

O60 (EI0417)

THE POTENTIAL OF OSTEOGENIC CELL SHEETS CO-CULTURED WITH ENDOTHELIAL CELLS FOR BONE TISSUE ENGINEERING

R.P. Pirraco^{1,2,3}, T. Iwata¹, A.P. Marques^{2,3}, M. Yamato¹, R.L. Reis^{2,3}, T. Okano¹

¹Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan; ²3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal

Objectives: Current strategies in the field of bone Tissue Engineering are limited by the use of scaffolds that present drawbacks such as cell necrosis at their bulk related to deficient vascularization after implantation. Cell sheet (CS) engineering has been proposed as a scaffold-free alternative for the regeneration of several tissues. This work proposes the use of this technology for bone regeneration by combining osteogenic CSs and endothelial cells.

Methods: Osteogenic CSs were fabricated by culturing male rat bone marrow cells (rBMSCs) in thermo-responsive culture dishes in osteogenic medium. Human umbilical vein endothelial cells (HUVECs) were seeded on the rBMSCs to create co-cultured CSs. The osteogenic CSs were recovered by lowering the temperature and then stacked on top of either a co-cultured or a similar osteogenic CS, and transplanted to female nude mice. Implants were recovered after 7 days and characterized by hematoxylin & eosin (H&E) and alizarin red (AR) stainings, immunohistochemistry for osterix, osteopontin, SRY (to identify transplanted male rat cells) and CD31, and calcium quantification.

Results and Discussion: H&E and AR stainings showed mineralized tissue formation in the implants both with and without HUVECs. Osterix and SRY immunostaining demonstrated the presence of host and donor osteogenic cells at the mineralization site showing recruitment of host osteogenic cells. HUVECs contribution to neo-vascularization was confirmed by identifying human CD31 cells in blood vessels. Furthermore, calcium quantification results showed a higher degree of mineralized tissue after the transplantation of the constructs with HUVECs.

Conclusions: This work confirmed the potential of transplanted osteogenic cell sheets for bone regeneration as well as the advantage of promoting cross-talk between osteogenic and endothelial cells for improved new tissue formation. The proposed approach avoids the constraints of scaffold use while successfully addressing the important issue of implant vascularization.