into 3D cell cultures. In addition, we demonstrated that similar to pure ELP hydrogels, the matrix stiffness and cell-adhesion ligand density could be tuned independently in this hybrid ELP-PEG hydrogel. Encapsulated human fibroblasts demonstrated high viability (>98%) after 7 days of culture and adopted a more spread morphology in gels with lower matrix stiffness and higher RGD ligand concentration. The good light transmittance, excellent cytocompatibility, as well as independently tunable biochemical and mechanical properties make this newly designed ELP-PEG hybrid gel an ideal platform for future cell-matrix interaction studies.

Modulation of the Secretome of hBMSCs by Tailoring the Macromolecular Gradient In Hydrogels to Generate Tissue-to-Tissue Interfaces

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Tissue-to-tissue interfaces exhibit structural, biological and chemical gradients serving a wide range of physiological functions (e.g.:load transfer mediation between two adjacent tissues and sustaining cellular communications to retain tissue's homeostasis) [1]. Cells have the capacity to sense and respond to physical and chemical stimulus, performing better when cultured in three-dimensional (3D) environments. Spatial and temporal 3D gradient hydrogels (GHs) better resemble the natural environment of cells in mimicking their extracellular matrix [2]. We hypothesize that differential functional properties can be engineered by macromolecules modulation in 3D GH systems. The aim is to assemble the GHs and evaluate the suitability for human Bone Marrow Stem Cells (hBMSC's) encapsulation. GHs solutions were prepared by blend of macromolecules: hyaluronic acid (MW 851 KDa-1.15 MDa, Lifecore) and collagen type II (MW 110–200 KDa, Symatese) at different ratios. Hydrogels were fabricated into moulds; higher ratio solutions assembled at the bottom and two other solutions consecutively on top-of-each-other. FITC-labelling macromolecules confirmed the gradient construction. AFM proved the different young modulus along the gradient. hBMSC cultures, P3 at 106 cells/ml within GHs were observed under confocal microscopy by Live/Dead® staining showing good viability. Secretory cytokine measurement for pro-inflammatory and angiogenesis factors was performed using ELISA. 3D GHs platform made of different macromolecules showed to be a suitable environment for hBMSC's supporting high cell survival and exhibited biofunctionality. A suitable 3D platform to modulate the secretome of hBMSC's, concerning their pro-inflammatory and angiogenic secretion, can be achieved by tailoring the macromolecular gradient environment.

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Efficient Formation of Size-regulated Hepatocyte Aggregates on Oxygen Permeable Microwell Sheets and the Size-dependency of Their Metabolic Capacities

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Three-dimensional hepatocyte aggregates are expected as a useful *in vitro* model for a number of assays. One of the common issues for hepatocyte aggregate formation is critical loss of oxygen supply to the culture system, because of the high oxygen consumption of hepatocyte and of the low gas permeability of culture environment. Here we report efficient method to obtain size-controlled hepatocyte aggregates using an oxygen permeable poly-dimethylsiloxane (PDMS) -based microwell sheet, which enables direct oxygenation to the culture environment. We prepared PDMS-based microwell sheet in different sizes and coated with 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer to prevent cellular attachment. Rat hepatocytes were cultured and formed size-controlled aggregates on their surface. Our microwell enhanced inoculum cellular density up to four times higher and accelerated aggregation compare to the conventional polystyrene tissue culture plate surfaces (TCPS). Less than 88 μm aggregates were overall higher functional than other sizes aggregates or monolayer culture in terms of several different subfamilies of P450s. This highly-metabolic size ranges of hepatocyte aggregates are smaller than the limited size decided by the oxygen diffusion and consumption (~150 μm). In addition, irinotecan toxic assays showed interesting size-dependency and more than 52 μm aggregates were effective for detoxification by CYP3A2. These results demonstrates the importance of the selection of hepatocyte aggregates sizes for accurate pharmacological and/or toxicological studies simply because observed metabolic rates of exogenous chemicals are the results of the diffusion, reaction and production of both the chemicals and their metabolite in the aggregates.

Improving the Multipotency of Mesenchymal Stem Cell During *In Vitro* Cell Expansion within the Grooves of a Micro-structure Pillar Array

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Multipotent stem cells such as mesenchymal stem cells (MSCs) have been extensively studied for the past decades to promote tissue repairs. Like any other cells, MSCs are home to 3-dimensional (3D) microenvironments where they constantly sense and respond to their environmental cues which governs their appropriate self-renewal and controlled differentiation. In regenerative medicine, donor MSCs are however culture on a flat substrate to facilitate cell expansion for achieving high number of cell population for the intended tissue repairs. The culturing on a flat substrate can possibly change their cellular morphology which affect their multipotency abilities. In this study, we investigated the effect of expanding MSCs within the grooves of micro-structural pillars (circles, rectangles and grills) on their multipotency. The grooves within the micro-structural pillars had demonstrate their capabilities to manipulate the MSCs morphology. Further investigation showed that the use of these MSCs expanded in different 3D microenvironment carried different capacities for osteogenic and chondrogenic differentiation. These findings gained us insights in creating novel cell culture platform for MSCs to improve their multipotency during cell expansion in the field of regenerative medicine.

In Vitro Models of SMCs Under Cyclic Mechanical Stimulation: a Comparative Study between 2D and 3D

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Herein, 2D (cell monolayers) and 3D (cellularized gels) cultures are directly compared as *in vitro* models for the investigation of the response of vascular smooth muscle cells (SMCs) to cyclic strain.

Human Umbilical Artery SMCs (HUASMCs) were cultured in monolayers and inside collagen gels cast in UniFlex® plates using specific molds and anchors. Uniaxial 7% cyclic strain was applied at 1 Hz for 2 and 5 days. For histology, 10 μm sections were stained by Masson's trichrome. For immunofluorescence, samples were stained with phalloidin-rhodamine and DAPI. Western blot was used to detect α-actin and calponin.