Mesenchymal Stem Cells and in situ Cross-linkable Hydrogel Scaffolds for Bone Tissue Engineering.

Maria Cornelissen¹, Evi Lippens¹, Geert Vertenten², Heidi Declercq¹, Ives Swennen³, Jan Luyten⁴, Lieven Vlaminck², Frank Gasthuys², Etienne Schacht³.

Corresponding Author: Ria.Cornelissen@UGent.be

¹Ghent University, Department of Basic Medical Sciences, Ghent, Belgium and ²Ghent University, Department Of Surgery and Anaesthesiology of Domestic Animals, Ghent, Belgium and ³Ghent University, Polymer Chemistry and Biomaterials Research Group, Ghent, Belgium and ⁴VITO NV, Mol, Belgium

Introduction

Thanks to their ability to integrate well with the defect sides, in situ cross-linkable biomaterials are promising materials to treat irregular bone defects. However, for large defects (critical size), a shortage of local bone forming cells can hamper bone regeneration. In this regard, the addition of in vitro pre-cultured and osteogenic differentiated cells could be beneficial. By preference, the cells should be pre-cultured on a cell delivery system, offering a substrate for the anchorage dependent cells and giving protection to the cells during the mixing and hardening of the materials. In this work, the use of cell loaded macroporous CultiSpher –S carriers and hydroxyapatite (HA) based hollow cylindric carriers in combination with an in situ cross-linkable hydrogel is evaluated, in vitro as well as in vivo.

Materials and Methods

Mesenchymal stem cells (MSC) were obtained from the bone marrow of goats. Cells were cultured in an osteogenic medium on commercially available gelatin based macroporous carriers (CultiSpher-S from Percell Biolytica) and hollow HA based cylinders made by VITO, Belgium (diameter 1mm, internal diameter of the cannal: 700 nm). Modified Pluronic F127 (Plu-ALA-L), photo-cross-linkable with Irgacure 2959 as photo-initiator was used as an in situ cross-linkable hydrogel. Cell viability of the pre-cultured cells encapsulated in the in situ forming biomaterial was assessed in vitro by MTT, MTS and live-dead fluorescent assays. Cell loaded carriers were implanted subcutaneously in goats in combination with the above mentioned Plu-ALA-L, in unicortical 6 mm tibial defects. Bone regeneration at the defect site was evaluated over a period of 8 weeks by histological analysis, using paraffin or Technovit embedded material. The following stainings were performed: HE, Masson’s Trichrome and von Kossa, as well as immunostainings using antibodies for cbfa-1 (an early osteogenic marker) and osteocalcin (a late osteogenic marker).

Results

MSC could easily be loaded and pre-differentiated into the osteogenic lineage on both carrier systems. Cell viability of the cells encapsulated in the Plu-ALA-L was tested in vitro over a period of 7 days. Compared to controls, viability percentages of 80% were observed after 7 days in culture. Subcutaneous implantation of the CultiSpher loaded carriers showed the presence of cuboidal cells, positive for cbfa-1, in the carriers and in their vicinity. No cell in- or outgrowth was observed in the HA carriers. Cells delivered into the unicortical tibial defects using Cultisphere-S as cell delivery system and Plu-ALA-L as cross-linkable material, enhanced bone formation. No bone formation or ingrowth of surrounding tissue was observed on the HA carriers. The low porosity and small pore size of the cylinder wall (overall porosity of 24%, pore size of 2 µm) could be the cause of the negative results obtained with the HA carriers.