TS36
Dextran sulphate and ficcol fail as macromolecular crowders to enhance extracellular matrix deposition

RP Pirraco[1,2], F Carvalho[1,2], RL Reis[1,2] and AP Marques[1,2]
[1]*3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine. AvePark, 4806-909 Taipas, Guimarães, Portugal;* [2]*ICVS/3B's – PT Government Associate Laboratory, Braga/ Guimarães, Portugal*

The development of strategies based on the use of scaffolds for Tissue Engineering and Regenerative Medicine(TERM) has been hindered by the inability of researchers to present solutions to overcome problems mainly related with the biocompatibility of materials and *in vivo* perfusion of the 3D structures. It is in this context that scaffold-free methodologies are being presented as increasingly attractive strategies for TERM. Scaffold-free approaches in general rely on the production of extracellular matrix(ECM) by the cells of interest. However, the creation of an ECM robust enough for use in TERM is many times a challenge. Therefore, there is an important need to develop protocols that boost cell's ability to produce ECM. One way to achieve this is to expose cells to an environment crowded with adequate macromolecules in order to mimic the physiological cellular milieu. Dextran sulphate(DxS) and Ficoll(Fc) have been suggested as compounds capable of increasing ECM deposition[1]. The involved mechanism is closely related to the increase in enzyme-mediated collagen deposition. In the present work, we hypothesized that the use of DxS or of a combination of Fc of different molecular weights (Fc70/Fc400) as crowders in culture medium could increase the robustness of the ECM produced by human fibroblasts(hFb) or human adipose-derived Stem Cells(hASCs). $5x10^4$ hFb or hASC were seeded on wells of 24 and 48 well plates, and cultured for 24 h in α-MEM supplemented wih10% FBS and 1% antibiotics. After the first 24 h, the medium was replaced by fresh medium supplemented with 1% FBS and a)50 μg/mL of Ascorbic Acid, b)50 μg/mL of Dextran Sulphate, or c)37.5 mg/mL of Fc70 + 25 mg/mL of Fc400. Cells were cultured for further 2 and 5 days. dsDNA quantification showed that in both conditions b) and c), and independently of the cell type, cell proliferation was significantly reduced. ECM production was evaluated by quantifying the deposited collagen using a semi-quantitative Sirius Red kit (Picrosirius, Chondrex, USA). Collagen quantification, normalized with dsDNA, demonstrated that for both cells types, the presence of either DxS or Fc70/Fc400 resulted in the decrease of ECM deposition. In conclusion, the use of DxS and Fc70/Fc under the conditions herein described failed to increase the ECM production by both hFb and hASCs.

Acknowledgements: This research has been funded by the EU Seventh Framework Programme under grant agreement FP7-KBBE-2010-4-266033-SPECIAL and by the Portuguese Foundation for Science and Technology (FCT)-funded project Skingineering (PTDC/SAU-OSM/ 099422/2008).

Reference:

[1]Chen et al *BJP* 2009