OUKA
The University of Osaka
Institutional Knowledge Archive

Title	Fabrication of poly(lactic acid/caprolactone) bilayer membrane for GBR application
Author(s)	Laranjeira Abe, Gabriela
Citation	大阪大学, 2022, 博士論文
Version Type	VoR
URL	https://doi.org/10.18910/87951
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

https://ir.library.osaka-u.ac.jp/

The University of Osaka

Abstract of Thesis

Name (Gabriela Laranjeira Abe)

Title: Fabrication of poly(lactic acid/caprolactone) bilayer membrane for GBR application
(ポリ乳酸/ポリカプロラクトン二層性GBRメンブレンの創製)

[Objective]

Biodegradable polymers, such as poly(lactic-acid) (PLA), are common base materials for barrier membranes essential to guided bone regeneration (GBR) protocols. However, controlling their degradability remains a challenge. In this study, fabrication of a barrier membrane with low degradation rate was attempted by copolymerization of PLA with poly(caprolactone) (PCL) that shows adjustable degradation. This novel barrier membrane was also designed with a bilayer structure, to provide efficient blocking functions against epithelial tissue and bacterial infection, further improving GBR outcomes. The purpose of this study was to evaluate usefulness of the experimental poly(lactic acid/caprolactone) (PLCL) bilayer membrane for GBR by *in vitro* and *in vivo* experiments.

[Materials and Methods]

Preparation of the experimental membrane

The experimental membrane (PLCL-membrane) was prepared using the mixed solution of PLA and PCL Briefly, the solution of PLA and PCL dissolved in 1,4-dioxane was copolymerized and freeze-dried to form a film of porous structure. Then, the solution was poured into a mold to form a compact layer, and the previously obtained porous film was positioned directly over it. Commercially available membranes fabricated with poly(lactic-co-glycolic acid) (GC membrane, GC, Japan; PLGA-membrane) and with porcine collagen (Bio-Gide; Geistlich Pharma AG; Col-membrane) were used as controls.

Characterization of the experimental membrane

Structural observation and surface roughness: The cross-section as well as surfaces of each membrane were observed by scanning electron microscopy (SEM). The roughness average (*Ra*) was measured at four random areas of the surface ($n = 4$).

In vitro degradability: Membranes (10 mm × 10 mm) were immersed in PBS for up to 52 weeks at 37°C. The weight ratio was calculated between the initial weight (W_0) and current weight (W_1) according to the formula; weight ratio (%) = $W_1/W_0 \times 100$ ($n = 4$).

Mechanical property: Membranes (20 mm × 3 mm) were fixed to the jigs of a tensile tester, and an axial tensile force was applied with a cross-head speed of 1.0 mm/min until sample rupture. Tensile strength and breaking strain were then calculated ($n = 4$).

Fitting property: Using an epoxy model to simulate a bone defect, the fitting of each membrane to the model was evaluated under dry and wet conditions. Membrane was sutured to the model and scanned by micro-computed tomography (micro-CT). Distances between the membrane and model at thirteen different points were measured ($n = 3$).

Cytocompatibility of the experimental membrane

Cell proliferation and morphology: Human bone marrow stem cells (hBMSCs) and human gingival epithelium progenitor cells (HGEPs) were seeded onto each membrane and cultured for 3, 7, or 12 days. Proliferation was assessed by Cell Counting Kit-8 and SEM observation ($n = 5$).

Mineralization: hBMSCs were cultured under osteogenic conditions to promote cell differentiation for 21 and 28 days. Deposition of mineralized matrices were observed by von

Kossa staining and semi-quantified by the color depth analysis (n = 3).

Barrier function of the experimental membrane against bacterial invasion

Porphyromonas gingivalis, *Streptococcus mutans* and multispecies bacteria from human saliva were seeded onto the membranes. Bacterial adherence was assessed after 6, 24 or 72 h (n = 5), and bacterial invasion was assessed after 24 or 72 h (n = 6).

Effectiveness of the experimental membrane *in vivo*

Subcutaneous biodegradation model: Membranes (10 mm × 10 mm) were implanted into subcutaneous pouches on the back of 9-week-old F344 rats. After 16 or 24 weeks, membranes were collected along with surrounding tissues for hematoxylin and eosin (HE) staining (n = 4).

Bone regeneration model: A bone defect (ϕ 5 mm) was created at the parietal bones of 10-week-old Sprague-Dawley rats, and each membrane was applied to treat the defect. Micro-CT analysis and histological observation were performed after 4 (n = 3) or 8 weeks (n = 5).

[Results and Discussion]

Structure and surface roughness: SEM observation confirmed the bilayer structure of the PLCL-membrane, comprising of a compact layer and a porous layer. PLCL-porous layer showed greater *Ra* (7.17 ± 0.18 μm) compared with compact layer (0.47 ± 0.06 μm), which showed the smoothest surface among all groups ($p < 0.05$, Kruskal-Wallis, Dunn's test).

In vitro degradability: PLCL-membrane showed significantly slower degradation in comparison with PLGA-membrane up to 26 weeks ($p < 0.05$, Kruskal-Wallis, Dunn's test).

Mechanical property: PLCL-membrane showed smaller tensile strength (2.03 ± 0.11 N/mm^2) than PLGA-membrane (3.57 ± 0.09 N/mm^2) and Col-membrane (6.00 ± 0.11 N/mm^2). However, the breaking strain for PLCL-membrane was approximately twenty times greater than that of other two materials, indicating its ability to endure deformation before rupture.

Fitting property: Dry PLCL-membrane had closer fitting compared with other two materials. All materials showed closer fitting under wet condition, but Col-membrane collapsed into the simulated defect, losing space-making ability ($p < 0.05$, Kruskal-Wallis, Dunn's test).

Cell proliferation and morphology: hBMSCs proliferation on PLCL-porous layer showed no significant difference from PLGA-membrane and Col-membrane, demonstrating a spindle-like morphology. HGEPs proliferation was significantly greater on PLCL-compact and -porous layers than on Col-membrane.

Mineralization: The greatest mineralization was observed on Col-membrane. While there was no difference between PLCL-porous layer and PLGA-surface, significantly less mineralization was observed on PLCL-compact layer than other groups ($p < 0.05$, Kruskal-Wallis, Dunn's test).

Bacterial adherence and invasion: For all bacteria tested, adherence was reduced on PLCL-compact layer ($p < 0.05$, Kruskal-Wallis, Dunn's test). PLCL-membrane blocked bacterial invasion completely, while invasion up to 80-μm deep into the structure was found for other two materials.

Subcutaneous biodegradation: At 24 weeks after implantation, PLCL-membrane was present in the tissue, while PLGA-membrane and Col-membrane were completely biodegraded.

Bone regeneration: After 8 weeks, bone defects treated with PLCL-membrane showed significantly greater regeneration (71.84 ± 14.57%) than other two materials ($p < 0.05$).

[Conclusion]

In this study, a bilayer-structured membrane composed of PLCL was successfully fabricated. The strongest features of this material are its slower degradation, prolonged support to bone regeneration, and blocking of undesirable tissue and bacteria. Overall, it was demonstrated that the novel PLCL bilayer membrane is useful for GBR application.

様式 7

論文審査の結果の要旨及び担当者

氏　名　（　Gabriela Laranjeira Abe　）		
	（職）	氏　名
論文審査担当者	主 査　教 授　今里　聡 副 査　教 授　池邉　一典 副 査　准教授　波多　賢二 副 査　講 師　犬伏　俊博	

論文審査の結果の要旨

本研究は、ポリ乳酸/ポリカプロラクトン共重合体を基材とした二層性メンブレンを試作し、その物理化学的性質、細胞や組織に対する影響、ならびに口腔細菌の侵入防御能を評価することで、GBR 用メンブレンとしての有用性について検討したものである。

その結果、試作メンブレンは、密と疎な二層構造を有し、骨再生に有利な生分解性を示しつつ、上皮組織や口腔細菌に対して高いバリア機能を発揮することで、骨再生を効果的に促進することが明らかとなった。

以上の研究成果は、GBR 法に有用な新規メンブレンの開発に成功したことを示すものであり、本研究は博士（歯学）の学位授与に値するものと認める。