Graduate School of Advanced Science and Engineering,
Waseda University

博 士 論 文 概 要
Doctor Thesis Synopsis

論 文 題 目

Enhancement of Hepatocellular Functionality and Cytotoxicity In-vitro by Nanostructure and Chemical Modification of Materials That Mimic In-vivo Environment

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Abstract

Living cells in their native environment are embedded in complex, well-defined organic matrices that incorporate macromolecules to provide the extracellular matrix (ECM). The ECM is assembled into a three-dimensional meshwork containing precisely contoured nanostructures. The architecture and composition of the ECM possess the essential physical cues and biochemical factors that trigger and control specific reactions and improve the cellular behaviors and functionality. Furthermore, the existence of such physical and chemical cues within native cellular microenvironment is a main reason for such observed decrease in cellular functionality between in-vivo- and in-vitro conditions. This represents a main challenge for the biomaterial fabrication technology especially in biosensor and drug development related applications.

Accordingly, an understanding of the relationship between human cells and their environmental native cues is very important especially for the biomaterial technology. Further recognition and mimic of such cues would provide the excellent mean for the optimization of interfacial characteristics of biomaterials and prediction of best mutable factors for a maximized functionality of human cell in-vitro applications.

This is particularly true for liver cells, as they need a specific architecture with distinctive surroundings and biochemical characteristics for the maximum cellular functionality. Since a decrease in such functionality generally occurs when liver cell are cultured on two-dimensional surfaces.

For the improvement of hepatocellular functionality and maximize their applications in-vitro culture conditions. I have hypothesized that artificially mimicking the ECM physical and chemical cues by the available advancement in nanofabrication techniques and study their interactions with hepatic cells in order to promote the hepatocellular behaviors and maintain the required in vivo architecture. Therefore, I focused in my PhD on the modifications of the superficial physical and chemical characteristics of TiO_2 nanopatterns as a key regulator of cellular behavior. Firstly, the role of physical cues variations was determined by recognizing the influence of different geometry and size of nano and submicro-topographical features of TiO_2 nanopattern on hepatocellular behavior. Secondly, Integration between the physical (TiO_2 pattern) and chemical (RGD, adhesive peptide motif) cues and the influence of such integration on cellular behaviors. Thirdly, we have determined the effect of such physical cues on the variations of cellular behavior in normal and cytotoxic condition to overcome some of discrepancies between cellular behaviors in-vivo and in-vitro. Such work is composed of 6 Chapters.

First Chapter: contains an introduction for ECM, its composition, various types of cues, and functions. How the presence of such complicated structures and cues orchestrate the cellular behaviors and response.

Second Chapter: Composes of background for different reported methodologies to mimikc ECM cues, biomaterial as a good candidate for such role, in addition to the resulted interactions between the biomaterials that mimick ECM cues and various types of human cells. Finally the relationship between our fabricated TiO_2 cues nanopattern and ECM cues, since the diameter of the nano-scale depressions and projections were chosen to fit within the dimensions of hierarchically-extended collagen nanofiber networks (one of ECM components).

Third chapter: describes the relationship between the manipulation of amorphous TiO_2 geometrical and dimensional characteristics and the optimization of hepatic cell line behaviors. The morphological and functional changes in the hepatocytes induced by such diversified nanofeatures were characterized using fluorescent immunostaining techniques. The expressions of functional proteins such as albumin, transferrin and cytochrome P450 were tested as functional markers. In addition, the change in cellular orientation, cell alignment and native extracellular matrix (ECM) assembly induced by these well-defined nanotopographies were also recorded. Data show that the control of the physical nanofeatures associated with a certain dimension and shape that closely mimics the natural ECM components could be critical for optimizing cellular behaviour.

Fourth Chapter: describes the integration of chemical and physical ECM cues. This was done by the incorporation of organic moieties with specified cellular functionality, such as RGD motifs, into the deposited TiO_2 inorganic film using an enzyme catalyzed oxidation reaction. The role of simultaneously combining such diversified cues on the cellular behavior was investigated. The results show a synergism is observed due to the combination such cues in a single substrate especially for the expression of liver-specific markers. Nonetheless, it emphasizes major role of chemical cues in enhancing hepatic functionality compared to substratum topography alone.

Fifth Chapter: describes the influence of TiO_2 nanopattern in especial that mimic ECM cues and cancer cell responses to cytotoxic agents. Comparisons between resulted hepatocellular morphological changes due to the presence of such nanofeatures after exposure to a cytotoxic agent “cisplatin” were performed. Since ECM physical cues and such cytotoxic agent provoke several signaling pathways and cellular cascades with further illustrations of converging points between such cascades. The findings show a significant increase in the % of dead cells upon the change of TiO_2 topography after cisplatin exposure relative to flat surface. This emphasizes the role of simulation of ECM intrinsic elements (as physical cues) in the alteration of cytotoxic response to cisplatin. Furthermore, Data Shows an alteration in substratum topography in presence of cisplatin induce a transformation in nuclear chromatin condensations, morphological variabilities and its directional modifications that were examined by different

techniques as fluorescent microscope and SEM.

Sixth Chapter describes final conclusions and future prospects.

In conclusion, I have been able to mimic physical and chemical ECM cues to efficiently improve hepatocellular behaviors in-vitro. Such ECM simulation was done by the alteration of TiO_2 nanopatterns surface characteristics.

1- The optimum topography of TiO_2 nanofeatures was determined, as TiO_2 nanofeatures that resemble one of the hierarchically-assembled ECM proteins (collagen) could be recognized by liver cell line allowing the proper cytoskeletal orientation and cellular integrity. Moreover, physical characteristics of TiO_2 nanofeatures as size and geometry are crucial regulatory determinant of cellular responses. Consequently, this work was not only able to recognize the tangible cues to improve hepatocellular behaviors but also utilize these results in the production of novel biomaterials that would potentially be of great benefit for tissue engineering and its future applications.

2- Mimicking of the diversified components of ECM cues in a TiO_2 single substrate by the integration of specific surface chemistry and well-defined nanotopographical features would promote further improvement of hepatocellular functionalities. Such integration of more than a single ECM cues in the fabricated biomaterials goes beyond hepatic cells as it can be extended to other types of cellular models in implant technologies as well as the determination of stem cells fate.

3- The biophysical characteristics of ECM are extremely important determinants of cellular response in a cytotoxic platform, since cells embedded in such nanopattern showed an alternative response compared to flat surface. Thus, an increased knowledge of the role extracellular matrix structural elements at the cellular level especially under cytotoxic conditions could be utilized as a future tool for the superior understating of cancer therapeutics and cellular behaviors.

Finally, this work could be used to for the development of novel biomaterials culture models since essential structural determinants of ECM cues required for the biomaterial optimization in liver cells' applications were identified that could be further exploited for tissue engineering and bioreactor technology. As mimicking of the recognized ECM elemental cues would be of great benefits for the efficient in-vitro testing strategies and their existing challenges. Since the role of biomaterial manipulation; superficial texturing, chemical composition and topographical features as excellent means to control hepatic cellular behaviors and functionality were emphasized.

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