Rotational Dual Chamber Bioreactor for Interfaced Engineered Tissue Models

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Statement of Purpose: 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 presents obvious ethical and cost advantages over in vivo models. In vitro models can also offer important scientific benefits in the study of biological mechanisms of action focusing on isolated variables/effects. Different 2D and 3D cell culturing systems, static (e.g., tissue culture polysterene with or without transwell) and dynamic (e.g., bioreactors), have been proposed along the years. However, the lack of suitable physical structures capable of supporting the generation of multilayered and complex tissues has been hampering the field of tissue engineering [1]. Among the challenges to attain multilayered structures, the creation of a continuous gradient that allow a smooth interface formation between layers is one of the most demanding [2]. In addition, improved cell culturing conditions in a 3D environment are still to be achieved and one of the biggest problems to be overcome. In this sense, this work refers to the development of a rotational dual chamber bioreactor adapted for cell culture in multi-layered scaffolds, providing an enhanced cellular homogenization inside of the layered 3D structure. Ultimately the developed system will allow creating an osteochondral 3D in vitro model.

Methods: A rotational dual chamber bioreactor capable of improving medium diffusion into 3D layered structures, of providing different culture medium to each layer and an homogeneous cell distribution in the scaffolds, as well as of introducing mechanical stimuli by 180º stirring and compression, was fabricated in-house. 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) 2%w/v-LAGG2%w/v/hydroxyapatite (HAp) 30%w/w spongy structures with and without Gelatin (1:1) were produced integrating cartilage- and bone-like layers. The developed cell-scaffolds constructs were cultured in static conditions as control of the dynamic cultures.

Results: A bioreactor comprising a multiposition magnetic stirred plate on which are connected dual chambers by magnetic attraction, was developed. The dual chambers can have rotational movement, which is horizontal and/or vertical. The size of the scaffold is dependent on the ability of the medium to enter and exit the interior of the 3D structure. By introducing the stirring movement over the cell culture, the diffusion potential increases. This allows the use of bigger 3D scaffold consequently enhancing the ability to produce larger tissues in vitro. Moreover, the multi-chamber bioreactor is adapted for co-culturing different cell types allowing the generation of multi-cell type tissues. The chamber cap allows cyclic compression over the respective cell-scaffold construct, creating a physical stimulus that intends to mimic the body weight. The compartments of the dual chamber allow flowing different culture medium per compartment or the creation of an air-liquid interface. The multi-chamber may further comprise a conductive material, as a coating or as a scaffold itself, allowing providing an electric pulsatile stimulus over the cells. In addition to this stimulus to the cell cultures it can be also used to promote transfection of the cells by microporation. The magnet present can be used to attract magnetics particles into the culture for transfection thus allowing compiling the advantages of magnetofection with microporation.

Figure1. A: Rotational Dual Chamber Bioreactor prototype [3]; B: Bilayered structures of LAGG-LAGG/HAp30% (left) and LAGG/Gelatin-LAGG/Gelatin/HAp30% (right).

The freeze-dried bilayered scaffolds composed of 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 are being performed to compare static and dynamic cultures.

Conclusions: The described technology has the potential to be used in the development of different in vitro tissue model platforms. These can allow replacing or decreasing the animal models for scientific research and in the pharmaceutical field for drug screening and evaluation.

References:

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