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Progress of Bone Scaffold by Laser Rapid Prototyping

激光快速成型骨支架进展

Science Press
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内 容 简 介

本书系统介绍激光快速成型技术在人工骨支架制备中的研究现状，详细分析和归纳目前生物材料用于骨修复及再生的研究进展，全面总结骨支架材料的种类及特点，重点探讨不同骨支架材料的强度、韧性、生物相容性、降解性及其与组织细胞的相互作用规律，以期对骨组织缺损的修复与功能重建提供理论与技术指导。

本书可供生物制造、激光制造、组织工程、再生医学等领域的研究人员阅读。

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Preface

Bone defects have become a global healthcare problem and the associated loss of function considerably impairs the life quality of patients. Current clinical treatments have been mainly focused on repairing bone defect by autogenous or allogeneic bone grafts. However, autogenous bone grafts face on inherent drawbacks such as additional surgery, limited supply and increased morbidity of the donor site, while allogeneic bone grafts are associated with the risks of immunoreactions and disease transmissions. Therefore new treatment strategies for bone defects are needed in order to realize bone repair and regeneration.

Bone tissue engineering (BTE) has been emerging as a valid approach for bone repair and regeneration by using porous and bioresorbable scaffold seeded with cells. The scaffold plays an important role in BTE since it serves as temporary framework to guide cell attachment, differentiation, proliferation, and subsequent bone tissue regeneration. The scaffold will then be resorbed and replaced by, and in tune with, the newly regenerated bone tissue. Therefore, an ideal scaffold should possess the following characteristics: (i) good bioactivity, biodegradability, biocompatibility and adjustable rate of degradation; (ii) suitable porous structure to promote cell proliferation, vascular ingrowth and nutrient transportation; (iii) suitable surface morphology and physiochemical properties to encourage intracellular signaling; and (iv) customized shape to satisfy specific bone defects.

Bioactive ceramics, including hydroxyapatite (HAP), tricalcium phosphate (TCP), bioactive glass (BG) and calcium silicate (CS) are considered as the most promising scaffold material owing to their similar composition to the mineral phase of bone and excellent biocompatibility, degradability and osteogenesis. Hereinto, HAP is a major component of natural bone and can combine with it by chemical bonds to form new bone tissue after implanted. TCP has good bioactivity, biodegradability, biocompatibility and it can enhance the proliferation capacity of stem cells and guide bone regeneration. BG is able to promote gene expression and production of osteocalcin. CS has excellent bioactivity and the ability to bond with bone tissue. In particular, the Si ion is capable of inducing bone-like apatite formation in simulated body fluid.

Traditional sintering methods have been widely used in the fabrication of bone scaffold. However, it is difficult to manufacture customized bone scaffold by these methods because the scaffold shape is limited by the sintering mold. Conventional fabrication methods for porous structure mainly include pore-forming method, sintered microsphere method and chemical foaming method, etc. Nevertheless, these methods have poor controllability over pore structure (such as pore size and connectivity) and shape. Besides, the mechanical properties of fabricated scaffolds were far below those of weight-bearing bone (fracture toughness: 2–12 $MPa \cdot m^{1/2}$; compressive strength: 130–180 MPa) in the human body.

Selective laser sintering (SLS) is one of promising rapid prototype technologies and attracts great interest in the past decades. It is able to quickly manufacture customized parts with complicated internal structure based on computer-aided design (CAD) data without using any mold. These advantages pave the way for the fabrication of controllable porous structure and customized shape of bone scaffold. In addition, the recent developments of nanotechnology and composite materials provide new methods to improve the strength and toughness of bone scaffold, as well as the control and optimization of physicochemical and biological properties for BTE applications.

Our group was established under the support from the Foundation for the Author of National Excellent Doctoral Dissertation of China (201032), the Natural Science Foundation of China (51222506, 81372366), Project supported by the Fok Ying-Tong Education Foundation, China (131050), Hunan Provincial Natural Science Foundation of China (14JJ1006), Program for New Century Excellent Talents in University (NCET-12-0544), the Fundamental Research Funds for the Central Universities (2011JQ005, 2012QNZT015), Shenzhen Strategic Emerging Industrial Development Funds (JCYJ20130401160614372) and the faculty research grant of Central South University (2013JSJJ011, 2013JSJJ046).

Here we select some of our papers published in international journals to provide a review of the progress of bone scaffold by laser rapid prototyping. The subject matter covers the development of laser rapid prototyping system, types and characters of scaffold materials, fabrication techniques for bone scaffold with hierarchically porous structure and customized shape, toughening methods and corresponding mechanisms. Moreover, greater insights into the degradation behavior and bone formation ability of the scaffolds are provided, as well as the interaction between scaffolds and cells. Frankly speaking, it is still not the ideal time to sum-up all these fascinating areas of development for the work at hand. We hope this book will contribute to both scientific community and clinical application of bone scaffold.

We acknowledge the many wonderful graduate students who have helped to do experiments, data analysis and numerical simulation, and write down the original reports which are used in this book. They are: Chengde Gao, Pei Feng, Jingyu Zhuang, Yi Nie, Pengjian Li, Bo Yang, Zhongzheng Mao, Junjie Deng, Zikai Han, Tingting Liu, Yiyuan Cao and Huanlong Hu. We particularly acknowledge Prof. Jue Zhong (Central South

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Cijun Shuai
July 2014

Contents

Chapter 1
Hydroxyapatite-Based Bone Scaffolds

Hydroxyapatite (HAP) possesses both excellent bioactivity and osteoconductivity which made it a very attractive material for bone scaffolds. It can combine with bone tissues by chemical bonds after implantation. However, its applications are limited to non-bearing bone repair due to its poor mechanical properties, such as high brittleness and low strength.

In this chapter, nanotechnology was employed to improve the mechanical properties of HAP bone scaffold. A novel selective laser sintering (SLS) system for scaffold fabrication was developed based on rapid prototyping technology. Implement arbitrary complex movements were realized based on the Non-Uniform Rational B-Spline theory. The fast heating and fast cooling properties of laser were expected to inhibit grain growth during the sintering process. A mathematical model was also established to study the dynamic temperature field of selective laser sintering process with nano-HAP powder. The change rules of three-dimensional transient temperature field with the different speeds of the moving laser heat source were analyzed.

Serious micro-cracks often occur on the surface of bone scaffold prepared by SLS technology. We found that appropriate preheating before sintering can reduce and attenuate the cracks. Moreover, grain growth was greatly inhibited due to the improved thermal conductivity of nano-HAP after preheating. Besides, a small amount of biodegradable poly (L-lactide acid) (PLLA) was added into nano-HAP powder during sintering in order to improve the sintering properties. The molten PLLA filled in the gaps among HAP particles and may absorb thermal stress in laser sintering process, resulting in a rearrangement of HAP particles. PLLA was then excluded from the final sintered bone scaffold.

The sintering behaviors, microstructure and resulting mechanical and biological properties of nano-HAP were studied with X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy and Vickers hardness tester and in vitro simulated body fluid tests. The findings indicated that the HAP bone scaffold prepared by SLS possesses favorable mechanical properties and bioactivity for bone tissue engineering.

1.1 Structural Design and Experimental Analysis of a Selective Laser Sintering System with Nano-Hydroxyapatite Powder[1]

1.1.1 Introduction

Hydroxyapatite (HAP) has been used for bone repair and tissue engineering due to its biocompatibility, osteoconductivity, and osteoinductivity[2-4]. However, compared with natural hard tissues, its applications are limited to small, unloaded, or low-loaded implantation, powder, coating composites because of its biomechanical properties (high brittleness, low tensile strength, high elastic modulus, low fatigue strength, and low flexibility, etc). Natural bone tissue possesses a nanocomposite structure interwoven in a three-dimensional (3D) matrix, which plays a critical role in conferring appropriate physical and biological properties to the bone tissue[5]. Nano-HAP, which is produced with hydroxyapatite by nano-technology, is similar to bone apatite in size, phase composition, and crystal structure[6,7], and it possesses improved mechanical properties and superior bioactivity[8,9] compared with the ones with micron-size of the same material. Several ways were reported to prepare nano-HAP. Wang et al. reported that they used hydrothermal method to prepare the HAP single crystals. But it requires critical airtight equipment and it is difficult to control the reaction conditions[10]. It was reported that nanocrystalline hydroxyapatite was synthesized by using precipitation method. The nano-HAP particles are characterized by wide range of size distribution and low dispersion[11,12]. Nano-HAP used in this study was synthesized via sucrose-templated sol-gel route with calcium nitrate and ammonium hydrogen phosphate. The prepared nano-HAP has multiple advantages (small particle size, good crystallized activity, high phase purity, and excellent crystallinity, etc)[13,14]. Usually, raw materials are sintered for a few hours in the high-temperature furnace after compression and formation of the nano-HAP by conventional sintering method. In fact, nano-particles have formed into micron particles after sintering for several hours, which leads to a loss of nano-effect[15]. The injection moulding process was reported to prepare the hydroxyapatite for bone tissue repair by Wang et al. However, it has disadvantages of low intensity, and the acidic degradation products, which may cause inflammation[16,17].

Application of nano technology is more likely to overcome the shortcomings (high brittleness, small intensity, etc.) because the inner air bubbles and defect of the material can be minimized when the grain size is small. Furthermore, Nano material is not prone to transgranular fracture, which can dramatically increases the toughness and strength of materials. In addition, the surface area of grain boundaries are increased greatly, which makes grain boundary sliding more readily. Thus, it causes the deformation more easily, which facilitates the movement between grains, enhances plasticity, and reduces brittleness. The smaller particles usually have the higher the torsion modulus, tensile modulus and tensile strength[5,18,19]. However, to our knowledge, there is no bio-manufacturing technology available to maintain its nano structure after deformation process.

In this study, we developed a selective sintering system to fabricate a porous artificial bone scaffold with complicated three-dimensional structure. In this system, micron-sized spot of high power laser and nano-HAP were used as energy and material, respectively. Fast heating and cooling was applied in this laser sintering system and thus HAP could be heated rapidly and linked each other and then the laser could be rapidly removed, which prevented the further formation and growing of the particles and maintained its nano-size structure and properties. We found that that the crystals were linked to each other, fused and became bigger gradually. However, the crystals were more like granules instead of the original needle shape. And they could maintain their nano structure if the technical parameters were optimized.

1.1.2 Structural Design

1.1.2.1 Working Principle

The solid material was sintered in the system by laser power and the overlaying sintering layers were formed into the conformational structure required. The sintering process was shown in Fig. 1.1. The laser beam, which is focused into a 50 μm or smaller spot by an optical focus system, was used as power source. The nano-HAP particles (10–100 nm) were placed on a motion platform. The motion platform moved according to the requirements of bone cross-section profiles and the sintering interval distance (150–500 μm). When scanned by the laser beam, the particles were fused and linked to each other at the high-energy state due to high temperature, while the particles in non-sintered area were still separated. A layer with 50 μm or thinner wall formed. Then the next layer was fed and sintered again on the former layer. Finally, a three-dimensional porous artificial bone scaffold was formed.

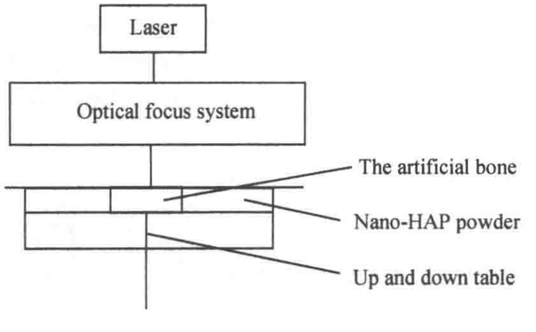


Fig. 1.1 The schematic of selective laser sintering system workflow. The nano-HAP particles are placed on a motion platform. The laser power sinters the powders while the motion platform moves.

1.1.2.2 Design Analysis

A novel laser sintering system was designed and shown in Fig. 1.2. The sintering platform moved with a two-dimensional (*X* and *Y*) direction and driven by a software. *X*- and *Y*- axial movements were controlled by ball screws, which were driven by servomotor. The resolution was 1 μm and the movement velocity was 0–100 mm/s. The velocity can be adjusted to meet the sintering time, sintering trace, and laser power by computer. The sintering platform was fixed on an up-down movement system (*Z*-axial), which drove the platform to move downwards by the height of a cross-section after the former one cross-section sintering finishes.

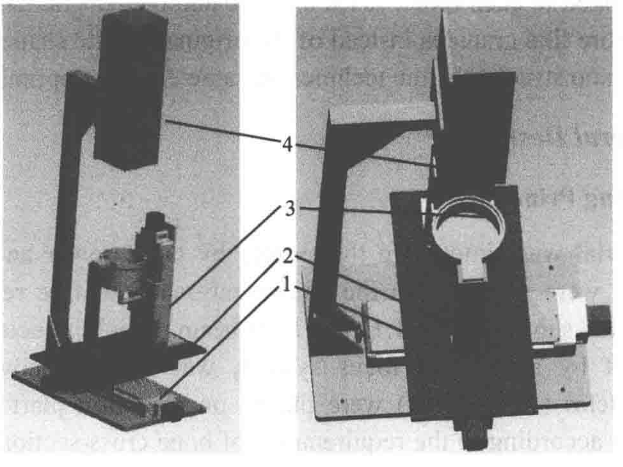


Fig. 1.2 Selective laser sintering system (1. *X*-axial motion unit; 2. *Y*-axial motion unit; 3. up-down motion unit of sintering platform; 4. laser power).

The structure of a high-power laser with micron-size spot was shown in Fig. 1.3. A variety spots of more than 50 μm in diameter could be obtained by controlling the distance from lens center to the heating region. The diameter of laser beam (5) was approximately 3 mm. The laser beam was calibrated with the parallel optical device (7) fixed on an installation mechanism (6) and a fixed mechanism (8). The laser beam can be moved by the up-down movement system which connects to an up-down micro

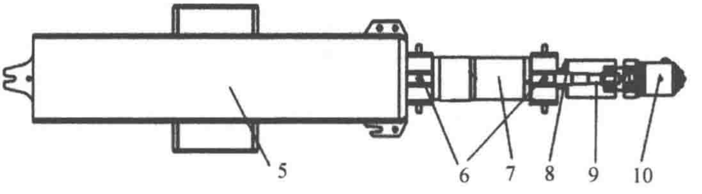


Fig. 1.3 Laser and its focus system (5. laser, 6. installation platform; 7. parallel optical devices; 8. fixed installment; 9. up-down movement unit; 10. convex lens).

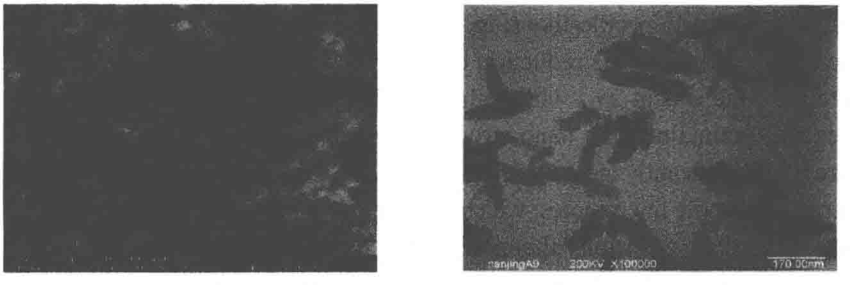
feeding unit (9). Finally, the laser beam focused into a spot of a minimum of 50 μm with a convex lens (10).

1.1.3 Material and Methods

Nano-HAP was purchased from Nanjing Emperor Nano Material Co. China. It was synthesized via sucrose-templated sol-gel route with calcium nitrate and ammonium hydrogen phosphate. Its performance parameters are shown in Tab. 1.1 and its microstructure was shown in Fig. 1.4. The laser (Model: FST100SFD) was purchased from Synrad Co. USA. Its parameters were shown in Tab. 1.2, and scan mode was shown in Fig. 1.5. The laser power was controlled by a PWM (Pulse Width Modulation) signal. The higher the laser power, the higher its duty ratio.

Tab. 1.1 The performance parameters of nano-HAP

Nano-hydroxyapatite	
Crystal form	Needle-like
Average width	20 nm
Average length	150 nm
Elements	$Ca_{10}(PO_4)_6(OH)_2 \geqslant 99.5\%$, Heave metal $\leqslant 8$ ppm
Drying Loss	0.59%
Cauterizing Loss	2.59%
White degree	96
PH value	7.41
Hydrochloric acid insoluble matter	0.02%
Application range	Artificial bone, artificial joint

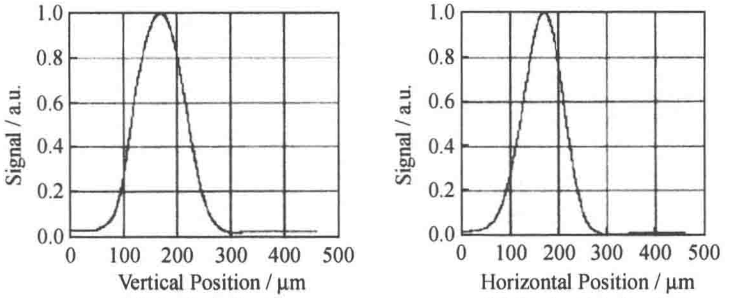


(a) (b)

Fig. 1.4 The microstructure of nano-HAP prior to sintering: (a) tested with SEM; (b) tested with TEM.

Tab. 1.2 Parameters of the laser

Parameter	Specification	Measured	Units
Min power	100.00	120.00	W
Max power	—	131.00	W
Average power output	>120.00	125.41	W
Power stability	±7.00	±4.38	%
Peak power	>131.00	41	W
Rise time	<75	46	μs
Fall time	<75	46	μs



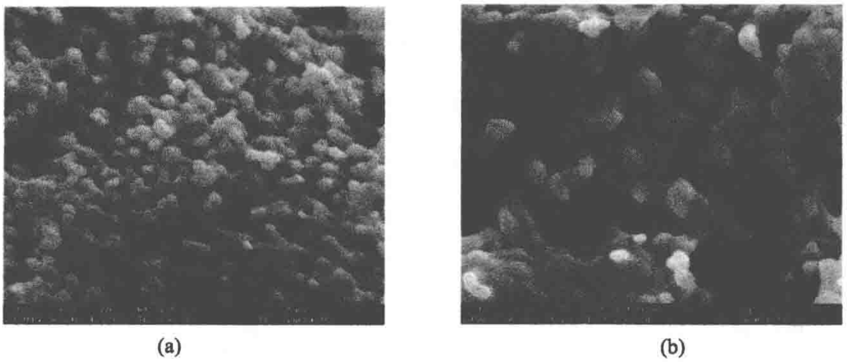
(a) Vertical direction (b) Horizontal direction

Fig. 1.5 The laser mode of FST100SFD. The laser mode scanning at vertical direction is similar to the laser mode scanning at horizontal direction. The laser power distribution is centrosymmetric.

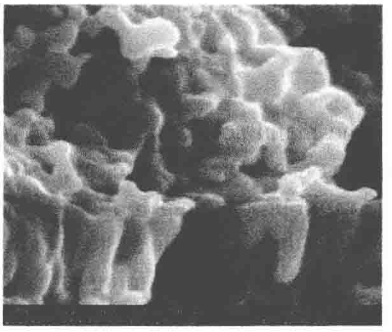
The artificial bone with arbitrary shape could be sintered through the movement of *X*, *Y* and *Z* axial platforms controlled by the software in computer. Feeding with material powder was implemented manually.

1.1.4 Results and Discussion

Interestingly, our study found that the microstructure of nano-HAP was changed after sintering and morphological characteristics was altered with increasing sintering time as well. The morphology of the sintered layer with 100 W laser power, 0.05 duty ratio, and at the different sintering times (15 s, 25 s, 35 s) measured under SEM was shown in Fig. 1.6. The shape of nano-HAP was changed to granule-like after the sintering for 15 s but not originally needle-like (Fig. 1.6(a)). Inadequate fusion caused the looseness of nano-HAP, leading to the reduction of the intensity after re-crystallization[20]. When the sintering time was at 25 s while other parameters were kept unchanged, nano-HAP particles still maintained the nano-structure, whereas they linked with each other and their size was significantly increased (Fig. 1.6(b)). The sintered nano-HAP was compact but it still contained some micro pores, which may be beneficial to the growth and regeneration of bone tissue. In addition, the nano-HAP particles were completely fused and re-crystallized when the sintering time was at 35 s (Fig. 1.6(c)). Under this condition,



(a) (b)



(c)

Fig. 1.6 Morphology of the sintered layer when the laser power is 100 W, the duty ratio is 0.05, and the sintering time is 15 s (a), 25 s (b), 35 s (c) respectively.

the sintered tissue became very compact and the intensity of nano-HAP was largely increased. Thus, this kind of sintered tissue is unlikely favorable for bone growth.

Selective laser sintering of nano-HAP is a process that the powder melts and solidifies rapidly and forms a sintered layer under the laser irradiation. Due to the loose structure, low density, low melting mobility and inefficient thermal conductivity of nano-HAP, it may cause the increasing temperature locally of sintered powder sharply, or fusion, even evaporation. Meanwhile, the air sealed in nano-HAP powders is heated constantly, which may expand, bounce and aggregate to form a bubble, or even break through the layer of the powder at certain scale, and in a few cases it may cause a dramatic splash of the powder.

The factors affecting the sintering of nano-HAP include laser power, diameter of laser spot, and the sintering time, etc. Therefore, it is so important to select the appropriate sintering parameters to maintain a stable sintering process of nano-HAP powder, which minimizes the air bubbles in the sintered layer or inhibits the intense vaporization and splashing of the powder, then to form an excellent sintered layer finally. Laser power, the diameter of laser spot can be determined according to the properties of different materials and the requirement of products. Sintering time is another critical parameter. Under the same conditions, when the sintering time is shorter, the nano-HAP particles may melt inadequately, and then the sintered tissue may be looser. When the sintering time is too long, the crystal grain may re-crystallize completely, and then the sintered nano-HAP may be too compact, which is also unfavorable for bone growth. Thus, the sintering time should be optimized carefully.

The sintering situation of nano-HAP power largely depends on the energy density, which is determined by both the spot diameter and laser power. When other parameters are fixed, smaller spot in diameter and higher laser power lead to higher energy density, which results in more sufficient sintering and less adverse effects, such as the inclusion in powder. However, some problems (vigorous vaporization or splash) may occur when spot diameter is too small and/or laser power is too high.

1.1.5 Conclusions

In this study, we designed and manufactured a laser-sintering system and further analyzed the effects of the molding parameters on the microstructure of nano-HAP powder. Our results showed that the rapid prototyping of nano-HAP with selective laser sintering system can be obtained by optimizing the system parameters. These findings will provide experimental basis for the rapid prototyping of nano-HAP.

1.2 Structure and Properties of Nano-Hydroxyapatite Scaffolds for Bone Tissue Engineering with Selective Laser Sintering System[21]

1.2.1 Introduction

One of the most advanced strategies in recent research on bone tissue engineering is that the osteocytes can grow and proliferate on three-dimensional scaffolds, and the scaffold material degrades gradually until being absorbed with bone regenerative growth[22]. The scaffold needs to be integrated with the surrounding bone tissue and provide the initial three-dimensional framework upon which cells may adhere, proliferate and eventually produce extracellular matrix (ECM) proteins. To seek an ideal bio-active material and optimized technology for the fabrication of bone scaffolds has been a hot and urgent issue in the bone tissue engineering field. So far, several biomaterials, including bioceramics, biopolymers, metals and composites have been used[23,24]. Of those materials, bio-ceramic materials including hydroxyapatite (HAP), bioglass, A-W glass ceramic, and β-tricalcium phosphate closely mimic bone tissue have gained much attention due to their superior properties[25-28].

Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) is the main inorganic component (about 77 % weight) in natural bone. It is widely recognized as a highly biocompatible and bioactive material which has degradable bioactivity, no toxicity and good osteoconduction[29-31]. Strong chemical bond between HAP and interface of bone with good osteoconduction can be formed after implantation of HAP into defective bone area. Therefore, hydroxyapatite was widely applied for bio active material for teeth, bones and joints. However, the limitations such as the high brittleness, low hardness, and bad performance in reliability resulted by conventional technology prevent the application of HAP in the weight-bearing bone scaffolds. This leads to the motivation for developing HAP-based composite stems from the requirement to fabricate materials with improved strength and toughness without compromising its biocompatibility. HAP-Mullite, PCL/HAP, and HAP/PLLA composites with combination of mechanical properties were well studied[32-34]. The composite improved the strength and anti-destroy ability of HAP scaffolds, however, it also result in a decrease of the biological activity and