Poster session 12: New technologies for biofabrication

P12.2

Surface design for selective cell catch-and-release using electrochemical trigger

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Aim: Sorting of specific cells from a heterogeneous suspension is indispensable in various applications such as regenerative medicine and cancer research. Dynamic control of the biointerface between adherent cells and materials may provide a promising approach for the detachment and manipulation of cells in vitro. In this study we developed the surface modified with zwitterionic oligopeptides and aptamers with which cells can be selectively attached and then rapidly detached by the application of an electrochemical potential (Figure 1).
Methods: We designed a zwitterionic oligopeptide CGGGKEKEKEK for preparation of cell repulsive surface. This peptide spontaneously bonded to a gold surface via a gold-thiolate bond and formed a dense molecular monolayer by the electrostatic force between neighboring molecules due to the alternating charged lysine (K) and glutamic acid (E) making the surface cell repulsive. To selectively catch cells the surface of peptide layer was further modified with an aptamer which has high affinity for Hep G2 cells. Cells selectively attracted on the surface were subsequently detached by electrochemically cleaving the gold-thiolate bond and releasing the peptide layer from the gold surface.
Results: Quartz crystal microbalance measurements revealed that the peptide densely adsorbed onto the gold surface and significantly reduced the non-specific adsorption of fibronectin or proteins in culture medium containing 10% FBS. When Hep G2 cells and normal human dermal fibroblasts (NHDFs) were exposed to the surface modified with the peptide none of them attached on the surface. On the surface further modified with the aptamer selective adhesion of Hep G2 cells was observed and the ratio of Hep G2 cells (Hep G2/NHDF) was 88%. Almost all the cells attached on the surface were electrochemically detached within 5 min.
Conclusions: The gold surface modified with designed zwitterionic oligopeptides and aptamers can be used for selective cell sorting.

P12.3

Patterning of endothelial cells by Laser-Assisted Bioprinting promotes the creation of capillary-like structures

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Aim: Development of microvasculature and microcirculation is critical for bone tissue engineering (1). To resolve the issue of a reduced vascular component the reproduction of local microenvironment and the organization of cells are regarded as ultimate goals (2). In biofabrication parallel to inkjet printing and extrusion-based deposition the Laser-Assisted Bioprinting (LAB) is an alternative method for the assembly and micropatterning of biomaterials and cells (3). This technology allows fabrication of 2 and 3 dimensional tissue engineering constructs. The objective of this work was to promote the creation of a vascular network by different methods based on LAB in order to optimize bone regeneration by tissue engineering.
Methods: The LAB workstation comprised a laser (λ=1064 nm 30 ns) focused on a quartz ribbon that was coated with a thin absorbing layer of gold (60 nm) and a 30 µm layer of laser bioink. Two strategies were developed to study the creation of capillary-like structures. The first method consisted in seeding HUVECs monoculture on collagen patterns printed onto agarose. The second method consisted in a coculture obtained by printing HUVECs patterns on collagen and mesenchymal stem cells (SCAPs).
Results: HUVECs were patterned in 10mm length lines and a width between 150 and 300 microns. In monoculture self-assembly of cells was observed in less than an hour. Capillary-like structures emerged in 48h. In coculture vascular network was obtained in 7 days. These results imply that LAB allows printing of collagen and cells with micrometric resolution to promote in vitro development of vascular-like structures by organizing HUVECs.
Conclusions: This study explored two strategies to organize HUVECs by LAB. The results demonstrate that LAB is a relevant method for micropatterning HUVECs and is adapted to promote the creation of vascular-like structures in a context of bone regeneration. These capillary-like structures obtained by LAB could be included into three-dimensional constructs in order to improve angiogenesis which is an essential prerequisite for bone healing. LAB could also allow development of vascularized bone grafts by in situ bioprinting of endothelial cells and osteoblast precursors. It would be a new therapeutic approach promoting bone regeneration.
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P12.5

Lonic liquid pumping with DC electric field

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Liquid pumping can occur along the outer surface of an electrode under a DC electric field. For biological applications a better understanding of the ionic solution pumping mechanism is required. Here we fabricated CNT wire electrodes (CWEs) and tungsten wire electrodes (TWEs) of various diameters to assess an ionic solution pumping. A DC electric field created by a bias of several volts pumped the ionic solution in the direction of the negatively biased electrode. The resulting electro-osmotic flow was attributed to the movement of an electric double layer near the electrode and the flow rates along the CWEs were on the order of picoliters per minute. According to electric field analysis the z-directional electric field around the meniscus of the small electrode was more concentrated than that of the larger electrode. Thus the pumping effect increased as the electrode diameter decreased. Interestingly in CWEs the initiating voltage for liquid pumping did not change with increasing diameter up to 20 µm. We classified into three pumping zones according to the initiating voltage and faradaic reaction. Liquid pumping using the CWEs could provide a new method for biological studies with adoptable flow rates and a larger 'Recommended pumping zone'.

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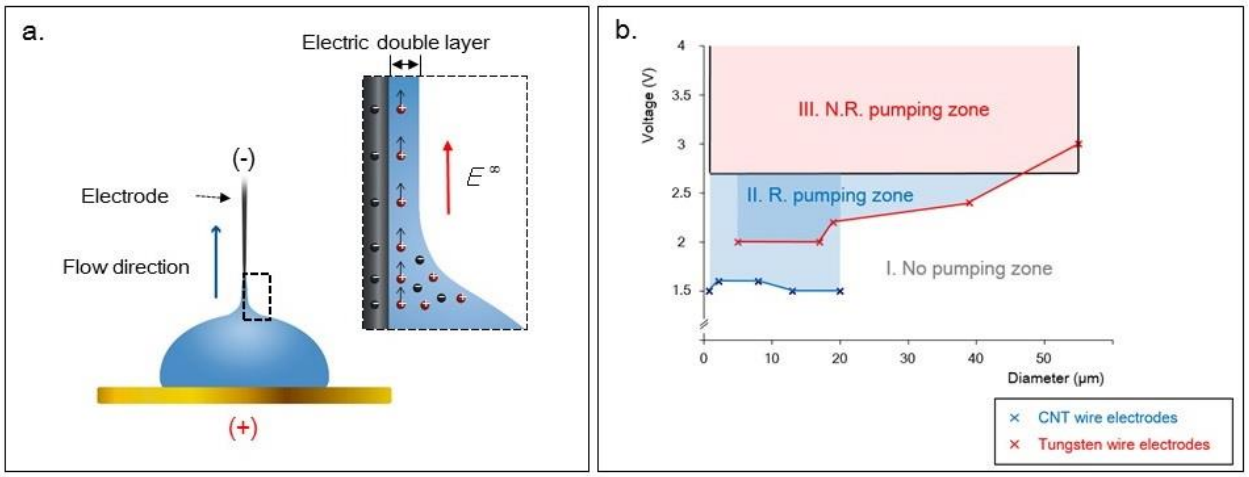

Electro-osmotic flow (EOF) by a DC electric potential

P12.9

Directional Foaming of scaffolds by integration of 3D Printing and Supercritical CO2 Foaming

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Aim: Tissue engineering has recently moved towards the attempt to produce biomimetical structure. A major drawback is the difficulty to produce defined hierarchical scaffold mimicking tissue as bones and cartilage. While many techniques as been applied in this sense to process biomaterials physical methods as supercritical foaming and Additive Manufacturing represent a clean way to control the exact composition of the final construct. However their limitation as low porosity and reproducibility still prevent us to reach a complex structure. This work represents a first attempt to combine the advantages of the two techniques while overcoming some of the main drawbacks producing 3D anisotropic size-controlled structures.
Methods: An Ultimaker Original+ was used to produce the raw scaffold by fusion deposition modelling from PLA Natureworks 4043D and 2003D. Fibers. Three 2D structures were printed for each PLA isomer with a dimension of 10mm x 10mm x 1mm and with inner windows of 3.5mm x 3.5mm. Then three 3D scaffolds were produced from each PLA (4.4mm x 4.4mm x 4.4 mm 0.4mm fibers and 0.6mm fibers spacing). Each 2D and 3D structure was then foamed with supercritical CO2 in a GMP medical autoclave from SITEC SIEBER Engineering AG (2).
Porosity pore size distribution interconnectivity and scaffold expansion post foaming were determined by µCT (Microcomputed Technologies Inc. Skyscan 1076 Belgium) and Scanning Electron Microscopy (Microscopy XLF30 microscope) (SEM). Compression behaviour was investigated with an Ultimate Tensile Strength machine (Test Machine Systeme Germany).
Results: The minimum architecture deformation were obtained by tuning the foaming parameters offering the desired cellular architectures with fibre directional porosity in the micro meter range.
A range of mechanical properties were obtained from solid to foamed cellular material reducing the stiffness of scaffolds with solid walls (2) introducing anisotropic properties related to the orientation of the fibres. A model of expansion from 3D printed structure to 3D foamed structure is finally proposed.
Discussion and Conclusions: The results shows a possibility to overcome the porosity limit of 3D printed scaffold and anisotropy control of foams. The process can be now extended to different biopolymers and to aim specific applications. A controlled anisotropy a homogeneous ma cro- and an oriented micro-porosity in 3D structures are obtained by combining 3D additive manufacturing and supercritical foaming two solvent-free processes which could integrate living cells.