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論　　文　　の　　要　　旨

Severe disease or loss of body structures are usually treated with drugs, artificial prosthesis or transplantation of tissues or organs. However, drugs neither fully heal the tissue with major damage nor achieve its long-term recovery. Artificial prosthesis can only restore partial function of a tissue and cannot sufficiently integrate with host tissue and need to be replaced when they are worn out. Organ transplantation has constraints such as shortage of donor tissues and organs for transplantation and chronic rejection produced by the immune system. Tissue engineering has emerged to overcome these constraints. In essence, cells are isolated from a fraction of human tissue and cultured *in vitro* to expand cell number. Then the cells are harvested and cultured with bioactive molecules in a three-dimensional (3D) scaffold material *in vitro*. During the *in vitro* culture, cells grow and produce extracellular matrix (ECM) within the 3D scaffold which turn into an engineered tissue. The engineered tissue is transplanted into human body to treat the diseased body structure. The 3D scaffolds support the cell adhesion, proliferation and ECM synthesis in a 3D environment and control the shape of engineered tissue or organs.

Porous scaffolds can be fabricated from biodegradable synthetic polymers or natural polymers. While synthetic polymer scaffolds often cause unwanted inflammatory responses, natural polymer scaffolds are more compatible with cells and tissues due to their natural origin. Therefore, in this study natural polymers were chosen to develop porous scaffolds. However, natural polymer scaffolds often have uncontrolled microstructures, which limits their application in tissue engineering. Scaffolds with homogenous, open-pore structure are necessary to facilitate cell infiltration, homogeneous distribution of cells and formation of tissue in the 3D material. Scaffolds with micropatterned structure such as the aligned pores can guide the

assembly and growth of cells into ordered tissues such as muscle and peripheral nerve. In the present study, porous scaffolds of natural polymers with controlled microstructures were designed and prepared for tissue engineering.

1. **Preparation of highly active porous scaffolds of collagen and hyaluronic acid by suppression of polyion complex formation**

Collagen-hyaluronic acid scaffolds with homogeneous pore structure, good mechanical property and high bioactivity are desirable for their applications in tissue engineering. However, in aqueous condition collagen and hyaluronic acid form polyion complex (PIC), which results in heterogeneous structures and poor mechanical properties of the scaffolds. In this part, we used low molecular weight salts to suppress PIC formation in collagen-hyaluronic acid suspensions during scaffold preparation. The suppression of PIC formation was studied by using turbidimetry, viscosity measurement and infrared analysis. The effects of PIC formation suppression on the morphology and mechanical properties of the scaffolds were examined with scanning electron microscopy and compression tests. PIC formation was found to be dependent on the ionic strength of the suspension. The suppression of PIC formation resulted in collagen-hyaluronic acid scaffolds with homogeneous pore structure and enhanced mechanical property. Collagen-hyaluronic acid scaffolds prepared under suppression of PIC formation promoted proliferation of dermal fibroblasts and upregulated the expression of genes encoding EGF, VEGF and IGF-1. Using low molecular weight salts to suppress PIC formation could aid in the design of collagen-glycosaminoglycan scaffolds for tissue engineering.

2. **Effect of high molecular weight hyaluronic acid on chondrocytes cultured in collagen/hyaluronic acid porous scaffolds**

Engineering cartilage tissue by culturing chondrocytes in porous scaffolds is one promising method to repair or restore the functions of diseased cartilage. Hyaluronic acid (HA) is used in porous scaffolds or hydrogels to promote the proliferation of chondrocytes and synthesis of cartilage extracellular matrix (ECM). However, whether HA in porous scaffolds has a beneficial effect on chondrocytes remains uncertain, possibly due to the uncontrolled pore structure and inhomogeneous HA in scaffolds. In this part, homogeneous collagen/HA scaffolds with well-controlled and interconnected pore structure were prepared by suppression of polyion complex formation between collagen and HA and using ice particulates as porogen. The pore structure and mechanical property of collagen/HA scaffolds and collagen scaffolds could be well controlled. High molecular weight HA in collagen/HA scaffolds inhibited the cellular proliferation, synthesis of sulfated glycosaminoglycan (sGAG) and cartilage ECM, compared with the results of collagen scaffolds. The results should provide additional information on the effects of HA in porous scaffolds on the chondrocyte behaviour in 3D culture.

3. **Gelatin scaffolds with controlled pore structure and mechanical property for cartilage tissue engineering**

For engineering of cartilage tissue with porous scaffolds, nonuniform cell distribution, heterogeneous tissue formation and weak mechanical property of *in vitro* engineered cartilage limit their clinical

applications. Porous scaffolds with desirable pore structures and mechanical property should be designed to solve these problems. In this part, gelatin porous scaffolds with homogeneous and open pores were prepared by using ice particulates and freeze-drying. The scaffolds were used to culture bovine articular chondrocytes to investigate the influence of the gelatin porous scaffolds on the functions of chondrocytes. The pore structure and mechanical property of gelatin scaffolds could be well controlled by using different ratios of ice particulates to gelatin solution and different concentrations of gelatin. Gelatin scaffolds prepared from ≥ 70% ice particulates enabled homogeneous seeding of bovine articular chondrocytes throughout the scaffolds and formation of homogeneous cartilage ECM. While soft scaffolds underwent cellular contraction, stiff scaffolds resisted cellular contraction and had significantly higher cell proliferation and synthesis of sulfated glycosaminoglycan. Compared with the gelatin scaffolds prepared without ice particulates, the gelatin scaffolds prepared with ice particulates facilitated formation of homogeneous cartilage tissue with significantly higher compressive modulus. The gelatin scaffolds with highly open-pore structure and good mechanical property can be used to promote chondrocytes functions for *in vitro* tissue-engineered cartilage.

4. Preparation of 3D microgrooved collagen scaffolds for multi-layered skeletal muscle tissue engineering

Preparation of 3D micropatterned porous scaffolds remains a great challenge for engineering of highly organized tissues such as skeletal muscle tissue and cardiac tissue. Two-dimensional (2D) micropatterned surfaces with periodic features (several nanometers to less than 100 μm) are commonly used to guide the alignment of muscle myoblasts and myotubes and lead to formation of pre-patterned cell sheets. However, cell sheets from 2D patterned surfaces have limited thickness, and harvesting the cell sheets for implantation is inconvenient and can lead to less alignment of myotubes. 3D micropatterned scaffolds can promote cell alignment and muscle tissue formation. In this part, we developed a novel type of 3D porous collagen scaffolds with concave microgrooves that mimic muscle basement membrane to engineer skeletal muscle tissue. Highly aligned and multi-layered muscle bundle tissues were engineered by controlling the size of microgrooves and cell seeding concentration. Myoblasts in the engineered muscle tissue were well-aligned and had high expression of myosin heavy chain and synthesis of muscle extracellular matrix. The microgrooved collagen scaffolds could be used to engineer organized multi-layered muscle tissue for implantation to repair/restore the function of diseased tissues or be used to investigate the cell-cell interaction in microscale 3D topography.

5. Biomimetic assembly of HUVECs and muscle cells in the microgrooved collagen scaffolds

Engineering 3D thick tissues and maintaining their viability *in vivo* necessitate the vascularization of engineered tissues. Vascularization of engineered tissue can enable the delivery of nutrients and oxygen and removal of waste matter from living tissue. In addition, many types of tissues such as skeletal muscle and limb bones have well ordered tissue structures and vasculatures to maintain the viability of the tissues. Engineering vascularized 3D tissue with well ordered structure remains a challenge in tissue engineering. In this part, the microgrooved collagen scaffolds with parallel, concave microgrooves were used for

coculture of skeletal muscle myoblasts and vascular endothelial cells. When the cells were seeded at a proper ratio and concentration, the unique microstructure of microgrooved scaffolds triggered the spontaneous assembly of cells into vascularized, well-ordered muscle tissue that mimicked the normal muscle tissue organization.

審　　査　　の　　要　　旨

〔批評〕

本論文では、生体組織を再生するための細胞足場材料として材料組成及び空孔構造を制御した天然高分子の多孔質材料を設計し、作製した。まず、コラーゲンとヒアルロン酸からなる高分子混合溶液のイオン強度を塩で調整することにより、混合溶液におけるイオンコンプレックス凝集体の形成を抑制し、コラーゲンとヒアルロン酸を分子レベルで複合化した多孔質足場材料を作製した。作製した多孔質足場材料は、従来の方法で作製した材料にくらべ均一な多孔質構造と高い力学強度を有し、皮膚由来の線維芽細胞の増殖といくつかの細胞成長因子遺伝子の発現を促進した。また、本複合多孔質足場材料の多孔質構造を精密に制御するために、多孔質体の形成プロセスにおいて、予め作製した氷微粒子を空孔形成の鋳型として用いた。その結果、足場材料の多孔質構造は氷微粒子の形状やサイズに応じてより精密に制御できることができた。本複合多孔質足場材料を用いてウシ軟骨細胞を培養し、分子量の異なるヒアルロン酸による軟骨細胞機能への影響を明らかにした。また、氷微粒子の添加率及びゼラチンの濃度を検討することにより、多孔質構造と力学強度が異なるゼラチンの多孔質材料を作製することができた。本ゼラチン多孔質材料を用いてウシ軟骨細胞を培養したところ、軟骨組織を再生することができた。さらに微量液体ディスペンサーで描画した氷のパターンを鋳型として利用することにより、ストライプ状のマイクロ溝を導入したコラーゲン足場材料を作製した。本マイクロパターン化足場材料を用いて筋芽細胞を培養したところ、細胞は配向・融合し、さらにバンドル化した細胞集団からなる筋肉様組織が再生された。また、筋芽細胞と血管内皮細胞を共培養すると、この二種類の細胞はそれぞれ集合し、バンドル化した筋芽細胞の集団に血管様組織が形成された。一連の研究で得られた多孔質足場材料は、独創性に富んだ発想にもとづいて設計・作製されたもので、再生医工学における材料技術に対して重要な学術的貢献を果たすものである。よって、本論文は博士（工学）の学位論文として十分な学術的価値をもつものと認める。

〔最終試験結果〕

平成28年2月12日、数理物質科学研究科学位論文審査委員会において審査委員の全員出席のもと、著者に論文について説明を求め、関連事項につき質疑応答を行った。その結果、審査委員全員によって、合格と判定された。

〔結論〕

上記の論文審査ならびに最終試験の結果に基づき、著者は博士（工学）の学位を受けるに十分な資格を有するものと認める。