論 文 の 内 容 の 要 旨

論文題目

Fabrication of Cell-hybridized Polymeric Hydrogels for Fusing with Soft Tissues

（生体軟組織と融合する細胞複合化ポリマーハイドロゲルの創製）

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Motivation and objective

Tissue-material integration is required in tissue regeneration and clinical medical treatments such as percutaneous medical devices. However, the low surface biocompatibility of the materials brings complications to the bordered tissues, which arouses the separation of tissue material. These complications directly or indirectly lead to tissue necrosis and device failure. This research aims to fabricate a tissue-mimicking hybrid material that can integrate with the tissue to solve these problems and complications. 2-methacryloyloxyethyl phosphorylcholine (MPC) hydrogel (PMBV/PVA) has shown fascinating properties in 3D cell culture, such as the high cytocompatibility or unifying the period of the laden cells, is an ideal candidate matrix. In this research, the hydrogel was newly designed with cytokine conjugation. Cell immobilized in the hydrogel formed an ECM hybrid hydrogel structure similar to natural tissue and connected to tissue by wound healing mechanism. Consequently, it aims to construct a material with a tissue-mimic surface and high biocompatibility to integrate with natural tissue for the long period implant medical device (Fig. 1).

Introduction

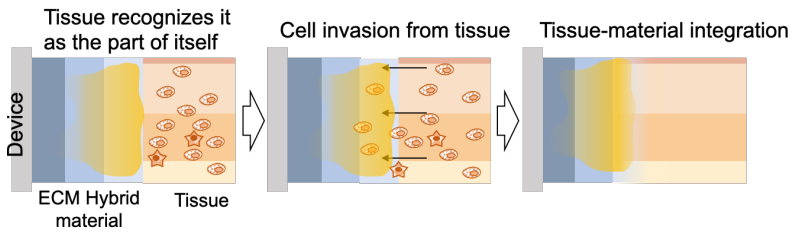

Figure 1 Tissue integration mechanism.

Percutaneous medical devices are widely used in clinical, such as the catheter, implantable sensors, etc. However, the low cytocompatibility of long-period-use implantable medical device material arouses rejection reactions on the interface between material and tissue. The separation occurs on the material-tissue

interface, accompanied by infection, inflammation, and even tissue necrosis and device failure. The ideal solution to these problems is that a material with a tissue-like surface can tightly integrate with natural tissue. Natural tissue treats the material as apart of itself and fuses following the wound healing mechanism. That means the material should have high cytocompatibility and designability to suit complex requirements.

Among existing biomaterials, MPC polymers with cell membrane-similar chemical structures have ultra-high cytocompatibility and are wildly utilized in clinical medical treatments. PMBV/PVA hydrogel is a reversible hydrogel fabricated by mixing MPC polymer (PMBV) and poly(vinyl alcohol) PVA solution. PMBV/PVA hydrogel has the same high cytocompatibility as MPC polymers and can form a hydrogel in the cell culture condition. It is reported that PMBV/PVA hydrogel can immobilize cells with high cell viability and allow for cellular activities like proliferation or differentiation. The immobilized cells can also be controlled by the physical properties of PMBV/PVA hydrogel. Like the cell cycle, it can be unified at a particular modulus of the PMBV/PVA hydrogel. PMBV/PVA hydrogel crosslinked by the connection between the boronic acid on the PMBV and hydroxyl groups on PVA. Moreover, this connection is reversible with the addition of saccharide. That means the immobilized cells can be recollected from PMBV/PVA hydrogel network. For all these characteristics, PMBV/PVA hydrogel shows excellent potential as a scaffold material for percutaneous medical device-surface modification.

In this research, PMBV/PVA hydrogel was utilized as the base matrix for tissue-like surface formation. Three elements of tissue are required: cells, cytokines, and scaffold. To maintain cytokines in the matrix, cytokine-PMBV/PVA hydrogel was newly fabricated for cell immobilization. After cellular activities like proliferation and extracellular matrix (ECM) secretion, the hydrogel is fulfilled with ECM and form a tissue-like structure.

PMBV/PVA hydrogel can be fixed on the material surface with a chemical connection. However, the considerable difference of properties between tissue and device will arouse stress concentration on the interface, one of the leading causes of connection collapse. Therefore, a layer-by-layer hydrogel structure is designed to eliminate the stress concentration on the interface between the tissue-like layer and device surface, with gradually changing properties from the device side to the tissue side. This research aims to fabricate a tissue-like structure in vitro and realize tissue integration as well. This scaffold material can not only connect to natural tissue and give a new glimpse towards tissue regeneration and organization.

Experimental

(1) Scaffold Cytokine-PMBV/PVA hydrogel fabrication

For cell proliferation, the necessary cytokines are required. Therefore, *N*-hydroxysuccinimide units were added into the PMBV chain during polymerization (PMBVS). PMBVS was synthesized with traditional free radical polymerization before cytokines conjugation. The conjugation between cytokines and PMBVS were conducted in PBS. Cytokine-PMBV and PVA solutions were prepared in PBS or DMEM. Cytokine-PMBV/PVA hydrogel was prepared by simply mixing the solutions tens of seconds at room temperature.

(2) PMBVS/PVA hydrogel conjugation on the material surface

There are multiple ways to conjugated PMBVS/PVA hydrogel on different substrates. Here, poly(ether ether ketone) (PEEK) was selected as the representative substrate. PMBVS was dissolved in ethanol with the desired concentration. Spin/dip-coated the PMBVS solution on the PEEK surface. After drying, UV irradiated, and washed the substrate with ethanol and water. Before drying out, laid a Cytokine-PMBV/PVA hydrogel on the top of the substrate to conjugate the hydrogel on the PEEK surface.

(3) Construction of cell-immobilized cytokine-PMBV/PVA hydrogel system

L929 cells, human dermal fibroblasts (NHDFs), and mouse iPS cells were immobilized into the cytokine-PMBV/PVA hydrogel with the steps below: collect the cells with trypsin treatment and count desired cell density. Finally, add PVA solution with thorough mixing to get a cell immobilized hydrogel.

(4) Fabrication and evaluation of ECM hybrid hydrogel

Cell immobilized cytokine-PMBV/PVA hydrogel was cultured for 1 week. Cell proliferation was confirmed by recollected cells from the hydrogel and counted. The differentiation state of mouse iPS cells was confirmed by PCR. ECM secreted by the laden cells was stained with fluorescence for the observation of distribution and quantified by PCR.

Results and discussion

(1) Cytokine-PMBV was synthesized successfully with the desired molecular weight and composition. Cytokine-PMBV and PVA hydrogels were successfully fabricated and the modulus can be adjusted by varying the concentrations of two solutions. Hydrogels formed a layer-by-layer structure by laying one layer of the hydrogel on the top of another. Two layers of the hydrogel can spontaneously connect, due to the reaction between boronic acid and hydroxyl groups on the hydrogel surface.

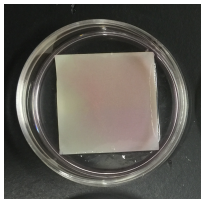

Figure 2 Cytokine-PMBV/PVA hydrogel on PEEK surface.

(2) PMBVS polymer was conjugated on the PEEK surface successfully with chemical connections. The conjugation of PMBVS was verified. And PMBVS layer helps the hydrogel to conjugate on the PEEK surface, that the PMBV/PVA hydrogel was simply laid on PMBV modified PEEK surface and

formed a hydrogel-on-surface structure (Fig. 2).

(3) Cytokine-PMBV/PVA hydrogel can be fabricated with different types of necessary cytokines that laden cells required. L929, NHDFs, and mouse iPS cells were well immobilized in optimized cytokine-PMBV/PVA hydrogel. Immobilized cells got high cell viability, and under the stimulation of conjugated cytokines, cells proliferated well in the hydrogel network (Fig. 3).

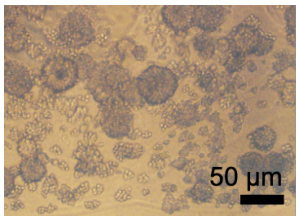

Figure 3 L929 cell morphology in the hydrogel.

(4) With the stimulation of cytokines and cellular activities, fibroblasts secreted ECM in the hydrogel. The ECM secretion was confirmed by PCR. From the results, cells secreted more ECM in an optimized hydrogel with the conjugation of necessary cytokines. Fig. 4 shows the 3D structure ECM distribution in 7-day cultured L929 cell immobilized ECM hybrid hydrogel. Moreover, mouse iPS cells kept in high differentiation state in the hydrogel network.

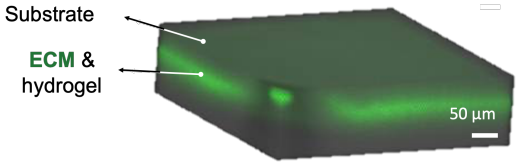

Figure 4 ECM hybrid structure of the hydrogel.

Conclusions and perspectives

Cytokine-PMBV/PVA hydrogel was fabricated successfully with the desired properties. PMBVS/PVA hydrogel was conjugated on PEEK successfully and formed a layer-by-layer structure by laying one layer of the hydrogel on the top of another. Cytokines were conjugated on the hydrogel network successfully. L929 cells, NHDFs, and mouse iPS cells were immobilized well in an optimized hydrogel with high cell viability. Under the stimulation of conjugated cytokines, fibroblasts proliferated in the hydrogel and secreted ECM after several days of culture. The 3D structure of the ECM hybrid hydrogel was confirmed on the PEEK surface as well. Also, mouse iPS cells kept in the high undifferentiated state give the cell-laden hydrogel a possibility to mimic the complex cellular environment in natural tissue.

The mouse iPS cells are kept undifferentiated and may have a higher differentiation ability than standard 2D culture. The gradual dissociation of the PMBV/PVA hydrogel can solve the clinical problem of iPS cell transplantation-tumor formation. Meantime, the ECM hybrid hydrogel requests animal test to verify the tissue integration. The degradation and the replacement to natural tissue of this hydrogel and dissociation of the hydrogel network require verification as well.

Reference:

[1] *Macromol. Biosci.*, 2020, **20,** 2000114.

[2] *Biomater. Sci.*, 2019, **7,** 2793–2802.