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研究課題名（和文）Development of immunomodulatory scaffolds for tissue regeneration

研究課題名（英文）Development of immunomodulatory scaffolds for tissue regeneration

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：本研究では、動物由来材料を用いた従来のスキャフォールドに代わる新規高分子スキャフォールドを開発することを目的として、ポリ(乳酸/カプロラクトン)共重合体（PLCL）を用いた多孔性スキャフォールドを創製した。PLCLスキャフォールドの物理化学的性質および生物学的特性を評価したところ、動物性コラーゲンを組成とするスキャフォールドと比較して、生分解性が低く、より長期間にわたりスキャフォールドとして機能することが分かった。本研究の結果、PLCL多孔性スキャフォールドは、動物由来材料を組成とするスキャフォールドの代替材料として応用できる可能性が示された。

研究成果の学術的意義や社会的意義
Scaffolds fabricated from synthetic polymers are essential biomaterials in dentistry. These biomaterials can replace animal collagen-derived scaffolds and contribute to tissue regeneration. The physical and biological properties of PLCL scaffolds make this material a novel treatment option.

研究成果の概要（英文）：Poly(lactic acid/caprolactone) copolymer (PLCL) was used to fabricate a synthetic scaffold biomaterial. Physical and biological properties of PLCL scaffolds were assessed and compared with commercially available scaffolds. In vitro, human fibroblasts seeded onto the PLCL scaffolds proliferated and expressed higher levels of genes associated with wound healing in comparison with commercial scaffolds. PLCL scaffolds also showed biocompatibility and slow degradation in vivo, which is considered an advantage for treatments targeting bone regeneration. Therefore, an animal model of bone regeneration was implemented. Slow degrading PLCL scaffolds provided scaffold function for longer time when compared to collagen-derived scaffolds, resulting in significantly greater volume of regenerated bone. The results obtained in this study indicate that a PLCL scaffold is a useful and safe alternative to animal collagen-derived scaffolds.

研究分野：Dental Biomaterials

キーワード：Scaffolds　Biomaterials　Synthetic Polymers　Cells　Inflammation

１．研究開始当初の背景　(Background of the study)

Scaffolds are temporary structures that help support tissue regeneration. Animal collagen-derived scaffolds are commercially available and commonly used in regenerative treatments. However, the fabrication process may not completely remove potential antigens that trigger the immune system. Making these materials an inherent biosafety risk regarding disease transmission and immune reaction. The body's immune reaction plays a crucial role in tissue healing, local immune cells can exacerbate inflammation leading to tissue fibrosis and failure to regenerate functional tissue. Therefore, **synthetic scaffolds appear as desirable alternatives to animal collagen-derived scaffolds**, given their biocompatibility and lack of animal-derived components. In this study, a copolymer of poly(lactic acid/caprolactone) is used as a candidate synthetic polymer to fabricate a new scaffold. Poly(caprolactone) is an aliphatic polyester capable of copolymerization with other polymers, approved by the FDA for medical use, and regarded as a safe medical biomaterial. By copolymerizing poly(caprolactone) with poly(lactic acid), a biocompatible synthetic scaffold (PLCL scaffold) was fabricated (Figure 1).

２．研究の目的　(Research objectives)

In this study, the objectives are to fabricate a synthetic scaffold of poly(lactic acid/caprolactone) copolymer and evaluate the biocompatibility and regenerative ability of PLCL scaffolds.

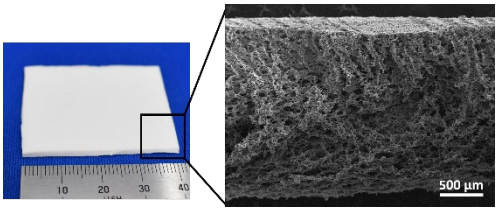

Figure 1. Visual aspect of the PLCL scaffold (left) and cross-sectional SEM image (right) showing the porous structure of PLCL scaffold.

３．研究の方法　(Research methods)

(1) PLCL scaffold preparation: PLCL copolymer is dissolved in 1,4-dioxane and submitted to freeze-drying to result in PLCL porous scaffold.

(2) Evaluation of physical properties: samples of PLCL scaffold were prepared for assessment of tensile strength, suture pull-out strength and surface roughness.

(3) Evaluation of biological properties: proliferation of human gingival fibroblasts (HGF-1) was assessed, followed by mRNA isolation and quantification of relative expression of fibroblast growth factor 7 (FGF7).

(4) Biocompatibility and regenerative ability: PLCL scaffolds were implanted into the subcutaneous space of mice, and the tissue response was observed after 16 and 24 weeks. Additionally, critical bone defects were created in the calvaria of Sprague-Dawley rats, treated with the PLCL scaffolds and compared to a commercial synthetic scaffold composed of poly(lactic-glicolic-acid) (PLGA) and a commercial animal collagen-derived scaffold (Col).

４．研究成果　(Research results)

The freeze-drying process resulted in a PLCL scaffold with 2 mm thickness and three-dimensional interconnected porous structure (Figure 1). The physical properties of PLCL scaffold indicate that this biomaterial can be applied in regenerative strategies that target bone regeneration and soft tissue regeneration. When tested *in vitro* in a cell culture model, PLCL

scaffold promoted HGF-1 proliferation and expression of FGF7. These results may be due to the porous structure of the scaffold, which stimulated cell attachment.

In vivo, implanted PLCL scaffolds showed reduced degradation in comparison to both commercially available scaffolds (Figure 2). At week 16, collagen-derived scaffolds were completely absorbed by the host, while the synthetic PLGA scaffold was severely degraded. At week 24, PLCL scaffolds were still present, showing structural degradation, but still serving as scaffold for host cells. These results indicate that our synthetic PLCL scaffolds can provide scaffold function for longer time, which is an advantage for regenerative treatments targeting bone. Therefore, a critical bone defect model was implemented. Treatment with PLCL scaffolds significantly promoted bone regeneration, quantified by the volume of new bone, in comparison with both commercially available scaffolds (Figure 3).

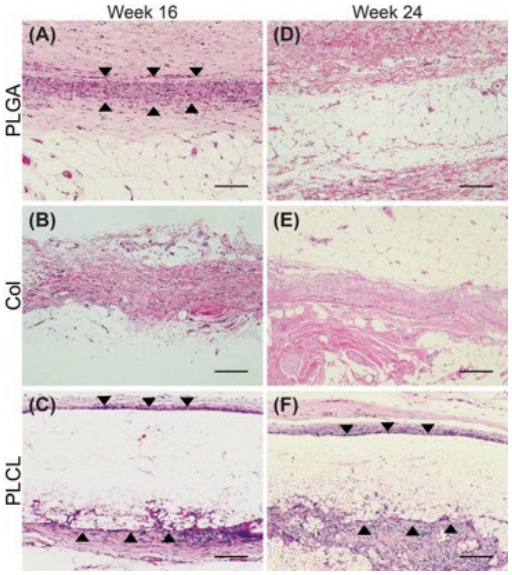

Figure 2. PLGA, Col, and PLCL scaffolds implanted into the subcutaneous space of mice. Black arrowheads indicate the limits of the scaffolds. At week 16, Col is completely degraded. At week 24, only PLCL still provides scaffold function.

CONCLUSIONS

A novel PLCL scaffold was fabricated, showing adequate physical properties and biocompatibility. The PLCL scaffold promoted bone regeneration *in vivo* in comparison to other commercial materials. **The main advantages of this material are the lack of animal components and the slow degradation, providing scaffold function for longer time.**

Tissue regeneration is considered successful when functional tissue is restored, and fibrotic tissue is avoided. The results of this study indicate that **the synthetic PLCL scaffold is a useful alternative to animal collagen-derived scaffolds**. It is also expected that the copolymer of poly(lactic acid/caprolactone) can be applied to fabricate other scaffolds showing different structural designs, which may serve different applications.

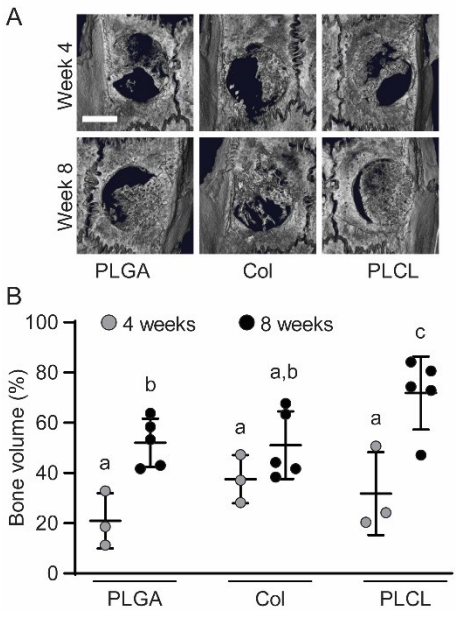

Figure 3. All scaffolds can promote the regeneration of bone defects (A). However, PLCL scaffolds showed significantly greater bone volume compared to other materials (B). Two-way ANOVA, $p < .05$.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Abe Gabriela L.、Sasaki Jun‐Ichi、Tsuboi Ririko、Kohno Tomoki、Kitagawa Haruaki、Imazato Satoshi	４．巻 112
２．論文標題 Poly(lactic acid/caprolactone) bilayer membrane achieves bone regeneration through a prolonged barrier function	５．発行年 2023年
３．雑誌名 Journal of Biomedical Materials Research Part B: Applied Biomaterials	６．最初と最後の頁 e35365
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/jbm.b.35365	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　1件／うち国際学会　2件）

１．発表者名 Laranjeria Abe, Gabriela
２．発表標題 Development of synthetic polymer-based grafting material to substitute autogenous connective tissue grafts
３．学会等名 2nd International Conference for Future Dentistry（国際学会）
４．発表年 2024年

１．発表者名 Laranjeria Abe, Gabriela
２．発表標題 Poly(lactic acid/caprolactone) as an alternative to natural polymers
３．学会等名 Online Seminar - Minnesota Dental Research Center for Biomaterials and Biomechanics（招待講演）
４．発表年 2023年

１．発表者名 Laranjeria Abe, Gabriela
２．発表標題 Fabrication of a synthetic bioresorbable poly(lactic acid/caprolactone) grafting material as an alternative to autogenous connective tissue graft
３．学会等名 Academy of Dental Materials Conference（国際学会）
４．発表年 2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関