Preparation of Salmon Atelocollagen Fibrillar Gel and Its Application to the Cell Culture

Nobuhiro Nagai[1], Shunji Yunoki[2], Takeshi Suzuki[3], Yasuharu Satoh[1], Kenji Tajima[1], and Masanobu Munekata[1]

[1]Division of Molecular Chemistry, Graduate School of Engineering, Hokkaido University, N13-W8, Kita-ku, Sapporo, Hokkaido 060-8628, Japan [2]National Institute for Materials Science Namiki 1-1 Tsukuba, 305-0044 Japan [3]Ihara & Company Ltd., 3-263-23 Zenibako, Otaru, Hokkaido 047-0261, Japan

Abstract: Salmon atelocollagen (SAC) has not been used as biomaterials due to its low denaturation temperature (19 degrees C). In the present study, we succeeded in preparation of SAC fibrillar gel stable at an actual physical temperature of human by cross-linking during fibril formation, and in cultivating human periodontal ligament cells on the cross-linked SAC fibrillar gel.

Key words: collagen, salmon, fibrillar gel, denaturation temperature, cell proliferation

1. INTRODUCTION

Generally, collagens for biomaterials are prepared from mammalian sources, such as bovine and porcine skins. However, the use of mammalian sources has to be reconsidered and limited because of the risks of pathogens such as bovine spongiform encephalopathy (BSE). Although fish collagen is thought to be safe (1), it has not been widely used for biomaterials due to its low denaturation temperature.

Large quantities of fish skin are discarded as waste in the food industry. Collagens are easily extracted from wasted fish skins with high yield. The use of fish collagens, therefore, could contribute to the recycling of natural unutilized resources. Fish collagens have the potential to be used as a novel source for collagen biomaterials if the thermal stability could be improved.

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Recently, we have reported the fabrication of collagen sponges from salmon atelocollagen (SAC) using UV irradiation, dehydrothermal treatment (2), and chemical cross-linking (3). It was found that the thermal stability of SAC sponges was comparable to that of bovine atelocollagen sponges. Next, we tried to prepare the SAC fibrillar gel stable at an actual physical temperature of human.

Collagen molecules self-assemble into cross-striated fibrils in tissues. The aggregation and alignment of molecules improves the thermal stability and mechanical strength of collagen matrix. The collagen fibrils, therefore, provide the major biomechanical scaffold for cell attachment, allowing the shape and form of tissues to be maintained. We hypothesized that the introduction of cross-linking among collagen fibrils during fibril formation would result in a further increase in the thermal stability of SAC fibrillar gel.

As a cross-linking reagent for SAC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), a water-soluble-carbodiimide, was used. EDC has become popular as a cross-linking reagent for collagens due to ease of handling and potentially low cytotoxicity (4). Here, we show the increase in the thermal stability of SAC fibrillar gel and its application to the cell culture.

2. EXPERIMENTAL

SAC was prepared from fresh skin of chum salmon (*Oncorhynchus keta*) (2) and the purified SAC was found to be mainly composed of type I collagen. The introduction of EDC cross-linking during fibril formation was performed by mixture of acidic SAC solution and EDC solution in pH 6.8 at 4 degrees C (5). Porcine atelocollagen (PAC) was purchased from Nitta Gelatin (Cellmatrix Type I-P, Japan) and the PAC fibrillar gel was prepared (5). The thermal stability, the mechanical strength, and the fibril structure of the gels were evaluated (5). The cell proliferation on the collagen fibrillar gels was measured *in vitro* using human periodontal ligament cells (HPDL cells). The cell number was directly counted by hemocytometer after digestion of gels with collagenase and trypsin. Alkaline phosphatase (ALP) activity, a differentiated cell function of HPDL cells, was measured (3).

3. RESULTS AND DISCUSSION

The introduction of EDC cross-linking during fibril formation resulted in further increase of thermal stability of SAC fibrillar gel, and the denaturation temperature was found to be 55 degrees C at an EDC concentration of 60 mM. At an EDC concentration of more than 60 mM, the fibril formation was

inhibited, and the denaturation temperature decreased. These results indicate that the maximum synergistic effect of the EDC cross-linking and the aggregation of collagen molecules is obtained at 60 mM. We have reported that the maximum denaturation temperature of SAC fibrillar gel was 47 degrees C at an EDC concentration of 50 mM (5). In the present study, we investigated the condition of EDC concentration in more detail, and it was found that the denaturation temperature reached maximum values at 60 mM.

The mechanical strength of SAC fibrillar gel was five times higher than that of PAC fibrillar gel. Scanning electron microscopy observation showed that SAC fibrillar gel had thin fibrils and well-developed fibril network compared with PAC fibrillar gel. The fibril formation rate of SAC was found to be faster than that of PAC. It was considered that the higher the fibril formation rate the thinner the fibril size. The well-developed fibril network and the cross-links could contribute to the mechanical strength of SAC fibrillar gel.

The proliferation rates of HPDL cells cultured on SAC fibrillar gel were faster than that cultured on PAC fibrillar gel. The mechanism of high cell proliferation rate was unclear. The high mechanical strength and well-developed fibril network could influence the cell proliferation. On the other hand, ALP activities of HPDL cells cultured on PAC fibrillar gel were higher than those on SAC fibrillar gel. The differences of cell activities between SAC and PAC fibrillar gel are under consideration.

SAC fibrillar gel could have the potential being used for biomaterials such as a scaffold for tissue engineering. We have reported the application of SAC fibrillar gel to cell-sheet preparation using collagenase digestion (6). Cell-sheet engineering is a novel method to develop the artificial tissues by layering cell-sheets. We are studying to prepare the layering cell-sheets using SAC fibrillar gel.

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