Characterization of Compressive Deformation Behavior and Biocompatibility of Bioabsorbable Layered PLLA Scaffolds

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Abstract— **Development of scaffolds with porous structures for cell seeding and growth has been one of the most important issues in tissue engineering. Typical biodegradable polymer PLLA is widely utilized for such scaffolds due to biodegradability and biocompatibility. When PLLA scaffolds are used for regeneration of bone tissue, it is preferable that they have mechanical compatibility with the bone tissue to be regenerated. However, the mechanical properties of porous PLLA structures tend to be much lower than those of the bone tissue. In the present study, therefore, a new concept for PLLA scaffold, called layered structure, was introduced to improve the mechanical properties. Experimental results showed that the elastic modulus and strength under compression were effectively improved due to the layered structure. Deformation mechanism for catastrophic failure in the layered scaffold was found to be buckling of the outer layer, while in the standard scaffold, such critical mechanism of deformation was micro-buckling of the wall structures surrounding pores. It was also found that MC3T3-E1 cells well adhered to the surface regions of the scaffolds.**

Keywords— **Scaffold, Biodegradable polymer, Bone regeneration, Tissue engineering.**

I. Introduction

Autografts and allografts have mainly been used to reconstruct damaged bone tissues. However, for autografts, limited amount of available bone tissues and invasion to healthy bones and for allografts, donor shortage, potential risk of transmitting diseases and immunological response have been critical issues in bone regeneration [1,2]. Therefore, artificial bone substitutes mainly consisting of bioactive ceramics have actively been investigated and clinically been applied in orthopedics. However, slow rate of bone regeneration, low mechanical properties and non-resorbability have been problems. Recently, therefore, bone tissue formation in *in vitro* conditions has widely been investigated by using osteoblast or mesenchymal stem cells with scaffolds under suitable proliferation conditions [3,4].

In such regeneration technology of bone tissue, scaffolds with three-dimensional porous structures play an important role to construct new tissues with proper mechanical properties to be fitted for damaged regions of patients. Biodegradable polymers such as PLLA, PGA and PCL and bioceramics such as HAp, β-TCP and α-TCP are widely utilized to fabricate such scaffolds due to bioabsorbability, biocompatibility and bioactivity [5,7,8]. As a scaffold for bone tissue regeneration, appropriate porosity and size of pores and furthermore suitable mechanical properties such as modulus and strength are required to regenerate desired new tissue from cells and keep mechanical compatibility with the surrounding tissues when the scaffold with regenerated tissue is grafted directly into the damaged region of human body. Thus, fabrication technology for scaffolds with controllable mechanical properties needs to be developed.

The primary aim of the present study is therefore to develop a new technique to improve the compressive mechanical properties of a mono-structural PLLA scaffold with homogeneous pore distribution by introducing a new reinforcement such as layered structure. Porous cylindrical PLLA scaffolds having a solid outer layer were fabricated by the solid-liquid phase separation method and freeze-drying technique. Compressive mechanical properties were then evaluated to confirm the effectiveness of the layered structure. Cell adhesion and proliferation behavior and toxicity were also assessed by using MC3T3-E1 osteoblast-like cells under a typical culture condition.

II. Experimental

A. Materials and Specimen preparation

PLLA pellets (Lacty #5000, Shimadzu Co., Ltd) with T_G=69.72ºC, T_m=174.9ºC and the average molecular weight of 3.51gmol^{-1} were used to fabricate scaffolds. Porous structures of PLLA were fabricated by using the solid-liquid phase separation and freeze-drying methods [8-10]. Firstly, PLLA pellets were dissolved in 1, 4 Dioxane solvent in a PP cylindrical tube and then frozen by dipping into liquid nitrogen at a constant rate. The frozen solution was dried at -5ºC using a vacuum pump. The concentrations of the solutions were chosen to be 3, 5 and 7wt%. For layered PLLA scaffolds, PLLA solid films of about 250mm were fabricated by using a thermal-press technique, and then

C.T. Lim and J.C.H. Goh (Eds.): WCB 2010, IFMBE Proceedings 31, pp. 1230–1233, 2010.
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inserted into the PP tube so that the PLLA-Dioxane solution was surrounded by the cylindrical film. The freeze-drying method was then applied to fabricate the layered scaffolds. The standard type of PLLA scaffold with mono-porous structure was also fabricated for comparison.

The porosity values, $\varepsilon_{porosity}$, of the scaffolds fabricated were estimated from the following formula:

$$\varepsilon_{porosity} = \left(1 - \frac{V_{PLLA}}{V_{total}}\right) \times 100 \qquad (1)$$

where V_{Total} is the measured volume of a scaffold specimen and V_{PLLA} is the volume of bulk PLLA in the scaffold.

B. Compression test

The compressive mechanical properties such as elastic modulus and critical stress were evaluated by conducting compression tests of cylindrical specimens of the scaffolds using a conventional mechanical testing machine at a displacement rate of 1mm/min. The elastic modulus was calculated from the slope of the initial liner elastic region of the compressive stress-strain curve. The critical stress was defined as the stress that break the linear elastic deformation and therefore, it can be obtained at the end of the linear elastic portion of the stress-strain curve.

C. Microstructural characterization

The cross-sectional areas of the scaffolds were observed by a field emission scanning electron microscope (FE-SEM) (S-4100, Hitachi, Japan) to characterize their micro-porous structures. The longitudinal cross-sectional areas of the scaffolds deformed up to the critical stress were also observed to characterize the deformation mechanism. As the sample preparation for the FE-SEM observation, the scaffolds were cut about 5mm by a flesh razor blade after frozen in liquid nitrogen for several minutes, and the cutting-surfaces were coated by Pt-Pd by using an Ion sputter coater (E-1030, Hitachi, Japan).

D. Evaluation of Biocompatibility

MC3T3-E1 osteoblast-like cells were used to assess the biocompatibility of the scaffolds. The cells were cultured in ascorbic-free α-MEM supplements with 1% penicillin-streptomycin and 10% FBS in a 60mm^2 cell culture dish, and the culture medium was changed every two days. The cells were placed in a humidified incubator at 37ºC with 95% air/5% CO_2 (v/v) during cell culturing. The MC3T3-E1 cells were counted using a hemacytometer every subculture day. The cultured MC3T3-E1 cells were suspended at the concentration of 5x10^4 cells/μl in fresh medium to seed on the scaffolds. In advance, the scaffolds were submerged in ethanol for 1hour and then soaked in PBS three times (30minutes each). After the sterilizing process, the scaffolds were then washed in prepared culture medium twice (2hours each) [10]. The cell suspensions of high concentration of 10μ*l* were then seeded onto the surface of the pre-wetted scaffolds. The scaffolds with cells were then placed in the humidified incubator and the medium was changed every 2 days. The adhesion behaviors of the cells on the surface of the scaffolds after 12days of cell seeding were observed by FE-SEM after freeze-drying the scaffold samples.

III. Results and Discussions

A. Porosities and Microstructures of scaffolds

The porosities of the scaffolds estimated from Eq.1 are shown in Fig.1. The porosities tended to be monotonically decreased as PLLA contents increased. It was shown that the average porosities of the layered-structural scaffolds were much lower than those of the mono-structural scaffolds, as a result of existence of the solid outer-layer. It was also found that the porosities of the inner cores were kept at the same levels of the porosities of the mono scaffolds.

FE-SEM micrographs of the microstructures of the 7wt% mono and layered scaffolds are shown in Fig.2. The mono scaffold showed homogeneous distribution of pores. The larger pores are known to be solvent exhaust holes. The layered scaffold exhibited an inner core region with homogeneously distributed pores and a dense outer-layer as shown in Fig.2(b). It was also found that the pores in the region between the outer layer and the core region were smaller than those in core region, as a result of dissolution of the outer layer by 1,4 Dioxane into the solution during fabrication process.

B. Mechanical properties and deformation mechanisms

Compressive mechanical properties are shown in Fig.3. Both the elastic modulus, E, and the critical stress, $\sigma_{Critical}$, increased as the PLLA concentration increased. It is noted that both the properties were effectively improved by introducing the layered structure. The properties of the layered scaffolds were found to be 4 to 6 times larger than those of the mono scaffolds.

Deformation behaviors on the longitudinal cross-sectional areas are shown in Fig.4. In the mono scaffold, the deformation at the critical point was characterized as micro-buckling of the cell walls under compression. On the other

hand, in the layered scaffold, the primary mechanism of deformation at the critical point was buckling of the outer solid layer. It is also seen that the inner core region could maintain the initial porous structure because the outer layer functioned as the load bearing support during compressive loading. It is thus considered that such initiation mechanism of failure in the layered scaffold require higher stress level than that in the mono scaffold, resulting in the effective improvement of the mechanical properties as shown in Fig.3.

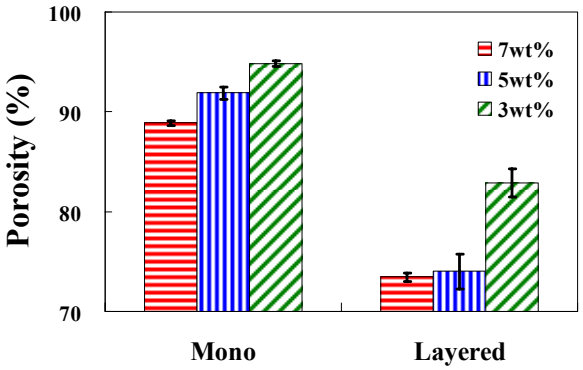
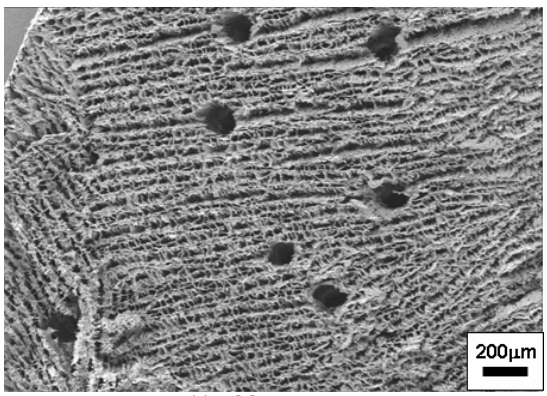
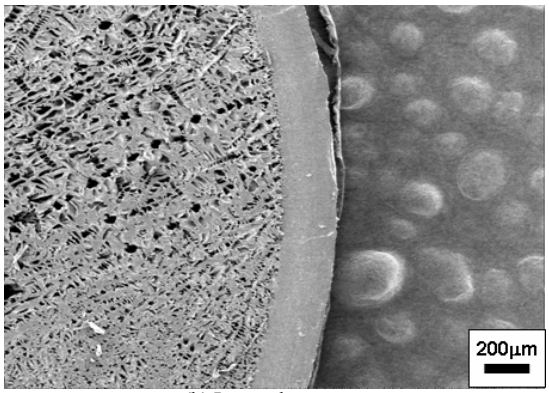


Fig. 1 Porosities of Scaffolds

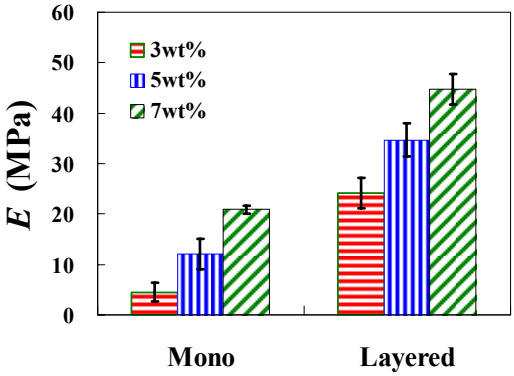


(a) Mono-structure

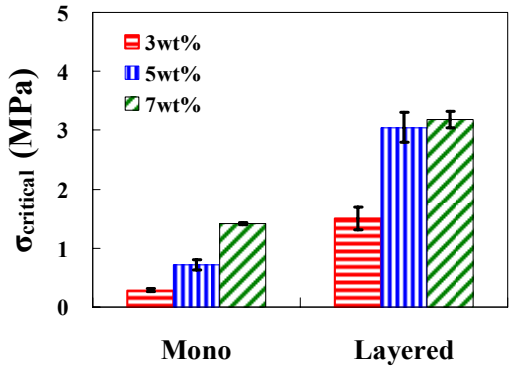


(b) Layered-structure

Fig. 2 Microstructures of PLLA scaffolds

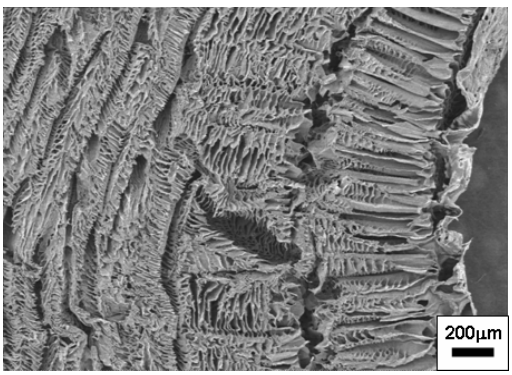


(a) Elastic modulus



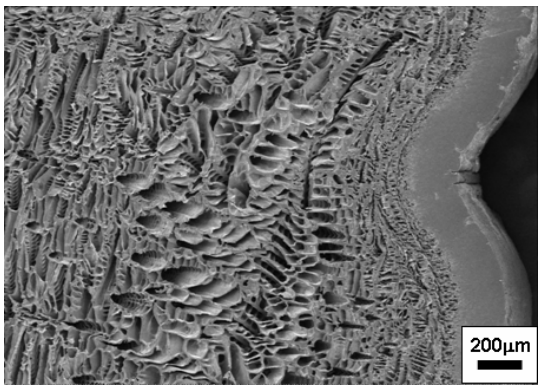
(b) Strength

Fig. 3 Compressive mechanical properties



(a) Mono-structure

Fig. 4 Deformation behaviors of PLLA scaffolds



(b) Layered-structure

Fig. 4 (continued)

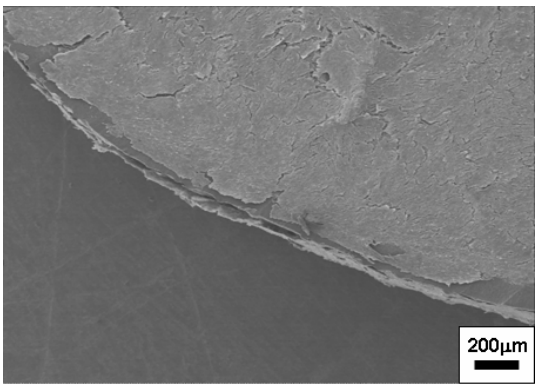


Fig. 5 Adhesion behavior of MC3T3-E1 cells on layered scaffold

C. *MC3T3-E1 cell adhesion and proliferation*

Adhesion behavior of MC3T3-E1 cells on the surface of the layered scaffold is shown in Fig.5. It was found that MC3T3-E1 cells well adhered on the surface regions of all the scaffolds. This successful adhesion and proliferation of MC3T3-E1 cells cultured for 12 days indicate that the newly developed layered scaffolds can be applied to bone tissue regeneration with non-toxicity.

IV. Conclusions

Layered structure was successfully introduced into bioabsorbable PLLA scaffolds. The scaffolds were fabricated by the solid-liquid phase separation and the freeze-drying methods. The outer solid layer was made by the thermal pressing and combined with the PLLA-Dioxane solution prior to the freeze-drying process. The average porosity of the layered scaffold was lower than the standard mono-structure scaffold; however, the porosity of the inner core was kept at the same level with the mono scaffold. The results of the compressive mechanical testing clearly showed that the mechanical properties such as the elastic modulus and the critical stress were effectively improved by the introduction of the layered structure. The primary mechanism of such improvement of the mechanical properties was found to be the load bearing effect of the outer layer. It is thus expected that further improvement of the mechanical properties may be achieved by introducing thicker outer layers. Adhesion behavior of osteblast-like MC3T3-E1 cells on the scaffolds were also assessed and it was found that those cells well adhered on the surfaces of the scaffolds.

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