Susceptibility of macroporous PHEMA hydrogels for growth of mouse embryonic stem cells

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Statement of purpose: Synthetic macroporous hydrogels are widely used in tissue engineering research studies and medical applications as versatile and tunable 3D substrates for cell cultivation. For successful cell growth on and into these scaffolds, certain requirements must be met such as an appropriate pore size and shape, certain matrix softness/rigidity as well as the connectivity of pores should be secured. This study aims at development of 3D macroporous poly(2-hydroxyethyl methacrylate), (PHEMA) hydrogel scaffolds for cultivation of the mouse embryonal stem cells. Particularly, we addressed the question how the pores morphology and connectivity affect susceptibility for the cell adhesion, growth and viability. The aim of this study is to discern clearly the effect of the hydrogel morphology from the other parameters such as hydrogel matrix chemical composition and matrix stiffness.

Methods and strategy: We prepared four types of synthetic reference hydrogels of PHEMA matrix, i.e. the gels were of the same chemical composition differing only by their microstructure: Gel A has only small pores (2-5 um) formed by spaces between the fused spheres of gel matrix. Gel B contains only large pores made by washing out soluble template particles, these pores were not fully connected. Gel C contained large pores like those of the gel B but separated by the permeable gel walls made of the gel A with tiny pores for perfusion of metabolites and nutrition. Finally, we prepared the gel matrix D without the pores. The total pore volume and pore connectivity of gels using their swelling values and their hydraulic permeability were characterized. The morphology of the samples in their swollen state was visualized using light microscopy (LM) and the morphology of the flash-frozen samples was visualized by cryo-low-vacuum scanning electron microscopy (cryoLVSEM). The mechanical properties of the gels were measured using the oscillatory shear rheometry. For the cell cultivation studies, the ESD3 mouse embryonic stem cells (ATCC, LGC Standards Sp. z.o.o., Poland) were used. ESD3 were cultivated with the scaffolds for four days. Viability and proliferation of cells were evaluated every day. The cells attached to the gels were labeled with Hoechst 33258 and visualized using the Olympus multi-photon laser scanning confocal microscope FV10-ASW (Olympus, Japan).

Results: The morphology study of the hydrogels studied both in the swollen state (light microscopy) and in the frozen state (cryoLVSEM) proved that the gel C possessed the dual porosity: fine and large, while the fine porosity was attained by employing the mechanism of reaction induced phase separation during gel polymerization. The hydraulic permeability confirmed that this gel provides the highest connectivity of pores throughout the matrix (highest value of all four) while mechanically, the gel C behaved similarly as the gel B that was rather stiff gel with some non-communicating large pores. The ESD3 cells attached well on the all gel types (A,B,C, and D) while the cells proliferated only on the scaffold of the gel C (i.e. the dual porosity hydrogel). The cultivation tests showed clearly the importance of porosity arrangement within the matrix. The cells were able to proliferate for five days of cultivation with viability between 90-100%. The LSCM showed characteristics of embryonic cells growth on the gel C (Fig.1)

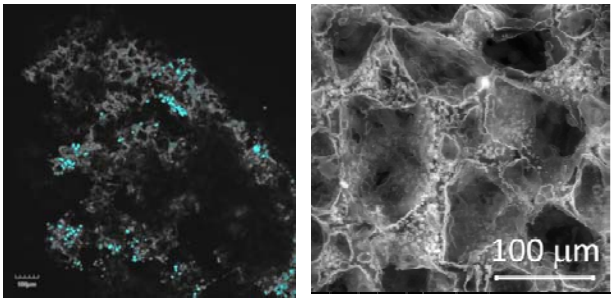

Figure 1. The ESD3 cells growing on the gel scaffold (type C visualized using the cell nuclei fluorescence (blue spots) on the 4th day of the cultivation and picture of the structure of gel C.

Conclusions: We demonstrated the key role of highly communicating pores embedded in the synthetic macroporous hydrogels for cell cultivation and proposed a simple way of fabrication of such "dual porosity" hydrogels. The hydrogel of dual porosity prepared using combination of templating and micro-phase separation had hierarchical pore arrangement in space, showed high total pore volume while maintaining sufficient mechanical coherence and stiffness important for adherence, growth and proliferation of embryonal cells. The concept of dual porosity gels for hosting the cells proved viable; it enabled sufficient supply of nutrients and thus is promising for advanced tissue engineering materials fabrication, especially for successful growth of embryonic stem cells. The outlook for the further study in our laboratory is to functionalize the inner pore surfaces by incorporating electrostatically charged monomers and/or by attaching growth factors to enhance the specific cell attachment and growth.

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