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The Effects of Cyclic Strains on the Hybrid Aligned Nanofibrous Scaffold Seeded with Smooth Muscle Cells

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1 Introduction

The objective of this study was to investigate the effect of cyclic tension on multi-layered scaffold of polyurethane and poly(ethylene oxide) for vascular grafts.

2 Materials and Methods

To fabricate multi-layered align scaffold, solution of polyurethane (PU; Pellethane 2102-75A, DOW Chemical Corp., MI, USA) was prepared with *N,N*-demethyle formamide (DMF; Junsei Chemical Co., Japan) and that of poly(ethylene oxide)(PEO; Sigma, MW : 100,000) was dissolved in deionized water at a same concentration of 20 wt%. Electric potential was set 0.75 kV/cm and 1.2 kV/cm, respectively. Spinning time of PU solution and PEO solutions was set as 30 min. To make aligned scaffold, rotating target was adapted. For the cell penetration, each multi-layered scaffold was soaked in deionized water for 12h and dried in a vacuum dryer for 24h to eliminate PEO. Each scaffold was cut into 0.5cm × 3cm. Four passaged human aortic smooth muscle cells (Cascade Biologics, USA) were used in this study. To apply cyclic strain, the modified Flexcell system was employed. The system was controlled by LabVIEW program (National Instrument Corp., version 6.0, USA). The multi-layered sheet was glued to the Flexcell plate by silicon. To apply uniaxial strain, a vacuum was applied repeatedly to the rubber-bottomed dishes via the base plates, in a humidified incubator with 5% CO_2 at 37°C. We applied mechanical stretch to multi-layered scaffold for 2h,, twice a day, over 2 days, at a frequency of 1 Hz and strain magnitudes of 7% and 10% sequentially. Cell density was 5×10^4 cells/cm^2 and each specimen was harvested at 1 and 4 days after mechanical stimuli. SMC proliferation was assessed by measuring the total DNA contents. The quantity of DNA in the cells was determined using a PicoGreen dsDNA Quantitation Reagent Kit (Molecular Probes, OR, USA). Cell activity was estimated by measuring total collagen (Sircol total collagen kit, Biocolor, UK) and glycosaminoglycan (GAG; Blyscan sulfated glycosaminoglycan assay, Biocolor, UK).

3 Results

As a result, group of parallel aligned scaffold with stimuli was maintained similar level of DNA content compared with those of groups without stimuli. But group of vertically aligned with stimuli shows smaller DNA amounts (**Fig. 1**). However, amount of collagen and GAG synthesis was higher with stimuli groups at day 4 than the groups without stimuli even though amount of synthesis was decreased at one day after stimuli (**Fig. 2 and 3**).

We applied cyclic stimuli to the multi-layered scaffold. The aligned multi-layered scaffold and cyclic stretch were used to mimic vascular mimetic environment in vitro.

4 Conclusion

This study shows that this cyclic tension affects ECM production of the smooth vascular cells; however, fiber direction does not affect cell activity significantly.

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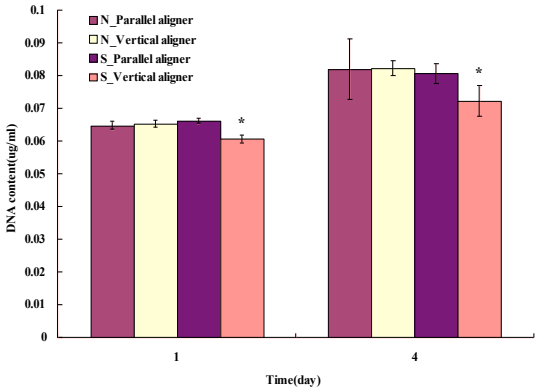

Figure 1 : Proliferation of SMCs on hybrid scaffold.

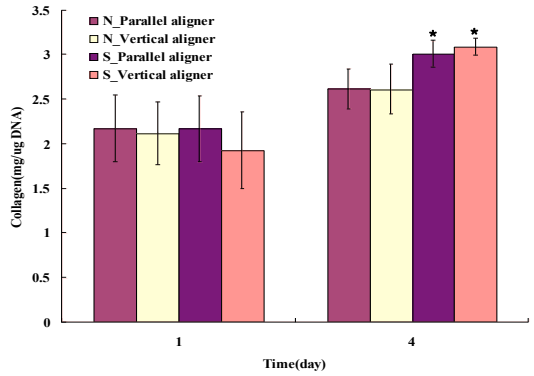

Figure 2 : Collagen synthesis on the hybrid scaffold.

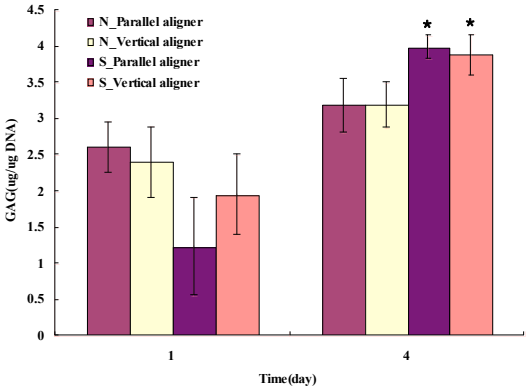

Figure 3 : Amount of GAG on the hybrid scaffold.