Electronic Supplementary Information for

Improved cellular infiltration into 3D interconnected microchannel scaffolds formed by using melt-spun sacrificial microfibers

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Experimental Section

Materials Poly(lactide-co-glycolide) (PLGA, LA:GA=75:25, Mw=100,000) was donated by Changchun Sinobiomaterials Co., Ltd. 3-(4,5-dimethyl-2-thiazolyl)- 2,5-diphenyl-2-H -tetrazolium bromide (MTT), 4',6-diamidino-2-phenylindole (DAPI) and Toluidine blue were purchased from Beijing Solar-bio Science& Technology Co., Ltd. All the solvents and reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. All chemicals were of analytical grade or higher. Deionized water was fabricated with Milli-Q Ultrapure Water System (18.2 MΩ, Millipore) and utilized in the experiments.

Scaffold fabrication and characterization. Sucrose microfibers were obtained by melt-spinning which have been described in our previous study [1]. Briefly, sucrose was loaded into a homemade disc which has five holes equidistantly distributed on its rising edge. The sucrose were heated under 200 ˚C and the disc was rotated up to 1000 rpm by rotating electrical machines. Then the melt was escaped from holes and fibers were generated as outside temperature decrease. The microfibers were collected by rotating hairbrush with a speed of 100 rpm (Fig. 1A). The microfibers were cut into small pieces and used as follows.

The microchannel scaffolds were prepared with sacrificial microfibers by phase separation as described in our previous study [2]. Briefly, 5% (w/v) PLGA solution was prepared by dissolving PLGA in 1,4-dioxane. Different amounts of sucrose microfibers (PLGA: microfibers =1:1, 1:2 and 1:4 in weight, abbreviation for 50 wt.%, 67 wt.% and 80 wt.% fibers, respectively) were mixed with the above PLGA solutions and gently rotated for even distribution. Then the solution was placed at -20 ˚C to freeze. The frozen samples were placed in a freezer and 1,4-dioxane was removed using vacuum drying at -4 ˚C. The scaffolds fabricated with no sacrificial microfibers were abbreviated as porous scaffold. Environmental scanning electron microscope (ESEM, XL30 FEG, Philips) was used to characterize the morphology of the scaffolds.

Determination of the porosity. The porosity of the scaffolds was measured by the method of modified liquid displacement with absolute ethanol according to the

literature [3]. Briefly, a scaffold sample was immersed in a graduated test tube containing a known volume (V_1) of ethanol. The sample was kept in ethanol for 5 min and then vacuumed to evacuate the air, allowing the ethanol into pores of the scaffold. This process was continued until no air bubbles emerged from the scaffold. The total volume of the ethanol and scaffold was then recorded as V_2. The ethanol-impregnated scaffold was removed carefully from the test tube and the residual ethanol volume in the test tube recorded as V_3. The volume of the ethanol held in the foam was (V_1-V_3), which was determined as the void volume of the scaffold. The total volume of the scaffold was (V_2-V_3). The porosity of the scaffold (ε) was calculated by: $\varepsilon = (V_1-V_3)/(V_2-V_3)$. The average of three measurements was taken for each sample.

Mechanical testing. Rectangular bars of Φ10 mm×20 mm were used for mechanical strength tests measured by a universal testing machine (Instron 1121, UK). The compressive strength was measured at a crosshead speed of 2 $mm \cdot min^{-1}$. Three replicates were tested for each group (n=3).

Cell viability. The cell viability of chondrocytes in the scaffolds was assayed using MTT method. Rabbit cartilage chondrocytes were obtained from articular cartilage and grown in the recommended medium: DMEM plus 10% FBS. All the scaffolds were cut into thin slices about 1.5 mm thick and placed in a well of 24-well tissue culture plate. The bottom surface of the wells was fully covered by the scaffolds and sterilized using 70% ethanol followed by three washes with PBS. Then cell suspension with 2×10^6 cells was seeded onto the scaffolds. After seeding, the medium was changed every other day and the cultures were maintained for 3 and 7 days at 37 ˚C under 5% CO_2.

Four hours before each culture interval, 80 μl of MTT (5 $mg \cdot ml^{-1}$ in PBS) was added to each well and the cells were incubated for an additional 4 h. The medium was removed and 800 μl of acidified isopropanol (0.2 ml of 0.04 N HCl in 10 ml of isopropanol) was added to each well to solubilize the converted dye. The solution (200 μl) in each well was mixed and transferred to a 96-well plate, and optical density was measured at 540 nm wavelength on a Full Wavelength Microplate Reader (Infinite M200, TECAN). The mean value of the six readings for each sample was

used as the final result.

Meanwhile, the cell-laden scaffolds were collected after incubation for 7 days and fixed with 25 $g \cdot L^{-1}$ glutaraldehyde for 2 h at room temperature. The samples were washed with distilled water for 3 times, dehydrated through a graded series of ethanol and freeze-dried for 48 h before ESEM observation.

DAPI and Toluidine blue staining. To visualise the cell distribution through the cross section of the scaffolds, cell-laden scaffolds were dissected longitudinally using a frozen section machine (Leica CM1900, German) and each section was evaluated for the cell nuclei and glycosaminoglycan using DAPI and Toluidine blue staining. The cell-laden scaffolds cultured for 7 days were washed for 3 times with PBS and fixed by frozen in OCT embedding agent. Then the sections were made and stained with DAPI to counterstain the cell nuclei. Toluidine blue staining was also performed for the secretion of glycosaminoglycan (GAG) within the sections.

Statistics analysis. All data were expressed as the mean ± standard deviation. Statistical comparisons were carried out using analysis of variance (ANOVA). The significant level was set to $p<0.05$.

References:

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