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Effects of Mechanical Stress on the in vitro Degradation of Porous Composite Scaffold for Bone Tissue Engineering

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Abstract. In bone tissue engineering, porous scaffolds served as the temporary matrix are often subjected to mechanical stress when implanted in the body. Based on this fact, the goal of this study was to examine the effects of mechanical loading on the in vitro degradation characteristics and kinetics of porous scaffolds in a custom-designed loading system. Porous Poly(L-lactic acid)/β-Tricalcium Phosphate (PLLA/β-TCP) composite scaffolds fabricated by using solution casting/compression molding/particulate leaching technique (SCP) were subjected to degradation in simulated body fluid (SBF) at 37°C for up to 6 weeks under the conditions: with and without static compressive loading, respectively. The results indicated that the increase of the porosity and decrease of the compressive strength under static compressive loading were slower than that of non-loading case, and so did the mass loss rate. It might be due to that the loading retarded the penetration, absorption and transfer of simulated body fluid. These data provide an important step towards understanding mechanical loading factors contributing to degradation.

Introduction

Bone is a dynamic, highly vascularised tissue. Its main role is to provide structural support for the body. Bone is generally exposed to a dynamic external environment in which functional adaptation is necessary for survival [1]. Osseous tumors, trauma, disease, and birth defects can create a need to fill defects in the skeletal system. Tissue Engineering has been emerging as a valid approach to the current therapies for bone regeneration or substitution.

Scaffolds, one of the key issues in bone tissue engineering research, will act as a temporary matrix for cell proliferation and extracellular matrix deposition. It is now believed that the influence of mechanical stress on biodegradation is an important factor [2]. Some researches [3,4] have used biodegradable scaffolds to evaluate the degradation under stress condition. Several studies [5,6] have shown that stress conditions are likely to affect the degradation kinetics of biodegradation and this may have significant implication on the bone regeneration. However, relatively few studies are available in the literature about biodegradation properties of PLLA/β-TCP scaffold in SBF under compressive loading. There is a need to use external compressive loading to test the degradable characteristics of scaffold for evaluation of feasibility in clinical applications.

The main objective of this work is to study the influence of mechanical loading over the degradation kinetics of PLLA/β-TCP porous scaffold in vitro when withstanding static loading in SBF. A simple method allowed compressive loading experiments to be performed in a customized loading environment that simulates the situation in human body. The results show some important differences in the degradation kinetics between static loading and non-loading case.

Materials and method

Preparation of PLLA/β-TCP scaffold. β-TCP powder was prepared in our laboratory by the most common synthesis route; PLLA (η=7g/ml, Mw=620,000) was provided by Chengdu organic chemicals co. LTD. Chinese academy of sciences. Scaffolds were fabricated using SCP technique [7]. In brief, PLLA was dissolved in chloroform and then β-TCP and porogen NaCl(200μm≤size≤400μm) were added into the polymer solution. The polymer solution was mixed up under continuous stirring and dispersed by ultrasonic wave. Next, the solution was extended out on the surface of glass to form a slim layer of film. After the solvent evaporated, the composite film was taken off and cut into microparticles. These mixture particles were loaded into a 5mm diameter stainless steel mold which was then heated up to 150°C at a pressure of 6Mpa for 5min.Then the shaped specimens were demolded and immersed in de-ionized water for 48 hours to leach porogen NaCl followed by drying in a vacuum at 60°C for 24 hours. Group I、II、III were fabricated in exactly the same procedure, but the weight ratios of β-TCP:PLLA were different as 1:2,1:1 and 2:1.

In vitro degradation under loading. Altogether there were 30 scaffolds in each of the three groups and the 30 scaffolds were further divided into 3 subgroups: control (6 specimens), under loading (12 specimens) and non-loading (12 specimens). The control specimens were not subjected to degradation but tested for the original properties. Scaffolds in the loading subgroups underwent degradation under a constant compressive loading in a specially designed loading system that was immersed in SBF (Fig.1), while scaffolds in non-loading group were only immersed in SBF. The constant compressive loading was measured to be 0.32MPa referring to the contact pressures of the human hip joint ranging from 0.1 to 4MPa while walking [8]. SBF was used for the experiment as it had ion concentrations that were nearly the same with those of human blood plasma [9]. Scaffolds were immersed in SBF at 37°C for various periods of degradation time: 1, 2, 4 and 6 weeks. At the end of each test time point, a set of scaffolds were removed from the system and subjected to analysis as briefly described below:

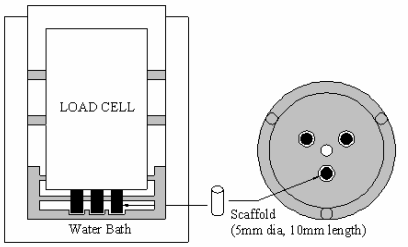

Figure 1 schematic configuration of the experiment apparatus used to perform biodegradation in vitro under static compressive loading.

Mass loss rate. the mass loss rate of each scaffold was determined by the equation: $W_l\%=100(W_0 - W_r)/W_0$ (W_0:original weight, W_r: remaining dried weight)

Porosity. Porosity was conducted by using a conventional Archimedes immersion technique [10]. The average value of three determinations was taken as the porosity of scaffold.

Compressive strength. The strength of scaffold was tested using AG-10TA electronic apparatus (Japan). The speed of loading was 5mm/min, test temperature: 24°C, and humidity: 80%.

Results and discussion

SEM Morphology. Micrographs of specimens from the three groups are shown in Fig.2. It can be seen that the specimens tested under two conditions show the different degree of change. The pore of specimens under loading case (Fig.2c) became narrow, which may retard the diffusion of SBF to a certain degree.

Porosity. Generally, for all three groups and for two conditions, the porosity of the scaffolds increased as a function of degradation time. For group I, the porosity under loading was lower than that under non-loading except that it was higher at week 2. The porosity in group II under loading was higher than that under non-loading up to week 2 and then was lower after week 2 (Fig.3A).

In group III, the porosity of scaffolds under non-loading increased faster than that under loading case, which had statistically significance ($P<0.05$) (Fig.3B). The pore diameter of the scaffolds under loading group would become compressed. Therefore, the increase of porosity was also restrained to a certain degree.

Mass loss rate. The mass of the scaffolds in all three groups decreased as a function of degradation time under both two test conditions. In general, mass loss rate of the scaffolds with

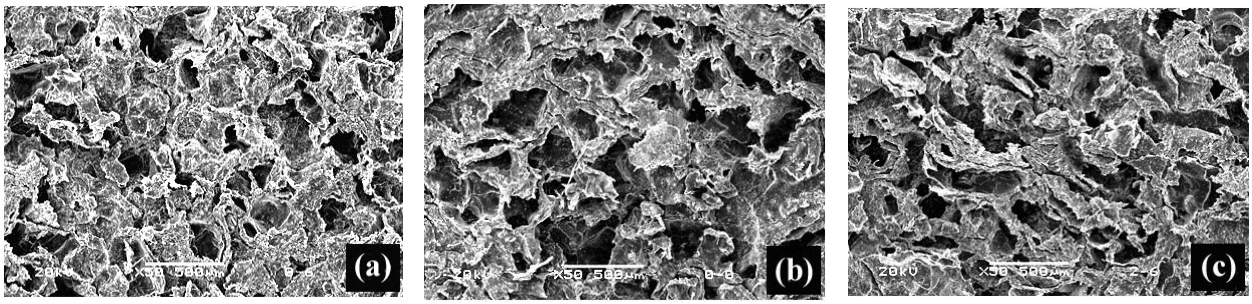

Figure 2 SEM showing representative scaffolds from the three groups: (a) original morphology, (b) non-loading group after 6 weeks, and (c) loading group after 6 weeks.

degradation time was fast up to week 2 and slower thereafter. It can be seen from Fig.4A that the mass loss rate of the loading group is lower than that of non-loading group at any degradation time.

In general, the mass remaining at any degradation time point under the stress condition was least for the group I and highest for the group III (Fig.4B). PLLA degradation process is a hydrolysis process of irregular and random fission of ester chain in PLLA molecular chain [11]. Being alkalescent, β-TCP can neutralize the acidic environment caused by PLLA hydrolysis. Therefore, adding β-TCP restrains autocatalysis of PLLA. This can be seen from Fig.4B as a result of different content of β-TCP, that changes in mass loss rate of materials after degradation are different.

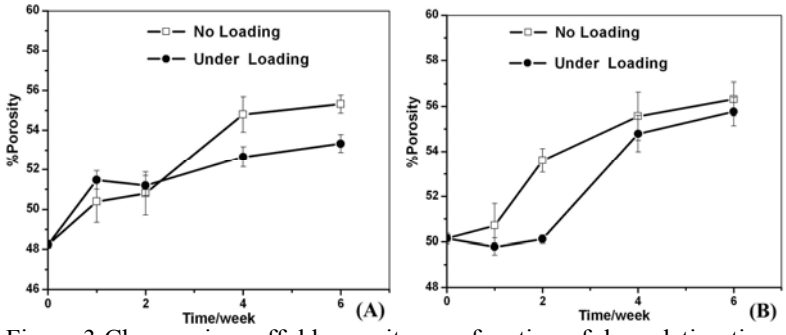

Figure 3 Changes in scaffold porosity as a function of degradation time under test conditions for group II (A) and group III (B)

Compressive strength. The compressive strength decreased continually with degradation time for all the three groups, and the decreasing speed of compressive strength under loading condition was lower than that of non-loading case (Fig.5A). The result of this study further indicates that compressive strength decrease is retarded under loading case as shown in Fig.5B.

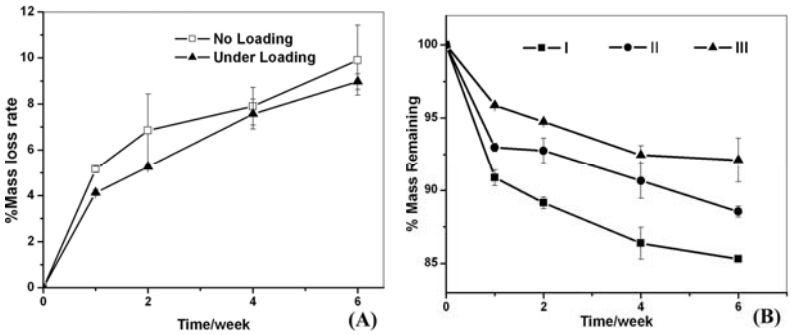

Figure 4 Changes of mass loss as a function of degradation time under test conditions for group III (A) and mass remaining under loading conditions for three groups scaffolds (B).

The declining rate of compressive strength under loading is slower than that non-loading. This may be because of the counteraction of polymer molecular chain. The three dimensional space of the polymer under loading may become tighter by resisting compressive stress, which can improve the ability of the material resisting the decrease of strength. During degradation, because the material absorbed SBF, polymer in the material became plastic, and therefore the chain may get back toughness to a better condition. From this point, this meets the demand of making the β-TCP become more toughness with PLLA, and it is confirmed that we make β-TCP combined with PLLA to overcome the disadvantage of each.

In this paper, in vitro degradation of PLLA/β-TCP porous scaffolds under loading was the focus of investigation. However, relatively few studies and little information are available in the literature about biodegradation properties of this porous scaffold in SBF under compressive loading. In order to go deep into investigation the degradation behavior under loading, it is necessary to perform a simple compressive loading test firstly. Based on these results, other degradation experiment under dynamic cycle loading may be performed later.

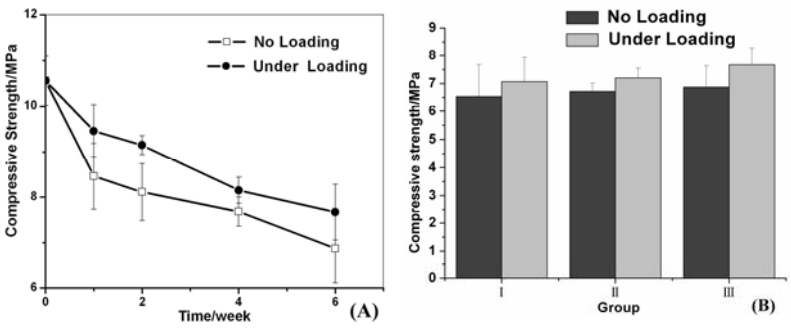

Figure 5 Changes in compressive strength under two test conditions for group III (A); and changes in compressive strength at week 6 as a function of groups under two test conditions (B).

Conclusions

From the degradation under static mechanical stress, conclusions would come to that (1) under static compressive loading, the scaffold degradation in vitro has significant differences from that in non-loading cases; (2) Under loading, the loss of weight, the increase of porosity and the decrease of compressive strength are slower than that in non-loading cases, which is related to the SBF absorption of the material; (3) The degradation rate is also related to the scaffolds composition: adding β-TCP neutralizes the acidic products of PLLA and slows down the degradation rate.

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