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Development of layered PLGA membranes for periodontal tissue regeneration

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ABSTRACT

Objective. Various commercial products are available for guided tissue regeneration (GTR) therapy; however, they do not combine biosafety with the ability to control cell function. The purpose of this study was to evaluate the physicochemical and biological characteristics of the novel bilayer biodegradable poly(lactic-*co*-glycolic acid) (PLGA) membrane, and to assess whether the bilayer PLGA membrane could be used for periodontal tissue regeneration.

Methods. Bilayer biodegradable membrane was fabricated thorough a two-step freezing and lyophilization process using PLGA solution. The characteristics of bilayer membranes were evaluated with respect to surface morphology, stability, mechanical strength, and operability for clinical use. Cell proliferation and osteogenic differentiation were investigated on the each surface of bilayer membrane. Then, these membranes were implanted to the rat calvaria bone defect models and evaluated their capability for tissue regeneration.

Results. Biodegradable membranes composed of the solid and porous layer were successfully prepared and the surface morphologies analyzed. Physicochemical analyses revealed that the membranes possessed enough stability and mechanical properties for clinical use. It was also confirmed that the solid layer inhibited cell proliferation and subsequent connective tissue invasion, while the inner layer promoted proliferation and osteogenic differentiation, thus resulting in bone regeneration *in vivo*.

Significance. The layering technology used to fabricate the bilayer polymer membrane could be applied in the developing of other novel biomaterials. The present study demonstrates that the bilayer biodegradable polymer membranes facilitate tissue regeneration *in vivo*, and therefore represent a prospective biomaterial for GTR therapy.

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1. Introduction

Guided tissue regeneration (GTR) is a clinical approach for periodontal tissue regeneration by guiding periodontal ligament-derived cells and osteoblasts following application of a barrier membrane to the periodontal tissue defect [1,2]. The membranes used in GTR therapy are classified into two types: biodegradable (including animal origin constituents [3–5] and polylactic acid (PLA) [6–8]) and non-biodegradable (including polytetrafluoroethylene (PTFE) and titanium) [9–11].

Recently, the use of biodegradable membranes is mainstream in GTR therapy [12,13], because secondary surgery to remove the remaining membranes is unfavorable after tissue regeneration [14,15]. Biodegradable membranes composed of animal collagen, PLA, polyglycolic acid (PGA), and poly(lactic-*co*-glycolic acid) (PLGA) are commercially available and are in widespread dental clinical use. Collagen-derived membranes possess high biocompatibility and biodegradability [16,17]; however, they might contain infectious agents following their generation from an unspecified number of swine and cows [18,19]. Furthermore, collagen membranes have low mechanical strength [20] and their degradation rate is difficult to control [21,22]. By contrast, membranes composed of biodegradable polymer are superior in biodegradability [23,24] and mechanical property [25]. In addition, biodegradable polymer membranes are eventually degraded into carbon dioxide and water, therefore they are believed to be safe within the body. Despite this, biodegradable polymer membranes are reported to have low cytocompatibility [24,26,27] and operability for clinical use [28].

Clinical reports indicate that GTR therapy remains problematic for periodontal regeneration. For example, Tanner et al.[29] reported that collagen membranes are insufficient to block epithelial tissue invasion, and consequently induce periodontal recovery with non-native epithelial attachment. When applying PTFE membranes, some reports show that periodontal tissue regeneration is impeded by penetrating epithelial cells between the membrane and regeneration site [30,31]. Thus, it is believed that selective control capability, to block tissue invasion at the epithelial side and promote cell proliferation and differentiation at the tissue regeneration site, is necessary for biodegradable polymer membranes.

A number of studies have investigated cellular behavior on biodegradable polymers [32–34]. Osteoblast attachment is reported to be good on PLGA nanofibers but not on solid materials [32]. In addition, porous PLA films are reported to promote proliferation of fibroblasts while a smooth surface inhibits cell growth [33]. A study using epithelial 293T cells showed that tight PLA/PLGA surfaces inhibit cell invasion into the membrane [34]. Based on these findings, bilayer membranes with differing surface morphologies are thought to enable better control of cell function, as well as tissue behavior. In fact, a previous report has proposed the use of bilayer membranes [35]; however the effect of the bilayer structure on tissue regeneration remains unclear.

In this study, we fabricate a bilayer membrane using PLGA, a commonly used and well known biomaterial [36–38], and evaluate its usability as a bilayer PLGA membrane for GTR therapy *in vitro* and *in vivo*. We also tested the null hypothesis: that there would be no difference in tissue regeneration using various thicknesses of each layer. The purpose of this study was to evaluate the physicochemical and biological characteristics of bilayer biodegradable PLGA membrane, and to assess whether the membrane could be used for periodontal tissue regeneration.

2. Materials and methods

2.1. *Preparation of bilayer PLGA membrane*

PLGA (PLA:PLGA = 75:25; Molecular weight: 25 kDa) was dissolved in 1,4-dioxane (Wako, Osaka, Japan) at 2.7 wt%. Two types of layered-membrane (LM-1 and LM-2) were prepared as follows: PLGA solution was poured into the fluoroplastic-coated mold. Then, the base of the plate was cooled to either −30 °C (LM-1) or −80 °C (LM-2) for 10 s and the PLGA solution was frozen partially. The mold was then covered with a brass plate (cooled to −80 °C) and the PLGA solution was slowly and completely frozen. The frozen PLGA solution was then lyophilized using a freeze-dryer (FDU-1110, EYELA, Tokyo, Japan), and then formed as membrane by pressing at 300 kgf/cm^2 for 15 s using a hydraulic press machine (JP-1T, Nissin Kagaku, Osaka, Japan). The obtained PLGA membranes were then treated with gamma sterilization and kept in a nitrogen atmosphere at 4 °C prior to experimentation.

The solid layer formed by prior freezing and its opposing side were referred as the outer layer and internal layer, respectively. Commercial PLGA membrane (GC membrane, GC, Tokyo, Japan), which was the same in composition to bilayer membrane fabricated, was used as a control.

2.2. *Characteristics of the bilayer PLGA membrane*

2.2.1. *Surface structure*

The surface and cross-sectional surface of each membrane were coated with gold and observed using a scanning electron microscope (SEM; JSM-6390, JEOL, Tokyo, Japan) at 5 kV. Surface morphology and roughness were evaluated using a laser microscope (VK-X250, Keyence, Osaka, Japan). Roughness average (Ra) was calculated from the three areas of arbitrary surface (n = 7).

2.2.2. *Stability analyses*

To evaluate the water contact angle, each membrane was cut into 5.0 × 5.0 mm samples. Ten microliters of phosphate-buffered saline (PBS; Nissui, Tokyo, Japan) was then dropped onto the membrane, and a picture was immediately taken using a single-lens reflex camera (D5500, Nikon, Tokyo, Japan) in a horizontal direction. Contact angle was measured as the tangent line from the end of the droplet (n = 5).

To investigate dimensional change, each membrane was cut into 10 × 10 mm samples and immersed into PBS at 37 °C. After 1, 3, 7, and 21 days, margin length and thickness were measured using a digital caliper (CD-15CP, Mitutoyo, Kanagawa, Japan) (n = 4).

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