The Development and Collagen Ingredients Analysis of Submandibular Gland Acellular Matrix

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Abstract: To develop submandibular gland acellular matrix and observe its structure and analyze its histological ingredients. METHODS: The fresh submandibular glands of SD mice were cell-extracted by chemical detergent and histological ingredients were analyzed by immunohistochemistry and ultra structure observed. RESULTS: Submandibular gland cells disappeared, matrix ingredients appeared network structure under microscopy and The collagen fibers showed different diameter and directions and formed complicated network structure and SEM. I, II and III type collagen proteins of submandibular gland before and after cell-extracted were positive under immunohistochemistry. CONCLUSIONS: The main ingredients of submandibular gland acellular matrix were I, II and III type collagen proteins.

Key words: Submandibular gland, Acellular, Immune histochemistry, Collagen proteins.

Introduction

Tissue engineering is an emerging multidisciplinary filed involving biology, medicine and engineering that is studying on restoring, maintaining, or enhancing tissue and organ function [1]. Tissue engineering research includes the following four areas: seeding cells, scaffold materials, construction of organs, and clinical application in which seeding cells and scaffold materials are more important. Along with the tissue engineering research development, the technology of isolating and culturing submandibular glands seeding cells is well development, however, no great breakthrough has been made on the research of the scaffold materials till now. Materials achieved by biotissue processed are called bioderived scaffold materials. Bioderived scaffold materials derived from organs are fairly ideal tissue engineering scaffold materials because of the normal network structure and good histocampatibility.

The purpose of this study was to get natural bioderived materials by submandibular glands after acellular process. The histological ingredients after acellular process were analyzed by histological and histochemistry study. Thus the suitable scaffold material for submandibular glands tissue engineering may be found.

Materials and Methods

2.1 Experimental animals

Under the approval of the Animal Ethics Committee of China medical university health science center, twenty SD mice aged 8 weeks were used in the present study.

2.2 Reagents

TritonX-100, proteinase inhibitor (PMSF), DNase, RNase and the goat polyclonal antibodies raised against mouse type I~V collagen were all purchased from Sigma company, USA.

2.3 Preparation of submandibular glands acellular matrix (SGAM)

The submandibular glands of SD mice were extracted after all the animals were anesthetized. Then the glands were mounted to 5mm×5mm×2mm pieces, which were cleaned by PBS and loaded into 5 mmol/L Tris-HCl buffer with 0.25g proteinase inhibitor and 2×10^5 units gentamicin, and maintained 4 days at 4ºC. The samples were cell-extracted by Tris-HCl buffer contained 3% TritonX-100 (V/V), proteinase inhibitor, and gentamicin, maintained 7 days at 4ºC, and cleaned by distilled water continuously 24 hours. Then the samples were disgested with DNase and RNase 12 hours, and cell-extracted again with Tris-HCl buffer 24 hours, thus the SGAM were obtained.

2.4 Examination of submandibular glands acellular matrix

2.4.1 Histological observation

The SGAM and fresh submandibular glandswere fixed in 10% formaldehyde and then embedded in paraffin. All the hematoxylin eosin stained specimens were observed by microscope.

2.4.2 Immune histochemical test

The specimens were hydrated by PBS after fixed in 10% formaldehyde, all the immunohistochemical stained specimens by means of ABC with antibody of I, II, III, IV and V type collagen were observed by microscope.

2.4.3 Scanning electron microscopic observation

The SGAM and normal submandibular glands were fixed in 2.5%glutaraladehyde£¬rinsed by 0.1M PBS, fixed in 1% osmium acid£¬dehydrated by alcohol and embedded in Epon812, The ultra-thin sections were cut and dried at critical point, sprayed in vacuum, then were observed by SEM (JSM-T300,Japan).

Results

3.1 Histomorphological observation

After the submandibular glands were cell-extracted, there were no cellular ingredients in the SGAM, the pink collagen aligned loosely, with big space and network structure.

3.2 immunohistochemical test

In normal submandibular gland tissue, type I, II and III collagens expressed positively with deeply brown color (As shown in Fig. 1), and type IV and V collagens expressed negatively or rarely positively. In SGAM tissue, the cellular ingredients of submandibular glands were completely deleted and the network frame contained type I, II and III collagens were maintained and expressed positively (As shown in Fig. 2-4).

3.3 Scanning electron microscopic observation

SGAM after cell-extracted by 3% TritonX-100, cells disappeared absolutely, only empty fossa remained. The collagen fibers showed different diameter and directions and formed complicated network structure (Fig.5).

Discussion

Along with the development of tissue engineering study, the emphasis of the scaffold materials of tissue engineering was

transferred to founding the materials like natural structure and function[2]. Bioderived materials were called natural bioderived materials which were maintained all or some network structure without antigen by cell-extracted. There was no report about the study of SGAM in civil and overseas. This study obtained SGAM from submandibular glands cell-extracted by chemical detergent and amylase digestion. The SGAM showed network structure observed by microscope. The network structure were type I, II, III collagen mainly by immunohistochemical staining.

Collagens exist as the ingredients of supplying structure collagen proteins are the richest protein in animals. AS the scaffold materials of tissue engineering, collagens not only provide supply and protection for cells but also related tightly with the adhesion, proliferation, differentiation and metabolism. Collagens as natural biomaterials have some other virtues, such as: excellent biocompatibility, digestible and absorbable, low antigen, accelerating tissue renewing and no excluding reaction, etc.

The main ingredients of the SGAM obtained from this study were type I, II and III collagen whose structure and ingredients were completely accorded with the request of tissue engineering scaffold materials. So, the SGAM can be used as a new scaffold material in submandibular glands tissue engineering.

References

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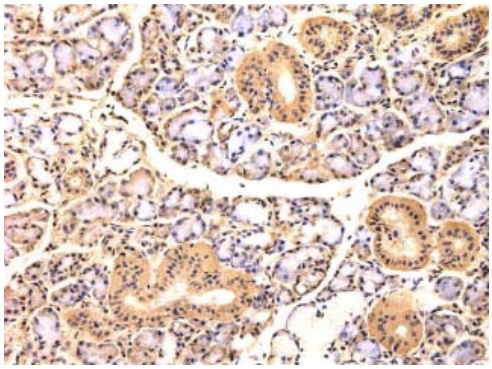

Figure 1. II type collagen proteins of normal submandibular gland were strongly positive (immunohistochemistry, ×40)

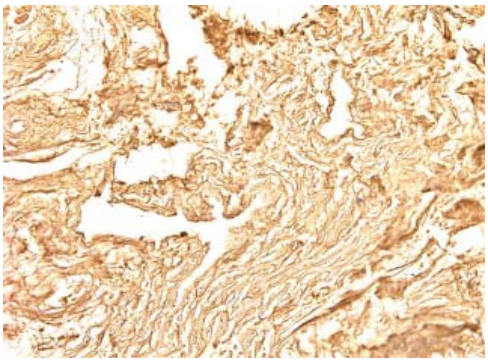

Figure 2. I type collagen proteins after cell-extracted were strongly positive (immunohistochemistry, ×40)

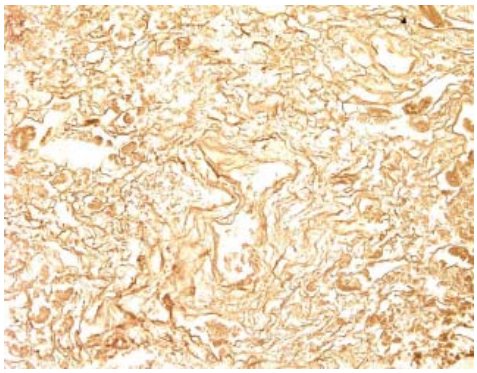

Figure 3. I type collagen proteins after cell-extracted were strongly positive (immunohistochemistry, ×40)

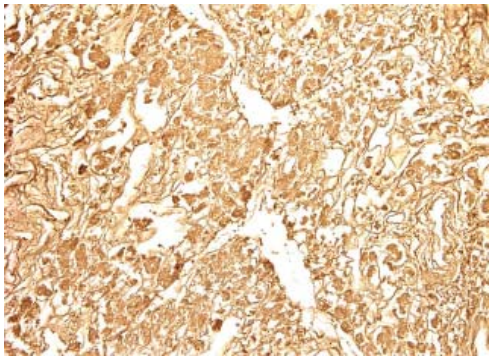

Figure 4. III type collagen proteins after cell-extracted were strongly positive (immunohistochemistry, ×40)

Figure 5. SEM photo of SGAM after cell-extracted (×2000).