

An *in vitro* model of bone organ starting from progenitor cell populations

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INTRODUCTION: The ultimate goal of this work is to generate a 3D osteoblastic-osteoclastic-endothelial co-culture system, as an *in-vitro* model to mimic the process of bone matrix deposition and remodelling. Here, we investigate the feasibility to generate the three cell lineages starting from fresh human adipose tissue(AT) derived cells and monocytes(CD14+ from peripheral blood(PBM)). Additionally, non-invasive methods have been tested to monitor the functionality of the system, in terms of matrix formation and resorption.

METHODS: AT or AT with CD14+ cells (AT/CD14+) were seeded and cultured on 3D ceramic scaffolds using a perfusion bioreactor in presence of osteoclastogenic medium (MCSF and RANKL) for 19 days. Cell phenotypes were assessed by cytofluorimetry and Tartrate Resistant Acid Phosphatase (TRAP) staining. Qualitative and semi-quantitative assessment of extracellular matrix (ECM) deposited on the scaffolds has been performed by means of electron microscopy (SEM) and quantitative magnetic resonance imaging (qMRI). Supernatant analysis has also been performed to monitor the process of matrix formation (C-terminus procollagen-I, PICP) and resorption (TRAP5b and phosphate concentration).

RESULTS: Only in AT/CD14+, cytofluorimetry analysis showed the co-existence of cells of the endothelial (1.5% of CD34⁺/CD31⁺ cells), pre/osteoclastic (13% of CD14⁺/CD31⁺ cells) and osteoblastic lineages at different stages of differentiation (21% STRO-1⁺/ALP⁻ (early), 22% STRO1⁺/ALP⁺ (late)). Osteoclastic cells (TRAP⁺) were attached to the pores of scaffolds in contact with ECM (Fig1A). In AT/CD14+, SEM (Fig 1B) and qMRI indicated lower extent of ECM deposited on the scaffolds as compared to the AT constructs, possibly due to osteoclastic resorption activity. In this regard, supernatant analysis showed an osteoblastic activity in terms of collagen-I production (Fig.2 PICP) in both groups, while only AT/CD14+ showed a

temporal increase in osteoclastic activity (Fig.2 TRAP5b) and elevated phosphate levels.

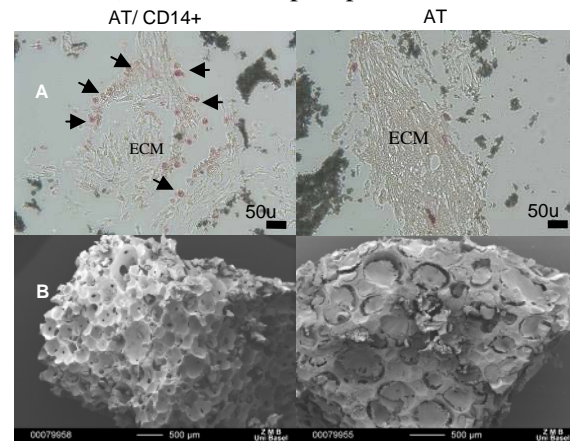


Fig. 1: Characterisation of *in-vitro* generated constructs by means of histology(A) and SEM (B); arrows indicate TRAP⁺ osteoclasts in contact with ECM.

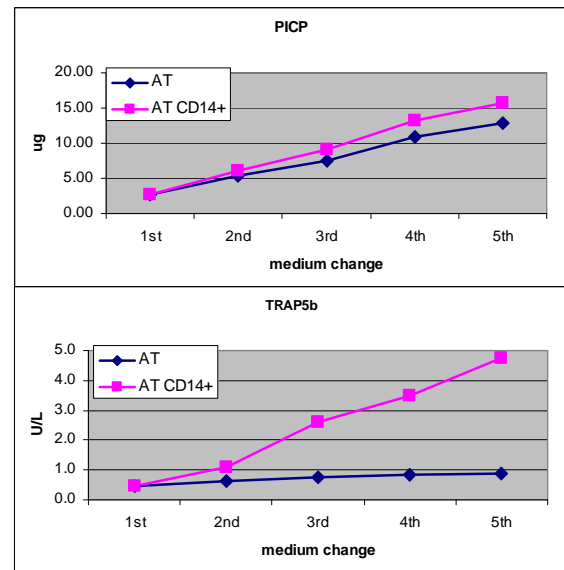


Fig. 2 Supernatant analysis to monitor osteoblastic (PICP) and osteoclastic (TRAP5b) activity.

DISCUSSION & CONCLUSIONS: This study indicates that cells from human AT and PBM, within a 3D perfusion-based culture environment, can be used to establish an *in vitro* system of osteoblastic, osteoclastic and endothelial lineage cells, which can be effectively monitored in a non-invasive way. The model is planned to be used for testing the effects of biochemical and biomechanical factors on bone homeostasis.