Proliferation of HDFs on gelatin based three-dimensional scaffolds by 3D bioprinting

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Statement of Purpose: Full replacement, restoration, or regeneration of defective or injured functional living tissues is important goal in tissue engineering. In order to achieve these goal, 3D shape of the biomedical scaffold should be highly porous and with an appropriate pore size, pore interconnectivity, and exhibit a high surface area-to-volume ratio[1]. In particular, uncontrollable pore size and porosity may obstruct successful tissue regeneration. 3D bioprinting technologies is appropriate method to fabricate these 3D scaffold. Natural biopolymers, such as gelatin, chitosan and alginate, have been widely used as 3D bioprinting[2-3]. In this study, we fabricate pore size controlled 3D gelatin scaffolds by using 3D bioprinting and to evaluate their biological properties. The pore sizes of 3D scaffolds were controlled in the range of 600 to 1,200 μm. We successfully fabricated 3D gelatin scaffold with various pore size by using 3D bioprinting system with a cryogenic plate. To evaluate the feasibility of this structure as substrates for scaffold, human dermal fibroblast (HDFs) were cultured on the scaffold and the cellular response was compared with that from various mean pore sizes (600, 800, 1,000, 1,200 μm) of the scaffold.

Methods: A 3D printer (3DISON PRO; ROKIT, Korea) included a cryogenic refrigeration system and air dispenser (ML-500, MUSHASHI, Japan). To fabricate 3D scaffolds, 5.0 % (weight/volume; w/v) gelatin (Bloom 300, type A, Sigma, USA) solution is plotted on a cryogenic plate to maintain the temperature at −10 °C. The square shape of the gelatin scaffolds was designed by CAD (Solidworks) and scaffold size was fixed at 16.5 × 16.5 × 3 mm^3. In this process, the pore size of the scaffolds, which is defined by the distance between the strands, was 600 μm, 800 μm, 1,000 μm and 1,200 μm (G6, G8, G10, G12). The fabricated 3D scaffolds were cross-linked with 10mM 1-ethyl-3-(3-dimethylaminopropy) Carbodiimide hydrochloride (EDC) (DAE JUNG, Korea) and 5mM N-hydroxysuccinimide (NHS) (Sigma, USA) solution for overnight. 3D gelatin scaffolds morphology was observed under the scanning electron microscope (SEM, Coxem, Korea). The strand and pore sizes of 3D gelatin scaffold were measured using a digimatic caliper (Mitutoyo, Japan). HDF were used to observe cellular behavior on the 3D gelatin scaffolds. Aliquots of 100 μl of cell suspension (3×10^4cells/scaffold) were seeded on the pre-wetted scaffolds. Medium were added at 3h and incubated in an atmosphere of 5 % CO_2 at 37 °C. Cell growth was determined using an Cell counting kit-8 (WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)- 5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt]) assay (Dojindo, Japan).

Results: SEM images showing the various pore size and cross-sectional morphology of the 3D gelatin scaffolds. We confirmed that the designed pore size was well sustained after the 3D bioprinting process. The size of CAD designed strand was 350 μm and the strand size of 3D gelatin scaffold was swelled slightly (426 ± 10 μm). The 3D gelatin scaffolds pore sizes were approximately 30 % reduced than that of a designed pore sizes; 436 ± 41 μm (G6), 580 ± 79 μm (G8), 654 ± 89 μm (G10), 777 ± 109 μm (G12). The cross-sectional pore sizes of 3D scaffolds were approximately 50 % reduced than that of a designed pore size; 302 ± 69 μm (G6), 409 ± 87 μm (G8), 486 ± 84 μm (G10), 591 ± 98 μm (G12). The biological property of 3D gelatin scaffold was evaluated by MTT assay after 1, 3, and 7 days of culture. The HDFs proliferation was evaluated on four different structured gelatin scaffolds (G6, G8, G10, G12) and the 2D culture plate. The optical density (O.D) value of the 3D gelatin scaffolds (G6, G8, G10, G12) was lower than that of the 2D culture plate after 7 days. This is due to initial attachment onto the structured scaffolds and surface area was lower than 2D culture plate. The O.D value of various 3D gelatin scaffolds (G6, G8, G10, G12) was different after 7 days of culture. The growth rate of HDFs from G8 to G12 scaffolds show higher than that of the G6 scaffold.

Conclusions: Highly porous and interconnected 3D gelatin scaffolds were fabricated using a 3D biopriting, coupled with a cryogenic system. The scaffold pore size was readily controlled by tailoring process parameters and the gelatin scaffold remained extremely porous. The HDFs proliferation rate on 3D gelatin scaffolds with the range of 800 to 1,200 μm pore sizes was faster than that on the scaffold with 600 μm after 7 days of cell culture. These results suggest that the pore size is important parameter in fabrication of 3D scaffold.

References:

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