Antibacterial Ability and Physical Properties of Poly (L-lactic acid) Film Blended with Baked Shell Powder

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Abstract: A poly (L-lactic acid) (PLLA) film, which was blended with baked shell powder, was made and the antibacterial ability, biodegradability and physical properties of it were evaluated. The PLLA film blended with the powder had no antibacterial ability although the PLLA itself had strong antibacterial ability. Moreover the biodegradation rate was faster by blending the powder in PLLA film, and in the thermal and mechanical properties, which are obtained by differential scanning calorimeter (DSC) and tensile tests, there was slightly difference between the unblended PLLA film and the blended PLLA film. So, these results suggest that the antibacterial ability of PLLA is caused by lactic acid which is created in hydrolysis and that the powder neutralizes the lactic acid.

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1. Introduction

Poly (L-lactic acid) (PLLA) is made from lactic acid which is obtained by fermentation of starch [1]. PLLA is easily hydrolyzed to lactic acid oligomer and lactic acid in the alkaline condition, and they are degraded to carbon dioxide and water by the metabolism in microorganisms. Moreover PLLA is harmless to a human body because humans can slowly metabolize it.

In the environment, PLLA is hydrolyzed into oligomer, and finally biodegraded to carbon dioxide and water by microorganisms [2]. In addition, it is reported that lactide and lactic acid is made by the hydrolysis of PLLA [3, 4]. Moreover, PLLA can be monomer recycled and of interest as not only medical materials but also environmental protection materials because it is degraded into lactide or lactic acid.

In recent years, it is reported that PLLA itself has antibacterial ability [5]. So, PLLA is of interest as an antibacterial polymer, whereas it was considered that the antibacterial ability decreases the biodegradability of PLLA.

On the other hand, a large quantity of shell, such as a scallop or oyster, is dumped and many researchers try to recycle that. In recent years, baked shell is known as a natural antibacterial material [6]. Shell is mainly made from calcium carbonate and turns into calcium oxide by baking at the temperature above 1000°C. Although this baked shell has antibacterial ability, it is only used practically in food sterilizing agent and food additives.

It is thought that one of causes of the antibacterial mechanism of baked shell is pH because it changes into calcium hydroxide and alkalinizes in water. As a result, when about 1.5g baked shell dissolves into 1 liter water to make a saturated solution, the pH of the solution is about 11~12.

Recently, it is reported that reactive oxygen species (ROS) are generated from calcium oxide, so, baked shell is considered to sterilize Bacillus subtilis spores [6, 7].

In this study, PLLA was blended with the powder, which was harmless and functional material, and the property of PLLA film blended with baked shell was investigated.

2. Experimental

2.1 Materials

2.1.1 PLLA

LACEA H-100 (Mitsui Chemical Co., Ltd.) was used as PLLA powder. Number-average molecular weight of PLLA was about 78,000 and melting point was about 160°C.

2.1.2 Baked shell powder

Baked shell powder was purchased from Calcine Co., Ltd. This baked shell powder was made of a scallop shell and the average diameter of the powder was 75~80 μm.

2.2 Methods

2.2.1 PLLA film blended with baked shell powder

In this study, the contents of the powder blended in PLLA film used were 1 %, 5 %, 10 % and 40 %, respectively.
At first, the powder was put into PLLA with various blend ratio in a mixing roll (Imoto Co., Ltd) at 185°C for 10 min and the mixed sample was cooled at room temperature. Next, that sample was held between the iron plates which were heated to 185°C for 4 min, then pressed for 1 min to mold film and took out it. Lastly, the sample was rapidly cold-pressed for 2 min.

### 2.2.2 Autoclave treatment

In this study, the PLLA film was sterilized by autoclave treatment (HIRAYAMA Co. Ltd, HA300-M). The autoclave treatment condition was 121°C under 2 atm for 15 min.

### 2.2.3 Strains

*Staphylococcus aureus* (IAM No. 12119 T) and *Klebsiella pneumoniae* (IAM No.12015) were used to estimate the antibacterial ability of PLLA film in which baked shell was blended in this study. These strains were provided by the Institute of Molecular and Cellular Biosciences of University of Tokyo.

### 2.2.4 Medium

The nutrient medium was used for the antibacterial test. This nutrient medium was composed of 3 g Bacto Beef Extract (DIFCO), 5 g Bacto Peptone (Wako) and 1000 ml water.

### 2.2.5 Antibacterial test

The qualitative test in the antibacterial test was performed by the same method as the previous paper [8], while the quantitative test was performed by the method of JIS Z 2801. JIS is an abbreviation for Japanese Industrial Standard which determines the various test methods. It is considered that the antibacterial test was valid when increase value was more than 1.5. The number of colony forming unit (CFU) was used to calculate increase value, values of inhibitory activity and sterilizing activity.

In this test, minimal value of CFU was 10. The calculation method of each value is following equations.

Increase value = \[ \log \left( \frac{\text{CFU (ST inc 18)}}{\text{CFU (ST inc 0)}} \right) \]  

Value of inhibitory activity = \[ \log \left( \frac{\text{CFU (ST inc 18)}}{\text{CFU (SA inc 18)}} \right) \]  

Value of sterilizing activity = \[ \log \left( \frac{\text{CFU (ST inc 0)}}{\text{CFU (SA inc 18)}} \right) \]  

where CFU (ST inc 18) is CFU in standard plastics after 18 h incubation, CFU (ST inc 0) is CFU in vaccination to standard plastic, and CFU (SA inc 18) is CFU in sample after 18 h incubation, respectively.

### 2.2.6 Cotton textile put with the baked shell powder

To estimate the antibacterial ability, the 0.5g baked shell powder was put on cotton textile (4cm × 5cm) uniformly. The cotton textile used in this study was the standard adjacent fabrics for color fastness test (JIS L0803).

### 2.2.7 Biodegradation test by enzyme

*Tritirachium album* proteinase K was used to estimate the degradability of PLLA film. The proteinase K was diluted with buffer, which was 100mmol Tris-HCL (pH8.5), and PLLA film blended with the powder was put into that buffer, and retained at 37°C for 6 days. The enzyme and buffer were changed each day, and at the same time, for comparison, the blank test was done in the buffer without the enzyme.

The weight loss was calculated using the following equation:

\[ \text{Weight loss} \% = \left( \frac{W_0 - W_d}{W_0} \right) \times 100 \]  

where \( W_0 \) is the weight of film before the degradation test and \( W_d \) is the weight of film after the degradation test.

In this study, glass beads of similar particle size to the powder was used as a control.

### 2.2.8 Differential scanning calorimetry

Thermal properties were measured by a Differential Scanning Calorimetry DSC-60 (Shimadzu). This was performed in a nitrogen environment, and the sample weight was 4.5 ± 0.1 mg. The blended PLLA film was heated to 185°C and cooled from 185°C to 30°C at a heating and cooling rate of 10°C/min, respectively.

### 2.2.9 Tensile test

The stress-strain curves of the films were obtained by Tensilon/UTM-III-500 (Toyo Baldwin Co. Ltd.) at room temperature. The tensile strength at break, elongation at break and Young’s modulus were calculated. The crosshead speed was 2 mm/min and the gauge length was 25 mm. The tensile test under the same condition was repeated 10 times and the average value was calculated from each sample datum.

### 3. Results and Discussion

#### 3.1 Antibacterial ability of PLLA and baked shell powder

At first, the antibacterial ability of PLLA and the powder themselves was measured. PLLA was very sensitive at high temperature because of resulting further crystalization. For comparison, before the antibacterial test of PLLA, the PLLA was sterilized by ethanol at room temperature. The antibacterial ability of the PLLA was measured, and it had the antibacterial ability (date not shown). For the antibacterial test, the PLLA film was sterilized by an autoclave treatment at the condition of 121°C and 2 atm for 15 min. Ogawa et al. reported that...
there are two steps in the hydrolysis of PLLA [9, 10]. At the first step, decrease in the molecular weight of PLLA was observed, but the weight loss of that was not observed. At the second step, if the hydrolysis becomes advanced, the soluble oligomer formed from PLLA elutes and that results in the weight decrease of PLLA [9, 10]. In case of the autoclave treatment, although the weight decrease of PLLA film was not detected, the film became translucent. Therefore, the crystallization of the film might be occurred (data not shown).

The weight-average molecular weight (Mw) of the PLLA film decreased from 85,000~110,000 to 60,000~65,000 and the number-average molecular weight (Mn) of the PLLA decreased from 60,000~70,000 to 30,000~40,000 by autoclave treatment (Table 1).

Moreover, the molecular mass of the PLLA film decreased, as the condition of autoclave treatment became severer. Therefore, in the autoclave treatment, the first step of hydrolysis is considered to occur.

It was confirmed that PLLA had antibacterial ability (Table 2).

The biodegradation of PLLA in the environment is resulted after hydrolysis. Decrease in the weight average molecular weight (Mw) by hydrolysis was considered to cause increase in the number of carboxyl group. The antibacterial ability of PLLA is considered to be caused by carboxyl group because of its acidic property. However, in the environment, there are only a few microorganisms which can degrade PLLA [11]. Therefore, there is a possibility that this antibacterial ability obstructs the biodegradation of PLLA in the environment because the lactic acid suppresses the growth of most microorganisms.

The baked shell powder also has antibacterial ability (Table 3). Generally, in the antibacterial qualitative test, the region where a microorganism can not grow, so-called halo, is not formed by an antibacterial material which is insoluble. Only 1.5 g of the powder could be dissolved into 1 liter of water. Therefore, it is considered to be hard for the powder to dissolve into the medium in the antibacterial qualitative test. So, the powder was dusted on a cotton textile as described in 2.2.6, which was used to evaluate the antibacterial ability.

### 3.2 Antibacterial ability of the PLLA film blended with baked shell powder

The antibacterial ability of PLLA film blended with the powder was measured after sterilization by ethanol and autoclave treatment. In this test, because the increase values are 2.59 (Staphylococcus aureus) and 2.31 (Klebsiella pneumoniae), respectively, the quantitative tests are valid experimentally.

The result of the antibacterial test is shown in Table 4, and the PLLA film blended with 1% the powder had been sterilized by ethanol. When it was sterilized by ethanol, the PLLA blended with the powder maintained the strong antibacterial ability.

When it was sterilized by autoclave treatment, the PLLA film blended with the powder had no antibacterial ability unexpectedly (Fig. 1). This result suggests that

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**Table 1** The molecular weight of PLLA film before and after autoclave treatment.

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight-average molecular weight (Mw)</td>
<td>85,000~110,000</td>
<td>60,000~65,000</td>
</tr>
<tr>
<td>Poly disperse</td>
<td>1.50</td>
<td>1.79</td>
</tr>
</tbody>
</table>

**Table 2** Quantitative test for antibacterial ability of PLLA film. The film was autoclaved before the antibacterial test.

<table>
<thead>
<tr>
<th></th>
<th>S. aureus</th>
<th>K. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU in vaccination</td>
<td>(7.16 \times 10^5)</td>
<td>(9.49 \times 10^5)</td>
</tr>
<tr>
<td>CFU of cotton textile (control)</td>
<td>(9.52 \times 10^5)</td>
<td>(2.37 \times 10^6)</td>
</tr>
<tr>
<td>CFU of PLLA film</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Increase value</td>
<td>2.12</td>
<td>2.40</td>
</tr>
<tr>
<td>Value of inhibitive activity</td>
<td>4.97</td>
<td>5.37</td>
</tr>
<tr>
<td>Value of sterilizing activity</td>
<td>2.85</td>
<td>2.98</td>
</tr>
</tbody>
</table>

**Table 3** Antibacterial ability of baked shell powder.

<table>
<thead>
<tr>
<th></th>
<th>S. aureus</th>
<th>K. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU in vaccination</td>
<td>(8.22 \times 10^5)</td>
<td>(6.68 \times 10^5)</td>
</tr>
<tr>
<td>CFU of PLLA film with 1% the powder</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

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PLLA was neutralized by the powder. It is expected that the lactic acid produced by hydrolysis of PLLA is neutralized by the powder whose main composition is calcium hydroxide.

While, when PLLA is sterilized with ethanol, PLLA is hardly hydrolyzed and little the powder appears at the surface area of PLLA film. However, when sterilized by autoclave treatment, PLLA is hydrolyzed and the molecular weight decreases (Table 1).

So, the powder is considered to appear to the surface of shrinked or cracked PLLA film. Therefore, the appearance of the powder on the surface of PLLA film depends on the sensitivity of the autoclave treatment condition.

As a result, it is considered that the oligomer produced by the hydrolyzation of PLLA is neutralized by the powder and the antibacterial ability of PLLA decreases. However, the growth of microorganisms was not changed when the contents of the blended powder in PLLA was more than 5%. These results suggest that the antibacterial ability of PLLA is lost even in a small amount of the powder, such as 1%. Probably, it is considered that the powder exists too much compared with carboxyl group produced by the hydrolysis of the PLLA. Therefore, there is a possibility that PLLA becomes aware of still more ecological material by blending the powder because the method to lose the antibacterial ability of PLLA was found in this study.

3.4 DSC of the PLLA film blended with the baked shell powder.

The antibacterial test of the PLLA film blended with the powder was carried out, however, for practical use, it is necessary to investigate the physical property of them. At first, thermal properties of these films were measured by DSC. These results are shown in Fig. 2.

The melting point and glass transition temperature of PLLA film slightly shifted to higher temperature by blending the powder. These changes might include somewhat experimental errors, however, they are possibly caused by blending the powder which plays the role of nucleating agents during the crystallization of PLLA in cooling. The area of melting peaks observed in Fig. 2 decreased slightly as concentration of the powder increased because of the relative decrease of PLLA content.

3.5 Tensile test of the PLLA film blended with baked shell powder.

To estimate the practical use of the PLLA products blended with the powder, we measured stress-strain curves of PLLA and calculated the tensile strength, the elongation at break and the Young’s modulus (Table 5).

When the content of the blended powder into PLLA was 1%, the tensile strength of the film was a little stiffer than that of the film without the powder. This result suggests that the PLLA film was slightly reinforced by blending the powder, while the tensile strength of the PLLA film blended the powder decreased lower by degree as the powder content increased.

The elongation at break of the PLLA film also increased slightly by blending a small amount of the powder, however, it began to decrease gradually at the larger contents.
Young’s modulus of the PLLA film blended with the powder increased with increasing the content of powder. This increase might be caused by the reinforcement effect of the powder which makes PLLA harder. These changes in tensile test might include somewhat experimental errors, however, these results indicate that the physical property of the PLLA film changes a little harder by blending the powder. So, it is necessary to investigate the change of physical property in the blended PLLA film under a certain temperature and humidity for long time. The physical property of the PLLA film blended with the powder may become more fragile because the hydrolysis of the PLLA is promoted by blending the powder.

3.6 Biodegradation test

The biodegradation of the PLLA film blended with the powder was measured by enzymatic degradation test. In the enzyme method, because the enzyme which can degrade the PLLA is superior in quantifiability and reactivity, it is possible to obtain the experimental data for short time. This result is shown in Fig. 3.

The result indicates that the weight loss of the PLLA film blended with the powder increases as the powder content increases. While, in the blank test, which is the test without enzyme, the weight loss of the film was not observed (data not shown). It is considered that there are two reasons why the weight loss of the film blended with the powder was promoted.

First, hydrolysis of PLLA was considered to be promoted by the alkaline solution which was prepared from the powder because PLLA was sensitive to alkaline condition.

In addition, the most suitable pH of proteinase K origined from *Trichirachium album* is pH 8.0-11.5. In case of the PLLA film without the powder, the pH value in the reaction buffer depressed (data not shown) and the enzyme activity declined when the PLLA was degraded and changed to lactic acid and oligomer of lactic acid. In case of the PLLA film blended with the powder, pH value in the reaction buffer does not decrease because lactic acid and oligomer of lactic acid produced by the hydrolyzation of the PLLA neutralizes the solution with the powder. Therefore, the result of enzymatic degradation test may depend on the enzymes which are used in biodegradation test. However, it is cleared that, at least, the degradation rate of the PLLA film blended with the powder is faster than that of the PLLA film without the powder whenever the suitable pH value of the enzymes is high.

Second, because the surface area of PLLA film was increased by blending the powder or the glass beads, it might be degraded by the enzyme more rapidly. The weight loss rate of the PLLA film was slightly increased by blending glass beads. The glass bead itself is not alkaline material and is considered not to react with the PLLA film. So, the increase of the surface area of PLLA film may promote the degradation by the enzyme.

However, there was a possibility that some of PLLA fragments fell off was contained in the weight loss value of the film. So, it is necessary to measure the real weight of PLLA fragment fell off in the buffer, and the quantity of lactic acid dissolving in the buffer by HPLC.

This result suggests that there is a possibility to control the biodegradation rate by blending the powder into the PLLA. Although the weight loss of the PLLA film with the powder is not always equal to the weight of the degraded film, it often includes the weight of fragments of the film fell down.

In addition, it is necessary to not only inspect the enzymatic degradation but also soil burial test.

**Table 5** Mechanical properties of PLLA blended with baked shell powder.

<table>
<thead>
<tr>
<th></th>
<th>Tensile strength (MPa)</th>
<th>Elongation at break (%)</th>
<th>Young’s modulus (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLLA</td>
<td>42.2</td>
<td>2.07</td>
<td>2.03</td>
</tr>
<tr>
<td>PLLA with 1% powder</td>
<td>49.0</td>
<td>2.26</td>
<td>2.19</td>
</tr>
<tr>
<td>PLLA with 3% powder</td>
<td>46.1</td>
<td>2.10</td>
<td>2.20</td>
</tr>
<tr>
<td>PLLA with 5% powder</td>
<td>41.8</td>
<td>1.91</td>
<td>2.20</td>
</tr>
<tr>
<td>PLLA with 10% powder</td>
<td>38.0</td>
<td>1.53</td>
<td>2.45</td>
</tr>
</tbody>
</table>

**Fig. 3** Weight loss curves of enzymatic degradation of PLLA films blended with baked shell powder.

- ● : PLLA (Control)
- ◇ : PLLA with 1% glass beads
- ■ : PLLA with 1% baked shell powder
- ▲ : PLLA with 10% baked shell powder
- □ : PLLA with 20% baked shell powder
4. Conclusions

In this study, it was cleared that the antibacterial ability of PLLA is suppressed when the contents of the powder blended in the PLLA film was more than 1%. Moreover, it was indicated that the biodegradation of PLLA was promoted by blending the powder. As a result, it is possible to give PLLA which is remarked as biodegradable plastic a new function by blending the powder origined from nature. These results suggest that the PLLA film blended with the powder is able to be applied to food container, packing bag, vegetation mat and medical supplies and so on.

Although PLLA is known as ecological friendly material for industrial and medical use, the behavior or mechanism in the degradation is not known completely. Therefore, to practical use, it is necessary to accumulate the fundamental data about the behavior in various conditions over a long term and to clarify the degradation mechanism.

References