学 位 論 文 要 約

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題　　目　　心筋虚血からの組織修復を目指したシルクフィブロイン足場材料の基礎的研究

培養細胞や患者由来の細胞を用いて組織の再構築を目指す再生医療技術が注目を集めており，本技術を基盤として開発された組織修復デバイスの臨床応用も進められている。しかしながら,この組織修復デバイスの作製には専用の細胞培養施設に加え,複雑で高度な技術が必要とされ,このことがデバイス生産の商業化を難しいものにしている。本研究では,この課題の解決を目指し,作製過程が簡素で経済性･汎用性に優れている人工材料からなる心筋修復デバイスについて基礎的検討を行った。

第 1 章では，足場材料であるシクロフィブロイン(SF)の細胞反応性・分解性を評価するために，エレクトロスピンニング法を用いて血管修復シートを作製し，ラット腹部大動脈への移植を実施した。SF 単体のシートの硬くて脆いという欠点を，ポリウレタン(PU)を混合することで改良し，既存の非生体吸収性シート(ePTFE シート)と比較した。組織学的検査の結果から，作製された SF/PU シートは，自己血管に対し修復反応を誘導し，足場として血管構造を再生させた。さらに，ePTFE シートでは石灰化を生じたが，SF/PU シートはシート内への細胞浸潤が観察され，SF による細胞反応性・分解性が示唆された。しかしながら，臨床応用には細胞反応性・分解性の向上が必要であると判断された。

第 2 章では，SF/PU シートの細胞反応性・分解性の向上のために，生理活性物質に着目した。生理活性物質として，血管内皮細胞の接着，遊走性，管腔形成を促進する血管新生ペプチド(SVVYGLR:SV)を基盤材料に混合し SF/PU/SV シートを作製し,ラット腹部大動脈への移植を実施した。組織学的検査の結果から，SV の付与によりマクロファージがシートへ集簇し，細胞反応性がシートへ集中している様子が観察された。また，修復組織内に微小血管が顕著に観察され,これは SV の効果の一つである管腔形成能によるものと考えられた。SF/PU シートに対し，血管新生ペプチドを付与することで，組織修復デバイスとしての有用性が高まると考えられた。しかしながら，SF/PU/SV シートは基盤材料と SV の溶液を混合することで作製されており，SV の足場材料への固定化が実施されていないため，作製段階での SV の漏出や遊離の可能性が指摘された。また,最終目的である心筋修復デバイスとしての臨床応用性を考慮すると，シート形態が不適切であると考えられ，心臓の運動を阻害しないデバイスデザインの検討が必要と判断された。

そこで，第 3 章では血管新生ペプチドを足場材料へ固定化し，デバイスをフィルム状へ加工することで，解決を試みた。SF 水溶液から SF フィルムを作製し，ポリエチレングリコール（PEG）を架橋させた SF-PEG フィルムに，SV および KGHK の 2 種の血管新生ペプチドを架橋させた。*in vitro* 試験では，SF-PEG-SV フィルムが高い細胞接着性および管腔形成能を示した。また，*in vitro* にて良好な結果を示した SF-PEG-SV フィルムを薄膜化し,

心筋梗塞ラットモデルへ応用した。心臓超音波検査にて，薄膜フィルムデバイスによる心機能への悪影響は認められず，また心臓へ設置する際のハンドリングも極めて良好であったことから，本デバイスデザインは臨床応用性の観点から適当であると判断された。

以上より，SF を足場材料として血管新生ペプチドを付与した組織修復デバイスは，優れた修復反応を有する上に，修復した組織中に酸素や栄養を供給する微小血管を誘導することで，組織の再生を促進することが期待された。また，組織工学において重要となるデバイスデザインに関しても，薄膜フィルム形態が臨床応用性の観点から優れていると判断された。これらの結果は，新規心筋梗塞治療デバイス開発の一助となるものと考えられた。

学 位 論 文 要 約

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題　　目　　Basic Study of Silk Fibroin Scaffold Materials for Tissue Repairing from Myocardial Ischemia
(心筋虚血からの組織修復を目指したシルクフィブロイン足場材料の基礎的研究)

Myocardial infarction (MI), which potentially induces ischemia and necrosis resulting from coronary embolism or stenosis, is known as a cause of death in worldwide. Thrombolytic drug, surgical intervention, stent implantation, and coronary artery bypass graft surgery have been currently performed to prevent and manage the ischemic necrosis. Necrotic myocardium, however, is refractory to compensate the reduced function, and the effective application of MI is also being researched.

Regenerative medicines, such as artificial materials and cell engineering, have been developed rapidly. The treatments using regenerative techniques have been reported. Especially, self-pulsating myocardial sheet has been developed and used for MI patients. In general, regenerative medical techniques require the dedicated facilities to handle the cultured cardiomyocytes, autologous cells and complex biological factors as well as it also takes long production time for adaptation. From these reasons, regenerative medical treatments are inconvenient and not suitable for mass production. In the present study, artificial materials were produced based on tissue engineering technique. By using artificial materials, the production process would be simple, stable, and reasonable.

Tissue engineering technique contains three elements, a scaffold to grow the cells, bioactive substances, and cell colonization. These elements are essential for tissue regeneration. This experiment, silk fibroin (SF) having highly cell reactivity and biodegradability was selected and used for the scaffold material. SF can also be chemically modified for the reason that it composes of amino acids with reactive functional group as well as its aqueous solution can be processed into various forms such as films, sponges and gel. The aim of this study is to develop a SF-based tissue engineering device suitable for MI treatment.

In chapter 1, SF based vascular repairing sheet made by electro-spinning method was implanted onto rat abdominal aorta to evaluate the effectiveness of SF as a scaffold material. Because of the difficulty in implantation of pure SF sheet, as it has high hardness and fragility, polyurethane (PU) was mixed with SF aqueous solution to enhance the flexibility. Moreover, SF and PU blended sheet (SF/PU sheet) was produced by electro-spinning method. Commercial non-bioabsorbable sheet (ePTFE sheet) was

implanted as a control group. Histopathological evaluation revealed that SF/PU sheet induced repairing responses to damaged autologous blood vessels, and neovascularization was noticed under the SF/PU sheet. On the other hand, in ePTFE sheet, these reactions were not observed and a calcification also occurred at 3 month after implantation. It was suggested that SF/PU sheet could be potentially replaced with self-organization in the future because large amounts of cells infiltrated into the sheet. However, the cell reactivity and biodegradability of SF/PU sheet were ineffective than expected.

In chapter 2, the bioactive substances in tissue engineering were focused in order to improve the cell reactivity and the biodegradability of SF/PU sheet. In the present study, angiogenic peptide was focused as a bioactive substance because it could be synthesized more easily and stable than other angiogenic-promoting factors. The angiogenic peptide, SVVYGLR (SV), was reported to promote the adhesion and migration of endothelial cells and lumen formation. SF/PU/SV sheet was prepared by electro-spinning method and implanted as same as chapter 1. Macrophage aggregation and continuous cell reactivity were induced in SF/PU/SV sheet. Moreover, small vessels were formed in regenerated tissue surrounding the sheet. These vessels were expected to have an effect of supplying oxygen and nutrition for regenerated tissue. By adding angiogenesis peptide to SF-based scaffold, the usefulness of tissue engineering device was appropriately increased.

In chapter 2, it was suggested that SV might leak from the sheet during the production process, because SF/PU/SV sheet was made only of the mixed solution of SF, PU and SV. Therefore, angiogenic peptides were required to immobilize into SF-based scaffold. In addition, because the sheets in chapter 1 and 2 were not suitable for using as an application of MI heart, the design of more applicable device should be considered. In chapter 3, SF-based films were prepared with two types of angiogenic peptide and evaluated their effectiveness. SF films were produced by spreading SF solution on the dish, and polyethylene glycol (PEG) was added to immobilize SF film by the cross-linking reaction and improved the accessibility of the terminal group. Angiogenic peptides of SV and KGHK were immobilized to these SF-PEG films using cross-linked reaction. As a result of *in vitro* studies, cross-linked SF-PEG-SV films significantly promoted cell adhesion and lumen formation. Applying to MI rat models, SF-PEG-SV films were thin enough to have no negative impact on the cardiac function based on echocardiographic analysis.

In this study, SF was useful for tissue engineering as a scaffold material, and SV and KGHK were also useful to promote the cell reactivity as bioactive substances. These new devices were expected to have excellent tissue repairing ability and induced neovascularization to supply oxygen and nutrition for regenerated tissue. The results obtained in this study can provide a new prospect for MI treatment and device design of tissue engineering in the future.