Investigation of pore size effect on adipose stem cell differentiations using a pore size gradient scaffold

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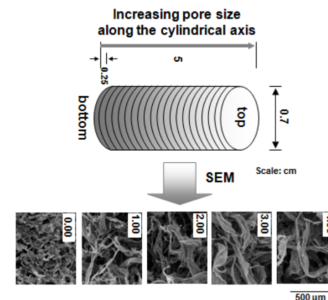
Statement of Purpose: Tissue engineering with the use of stem cells that can be self-regenerated, expanded to high cell numbers and differentiated into certain cell types is a rapidly emerging field for the regeneration or reconstruction of a variety of tissues and organs [1]. It is well recognized that the efficient stem cell differentiation into a target cell is an essential requirement for clinical applications. To effectively induce the differentiation of the stem cells, the chemical cues including growth factors have been commonly adapted, and encouraging outcomes have been reported. Although it is widely believed that the physical cues, such as pore size and porosity in three-dimensional (3-D) scaffolds, can also influence the cell differentiation, there is scattered data available indicating which pore size or porosity is favorable for stem cell differentiation into a specific cell type. In this study, we fabricated polycaprolactone (PCL) cylindrical scaffolds with a pore size gradient (i. e., gradually increasing pore size along the longitudinal direction) by a modified centrifugation method [2]. The *in vitro* specific cell (osteogeinc, chondrogenic, myogenic, and neuronic cell) differentiations of adipose stem cells (ASCs) in the pore size gradient scaffold in terms of scaffold pore sizes were investigated.

Methods: To prepare PCL scaffold having a pore size gradient, hot PCL solution [10 wt% (in tetraglycol), 90 ^{o}C] was slowly dropped into water at room temperature with vigorous agitation using a homogenizer to obtain fibril-like PCLs. Then it was washed in excess water and freeze-dried. The obtained fibril-like PCLs were resuspended in a cold Pluronic F127 solution (20 wt%, ~4 ^{o}C) to be PCL/F127 solution, 1/50 ratio (w/v). The mixture solution was poured into a polypropylene cylindrical mold and then the mold was centrifuged (3,000 rpm) for 5 min. The pore size gradient PCL scaffold was eventually obtained *via* a fibril-bonding process. The *in vitro* specific cell differentiation behaviors of ASCs in the pore size gradient scaffold were assessed by DNA, gene expression, and histological examinations.

Results: It was observed that the pore size and porosity of the scaffold gradually increased along the cylindrical axis, as expected, because of the gradual increment of the centrifugal force along the longitudinal axis in the cylindrical mold containing fibril-like PCLs during the centrifugation (Fig. 1). As the centrifugal speed, 3000 rpm, was applied to the cylindrical mold for 5 min, the pore size and porosity ranges in the prepared scaffold along the cylindrical axis were observed from ~ 90 to ~ 400 μm and from ~ 80 to ~ 97 %, respectively. From these observations, we recognized that the prepared pore size gradient scaffold can be useful for the investigation of pore size effect on cell differentiation, because the scaffold fabrication step is not complicated and the pore size ranges in the scaffold are reasonably broad. From the

in vitro cell culture to estimate the effect of pore size on specific cell differentiations of ASCs, it was observed that the scaffold sections having the pore size ranges of 300 ~ 320 μm, 370 ~ 400 μm, and 90 ~ 105 μm provides an optimum environment for the osteogenic, chondrogenic,



and myogenic/neurogenic differentiations of ASCs, respectively (Fig. 2).

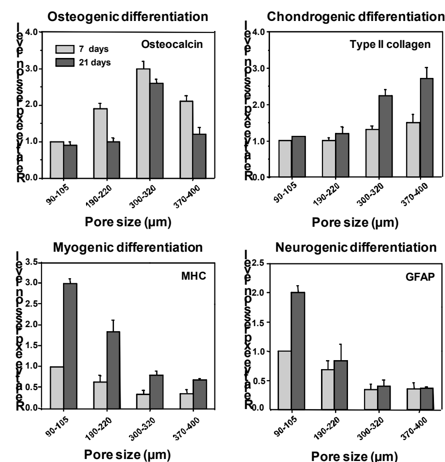


Figure 1. SEM photographs of pore size gradient PCL scaffold (x100).

Figure 2. Real-time PCR after 7 and 21 days of *in vitro* ASC culture in the PCL scaffold sections with different pore size ranges.

Conclusions: From the results, we can suggest that the pore size gradient scaffolds can contribute to the determination of optimum pore size ranges for a variety of stem cell differentiations to specific cell types, and can provide basic but revealing information on tissue engineering fields using stem cells.

References:

1. Kent JN et al., Otolaryngol Clin North Am. 1984;17: 273-285.
2. Oh SH et al., Biomaterials. 2007; 28:1644-1671.

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