Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Study on the control of the double network hydrogel surface-bulk transition and its effect on mesenchymal stem cells

ダブルネットワークゲル表面-バルク転移の制御およびその間葉系幹細胞への影響に関する研究

Recently, the double network (DN) hydrogel, an interpenetrating hydrogel out of a brittle polymer, the first network and a ductile polymer, second network, was found applicable in the biomedical field. A study described that the implantation of DN hydrogel induced spontaneous articular cartilage regeneration in osteochondral defects. Based on such regenerative potential, it has been argued that the DN hydrogel might be involved in inducing stem cells and therefore could be used for in vitro stem cell expansion as well, which still requires experimental evaluation. The engineering of cell scaffolds that preserve pluripotent stem cells in large scale is challenging but necessary to improve the accessibility of stem cell therapy and regenerative medicine for patients. The development of such scaffolds requires a deep understanding of underlying cell-substrate interactions that is prohibited unless the normally coupled physical and chemical properties of soft matter scaffolds are individually tunable.

In this study we at first focused on the development and characterization of DN hydrogels for biomedical purposes. Here we tried to identify how the hydrogel synthesis molding material affects the chemical structure of the DN hydrogel at the surface-bulk transition. Therefore, we used hydrophilic and hydrophobic synthesis molds and characterized the surface and bulk structure via combined ATR-FTIR, AFM, and a newly developed microelectrode technique that probes the electric potential distribution inside a hydrogel. We found that the molding substrate properties, namely the surface energy and charge, regulate the chemical structure on the DN hydrogel surface. By controlling the second network polymerization condition and molding substrate, the surface-bulk transition region was modified, so that only the second network or both networks are present at the double network hydrogel surface. Through these findings we gained a new insight on the structure formation at the DN hydrogel surface, that allowed us to modify the polymer surface density of the first and second network individually so that cell adhesion or repulsion at the hydrogel surface is regulated.

Second, we investigated the applicability of the introduced hydrogels as cell scaffolds in stem cell therapy and regenerative medicine. In cell-based therapies and related in vitro stem cell expansion a key factor is the maintenance of pluripotency. Therefore, we monitored by RT-qPCR, microarray transcriptome profiling and western blotting how the expression of pluripotency marker genes in stem cells differs between cultivation on the hydrogels and polystyrene dishes.

To investigate if mechanical or chemical hydrogel properties affect stem cell pluripotency, we utilized the two-component nature of the DN hydrogel, so that both networks have an ability to influence the stiffness and therefore local and global mechanical properties can vary dramatically. For DN hydrogels, the bulk stiffness is regulated by varying the crosslinking degree of the first network, while polymer density at the surface is maintained through a constant second network crosslinker density.

Finally, the study has indicated that the gene expression patterns correlate with the hydrogel bulk stiffness, independent of polymer surface density. This phenomenon was found to be independent of polymer chemistry as different types of monomers were used to synthesize the second network. Further there are hints, that residual monomers from the first network polymerization and the cultivation time affect the pluripotency marker gene expression on a minor level. These findings contribute towards the understanding of the DN hydrogel surface chemistry and the hydrogel-based regulation of the in vitro pluripotent stem cell state.