Changes in mechanical properties of poly-l-lactic acid mini-plate under functional load simulating sagittal splitting ramus osteotomy

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Changes in mechanical properties of poly-L-lactic acid mini-plate under functional load simulating sagittal splitting ramus osteotomy

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Key words
poly-L-lactic acid, mechanical properties, fatigue test,
simulated osteosynthesis model, sagittal splitting ramus osteotomy
Abstract

The purpose of this study was to investigate how the characteristics of a poly-L-lactic acid mini-plate changed with dynamic loading in an environment with hydrolytic degradation.

We prepared a mandible osteosynthesis model with specimen poly-L-lactic acid mini-plates to simulate sagittal splitting ramus osteotomy. The model was then subjected to dynamic loading, and changes in specimen shape and surface quality were observed. Specimen bending strength was then measured, and degree of hydrolytic degradation estimated. Statistical studies included an analysis of variance (ANOVA).

Neither dynamic loading nor degree of load clearly affected degree of hydrolytic degradation. The specimens maintained their original shape and bending strength for up to 4 weeks with dynamic loading of 40 N or less in an environment with hydrolytic degradation. However, at 8 weeks, under the same conditions, the specimens showed cracks or fractures, or both, together with a clear decrease in bending strength.

The results suggest that dynamic loading causes cracking in a poly-L-lactic acid mini-plate, and that growth of those cracks decreases bending strength over time, leading to fatigue fracture.
Introduction

Poly-L-lactic acid (PLLA) is often used in osteosynthesis as it does not have to be removed following surgery. Since Kulkarni et al. first reported on polylactic acid (PLA) in 1966, much research has been done on PLA, PLLA and other such bioabsorbable materials used in osteosynthesis. Some have evaluated the stability of bone segments after employment of PLLA in oral and maxillofacial surgery. Others have evaluated the dynamic and chemical properties of these materials, or changes in their physical properties with hydrolytic degradation. Some found that PLLA retains about 80% of its original bending strength for 12 weeks after hydrolytic degradation sets in, quickly decreasing in strength thereafter. Many of these studies examined the effects of a static load. However, none of these studies have investigated how dynamic functional loading (e.g., muscle force) affected bioabsorbable materials in an environment subjected to hydrolytic degradation, such as the human body. Functional loading of the jawbones by the masticatory muscles is believed to affect the strength of a PLLA mini-plate.

Therefore, the purpose of this study was to investigate how the characteristics of a poly-L-lactic acid (PLLA) mini-plate changed with dynamic loading in an environment with hydrolytic degradation by preparing mandible osteosynthesis model with a PLLA mini-plate to simulate sagittal splitting ramus osteotomy (SSRO).
Materials and methods

Specimens

PLLA mini-plates (FIXSORB®-MX, TAKIRON Co., Ltd., Tokyo, Japan) were used. The devices are produced by a forging process involving a unique method of compression molding and machining. The plates were short and straight (22 mm long, 4.5 mm wide, and 1.5 mm thick) and had 4 holes. Screw hole diameter was 1.6 mm, and the interval between screw holes was 4 mm.

Mandible osteosynthesis model

A mandible osteosynthesis model simulating a SSRO was used to investigate the effect of loading on the PLLA mini-plate (Figure 1 and 2). The two bone segments were simulated by acrylic blocks, each 35 mm long, 10 mm wide, and 7 mm thick. The blocks were joined together by the PLLA mini-plate and four titanium screws (2.0 mm Cortex screw, SYNTHESE Co., Tokyo, Japan) so that the middle of the mini-plate was aligned with a 2 mm space between the blocks, representing the divide of the mandibular angle. In this study, titanium screws were used, as we wanted to determine changes in the mechanical properties of the PLLA mini plate alone. The assembly was incorporated into a custom-made stainless-steel jig, to allow dynamic loading. The blocks were supported at either end of the jig. The block to which the load was applied was labelled the “loaded block”, and the other one, which was not loaded, was labelled the “unloaded block.” A support was placed between the unloaded block and bottom of the jig to prevent the block from rotating downwards. Load was applied to
the upper face of the loaded block, near the interface between the two blocks.

**Fatigue test**

A pneumatic dynamic load testing apparatus (Kondo Technology, Co., Tokyo, Japan) was used for dynamic loading. The apparatus (Figure 3) allows a load to be adjusted to any value from 0.5 to 50 kgf, and load is applied by means of a supporting spring and air pressure applied from above via a cylinder. It allows loading of up to a million times at 1 Hz. It has 5 axes and, therefore, allows fatigue testing on up to 5 specimens simultaneously, all within the same environment. In addition, it has a chamber which allows tests to be conducted in solution.

The mandible osteosynthesis model was placed inside the chamber of the fatigue test apparatus. Inside the chamber, a phosphate buffered saline solution (PBS, 0.1 mol, pH 7.4) was maintained at 37°C. The PBS was circulated to allow the mini-plate to be subjected simultaneously to both hydrolytic degradation and dynamic loading.

Dynamic loading was presupposed to simulate the force of the masticatory muscles against the mandibular angle. The force of loading at the site of an osteosynthesis has yet to be clarified. It was found that the maximum occlusal force of a dental arch was 40 to 60 N using a Dental Prescale one month after SSRO\(^9\). The loads in this study were set at 0 N to represent intermaxillary fixation, and at 20 N or 40 N to represent non-intermaxillary fixation. Six groups of five specimens each were used; each group was subjected to different conditions of dynamic loading (table 1).
fresh PLLA mini-plate was also used as a control to which no hydrolytic degradation or dynamic load were applied.

Load was applied and adjusted with a load cell as a dynamic load at 1 Hz and 300 sec. in a single round. The test was run 3 times every day, starting at 8:30 am, 1:00 pm, and 6:00 pm. Based on clinical experience of 4-week intermaxillary fixation and data from earlier reports\textsuperscript{7,8,13,15}, we continued testing for either 4 or 8 weeks.

Analysis of physical properties

After completion of the fatigue test, we first determined changes in shape and surface quality in each specimen. A three-point bending test was performed to determine degree of remaining strength in each specimen, after which, and the degree of hydrolytic degradation was estimated.

The specimens were observed with a charge-coupled device (CCD) microscope (VH5000, Keyence Co., Tokyo, Japan) and a scanning electron microscope (SEM) (JSM-6340F, JEOL Ltd., Tokyo, Japan). Au-Pd was deposited over the specimens for observation with the SEM.

The specimens were subjected to a three-point bending test (Span: 30 mm, Crosshead speed: 1 mm/min., Punch rod diameter: 4 mm, Support point rod diameter: 4 mm) using an autograph (DCS-5000, Shimadzu Co, Kyoto, Japan), and the load-displacement curve (range: ×20) was recorded (Figure 4).

The peak of the load-displacement curve was defined as maximum load (N). Mean load per 1 mm of displacement (N/mm) was calculated within the range of 10 N
to 20 N along the straight portion of the load-displacement curve, which denoted elastic
deformation as derived from the three-point bending test. This value was considered
to represent apparent degree of elasticity.

Degree of hydrolytic degradation was estimated by comparing the average
molecular weights, melting points, and degree of crystallization between the 4-week and
8-week groups. The PLLA mini-plate was divided into four sections according to the
screw hole positions, and the values for each section determined. These four sections
were numbered “1,” “2,” “3,” and “4,” commencing from the unloaded end of the block
(Figure 5).

The viscosity average molecular weight was obtained by the viscometric
method\textsuperscript{14}, which is used to measure the molecular weights of very large molecules.
Part of each specimen was dissolved in a 25°C chloroform solution, and an Oswald
viscometer was used to obtain limiting viscosity [\eta]. To obtain molecular weight [M_v],
Mark-Houwiks formula was applied to [\eta]\textsuperscript{16}.

Following the method defined in the specifications (JIS K-7121) for
measurement of transition temperatures in plastics\textsuperscript{5}, we calculated melting point using
differential scanning calorimetry (DSC). A small sample from each section was
melted at 10°C/min within a temperature range of 20°C to 220°C, and the DSC curve
was obtained using a heat flux differential scanning calorimeter (DSC6200, Seiko
Instruments Inc., Tokyo, Japan). Melting point was then calculated from the peak of
the DSC curve.

Reaction calorie (\Delta H_{TM}), defined as the peak area of the melting curve
obtained with DSC, was then used to obtain the degree of crystallization (%) by the following formula: \( \frac{\Delta H_{TM}}{93.7 \times 100} \).

**Statistical analysis**

The data for maximum load, apparent degree of elasticity, average molecular weight, melting point, and degree of crystallization were evaluated using a two-way analysis of variance (ANOVA) and the Tukey post hoc test at a 95% significance level (P<0.05) with the SPSS software program (SPSS 14.0J, SPSS Japan Inc., Tokyo, Japan) to establish the effects of dynamic loading and test time length.
Results

Changes in shape and surface quality of specimens (Figure 6, Table 2)

A comparison of the shapes and surface qualities of the specimens among Groups A1, A2, B1 and the control group showed no marked differences. In Group C1, white areas were observed in 3 of the 5 specimens. In Group B2, 3 of the 5 specimens had cracks beside the screw hole, directly below where dynamic load was applied. Furthermore, 2 of the 5 specimens in Group B2 and all 5 specimens in Group C2 showed fractures directly beneath where dynamic load was applied. White areas were observed around the cracks and fractures.

Magnified images of these white areas (Figure 7, 8) revealed microcracks running vertically. Furthermore, the fracture surfaces showed striations spreading upwards. All fracture surfaces revealed similar patterns (Figure 9).

Bending strength according to three-point bending test (Table 3)

Maximum load was 34.6 N in the control group, 34.4 N in Group A1 and 34.3 N in Group A2. In other words, regardless of length of time, immersion in PBS had no effect on maximum load. Maximum load was 35.9 N for Group B1 and 34.6 N for Group C1. These two groups were immersed in PBS for 4 weeks and subjected to dynamic loads of 20 N and 40 N, respectively. This means that there was no difference in maximum load between the specimens subjected to a dynamic load of 20 N and those subjected to a load of 40 N, both for 4 weeks. The 3 cracked specimens in Group B2 had a maximum load of 5.9 N; the remaining 2 specimens in the same group
and all 5 plates in Group C2 fractured and could not be measured. Thus, the groups subjected to 8 weeks of dynamic loading showed a decrease in maximum load.

The two-way ANOVA revealed a correlation between dynamic load and test time length for maximum load (ANOVA, F=234.0, df =4, P<0.01). The above results showed that the bending strength of the PLLA mini-plates decreased with, or without, dynamic loading and regardless of degree of loading over time. Bending strength showed no decrease after 4 weeks of dynamic loading of 40 N or less.

Apparent degree of elasticity was 22.8 N/mm in the control group, 21.4 N/mm in Group A1 and 23.4 N/mm in Group A2. Thus, there was no difference in the degree of elasticity, regardless of length of time of immersion in PBS. Furthermore, no difference was observed in apparent degree of elasticity between Group B1 (21.7 N/mm) and Group C1 (23.1 N/mm), both immersed in PBS for 4 weeks under dynamic loading. However, among the specimens immersed in PBS for 8 weeks under dynamic loading, the elasticity of 3 specimens in Group B2 could not be measured, as their cracks grew larger and showed no elastic deformation. Degree of elasticity could not be measured in 2 specimens in Group B2, or in any of the specimens in Group C2, as they were fractured.

The two-way ANOVA revealed a correlation between dynamic load and test time length for apparent degree of elasticity (ANOVA, F=189.6, df=4, P<0.01). This confirmed that dynamic loading reduced apparent degree of elasticity in the PLLA mini-plates over time.
**Degree of hydrolytic degradation (Table 4)**

Average molecular weight was 283 kDa in the control group, 227 kDa in Group A1 and 171 kDa in Group A2. In other words, immersion in PBS resulted in a decrease in average molecular weight over time. For specimens under a dynamic loading immersed in PBS for 4 weeks, average molecular weights were 216 kDa in Group B1 and 204 kDa in Group C1. For the two groups under dynamic loading immersed in PBS for 8 weeks, average molecular weights were 170 kDa in Group B2 and 164 kDa in Group C2. These results revealed no difference in molecular weight as a result of differences in dynamic loading between the 4-week and 8-week groups. Furthermore, the average molecular weights of any section were similar to that of the whole specimen in all groups.

The two-way ANOVA revealed no correlation between dynamic load and test time length for average molecular weight (ANOVA, F=0.1, df=4, P=0.99). Average molecular weight was not affected by dynamic load (ANOVA, F=0.2, df=2, P=0.83), but was affected by test time length (ANOVA, F=128.8, df=2, P<0.01). Tukey post hoc test on test time lengths revealed a significant difference between the control and the 4-week groups (P<0.05), and between the control and the 8-week groups (P<0.05). Thus, although average molecular weight decreased with time of immersion in PBS, it showed no significant difference with dynamic loading, regardless of degree.

The melting point of the specimens was 179.7°C in the control group, 179.8°C in Group A1 and 179.6°C in Group A2. In other words, immersion in PBS showed no effect on melting point, regardless of length of time of immersion. For the two groups
under dynamic loading immersed in PBS for 4 weeks, melting points were 179.5°C in 
Group B1 and 179.8°C in Group C1. For the two groups under dynamic loading 
immersed in PBS for 8 weeks, melting points were 179.9°C in Group B2 and 179.8°C 
in Group C2. These results revealed no difference in melting point between the 
different degrees of dynamic loading in either the 4-week or 8-week groups. 
Furthermore, in all groups, the melting points of any section were similar to that for the 
whole specimen.

The two-way ANOVA revealed no correlation between dynamic load and test 
time length for melting point (ANOVA, F=0.9, df=4, P=0.47). No effect was observed 
on melting point by dynamic loading (ANOVA, F=1.0, df=2, P=0.40) or test time length 
(ANOVA, F=0.4, df=2, P=0.70).

Degree of crystallization was 43.9% in the control group, 47.2% in Group A1 
and 47.9% in Group A2. In other words, immersion in PBS raised degree of 
crystallization over time. For the two groups under dynamic loading immersed in PBS 
for 4 weeks, degree of crystallization was 45.7% in Group B1 and 44.1% in Group C1. 
For the two groups under dynamic loading immersed in PBS for 8 weeks, degree of 
crystallization was 47.5% in Group B2 and 48.4% in Group C2. These results 
revealed no difference in degree of crystallization between the different degrees of 
dynamic loading in either the 4-week or 8-week groups. Furthermore, the degree of 
crystallization of any section was similar to that for the whole specimen in all groups.

The two-way ANOVA revealed no correlation between dynamic load and test 
time length for degree of crystallization (ANOVA, F=0.2, df=4, P=0.93). Degree of
crystallization was not affected by dynamic load (ANOVA, F=0.2, df=2, P=0.83), but was affected by test time length (ANOVA, F=31.7, df=4, P<0.01). Tukey post hoc test on test time lengths revealed a significant difference between the control and the 4-week groups (P<0.05), and between the control and the 8-week group (P<0.05). Thus, although degree of crystallization increased with time of immersion in PBS, it showed no significant difference with dynamic loading, regardless of degree.
Discussion

With increase in hydrolytic degradation, PLLA tends to show a decrease in average molecular weight, a slow rise in melting point, and an increase in degree of crystallization\textsuperscript{7,8,13,15}. In this study, a decrease in average molecular weight and an increase in degree of crystallization also occurred over time, indicating an increase in hydrolytic degradation and embrittlement. However, when no load was applied to a specimen immersed in PBS, there was no obvious difference over time in surface quality or shape of specimen. Moreover, there was no obvious difference in maximum load or apparent degree of elasticity. Therefore, we concluded that immersion in PBS for at least 8 weeks did not affect bending strength, but might have slightly reduced average molecular weight and increased degree of crystallization.

A dynamic load of 20 N applied for 4 weeks resulted in no significant change in surface quality or shape. However, a 40 N load applied for the same number of weeks resulted in a clouded white area. There was no obvious difference in apparent degree of elasticity or maximum load between different dynamic loads. In specimens subjected to dynamic loading for 8 weeks, a load of 20 N created cracks or fractures, or both, directly below the load point in some specimens. Similarly, all specimens fractured at the same place under a dynamic load of 40 N. An obvious decrease in maximum load was noted in the specimens that cracked. Furthermore, statistical analysis indicated a correlation between degree of dynamic load and length of time of test. In short, a larger dynamic load and a longer time of loading induced cracks, after which, there was a marked reduction in bending strength.
In both the 4-week and 8-week groups, there was no significant difference in average molecular weight, melting point or degree of crystallization, regardless of degree of dynamic load. Furthermore, in all mini-plates, under all conditions, there was no significant difference in average molecular weight, melting point, or degree of crystallization between the point immediately below the dynamic load and any other point. Therefore, we concluded that neither dynamic load nor degree of load affected increase in hydrolytic degradation.

Each crack or fracture was located within a white area characterized by microcracks. Predictably, these cracks and fractures were found immediately below the point where dynamic load was applied and where tensile load was concentrated. Some of the fracture surfaces showed seashell patterns running in a fan-shape from the bottom towards the upper part of the specimen. These seashell patterns were striated, a characteristic of fatigue fractures\(^3,10\). Furthermore, there was no difference in degree of hydrolytic degradation between the fractured portions and any other portion of the same specimen. Therefore, we concluded that the fractures in some of the PLLA mini-plates were caused by the spread of cracks ascribable to fatigue, rather than by hydrolytic degradation.

When a mini-plate fracture occurs after a SSRO, it may be the result of dynamic loading. In this study, we found that the larger the dynamic load, the larger the possibility of fractures. In addition, generally, reducing dynamic load is thought to lengthen the time before a fatigue fracture will break a plate. Therefore, if loading on a mini-plate during the early postoperative period is kept to a minimum, the risk of it
breaking may be decreased. A load of 40 N or less for up to 4 weeks may result in no deformation, as the plate retains its bending strength. This suggests that, if an occlusal force of 40 N or less is applied when using a PLLA mini-plate (straight type) for osteosynthesis, bending strength will be maintained for 4 weeks following removal of intermaxillary fixation.
Acknowledgments

We would like to thank Associate Professor Jeremy Williams, Tokyo Dental College, for his assistance with the English of this manuscript. We wish to express our sincere gratitude to TAKIRON Co., Ltd. for their technical assistance.
References


Figure legends

Figure 1. Mandible osteosynthesis model with PLLA mini-plate to simulate SSRO. (A) Force of masticatory muscles. (B) Distal bone support of teeth. (C) PLLA mini-plate. (D) Mandibular body. (E) Mandibular ramus.

Figure 2. Mandible osteosynthesis model. Two acrylic blocks joined together by PLLA mini-plate and four titanium screws. 2 mm space between blocks was aligned with the middle of PLLA mini-plate. Total incorporated into custom-made stainless-steel jig to allow dynamic loading. Blocks supported either end of jig. Support placed between unloaded block and jig. Load applied to upper face of loaded block near interface between two blocks. Dynamic load simulates force of masticatory muscles. Support simulates distal bone support of teeth. Loaded block simulates mandibular ramus. Unloaded block simulates mandibular body.

Figure 3. Pneumatic dynamic load testing apparatus. Load applied to assembly in chamber.

Figure 4. Load-displacement curve of three-point bending test (span: 30 mm, crosshead speed: 1 mm/min., punch rod diameter: 4 mm, support point rod diameter: 4 mm, range: ×20). Maximum load (N) is peak of load-displacement curve. Apparent degree of elasticity (N/mm) is load per 1 mm of displacement within range of 10 N to 20 N on straight portion, which denotes elastic deformation on load-displacement curve.
Mean load per 1 mm of displacement (N/mm) was calculated within range of 10 N to 20 N along straight portion of load-displacement curve, which denotes elastic deformation as derived from three-point bending test. This value was considered to represent apparent degree of elasticity.

**Figure 5.** Four sections of PLLA mini-plate. Sections numbered “1,” “2,” “3,” and “4,” commencing from end of unloaded block.

**Figure 6.** CCD photomicrograph of PLLA mini-plate after fatigue test (×25). Three of five plates in Group C1 showed white area. Three of five plates in Group B2 showed crack beside screw hole, directly below where dynamic load applied. Two of five plates in Group B2 and all five plates in Group C2 fractured and showed white area.

**Figure 7.** CCD photomicrograph after fatigue test (×50). Arrows indicate white areas. (A) White area (B) Crack (C) Fracture.

**Figure 8.** SEM images of PLLA mini-plate surfaces. White area with microcracks (×500). Arrows indicate microcracks. (A) Control (B) White area.

**Figure 9.** SEM images of fracture surface (×25). All fracture surfaces showed striations spreading upwards on mini-plate. Arrow indicates site of a fracture surface.
Figure 1.
Figure 2.
Figure 3.
Figure 4.

Maximum load = \( A \) (N)

Apparent degree of elasticity = \( \frac{10}{B} \) (N/mm)
Figure 5.
Figure 6.
<table>
<thead>
<tr>
<th>Group</th>
<th>Dynamic load</th>
<th>PBS conditions</th>
<th>Test time lengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0 N</td>
<td>37°C, pH 7.4</td>
<td>4 weeks</td>
</tr>
<tr>
<td>A2</td>
<td>0 N</td>
<td>37°C, pH 7.4</td>
<td>8 weeks</td>
</tr>
<tr>
<td>B1</td>
<td>20 N, 1 Hz, 300sec. 3 times/day</td>
<td>37°C, pH 7.4</td>
<td>4 weeks</td>
</tr>
<tr>
<td>B2</td>
<td>20 N, 1 Hz, 300sec. 3 times/day</td>
<td>37°C, pH 7.4</td>
<td>8 weeks</td>
</tr>
<tr>
<td>C1</td>
<td>40 N, 1 Hz, 300sec. 3 times/day</td>
<td>37°C, pH 7.4</td>
<td>4 weeks</td>
</tr>
<tr>
<td>C2</td>
<td>40 N, 1 Hz, 300sec. 3 times/day</td>
<td>37°C, pH 7.4</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Control</td>
<td>Fresh PLLA mini-plate, no dynamic load were applied, no hydrolytic degradation</td>
<td></td>
<td></td>
</tr>
</tbody>
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Table 2. Changes in shape and surface quality of PLLA mini plates with fatigue test

<table>
<thead>
<tr>
<th>Group</th>
<th>White areas</th>
<th>Cracks</th>
<th>Fractures</th>
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<tbody>
<tr>
<td>Group A1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group A2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group B1</td>
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<td>Group C1</td>
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<tr>
<td>Group C2</td>
<td>5</td>
<td>0</td>
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</table>
### Table 3. Maximum load and apparent degree of elasticity of PLLA mini-plates with three-point bending tests

<table>
<thead>
<tr>
<th>Group</th>
<th>Maximum load (N) Mean±SD (N=5)</th>
<th>Apparent degree of elasticity (N/mm) Mean±SD (N=5)</th>
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</thead>
<tbody>
<tr>
<td>Group A1</td>
<td>34.4±0.5</td>
<td>21.4±0.6</td>
</tr>
<tr>
<td>Group A2</td>
<td>34.3±1.2</td>
<td>23.4±1.7</td>
</tr>
<tr>
<td>Group B1</td>
<td>35.9±1.4</td>
<td>21.6±0.8</td>
</tr>
<tr>
<td>Group B2</td>
<td>5.9±0.0 (N=3) / Not completed (N=2)</td>
<td>Not completed *</td>
</tr>
<tr>
<td>Group C1</td>
<td>34.6±1.8</td>
<td>23.1±2.0</td>
</tr>
<tr>
<td>Group C2</td>
<td>Not completed *</td>
<td>Not completed *</td>
</tr>
<tr>
<td>Control</td>
<td>34.6±0.9</td>
<td>22.8±1.5</td>
</tr>
</tbody>
</table>

* Not completed due to crack or breakage
Table 4. Average molecular weight, melting point and degree of crystallization of PLLA mini-plates

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean of all sections</th>
<th>Four sections</th>
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<tr>
<td><strong>Average molecular weight (KDa)</strong></td>
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<tr>
<td>Mean±SD (N=5)</td>
<td>Group A1</td>
<td>227±27</td>
</tr>
<tr>
<td></td>
<td>Group A2</td>
<td>171±13</td>
</tr>
<tr>
<td></td>
<td>Group B1</td>
<td>216±22</td>
</tr>
<tr>
<td></td>
<td>Group B2</td>
<td>170±27</td>
</tr>
<tr>
<td></td>
<td>Group C1</td>
<td>204±23</td>
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<tr>
<td></td>
<td>Group C2</td>
<td>164±12</td>
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<tr>
<td></td>
<td>Control</td>
<td>283±18</td>
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<tr>
<td><strong>Melting point (°C)</strong></td>
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<td></td>
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<tr>
<td>Mean±SD (N=5)</td>
<td>Group A1</td>
<td>179.8±0.1</td>
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<tr>
<td></td>
<td>Group A2</td>
<td>179.6±0.2</td>
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<tr>
<td></td>
<td>Group B1</td>
<td>179.5±0.4</td>
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<tr>
<td></td>
<td>Group B2</td>
<td>179.9±0.2</td>
</tr>
<tr>
<td></td>
<td>Group C1</td>
<td>179.8±0.4</td>
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<tr>
<td></td>
<td>Group C2</td>
<td>179.8±0.3</td>
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<tr>
<td></td>
<td>Control</td>
<td>179.7±0.5</td>
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<td><strong>Degree of Crystallization (%)</strong></td>
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<tr>
<td>Mean±SD (N=5)</td>
<td>Group A1</td>
<td>47.2±4.4</td>
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<td></td>
<td>Group A2</td>
<td>47.9±0.5</td>
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<tr>
<td></td>
<td>Group B1</td>
<td>45.7±0.8</td>
</tr>
<tr>
<td></td>
<td>Group B2</td>
<td>47.5±0.8</td>
</tr>
<tr>
<td></td>
<td>Group C1</td>
<td>44.1±0.9</td>
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<tr>
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<td>Group C2</td>
<td>48.4±0.8</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>43.9±1.8</td>
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