADVISOR : ASST. PROF. NITINAT  
SUPPAKARN, Ph.D., 139 PP.

POLY(LACTIC ACID)/ HYDROXYAPATITE/ COMPOSITE/ SILANE  
COUPLING AGENT/ BOVINE BONE

In this thesis, hydroxyapatite (HA) powder was produced from bovine bones in order to use as a filler for poly(lactic acid) (PLA) composites. Scanning electron microscope (SEM), X-ray diffractometer (XRD) and Fourier transform infrared spectrometer (FTIR) were used to characterize the obtained powder. SEM micrographs, XRD pattern, and FTIR spectrum of calcined bovine bone powder revealed that the obtained powder was in a form of crystalline carbonated HA, and highly agglomerated. So, the calcined bovine bone powder was called u-HA in this study. u-HA/PLA composites at various contents of HA were prepared by either melt-mixing or solution-mixing techniques. The u-HA/PLA composites prepared by melt-mixing exhibited the more homogeneous distribution of u-HA in PLA matrix as compared with the composites prepared by solution-mixing technique. In comparison, tensile modulus, tensile strength, and impact strength of the melt-mixed composites were higher than those of the solution-mixed composites. Moreover, decomposition temperatures of the melt-mixed composites were higher than those of the solution-mixed composites. Nonetheless, average molecular weights of PLA in the solution mixed composites, as confirmed by GPC, were significantly higher than those in the

melt-mixed composites. The surface of HA powder was modified with either 3-aminopropyltriethoxysilane (APES) or 3-methacryloxypropyltrimethoxysilane (MPTS). FTIR and EDXRF results confirmed the appearance of APES and MPTS on the HA surfaces. SEM micrographs of silane-treated HA/PLA composites revealed that modification of HA with APES or MPTS eased distribution of HA powder in PLA matrix and enhanced interfacial adhesion between both phases. Based on the results, the mechanical properties of silane-treated HA/PLA composites were better than those of untreated HA/PLA composites. Moreover, TGA and GPC results showed that the incorporation of silane-treated HA into the PLA matrix significantly increased thermal stability of the composite and decreased the thermal degradation of PLA chains. Additionally, *in vitro* degradation behaviors of untreated HA/PLA and silane-treated HA/PLA composites were also analyzed. The results showed that the stronger interfacial bonding between silane-treated HA and PLA matrix significantly delayed the *in vitro* degradation rate of the PLA, after immersing in PBS. Moreover, the results of bioactive study showed that the incorporation of u-HA into the PLA matrix significantly induced the formation of calcium phosphate compounds on the composite surface as evaluated by means of SEM, EDX, FTIR and XRD. In addition, *in vitro* cytotoxicity tests indicated that the extracts from all HA/PLA composites had no toxicity to human osteoblast cell.

School of Polymer Engineering

Academic Year 2009

Student's Signature\_\_\_\_\_

Advisor's Signature\_\_\_\_\_

Co-advisor's Signature\_\_\_\_\_

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## ACKNOWLEDGEMENTS

I wish to acknowledge the financial supports from Suranaree University of Technology and National Center of Excellence for Petroleum, Petrochemical and Advance Materials.

I am deeply indebted to my thesis advisor, Asst. Prof. Dr. Nitinat Suppakarn, who always gives me a kind suggestions and supports me throughout the period of the study. Furthermore, the grateful thanks and appreciation are given to the thesis co-advisor, Asst. Prof. Dr. Yupaporn Ruksakulpiwat and Asst. Prof. Dr. Wimonlak Sutapun for their valuable suggestions.

My thanks go to Asst. Prof. Dr. Utai Meekum, Assoc. Prof. Dr. Jatuporn Wittayakun, Asst. Prof. Dr. Visit Vao-soongnern, Asst. Prof. Dr. Sirirat Tubsungneon Rattanachan for their valuable suggestion and guidance given as committee member.

I would like to express my a appreciation to the faculty and staff members of the School of Polymer Engineering and the Center for Scientific and Technological Equipment of Suranaree University of Technology. Special thanks are extended to Mr. Chalermpan Keawkumay and all friends for their help, support and encouragement.

Last but not least, I would like to thank my parents, Mr. Sittisak and Mrs. Jutarut Rakmae, and my younger brother, Mr. Suriyong Rakmae, who give me a valuable life and protect my mind from an evil with their unconditional loves.

Suriyan Rakmae

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## SYMBOLS AND ABBREVIATIONS

%	=	Percent
°	=	Degree
°C	=	Degree Celsius
μm	=	Micrometer
APES	=	3-aminopropyltriethoxysilane
cm	=	Centimeter
EDX	=	Energy dispersive X-Ray spectroscopy
EDXRF	=	Energy dispersive X-Ray fluorescence spectroscopy
eV	=	Electron volt
FTIR	=	Fourier transform infrared spectrometer
g	=	Gram
GPa	=	Gigapascal
h	=	Hour
HA	=	Hydroxyapatite
J	=	Joule
keV	=	Kilo electron volt
kV	=	Kilo volt
m <sup>2</sup>	=	Square meter
m <sup>3</sup>	=	Cubic meter
mg	=	Milligram
min	=	Minute

**SYMBOLS AND ABBREVIATIONS (Continued)**

mm	=	Millimeter
mol	=	Mole
M	=	Molar
$\bar{M}_n$	=	Average molecular weight by number
$\bar{M}_w$	=	Average molecular weight by weight
MPa	=	Megapascal
MPTS	=	3-metacryloxypropyltrimetoxysilane
N	=	Normal
P	=	Pascal
PBS	=	Phosphate-buffered solution
PLA	=	Poly(lactic acid)
psi	=	Pound per square inch
rpm	=	Revolution per minute
SBF	=	Simulated body fluid
SEM	=	Scanning electron microscope
wt%	=	Percent by weight
vol.%	=	Percent by volume
XRD	=	X-ray diffractometer

# CHAPTER I

## INTRODUCTION

### 1.1 Background

Hydroxyapatite [HA:  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] is a form of calcium phosphate which is similar to a major inorganic component in hard tissues of human body. HA has been considered as a biomaterial due to its biocompatibility and osteoconductivity (Chandrasekhar, Shaw, and Mei, 2005; Hasegawa *et al.*, 2006). Therefore, HA has been synthesized and used in various medical applications such as fillers, spacers and bone graft substitutes in orthopedic, maxillofacial applications and bone replacement. HA can be synthetically prepared or derived from natural sources, *e.g.* coral, bovine bone, swine bone. (Chandrasekhar *et al.*, 2005; Shikinami and Okuno, 2001; Ruksudjarit, Pengpat, Rujijanagul, and Tunkasiri, 2008). However, the major drawbacks of using HA in a form of single component, *e.g.* dense HA, porous HA, are its brittleness and the difficulty of processing. These problems have been solved by using HA as a filler for polymer matrices.

The synthetic HA powders are frequently used in biomaterial studies as a reinforcing filler for polymer composites. However, the synthetic HA is quite expensive. Also, its chemical composition and properties extremely depend on the preparation condition. In recent years, several attempts have been done to produce HA from natural sources for biomedical applications since the natural HA is less expensive and more compatible to human hard tissues (Chandrasekhar *et al.*, 2005; Shikinami *et al.*, 2001; Ruksudjarit *et al.*, 2008). In Thailand, there are high volumes

of bovine bone as a livestock waste. Normally, the bone is used in fertilizer, animal foods, and making porcelain (*i.e.* bone china). Using the bovine bone as a raw material for producing HA not only increase added value of the bovine bone but also reduce volumes of the livestock waste. As another approach to utilize bovine bone, this research aims to prepare bovine bone based HA powder and further use the powder as a filler for a polymer composite.

HA/polymer composites have become an interesting topic for many research groups because of the flexibility in tailoring properties of the composites, *e.g.* by varying types of polymer matrix. For an example, HA reinforced HDPE composite has been developed and successfully used in orbital surgery as a hard tissue replacement material (Wang, Deb, and Bonfield, 2000). Nevertheless, some orthopaedic implants such as bone screw, bone plate, *etc.* need temporary materials that stay intact until the healing process in the body is complete. After that, the materials must be degraded by hydrolytic or enzymatic action and excreted from the body as waste products. Thus, HA/bioresorbable polymer composites become important representatives of those materials since they combine the osteoconductivity and the bone bonding ability of HA with the resorbability of the biodegradable polymer. In addition, HA/bioresorbable polymer composites are also easy to process into required shapes (Zhang *et al.*, 2005; Ooi, Hamdi, and Ramesh, 2007; Hasegawa *et al.*, 2006; Deng, Hao, and Wang, 2001).

Many types of bioresorbable polymers have been developed and used in medical applications such as poly(lactic acid) (PLA), poly(3-hydroxybutyrate) (PHB), poly(glycolic acid) (PGA), poly(lactide-co-glycolide) (PLGA), poly(anhydrides), collagen, chitosan and poly(hydroxyalkanoates). Among these polymers, poly(lactic

acid) is a good candidate because it is biodegradable and essentially yields nontoxic byproduct after degradation (Russias *et al.*, 2006). From various research works, it is well known that degradation of pure PLA implanted *in vivo* produces intermediate acidic products, such as lactic acid, via hydrolysis of the polyester bonds. Subsequently, these acidic products are processed through normal metabolic pathways and are eliminated from the body as carbon dioxide (Suganuma and Alexander, 1993; Agrawal and Athanasiou, 1997; Horst, Robert, Suzanne, and Antonios, 1995). Hence, the composite between PLA and HA is a good alternative to be used as a biomaterial since it is combining bone-bonding potentials, strength and stiffness of HA with flexibility and bioresorbability of PLA. During healing process in human body, the polymeric part is metabolized and excreted while the HA is retained in the body and being a supporter for the growth of osteoblast cell (Furukawa *et al.*, 2000).

However, PLA matrix has methyl units ( $-\text{CH}_3$ ) as side groups, therefore, PLA surface is hydrophobic in nature. This is in contrast to HA surface, which exhibits hydrophilic character. Because of the polarity difference between PLA and HA surfaces, the agglomeration of HA is observed in a HA/PLA composite system. Also, the composite failures mainly occur at the interface of HA and polymer matrix. These lead to poor mechanical properties of HA/PLA composites (Ignjatovic, Suljovrujic, Simendic, Krakovsky, and Uskokovic, 2001; Chandrasekhar *et al.*, 2005).

Mechanical properties of the composite are the important factors that relate to applications of the material. To use a HA/PLA composite as a biomaterial, its mechanical properties need to be adjusted to be close to those of the replaced tissue, *e.g.* natural cortical bone has modulus of elasticity of 3-30 GPa. (Kasuga, Ota, Nogami, and Abe, 2001). To achieve the requirements imposed on the mechanical

properties of the composite, a good distribution of HA in PLA matrix and good adhesion between HA and PLA must be taken place. So, a lacking of effective adhesion between the HA and the PLA matrix needed to be solved. Therefore, an improvement of the interfacial adhesion between HA and PLA matrix has become an important area of studies. To improve the interfacial adhesion between HA and PLA matrix, the hydroxyl group presenting on the HA surface can react with organic molecules such as silane coupling agents, zirconyl salt, poly acids, poly(ethylene glycol), isocyanate and dodecyl alcohol (Liu, Wijn, Groot, and Blitterswijk, 1998). Generally, treating HA surface with a silane coupling agent is an effective method to improve the interfacial adhesion between HA particles and polymer matrix (Arami *et al.*, 2008).

*In vitro* experiment is a primary method usually used to evaluate cytotoxicity and bioactivity performances of biomaterials. This method refers to the technique of performing a given experiment in a controlled environment outside of a living organism, for example in a test tube (Tsuji and Ikarashi, 2004). Furthermore, to study the simultaneous degradation of HA/PLA composites in human body, the experiment is designed by simulating the condition similar to human body. The degradation of the composites is studied by immersing the composites in a buffer solution having properties similar to human body fluid. Various buffered solutions, for example, phosphate-buffered solution (PBS), Hank's balance salt solution (HBSS), sodium citrate buffered solution can be used for an *in vitro* study. (Tsuji *et al.*, 2004; Verheyen *et al.*, 1993). The degradation of PLA is a complex process involving numerous variables, such as the crystallinity and molecular weight of PLA, processing conditions, shape and size of specimens, *etc.* Additionally, the

incorporation of an HA phase into the PLA matrix is an interesting factor that would affect *in vitro* degradation of HA/PLA composites (Navarro, Ginebra, Planell, Barrias, and Barbosa, 2005).

In this study, natural HA powder was produced from bovine bone by thermal treatment. The HA powder was treated with either 3-aminopropyltriethoxysilane (APES) or 3-methacryloxypropyltrimethoxysilane (MPTS). Then, natural HA powders with different surface modification were used to reinforce PLA. Effects of surface modification and filler content on morphological and mechanical properties of HA/PLA composites were examined. In addition, the degradation behavior of HA/PLA composites in buffer solution, *in vitro* testing, were investigated.

## **1.2 Research objectives**

The objectives of this study are as follows:

- (i) To investigate characteristics of HA powder prepared from bovine bone.
- (ii) To investigate effect of mixing process, melt-mixing and solution-mixing, on thermal properties, mechanical properties, morphological properties of natural HA/PLA composites.
- (iii) To investigate effects of natural HA content and natural HA surface modification on thermal properties, mechanical properties, morphological properties and degradation behavior of natural HA/PLA composites.

### 1.3 Scope and limitation of the study

In this study, natural HA powder was produced from bovine bone by thermal treatment. The obtained HA was separated into two groups, *i.e.* untreated HA and silane-treated HA. Surface modification of HA powder was done using either 3-aminopropyltriethoxysilane (APES) or 3-methacryloxypropyltrimethoxysilane (MPTS). The amount of silane used is 2.0% based on the weight of HA powder. Untreated HA and silane-treated HA were investigated by a Fourier transform infrared spectrometer (FTIR), an X-ray diffractometer (XRD), a scanning electron microscope (SEM) and a thermogravimetric analyzer (TGA).

HA/PLA composites at various contents of HA powder were compounded through either solution-mixing or melt-mixing technique using an internal mixer and then the composites specimens were prepared by compression molding. Effects of HA content and surface modification on mechanical, thermal and morphological properties of natural HA/PLA composites were determined. A universal testing machine and an impact testing machine were used to measure the mechanical properties of the HA/PLA composites. Tensile fracture surfaces of the HA/PLA composites were evaluated using a SEM.

*In vitro* degradation behavior of HA/PLA composites was determined. The composites were immersed in a phosphate buffer solution (PBS) and simulated body fluid (SBF) at 37°C for various time periods. The weight change and the thickness change of the composite specimens after immersing in PBS were measured. Also, the changes in pH of PBS solution were recorded. Additionally, molecular weight of PLA in neat PLA and HA/PLA composite before and after hydrolytic degradation in PBS were evaluated by a gel permeable chromatography (GPC). The surface of the

composites after immersing in SBF were characterized using SEM, EDX, XRD and FTIR.

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## CHAPTER II

### LITERATURE REVIEW

#### 2.1 Preparation of natural hydroxyapatite

In recent years, several researchers investigated possibilities of producing natural hydroxyapatite from bovine bone. Ooi *et al.* (2007) studied the properties of HA bioceramic produced by heat treatment of bovine bone. They have found that the nanocrystalline apatite was observed when the annealing temperature was above 700°C and up to 1000°C. However, annealing beyond 1000°C resulted in partial decomposition of the HA phase to form a minor phase of  $\beta$ -tricalciumdiphosphate ( $\beta$ -TCP). As the annealing temperature increased to 1200°C, the intensity of the XRD characteristic peaks of  $\beta$ -TCP gradually increased.

Yoganand, Selvarajan, Wu, and Xue (2009) prepared HA by heat treatment of bovine bone at 850°C for 5-6 h. The XRD spectrum indicated that the obtained product was hexagonal-phase HA. Then, the HA was powdered. SEM micrograph showed that HA powders were in highly irregular shapes, *e.g.* edges, angular, rounded, circular, dendritic, porous and fragmented morphologies. In addition, after heat treatment, a disease-causing agent was not observed. (Ozyegin, Oktar, Goller, Kayali, and Yazici, 2004).

Benmarouanea, Hansena, and Lodini (2004) studied effect of heat treatment on microstructure of bovine bone based HA. The neutron diffraction pattern indicated

that heating the bone at 625°C for 3 days did not change the orientation of HA crystallites, which still directed along the axis of the bone.

Ruksudjarit *et al.* (2008) used vibro-milling technique to produce HA nanopowders with high purity from bovine bone. The dried bone was calcined at 800°C for 3 h and ground by vibro-milling at various times. XRD patterns of all ground bone powders corresponded to that of pure HA phase. SEM micrographs of HA powders after vibro-milling time of 2 h showed nanoneedle-like shape of HA powders with diameter less than 100 nm.

Lorprayoon (1989) synthesized HA and TCP using bone ash as a starting material. The bone was calcined at 700°C for 8 h. TEM micrographs showed that the obtained bone ash was nano-scale particles. Then, the bone ash was dissolved and precipitated at various pH. The precipitated bone was heated to 1230-1280°C for 2-3 h. The results showed that TCP was obtained when the precipitation was at pH of 8.0-8.5 while the single phase of HA was precipitated at pH of 9.7-10, confirmed by XRD patterns.

Haberko *et al.* (2006) investigated characteristics of HA produced from pig bone at elevated temperatures. They have found that the pig bone started to decompose at the temperatures over 700 °C. As a result, calcium oxide and carbonate groups were partially removed from the system. Simultaneously, crystallite growth became intensive, specific surface area of the powder decreased and compacts of such powder started to shrink.

## 2.2 Surface treatment of hydroxyapatite by silanization

To enhance the integrity of HA/PLA composite, the HA can be surface treated with a coupling agent, such as organofunctional silanes, which in general effectively improves adhesion between HA and polymer matrix.

Dupraz, Wijn, Meer, and Groot (1996) modified surface of HA powder with several methoxysilane coupling agents (Table 2.1), *i.e.* vinyltriethoxysilane (VS), N-methylaminopropyltrimethoxysilane (TRIAMO), 3-methacryloxypropyltrimethoxy silane (MPTS), 3-aminopropyltriethoxysilane (APES) and N-(2-aminoethyl)-3-aminopropyltriethoxysilane (DAS). Low concentration of silane solution was prepared to retain the coupling agent as monomer or dimer. X-ray photoelectron (XPS) spectra of all HA samples revealed the presence of a few monolayers of thin silane films on HA powder. This indicated that silane coupling agents were able to chemically bond on the HA surface since a thin coating of silane film remained after washing the powder with water for several time. The stability of the silane coating film in a wet environment was measured by extracting the coated powders with water at 37°C for 5 days. XPS results showed that VS, MPS and DAS coatings were able to withstand a mild water extraction. This was due to silane-silane crosslinking prevented silane molecules from water extraction. In contrast, AMMO and TRIAMO coatings were completely removed.

**Table 2.1** Chemical formular of organofunctional silane coupling agents

Name	Chemical formular
VS	$\text{CH}_2 = \text{CH-Si-(OCH}_3)_3$
MPTS	$\text{CH}_2 = \text{C(CH}_3\text{)COOCH}_2\text{-CH}_2\text{-CH}_2\text{-Si-(OCH}_3)_3$
APES	$\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{- Si-(OCH}_2\text{CH}_3)_3$
TRIAMO	$\text{CH}_2\text{-NH-CH}_2\text{-CH}_2\text{-CH}_2\text{- Si-(OCH}_3)_3$
DAS	$\text{NH}_2\text{-CH}_2\text{-CH}_2\text{- NH-CH}_2\text{-CH}_2\text{-CH}_2\text{- Si-(OCH}_3)_3$

Shinzato, Nakamura, Kokubo, and Kitamura (2001) studied effect of the 3-methacryloxypropyltrimethoxysilane content on bending strength of poly(methyl methacrylate) (PMMA)/bioactive glass bead (GCB) composites. The glass beads were treated with different amounts of silane coupling agent, *i.e.* 0 (untreated GCB), 0.1, 0.2, 0.5, and 1.0wt% of the glass beads (silane-treated GCB). The bending strength of the PMMA/GCB composites before and after soaking in water at 75°C for 5 days was measured. At initial condition PMMA/silane-treated GCB composites had significantly higher bending strengths than PMMA/untreated GCB composites, but there were no significant differences between various types of PMMA/silane-treated GCB composite. Moreover, after soaking in water and by treating GCB with 0.2wt% of silane, the obtained PMMA/silane-treated GCB composite had the highest bending strength. The result implied that the siloxane bonds could make the theoretical monolayer of the silane coupling agent at 0.2wt% since the monolayer silane was believed to bind strongly to glass beads with PMMA. When the amount of the silane coupling agent was less than 0.2wt%, the number of siloxane bonds decreased leading to the less interfacial interaction between the two phases. When the amount of the

silane coupling agent was greater than 0.2wt%, the layer of the coupling agent became thicker. The formation of weak hydrogen bonds in thick coupling agent layer was the cause of the weak bending strength of PMMA/silane-treated GCB composites.

Zhang *et al.* (2005) investigated interfacial adhesion between silane-treated HA and PLA matrix. The HA powders were treated with 3-aminopropyltriethoxysilane, 3-(2,3-cyclopropoxy)-propyltrimethoxysilane, 3-methacryloxypropyltrimethoxysilane. Thereafter, various silane-treated HA/PLA composites at 20vol.% HA were produced. IR spectrum showed the existence of silane molecules on surface of the modified HA. Interfacial adhesion between the HA and the polymer matrix can be improved by silane coupling agent since PLA bond chemically to silane coupling agents on HA surfaces. Moreover, SEM micrographs of the silane-treated HA/PLA composites indicated that the silane-treated HA particles were homogeneously dispersed in PLA matrix. Therefore, bending strength of silane-treated HA/PLA composites were higher than that of untreated HA/PLA composite.

Wen *et al.* (2008) developed a method to prepare HA/silicone rubber (SR) composite. HA was modified by vinyltriethoxysilane, poly (methylvinylsiloxane) (PMVS) and 2,5-bis(tertbutylperoxy)-2,5-dimethylhexane (DBPMH). SEM micrographs of silane-treated HA clearly showed that the HA powders uniformly dispersed within SR matrix and closely connected with the matrices with obscure interfaces.

Daglilar and Erkan (2007) modified surface of HA derived from bovine bone (BHA), synthetic HA and  $\beta$ -TCP with 3-methacryloxypropyltrimethoxysilane. Then, different amounts of the silane-treated filler were added to PMMA matrix. Compressive and three point bending properties of the composites showed that the

amount of ceramic filler was more effective in enhancing their mechanical properties than the silanation treatment itself.

Wang and Bonfield (2001) studied effect of adhesion between HA particles and HDPE matrix on mechanical properties of composites. HA surface were treated with 3-trimethoxysilylpropylmethacrylate before blending with HDPE. EDX pattern of the silane-treated HA showed bands of the carboxyl and SiO functional groups. The result indicated that the silane coupling agent was grafted on HA surface. The improvement in tensile strength and fracture strain of treated HA/HDPE composite were observed. This was due to the homogeneously distribution of silane-treated HA in polyethylene. Moreover, SEM micrograph of HA/HDPE composites showed HDPE fibril network formation on a HA particle.

Daglilar, Erkan, Gunduz, Ozyegin, Salman, Agathopoulos, and Oktar (2007) studied water adsorption behavior of PMMA reinforced with silane-treated bovine bone based HA powder. The HA powder was treated with 0.2wt% of 3-methacryloxypropyltrimethoxysilane. The experimental results showed that the addition of untreated HA powder in the polymer matrix enhanced water absorption of the composites. In contrast, the water absorption of silane-treated HA/PMMA composite was decreased.

Furuzono, Sonoda, and Tanaka (2000) developed a method for HA/silicone composite preparation. HA microparticles were surface modified by 3-aminopropyltriethoxysilane before adding into 10 ml of pure water. Subsequently, acrylic acid-grafted silicone sheet was immersed in the solution. FTIR spectrum of silane-treated HA particles confirmed that the silane coupling agent was covalently

bonded to the HA particles due to the appearance of the SiO stretching vibration at  $1043\text{ cm}^{-1}$ .

### **2.3 Preparation of HA/PLA composites**

Various methods have been successfully used to produce HA/PLA composite biomaterials, such as melt-mixing of HA and PLA, polymerization of lactide monomer onto HA particles, forging and hot pressing to obtain three-dimensional blocks of the composite. Nowadays, the composite specimens with mechanical characteristics close to the natural bone tissue are obtained only by forging and hot pressing methods.

Ingjatovic, Suljovrujic, Simendic, Krakovsky, and Uskokovic (2004) prepared specimens of HA/poly(L-lactide) (PLLA) composites using two-step procedure. Firstly, HA powder was prepared by precipitation of calcium nitrate and ammonium phosphate in an alkaline medium. PLLA was dissolved in chloroform, then, HA powder was added and then obtained mixture was vacuum evaporated. In the second step, the obtained powders were compacted by hot pressing to get specimens. The researchers have found that the hot pressing procedure was possible to change the porosity of HA/PLLA composites. The porosity of HA/PLLA composites were decreased by prolonging the hot pressing time. Moreover, SEM micrographs of HA/PLLA composites showed HA particles were finely distributed in the polymer matrix. In addition, hot pressing causes a decrease in crystallinity of PLLA in the HA/PLLA composite biomaterial as proved by wide-angle X-ray scattering (WAXS) and DSC analysis. The WAXS results indicate a significant decrease in PLLA crystallinity during hot pressing. DSC analyses confirmed these changes in

crystallinity degree and also indicated changes in glass transition, cold crystallization, and melting temperature of the PLLA polymer. In the given time interval of hot pressing from 0 to 60 min, insignificant qualitative changes in the HA and the PLLA phase were recorded by IR spectroscopy.

Shikinami *et al.* (1999) prepared HA/PLLA composites by fogging method. The small HA/PLLA granules of different amounts of HA (*i.e.* 20, 30, 40, and 50wt% of HA particles within a PLLA matrix) were collected by adding ethanol dropwise to a PLLA-HA/dichloromethane solution. The granules were then extruded to make a thick billet. Thereafter, this billet was forged at 103°C into a thin billet without fibrillation and cut on a lathe into required shapes. The mechanical testing results, *i.e.* bending, tensile, compression and impact properties, indicated that the forged HA/PLLA composites generally have much higher mechanical strength compared with the HA/PLLA composites produced from other techniques. The devices made of forged composites have a crystalline morphology of complex (multi axial) orientation. So, they showed extremely high strength uniformly in all directions.

## **2.4 Properties of HA/PLA composites**

### **2.4.1 Thermal properties**

Ignjatovic *et al.* (2004) investigated thermal behavior of hot pressed HA/PLLA composites. The HA/PLLA composites were hot pressed for different pressing times, *i.e.* 5, 15, 30, 45, and 60 min. DSC thermograms and wide-angle X-ray scattering (WAXS) patterns of the HA/PLLA composites prepared at various hot pressing time revealed the decrease in glass transition temperature ( $T_g$ ), melting temperature ( $T_m$ ) and percent of crystallinity of PLLA component with prolonging hot

pressing time. The observations were due to the rearrangement of PLLA chains on the HA surfaces during the composite preparation. In addition, GPC results confirmed that the polymer chains were broken due to thermo-mechanical factors. TGA results showed that thermal stability of the obtained composites decreased with increasing hot pressing time.

Deng, Sui, Zhao, Cheng, and Yang (2007) studied the preparation of HA/PLLA hybrid nanofibrous scaffolds. PLLA and HA/PLLA scaffolds were fabricated using electrospinning technique. From DSC results, the HA/PLLA scaffolds exhibited lower melting enthalpies (34.8 J/g) than the PLLA scaffolds (44.6 J/g). The decrease in melting enthalpy of the HA/PLLA scaffold indicated that hybrid scaffolds contained microcrystals between PLLA (Shikinami *et al.*, 2001).

Zheng, Zhou, Li, and Weng (2007) studied shape memory properties of poly(D,L-lactide) (PDLLA)/HA composites. They have found that HA content played a very important role in changing of  $T_g$  of the composites. DSC results revealed that the  $T_g$  of neat PDLLA and HA/PDLLA composites at weight ratios of HA of 25, 30, 45, 50% were 53.7, 55.6, 56.8, 57.2, 59.0°C, respectively. The  $T_g$  of the composite increased with increasing HA content may be due to the existing interfacial interaction of PDLLA and HA.

Gay, Arostegui, and Lemaitre (2008) determined the thermal characteristics of PLLA and HA/PLLA composites using DSC. Effect of HA content on crystallisation and melting temperatures of the composites were measured. In the second heating scan, glass transition, crystallisation and melting points of PLLA and HA/PLLA composite were observed. The experiment had two heating cycles. In the first step, the sample was heated from 25 to 200°C (increment rate of 15°C/min).

Then, it was quenched at a rate of  $-15^{\circ}\text{C}/\text{min}$  to  $25^{\circ}\text{C}$ . The second step, the quenched sample was run from  $25^{\circ}\text{C}$  to  $200^{\circ}\text{C}$ . The DSC results indicated that the glass transition of the composites increased slightly with HA content but there was no significant changes in the crystallization and the melting temperatures of the composites as compared with those of the neat PLA.

#### **2.4.2 Mechanical properties**

Chandrasekhar *et al.* (2005) synthesized porous HA/PLA scaffolds using a salt leaching technique. The compressive properties of HA/PLA composites were investigated as a function of HA content. The compression strength and the modulus of elasticity of the composites increased with increasing HA content. Additionally, the increases in compressive strength and elastic modulus of HA/PLA scaffolds with increasing HA content have also been observed in dense HA/PDLLA composites (Lin, Fang, Tseng, and Lee, 2007). In contrast, the bending strength of HA/PLA composites decreased with increasing HA content (Shikinami *et al.*, 1999). Thus, mechanical properties, such as strength and elastic modulus of PLA can be controlled by the amount of added HA particles in the composites.

Deng *et al.* (2001) studied the preparation and mechanical properties of nanocomposites of PDLLA with Ca-deficient HA nanocrystals (d-HA). Tensile testing results indicated that adding d-HA into the PLA matrix could significantly increase in the tensile modulus of d-HA/PLA composite. The yield stress of the composite slightly varied with d-HA loading. This is typically found when there is effective adhesion between the polymer matrix and the filler.

Kasuga, Ota, Nogami, and Abe (2000) studied the preparation and mechanical properties of PLA composites containing HA fibers (HAF). Tangent

moduli of elasticity were estimated using stress/strain curves obtained from the bending tests. The bending strength of HAF/PLA composite was insignificantly changed with the HAF content. On the other hand, the modulus of elasticity of the composites was improved with increasing HAF content. It was reported that the contents of HAF of 75wt% should be added into the PLA matrix in order to prepare the composites with modulus of elasticity close to that of natural bone.

Nejati, Firouzdor, Eslaminejad, and Bagheri (2009) studied effect of HA particle size on mechanical properties of HA/PLA composite. The mechanical properties of HA/PLA composites containing nano-scale HA were compared with those of PLA composites containing micro-scale HA. The compressive strength and the compressive modulus of the microcomposite and the nanocomposite scaffolds were higher than those of the pure PLLA scaffolds. The compressive strength of 50wt% nano-scale HA/PLA composite was significantly higher than that of the micro-scale HA/PLA composite. It may be attributed to the larger surface area of the nano-scale HA and the more uniform distribution of the nano-scale HA particles in PLLA matrix as compared with those of micro-scale HA particles. However, the compressive modulus of the microcomposites and the nanocomposite scaffolds were not statistically different.

### **2.4.3 Morphological properties**

Todo, Park, Arakawa, and Takenoshita (2006) studied the relationship between microstructure and fracture behavior of bioabsorbable HA/PLLA composites. Effects of particle size and particle shape of HA on fracture surface of HA/PLLA composites were determined by SEM. SEM micrographs of fracture surfaces of HA/PLLA composites showed that the HA with plate and micro-scale

shape exhibited relatively rough surfaces. This was due to the ductile deformation of the PLLA matrix during the debonding of the HA/matrix interfaces. On the contrary, the HA with spherical and nano-scale shape exhibited smooth surfaces that corresponded to brittle fracture behavior due to the nano-scale interaction between the PLLA fibrils and the HA particles.

Takayama, Todo, and Takano (2008) investigated effect of HA particle size on the mechanical properties and fracture energy of HA/PLLA composites. SEM of cryofracture surfaces of HA/PLLA composites showed the good dispersion of micro-scale HA particles in PLLA matrix. On the other hand, SEM micrograph of PLA composites containing nano-scale HA exhibited more agglomeration of HA particles with various size of agglomerated particles. This indicated that the nano-scale HA particles easily agglomerated compared with the micro-scale HA particles. The existence of these agglomerations resulted in the reduction of the bending strength and the fracture energy because these are easily fractured due to weak bonding between particles and such localized fracture becomes the initiation of fast global fracture of the composite material. This mechanism was supported by the brittle fracture behavior. The micrograph of nano-scale HA/PLLA showed very smooth and flat fracture surface, indicating very low dissipated energy. This was in contrast to the fracture surface of the micro-scale HA/PLLA which exhibited a rough surface with interfacial failure at the micro-scale HA and PLLA interface and localized plastic deformation in the surroundings of the debonded particles.

#### **2.4.4 *In vitro* degradation**

Russias *et al.* (2006) investigated effect of fine-commercial HA powder and coarse HA whisker on *in vitro* degradation behavior of HA/PLA

composites. The mechanical properties of the composites were evaluated both before and after storage in a Hanks' balanced salt solution (HBSS) at various periods of time. The results indicated that composites with ceramic contents ranging between 70 and 85wt% had similar mechanical properties to human cortical bone. However, the properties of these composites were deteriorated with immersion in HBSS due to the degradation of the polymer phase. Microstructure of the composite clearly confirmed that the polymer bridges have degraded or disappeared after immersion in HBSS, causing a significant degradation of the mechanical properties of the composites. The degradation was more pronounced in composites with higher ceramic content due to the dissolution of the trapped polymer chains between the ceramic particles. In comparison, both fine HA and coarse HA based composites showed similar degradation pattern in a simulated environment.

Verheyen *et al.* (1993) examined physico-chemical behavior of the neat PLLA, the composites containing 30wt% and 50wt% of HA and the one-side HA-coated PLLA immersed three different buffer solutions, *i.e.* citrate buffer, Gomori's buffer and phosphate-buffered solution, for 24 weeks. The results showed that the releases of calcium ions, phosphate ions and L-lactate from the specimens increased with increasing immersing time in all types of buffers. Among three buffer solutions, the specimens immersed in citrate buffer solution released the highest amounts of calcium and phosphate ions. This was because calcium citrate complexes probably prevented precipitation of calcium phosphate on specimen surfaces. The drop of pH of PBS solution was observed after the immersion of HA-coated PLLA specimens for 8 weeks. It was possible that hydrolysis of the polymer releases  $H^+$  ions, causing a decrease in the pH which led to an increase in the solubility of calcium

phosphates. This study indicated that the *in vitro* solubility of calcium phosphates depended on the surrounding medium as well as on the materials themselves. The pH change of the buffer solution and weight loss of HA/PDLLA composite during the hydrolysis process has been also observed. The results indicated that the composite with a higher HA/PDLLA weight ratio had a higher degradation rate. The lower pH value also implied that the higher amounts of degraded PLA dissolved in the buffer solution. Moreover, the surface area of PLA exposed to the solution increased with increasing HA content in the HA/PDLLA composites. From a chemical reaction viewpoint, this led to a higher reaction rate of hydrolysis of PLA with increasing HA content (Lin *et al.*, 2006).

Tsuji *et al.* (2004) investigated the hydrolysis of PLLA crystalline residues in PBS using gel permeation chromatography (GPC). To prepare PLLA crystalline residue, the PLLA films were melted at 200°C for 5 min and then crystallized or annealed at 160°C for 600 min followed by quenching to 0°C. To remove the PLLA chains in the amorphous regions, PLLA films was immersed in 100 ml of 0.15 M PBS at pH 7.4 and 97°C for 40 h. Then, the hydrolysis PLLA crystalline residues (20 mg) was performed in 100 ml of 0.15 M PBS at pH 7.4 and 37°C for the periods of time up to 512 days. PBS was replaced once a month. The average hydrolysis rates estimated from the changes in number average molecular weight ( $M_n$ ) and peak top molecular weight ( $M_t$ ) for the hydrolysis period from 192 to 512 days revealing that linear decreases of  $M_n$  and  $M_t$  in this period were 5.31 and 5.01 g mol<sup>-1</sup> day<sup>-1</sup>, respectively. The low hydrolysis rates indicated that the PLLA crystalline residues can remain for a long period. The hydrolysis of the PLLA crystalline residues

proceeded from their surface composed of very short chains with a free end along the chain direction but the hydrolysis from their lateral surface could not be traced.

Dueka, Zavaglia, and Belangero (1999) investigated effect of degree of PLA crystallinity on degradation process of PLA pin. Mechanical, thermal and morphological properties of PLA pin were evaluated before and after immersion in buffer solution. DSC result showed an increase in degree of crystallinity of PLA with increasing immersion time for all specimens. The PLA pin with lower crystallinity had higher bending strength than that with higher crystallinity.

Yuan, Mak, and Yao (2002) studied degradation of PLLA fibers in PBS (pH 7.4) and in a dilute NaOH solution (pH 11.0) at 80°C. They have found that the viscosity-average molecular weights of the PLLA fibres dropped sharply and decreased by over 90% after 6 days of degradation. The thermal behaviours of the fibres showed that after immersion in both medias, the melting temperature of PLLA decreased while their crystallinities increased. The fibres completely lost their mechanical strength after 5 days of degradation in PBS or in dilute NaOH solution at 80°C. In addition, SEM showed microcracks on the fiber surfaces across the fibre axis after degradation. These morphological defects confirmed the decrease in viscosity-average molecular weight of PLLA chain.

Kang *et al.* (2007) synthesized porous PLLA/ $\beta$ -TCP composite. The PLLA/ $\beta$ -TCP composite were immersed in dynamic simulated body fluid (DSBF) and in static simulated body fluid (SSBF) at 37°C for 24 weeks. SEM micrographs of all specimens indicated that a large number of apatite layer were formed on the scaffolds, especially in SSBF. The molecular weight of specimens under flow SBF was higher compared with those tested under static conditions. This might be that flow of SBF

retarded the autocatalysis of PLLA. The porosity and mass change was related to the apatite formation and SBF flow. The changes in mass and porosity contributed to the formation of large number of apatite on the surface and in the interior of the materials. The formation of apatite may compensate the loss of mass due to dissolution of  $\beta$ -TCP and the hydrolysis of PLLA. Moreover, the addition of  $\beta$ -TCP into PLLA affects the degradation rate of PLLA in the composite scaffolds. The degradation rate of scaffolds could be adjusted by the additional fraction of  $\beta$ -TCP. Results indicated the possibility to modulate the degradation rate of the composite scaffolds by varying the content of  $\beta$ -TCP added to the polymer.

Navarro, Ginebra, Planell, Barrias, and Barbosa (2005) studied chemical and morphological changes of PLA/soluble calcium phosphate glass (G5) composite during its degradation in simulated physiological conditions. They have found that the G5 incorporated in the polymeric matrix induced morphological changes, such as the formation of cracks in the composite, which accelerated the degradation of the material. Additionally, G5 reacted with the aqueous medium inducing the formation of a calcium phosphate precipitate, which could enhance the interaction between the material and the bone tissue.

Li, Feng, and Cui (2006) prepared nano-HA/collagen/PLLA composite reinforced by high-strength chitin fibers. To further strengthen the scaffold, linking between PLLA and chitin fibers were produced by oil-soluble dicyclohexyl carbodimide (DCC). To prepare the linked PLLA/chitin fibers, the chitin fibers, PLLA and DCC (1:4:2) were dissolved into dichloromethane at 0°C for 2 h. The linked PLLA/chitin fibers was taken out and washed by dichloromethane and dried. Thereafter, the reinforced nano-HA/collagen/PLLA composites were incubated at

37°C in PBS solution. They have found that the nano-HA/collagen/PLLA composite reinforced by linked PLLA/chitin fibers showed better mechanical properties than the composite without PLLA/chitin linking. These results denoted that the strong interfacial bonding strength of the scaffold with linking could decrease the *in vitro* degradation rate.

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