

## MULTI-LAYER SCAFFOLD FOR CULTURING BIPHASIC JOINT CARTILAGE *IN VITRO*

Chun-Jen Liao, Yu-Ju Lin, Chin-Fu Chen

Biomedical Engineering Center, Industrial Technology Research Institute, Taiwan

**INTRODUCTION:** Tissue engineering technique nowadays is limited to culturing single tissue, while in clinical application, tissues to be repaired are often composite tissues such as the human articular surface is a typical biphasic composite tissue. In our previous study, we found that the partly digested cartilage fragments could reconstruct cartilaginous tissues *in vitro*. For reconstructing two layer tissue in an orderly pattern, a specific multi-layer porous scaffold that containing of an upper cavity were prepared. Partly digested cartilage fragments were injected into the cavity, thereafter the bone marrow cells were seeded in the multi-layer scaffold, wherein cartilage tissues fragments and bone marrow cells, by taking advantage of their disparity in volume, are to be distributed into different layers. The specific multi-layer porous scaffold with two kinds of cells was cultured to form a biphasic composite tissue *in vitro*.

**METHODS:** A multi-layer of porous scaffold with an upper hollow cavity was prepared by solvent/merging particular leaching method<sup>1</sup>. Articular cartilage was harvested from a one-week old New Zealand rabbit and was cut and sieved to particles of size 520-860  $\mu\text{m}$ . 50 mg of tissue fragments were partly digested in 0.1 % type II collagenase solution for 2 hours at 37°C. A straight 18-gauge needle on a 1 mL syringe was used to inject the partly digested tissue into the cavity of the scaffold. Bone marrow cells were harvested from a New Zealand rabbit weighted 2 kg.  $5 \times 10^5$  bone marrow cells were seeded into the scaffold after injecting the cartilage tissue fragments. The scaffolds were placed in a 500 ml spinner flask to incubate for 2 weeks and 1 month. The samples were fixed in PBS solution containing 4% formalin, sectioning with the paraffin-embedded section method, and finally, staining with hematoxylin-eosin and safranin-O.

**RESULTS:** Neocartilage is observed to grow around the seeded tissue fragments after 2-week incubation inside of the hollow cavity. The cells of the newly formed tissue exhibit the typical spherical morphology of chondrocytes and were in lacunae, surrounded by the newly

formed of cartilaginous matrix. A layer of bone marrow mesenchymal cells can be observed at the inner side of the porous structure. Figure 1 shows a histological examination of a tissue after 4-week incubation. The neocartilage tissue has already fused together with seeded tissue fragments, with the size of the whole tissue block being 5.2 mm in width and 1.8 mm in depth. The underside of tissue block has grown from upper hollow cavity into the lower porous structure, forming an interface similar to that formed between cartilage and subchondral bone. At porous structure on the lower end of scaffold, the pores of porous structure are filled up with the proliferated bone marrow mesenchymal cells.

**DISCUSSION & CONCLUSIONS:** In the study two types of tissues can be cultured separately and reconstructed *in vitro* by taking advantage of their disparity in volume and the materials of the scaffold. The multi-layer porous scaffold can be utilized to culture multi-layer tissue. In the future, by taking only few amounts of cartilage tissue and bone marrow from patient, and incubating subsequently with the invented multi-layer porous carrier, one is able to grow large amounts of tissues that can be used as implants in transplantation surgery to treat the wide damaged area and full thickness defect.

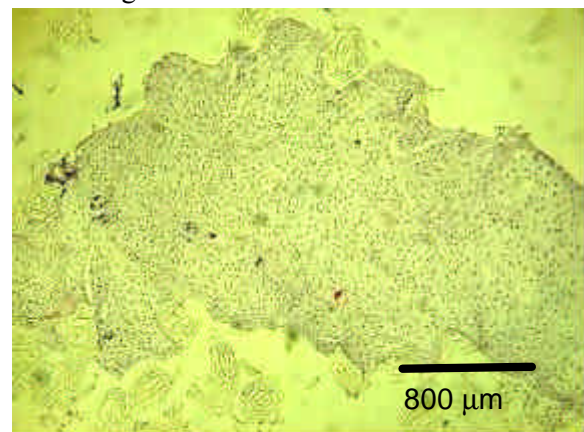


Fig.1 shows histological examination of biphasic tissue after 4-week incubation.

**REFERENCES:** <sup>1</sup> C.J. Liao, C. F. Chen, et al (2002) *J Biomater Mater Res* **59**: 676–81.

**ACKNOWLEDGEMENTS:** The authors would like to thank the Ministry of Economic Affairs (Taiwan) for financially supporting this research.