The Preparation of PLA/PLGA Composite Membrane and Its Application as Periodontal Barrier Membrane

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Abstract. We synthesized high molecular weight polymer PLA (polylactic acid) and low molecular weight polymer PLGA [poly (lactic-co-glycolic acid)]. Then we prepared PLA/PLGA composite membrane by blending these two polymers, and tested various properties, such as physical, mechanical and biological properties for periodontal barrier. When PLA/PLGA = 2:1 (w/w), degradation rate of the membrane was 63.7% within 32 weeks. The membrane showed aperture pores of 2 - 5 µm, the minimum tensile strength of 5.75MPa, and the porosity above 90%, which was beneficial to the entry of nutrients and the elimination of metabolic wastes. Under SEM (scanning electron microscope), we observed that gingival fibroblasts only grew on the positive surface, and didn’t grow on the negative side or the cross-section, indicating that the membrane played a good role of mechanical barrier.

Keywords: Polylactic acid; Poly (glycolide-co-lactide) copolymer; Synthesis; Membrane; Periodontal barrier.

1. Introduction
Periodontitis is one of the most destructive diseases that damage the tooth root and ultimately lead to tooth loss, then the gum will grow into the defect area and block the regeneration of the root [1-2]. To regenerate periodontal tissues, various regenerative modalities have been tried, including surgical approach, barrier membrane, bone graft. Among these approaches, barrier membrane for periodontal tissue regeneration prevails in these years [3-4].

Generally, these barrier membranes need to exhibit good biocompatibility, proper degradation rate, sufficient mechanical properties and adequate tensile strength [5-10]. The majority materials suitable for the membrane are degradable and resorbable synthetic polymers, such as PLGA [poly (lactic-co-glycolic acid)], PLA (polylactic acid), PGA (polyglycolic acid), PCL (polycaprolactone), PEG (polyethylene glycol), and their copolymers or composite [11-14].

In the previous study, the biodegradable PLA/PLGA membranes had been prepared by coating PLA and PLGA on PGA mesh, and was used for guiding periodontal tissue regeneration, however, it’s function as periodontal barrier wasn’t proved because of lack of mechanical properties [15]. In order to obtain membrane materials that can meet the characteristics of periodontal barrier, PLA with low molecular weight and PLGA with high molecular weight were designed and synthesized in this study. Then, the PLA/PLGA composite membrane was prepared by simply blending two polymers in various
PLA/PLGA molar ratios, and its mechanical and biological performances were evaluated. As a green material, the PLA/PLGA composite membrane had good biodegradability and no toxicity; furthermore it showed a decrease in tensile strength but an increase in both hardness and flexibility, as compared with the individual PLA and PLGA membrane, respectively.

2. Experimental

2.1. Chemical Regent and Measuring Apparatus
An Ubbelohde viscometer, Ping Xuan NDJ-9S (China), was used for calculating relative and intrinsic viscosity of PLA and PLGA polymers. The mechanical properties of three kinds of membranes, PLA, PLGA and PLA/PLGA composite membrane, were investigated with a tensile machine of Zwick Z400E (Germany) at speed of 10 mm/min. Infrared spectrum of PLGA were recorded at a spectrometer of Shimadzu IR Prestige-21 (Japan) and the region lay between 4000 and 500 cm\(^{-1}\). The molecular weight of PLGA was measured by EcoSEC HLC-8320 GPC (Japan) using the THF (tetrahydrofuran) as the solvent at 35°C, 0.6 ml/min. The HGF (hepatocyte growth factor) grown on dental membrane was observed by a Carl Zeiss SIGMA-300 SEM (Germany). The biological barrier effect was demonstrated by inoculation of gingival fibroblasts (China) on the membrane surface.

2.2. Synthesis and Characterization of PLA and PLGA
Low molecular weight PLA resin was synthesized from L-lactic acid under the presence of 0.01g/ml stannous octoate as the initiator catalyst, with a stannous octoate / lactic acid mass ratio of 0.0008. The reaction was carried out in an oil bath at 150°C under vacuum condition till no water is generated from the system. After the reaction, the product was cooled on the plate coated with teflon.

The high molecular weight PLGA was synthesized by ring-opening polymerization of 8.5g lactide and 1.5g glycolide without air contact under the presence of initiator catalyst with a stannous octoate / glycolide mass ratio of 0.0003, which was prepared by mixing 0.0195g/ml lauryl alcohol solution and 0.003g/ml stannous octoate alcoholic solution. The resulting system was heated to and kept at 150°C under vacuum for 3 hours, afterwards, the PLGA product was cured by putting into liquid nitrogen immediately.

2.3. The Fabrication of Periodontal Barrier Membrane
PLA/PLGA composite film was prepared by the solvent casting method. The PLA and PLGA powders was mixed in a PLA/PLGA ratio ranging from 2:1 to 10:1, then was dissolved into dichloromethane under stirring and heating, the final concentration was controlled at 15%. The film former was cleaned with acetone and the thickness was adjusted to 0.5 mm, then the resin solution was poured into the former. Periodontal barrier membrane was fabricated when the solution dried at room temperature for 2 days.

2.4. Mechanical Properties and Porosity of the PLA/PLGA Membrane
Tensile strength and the elongating rate at break of the composite membrane were tested at room temperature by universal materials testing machine (Zwick Z400E, Germany). The sample was rectangular with dimensions of 2 cm x 15 cm. The crosshead speed was set as 100 mm/min and applied until the specimen ultimate fracture. All data were the average of three measured values.

The porosity of sample was measured by liquid (anhydrous ethanol) displacement. A flask was filled with ethanol and weighed as W\(_1\) (g). The sample, weighed W\(_5\) (g) was dipped into the ethanol and degassed so that the ethanol filled the holes in the membrane. Then the sample was refilled with ethanol and weigh it as W\(_2\) (g). After the sample of ethanol-impregnated was taken out, the remaining ethanol and flask were weighed as W\(_3\) (g). The porosity was calculated as follows:

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P = \frac{(V_0-V)}{V_0} * 100\% = \frac{(W_2-W_3-W_5)}{(W_1-W_3)} * 100\%
\]

Where P represents porosity of the membrane (%); V\(_0\) and V represent volume of material in natural state (cm\(^3\)) and absolute compact volume of the material (cm\(^3\)), respectively. W\(_1\), W\(_2\), W\(_3\) and W\(_4\) represent the weight of the flask filled with ethanol (g), weight of sample (g), the total weight of the...
sample refilled with ethanol (g) and the weight of the remaining ethanol and the flask (g).

2.5. Biological Performance of the Periodontal Barrier Membrane in Vitro

The composite membrane, PLA: PLGA = 2:1, was cut into a small disk with a diameter of 6 mm and was sterilized with $^{60}$Co for 24 h. Before use, the samples were sterilized by ultraviolet (UV) irradiation for 1 h at 254 nm. The gingival fibroblast was seeded on the PLA/PLGA composite membrane at a concentration of $5 \times 10^3$/ml, then was cultured at a temperature of 37°C, a CO$_2$ flux of 50ml/L for about three days. The inoculation surface was marked as positive.

2.6. Degradation of The periodontal Barrier Membrane in Vitro

The degradation of the PLA/PLGA membrane was studied in vitro. After weighing, membranes measuring 2 x 5 mm were immersed in test tubes containing 17 ml of PBS (phosphate buffer saline; 0.2 M, pH 7.4) at 37 °C. After 1, 2, 4, 6, 8, 16, 32 weeks, the remaining membranes were weighed to determine the weight lost during hydrolysis. The composite membrane could maintain its form for 16 weeks and the degradation rate of the composite membrane was 4.7%, 8.7%, 13.5%, 14.1%, 14.6%, 20.1% and 63.7%, respectively.

3. Results and Discussion

3.1. Synthesis and Characterization of PLA and PLGA

The harmless initiator catalyst could make the polymerization easier and did not have to be separated from the product. In addition, excess catalyst might accelerate side reactions to reduce the molecular weight of target polymers. The result showed that low molecular weight PLA and high molecular weight PLGA could be synthesized, when the mass ratio of initiator catalyst / reactant was 0.0008 and 0.0003, respectively.

The infrared spectroscopy showed functional groups of PLA was at 3500.1 (ν OH), 2995.8 and 2945.8 (ν CH), 1757.3 (ν C=O), 1185.6, 1130.3 and 1091.7 (ν C-O) cm$^{-1}$. The number-average and the weight-average molecular weight were $1.9 \times 10^3$ (Mn) and $2.1 \times 10^3$ (Mw), respectively. The relative viscosity was 1.035, intrinsic viscosity was 0.138.

The infrared spectroscopy showed functional groups of PLGA was at 2943.6 (ν CH), 1746.4 (ν C=O), 1184.0 and 1080.7 (ν C-O-C) cm$^{-1}$. The number-average and the weight-average molecular weight were $4.2 \times 10^4$ (Mn) and $7.2 \times 10^4$ (Mw), respectively. The relative viscosity was 1.283, intrinsic viscosity was 0.995.

3.2. Mechanical Properties and Porosity of the PLA/PLGA Membranes

The bone pad should have aperture diameters less than 10 mm and the porosity above 90%, otherwise it was impossible for the pad to prevent gingival cells from the tooth defect area and meanwhile allow nutrients in. The results of porosity, thickness and aperture of those membranes were listed in Table 1.

| Material (molar ratio) | Thickness (mm) | Aperture (mm) | Porosity (%) |
|-----------------------|----------------|--------------|--------------|
| PLGA                  | 0.48           | 0.0024       | 90.5         |
| PLGA/PLA(1:2)         | 0.49           | 0.00232      | 93.0         |
| PLGA/PLA(1:4)         | 0.50           | 0.00223      | 93.6         |
| PLGA/PLA(1:6)         | 0.49           | 0.0021       | 92.0         |

Mechanical properties are important for the function as periodontal barrier of membrane [17]. PLA couldn’t form a complete membrane when the thickness was thinner than 0.5mm. Single component PLA membrane shows poor mechanical properties, especially low, elongation, while single component PLGA membrane has good film-forming property, good hydrophilicity and low hardness. PLA and PLGA were mixed to prepare composite membrane, which showed higher hardness than PLGA membrane, and higher tensile strength than PLA membrane. The PLA/PLGA composite membrane exhibited a minimum tensile strength at 5.75 Mpa, when the PLA/PLGA ratio was 2:1 (w/w), which
met the basic requirement of 5.04 Mpa. The maximum value of tensile strength was 38.7 Mpa, when the ratio was 6:1 (w/w). Afterward, with the increasing of the PLA/PLGA ratio, the tensile strength of the membrane was decreased (Fig 1).

![Figure 1](image)

**Figure 1.** Tensile strength of the PLA/PLGA composite membranes varying with different mixing ratio.

3.3. **Biological Performance of the Periodontal Barrier Membrane in Vitro**

Under SEM, it was observed that gingival fibroblasts only grew on the positive surface. The polygonal cells stretched out the long pseudopodia, spreading on the membrane surface (Fig 2 A). There were no cells grow on the negative side nor the cross-section (Fig 2 B), even the cross section, indicating that PLA/PLGA composite membrane can block the growth of human gingival fibroblasts and have a good barrier function. In addition, the contents of arsenic and tin were 0.036 and 139 mg/kg in the samples, respectively, no lead was detected, content of all the heavy metals were lower than the World Health Organization (WHO) standards.

![Figure 2](image)

**Figure 2.** SEM Photographs of the HGF (hepatocyte growth factor) grew on the dental membrane

- **A:** polygonal cells grew on positive side;
- **B:** the negative side was clean.

3.4. **The Degradation of the Periodontal Barrier Membrane in Vitro**

The porous nature of the composite membrane allowed water molecules enter the membrane and accelerate degradation. The porous structure formed with PLA was also beneficial to the discharge of acidic substances in the degradation process. The membrane degraded relatively fast in initial four weeks, afterward the degradation was not obvious during 4-8 weeks. Further, as the acid was accumulated in the degradation process and could in turn catalyzed the degradation of the membrane, the degradation was accelerated after 8 weeks (Fig. 3), and it would degrade completely soon. It was predicted that the degradation behavior of the PLA/PLGA composite membrane could ensure the time of healing periodontal tissues with the membrane as periodontal barrier.
Figure 3. The mass loss ratio of the PLA/PLGA composite membrane in degradation.

4. Conclusion
We have synthesized PLA and PLGA, with the number-average molecular weight of $1.9 \times 10^3$ (Mn) and $4.2 \times 10^4$ (Mn), and the weight-average molecular weight of $2.1 \times 10^3$ (Mw) and $7.2 \times 10^4$ (Mw), respectively, and prepared PLA/PLGA composite membrane by blending these two polymers. The mechanical property, biocompatibility and degradability of the membrane were investigated and the properties could be controlled by adjusting PLA/PLGA ratio. The PLA/PLGA composite membrane could block the growth of human gingival fibroblasts, playing a good role as barrier. The good tensile strength and the degradation time of the PLA/PLGA membrane maintained enough osteogenic space for bone repair. The PLA/PLGA membrane was expected to be a good biofilm that can be widely used clinically.

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