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学 位 論 文 題 名

A Study on the Effect of Hydrogel Scaffolds on the Behavior and Fate of Human Cells

(ハイドロゲルの物性によるヒト細胞の挙動と運命の制御)

学位論文内容の要旨

Many patients in need of organ transplants die while waiting for a suitable donor. Therefore, tissue engineering, which is a method by using scaffolds to expand the isolated cells or develop tissues from the isolated cells *in vitro*, has recently been proposed as an alternative treatment to whole organ transplantation, due to limited amount of donor organs available. In this case, the properties and functions of cells and tissues developed by tissue engineering *in vitro* were expected to be similar to that *in vivo*. In fact, isolated cells are usually expanded *in vitro* for tissue engineering by using a polystyrene (PS) scaffold, which is a widely used scaffold in cell culture *in vitro*. However, there are many researches showed that some cells lost their specific functions when cultured on this flat PS scaffold. The cells of human body reside in a complex environment whose nature is both biochemical and biomechanical. The complex environment surrounding each tissue *in vivo* is unique, and plays important role in regulating the development and maintaining the normal function of cells, which will influence the function of tissues. Therefore, it is necessary to find out the appropriate environment, which is called as scaffold in tissue engineering, for specific cell culture *in vitro*.

Hydrogels with three-dimensional network structure and viscoelasticity are similar to those of the macromolecular-based extracellular matrix ECM in biological tissue. As a result, hydrogels are commonly used not only as scaffolds for repairing and regenerating a wide variety of tissues and organs but also as substitute materials to develop artificial tissues and organs. A variety of naturally derived hydrogels can be used for tissue engineering scaffolds. However, these hydrogels usually show a poor cellular compatibility without modification with cell adhesive proteins. Our previous study showed that synthetic hydrogels with negative charges, such as poly (sodium p-styrene sulfonate) (PNaSS), poly (2-acrylamido-2-methylpropanesulfonic sodium) (PNaAMPS), could be used as good scaffolds for endothelial cells (ECs) culture without the need for any surface modification. Synthetic hydrogels have many advantages over natural hydrogels, for example, they are infection-free and low cost, can withstand high-temperature sterilization, have controllable and reproducible properties, can mimic several properties of natural ECM to regulate biospecific cell adhesion and cell migration.

Based on these results, in this study, we focus on the 2-dimensional cell culture by using hydrogels as scaffolds, and study on the effects of properties of hydrogels on the behavior and fate of human cells. Two kinds of cells, one is an anchorage independent cell, chondrocyte, which exists in a 3-dimensional environment *in vivo*, and the other one is an anchorage dependent cell, endothelial cell, which exists as mono-layer *in vivo*, were used in this study. The behaviors of these cells were investigated when cultured on the surface of several hydrogel scaffolds with different properties. The dissertation covers 4 chapters: chapter 1 is the general introduction, chapter 2 ~ 3 are the main text, and chapter 4 is the conclusions. The main results are given as follow:

In chapter 2, the spontaneous redifferentiation of chondrocytes was found on the surface of neutral hydrogels, such as poly (N, N'-dimethylacrylamide) (PDMAAm) etc., but was not on the surface of negative charged hydrogels, such as PNaAMPS etc. Furthermore, the redifferentiation of chondrocytes can be regulated by the charge density of hydrogel scaffolds. The normal human articular chondrocytes-knee (NHAC-kn) cells form cell colonies, show cartilage-specific morphology and high cartilage-specific gene expression on neutral hydrogel and hydrogels with low charge density. The results indicate that this redifferentiated behavior on chondrocytes is due to a proper weak adhesion of these hydrogels to adhesive protein existed in the cultivate solutions, which prevent a strong adhesion of the substrates to the cells. We assume that the weaker adhesion of the gel favors the 3D stacking of the cells, which is similar to the behavior *in vivo*, and this promotes the redifferentiation of the cells.

In chapter 3, The gene expression, glycocalyx content, and surface properties of human coronary artery endothelial cells (HCAECs) cultured on PNaSS hydrogels with various levels of elasticity ranged in 3 kPa ~ 300 kPa were measued. We found that all HCAECs reached confluence on these hydrogels while retaining the similar expression of EC-specific markers to that on PS, a widely used scaffold in cell culture *in vitro*. Polymerase chain reaction (PCR) and glycosaminoglycan assay showed that the amount of EC-specific glycocalyx secreted by HCAECs cultured on PNaSS was higher than that cultured on PS, and it increased with an increase of gel elasticity. Furthermore, the HCAECs cultured on PNaSS gels showed excellent property against platelet adhesion and low surface friction than that on PS. The platelet adhesion and surface friction of HCAECs cultured on PNaSS gels also depend on the elasticity of gels. The largest amount of EC-specific glycocalyx, excellent blood compatibility, and the lowest friction were observed when the elastic modulus of the gel was larger than 60 kPa. Overall, HCAECs cultured on these hydrogels have better surface properties than those cultured on PS scaffolds, demonstrating the PNaSS gels as potential tissue engineering material for blood vessels.

The results in this study promise a novel, less expensive, and efficient method to obtain the redifferentiated chondrocytes, and a novel culture system to get more similar functions of endothelial cells *in vitro* to that *in vivo*. It is expected that these synthetic hydrogels can be used as excellent scaffolds in fundamental medical research and tissue engineering in future.

学位論文審査の要旨

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博士学位論文審査等の結果について（報告）

ハイドロゲルは 3 次元網目の中に多量の水を含んでいるので、生体組織と似っていると言われている。そのため、細胞培養基盤として再生医療への応用を目指す研究が最近盛んに行われている。特に、化学架橋のハイドロゲルは化学種、電荷、弾性率など様々な物性が系統的に調整することが可能であるため、基盤の物性が細胞の増殖・分化への影響を検討する基礎研究、更には、諸ニーズに合わせた細胞培養基盤を開発する応用研究において、高い可能性を秘めている。従来の研究で用いたゲルは細胞に対する接着性が低く、ゲル表面に細胞接着タンパクの処理が必要であった。本学位申請者が所属する研究室では、アニオン性高分子ゲルを用いて、表面処理せずに内皮細胞を培養することに成功した。しかし、培養した内皮細胞の物性及びゲルによる他の細胞の制御などはまだ分かっていない。

本学位論文では、中性および負電荷を持つハイドロゲル上におけるヒト細胞の挙動について評価した。その際に、ゲルの弾性率、膨潤度、電荷密度などの物性を系統的に変化させた。細胞は非接着依存性細胞(軟骨細胞)と接着依存性細胞(血管内皮細胞)を用いて実験を行った。軟骨細胞においては、マイナス電荷を持つモノマーと中性なモノマーを共重合し、様々な電荷密度を持つハイドロゲル上での脱分化したヒト軟骨細胞の再分化性について調べた。その結果、ゲル上では脱分化した軟骨細胞が自発的に再分化する現象を発見した。これは二次元基盤上で軟骨細胞が再分化する初めての例である。また、ハイドロゲルの電荷密度による、軟骨細胞の再分化性が制御できることも明らかになった。すなわち、ハイドロゲルの電荷密度が低いほど、軟骨細胞の再分化が促進されることが分かった。一方、内皮細胞においては、マイナス電荷を持つモノマーを重合し、架橋剤の変化による弾性率が違うハイドロゲルを用いて、ヒト血管内皮細胞の挙動を調べた。その結果、ゲルの弾性率が上がると培養した内皮細胞が分泌した特徴的な糖鎖の量が多いことが明らかになった。また、ゲルの弾性率が上がると培養した細胞の表面血小板適合性がよくなり、ガラス基板上での滑り摩擦が小さくなることが分かった。さらに、ハイドロゲル上で培養した血管内皮細胞の表面物性は普通培養用ポリスチレン基盤上で培養したものより表面物性が良く、生体内の血管内皮細胞の表面物性と近いことが明らかになった。

本研究はさらに、なぜハイドロゲルの電荷密度や弾性率によって細胞の挙動が大きく異なるかについて、細胞と基盤との相互作用の観点から検討した。中性なゲル上で細胞を培養すると、基盤と細胞の相互作用が弱く、ゲル上での細胞運動が促進され、細胞と細胞の相互作用が強くなる。そのため、二次元基盤上で三次元な細胞構造が形成された。このような環境は非接着依存性細胞の生体内環境と近いため、非接着依存性細胞にとって最適な培養基盤となったと考えられる。それに対し、マイナス電荷を持つゲル上で細胞を培養すると、基盤と細胞の相互作用が強く、非接着依存性細胞が単層な細胞になってしまい、物性が劣化して行ったと考えられる。一方、マイナス電荷を持つゲル基盤と細胞の相互作用が強いため、接着依存性細胞は基盤上で強く接着し成長した。このような環境は接着依存性細胞の生体内

環境と近いため、接着依存性細胞にとって最適な培養基盤となったと考えられる。また、接着依存性の細胞は基盤への依存性が高いため、培養した細胞の物性がゲルの弾性率に強く影響されたと考えられる。

本研究で得られた成果は再生医療分野における基礎研究や応用研究に大きく貢献するものである。

よって著者は、北海道大学博士（理学）の学位を授与される資格あるものと認める。