Biomechanical properties of novel biodegradable poly ε-caprolactone–chitosan scaffolds

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biodegradable, biomechanical properties, chitosan, poly ε-caprolactone, scaffold.

Abstract
Aim: To investigate the biomechanical properties of poly ε-caprolactone (PCL)–chitosan (CS) scaffolds fabricated by the melt stretching and multilayer deposition technique.

Methods: The PCL–CS scaffolds containing CS at 0% (pure PCL), 10%, and 20% by weight were prepared. For the monolayer scaffolds, shear and blending tests simulating the reconstruction of orbital floor defects (situation A) and mandibular defects (situation B) were conducted. For the 3-D scaffolds, compression tests of their superior and lateral aspects were done.

Results: For the monolayer scaffolds, the pure PCL group had remarkably lower shear strength than the other groups (P > 0.05). In situation A, all groups withstood the forces without any significant difference. In situation B, the pure PCL group could withstand the forces remarkably lower than those of the other group (P < 0.05). The 3-D scaffolds of all groups could withstand compressive forces directed towards their superior aspects. However, they could not withstand the forces directed towards their lateral aspects at the limited strain.

Conclusions: The monolayer scaffolds were suitable for reconstruction of the orbital floor and mandibular defects under light load-bearing conditions. The 3-D scaffolds could be used in the high load bearing-areas only if the forces were directed at their superior aspects.

Introduction
Melt stretching and multilayer deposition (MSMD) is the novel technique developed by our institute for fabricating 3-D polymeric scaffolds. MSMD is named so because of the steps of processing; its concept is demonstrated in Figure 1. Poly ε-caprolactone (PCL)–chitosan (CS) composite scaffolds containing 0% (pure PCL), 10%, and 20% CS by weight have been successfully fabricated using the MSMD technique. The fabricating protocols of the pure PCL and PCL–CS scaffolds are described in our previous study.¹ The average pore size and porosity of the MSMD scaffolds were 536.90 ± 17.91 μm and 45.99 ± 2.8%, respectively. One of the major advantages of the MSMD scaffold system is that it provides both the monolayer and 3-D scaffolds available for several types of bone defects. In practice, these scaffolds should withstand forces in many directions; not only after grafting, but also during surgical manipulation. For example, the monolayer scaffolds should withstand compression and bending forces when they are used to reconstruct floors of orbits, the maxillary sinus, and the body of mandibles (Figure 2a
and 2b). In addition, the contact points of their filaments should withstand shear forces during surgical manipulations. For 3-D scaffolds, not only the vertical compression forces, but also forces from lateral sides, would affect their strength (Figure 2c). In our previous study, we found that increasing CS proportions tended to reduce tensile strength and elasticity of the PCL–CS filaments, whereas the compressive strength of the PCL–CS scaffolds was not affected. However, to confirm that the strength of the MSMD scaffolds was sufficient for reconstructing bone defects in several areas of the oral and maxillofacial region, the mechanical properties of the scaffolds have to be intensively investigated. The purpose of the present study was to evaluate the biomechanical properties of the MDMS scaffolds responding to the forces that were simulated for clinical circumstances.

**Materials and methods**

**Study groups and scaffold fabrication**

The MSMD scaffolds were divided into three study groups according to the percentage of CS by weight (pure PCL group: PCL 100%, PCL–CS groups: divided into PCL–10% CS group and PCL–20% group). In each group, the specimens were prepared as monolayer scaffolds and 3-D scaffolds ($n = 6$ group/testing). The specimens of the 3-D scaffolds were made by depositing three square-shaped monolayer scaffolds of $10 \times 10$ mm, with the lay-down pattern of $0^\circ/90^\circ$ and staggering between each layer (c,d). Regarding this, the regularity of the interconnecting structure and pore size is controllable.

**Mechanical testing**

The scaffolds were immersed in simulated body fluid (SBF) and incubated at 37°C for 24 h before the experiments. SBF was prepared in accordance with Kokubo et al.’s study, and consisted of ion concentrations nearly equal to those of human blood plasma. The specimens were tested in the wet stage using a universal testing machine.
machine (Lloyd, Bognor Regis, West Sussex, UK). The mechanical properties of the scaffolds were determined by the load-placement curves using data analysis software (NEXYGEN; UK).

Shear and bending tests of the monolayer scaffolds

For the shear test, the specimens were square-shaped monolayer scaffolds of 20 × 20 mm. The filaments of the scaffolds on the upper and lateral sides were immobilized using custom-made holders (Figure 3a and 3b). Vertical forces (250 N load cell) were applied to the scaffolds at a speed of 10 mm/min to pull out their filaments until all contact points were broken; maximum loads of the tests were then recorded.

For the bending tests, square-shaped monolayer scaffolds of 30 × 30 mm were used for testing. The testing was divided into two situations. In situation A, the reconstruction of orbital floor defects was simulated. The scaffolds were placed against the compressing probe (15.77-mm diameter) and immobilized using the custom-made holder (Figure 3c and 3d). In situation B, the reconstruction of mandibular defects was simulated. The scaffolds were bent and put into the slot of the custom-made holder against the blade-type compressing tip of 1.93 × 20 mm (Figure 3e and 3f). Vertical forces were applied to the mid-portion of the scaffolds at a cross-head speed of 10 mm/min until the scaffolds were deflected to 7 mm; maximum testing loads were then recorded.

Compression test of the 3-D scaffolds

For the superior aspects, the scaffolds were put on the flat testing platform against the compressing probe (15.77-mm diameter; 5-kN load cell) (Figure 4a and 4b). Vertical force from 0 to 300 N was applied to their superior aspects at a cross-head speed of 10 mm/min. The stress–strain behaviors were assessed by the load–displacement curve. For the lateral aspects, the lateral aspects of the scaffolds were put upright against the compressing probe (15.77-mm diameter; 250 N load cell) and secured using a vice grip (Figure 4c and 4d). Vertical force was applied
to their lateral aspects at the cross-head speed of 10 mm/min until their strain level reached 50%. The stress–strain behaviors were assessed by the load–displacement curve.

Statistical analysis
The data were analyzed using statistics analysis software (SPSS version 14.0; SPSS, Chicago, IL, USA). Morphologies of the scaffolds after applying the forces were investigated using descriptive analysis. Their mechanical data were assessed by parametric statistics. One-way ANOVA was applied to compare all parameters among the study groups. Multiple comparisons with the Tukey’s honestly significant difference (HSD) test were made where variances were homogeneous; otherwise Dunnett’s T3 test was performed. The level of statistical significance was set at \( P < 0.05 \).

Results
Monolayer scaffolds
The mechanical properties of the monolayer scaffolds are demonstrated in Table 1 and Figure 5. The results showed that the maximum loads for shearing the scaffolds among the study groups were not statistically different (ANOVA, \( F = 2.66 \), d.f. = 2; \( P = 0.11 \)). The highest maximum load was found in the PCL–10% CS group, followed by the PCL–20% CS group and the pure PCL group, respectively.

For the bending tests, the scaffolds could withstand the compression forces of both situations well. In situation A, some filaments of the scaffolds split out, and the scaffolds were slightly deformed after finishing the applied forces. Tendency of the filament splitting was found in the pure PCL scaffolds more than that in the PLC–CS scaffolds. The maximum loads for bending in situation A of all groups were not statistically different (ANOVA, \( F = 0.21 \); d.f. = 2; \( P = 0.81 \)). The highest maximum load in situation A was found in the PCL–20% CS group, followed by the pure PCL group and PCL–10% CS group, respectively. The maximum loads in situation B among the study groups were statistically different (ANOVA, \( F = 4.47 \); d.f. = 2; \( P = 0.041 \)). The highest maximum load in situation B was found in the PCL–10% CS group, followed by the PCL–20% CS group and pure PCL group, respectively. It was noted that the maximum load of the PCL–10% CS group was significant higher than that of the pure PCL group (Tukey’s HSD, \( P = 0.03 \)).

3-D scaffolds
The mechanical properties of the 3-D scaffolds are demonstrated in Table 2 and Figures 6 and 7. The scaffolds of all groups could withstand the forces in the superior direction very well. After applying the forces, the scaffolds could recover to their initial dimensions without distortion. The stress at maximum load of the 3-D scaffolds among the study groups was not statistically different (ANOVA, \( F = 3.44 \); d.f. = 2; \( P = 0.058 \)). The highest stress at maximum load was found in the PCL–20% CS group, followed by the PCL–10% CS group and pure PCL group, respectively. The strain at maximum loads among the study groups was statistically different (ANOVA, \( F = 7.44 \); d.f. = 2; \( P = 0.005 \)). The strain at maximum load of the PCL–20% CS group was significantly higher than those of the other groups (Tukey’s HSD, \( P = 0.036 \) and 0.006, respectively). The highest Young’s modulus was found in the PCL–10% CS group, followed by the PCL–20% CS group and pure PCL group, respectively, but there was no significant difference (ANOVA, \( F = 0.87 \); d.f. = 2; \( P = 0.44 \)). The scaffolds of all groups could not withstand the forces in the lateral direction. Most of the contact points between the layers of the scaffolds were broken before reaching their strain of 50%. However, each layer of the scaffolds was still intact. The maximum loads of the scaffolds among the study groups were statistically different (ANOVA, \( F = 4.53 \); d.f. = 2; \( P = 0.03 \)). The highest maximum load was found in the PCL–10% CS group, followed by the PCL–20% CS group and pure PCL group, respectively. The maximum load of the PCL–10% CS group was significantly higher than that of the pure PCL group (Tukey’s HSD, \( P = 0.023 \)). The stress at maximum load among the study groups was not statistically different (ANOVA, \( F = 0.164 \); d.f. = 2; \( P = 0.85 \)). Correspondingly, the highest Young’s modulus was found in the PCL–10% CS group, followed by the pure PCL group and PCL–20% CS group, respectively, but there was no significant difference among the groups (ANOVA, \( F = 0.21 \); d.f. = 2; \( P = 0.81 \)).

Table 1. Mechanical properties of the monolayer scaffolds

<table>
<thead>
<tr>
<th>Maximum load (N)</th>
<th>Groups</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pure PCL</td>
<td>PCL–10% CS</td>
</tr>
<tr>
<td>Shear force</td>
<td>4.32 ± 1.72</td>
<td>13.15 ± 6.63</td>
</tr>
<tr>
<td>Bending force;</td>
<td>39.53 ± 14.05</td>
<td>36.84 ± 9.46</td>
</tr>
<tr>
<td>situation A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(orbit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bending force;</td>
<td>3.43 ± 0.11</td>
<td>5.00 ± 0.61*</td>
</tr>
<tr>
<td>situation B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mandible)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CS, chitosan; PCL, poly ε-caprolactone.

*\( P < 0.05 \).
Figure 5. Bar graphs of the mean maximum loads applied to the monolayer scaffolds are demonstrated as shear force (a), and bending force situation A (b) and situation B (c), *P < 0.05, significant against the pure poly ε-caprolactone (PCL) group. CS, chitosan.

Table 2. Mechanical properties of the 3-D scaffolds

<table>
<thead>
<tr>
<th>Groups</th>
<th>Stress at maximum load (MPa)</th>
<th>Strain at maximum load (%)</th>
<th>Young's modulus (MPa)</th>
<th>Maximum load (N)</th>
<th>Stress at maximum load (MPa)</th>
<th>Young's modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PCL</td>
<td>2.78 ± 0.04</td>
<td>32.40 ± 2.24</td>
<td>13.05 ± 0.70</td>
<td>7.52 ± 1.23</td>
<td>0.52 ± 0.16</td>
<td>26.50 ± 7.00</td>
</tr>
<tr>
<td>PCL–10% CS</td>
<td>2.81 ± 0.04</td>
<td>30.47 ± 1.06</td>
<td>15.01 ± 1.34</td>
<td>12.25 ± 0.78*</td>
<td>0.54 ± 0.02</td>
<td>27.04 ± 4.22</td>
</tr>
<tr>
<td>PCL–20% CS</td>
<td>2.96 ± 0.07</td>
<td>38.14 ± 0.51*</td>
<td>13.82 ± 1.05</td>
<td>9.99 ± 1.25</td>
<td>0.48 ± 0.06</td>
<td>22.49 ± 5.16</td>
</tr>
<tr>
<td>P-value</td>
<td>0.058</td>
<td>0.005*</td>
<td>0.44</td>
<td>0.03*</td>
<td>0.85</td>
<td>0.81</td>
</tr>
</tbody>
</table>

CS, chitosan; PCL, poly ε-caprolactone.
*P < 0.05.

Figure 6. Bar graphs demonstrate the mechanical properties of the 3-D scaffolds when the compression forces were applied to the superior aspects, *P < 0.05, significant against the pure poly ε-caprolactone (PCL) group and PCL–10% chitosan (CS) group.
Discussion
This study tried to simulate the possible reconstruction situations of bone defects in the oral and maxillofacial region. Therefore, the scaffolds were tested under wet conditions after immersing in SBF and incubated at body temperature for 24 h. After that, forces similar to those in humans were applied to the scaffolds. A compression force of 300 N was applied to the superior aspects of the 3-D scaffolds, which simulated the human direct bite force. During the preliminary experiment, this force was found to be unsuitable for applying to the monolayer scaffolds, as well as lateral aspects of the 3-D scaffolds. The monolayer scaffolds were too flexible, resulting in their compression and reaching their maximum deflections by applying minimum loading forces. In addition, when applying that compressive force to the lateral aspects of the 3-D scaffolds, their contact points between the layers were broken before reaching this load level. Thus, these tests measured only the maximum loads at the limited deflections of the scaffolds.

The purpose of the shear test was to specifically assess the strength of the contact points of the monolayer scaffolds. The results showed that the strength of those of the PCL–CS scaffolds was much higher than that of the pure PCL scaffolds. The shear strength is important, due to the fact that the scaffolds should withstand the forces of surgical manipulation, especially when they are applied to small defects, such as periodontal defects and tooth sockets. In the cases of orbital floor and mandibular reconstructions, the tests limited the maximum deflection of the scaffolds at 7 mm, which was quite higher than what occurs in real circumstances. In addition, the distance was the average maximum displacement of the common orbital reconstruction materials. The result showed that the monolayer scaffolds of all groups had good flexibility and could withstand the compression forces at that limited deflection distance without collapsing. In situation A, the scaffolds could withstand the maximum load between the range of 36–47 N. Haug et al. measured the average weight of the human orbital contents, which was 42.97 ± 4.05 g. Thus, the strength of the scaffolds is adequate for the compression forces, due to the weight of the orbital contents. Similar to our study, Haug et al. also evaluated the ability of various commercial internal orbital reconstruction materials to resist loads under circumstances that resemble internal orbital reconstruction. The materials included resorbable materials (e.g. poly-l-lactic, polyglycolic acid and polytetrafluoroethylene sheet), titanium meshes, and dried calvarium. Comparatively, the strength of the MSMD monolayer scaffolds was more superior to those resorbable materials, due the better strength within the same range of deflection distance. The monolayer scaffolds can also be applied for reconstructing maxillary defects as the tray or framework for bone graft materials. Similarly, light forces seem to occur in this circumstance. In situation B, the maximum loads at the limited deflection distance were quite low (in the range of 3–5 N). This was due to the lateral aspects of the scaffolds not being fixed and still slightly mobilized, thus they could be compressed with light forces, due to their flexibility. However, none of these scaffolds deformed, and no split-out filament was found after applying the forces. In practice, the monolayer scaffolds are suitable for recon-
structing large three-wall defects, and can be used as a mesh tray or stent for holding a volume of bone graft materials (Figure 2b).

This application is similar to a previous study that used biodegradable poly (L-lactide) (PLLA) scaffolds as mesh sheets for reconstructing continuity defects in the mandible. The PLLA sheets were made by weaving filaments fabricated by spinning PLLA at 245°C and drawing it at 80°C. The sheets, combined with particulate cancellous bone and marrow, were successfully applied for reconstructing 2.5-cm continuity defects in the canine mandible. However, the sheets had to be stabilized with stainless steel wires on each side of the mandibular stumps due to their flexibility. In such a case, The MSMD scaffolds can be adapted and contoured on polyvinyl templates, which are duplicated from the defects in rapid prototyping models. The shapes of the scaffolds can be sustained by immersing them in warm water at 55°C. By using this technique, the scaffolds can maintain their shapes without any fixations. Nevertheless, in regard to the results, the application of the monolayer scaffolds should not be used in areas of direct bite forces.

For the 3-D scaffold, the design of the lay-down pattern of 0° /90° and staggering between each layer proved to be suitable for the growth and differentiation of osteoblasts. Depositing the monolayer scaffolds in 3-D was done by compressing them in warm water at 55°C. The result of this study showed that the strength of their contact points was excellent for withstanding the compression forces directed to the superior aspects of the 3-D scaffolds up to 300 N. It is presumed that the strength is sufficient to withstand wound contraction during the healing process and direct bite forces. Nevertheless, that strength was not strong enough to withstand the forces directed towards the lateral aspects of the scaffolds. Most of the contact points between the layers were broken by minimum forces (7–10 N). Therefore, contact points between the layers are the weak parts of the 3-D scaffolds, and should be manipulated carefully during adapting into the defects. This limitation might affect cases of reconstructing small defects, such as four-wall defects. We suggest that 3-D scaffolds be prepared in the actual sizes, assisted by computer tomography and rapid prototyping models, prior to the surgery. In addition, in some situations, it is not necessary to fuse the contact points of the layers together if the scaffolds do not receive bite forces directly.

Regarding the mechanical properties of human bone, compressive strength of cancellous bone lies in the range of 2–20 MPa, while Young’s modulus is in the range of 1–3 GPa. Therefore, compressive strength of the 3-D MSMD scaffolds is comparable to that of cancellous bone, but Young’s modulus is lower than that of the bone. However, mechanical strength of the scaffolds might not be necessary to be equal to bone, as new bone regeneration will enhance the time-dependent strength of the constructed bone. Correspondingly, Chim et al. demonstrated that stiffness of their PCL–hydroxyapatite scaffolds increased after 14 weeks of subcutaneous implantation in nude mice. Regarding the mechanical properties of commercial scaffolds, which are fabricated by the melting technique, the PCL scaffolds’ lay-down pattern 0°/90° fabricated by the fused deposition modeling technique have a compressive stiffness of 4–77 MPa, a yield strength of 0.4–3.6 MPa, and a yield strain of 4–28%. Therefore, the MSMD scaffolds are comparable in strength, but higher in elasticity compared to commercial scaffolds.

In conclusion, the present study showed that the biomechanical properties of the MSMD scaffold system were suitable for reconstructing common bone defects in the oral and maxillofacial region. However, the strength of the contact points between the layers of 3-D scaffolds is still a weak point. Preforming scaffolds aided by computed tomography and rapid prototyping models are suggested prior to the surgery to avoid wrong sizing, which leads to unintentional forces of adjustment during the operation.

Acknowledgment

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