Placenta Stem Cells Differentiation onto PU foams

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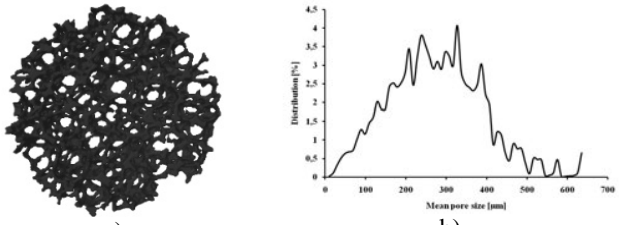
Statement of Purpose: Regeneration of living and functional bone tissue, by use of engineered constructs obtained by cells cultured into porous scaffolds, is an appealing strategy to treat bone defects and diseases. In this contest, mesenchymal stem cells (MSCs) seeded on scaffolds seem to be an encouraging approach. Different sources have been considered for harvesting human MSCs, such as bone marrow and adipose tissue; recently, human term placenta [1] has been recognized as an easily accessible source of pluripotent cells, also exempt from ethical debate.

This work was aimed at evaluating the proliferation and osteogenic differentiation of human amniotic (hA) and chorionic (hC) MSCs when cultured onto polyurethane (PU) foams, coated or not with α-tricalcium phosphates (αTCP).

Materials and Methods: A PU foam (PUf) was obtained with a gas foaming technique, by reacting a polyether-polyol mixture with MDI prepolymer (Bayer, Germany), using Fe-acetyl-acetonate as catalyst (0.001% w/w_{polyol}) and 2% w/w_{polyol} water as expanding agent, as previously described [2]. Morphological characterization of this PUf was performed by micro-CT (Skyscan 1172, Aartselaar, Belgium), by evaluating average pore size and distribution, porosity and pore interconnection. PUf samples (Ø=6 mm, h=2 mm), fixed in circular slots into a polymeric mesh, were coated by immersion in αTCP suspension under magnetic stirring [3]. hAMCs and hCMCs were isolated from human term placenta [1], seeded ($2.5x10^5$ and $5x10^5$ cells/well) onto PUf samples, αTCP-coated or not, and cultured in the presence of osteoinductive (NH Osteogenic Differentiation Medium, Miltenyi Biotec) or control medium (EMEM, Lonza) up to 20 days, using polystyrene tissue culture (PSTC) wells as control. Cell morphology was evaluated by Scanning Electron Microscopy (SEM), whereas scaffold colonization and in vitro cell differentiation were investigated by hematoxylin-eosin, Alizarin Red and von Kossa staining.

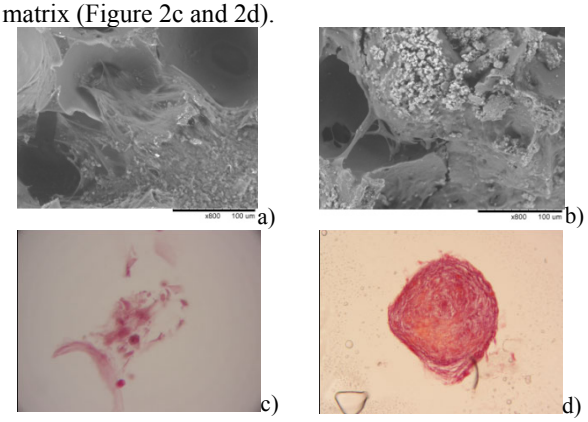
Results: The 3D model generated by micro-CT analysis demonstrates a homogeneous morphology of the foam and regular pore size, shape and distribution (Figure 1a). Micro-CT analyses also allowed to investigate the average pore size distribution (Figure 1b); foam pores showed a diameter size in the range of 150-400 µm, with an average pores size of 268 µm. Coated PUf exhibited a decrease only in pores interconnection. By SEM, a good cell colonization was observed both onto the PUf matrix (Figure 2a) and the α-TCP coated PUf (Figure 2b), with cells well adherent to the scaffold pores. Furthermore, the cells cultured onto αTCP-coated foam seem to aggregate around the αTCP particles (Figure 2b).



a) b)

Figure 1. PUf 3D model (a) and trend of the foam average pore size distribution (b) by micro-CT analyses

The osteogenic differentiation medium appeared to promote cell differentiation to the osteogenic phenotype, as highlighted by Alizarin Red staining performed on PUf matrix (Figure 2c and 2d).



a) b) c) d)

Figure 2: Representative SEM images of hCMCs cultured onto PUf matrix with EMEM (a) and hAMCs cultured onto αTCP-coated PUf using Osteogenic Medium (b) (seeding density of $5x10^5$ cells/well); scale bar = 100 µm and histological images of Alizarin red of hCMCs (seeding density of $2.5x10^5$ cells/well) cultured onto PUf matrix with EMEM (c) and Osteogenic Medium (d)

Discussion and Conclusions: Due to the ability to stimulate cell adhesion, scaffold colonization and osteoblast differentiation, the proposed PU foams appear good candidates as scaffolds for bone regeneration, also in agreement with previous results obtained with human MSCs from bone marrow [2]. In particular, αTCP-coated PUfs are able to promote hAMCs and hCMCs differentiation to the osteoblastic phenotype, probably due to the presence of CaPs particles onto the surface of the scaffold, that provide a good source for active bone forming cells.

References

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