Controlling the cell morphology in tissue engineering bilayer scaffold

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Introduction

Temporomandibular joint (TMJ) supports daily oral activities. The relevant diseases due to trauma or aging remains a clinic challenge in spite of surgical intervention. Tissue engineering can facilitate the healing of defect of TMJ condyle cartilage through the combine of biomaterials and stem cells. However, few works to date have taken notice of the hierarchical structure of the condyle articular cartilage. Here, a bilayered integrated scaffold is designed to reconstruct the different matrix of TMJ condyle cartilage under same induction condition, with the final goal of tissue repair. To achieve the bilayered integrated scaffold, poly (L-glutamic acid)-*g*-polycaprolactone (PLGA-*g*-PCL) and polyethylene glycol (PEG) were used to construct two layers of network in a whole by regulating the crosslink degree of each layer. It was found that the top layer was more hydrophobic than the bottom one after measuring their swelling ratio and distance between hydrophobic domains, which induced that cells would show a flat and spreading status in top layer and form spheroid in bottom layer. These two morphologies of cells showed different gene expression level under same chondrogenic condition after 14 days. Collagen type I (COL I) in top layer was 3.3 folds higher than that in bottom layer. And collagen type II (COL II) and Sox 9 in bottom layer were 1.67 and 3.7 folds higher that in top layer.

Results

In this study, a tissue engineering bilayer scaffold was developed to reconstruct the hierarchical structure of TMJ condyle articular cartilage. Data showed that the porosity and pore size of two layers of scaffolds were both suitable for nutrient transportation and cell growth. The water affinity of two layers was significantly diverse proved by swelling ratio, distance between microdomains and △H. The top layer was much more hydrophobic while the Bottom one was more hydrophilic, which had great influence on protein adsorption and, eventually, cell status. The stem cells adhered to the pore walls of top layer, while in the Bottom layer, the interaction between cells and scaffold was attenuated due to the hydrophilic property. Thus, the stem cells formed cell spheroid finally. Cell status plays an important role on differentiation of stem cells. Under same in vitro chondrogenic induction, cells in two layers expressed the feature of fibrocartilage and hyaline cartilage respectively. Based on the above results, the bilayer scaffold was believed an intelligent material in TMJ condyle cartilage tissue engineering, which can adjust the cell status spontaneously and mediate the differentiation of stem cells under same chondrogenic induction.

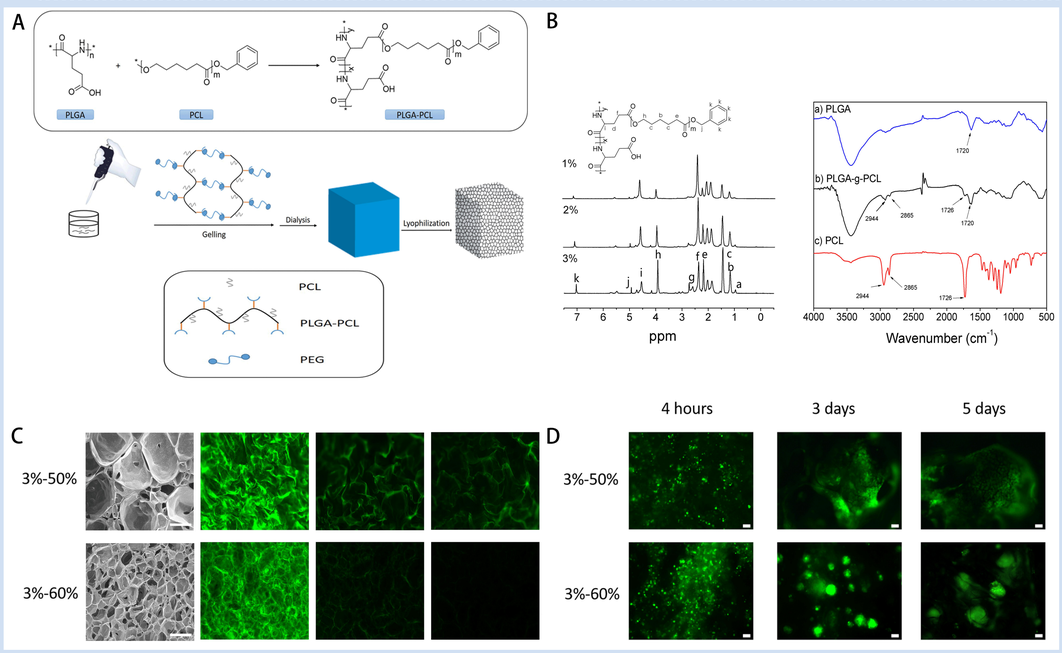

Fig.1 (A) Schematic of scaffold preparation. (B) 1H NMR and FTIR of PLGA-g-PCL copolymer. (C)BSA retention of two scaffolds. Bar: 200 μm. (D) In vitro cell culture in two scaffolds. Bar: 50 μm.

Experimental

To obtain bilayered integrated tissue engineering porous scaffolds, the initial gel was prepared by crosslinking PLGA-g-PCL copolymer with PEG400 in 2 ml DMSO under EDC chemistry. The bottom layer with 0.0274 g PLGA-g-PCL copolymer and 0.02 g PEG400 was pouring into cylindrical mold first. After curing for a few seconds, the top layer with 0.0274 g PLGA-g-PCL copolymer and 0.015 g PEG400 was pouring onto the bottom layer. Crosslink was continued for 24 h. The gel was then dialyzed against deionized water gradually to turn into hydrogel which was frozen at -20 oC for 8 h and lyophilized for 24 h. Besides, the gels with only one layer were used as control.

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