Enhanced cell proliferation on controlled pore size of chitosan nanofibers mat

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Statement of Purpose: Chitosan (CS) is prepared by N-deacetylation of chitin, which is the second most abundant polysaccharide found in nature. These properties make chitosan an ideal polymer for a wide variety of fields and industrial applications including wound dressing, drug delivery systems, and various tissue engineering applications.[1] Recently, many researchers have demonstrated polysaccharide CS as one of potentiality materials for tissue engineering applications because it has several distinctive biological properties including good biocompatibility and biodegradability.[2] An electrospinning method was able to fabricate biodegradable CS nanostructure matrix for tissue engineering. Nanofibers have amazing characteristics such as very large surface area-to-volume ratio and high porosity with very small pore size.[3] However, the size of pores in the polymer nanofibers mat is very small due to the random deposition of nanofibers. Therefore, this inevitable characteristic restricts cell infiltration and growth throughout the nanofibers mat. To overcome these limitations, we proposed that the thickness and porosity of nanofibers were controlled by ultra-sonication.

Methods: chitosan (Aldrich, USA) was dissolved in trifluoroacetic acid (TFA)/dichloromethane (DCM) to form a 5 wt% solution for electrospinning. The neutralizing agent with alkaline solutions as sodium hydroxide (NaOH) was purchased from Sigma Aldrich (USA). The neutralized CS nanofibers mat was immersed in distilled water under ice bath. Ultra-sonication of CS nanofibers was carried out using ultra-sonicator (VCX 750, Sonics, USA). The ultra-sonication time was controlled in the range from 1min to 4 min under 225 W power densities. The morphology and thickness of the chitosan nanofibers were observed using SEM. The porosity and pore size of sonicated chitosan nanofibers were measured by mercury porosimeter and their water adsorption properties (wettability) determined by contact angle analyzer. The viability of fibroblasts *in vitro* on the nanofibers was evaluated by CCK-8.

Results: To enhance cell viability, we used ultra-sonication to control the pore size and thickness of CS nanofibers mat. Figure 1 shows that cross sectional SEM image and surface morphology of CS nanofibers mat according to ultra-sonication treatment. Non-sonicated CS nanofibers mat had a thickness length of 78.4 ± 6.3 um and has a dense mesh structure with bead free and random nanofibers mat (inset image). On the other hand, the thickness of sonicated CS nanofibers mat was dramatically increased to 321.3 ± 23.2 um as shown in figure 1(b). Mercury porosimetry method for evaluating pore size distribution and porosity using non-sonicated and sonicated CS nanofibers mat were investigated. Pore size and porosity of non-sonicated mat had a maximum at about 470 nm and 79.9 %, while that of the sonicated mat had maximum at about 1,090 nm and 97.2 %, respectively.

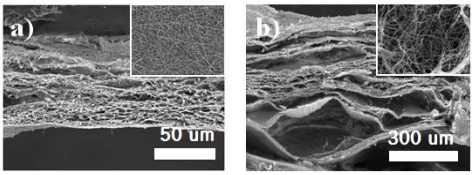

Fig 1. Cross-section SEM image of chitosan nanofibers. a) non-sonicated chitosan nanofibers sheet (thickness: 78 um), b) 4 min sonicated chitosan nanofibers sheet (thickness: 321 um). Inset images are surface morphology of chitosan nanofibers mat.

To investigate the effects of pore size on cells viability, we seeded normal human dermal fibroblast cells (NHDFs) on sonicated and non-sonicated CS nanofibers mat after 1, 4 and 7 days. We observed morphologies of cells grown on CS nanofibers mat by FE-SEM (data not shown). As shown in figure 2, the numbers of cell were determined by using a colorimetric CCK-8 assay. The cells on sonicated CS nanofibers mat increased the viability index by approximately 1.4-fold of those on non-sonicated CS nanofibers mat at the same culture condition.

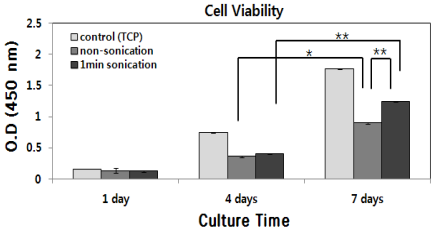

Fig 2. Normal human dermal fibroblast cells (NHDFs) proliferation on non-sonicated and sonicated chitosan nanofibers using CCK-8. ($^{*}p < 0.05$, $^{**}p < 0.01$)

Conclusions: Pure CS nanofibers were prepared by electrospinning and then controlled pore size and thickness using the ultra-sonication to enhance cell proliferation. The thickness, porosity and pore size of chitosan nanofibers were controlled according to time using ultra-sonication. And then we have analyzed various characteristics of chitosan nanofibers such as morphology, chemical and physical properties. Additionally, we confirmed that the cell viability of sonicated mat was higher than for non-sonicated mat. The CS nanofibers were found to be enhanced cell viability. Consequently, these chitosan nanofibers are expected to scaffold for tissue engineering applications.

References:

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