Melt electrowriting-based PCL engineered membrane as potential artificial periosteum scaffold

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INTRODUCTION: Periosteum is the connective tissue envelope that covers the bone surface and is important for bone remodeling and bone repair (*1*). Autologous periosteum transplantation is limited by its sources and the health status of patients (*1*). Tissue engineered periosteum is often used as a substitute for autologous periosteum and plays an important role in accelerating the repair of bone defects (*1*). The engineered periosteum scaffolds provide a three-dimensional structure for cell adhesion and a suitable microenvironment to support cell function and facilitate cell-cell interactions (*1*). Currently, electrospinning technology is the primary choice for the manufacture of artificial periosteum scaffolds (*1*). The organic solvents required for electrospinning solutions are often toxic and volatile. In particular, classically spun filaments are disordered and uncontrollable, which cannot be directed to guide cell growth. Compared with electrospinning technology, the melt electrowriting (MEW) technology does not require solvents, so there is no need to consider recycling or removing toxic organic solvents during the fiber manufacturing process. Importantly, its controllable microstructure can directionally guide cell growth along the filament. Therefore, it has significant advantages in manufacturing of engineered periosteum scaffolds. Interestingly, there have been few reports on the application of MEW to engineered periosteal scaffolds in recent years. Previous studies have found that MEW-based polycaprolactone (PCL) scaffolds with 30° angle between fibers have better performance in guiding cell growth than 90° (*2*). However, we speculate that the large difference in adjacent angles (30°-150°) may lead to poor mechanical properties in the perpendicular direction and poor cell-guiding performance. The purpose of this study was to develop a bidirectional engineered periosteal scaffold with optimized mechanical properties and directional guided cell growth and the hypothesis was that the optimizing bidirectional scaffold has better mechanical properties and cell guidance compared with the unidirectional scaffold based on MEW technology.

METHODS: PCL Purasorb ® PC12 (Corbion Purac, Amsterdam, The Netherlands; CAS 24980-41-4) was used to print the scaffold. The MEW operated at a high voltage field of 6 kV, a PCL temperature of 79 °C and an average metering pressure of 25 kPa. The printing speed was chosen to be 700 mm/min while the distance between the nozzle (Ø 250 µm) and the corresponding holder height was kept constant at 3.5 mm. The fabrication of the 30° diamond-shaped unidirectional scaffold was the same as previous study (*2*), and the bidirectional scaffold was obtained by rotating the scaffold printing direction 90° after the single-layer unidirectional scaffold was obtained. The PCL scaffolds of 5 mm × 5 mm × 8 layers and 10 mm × 10 mm × 8 layers were used for cell culture and mechanical experiments, respectively. Uniaxial compression tests were applied for mechanical testing using a Zwick-Roell Z010 (ZwickRoell, Ulm, Germany). Take the peak destructive force as the maximum breaking force (F_{max}) of the PCL scaffold. All scaffolds were hydrophilized in 1 M NaOH etching solution for 3 h prior to cell experiments. Then, immerse the scaffold in 70% ethanol twice for 20 min as a disinfection step. Scaffolds were then subjected to a 10 min wash step in DMEM (ThermoFisher, Waltham, MA, USA) containing 1% penicillin/streptomycin to remove hydrophilic/sterilizing residues. Then the bone marrow mesenchymal stem cells (BMSCs) were seeded on the scaffolds and cultured at 37 °C and 5% CO_2. After one week of incubation, the scaffolds were used for live-dead and Phalloidin/DAPI staining to observe cell growth on the scaffolds. Differences between multiple groups were tested using one -way ANOVA with Tukey's post hoc method and between two groups were tested using t-test. Differences were considered significant when $p < 0.05$.

RESULTS: The visual and the microscopic images showed that the filaments of the unidirectional and bidirectional scaffolds were uniformly distributed, and the filaments of the bidirectional scaffolds were more evenly distributed in all directions than the unidirectional scaffold **(Fig. 1)**. There was no significant difference in the diameter of the filaments of the two scaffolds (unidirectional vs. bidirectional scaffold: 15.6 ±1.2 vs. 14.9 ± 1.2 [µm] ($p = 0.51$)). As predicted, the unidirectional scaffold exhibited significantly greater deformation during the initial testing procedure and lower F_{max} when the force was applied along the 150° direction than the unidirectional scaffold when the force was applied along the 30° direction and the bidirectional scaffold. While the F_{max} of the unidirectional scaffold along the 30° showed no significant difference with the bidirectional scaffold group **(Fig. 2)**. The results of the cell experiment showed that no obvious cell adhesion was found on the two kinds of scaffolds at 4 hours after the BMSCs seeded under the light microscope. After 7 days of seeding, the Live/Dead and Phalloidin/DAPI staining under the fluorescent microscope showed that the cells adhered and grew at an angle of 30° on the unidirectional scaffolds. In the bidirectional group, the cells were evenly distributed throughout the entirely scaffold, and the number of cells was significantly more than that of in the unidirectional scaffold group **(Fig. 3)**.

DISCUSSION: This study showed that bidirectional scaffolds based on 30° angle have significant advantages over unidirectional scaffolds in terms of mechanics and cell guidance. The periosteum is traditionally divided into an outer fibrous layer rich in fibroblasts and an inner layer with significant osteogenic potential. The outer fiber layer has an anchoring effect that provides elasticity and flexibility, and allows resistance to pressure and tension (*1*). Therefore, the MEW-based 30° bidirectional scaffold of PCL in this study is an optimized periosteal scaffold that can provide the function of the natural periosteal outer fibrous layer. To further imitate the periosteum structure and endow the scaffold with osteogenic and angiogenic potential, we will compound suitable hydrogels in the next step to manufacture biomimetic engineered periosteum to promote the repair of bone defects.

SIGNIFICANCE/CLINICAL RELEVANCE: The MEW-based 30° bidirectional scaffold of PCL in this study is an optimized periosteal scaffold that can provide the function of the natural periosteal outer fibrous layer.

REFERENCES: 1. W. Zhang *et al.*, Periosteum and development of the tissue-engineered periosteum for guided bone regeneration. *J Orthop Translat* **33**, 41-54 (2022). 2. Richter, M. *et al.*, Influence of fiber orientation in MEW substrates on the growth of keratinocytes and fibroblasts.

Images:

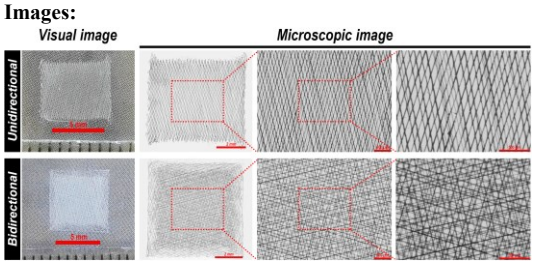

Fig. 1: The visual and microscopic images of the PCL periosteum scaffold. Unidirectional and Bidirectional indicate the unidirectional and bidirectional PCL scaffold.

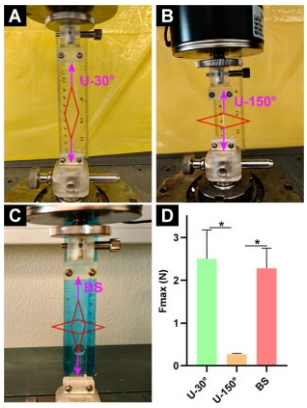

Fig. 2: The testing method **(A-C)** and results **(D)** of mechanical experiments. U-30° **(A)**, U-150° **(B)** and BS **(C)** indicate the fore was applied the unidirectional scaffold along the direction of 30°, the unidirectional scaffold along the direction of 30° and bidirectional scaffold for mechanical testing. Data are presented as means ± SD. *P < 0.05.

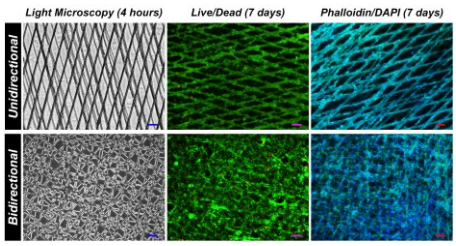

Fig. 3: The light microscopic images after BMSCs seeded on the PCL scaffold 4 hours (left). The fluorescence microscopic images of Live/Dead staining (middle) and Phalloidin/DAPI staining (right) after BMSCs seeded on the PCL scaffold 7 days. Unidirectional and Bidirectional indicate the unidirectional and bidirectional PCL scaffold. Scale bar = 100 µm.