pDNA-containing PLGA/Pluronic F127 porous scaffold for the effective chondrogenic differentiation of mesenchymal stem cells

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Statement of Purpose: Three dimensional scaffolds and growth factors have been proven to be effective stimulants for chondrogenic differentiation in articular cartilage tissue engineering. However, the short half-life of growth factors and the inability to restrict the protein to a localized area are still remained as limitations [1]. To overcome these, the combination of tissue engineering and gene therapy strategies by incorporating growth factor gene-transfected cells into 3D scaffold were introduced as a promising therapeutic technique [1]. However, it was reported that the prolonged expression of the growth factor from infected cells is also limited in the body, owing to the cell migration from the applied site and apoptosis [2]. More recently, the gene delivery from 3D porous scaffolds which can provide the continual transfection of cells by sustained release of gene, and thus more extended growth factor expression periods, is paid much attention to a new alternative [3]. In this study, we prepared plasmid DNA (Sox-9)-loaded porous scaffold to enhance the chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells (*h*BMMSCs). It is well known that the Sox-9 is master transcription factors for chondrogenesis. The *in vitro* characterizations to evaluate its potential use as a gene delivery system for 3D chondrogenesis were conducted.

Methods: For more effective transfection of plasmid DNA into cells, the plasmid DNA/polyethyleneimine polyethylene glycol (PEI-PEG) non-viral vectors complexes with different N/P ratios were prepared. Their particle size and zeta potential as well as cytotoxicity and transfection efficiency into *h*BMMSCs (*in vitro*) were evaluated to determine the optimum non-viral vector for pDNA. The pDNA/PEI-PEG complex-loaded hydrophilic porous scaffolds were fabricated by a precipitation/ particulate leaching method, developed by our laboratory, using poly(lactic-co-glycolic acid) (PLGA) and Pluronic F127. To this, PLGA/Pluronic F127 mixture powder dissolved in tetraglycol [15 wt%; PLGA/F127, 9.5/0.5 (w/w)] was mixed with pDNA/PEI-PEG complex dissolved in deionized water. The final concentration of pDNA/PEI-PEG complex in mixture solution was 10 μg/ml. The pDNA/PEI-PEG complex-containing PLGA/F127 solution was mixed with sodium chloride salt particles (sieved to sizes between 100 and 150 μm; 0.5/1, v/w), then cast into the silicone mold (diameter 7 mm, thickness 2 mm). The paste-type mixture-containing silicone mold was immersed into the excess ethanol to precipitate the polymer matrix. Finally, the pDNA/PEI-PEG complex-loaded PLGA/Pluronic F127 scaffolds were obtained after washing in excess 70% ethanol and freeze drying. Their morphology, mechanical properties, hydrophilicity, pDNA/PEI-PEG complex release behavior, cytotoxicity and transfection efficiency into *h*BMMSCs (*in vitro*) were evaluated.

Results: It was observed that the particle (complex) sizes decreased and the positive charge of complexes increased with the increasing N/P ratio, as expected. From the *in vitro* transfection experiment, the pDNA/PEI-PEG complexes with N/P ratios 8 and 16 were determined as optimum non-viral vectors for pDNA, based on the transfection efficiency into cells and cytotoxicity (*in vitro*). The prepared scaffold exhibited highly porous and open-cellular pore structures with almost same surface and interior porosities, which can provide good environment for cell proliferation and differentiation. The pDNA/PEI-PEG complexes were efficiently loaded into scaffold (> 90% of initial loading amount) and continuously released from the scaffold for 2 months (Figure 1). From the *in vitro* cell culture in the scaffolds for transfection, it was observed that the pDNA/PEI-PEG complex-loaded scaffolds have a better transfection efficiency of pDNA into *h*BMMSCs (both N/P ratio 8, 16) than the pDNA (w/o PEI-PEG non-viral vector)-loaded scaffold, and the cell viability has not significantly different among each group, indicating low toxicity of pDNA/PEI-PEG complexes, probably due to the sustained release of pDNA/PEI-PEG complex from the scaffold. From the results, we could suggest that the pDNA/PEI-PEG complex-loaded PLGA/F127 scaffolds can be a good tool as a gene delivery system for three-dimensional chondrogenesis of BMMSCs, owing to their high gene transfection efficiency and low toxicity.

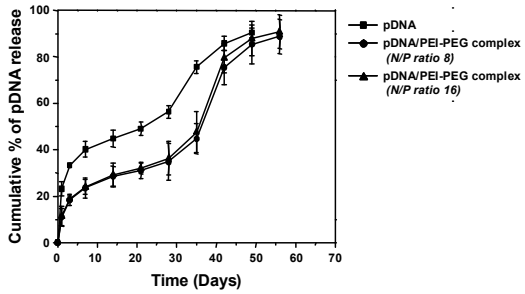

Figure 1. Release profiles of pDNA and pDNA/PEI-PEG complexes (N/P ratio 8 & 16) from the PLGA/Pluronic F127 scaffolds.

References:

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