

# 실크 피브로인 나노섬유-폴리글리콜산 하이브리드 스캐폴드를 사용한 토끼 두개골 결함의 재생

곽동민, 고윤제, 김은진\*, 권오형

금오공과대학교

ohkwon@kumoh.ac.kr

## Guided Regeneration of Rabbit Calvarial Defects Using Silk Fibroin Nanofiber-Poly(glycolic acid) Hybrid Scaffolds

Dongmin Kwak, Young-Gwang Yunjeh Ko, Eun Jin Kim and Oh Hyeong Kwon

Department of Polymer Science and Engineering, Kumoh National Institute of Technology, Gumi, Gyeongbuk 39177, Korea

Theracion Biomedical Company, Limited, Seongnam, Gyeonggi 13201, Korea\*

### 요약

Bone tissue engineering aims to regenerate defected bones by combining cells, scaffolds, and growth factors. We propose patient-customizable guided bone regeneration (GBR) and membrane-guided tissue regeneration (GTR) scaffold hybrid constructs for precise bone tissue restoration without dimensional collapse beyond the critical bone defect size. Silk fibroin (SF) nanofiber membranes and poly(glycolic acid) (PGA) scaffolds were fabricated using electrospinning and 3D printing methods. The initial attachment and proliferation of preosteoblasts on a PGA scaffold were analyzed. The regenerated bone volumes of control and SF-PGA hybrid scaffolds were 14.8 and 21.4%, respectively, after 8 weeks of in vivo rabbit calvarial defect regeneration. The SF-PGA hybrid scaffold group exhibits greater regeneration of bone tissue than the control and PGA scaffold groups, indicating that this is a promising material combination as a GBR-GTR agent.

### I. 서론

Clinical research on the replacement of damaged tissues and organs has progressed consistently over the past few decades. Recently, the importance of tissue regeneration has increased dramatically. Tissue engineering combined with regenerative medicine is one of the most promising treatment methods for repairing defected tissues[1-5]. The major components of tissue engineering are cells, scaffolds, and growth factors. The human body is composed of several different types of tissues, including skin, adipose, muscle, vascular, and bone tissue. Among these tissues, bone tissue plays a critical role as the skeletal framework for the body.

One of the critical considerations for bone tissue regeneration is the slower growth rate of bone compared to adjacent soft tissue. Guiding membranes or porous scaffolds are typically employed for bone tissue regeneration in clinical practice. Guiding membranes possess biocompatibility, biodegradability, and barrier properties that prevent soft tissue invasion. Recently, several guided tissue regeneration (GTR) agents have been commercialized. GTR scaffolds can effectively secure the dimensional integrity of the defected tissue. A porous GTR scaffold can provide a spatial framework for a defected area and facilitate osteoblast proliferation. Porous scaffolds can be fabricated using three-dimensional (3D) printing methods. 3D printing for biomedical applications is

the easy design of complex and uniform pore structures for patient customization and reproducibility.

The installation of a barrier membrane and guiding scaffold is a typical process for bone regeneration surgeries. In this paper, we propose the hybridization of a GBR membrane and patient-customizable GTR scaffold for bone tissue regeneration using electrospinning and 3D printing techniques. The base materials selected for this study were silk fibroin (SF) and poly(glycolic acid) (PGA). SF is a natural material with excellent tensile strength, biocompatibility, hydrophilicity, and biodegradability. PGA is one of the most widely used synthetic degradable polymers. The biodegradability and thermoplasticity of a PGA scaffold can enhance additive manufacturing processes and cell proliferation.

In this study, we prepared SF nanofiber membrane-PGA hybrid scaffolds and characterized their structures, biomechanical properties, biodegradability, cell attachment, proliferation, and in vivo bone tissue regeneration.

### II. 본론

Preventing soft tissue invasion and promoting hard tissue formation are important factors in the regeneration of bone defects. Nanofibrous barrier membranes can provide a physical boundary between soft and hard tissues. 3D printed porous PGA scaffolds can guide and facilitate

the proliferation of osteoblasts based on the critical sizes of bone defects. In this study, we prepared a hybrid construct with an SF nanofibrous membrane acting as a GBR membrane and a 3D printed PGA scaffold acting as a GTR framework for the advanced regeneration of defected bone.

In general, the essential properties of GBR membranes are flexibility, biomechanical strength, biocompatibility, and biodegradability. Additionally, a membrane should not only provide barrier properties for epithelial and soft tissues but also facilitate the permeability of nutrients, oxygen, and metabolic waste. Nanofibrous mats meet all of these criteria for GBR membranes. Therefore, we fabricated optimized SF nanofiber mats by adjusting electrospinning parameters. The electrospun nanofibers fabricated using a 4 wt % HFIP-SF solution exhibit randomly aligned nanofibrous structures and unimodal fiber diameter distributions without beads or microfibers. Therefore, we selected a 4 wt % solution as the optimal concentration for further experimentation.

The average diameter of the nanofibers fabricated using a 4 wt % solution was  $639 \pm 150$  nm. To guide bone proliferation, we fabricated porous PGA scaffolds using a 3D printer. The average strand diameter of the PGA scaffolds was  $207 \pm 11$   $\mu$ m. Four sizes of square pores were designed with mean side lengths of  $104 \pm 9$   $\mu$ m (P100),  $212 \pm 11$   $\mu$ m (P200),  $413 \pm 14$   $\mu$ m (P400), and  $802 \pm 24$   $\mu$ m (P800). All scaffolds contain sufficient interconnected pores to proliferate, migrate, form tissue, and deliver nutrients and metabolic materials.

To facilitate GTR along a scaffold frame, initial cell attachment is important for 3D printed PGA surfaces. To investigate initial cell attachment, preosteoblasts were seeded onto the P100, P200, P400, and P800 scaffolds. It was determined that the cell seeding efficiency decreases with an increasing pore size. Although many cells attached to the top surfaces of all scaffolds, the cell seeding efficiency was predominantly affected by the specific strand number of each PGA scaffold. Cell proliferation in the P100, P200, P400, and P800 scaffolds was monitored for 7 days. PGA serves a suitable substrate for proliferation and differentiation of progenitor cells and the formation of a 3D mineralized tissue. The cell proliferation gradient of P100 is diminished compared to those of P200 and P400 on day 7. However, the P800 scaffold consistently exhibits significantly lower cell proliferation compared to the other scaffolds. The large surface area per specific volume of a porous scaffold provides critical advantages in terms of initial cell attachment and proliferation. Also the proliferation rates of the P200 and P400 scaffolds after 7 days of incubation are close to that of the P100 scaffold. One of the important criteria for an engineered scaffold is pore size because pores facilitate the circulation of oxygen, nutrition, and waste. Additionally, an interconnected pore structure provides adequate spreading space for cells. For these reasons, we selected the P400 scaffold for our animal study.

Hybrid constructs for the regeneration of bone defects require membranes to prevent soft tissue invasion and porous scaffolds to guide bone tissue from defected surfaces. In this study, PGA scaffolds (P400) were attached to SF nanofibrous membranes using a PGA hot-melt process. The diameters of the SF nanofiber membranes were set to 10 mm

to cover the bone defects (8 mm) completely. The diameters of the PGA scaffolds were set to 7.5 mm to prevent tight implantation during surgery. Finally, the thicknesses of the PGA scaffolds were set to 1 mm to match the thickness of a rabbit calvaria ( $1.6 \pm 0.2$  mm,  $n = 10$ ).

To investigate the regeneration of calvarial defects, we conducted a rabbit model test. A thin fibroblast band could be observed in the control group on the top surfaces 4 weeks after the initial surgical operation. In vivo animal experiments revealed that the SF-PGA group exhibited greater bone formation compared to the control and PGA groups. This finding was confirmed by micro-CT images. The SF-PGA group exhibited excellent bone tissue coverage in the critical defect area, whereas the control group exhibited poor bone regeneration at the boundaries of the defect area. The SF-PGA hybrid scaffolds both promoted bone tissue regeneration and prevented soft tissue invasion.

### III. 결론

Nanofibrous SF membranes and porous PGA scaffolds were successfully fabricated by adjusting electrospinning and 3D printing parameters. The membranes and scaffolds were then used for guided bone tissue regeneration. The cell seeding efficiency of PGA scaffolds decreases with increasing pore length. PGA scaffolds with a pore length of 400  $\mu$ m exhibit superior proliferation of osteoblasts compared to other scaffolds. The biodegradation rates of PGA scaffolds and SF nanofiber membranes facilitate bone tissue formation. In an in vivo rabbit experiment, the SF-PGA scaffold group exhibited superior bone volume regeneration compared to the control and PGA scaffold groups. Overall, the SF-PGA hybrid scaffold constructs represent a promising solution for the guided regeneration of defected bone tissues.

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