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Effects of scaffold surface morphology on cell adhesion and survival rate in vitreous cryopreservation of tenocyte-scaffold constructs

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ABSTRACT

The purpose of this study was to investigate the effects of scaffold surface morphology on cell adhesion and survival rate in vitreous cryopreservation of tenocyte-scaffold constructs. Tenocytes were obtained from tail tendons of rats. Polydimethylsiloxane (PDMS) was used to fabricate three types of scaffolds with varying surface morphological characteristics, i.e., smooth, micro-grooved, and porous surfaces, respectively. The tenocytes were seeded on the surfaces of the scaffolds to form tenocyte-scaffold constructs. The constructs were cryopreserved in a vitreous cryoprotectant (CPA) with a multi-step protocol. The cell adhesion to scaffolds was observed with electronic scanning microscopy (SEM). The elongation index of the living tenocytes and ratio of live/dead cell number were examined based on a live/dead dual fluorescent staining technique, and the survival rate of tenocytes was studied with flow cytometry (FC). The results showed the shapes of tenocytes varied between the different groups: flat or polygonal (on smooth surface), spindle (on micro-grooved surface), and spindle or ellipse (on porous surface). After thawing, the porous surface got the most living tenocytes and a higher survival rate, suggesting its potential application for vitreous cryopreservation of engineered tendon constructs.

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1. Introduction

In tissue engineering, one of the most important scientific issues of translational medicine is effective preservation of engineered tissues. A conventional cryopreservation cannot ensure the effectiveness of engineered tissues. Comparing to the conventional cryopreservation, the vitreous cryopreservation could be the most hopeful method of cryopreservation [1]. Vitrification means non-crystal solidification of liquid. During the process of cooling, the movement of molecules in liquid is slow in accompanying with an increase in viscosity. The biggest advantage of vitrification is to avoid the cell damage that is caused by the ice crystals inside and outside the cells. Besides the preservation method, the morphology of scaffold surfaces is still an essential factor that affects the cell-scaffold constructs. Patntirapong et al. [2] confirmed that the cells' adhesion and proliferation increased along with the increased roughness of scaffold surfaces. Viswanathan et al. [3] found that the cells' attachment and behavior were affected by topology of three-dimensional scaffolds. The surface morphology of a scaffold, including the fibrous and porous shapes, could control the shapes of adhesive cells and further affect the behavior of the cells [4]. Whited et al. [5] discovered that, endothelial cells got an increased adhesion and formed a confluent endothelium when cultured on scaffolds with aligned topographies. Moreover, micropatterned surfaces could engineer focal adhesions and affect cell adhesion strengthening [6]. Taken together, it was hypothesized that the morphology of scaffold surfaces may affect the adhesion and survival rate of tenocytes in vitreous cryopreservation of tenocyte-scaffold constructs.

2. Materials and methods

2.1. The harvest and identification of tenocytes

Tenocytes were harvested from Sprague–Dawley rat (Sichuan academy of medical sciences institute of experimental animals) tail

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tendons. In brief, after anaesthetized, the tail tendons of SD rats were extracted with biarticular bone cutting forceps. Prior to being immersed into 10% FCS (Fetal calf serum, Hyclon Co., USA) + DF (DMEM + F12, Sigma Co., USA) medium, the tendons were washed by sterile PBS. Then the tendons were cut into small pieces and cultured in 5 flasks with 5 ml 10% FCS + DF medium.

As the markers of tenocyte identification, the collagen type I (Col I) and III (Col III) were detected with immunocytochemistry (ICC). Human prepuce derived fibroblasts (HPDFs) were used as a positive control in this assay. The tenocytes and HPDFs were seeded on 6-well plates for 48 h. After washing by PBS, each sample was fixed by 4% paraformaldehyde and then washed by deionized water. Lucifuge incubation was performed by 30% H_2O_2: methanol (1:50), and PBS washing was followed. After incubation with goat serum, the antibodies were used as follows: primary antibody (mouse anti-rat Col I and III, 1:200) for 2 h, second antibody (horse anti-mouse, 1:200) for 40 min, third antibody (horseradish peroxidase, 1:200) for 30 min. PBS washing was performed after incubation of each antibody. The prepared DAB reagent was added onto each sample for 5 min without light. The nuclei were stained by hematoxylin and eosin, and then each sample was observed by an inverted phase contrast microscope (Olympus Co., Japan).

2.2. Fabrication of three different scaffolds

PDMS mixed liquid (PML) was prepared by casting the liquid pre-polymers composed of a mixture of 10:1 (component A:B) silicone elastomer and the curing agent (sylgard 184, Dow Corning Co., Germany). PML was blended with NaCl and heated to 90 °C for 1 h to form the porous scaffold. Then the scaffold was split into small strips (2 cm length × 2 mm width × 2 mm height). For fabrication of the micro-grooved scaffold, PML was aspirated in vacuum for 20 min to eliminate bubbles. Then it was poured onto the lithography micro-patterned glass template (State Key Laboratory of Optical Technology for Micro Fabrication, Institute of Optics and Electronics, The Chinese Academy of Sciences) for 5–10 min. After this, the PML-template construct was heated to 90 °C for 40 min and then separated. The micro-grooved scaffold was cut into rectangles (25 mm length × 8 mm width). The reverse side of the micro-grooved scaffold was used as the smooth scaffold. Before seeding cells, all scaffolds were sterilized by UV.

2.3. Fabrication of tenocyte-scaffold constructs

For fabricating the constructs, the tenocytes were seeded onto the surface of each scaffold with a concentration of 2×10^5/scaffold. After 4 h incubation at 37 °C and 5% CO_2, then 10% FCS + DF medium was added into each sample. Medium was changed every other day.

2.4. Vitreous cryopreservation and thawing of tenocyte-scaffold constructs

DMSO (21%) was used as the vitreous cryoprotectant (CPA). A multi-step protocol was performed. In brief, 8 constructs of

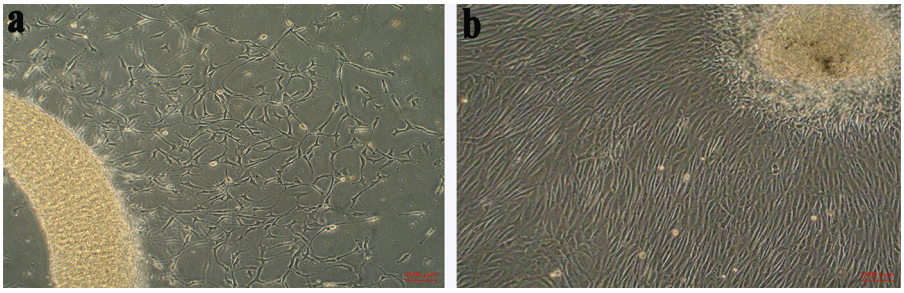

Fig. 1. Primary tenocytes culture: (a) 3 days, (b) 5 days. Scale bar = 200 μm.

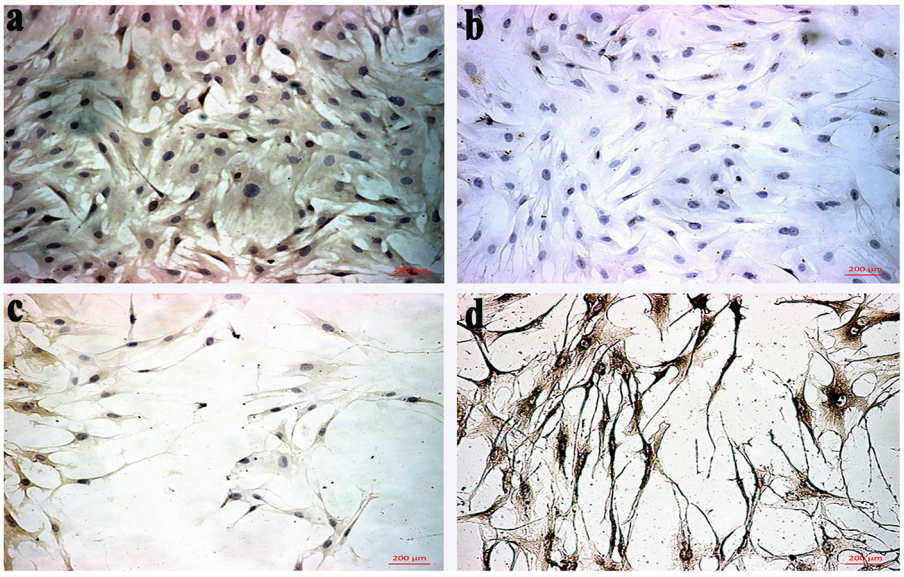

Fig. 2. Photographs showing the ICC assay of tenocytes and HPDFs. (a) Col I expression in tenocyte. (b) Col III un-expression in tenocyte. (c) Col I expression in HPDFs. (d) Col III expression in HPDFs. Scale bar = 200 μm.

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