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Cartilage tissue engineering: adult human mesenchymal stromal cells and collagen biomaterials

C. Sanjurjo-Rodríguez[†‡], A.H. Martínez Sánchez[†], T. Hermida-Gómez[†], I.M. Fuentes[†‡], J. Buján[§], F.J. De Toro[‡], S. Díaz-Prado[†‡], F.J. Blanco[†]

† INIBIC- Univ. Hosp. of A Coruña (CHUAC), A Coruña, Spain
‡ INIBIC Dept. of Med., Univ. of A Coruña, A Coruña, Spain
§ Dept. of Med. Specialties, Univ. of Alcalá de Henares, Madrid, Spain

Purpose: The purpose was to study chondrogenesis of adult human Mesenchymal Stromal Cells, isolated from bone marrow (hBMSCs), when culture on collagen (Col) biomaterials and to evaluate the suitability of the neotissue formed to use in Tissue Engineering.

Methods: hBMSCs were cultured during 30 days in chondrogenic medium with Transforming growth factor β-3 (TGFβ-3) on the following biomaterials: Col I, Col I+Col II, Col I+ heparan sulphate (HS), Col I+Col II+ heparan sulphate (HS), Col I+Col II+ chondroitin sulphate (CHS) and Col I+ heparin (OLH3). We have tested growth and cell morphology on the constructs by histochemical and Scanning (SEM) and Transmission (TEM) Electron Microscopy. Chondrogenic differentiation was evaluated in all the constructs by histochemical and immunohistochemistry analyses and molecular biology. Moreover, Col concentration was measured in supernatants along the culture.

Results: Histology showed that hBMSCs have grown through the scaffolds, being the cell percentage respect to the total area of the scaffold: >75% in Col I, Col I+Col II, Col I+HS, Col I+Col II+HS, Col I+Col II+CHS; < 50% in Col I+OLH3. It was observed a big amount of extracellular matrix (ECM), except in Col I+OLH3. ECM from Col I+Col II and Col I+HS showed the higher metachromasia for Safranin O proteoglycan staining, whereas Col I, Col I+Col II+HS, Col I+Col II+CHS showed an intermediate metachromasia (Figure). Col II immunostaining was highly positive in Col I and Col I+HS ECM and positive intracellularly in Col I+Col II, Col I+Col II+ HS, Col I+Col II+CHS (Figure). Col I+OLH3 did not show positivity for proteoglycans and Col II. By measurement of relative expression levels (REL), COL II gene expression was seen in cells all over the biomaterials, Col I (0.63 REL), Col I+Col II (1.00 REL), Col I+HS (0.47 REL), Col I+Col II+HS (0.93 REL), Col I+Col II+CHS (3.57 REL) and Col I+OLH3 (5.20 REL). AGG gene expression was higher in Col I+Col II+HS (6.47 REL) constructs than in Col I (0.00 REL), Col I+Col II (0.95 REL),Col I+HS (0.17 REL), Col I+Col II+CHS (0.00 REL) and Col I+OLH3 (0.00 REL). TEM analysis showed a big amount of mitochondria and oval/rounded shape cells (Figure). We could also see oval/rounded cells and ECM by SEM.

Col released (measured as μg/total volume) was detected in all the supernatants, finding the highest concentration in Col I+HS biomaterials cultures except at day 21: Col I (137.33), Col I+Col II (0.00), Col I+HS (0.00), Col I+Col II+HS (3.33), Col I+Col II+CHS (13.90) and Col I+OLH3 (0.00).

Conclusions: Data showed hBMSCs are able to attach and proliferate on all Col biomaterials, except in Col I+OLH3. These cells showed abundant ECM. Analysis showed differentiated cellular phenotype and an ECM similar to cartilage, in biomaterials composed by HS. The neotissue formed in the other biomaterials showed an intermediate phenotype. The neo-tissue formed in Col and HS biomaterials could be useful for cartilage tissue engineering. Acknowledgements: B. Parma (OPOCRIN S.P.A.); CAM (S2009/MAT-1472); CIBER BBN CB06-01-0040; Red Gallega de Terapia Celular (REDICENT); SAI-UDC; CSR is beneficiary of a fellowship from Diputación de A Coruña; P. Esbrit (Fundación Jiménez Díaz).

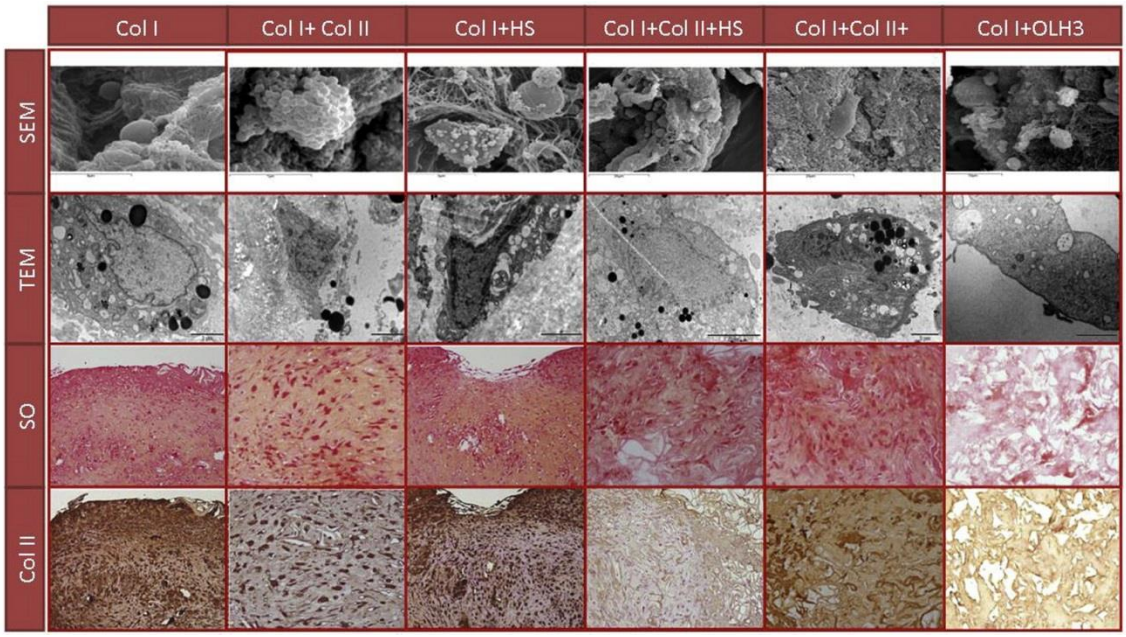

Figure: Images from the different constructs (columns). Ordered in rows are shown the different analysis: Scanning (SEM) and Transmission (TEM) Electron Microscopy, Safranin O staining (SO) and Collagen type II immunostaining (Col II).