DEVELOPMENT OF MULTI-LAYER COMPOSITE SCAFFOLD FOR ARTICULAR REGENERATION

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1. Introduction

Injury of multi-layered joint tissue consisting of articular cartilage and bone remains one of the major concerns in regenerative medicine [1-5]. However, the medical ability of various techniques and the repair of the cartilage and bone have limited healing capacity. The artificial joint connects many other tissues and its features reflect compositional function. These complex mechanical functions [3-4] provide the essential mechanism for load-support and stress relaxation on the joint [3]. Also, the regenerative treatment of cartilage and bone has many problems, such as meronecrosis, consequent lack of the thickness, and complex injuries and therefore, there are needs to improve current treatment. Recently, a layered scaffold has newly been developed for regeneration of articular layered tissue by using collagen and bioactive ceramics [4]. The tissue engineering approach using the layered scaffold has great potential in biological and functional regeneration of articular tissues such as cartilage and bone. However, detail of the mechanical properties and deformation behavior has not clearly been understood yet.

In this study, a novel multi-layer scaffold consisting of a porous PCL layer for cartilage regeneration and a porous PLLA/HAp composite layer for bone regeneration was developed. Compressive tests were also performed to understand the stress-strain behavior and the deformation mechanism. The linear elastic theory was applied to characterize the initial elastic deformation behavior and the finite element analysis was also performed to understand the linear elastic and the nonlinear deformation behavior under compression. The porous microstructures were characterized by a field emission scanning electron microscope (FE-SEM).

2. Materials and Methods

2.1 Specimens and characterization

The multi-layer scaffolds were fabricated by the solid–liquid phase separation and the freeze drying methods. The fabrication process is schematically shown in Fig.1. Firstly, PCL and PLLA pellets were firstly dissolved in 1,4-dioxane solution in a beaker (50 mL, warmed at 60°C) and mixed by a magnetic stirrer for 3 hours at 600 RPM with the solute concentrations of 3 and 7wt%. HAp powders were carefully mixed with the PLLA solution to obtain PLLA/HAp mixture. The solutions were then filled into polypropylene boxes and frozen at -180°C in liquid nitrogen atmosphere or at -30°C in a refrigerator. These frozen scaffolds were then orthogonally aligned and stacked to obtain a layered structure. The top was the porous PCL and the bottom was the porous PLLA/HAp composite. A contact pressure of 0.6-1 kPa was applied on the top side by placing weights. The layered scaffolds were maintained at -30°C in the refrigerator for 24h and then dried using a vacuum pump at -5°C in an ethanol bath.

The porous microstructures of the scaffolds were observed by FE-SEM. Compression tests were conducted using a universal test machine in order to examine the deformation behavior. Load was applied on the top of the specimens until they were compressed by about 80% of the height. Compressive moduli were measured from the stress-strain relations obtained.

2.2 Theoretical and finite element analyses

The simple linear elastic theory was introduced to predict the initial first elastic moduli evaluated from the compression tests. Two theoretical models were

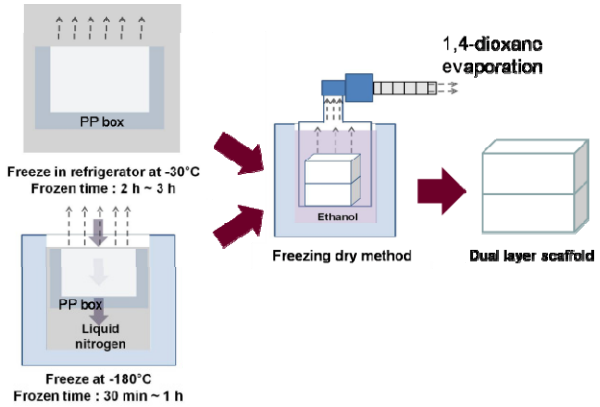


Fig.1 Schematic of fabrication process.

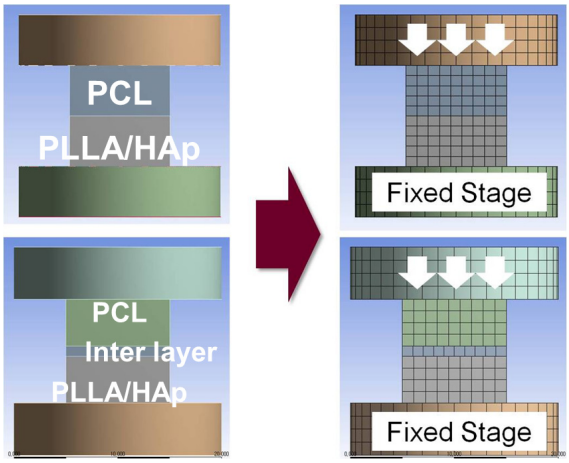


Fig.2 Theoretical and finite element models.

developed as shown in Fig.2: the first model is called the double layer model in which the multi-layer model was assumed to be composed of two layers, i.e., a PCL layer and a PLLA/HAp composite layer, and the second model is the triple layer model in which an interlayer was assumed to exist between the PCL and the composite layers. For the double layer model, the first elastic modulus, E_C, of a layered scaffold is given by:

$$E_C = \frac{hE_A E_B}{h_B E_A + h_A E_B} \qquad (1)$$

where E_A and E_B are the elastic moduli of the layer A and B, respectively. h is the total thickness of the scaffold. h_A and h_B are the thicknesses of the layer A and B, respectively. For the triple-layer model, E_C can be expressed by:

$$E_C = \frac{hE_A E_B E_{Inter}}{h_B E_A E_{Inter} + h_{Inter} E_A E_B + h_A E_B E_{Inter}} \qquad (2)$$

where h_{Inter} and E_{Inter} are the thickness and the elastic modulus of the inter layer.

The finite element analysis was also performed to predict the first elastic modulus as well as the linear elastic theoretical approach. Three-dimensional models of the double and the triple layer models were constructed as shown in Fig.2, and the elastic moduli were determined from experiments as shown in Table 1. The Poisson's ratio was set to be 0.45 for all the layers. The moduli of the interlayers were obtained as the average values of the top and the bottom layers.

The nonlinear deformation behaviors of the multi-layer scaffolds were also tried to be predicted by introducing a simple elastic-plastic model shown in Fig.3. In this model, the plastic deformation was assumed to be expressed as a kind of perfectly plastic model in which there is no stress hardening behavior.

Table 1 Modulus values for FEA.

Layers	Elastic modulus (MPa)
PCL3(-30)	0.178
PCL7(-30)	1.22
PLLA/HAp3(-30)	0.995
PLLA/HAp7(-30)	7.96
PCL3(-180)	0.42
PCL7(-180)	2.26
PLLA/HAp3(-180)	3.55
PLLA/HAp7(-180)	10.50

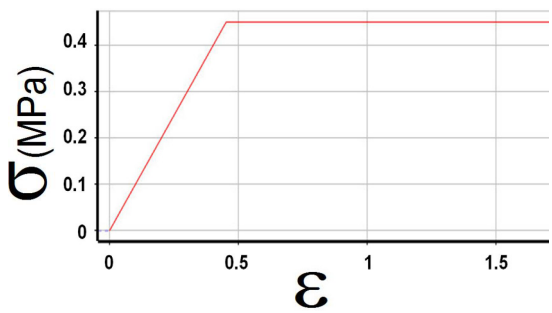


Fig.3 Elastic-plastic model for nonlinear FEA.

3. Results and Discussion

3.1 Microstructures

The FE-SEM micrographs of the porous microstructures are shown in Fig.4. By comparing the two micrographs, it is understood that the lower fabrication temperature, -180°C, results in much denser porous structure than -30°C. It is also noted that the higher concentration, 7wt%, of the PCL and PLLA/HAp solutions results in the denser porous structure than 3wt%. It is thus understood that the frozen temperature and the concentration of solution can be used as the parameters to control the size and the porosity of the scaffolds. It is also noted that for each of the scaffolds, an interlayer exists between the PCL and the composite layers. The thickness of the interlayer was estimated to be roughly 600 to 800 μm. The interlayer is thought to be constructed by PCL/PLLA/HAp composite materials.

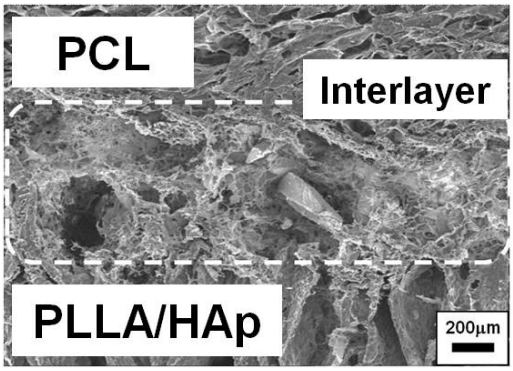
3.2 Compressive deformation behavior

Typical stress-strain relations obtained from the compressive tests are shown in Fig.5. It is noted that all the stress-strain curves basically exhibited double-linear elastic deformation behavior. The first elastic deformation is thought to be mainly related to the elastic deformation of the PCL layer that has much lower modulus than the composite layer. On the other hand, the secondary elastic deformation behavior appears to be related to the elastic deformation of PLLA/HAp layer and partially the deformation of the interlayer and, may be, the elastic deformation of the solidified PCL layer.

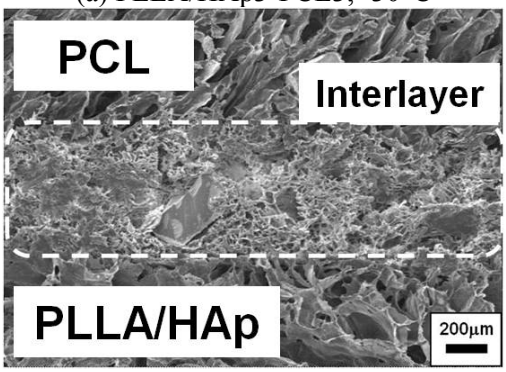
The dual modulus values of the multi-layer scaffolds are shown in Fig.6. It is easily seen that E1 that is related to the first elastic deformation is always lower than E2, which is related to the secondary deformation. The moduli of the scaffolds made from the 7wt% solution are much larger than those of the scaffolds made from the 3wt% solution. It is also noted that the moduli of the scaffold fabricated at -180°C are much larger than those of the scaffold fabricated at -30°C because of the denser porous structure.

3.3 Theoretical analysis and FEA

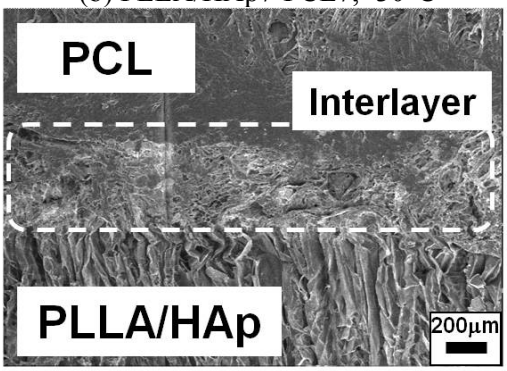
Comparison of E1 values of PLLA/HAp3-PCL3 obtained from the experiment, theory and FEA is shown in Fig.7. It is clearly seen that they coincided well each other. It is however noted that the theory 2



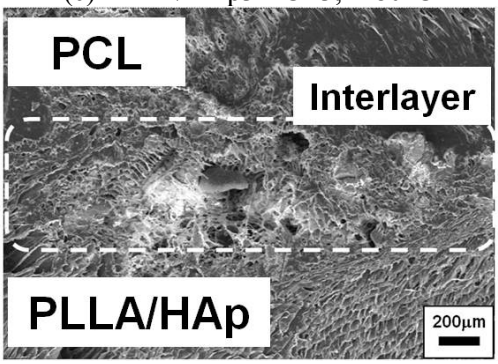
(a) PLLA/HAp3-PCL3, -30°C



(b) PLLA/HAp7-PCL7, -30°C



(c) PLLA/HAp3-PCL3, -180°C



(b) PLLA/HAp7-PCL7, -180°C

Fig.4 FE-SEM micrographs of porous structures of the multi-layer scaffolds

and the FEA 2, these are the triple layer models, tend to overestimate the E1 values compared to the dual layer models. The reason has not been clarified yet although the actual structure of the scaffolds is close to the triple layer model. It is also noted that the theoretical results are slightly better than the FEA results although they used the same material constants, i.e. Young's moduli and Poisson's ratios. This overestimation by FEA could be related to the boundary condition used in the current analysis.

The stress-strain relations obtained from the nonlinear FEA are shown in Fig.8. Although the predicted relations do not perfectly fit to the actual behaviors experimentally obtained, it should be noted that the dual elastic deformation behaviors were reasonably predicted by the simple elastic-plastic model introduced in the current analysis. This result clearly indicates that the nonlinear stress-strain behavior can well be predicted by introducing a suitable elastic-plastic model.

4. Conclusion

Multi-layer scaffolds were successfully developed for regeneration of articular joint tissues consisting of cartilage and bone. Characterization of the compressive deformation behavior was performed and the following results are obtained:

(1) The compressive stress-strain relation showed two different linear elastic deformations. The first elastic deformation is thought to be mainly related to the deformation of the PCL layer and the second one is related to the deformation of both the PLLA and the interfacial layers.

(2) The second modulus E2 is always much higher than the first modulus E1 since the E2 is related to the deformation of the harder layer. It was also found that the moduli depend on the frozen temperature and the concentration of the solution since these parameters affect the porous microstructures of the scaffolds.

(3) The linear elastic theory and 3D-FEA were successfully introduced to characterize the initial elastic deformation behavior under compression. Especially, these theoretical models well predicted the first modulus E1 values.

(4) A nonlinear elastic-plastic model was used to predict the nonlinear stress-strain behavior of the scaffolds. The FEA results were not perfectly fitted to the experimental results; however, the model could express the dual deformation behaviors.

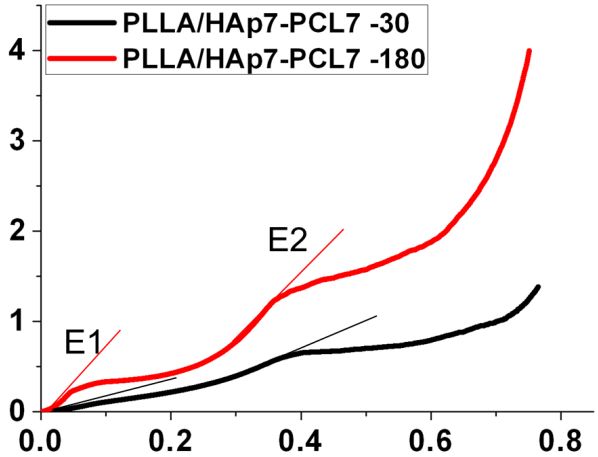
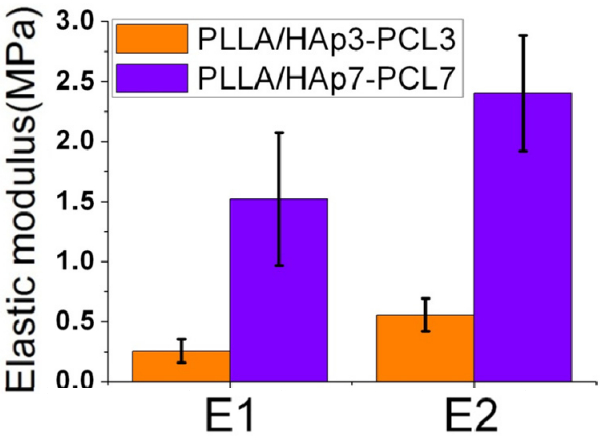
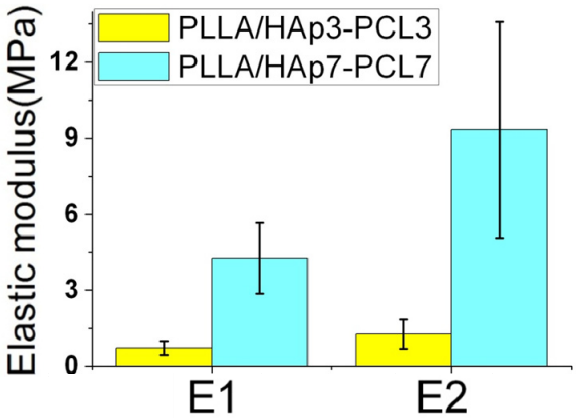


Fig.5 Typical stress-strain relation.

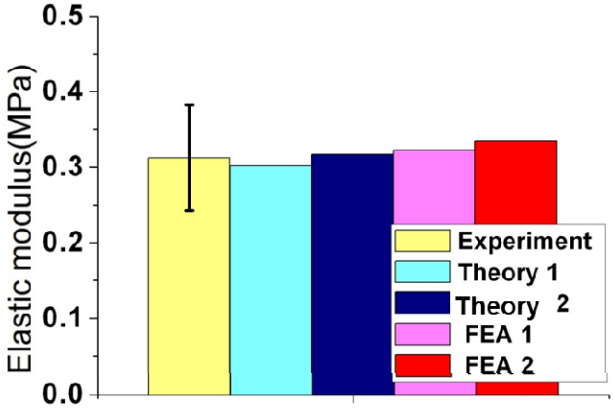


(a) Frozen at -30°C

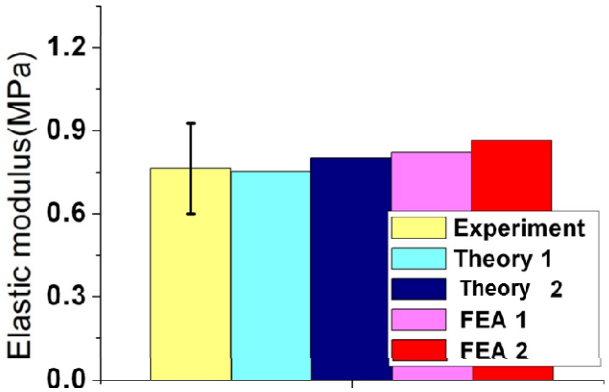


(b) Frozen at -180°C

Fig.6 Dual moduli of multi-layer scaffolds.

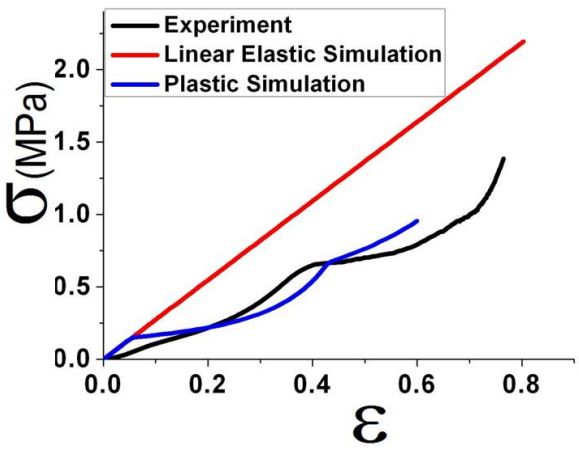


(a) Frozen at -30°C



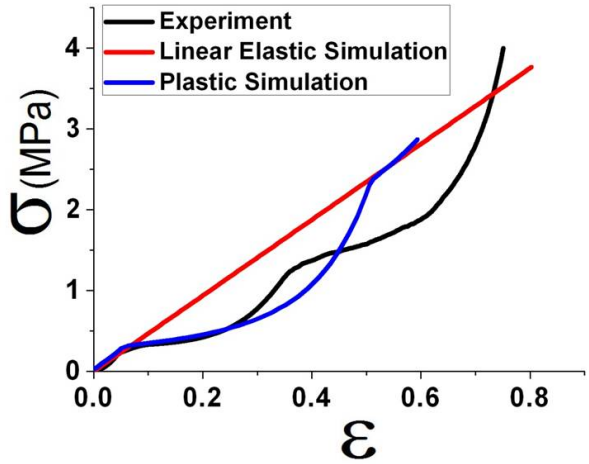
(b) Frozen at -18°C

Fig.7 Comparison of E1 values of PLLA/HAp3-PCL3 obtained from experimental, theory and FEA.



(a) Frozen at -30°C

Fig.8 Nonlinear stress-strain behavior of PLLA/HAp7-PCL7 with FEA result.



(b) Frozen at -180°C

Fig.8 (continued)

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