Physical Characteristics and Biocompatibility of 3D-printed PLGA Membranes with Different Ratios of Lactic and Glycolic Acid Used for Guided Bone Regeneration: A Comparative Study

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Abstract

Commercial resorbable membranes for guided tissue regeneration (GBR) are costly and primarily composed of porcine collagen, which may be prohibited for use in certain patients due to religious beliefs. Consequently, this investigation aimed to address these challenges and create an osteoconductive membrane composed of PLGA (Poly-lactic-co-glycolic acid). To compare the physical properties and biocompatibility of the three-dimensional (3D)-printed membranes made of PLGA with three different ratios of lactic acid (LA) to glycolic acid (GA). The PLGA membranes were fabricated using a 3-D bio-printer and categorized into three groups based on the LA: GA ratios, including Group A; 50:50, Group B; 70:30, and Group C; 85:15. Morphologies, surface wettability, mechanical properties, degradation behaviors, and cytotoxicity of the membranes were comparatively assessed. The membranes had different microscopic surface morphologies between their upper and lower sides. The membranes of all groups exhibited hydrophilic surface properties, as evidenced by water contact angles of less than 90 degrees. Compared to the other groups, Group C exhibited the lowest contact angle and the highest tensile strength ($p < 0.05$). The membranes of Group A degraded faster and produced more acidic by-products than the other groups. The membranes of all groups had no indirect cytotoxicity to osteoblasts and fibroblasts. However, the cells could attach and proliferate on the surfaces of the membranes of Group C better than the other groups. PLGA (LA: GA= 85:15) was suitable for fabricating the resorbable membranes for GBR.

***Keywords*:** *polylactic-co-glycolic acid, three-dimensional printing, membrane, guided bone regeneration*

1. Introduction

Alveolar ridge augmentation is a commonly executed surgery designed to rehabilitate bone loss after tooth extraction. Guided bone regeneration (GBR), using a barrier membrane, is a frequently used therapy (Liu, & Kerns, 2014). This method employs a barrier membrane to inhibit the infiltration of soft tissues, including epithelium and connective tissues, into the bone defect region (Wang, & Boyapati, 2006). This isolation is crucial for osteoprogenitor cells to grow and differentiate without interference from fast-growing soft tissues (Yang et al., 2022). Barrier membranes are categorized into two categories: non-resorbable and resorbable. Non-resorbable membranes can sustain and maintain the regeneration site. However, they have some disadvantages, such additional procedures to remove the membrane, a higher risk of membrane exposure, and wound dehiscence, which may cause patient discomfort. Moreover, the success of bone transplantation may be affected by bacterial contamination, leading to post-surgical infections and potentially suboptimal bone regeneration (Schenk, 1994; Soldatos et al., 2017; Zitzmann et al., 1997). Resorbable membranes are widely utilized in clinical practice owing to their numerous advantages, including eliminating the need for membrane removal and reducing patient morbidity (Hämmerle, & Jung, 2003). Despite the preference for resorbable collagen membranes, they often exhibit insufficient stiffness and have an indeterminate resorption time. Resorbable membranes can be produced by synthetic methods or derived from natural materials. Synthetic biodegradable polymers are used in biomedical applications, such as drug delivery, sutures, and tissue engineering, due to their customizable physicochemical properties achieved through changes in composition and manufacturing.

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Polycaprolactone (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers are the most commonly used due to their FDA-approved biocompatibility and biodegradability (Makadia, & Siegel, 2011; Song et al., 2018). PLGA consists of lactic acid (LA) and glycolic acid (GA). The ratios of these copolymers significantly influence their degradability (Wong et al., 2019). In principle, GA exhibits more hydrophilicity than LA; hence, the degradation rate of PLGA may be enhanced or reduced by varying the amounts of GA or LA. (Kapoor et al., 2015; Wang et al., 2016). In the present study (Petposri et al., 2023), the physical characteristics and biocompatibility of PLGA membranes with lactic acid-to-glycolic acid ratios of 70:30 (high LA–low GA) and 10:90 (low LA–high GA) were comparatively assessed to identify the ideal ratio for fabricating the GBR membranes. The results indicated that PLGA (10:90) membranes exhibited a higher degradation rate than PLGA (70:30) membranes. Furthermore, the degradation of glycolide and lactide polymers in PLGA (10:90) released more acid, creating an unsuitable environment for cell survival. Conversely, the PLGA (70:30) membranes demonstrated poor mechanical strength and excessive swelling when used for GBR in rat models.

This study aimed to reassess the physical properties and biocompatibility of PLGA membranes with varying ratios of lactide and glycolide in vitro to identify the optimal raw material for producing GBR membranes suitable for clinical applications.

2. Objective

To compare the physical properties and biocompatibility of 3D-printed PLGA membranes with LA:GA ratios of 50:50, 70:30, and 85:15.

3. Materials and Methods

3.1 Materials

The raw materials utilized were medical grade PLGA based on the LA: GA ratios, including 50:50 mol% (Group A), LA:GA = 70:30 mol% (Group B), and LA:GA = 85:15 mol% (Group C) (CMU-Bioplasorb® PLG, BPLCMU, Thailand).

3.2 Fabrication of 3D-printed Membranes

The materials for all groups were melted in a hot-melt pneumatic dispenser of a bio-printer (Dr. INVIVO 4D2, ROKIT Healthcare, Inc., Seoul, Republic of Korea) at 180-200 °C and then extruded through a 200 µm-nozzle tip to form the membranes. The architecture of the membranes was designed in a grid infill pattern at 0° and 90° of filament rows, with a layer height set to 0.2 mm for printing in two layers per membrane. The membranes were stored in sterilization pouches, and the packaging was sterilized using gamma irradiation at 25 KGy 2 weeks before the experiments.

3.3 Surface Morphology

The surface morphologies of the upper (bone side) and lower (soft tissue side) sides of the 10 mm × 10 mm membranes were assessed using a scanning electron microscope (SEM, JOEL Ltd., Tokyo, Japan).

3.4 Mechanical Properties

Tensile testing was conducted to assess the mechanical properties using a universal testing machine (Lloyd Instruments Ltd., West Sussex, UK). All membrane groups were soaked in simulated body fluid (SBF) at room temperature for 3 hours before testing. In its wet state, the specimen was placed at an initial distance of 30 mm between the grips. A 250 N load cell was employed, and tensile force was applied at a crosshead speed of 3 mm/min until the specimen fractured.

3.5 Surface Wettability

The surface wettability of the membranes was assessed using an optical contact angle analyzer (OCA25, Data Physics Instruments GmbH, Filderstadt, Germany). The sessile drop method was employed to

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measure the water contact angles (WCA) by placing 5 µL deionized water on the surfaces of the 10 mm × 10 mm membranes.

3.6 Degradation properties

The membranes were immersed in SBF to evaluate their weight loss. Each membrane was weighed (Wd0) using an analytical balance (Sartorius, Goettingen, Germany) before being immersed in 4 mL of SBF per well in 12-well plates (Corning, Merck KgaA, Darmstadt, Germany). The membranes were incubated at 37 °C, and SBF was added every 10 days to maintain a constant volume. To measure the weight loss on days 15, 30, 60, and 90, the membranes were collected and freeze-dried in a freeze dryer (LaboGene, Lillerød, Denmark) for 3 hours. Their dry weights (Wdt) were measured, and their weight loss was calculated as:

$$\% \text{ Weight loss} = 100 \times (Wd0 - Wdt)/Wd0$$

3.7. Cytotoxicity

To evaluate the membranes' cytotoxicity, the mouse osteoblast cell line (MC3T3-E1, ATCC, Manassas, VA, USA) and mouse fibroblast cell line (L929, ATCC, Manassas, VA, USA) were seeded on each side of the membranes at a density of 1×10^4 cells/cell type/side. The viability of the cells was quantitatively assessed using Presto Blue (Invitrogen, Thermo Fisher Scientific, USA) at an optical density of 600 nm on day 1 and day 3.

3.8 Statistical Analysis

The data were analyzed using one-way ANOVA and Tukey's HSD test. The results from sample groups (n = 5) are shown as mean ± SD, with significance set at $p < 0.05$.

4. Results

4.1 Surface Morphology

Figure 1 displays the surface morphologies of the 3D-printed membranes under SEM. The SEM images reveal distinct surface morphologies between the tissue and bone sides of the membranes. Small dimples are distributed across the smooth surface, while the rough sides show irregular and rough textures. The membranes from the groups presented similar morphologies.

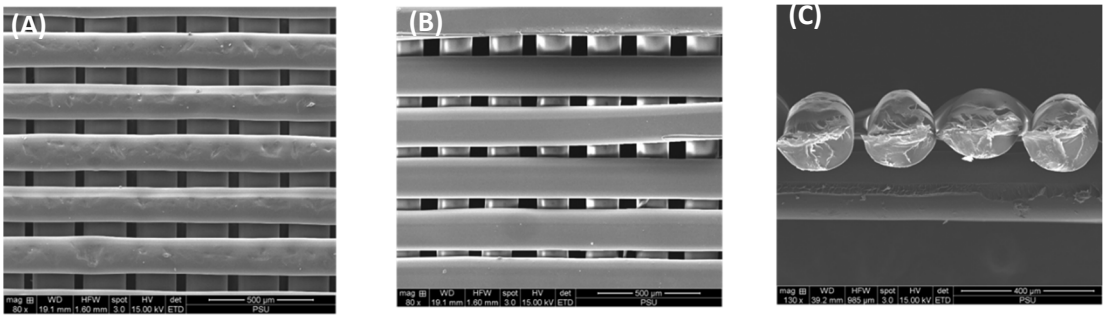

Figure 1 The SEM images of PLGA membranes (A) Superior aspect, (B) Inferior aspect, and (C) cross-sectional aspect

4.2 Mechanical Properties

The tensile testing results of the membranes indicate that both the maximum load and Young's modulus tend to decrease with an increase in the GA amount (Figure 2). Group C exhibited significantly higher tensile strength than Group A and Group B ($p < 0.05$). However, there was no statistically significant difference in Young's modulus among the groups.

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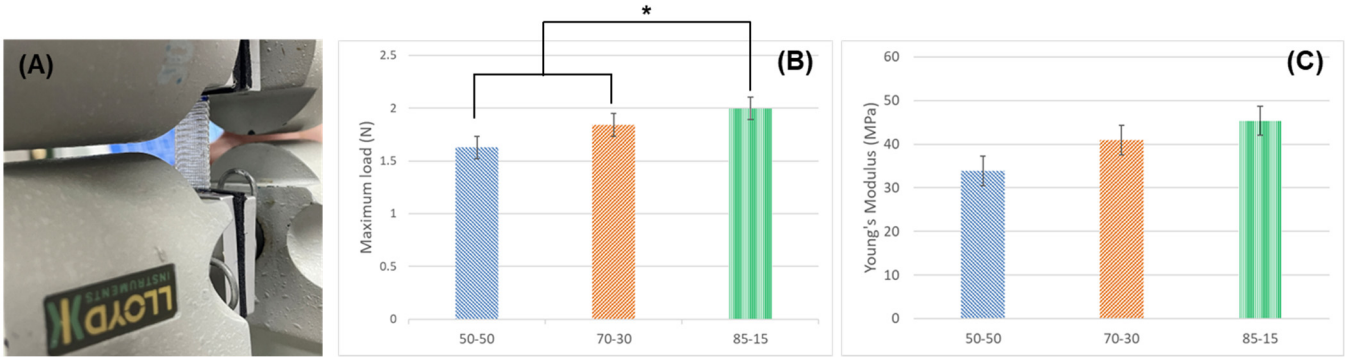

Figure 2 (A) The tensile testing on the PLGA membranes. (B) Maximum load, and (C) Young's modulus of each group (* = $p < 0.05$)

4.3 Surface Wettability

The sessile drop contact angle measured on the PLGA membranes is illustrated in Figure 3. The data indicate a general decrease in contact angle values with an increased LA content, from 56.3° in Group A (50:50) to 55.4° in Group B (70:30) and 46.8° in Group C (85:15). Group C had a significantly lower contact angle than the other groups ($p < 0.05$), indicating increased hydrophilicity.

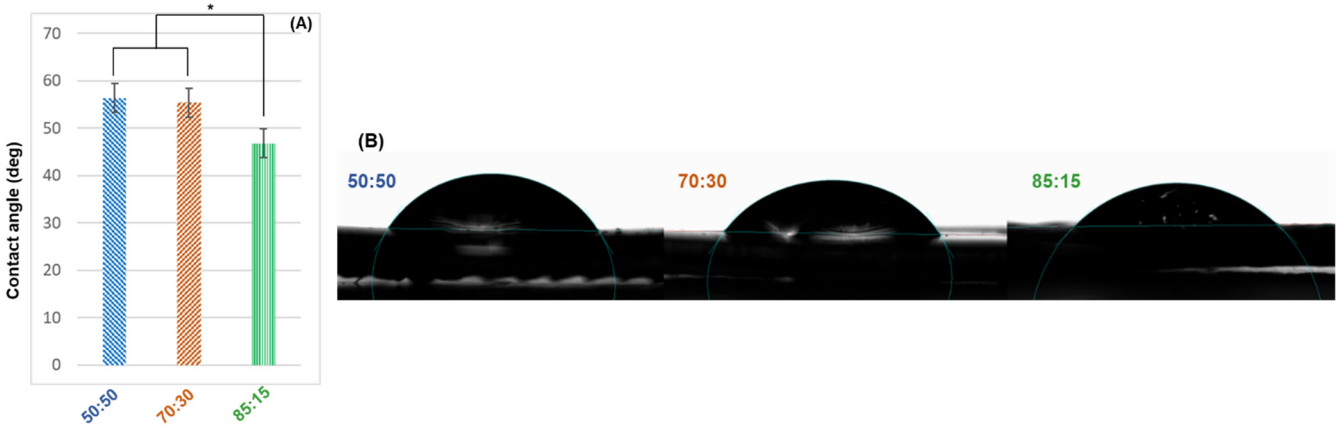

Figure 3 Water contact angles on the PLGA membranes. (A) Contact angle values, (B) The optical images of water contact angles (* $p < 0.05$)

4.4 Degradation Properties

The degradation of the PLGA membrane was assessed up to day 90 (Figure 4A). The results indicated that the rate of degradation increased over time. The results from day 14 to day 90 demonstrated that the degree of degradation was significantly greater in Groups A and B compared to Group C. Figure 4(B) shows the changes in pH of SBF containing the membranes. The pH of Group A was significantly lower than that of Groups B and C. It was observed that the pH of Groups B and C slightly changed over seven days. In contrast, the pH of group A decreased rapidly during the first seven days, and all groups had similar pH levels on day 21. The final pH of Group A was 2.61, Group B was 2.78, and Group C was 3.66.

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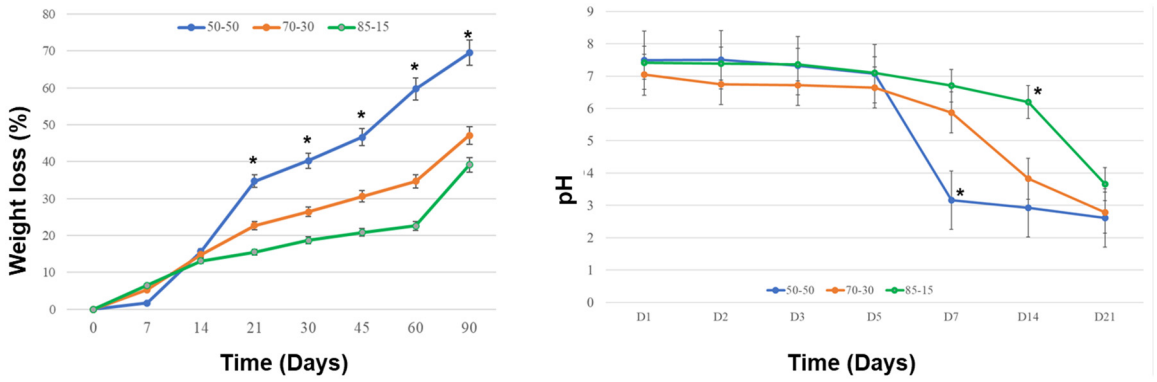

Figure 4 The graphs demonstrate the degradation (A) and the pH change (B) of the PLGA membranes. (* $p<0.05$ when comparing the groups at each time point

4.5 Cytotoxicity

The cytotoxicity test using L929 and MC3T3-E1 cells is demonstrated in Figure 5. There was no statistical difference in the proliferation of osteoblasts and fibroblasts among the groups on days one and three. Cell attachment and morphology of the L929 fibroblasts on the smooth surface and the MC3T3-E1 osteoblasts on the rough surfaces of each membrane were examined (Figure 6). After seeding the cells on the membranes and culturing them for three days, the SEM images indicated good cell attachment of both the fibroblasts and the osteoblasts on the surfaces of the membranes. There was no substantial difference in the proliferation of osteoblast and fibroblast cells between the groups on days 1 and 3.

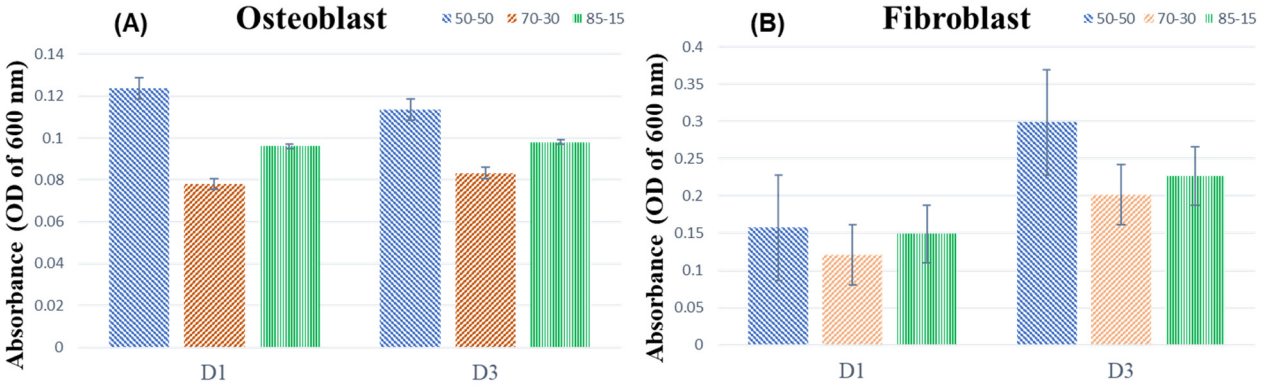

Figure 5 The graphs demonstrate the cytotoxicity results for osteoblasts (A) and fibroblasts (B)

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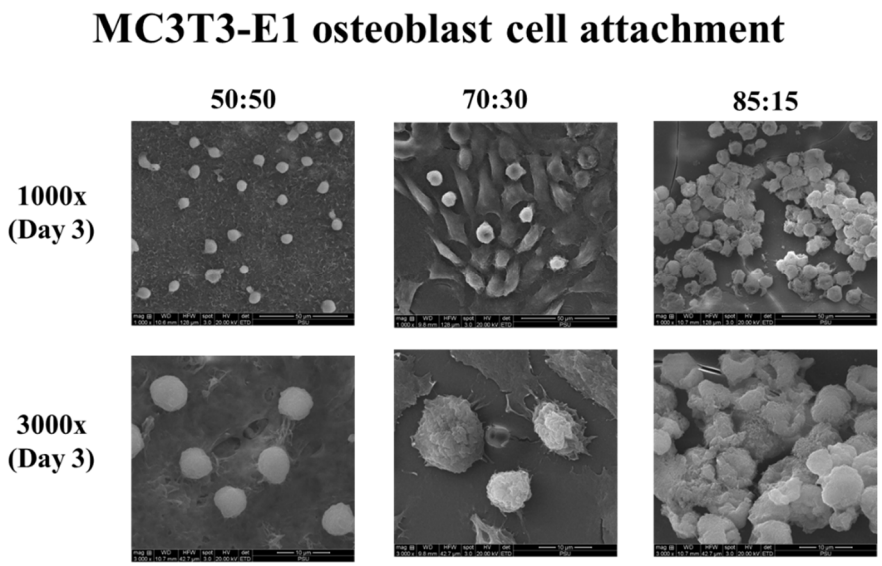

Figure 6 SEM images show the behavior of osteoblasts attached to the top surfaces of the PLGA50:50, 70:30, and 85:15 membranes on day 3 at magnifications of x1000 and x3000

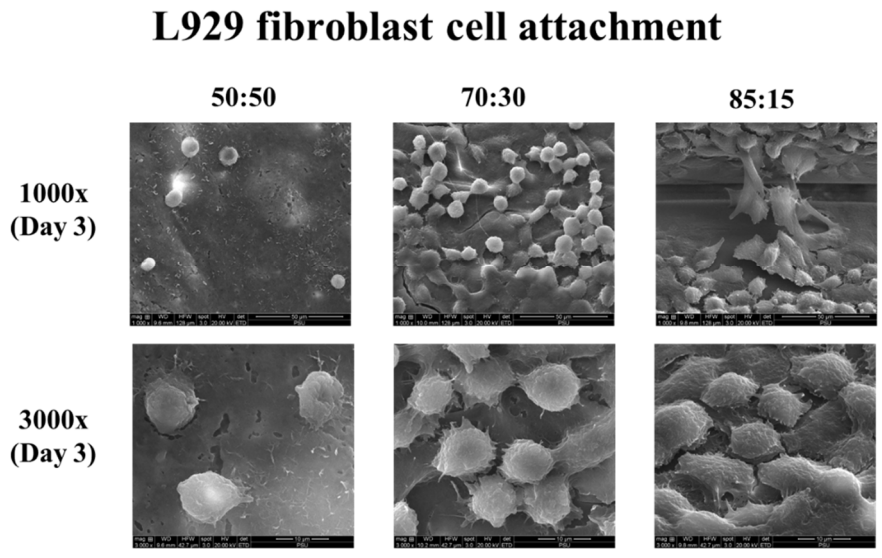

Figure 7 SEM images show the behavior of fibroblasts attached to the bottom surfaces of the PLGA50:50, 70:30, and 85:15 membranes on day 3 at magnifications of x1000 and x3000

5. Discussion

This study prepared three distinct ratios of PLGA polymer blends for membrane fabrication using extrusion-based 3D printing. The tensile tests demonstrated that membranes with a higher glycolic acid content exhibited lower strength than those with a lower proportion of glycolic acid. As a semi-crystalline polymer, the degree of crystallinity in PLGA significantly influences its mechanical properties. Glycolic acid units tend to disrupt crystalline structure, resulting in decreased crystallinity. Reduced crystallinity is typically associated with lower tensile strength and modulus. This increased flexibility can benefit applications

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requiring softer materials but may not be suitable where rigidity is needed (dos Santos et al., 2017). A study found that the contact angle decreases as lactic acid concentration increases, indicating that lactic acid can improve the hydrophilic properties of membranes. The enhanced hydrophilicity may facilitate the initial attachment of water on membrane surfaces, which is biocompatible and supports cell adherence (Subedi, 2011). In this context, the 85:15 PLGA membranes exhibited significantly higher tensile strength than Group A and Group B. In addition, the membranes exhibited a contact angle of 46.8°, which was substantially lower than those observed in the other groups. Therefore, the surfaces of the membranes are confirmed to be hydrophilic, making them suitable for both cell types. The cytotoxic properties of the PLGA membranes were characterized by cell proliferation using the Presto Blue assay. Cell attachment was evaluated through SEM images, which demonstrated that both fibroblast and osteoblast cells can adhere to the membrane's surface. Enhancing the hydrophilicity of PLGA membranes improves their interaction with osteoblasts, leading to better cell adhesion, proliferation, and differentiation. Some previous studies support incorporating more hydrophilic components into PLGA membranes, which has created a more favorable microenvironment for osteoblasts, resulting in reduced cytotoxic effects and improved cellular responses (Fu et al., 2017; Rocha et al., 2022).

After seven days, the acid release rate significantly increases, creating a more acidic environment unsuitable for cell viability. PLGA 50:50 degrades faster than other compositions, causing a quicker drop in pH due to acidic degradation products. All groups show similar acidic environments from accumulated degradation products. However, the less pronounced pH decreased in Group C (PLGA 85:15) indicates a slower degradation rate, which may be beneficial for applications needing longer-term material stability. Regarding degradation behaviors, the membranes in the 50:50 PLGA group degraded by more than 70% within 90 days, while other groups showed degradation rates exceeding 50%. This rate of degradation appears to align with the bone healing process (Roden Jr, 2010). The reduction in crystallinity enhances the hydrolytic degradation of PLGA, as amorphous regions are more prone to water penetration and subsequent breakdown. A higher glycolic acid content generally leads to faster degradation rates (Gentile et al., 2014).

However, further in vivo experiments are necessary to thoroughly evaluate the fibroblast cell occlusive property and osteogenic potential of the membrane. These studies are crucial for confirming the material's effectiveness in barrier function and its capability to support vascularization and membrane ossification within a physiological environment.

6. Conclusion

Polylactic-co-glycolic acid (PLGA) membranes were successfully fabricated using 3D printing. The PLGA membranes (LA:GA= 85:15) exhibited better mechanical properties, degradability, and biocompatibility than those with ratios of 50:50 and 70:30.

7. Acknowledgements

The authors thank Fundamental Fund 2568 for the grant and the Cranio-Maxillofacial Hard Tissue Engineering Center at Prince of Songkla University's Faculty of Dentistry.

8. References

dos Santos, F. A., Valle Iulianelli, G. C., & Bruno Tavares, M. I. (2017). Development and properties evaluation of bio-based PLA/PLGA blend films reinforced with microcrystalline cellulose and organophilic silica. *Polymer Engineering & Science*, *57*(4), 464-472.

Fu, C., Bai, H., Hu, Q., Gao, T., & Bai, Y. (2017). Enhanced proliferation and osteogenic differentiation of MC3T3-E1 pre-osteoblasts on graphene oxide-impregnated PLGA–gelatin nanocomposite fibrous membranes. *RSC Advances*, *7*(15), 8886-8897.

Gentile, P., Chiono, V., Carmagnola, I., & Hatton, P. V. (2014). An overview of poly (lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. *International Journal of Molecular Sciences*, *15*(3), 3640-3659.

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Hämmerle, C. H., & Jung, R. E. (2003). Bone augmentation by means of barrier membranes. *Periodontology 2000*, *33*(1), 36-53.
Kapoor, D. N., Bhatia, A., Kaur, R., Sharma, R., Kaur, G., & Dhawan, S. (2015). PLGA: a unique polymer for drug delivery. *Therapeutic Delivery*, *6*(1), 41-58.
Liu, J., & Kerns, D. G. (2014). Suppl 1: Mechanisms of guided bone regeneration: A review. *The Open Dentistry Journal*, *8*, 56.
Makadia, H. K., & Siegel, S. J. (2011). Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers*, *3*(3), 1377-1397.
Petposri, S., Thuaksuban, N., Buranadham, S., Suwanrat, T., Punyodom, W., & Supphaprasitt, W. (2023). Physical Characteristics and Biocompatibility of 3D-Printed Polylactic-Co-Glycolic Acid Membranes Used for Guided Bone Regeneration. *Journal of Functional Biomaterials*, *14*(5), 275.
Rocha, C. V., Gonçalves, V., da Silva, M. C., Bañobre-López, M., & Gallo, J. (2022). PLGA-based composites for various biomedical applications. *International Journal of Molecular Sciences*, *23*(4), 2034.
Roden Jr, R. D. (2010). Principles of bone grafting. *Oral and maxillofacial surgery clinics of North America*, *22*(3), 295-300.
Schenk, R. (1994). Bone regeneration: biologic basis. *Guided bone regeneration in implant dentistry*.
Soldatos, N. K., Stylianou, P., Koidou, V. P., Angelov, N., Yukna, R., & Romanos, G. E. (2017). Limitations and options using resorbable versus nonresorbable membranes for successful guided bone regeneration. *Quintessence International*, *48*(2).
Song, R., Murphy, M., Li, C., Ting, K., Soo, C., & Zheng, Z. (2018). Current development of biodegradable polymeric materials for biomedical applications. *Drug Design, Development and Therapy*, 3117-3145.
Subedi, D. (2011). Contact angle measurement for the surface characterization of solids. *Himalayan Physics*, *2*, 1-4.
Wang, H.-L., & Boyapati, L. (2006). "PASS" principles for predictable bone regeneration. *Implant Dentistry*, *15*(1), 8-17.
Wang, J., Wang, L., Zhou, Z., Lai, H., Xu, P., Liao, L., & Wei, J. (2016). Biodegradable polymer membranes applied in guided bone/tissue regeneration: a review. *Polymers*, *8*(4), 115.
Wong, C., Yoganarasimha, S., Carrico, C., & Madurantakam, P. (2019). Incorporation of fibrin matrix into electrospun membranes for periodontal wound healing. *Bioengineering*, *6*(3), 57.
Yang, Z., Wu, C., Shi, H., Luo, X., Sun, H., Wang, Q., & Zhang, D. (2022). Advances in barrier membranes for guided bone regeneration techniques. *Frontiers in Bioengineering and Biotechnology*, *10*, 921576.
Zitzmann, N. U., Naef, R., & Schärer, P. (1997). Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. *International Journal of Oral & Maxillofacial Implants*, *12*(6).

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