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Ke   ords: Porous scaffold; Infiltrated cell; Scale-down design; Glass capillary; Tissue culture; *In situ* assessment; Fibroblast

Introd ction

Tissue Engineering (TE) aims to apply the principles of engineering and life sciences toward the manufacturing of bio-substitutes for clinical or diagnostic applications [1,2]. Even though dramatic advances and developments have been made in this research field, it is still a challenge task to manufacture fully functional tissues due to several translational challenges [3,4]. First of all, the mechanistic understanding of the

Citation:

Materials and Methods

Cell c lt re

The methodologies for media preparation, cell isolation and culture were as described previously [18]. Briefly, HDFs were cultured in flasks with Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% (v/v) new born calf serum (NBCS, GIBCO), 2×10^{-3} mol l^{-1} glutamine (Sigma), 100I U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (Sigma), monitored using a phase contrast microscope (Nikon TS100, Japan), and detached for experiments when approximately 90% confluent.

Fabrication of the scale-do n c lt re models (SDCMs)

Commercially available medical glass capillaries with inside diameters (IDs) from 0.3, 1.0, 1.5, 1.7 to 2.0 mm were used to fabricate three types of SDCMs. In the horizontal SDCM (Figure 1a), glass capillaries with different lengths (from 5 to 80 mm) were used to simulate the interconnected pore structures in thick porous scaffolds. Both ends of each glass capillary (1) were inserted into the small holes (same size as the capillary outside diameter) created in the centers of two square silicone chips (4 mm × 4 mm, 0.5 mm thick) (2), so the capillary was suspended horizontally in a plastic petri-dish (3). Cell suspension (2×10^5 cells ml^{-1}) (4) was added gently into the capillary and incubated at 37°C and 5% CO_2 for 2 hours for cell attachment; fresh media (5) were then added into the petri-dish to submerge the capillary for subsequent cell culture. To understand the influences of oxygen gradients in thick scaffolds on infiltrated cells, a horizontal SDCM with connected capillaries (Figure 1b) was designed. Short capillaries (5-10 mm) (1) were connected into longer ones using oxygen permeable silicone tubes (2), then suspended horizontally using 2 pieces of square silicone chips (4 mm × 4 mm, 0.5 mm thick) (3) in a petri-dish (4). Cell suspension (2×10^5

Li e cell fl orescent and confocal microscop sing Cell Tracker™

The live-cell staining protocol for monolayer cell culture was adopted with slight modifications. Briefly, the media inside the capillaries were gently removed and replaced by the same amount of fresh media with 10 μM fluorescent probe Cell Tracker™ Red CMTPX (C34552, Invitrogen). After incubated for 24 hours at 37°C and 5% CO_2, the Cell Tracker™ reagent was replenished with fresh media. Cell culture was then continued and the fluorescently labeled cells were analyzed using fluorescent (Nikon Ti, Japan) or confocal microscopes (Nikon Cl, Japan) at λ_{ex}= 580nm, λ_{em}= 650 nm (for TRITC / Cell Tracker™ visualization). After culture, the cells were fixed in 4% (w/v) paraformaldehyde in PBS for further microscopic analysis.

Imm no-fl orescent microscop

The conventional immune-staining protocol with Phalloidin-FITC for monolayer cell culture was also modified for the SDCMs.

Briefly, the media in each of the capillaries were gently removed after cell culture. The cells inside were washed with PBS (×3), fixed in 4% (w/v) paraformaldehyde for 30 min, and permeabilised with 0.2% (w/v) Triton X-100 in PBS for 30 min, washed gently with PBS (×3) for 24

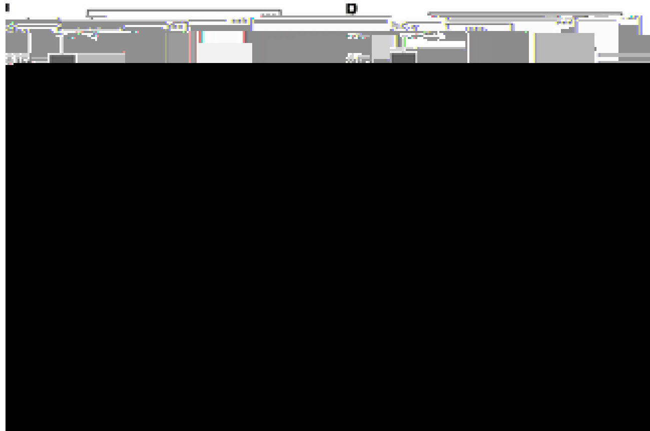

Figure 4: MVV çãæàãlãcˆ æ••æˆ [~ @˘ { æ} å^¦ { æ| ,à¦ [à|æ•c• (HDF•) &˘|c˘¦^å ã} c@^ @[¦ã: [}cæ| ÙDCM•. HDF• (2 10^5 cells ml^{-1}) •^^å^å ã} c@^ &æ]ã||æ¦ã^• ¸ãc@ çæ¦ˆã}* |^}*c@• (10, 30, 60, 80 { {) æ}å å^, }^å ã}•ãå^ åãæ { ^c^¦ (1.0 { {), &˘|c˘¦^å ~[¦ 20 åæˆ•, •cæã}^å ¸ãc@ MVV, **(a)** çæ¦ˆã}* ç[|˘ { ^• [~ DMÙO (200, 600, 1200, 1600 μl) ¸^¦^ ˘•^å c[åã••[|ç^ ~[¦ { æ:æ} ~¦[{ &æ]ã||æ¦ã^• ¸ãc@ çæ¦ˆã}* |^}*c@• (10, 30, 60, 80 { {) ~[¦ MVV æ••æˆ; **(b)** å^, }^å ç[|˘ { ^• [~ DMÙO (200 μl) ¸^¦^ ˘•^å c[åã••[|ç^ ~[¦ { æ:æ} ã} ^æ&@ &æ]ã||æ¦ˆ, à˘c c@^ O.D. çæ|˘^• æç^¦æ*^å c[10 { { [~ c@^ &æ]ã||æ¦ã^• ¸^¦^]|[cc^å. **(c)** HDF• (2 10^5 cells ml^{-1}) •^^å^å ã} c@^ &æ]ã||æ¦ã^• (L^}*c@: 80 { { , ID: 1.0 { {) æ}å &˘|c˘¦^ ~[¦ &˘|c˘¦^å ~[¦ åã~^¦^}c cã { ^]^¦ã[å• (~¦[{ 1 c[65 åæˆ•), æ}å æ••^••^å ˘•ã}* MTT. **(d)** HDF• (2 10^5 cells ml^{-1}) •^^å^å æ}å &˘|c˘¦^å ã} &[}}^&c^å &æ]ã||æ¦ã^• ¸ãc@ çæ¦ˆã}* |^}*c@• (Y@ãc^: 10 { { , Lã*@c *¦^ˆ: 30 μl)

with silicone tubes (oxygen permeability coefficient at 25°C: 600 Barrer, approximately 400 times higher than that butyl rubber, and 60 times higher than that of polystyrene), and used to culture HDFs for 20 days. The short glass capillaries were then disconnected and analyzed separately using MTT assay. As shown in Figure 4d, viable cells were detected across all the connected capillaries, which was confirmed by microscopic analysis (images not shown).

e in ences of interconnected pore si es on cell s r i al

To investigate the influences of interconnected open pores within thick scaffolds on the infiltrated cells, HDFs were seeded in capillaries with defined length (80 mm) and varying IDs (0.3, 1.0, 1.7, 2.2 mm) and cultured for 20 days for confocal fluorescent microscopy. The IDs demonstrated obvious influences on cell survival as cells were detected in more areas deep inside the capillaries as the ID increased from 1.0 to 2.2 mm (Figure 5). Cells with normal spindle morphologies were even observed in the central areas of the thick capillaries (ID: 2.2 mm) as shown in Figure 5d-iii, while almost no cell was detected in the central areas of thin capillaries (ID: 0.3 mm) as illustrated in Figure 5a-iii.

e in ences of media replenishment on cell s r i al

HDFs seeded in capillaries (ID: 1.0 and 1.5 mm, Length: 80 mm) were divided into 3 groups. The media inside the capillaries for the first and second groups were deliberately replenished with fresh media every day (CED) and twice a week (CTW) respectively, while in the third group the media inside the capillaries were not replenished (NC) during the whole culture time period. For all these 3 groups, the media in the petri-dishes were replenished twice a week. After cultured for 20 days, the cells inside all the capillaries were immune-stained for confocal fluorescent microscopy. As shown in Figure 6, cells with normal morphologies were detected across the capillaries in CED/

various cell behaviours [17,22,23]. Our mechanistic understanding of these regulatory factors within 3D scaffolds is still very limited [2,5,13],

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