HOKKAIDO UNIVERSITY

Title	Study on the control of the double network hydrogel surface-bulk transition and its effect on mesenchymal stem cells [an abstract of dissertation and a summary of dissertation review]
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Degree Grantor	北海道大学
Degree Name	博士(生命科学)
Dissertation Number	甲第13826号
Issue Date	2019-12-25
Doc URL	https://hdl.handle.net/2115/76598
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	doctoral thesis
File Information	Martin_Frauenlob_review.pdf, 審査の要旨

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Doctoral Dissertation Evaluation Review

Degree requested: Doctor of Life Science Applicant's name: MARTIN FRAUENLOB

Examiner :

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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）

Results of Evaluation of the Doctoral Dissertation (Report)

Recently, studies on the application of the double network (DN) hydrogels in the biomedical field are being actively done. Previously, it was demonstrated that the implantation of DN hydrogels into osteochondral defects induced spontaneous cartilage tissue regeneration, leading to the assumption that there are certain cell-DN hydrogel interactions present that guide the stem cell fate. In order to understand the interaction of DN gels with cells for future application of DN gels, it is of outermost importance to establish a method to characterization and regulate the DN hydrogel surface.

The aim of this study is to establish a systematic method to characterize and control the surface structure of the DN hydrogels, and to elucidate the effect of DN gel surface structure on the behaviors of mesenchymal stem cells.

In chapter 3 the author established systematical protocols to characterize the DN hydrogel surface structure and the pluripotent properties of stem cells on DN gels, via chemical analysis such as infrared spectroscopy and electric potential measurement and mechanical analysis via AFM and microindentation. The author revealed that a microscale surface layer of the second network polymer is formed on top of the DN gel prepared by conventional method. To clarify the mechanism of this surface layer formation, the author proposed a model based on the interfacial repulsion of the negatively 1st network gel and the glass molds for synthesizing the 2nd network. This model was confirmed by altering the synthesis conditions of the 2nd network, i.e., by using a low energy synthesis mold or by compression of the glass synthesis mold during the 2nd network polymerization. Based on this understanding, the author established methods to control the thickness of the surface-bulk transition.

In chapter 4, the author investigated the reason of the observed inconsistency from infrared spectroscopy and electric potential measurements between single network polyacrylamide and the corresponding DN hydrogel with its surface covered with the second network. The author clarified that the extremely small amounts of monomers, not reacted in the 1st polymerization step, are incorporated in the 2nd network and affect the potential of the DN hydrogel surface layer. Such small amount of charges in the surface layer was

only detected by the electric potential technique that has a very high sensitivity but could not be detected by the conventional infrared spectroscopy.

In chapter 5 and Chapter 6, the author designed an experiment to synthesize hydrogels with independently controlled surface chemistry and mechanical properties, and confirmed that the gene expression of cell spheroid cultures is affected by elastic modulus and polymer surface density of hydrogels.

In conclusion, the author has established a systematic approach to characterize and control the surface layer of DN hydrogels and confirmed that the surface layer chemistry of the DN hydrogels regulate the cell adhesion which in turn affects stem cell pluripotency. The author discovered that the 2nd network, synthesized by the conventional DN hydrogel polymerization protocol, contains residual monomers of 1st network. These fundamental findings are essential and important for the applications of DN gels in the biomedical field.

Therefore, we acknowledge that the author is qualified to be granted a Doctorate of Life Science from Hokkaido University.