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Development of novel bilayered structures to be used as an osteochondral in vitro model

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Proper *in vitro* models to evaluate the performance of relevant tissue engineered constructs are still a major demand of the field. The use of *in vitro* platforms in research presents obvious ethical and cost advantages over *in vivo* models. *In vitro* models can also offer important scientific advantages, as for example, the study of biological mechanisms of action because it is easier to isolate an experimental variable and measure its impact on a simple, well controlled system. The aim of this project is to create an osteochondral *in vitro* model. As proof-of-concept, two different bilayered sponge-like scaffolds were developed to act as a template for co-culturing rabbit adipose stem cells (rASCs)-derived osteoblasts and chondrocytes. Bilayered low acyl gellan gum (LAGG)-LAGG/hydroxyapatite (HAp) spongy structures with and without Gelatin were produced respectively integrating cartilage- and bone-like layers. The freeze-dried bilayered scaffolds composed by LAGG2%(w/v)-LAGG2%/HAp30% (w/w) and LAGG/Gelatin 1:1 2%(w/v)-LAGG/Gelatin 1:1 2%/HAp30% (w/w) have a gradient of HAp in the bone-like layer that, unlike cartilage-like layer, present a bioactive behavior. The bilayered structures possess about 90% porosity, 500 μm of pore size and 85% interconnectivity as determined by Micro-CT analysis. Swelling and degradation tests revealed that the structures can absorb about 120% of their weight and lost 10% of their mass after 30 days in phosphate buffered saline solution. *In vitro* studies with rASCS from Fat Pad (knee) are being performed to study cell adhesion and proliferation. A rotational dual chamber bioreactor was fabricated in-house to improve medium diffusion into the structures, to allow the use of two different culture mediums for each layer, to homogenize the cell distribution in the scaffolds, as well as to introduce mechanical stimuli by 180º stirring and compression of the top layer. So far, the results have shown that the developed bilayered scaffolds have a great potential for finding application as a screening platform of new therapeutic approaches for the treatment of osteochondral tissue disorders.

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