B.10 ULTRASTRUCTURAL ANALYSIS OF MESENCHYMAL DIFFERENTIATION INTO CARTILAGE INDUCED BY PEA/PHEA SCAFFOLD

Viñuela-Prieto JM[1], Panadero JA[2,3], Antolinos C[3], Ribeiro C[2,4], Gómez-Tejedor JA[3], Lanceros S[2,4], Gómez-Ribelles JL[3,5], Carda C[1]

[1]Faculty of Medicine and Dentistry, Unit of Histology, Department of Pathology, University of Valencia.
[2]Centro/Departamento de Física da Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal.
[3]Centro de Biomateriales e Ingeniería Tisular, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain
[4]International Iberian Nanotechnology Laboratory - INL, Avenida Mestre José Veiga s/n, 4715-330, Braga, Portugal
[5]Ciber en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Valencia, Spain

A scaffold biomaterial was developed to allow simple cell seeding and to analyze the samples reducing the further processing. The objective of the present work was to observe morphological changes in mesenchymal stem cells during proliferation and differentiation into cartilage.

The mesenchymal cells employed in the present work corresponded to the KUM5 cell line from murine bone marrow. For the construction of the scaffolds a PEA 90%/PHEA 10% copolymer was developed. Meanwhile, the cells were expanded in DMEM 4,5 g/L glucose with 10% FBS and 1% penicillin/streptomycin. After trypsinization and centrifugation, the samples were resuspended either in the same culture medium employed for expansion or in chondrogenic medium: DMEM 4.5 g/L glucose containing L-proline 50 µg/ml, ascorbic acid 50 µg/ml, dexamethasone 10^{-7} M, ITS+Premix 1%, penicillin/streptomycin 1% and TGF-β1 10 ng/ml. Posteriorly, 25 µL containing around 50000 cells were seeded in each surface of the scaffold, and held for 40 minutes in standard culture conditions. Finally, the scaffolds were submerged either in expansion medium or in chondrogenic medium. The samples were retrieved for analysis at days 1 and 3 of culture. The fixation of the samples was performed in glutaraldehyde 2,5% for 1h at 4 ^{o}C, and histological analysis was carried out by means of optical semi-thin sections and transmission electronic microscopy (TEM).

The histological analysis performed on the samples cultured in expansion medium at day 1 revealed a large number of mesenchymal cells that were closely related to the biomaterial (Fig. 1). Meanwhile, in the samples cultured in chondrogenic medium, apart from the small, reticular mesenchymal cells associated to the biomaterial, a more differentiated population of bigger and rounder cells occupying the interstitial spaces of the scaffold was observed. When assessed with TEM, these cells expressed active-synthesis morphology, with bigger cytoplasm and dilated endoplasmic reticulum cisternae, but without evidence of any newly-generated extracellular matrix component. Both mesenchymal and synthetizing cells showed a marked tendency to cover the biomaterial through cytoplasmic expansions.

At day 3 of culture, the samples in expansion medium showed a smaller cell number with respect to the samples from day 1, appearing in a more dispersed and isolated pattern. On the other side, the samples cultured in chondrogenic medium showed a higher cell number and a higher tendency to differentiation. Additionally, voluminous round cells co-exist with small reticular mesenchymal cells, the first occupying preferably the interstitial spaces of the porous scaffold, and the second in close relation to the biomaterial surface. Evidence of a newly synthetized fibrillar material was identified with TEM both in the cytoplasm of those cells showing active-synthesis morphology and in the extracellular compartment.