膠原蛋白基質作爲敷料之開發及酵素交聯法之應用

Development of Collagen- Based Matrix as a Wound dressing and Characterization of an Enzymatic Cross-Linking Method

中文摘要

近幾年來有許多作爲癒傷敷料的膠原蛋白製品研發出來，其中有些產品已經通過臨床試驗並且已經上市，然而目前這些產品仍有一些待改進的空間，例如材質的脆弱、來自交聯劑的潛在毒性以及結合兩種材質的優點之可能性。因此在本研究的第一部分，我們探討自 Streptoverticillium mobaraense 篩選的酵素，微生物麩胺酸轉移脢 (Microbial transglutaminase; MTGase)作爲交聯劑的效用。由實驗結果顯示，MTGase 所催化的交聯作用可提高膠原蛋白溶液的黏度，並且膠原蛋白溶液的黏度會隨著 MTGase 添加量的增加而提高；但是當作用溫度提高到 30℃時，膠原蛋白溶液之黏度反而有顯著下降的趨勢，其可能的原因是膠原蛋白纖維的析出所致。MTGase 在低 pH 值下交聯作用所得的膠原蛋白基質之拉張力較在高 pH 值下的作用爲高，且經過酵素作用的膠原蛋白基質之變性溫度(Td)均有提高的現象。另外，經過 MTT 試驗的結果顯示，以 MTGase 作用的膠原蛋白基質並無細胞毒性的產生；因此，使用 MTGase 催化膠原蛋白產生交聯作用的方法極具相當的潛力。

在本研究的第二部分中，我們嘗試以 N,O-羧甲基幾丁聚醣(N,O- carboxymethyl chitosan; NOCC)結合膠原蛋白作爲癒傷敷料的基質，並添加軟骨素硫酸鹽(Chondroitin Sulfate; CS)及去細胞基質(Acellular Dermal Matrix; ADM)作進一步的探討。由實驗結果發現，纖維原母細胞會隨著 NOCC 濃度的增加產生顯著的遷移(Migration)現象。而在動態機械分析儀的實驗中，以 NOCC/ CS/ COL 爲基質所產生的拉張力較 NOCC/ ADM/ COL 基質爲高。另外，觀察在含有 NOCC 基質上培養人類纖維原母細胞的生長狀況，發現其生長速率有提高的現象。將材質應用於動物實驗中，發現覆蓋含有 NOCC 基質的傷口，其傷口癒合的速度較快。因此，含有 NOCC 的基質是一個相當有潛力的癒傷敷料.

英文摘要

Although many collagen products for wound healing have been developed in the past few years, and some of them have been approved and are now commercially available. However there is still room for improvement, such as mechanical fragility of material, potential cytotoxic effect caused from crosslinking reagent and the possible advantage of combination of two materials. Accordingly, in the first part of the study, we examined the effect of Microbial transglutaminase (MTGases), isolated from a culture medium of Streptoverticillium mobaraense, as a crosslinking reagent on collagenous

matrices. As the results revealed, MTGase exhibited a crosslinking action that raised the viscosity of the collagen solution, and the viscosity rose with an increase in the amount of enzyme present. The final viscosities of the treatments incubated at 30 °C were markedly decreased as a result of isolation of collagen fibers from the solution. Matrices crosslinked with MTGase at the low pH values of pH 3 and 4 exhibited higher tensile strengths than those at high pH values. In comparison with untreated matrices, the denaturation temperatures of the corresponding matrices shifted toward higher temperatures. These enzyme-catalyzed crosslinked matrices were proven by MTT assay to be non-cytotoxic. In the second part, N,O-carboxymethyl chitosan (NOCC) was incorporated into the backbone of a collagen (COL) matrix without or with chondroitin sulfate (CS) or an acellular dermal matrix (ADM). In this paper, the preparation of the NOCC/COL matrices is reported, and their physical properties are characterized. Moreover, the enhancing effect of NOCC/COL matrices on wound healing was examined using an in vivo animal model. The result of a cell migration study demonstrated that the migration of fibroblasts was significantly enhanced by NOCC in a concentration-dependent manner. In the analysis of dynamic mechanical analyzer, NOCC/CS/COL matrices presented higher tensile strengths than did NOCC/ADM/COL matrices. Skin fibroblasts cultured on the matrices containing NOCC showed increased proliferation and secretion of 3 kinds of cytokines compared with the control. Results of the in vivo wound healing study showed that matrices incorporating NOCC showed markedly enhanced wound healing compared with the control.

In conclusion, the enzymatic method of using MTGase provides an alternative potential way for crosslinking collagen-based biomaterials. Furtheremore, NOCC/COL matrices containing CS or ADM can be potential wound dressings for clinical applications.