Cartilage Tissue Engineering with Silk Fibroin Scaffolds Fabricated by Indirect Additive Manufacturing Technology

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Statement of Purpose: Cartilage engineering for aesthetic or reconstructive purposes in craniofacial surgery relies on the same foundation of scaffold and cell interaction found in all tissue engineering fields. PCL is a polyester polymer that has been frequently used in the tissue engineering. However, reports have demonstrated that there was poor biological adhesion between cells and the naked scaffold. Hence, the PCL material may require further optimization through manufacturing or modification techniques. To investigate the effects of two biocompatible surface modification materials and the use of rapid prototyping techniques in cartilage tissue engineering, we designed a study to further optimize the process of cartilage tissue engineering through these techniques.

Methods: The PCL scaffolds used in this study were fabricated via SLS, rapid prototyping machine. Surface modification of scaffolds was performed by using gelatin and collagen. Three groups of scaffold were produced including naked PCL scaffold group (PCL), gelatin-modified PCL scaffold group (GEL+P), and type II collagen-modified PCL scaffold group (COL+P). The chondrocytes were isolated by enzymatic digestion of the knee joint articular cartilage from hind limbs of swine. To determine the viability and proliferation of cells cultured in the PCL scaffolds, MTT test was carried out. Constructs were examined after 2 and 4 weeks under SEM. A live and dead assay was used to evaluate cells survival and distribution on the scaffolds. Glycosaminoglycan (GAG) levels and the type II collagen (COL II) in the constructs were determined. For in vivo studies, a subcutaneous implantation protocol of nude mice was used. The specimens were harvested and were evaluated by immunohistochemical (IHC) staining after 3 months.

Results: Scaffolds fabricated through selective laser sintering included twelve layers at repeating stack angles of 0°/45°/90°/135°. Coating the PCL scaffold through immersion method reduced the contact angles by 23.84% and 41.89% for gelatin and type II collagen, respectively. The swelling ratios of PCL, GEL+P and COL+P scaffolds were 357.14±0.00%, 375±34.09% and 400±12.50%. MTT assay showed a significant increase of cell attachment to the COL+P scaffolds. By week 4, a significant difference in ECM deposition can be noted between groups, with scarce regions on the naked scaffold and larger patches for GEL+P and COL+P scaffolds. The confocal images of GEL+P groups at two and four weeks show a visible difference in green fluorescent intensity. COL+P groups demonstrate much higher intensity and cell cluster number when compared to naked PCL and GEL+P, showing that type II collagen improves the biointeractive properties of PCL scaffolds. At eight weeks, both modified scaffold groups already had a significantly higher amount of GAGs and COL II. For subdermal implantation of animal study, the constructs were retrieved after 12 weeks for histological and immunohistochemical staining. No apparent inflammatory infiltrations were seen on the H&E stains. COL+P scaffolds showed more evenly distributed staining than the other two groups. IHC stain indicated positive staining for COL II also showed a similar pattern, with less intense staining in porous regions of the scaffold.

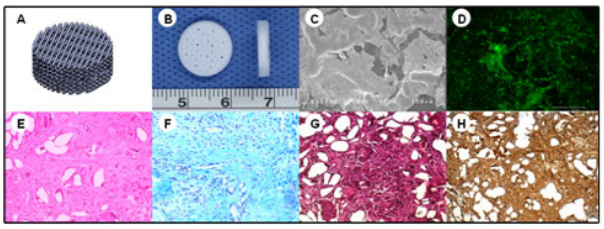

Figure (A) Computer-aided design of PCL scaffolds; (B) Gross view; (C) SEM of COL+P constructs at 4 weeks; (D) Confocal microscopy; Implanted constructs at 3 months (E) HE staining; (F) Alcian blue staining; (G) Safranin-O staining; and (H) IHC staining of COL II

Conclusions: Through the combination of SLS manufacturing technology and surface modified PCL, we provide a report on the tissue engineering of cartilage for aesthetic reconstruction. SLS provides us with the ability to optimize macrostructure and geometry of the scaffold, and produces reproducible in scaffold characteristics. Surface modification of PCL can balance out the less ideal properties of the PCL material itself. Collagen as a surface modification material is superior to gelatin in terms of supporting cells growth and stimulating ECM protein secretion. With further adjustments in these parameters, an ideal scaffold for cartilage tissue engineering aimed at reconstructive purposes may be achieved.

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