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Author(s)	Laranjeira Abe, Gabriela; Sasaki, Jun-ichi; Imazato, Satoshi
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A poly(lactic acid/caprolactone) bilayer membrane for guided bone regeneration

Gabriela L Abe^a, Jun-Ichi Sasaki^a, Satoshi Imazato^{a,b}

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Introduction

Bone augmentation is often necessary to improve the outcomes of dental implant treatments^{1,2}. In the past decades, a variety of protocols for bone augmentation have been developed, however guided bone regeneration (GBR) is the most widely adopted by dentists³. GBR applies surgical techniques and biomaterials such as barrier membranes to secure space for bone growth and exclude non-osteogenic cells from the defect area⁴.

Barrier membranes can be categorized into biodegradable, composed of collagen or synthetic polymers such as poly(lactic-glycolic-acid) (PLGA)⁵; or non-biodegradable, such as poly(tetrafluoroethylene)⁶. Non-biodegradable membranes require a second surgery for removal⁷, therefore, whenever possible, biodegradable membranes are preferred in GBR treatment⁸.

The ability of a barrier membrane to block connective tissue on one side and support cell adhesion, proliferation, and osteogenic differentiation on the other side is favorable for GBR⁹. Ideally, barrier membranes should provide a gradual variation in composition or structure across the material, which results in changes to its properties¹⁰. Based on this principle, we previously developed a bilayer membrane composed of PLGA that combined a solid layer and a

porous layer, which respectively provided a barrier function and cell support¹¹. It was shown that the PLGA bilayer membrane could promote bone regeneration *in vivo*, however, degradation rates were comparable to those of a monolayer membrane, not providing a significant improvement over commercially available materials. A degradation rate that is proportional and comparable to the local tissue regeneration is highly desirable¹². In the case of bone tissue, GBR membranes are required to provide cell support and barrier function for an extended period.

Recently, we have advanced on developing a slow degradation barrier membrane¹³. The previous bilayer membrane was improved when fabricated from a copolymer of poly(lactic-acid) and poly(caprolactone) (PLCL). Poly(caprolactone) is an aliphatic polyester capable of copolymerization with many other polymers¹⁴. It takes several months to several years for poly(caprolactone) to completely biodegrade, depending on the degree of crystallinity, molecular weight, and conditions of degradation such as temperature and the presence of enzymes¹⁵. By copolymerizing poly(caprolactone) with poly(lactic-acid), the rate of polymer degradation can be reduced¹³, resulting in a more durable and biocompatible bilayer membrane for GBR application. Here we summarize the various properties of a novel PLCL bilayer membrane which we have developed.

^a Department of Biomaterials Science, Osaka University Graduate School of Dentistry

^b Department of Advanced Functional Materials Science, Osaka University Graduate School of Dentistry

Physical characteristics of the PLCL bilayer membrane

To obtain a bilayer membrane, each layer was prepared separately by different processes based on our previous study¹¹). Briefly, PLCL was dissolved in 1,4-dioxane, whereupon the solution was transferred into a mold and submitted to freeze drying to result in a polymer film with a porous structure. A new solution of PLCL in 1,4-dioxane was then prepared and placed into a mold, and the previously obtained porous film was positioned directly over the new solution. This combined set was allowed to dry at 60°C and peeled off the mold to produce a bilayer membrane. The membrane was sterilized by γ -radiation and stored at 4°C. A commercially available monolayer PLGA biodegradable membrane (GC membrane, GC, Tokyo, Japan)

was used as a control.

Scanning electron microscope (SEM) observation confirmed that PLCL membrane is comprised of a bilayer structure, with compact and porous layers, while control membrane exhibited a monolayer with a uniform porous structure (Fig. 1Aa–e). The PLCL compact layer showed smooth texture on its surface and the porous layer exhibited wider pores than those of the control membrane.

Surface roughness of PLCL membrane was significantly greater for the porous surface than that of the compact surface and the control membrane (Fig. 1Ba). Additionally, conventional tensile tests showed that PLCL membrane had a significantly smaller tensile strength compared with the control membrane (Fig. 1Bb). However, the breaking strain of PLCL membrane was more than twenty times greater than

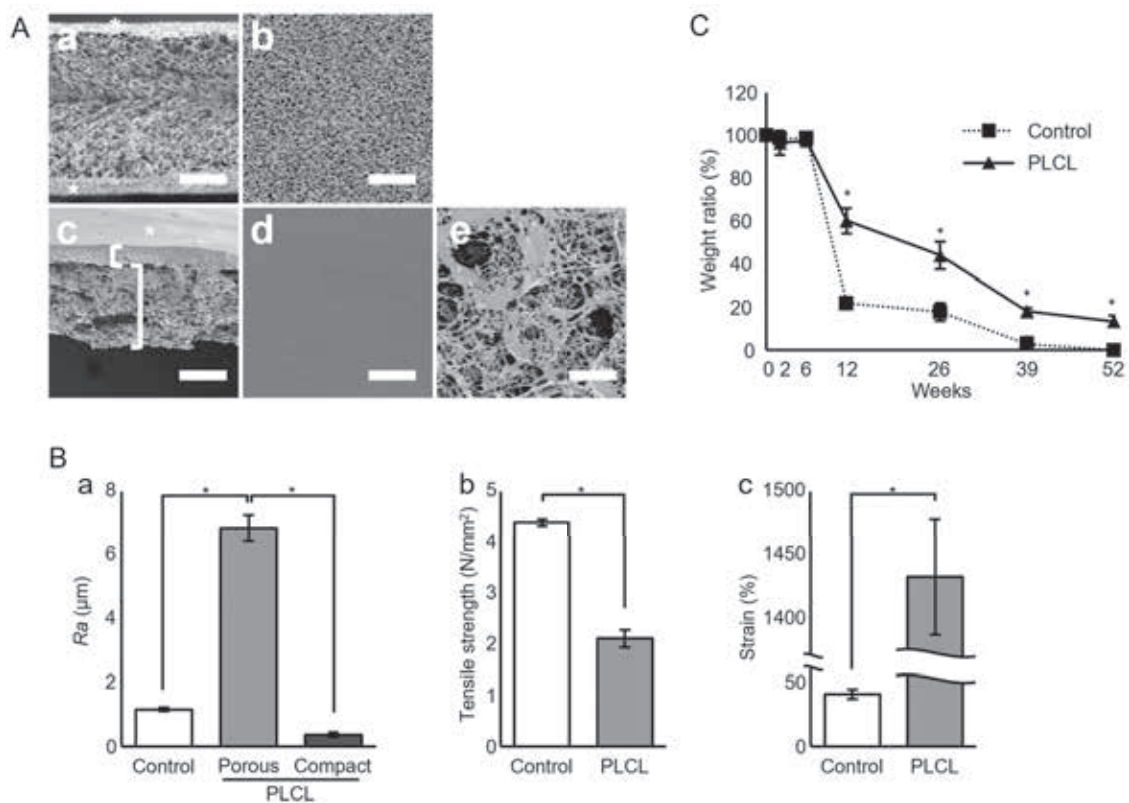


Figure 1 Physical characteristics of the PLCL bilayer membrane. (A) SEM images of control membrane (a,b) and PLCL bilayer membrane (c–e). Brackets in PLCL membrane indicate compact (upper) and porous (lower) layers. Asterisks indicate membrane surfaces. Scale bars: 100 μm . (B) Surface roughness (a), tensile strength (b), and strain analysis (c) of PLCL in comparison to control. (C) Membrane degradation in PBS up to 52 weeks. (B, C) Asterisks indicate statistically significant difference among groups ($*p < 0.05$; mean \pm SD, $n = 4$). Adapted from Abe *et al.*¹³), Copyright 2020, with permission from Elsevier.

that of control membrane, indicating that PLCL membrane underwent a greater deformation prior to rupture (Fig. 1Bc).

Membrane degradation was also evaluated, by recording the weight loss of membranes immersed in phosphate buffered saline (PBS) for up to 52 weeks (Fig. 1C). No change in weight was detected in both membranes up to 6 weeks, however at 12 weeks, the control PLGA membrane lost $79.2 \pm 2.7\%$ of its weight while PLCL membranes maintained $60.9 \pm 5.9\%$ of its weight. Hydrolytic degradation continued gradually, and the PLCL membrane preserved $13.5 \pm 2.5\%$ of its weight after 52 weeks of immersion. The control membrane, however, was totally dispersed in PBS after 52 weeks. The slower degradation of the PLCL may contribute to prolong the barrier function and the scaffold function of this bilayer membrane.

Biocompatibility of the PLCL bilayer membrane

Human bone marrow-derived stem cells (hBMSCs) were seeded at 4.0×10^4 cells/well onto membranes fixed to the bottom of 12-well culture plates and cultured for 3, 7, and 12 days to evaluate cell proliferation.

SEM images of the membranes at 12 days of culture showed that the attached cells presented branched shapes with cell membrane extensions on control and PLCL porous layer (Fig. 2Aa, b), while a spindle-like morphology was exhibited on compact layer (Fig. 2Ac). Cell proliferation assays showed that hBMSCs were able to proliferate on the porous layer of PLCL bilayer membrane as much as on control membrane at day 12, though the hBMSCs on the compact layer were significantly less in number than on the control membranes (Fig. 2B).

Additionally, hBMSCs were cultured in osteogenic environment for 21 and 28 days, and the deposition of mineralized matrices was revealed by von Kossa staining (Fig. 3). All membranes showed deposition of mineralized matrix, however, control and PLCL porous layer showed more intense staining in comparison to PLCL compact layer (Fig. 3A). A color depth analysis

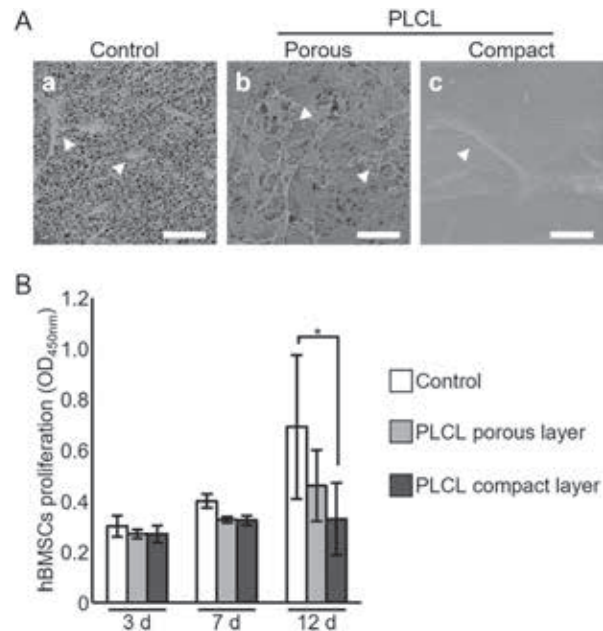


Figure 2 Biocompatibility of the PLCL bilayer membrane. (A) SEM images of hBMSCs at 12 days of culture. Arrows indicate attached cells on the membrane. Scale bars: 100 μm . (B) Proliferation of hBMSCs on PLCL membrane in comparison to control. ($*p < 0.05$, mean \pm SD, $n = 4$). Adapted from Abe *et al.*¹³, Copyright 2020, with permission from Elsevier.

was performed using images obtained after von Kossa staining (Fig. 3B). The result indicated that a greater amount of mineralized matrix was deposited on the control than on the porous layer of PLCL membrane at day 21. However, at day 28, no difference was found between control and PLCL porous layer. Furthermore, the surface of compact layer showed significantly less matrix deposition compared with the other two groups.

Both porous and compact layers of PLCL membrane supported growth of hBMSCs. On the porous layer, as well as on the control, there was significantly greater deposition of mineralized matrix compared to that on the compact layer. Casillo *et al.*¹⁶ have reported that a stronger cell-cell interaction occurs when the cells are allowed to grow on rough surfaces, which was identified by the increased production of extracellular matrices. Our results are in accordance with reports showing that surface roughness can influence hBMSC differentiation and prolifer-

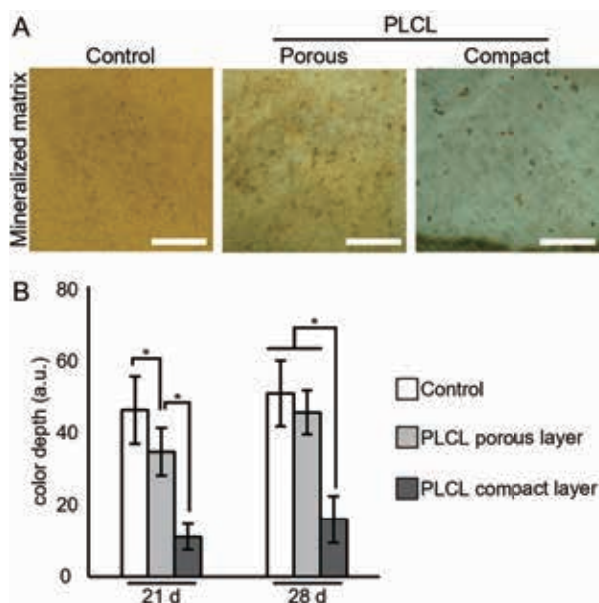


Figure 3 Deposition of mineralized matrix on the membranes. (A) Mineralized matrices were revealed by von Kossa staining after 28 days of osteogenic differentiation. Scale bars: 1 mm. (B) Color depth analysis of von Kossa staining. Asterisks indicate statistically significant difference among groups ($p < 0.05$, mean \pm SD, $n = 4$). Adapted from Abe *et al.*¹³⁾, Copyright 2020, with permission from Elsevier.

ation, causing an increased deposition of mineralized extracellular matrix^{16,17)}. On the compact layer, even though the mineralization was produced at significantly smaller amounts, hBMSCs could adhere and proliferate. Altogether, the results indicated that the PLCL bilayer membrane possessed biocompatibility.

Conclusion

A PLCL bilayer membrane with a reduced degradation rate along with suitable mechanical behavior and biocompatibility was successfully fabricated. The strongest feature of this material is its slower degradation compared to commercial PLGA membranes, which indicates the prolongation of its functions to support wound healing, facilitate bone regeneration, and block undesirable tissue invasion. Overall, it was demonstrated that the novel PLCL bilayer membrane is useful for GBR application.

Acknowledgments

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Conflicts of interest

The authors declare no conflict of interest.

References

- Behring, J., Junker, R., Walboomers, X. F., Chessnut, B., Jansen, J. A. (2008): Toward guided tissue and bone regeneration: morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. *Odontology*, **96**: 1–11.
- Elgali, I., Omar, O., Dahlin, C., Thomsen, P. (2017): Guided bone regeneration: materials and biological mechanisms revisited. *Eur. J. Oral Sci.*, **125**, 315–37.
- Jensen, A. T., Jensen, S. S., Worsaae, N. (2016): Complications related to bone augmentation procedures of localized defects in the alveolar ridge. A retrospective clinical study. *Oral Maxillofac. Surg.*, **20**: 115–22.
- Polimeni, G., Xiropaidis, A. V., Wikesjö, U. M. (2006): Biology and principles of periodontal wound healing/regeneration. *Periodontol. 2000*, **41**: 30–47.
- Florjanski, W., Orzeszek, S., Olchowy, A., Grychowska, N., Wieckiewicz, W., Malysa, A., *et al.* (2019): Modifications of polymeric membranes used in guided tissue and bone regeneration. *Polymers*, **11**: 782–92.
- Lee, S. W., Kim, S. G. (2014): Membranes for the guided bone regeneration. *Maxillofac. Plast. Reconstr. Surg.*, **36**: 239–46.
- Bottino, M. C., Thomas, V. (2015): Membranes for periodontal regeneration – a materials perspective. *Front. Oral Biol.*, **17**: 90–100.
- Ivanovic, A., Nikou, G., Miron, R. J., Nikolidakis, D., Sculean, A. (2014): Which biomaterials may promote periodontal regeneration in intrabony periodontal defects? A systematic review of preclinical studies. *Quintessence Int.*, **45**: 385–95.
- Bottino, M. C., Thomas, V., Janowski, G. M. (2011): A novel spatially designed and functionally graded electrospun membrane for periodontal regeneration. *Acta Biomater.*, **7**: 216–24.
- Iviglia, G., Kargozar, S., Baino, F. (2019): Biomaterials, current strategies, and novel nano-technological approaches for periodontal regeneration. *J. Funct. Biomater.*, **10**: 3.

- 11) Yoshimoto, I., Sasaki, J.I., Tsuboi, R., Yamaguchi, S., Kitagawa, H., Imazato, S. (2018): Development of layered PLGA membranes for periodontal tissue regeneration. *Dent. Mater.*, **34**: 538–50.
- 12) Xue, J., Shi, R., Niu, Y., Gong, M., Coates, P., Crawford, A., *et al.* (2015): Fabrication of drug-loaded anti-infective guided tissue regeneration membrane with adjustable biodegradation property. *Colloids Surf. B: Biointerfaces*, **135**: 846–54.
- 13) Abe, G. L., Sasaki, J. I., Katata, C., Kohno, T., Tsuboi, R., Kitagawa, H., Imazato, S. (2020): Fabrication of novel poly(lactic acid/caprolactone) bilayer membrane for GBR application. *Dent. Mater.*, **5**: 626–34.
- 14) Woodruff, M. A., Hutnacher, D. W. (2010): The return of a forgotten polymer—Polycaprolactone in the 21st century. *Prog. Polym. Sci.*, **35**: 1217–56.
- 15) Lin, W. C., Yao, C., Huang, T. Y., Cheng, S. J., Tang, C. M. (2019): Long-term *in vitro* degradation behavior and biocompatibility of polycaprolactone/cobalt-substituted hydroxyapatite composite for bone tissue engineering. *Dent. Mater.*, **35**: 751–62.
- 16) Casillo, S. M., Peredo, A. P., Perry, S. J., Chung, H. H., Gaborski, T. R. (2017): Membrane pore spacing can modulate endothelial cell-substrate and cell-cell interactions. *ACS Biomater. Sci. Eng.*, **3**: 243–8.
- 17) Sonomoto, K., Yamaoka, K., Kaneko, H., Yamagata, K., Sakata, K., Zhang, X., *et al.* (2016): Spontaneous differentiation of human mesenchymal stem cells on poly-lactic-co-glycolic acid nano-fiber scaffold. *PLoS One*, **11**: e0153231.