56.P29
Hydrogel microsphere cell carriers for cell expansion in suspension culture: implications for stem cell therapeutics

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The development of scalable methodologies for handling human stem cells (hSC) necessitates an approach premised on the use of three dimensional (3D) suspension cultures that are readily adaptable to large-scale bioreactors. In this project, encapsulating fibrinogen based hydrogel biomaterials were used for developing a 3D bioprocessing methodology for stem cells through a straightforward cell inoculation into microspheres, in situ self-renewal in the microsphere culture system and mild cell recovery into a solution phase. For creating the microsphere carriers, we employed a temperature-responsive semi-synthetic hydrogel material produced by the conjugation of poloxamers to fibrinogen. Mesenchymal cells were encapsulated and cultivated in the hydrogel microspheres using suspension bioreactors for up to 14 days. The viability, cell cycle and proliferation were characterized and indicated comparable expansion results to 2D tissue cultures on petri dishes. Cell recovery from the hydrogels was accomplished enzymatically with high yields and minimal disruption to cell viability. We conclude that a biomaterial-based method can be successfully applied for suspension expansion of cells with controlled cell proliferation through cell-cell and cell-material interactions. Moreover, hydrogel microsphere cell carriers such as the ones described herein may offer a tangible solution to the growing demand for commercial-scale stem cell bioprocessing practices.

56.P30
Development of bioreactor for culturing 3D hydrogel structures containing living cells

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It is essential to keep sufficient delivery of nutrients and oxygen to the periphery in engineering large sized tissues. For this purpose, the bioreactors for perfusion culture play an important role. Various 3D hydrogel structures containing living cells can be designed and fabricated using 3D bio-printer we have ever developed. 3D structures with multiple perfusion canals can be designed and fabricated. Then, the custom-made bioreactors were developed to culture such 3D bio-constructs. In order to achieve a fluent flow, flow-canals with uniform multiple micro channels of 180 μm in diameter were installed in the culture chamber. The bioreactor circuit was composed of a peristaltic pump, air trap, a gas exchanger and the designed culture chamber. Then, we investigated the flow in the chamber by flow visualization using fluorescent ink and the samples of non-woven mesh. As a result of the flow visualization, gentle and uniform flow was observed in the chamber, which is thought due to micro-channel. The trace of the flow was decided by the thickness of the fluorescent inks remained in the non-woven mesh. Compared to the chamber without micro-channels, the distribution of stain was significantly uniform. In conclusion, the bioreactor system in which fabricated 3D tissues can be cultured with uniform and gentle flow was developed. Using this bioreactor, fragile 3D hydrogel structures can be cultured towards culturing large sized thick artificial tissues.

56.P31
Site-directed cell differentiation on a single multicellular spheroid using multilayer microfluidic system

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Multicellular spheroids which are formed following rearrangement and compaction of cell aggregates, have been widely use as a 3-dimentional culture model to supplement traditional monolayer cultures. Because of their tissue-like characteristics, spheroids culture is suitable for various applications, including tumor metastasis and invasion research, drug screening, and serving as building units for tissue engineering. This abstract presents a microfluidic system which can induce cell differentiation at a specific site on a single spheroid. A thin PDMS membrane contained multiple orifices (200 μm) was bonded between two PDMS micro-channels to create a multilayer microfluidic chip. The designed geometry could trap a spheroid, partitioning the spheroid into two parts which allows different parts of a spheroid exposing to different stimuli and later differentiating into different cell types. Our preliminary results showed that spheroids could be trapped on the membrane under continuous flow. To mimic the different stimuli, two fluorescent dyes, CMFDA (green) and CMTMR (red), were injected into different channels. After treatments, a chimeric spheroid was shown one part with red and the other part with green fluorescence. In conclusion, cell differentiation cues can be delivered by fluid flow to a selected part of the spheroid. This technique may contribute to tissue engineering, drug screening, and biological studies.

56.P32
Perfusion seeding of porous scaffolds: a bioreactor-based procedure suitable for the establishment of uniform 3D-structured co-cultures

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Interstitial perfusion has been proved successful for the development of 3D tissues, whereby it is crucial to provide a high initial number of viable cells with a homogeneous distribution throughout scaffolds. The present study aims at characterizing and enhancing a perfusion seeding technique based on a previously validated Oscillating Perfusion Bioreactor (OPB). Design of Experiments: statistical methods were used to assess the effects of process parameters, considering flow speed, cell seeding density and seeding time as process variables and cells and commercial scaffolds as inputs. OPB enabled to reach up to 70% seeding efficiency associated to significantly higher cell viability as compared to static controls (170% relative to static, $P < 0.0001$) for densities up to 4×104 cells/mm^3. Scaffold cross-sections showed higher uniformity for perfusion seeded compared to statically seeded constructs. In order to assess the possibility to guide the structural organization of the construct obtained with our procedure, we performed preliminary experiments with a two steps sequential seeding method of MSC and HUVEC. With our procedure we generated constructs with HUVEC networks embedded on uniformly MSCs-lined scaffolds, as opposed to statically or one-step seeded mixed populations constructs. In conclusion results demonstrate that our bioreactor-based seeding procedure can represent a step forward in the achievement of structural control in organized 3D multicellular systems.