Ovine mesenchymal stromal cells for osteochondral tissue engineering

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Purpose: Tissue engineering animal models with similar joints to humans are needed before its clinical application. The purpose of this study was to isolate and characterize ovine mesenchymal stromal cells (MSC) from bone marrow aspirates and to assess their usefulness for osteochondral repair using scaffolds.

Methods: Firstly, MSC from ovine bone marrow were isolated and characterized by flow cytometry and multipotent assays. Secondly, osteogenic differentiation capability of ovine MSC on collagen type I (Col-I) and β tricalcium phosphate (β-TCP) scaffolds was tested after culturing them in differentiation medium during 30 days. Finally, the capability of ovine MSC cultured on Col-I scaffolds to repair chondral lesions generated in the ovine articular cartilage was assessed in an *in vitro* model.

Results: MSC cell population was homogeneous and presented a fibroblastic-like morphology. Cytometry analysis showed a defined cell population positive for CD29 (87.58% ± 11.70%), CD44 (81.08% ± 16.68%), CD166 (66.85% ± 8.79%) and SSEA4 (11.67% ± 11.31%), and not for the hematopoietic marker CD45. Histological and immunohistochemical analysis showed that ovine MSC seeded on Col-I scaffolds gave rise to osteogenic-like neotissue, rather than ovine MSC seeded on β-TCP. Cells presented an osteoblast-like morphology on Col-I constructs and their extracellular matrix showed more type I collagen and osteocalcin on Col I constructs (Figure 1A, 11.85% ± 1.07% and 12.22% ± 1.46%, respectively) than on β-TCP (2.47% ± 0.61% and 5.51% ± 0.51%, respectively). Osteogenic constructs of Col-I sponges analyzed by transmission electron microscopy showed precipitates composed by carbon (47.95% ± 4.93%) and oxygen (18.77% ± 0.41%), followed by calcium (11.87% ± 0.41) and phosphate (6.51% ± 0.19%). On the other hand, ovine MSC seeded on Col-I sponges allowed neotissue formation inside the cartilage focal lesion. Histological analysis (Figure 1B) and the assessment by the modified ICRS II scale revealed that a neotissue with fibrocartilage/hyaline cartilage characteristics was obtained.

Conclusions: Isolated ovine cells were demonstrated to be ovine MSC. Ovine MSC cultured on Col-I sponges successfully synthesized osteochondral tissue. Data obtained suggest that ovine MSC have potential to be used in preclinical models prior to human clinical studies. Acknowledgements: OPOCRIN S.p.A.; ICIRO; CIBER-BBN; REDICENT and GPC (Xunta de Galicia); FER; UCEX; UDC.

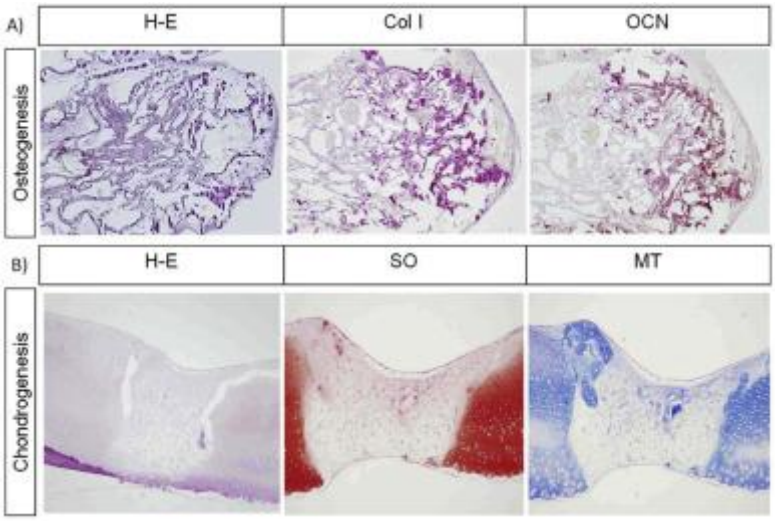

Figure 1 A) Osteogenic Col-I constructs stained with hematoxilyn-eosin (H-E) and immunostained with type I Col (Col I) and osteocalcin (OCN). Magnification ×l00. B) Chondrogenic Col-I constructs stained with H-E, Safranin O (SO) and Masson's Thricrome (MT). Magnification ×40.