Compressive deformation behavior of multi-layer scaffold for bone-cartilage interface tissue regeneration

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1. Introduction
Tissue engineering has successfully applied to regeneration of hard tissue such as bone, while the regeneration of articular tissue has a limited capacity for self-repair and mechanical properties. The articular joints are composed of different kinds of tissues such as cartilage and bone, and its features are reflected by the compositional functions of these tissues. The current regenerative treatments for articular tissues have some problems such as nocicecence, lack of thickness and complex damages, and therefore regeneration technology has been tried to be improved.

Recent trend in this field has aimed at creating multi-layer scaffolds with the same functions as articular joints. In this study, poly-L-lactic acid (PLLA) and poly(e-caprolactone) (PCL) scaffolds were used to create biocompatible and biodegradable layered scaffolds with porous structure. The scaffolds were designed with a separate inner structure to achieve similar mechanical properties of articular joints.

2. Experiment
Two different polymers were used to fabricate dual-layer scaffold. The layered scaffold consists of the porous layer on the top and the reinforced layered structure on the bottom. The top layer was fabricated from PCL pellet (Daiced Chemistry Industries Co.). On the other hand, the bottom layer was fabricated from PLLA pellets (TOYOTA Co.). The molecular weight of PCL is $M_w=1.4 \times 10^4$ g/mol, the glass transition temperature $T_g=-62.62^\circ$C and the melting point $T_m=65.4^\circ$C. The molecular weight of PLLA is $M_w=2.2 \times 10^5$ g/mol, with glass transition temperature $T_g=68.28^\circ$C and melting point $T_m=173.03^\circ$C.

To fabricate the solid disk reinforcement for the bottom layer, PCL pellets were filled in a glass bottle and baked in an oven at 190°C for 6 hours. After the bottle was cooled at room temperature, the melted PCL was removed and cut into 10 mm lengths. Each piece of PCL was then drilled to create a hole to insert porous core. The outer and inner diameters and height of cylindrical shell were about 8 mm, 4 mm and 10 mm, respectively.

The PCL-PLLA layered scaffolds were prepared by dissolving each polymer in 1,4-dioxane with solute concentrations at 3 and 7 wt%. Each solution was filled into separate polypropylene boxes and frozen in -30°C refrigerator. The PLLA solid-disk was placed in the box so that reinforced structure was created. Once frozen, the porous PCL and PLLA were orthogonally stacked on top of each other, and a contact pressure of 0.6-1 kPa was applied on the top side by placing weights. The layered scaffolds were again placed in the -30°C refrigerator for 24 hours and transferred to a freeze-drying vacuum vessel to form the porous structure. Overview of the layered scaffold is shown in Fig.1. The thickness of the top and bottom layers was about 10 mm each.

Compression test of the layered scaffold was performed to estimate the mechanical properties. The test was conducted using a universal testing machine with a 1 kN load cell. The load was applied on top of each specimen until the specimen was compressed by 80% of the original height. The compressive modulus was then obtained from the stress-strain relation. Field emission scanning electron microscope (FE-SEM) was also used to characterize the micro structure of the dual-layered scaffolds.

Porcine hip and knee joints were used to characterize the compressive deformation behavior of real osteochondral tissues. They were firstly placed in an ethanol bath for 24 hours to remove trabecular tissue and marrow, and rinsed in a distilled water bath for 2-3 hours. The joints were then cut into cylindrical specimens 10 mm in length and 8 mm in diameter. Overviews of porcine knee joint and an osteochondral specimen are shown in Fig.2. Compressive test was performed to characterize stress-strain behavior and the microstructures were also observed by FE-SEM.
PLLA existed in the interfacial region between the PCL and PLLA layers. On the other hand, the thickness of cartilage layer was about 0.8mm for knee joint and 0.3mm for hip joint. Hard cancellous bone layer exists just beneath the cartilage layer.

Typical stress-strain curves under compression are shown in Figs.4(a) and (b). The knee and hip layered tissues showed initial low-stress region with low elastic modulus corresponding to the deformation of soft cartilage tissue. The subsequent high-stress region was generated by the deformation of cancellous bone. The stress-strain behaviors of the layered scaffolds are very similar to those of osteochondral tissues, although the stress level was much higher. The difference of stress level may be caused by the geometrical difference since the thickness of PCL layer (about 10mm) is much thicker than that of cartilage layers (less than 1mm).

The initial slope and the secondary slope of the stress-strain curves shown in Fig.4 are understood to be the elastic moduli related to cartilage or PCL layer and bone or PLLA layer, respectively. The elastic moduli are summarized in Table 1 with the moduli of human cartilage and cancellous bone. It is noted that the initial modulus of the tissue specimen corresponding to the cartilage layer lies between the initial moduli of PCL3 and PCL7. This clearly suggests that such cartilage modulus can be imitated by controlling the concentration of PCL solution with 1,4-dioxane. It should be also noted that the secondary moduli of the scaffolds are comparable to the elastic modulus of human cancellous bone.

4. Conclusion
In summary, dual-layer scaffolds with similar compressive mechanical properties as porcine osteochondral tissues were developed from biocompatible and biodegradable PCL and PLLA polymers. The top soft layer is porous PCL layer imitating cartilage tissue. The bottom layer has a solid PLLA disk insert as reinforcement to improve the stiffness that is comparable to cancellous bone.

Reference

![Dual-layer scaffold](image1)

![Osteochondral tissues of porcine knee joint](image2)

Fig.3 Microstructures of scaffold and tissue specimen.

![Stress-strain curves](image3)

(a) Osteochondral specimens

(b) Dual-layer specimens

Fig.4 Stress-strain curves under compression.

Table 1 Initial and secondary elastic moduli (unit : MPa)

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<thead>
<tr>
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<th>Initial modulus</th>
<th>Secondary modulus</th>
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<tbody>
<tr>
<td>Porcine knee joint</td>
<td>1.24</td>
<td>144</td>
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<tr>
<td>Porcine hip joint</td>
<td>0.40</td>
<td>91.5</td>
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<tr>
<td>PCL3-PLLA3/disk</td>
<td>0.18</td>
<td>287</td>
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<tr>
<td>PCL7-PLLA7/disk</td>
<td>2.05</td>
<td>279</td>
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<tr>
<td>Human cartilage</td>
<td>0.4-0.8 [4]</td>
<td>-</td>
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<tr>
<td>Human cancellous bone</td>
<td>-</td>
<td>247-356 [5]</td>
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