

Micro-Folding Culture: A New Method of Making Multicellular Aggregate

R.Takaya¹, N.Kachi¹, N.Tomita¹

¹ Graduate School of Engineering, Kyoto University, Kyoto, Japan

Corresponding: ntomita@iic.kyoto-u.ac.jp

INTRODUCTION: Formation of multicellular aggregates is of particular interest, since aggregates show not only morphological but also functional similarities to tissues and organs; unlike conventional monolayer cell cultures [1-2]. In this study, a new method of making (histologically) homogeneous cell aggregates [3] is introduced. In this method, single cells are cultured on micro-patterned non-adhesive substrate. The purpose of this study is to evaluate the performances of this method using chondrocytes and pitted substrate as an example.

METHODS: [Micro-Folding Culture] Micro-patterned polydimethylsiloxane (PDMS) substrates with two-dimensional arrays of pits were prepared by using simple soft-lithography processes. Depth of the pit was approximately 100 μ m. Two types of substrates with different pit diameter were prepared: Large; D =200 μ m, S=80 μ m, Small; D=100 μ m, S=40 μ m, D represents diameter of pit, S represents spacing between pits. Chondrocytes were harvested from humerus, femur and tibia of 4-weeks-old Japanese white rabbits. These chondrocytes were seeded into pits and cultured for 48 hours. [Cartilage regeneration] Aggregates were inoculated into the fibroin sponge at a cell concentration of 1.25×10^7 cells/ml. Fibroin sponge (diameter: 8 mm, thickness: 1 ± 0.2 mm) made of fibroin hydro gel was used as a scaffold for cartilage regeneration. [Chondroitin sulfate assay] Chondroitin sulfate was measured by using DMB method.

RESULTS: Chondrocytes seeded into the pits of micro-patterned substrate formed multicellular aggregates (Fig. 1). Mean diameter of aggregate was 56.3 μ m (Small) and 84.8 μ m (Large). Amount of ECM synthesis was shown in Fig. 2, where the small-aggregates group tends to show higher EMC synthesis. This advantage become clearer after serial subcultivation (when passage-2 cells were used).

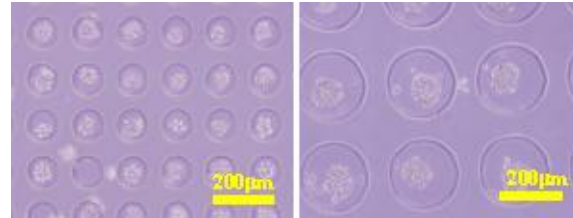


Fig. 1: Aggregates in pits of micro-patterned substrate (48h after seeding): Small (left), Large (right). Mean diameter of aggregate was 56.3 μ m (Small) and 84.8 μ m (Large).

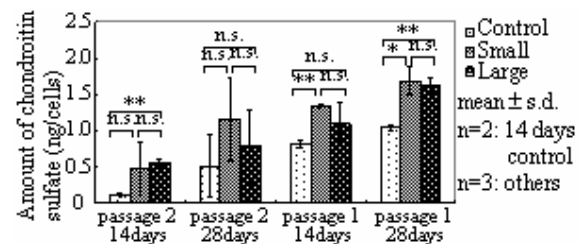


Fig. 2: Amount of chondroitin sulfate per one chondrocyte seeded into fibroin sponge. (*: $P < 0.05$, **: $P < 0.01$, n.s.: $P > 0.05$, by t-test)

DISCUSSION & CONCLUSIONS: Diameter of aggregate could be controlled by using substrates having pits of different diameter. The aggregates made by the Micro-Folding Culture showed higher performance of ECM synthesis. This tendency seems to be more prominently seen when proliferated cells were used. We are now evaluating the tribological functions of the Micro-Folding Culture derived cartilage tissue.

REFERENCES: ¹ H. Suenaga, K. S Furukawa, T. Ushida, T. Takato and T. Tateishi, (2004) *Materials Science & Engineering*, 24:421-424. ² R. Glicks, J. C. Merchuk and S. Cohen (2004) *Biotechnology and Bioengineering*, 86-6:672-680. ³ N. Kachi, (2005) *JSME conference*

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