Poster session 6: Bottum-up approaches

P6.1

A novel approach for the fabrication of micro-objects as cell spacers in regenerative medicine applications

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Bottom-up tissue engineering makes use of micro-modules which serve as building blocks to construct the tissue from the bottom up. Thereby micrometer-sized objects can serve as cell spacers to limit the amount of cells needed per construct. These micro-objects can for example be made by photolithography (Leferink Schipper et al. 2014). However this technique comes with the disadvantage of producing objects from photoresists containing toxic agents. Different object-geometries can be engineered with this technique making it possible to influence the cellular response when the objects are combined with cells in vitro and eventually in vivo.
To overcome the problem that arises from photolithography we developed a novel fabrication method which does not rely on photo curable resins containing toxic crosslinking agents. Instead micro-objects were made of the thermoplastic polymer poly-(D L-lactic acid) (PDLLA). A silicon mold with cube-shaped features with a dimension of 25x25x15 µm (w x l x h) was made by cryogenic etching. By hot embossing a 5 µm thin dip-coated PDLLA film fully biocompatible PDLLA micro-objects were produced. To prevent the polymer from sticking to the mold an anti-sticking layer was vapor-deposited onto the mold. The substrate silicon wafer for the hot embossing process was pre-treated by spinning a water-soluble layer of poly(vinyl alcohol) (PVA) onto an Omnicoat™-treated silicon wafer. After imprinting the residual layer in between the embossed PDLLA micro-objects was removed by reactive ion etching using oxygen plasma. The free-standing objects were released from the substrate wafer by dissolving the PVA layer in dH2O. The oxygen plasma treatment reduces the hydrophobicity of the objects and introduces a micro-roughness on the surface. An increased hydrophilicity is known to improve cell attachment which is necessary for cell proliferation and differentiation. We were able to produce up to 3 million micro-objects with the same controlled geometry on a single wafer. Different shaped objects (donut- or LEGO®-type blocks) made from PDLLA were designed and produced using the production method described above. In conclusion it is possible to create a whole library of shapes and thermoplastic materials to be used as cell spacers in bottom-up tissue engineering.

P6.2

Rheological characterization of gelatin hydrogels for 3D cell printing with high cell viability and printability

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Aim: Three dimensional (3D) cell printing has provided a promising tool to fabricate 3D tissue constructs for tissue engineering and in vitro tissue/ pathological models. However it still remained a challenge that printing hydrogels with high viscoelasticity are required to support the 3D printed constructs but still retaining the fluidity during printing process and high cell viability after printing.
Gelatin and gelatin derivative hydrogels with good biocompatibility high viscoelasticity and temperature-sensitive properties have been widely used as good biomaterials for 3D printed tissue constructs but two main hurdles limit the applications of gelatin hydrogels. 1) Natural biomaterials have unstable differences between different batches. 2) The viscoelasticity of gelatin is relied on holding time which result in the changes of cell viability and printability during printing process. Herein we proposed to measure rheological characterization of gelatin hydrogels during the printing process to predict the cell viability and printability as well as to provide a more stable printing process.
Methods: All the rheological measurements were performed with a rotational rheometer operating in the oscillatory mode with a strain of 0.1% and a frequency of 1Hz. The temperature sweep and the time sweep were conducted to analyze the rheological characterization of gelatin hydrogels. Cell survival rate was tested immediately after printing. Grid structures of 10mm*10mm*4 layers were printed at different time to test the printability of the gelatin hydrogels.
Results: The results showed that increasing the gelatin concentration decreasing holding temperature and increasing holding time under gel point increased the hydrogel viscoelasticity. And increasing the viscoelasticity of the gelatin hydrogel decreased the cell survival rate after printing. Different gelatin hydrogels with different concentration but similar viscoelasticity showed similar cell viability. Good printability of gelatin hydrogels was conducted during the middle term of the holding time.
Conclusions: Rheological measurements were conducted to optimize the printing process of gelatin hydrogels. According to the results we further provided a protocol of characterizing temperature-sensitive gelatin hydrogels for 3D cell printing with high cell viability and printability. Researchers in 3D cell printing could use this protocol to adjust the printing process quickly and develop new temperature-sensitive hydrogels for 3D cell printing.