T3-017

Ultrastructural Visualization of Chondrocytes Extracellular Matrix Distribution by Using Electron Microscopy

[1]S. Ab-Rahim, [1]T. Kamarul, [2]L. Selvaratnam

[1]*Department of Orthopaedic Surgery, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[2]*School of Medicine and Health Sciences, Monash University, Sunway Campus, 46150 Selangor*

The limited healing capability of articular cartilage has primarily been associated with changes in chondrocyte phenotype. Chondrocytes, resident cells in articular cartilage, are responsible for the control of extracellular matrix turnover. On the other hand, ECM homeostasis plays important role in ensuring appropriate cartilage tissue function. Hence, to achieve this state of balance, 2 different culture systems were developed in order to establish optimal conditions for chondrocyte growth and to warrant chondrocyte phenotype constancy. In the first system, cells were cultured as monolayer sheets in culture flasks. In the second system, a 3-dimensional construct was developed utilizing alginate hydrogel beads. Alginate, a well established biomaterial commonly used for cell encapsulation and transportation, is known to improve cellular expression in culture. Thus, this study investigates the organization and distribution of ECM and collagen type II utilizing transmission and scanning electron microscopy (TEM/SEM). TEM results demonstrated that although the ultrastructure of cells in both culture systems were similar and possessed large nuclei with several mitochondria distributed intracellularly, ECM production and organization is markedly differed. SEM imaging revealed monolayer cultures containing flattened chondrocytes distributed evenly as single sheets with sparse extracellular matrix fibres noted immediately surrounding these cells. In contrast, chondrocytes cultured within alginate constructs grew as dense colonies, not only located on bead surfaces, but also located within the pores of the scaffold. Extracellular matrix was also clearly observed surrounding the colonies as dense collagen networks comprising irregular stringy fibres. Based on this study, it is clearly evident that chondrocyte growth and ECM production is clearly superior in 3-D alginate constructs which more closely approximates the cartilage environment *'in vivo'* than monolayer constructs.