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Feasibility of chitosan-based hyaluronic acid hybrid biomaterial for a novel scaffold in cartilage tissue engineering ☆

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Abstract

In this study, we hypothesized that hyaluronic acid could provide superior biological effects on the chondrocytes in a three-dimensional culture system. To test this hypothesis, we investigated the in vitro behavior of rabbit chondrocytes on a novel chitosan-based hyaluronic acid hybrid polymer fiber. The goal of the current study was to show the superiority of this novel fiber as a scaffold biomaterial for cartilage tissue engineering. Chitosan polymer fibers (chitosan group) and chitosan-based hyaluronic acid hybrid polymer fibers (HA 0.04% and HA 0.07% groups, chitosan coated with hyaluronic acid 0.04% and 0.07%, respectively) were originally developed by the wetspinning method. Articular chondrocytes were isolated from Japanese white rabbits and cultured in the sheets consisting of each polymer fiber. The effects of each polymer fiber on cell adhesivity, proliferation, morphological changes, and synthesis of the extracellular matrix were analyzed by quantitative a cell attachment test, DNA quantification, light and scanning electron microscopy, semi-quantitative RT-PCR, and immunohistochemical analysis. Cell adhesivity, proliferation and the synthesis of aggrecan were significantly higher in the hybrid fiber (HA 0.04% and 0.07%) groups than in the chitosan group. On the cultured hybrid polymer materials, scanning electron microscopic observation showed that chondrocytes proliferated while maintaining their morphological phenotype and with a rich extracellular matrix synthesis around the cells. Immunohistochemical staining with an anti-type II collagen antibody demonstrated rich production of the type II collagen in the pericellular matrix from the chondrocytes. The chitosan-based hyaluronic acid hybrid polymer fibers show great potential as a desirable biomaterial for cartilaginous tissue scaffolds.

Keywords: Chitin/chitosan; Chondrocyte; Hyaluronic acid/hyaluronan; Cell culture; Cell adhesion

1. Introduction

In Living organisms, the authentic substrate for most cells is the extracellular matrix (ECM). The ECM adheres to cells via integrins, which are membrane-spanning heterodimeric receptors [1]. Through the cell–matrix adhesions, the ECM transduces physiological signals regulating cell growth, cell proliferation, cell differentiation, and matrix remodeling to the cells [1]. Therefore, the ECM plays an important role in living tissue development and regeneration.

In a tissue engineering technique, tissue regeneration is achieved by culturing isolated cells on biocompatible and biodegradable materials as scafollds onto which cells are seeded. A large number of studies have shown the importance of selecting the appropriate biomaterials as scaffolds for the cell adhesion and supporting the proliferation [2–10]. For the reason given above, the ideal scaffold material should be one which closely mimics the natural environment in the tissue-specific ECM [9].

Once damaged, the articular cartilage consisting of hyaline cartilage tissue has little capacity for spontaneous

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healing. Although the limited potential for self-repair of the articular cartilage necessitates operations to treat injured cartilage, no current procedures for cartilage repair have successfully regenerated long-lasting hyaline cartilage tissue to replace a cartilaginous lesion [11,12]. To solve this limitation, tissue engineering techniques by culturing isolated chondrocytes on a variety of scaffold materials, including naturally occurring and synthetic, have been developed [4–10]. However, there have been no ideal materials for cartilage tissue engineering.

One of the considerable characteristics in the cartilage tissue is that a small number of chondrocytes are embedded in the rich ECM. Therefore, cell–matrix interactions play a crucial role in the development and regeneration of the cartilage tissue. To successfully achieve cartilage tissue regeneration, a cell-carrier substance which closely mimics the natural environment in the cartilage-specific ECM must be developed. In the current study, hyaluronic acid, which is a main component of the proteoglycans (PGAs) in the cartilage, was applied to chitosan as a fundamental biomaterial.

Recently, several studies have demonstrated that cellular functions differ in two-dimensional and three-dimensional (3D) systems [13,14]. In cartilage tissue engineering, a closer approximation to in vivo environments should be attained by culturing cells in 3D materials. Additionally, the articular cartilage must be considered for its mobility as an excessively stressed tissue. To structurally mimic the environments of the cartilage tissue, the fundamental structure of a scaffold should be a 3D system with adequate mechanical strength. In the current study, the authors have structurally developed a novel polymer fiber—chitosan-based hyaluronic acid hybrid fiber—as a biomaterial to easily fabricate 3D scaffolds.

In this study, we hypothesized that hyaluronic acid could provide superior biological effects on the chondrocytes in a 3D culture system. To test this hypothesis, we investigated the in vitro behavior of rabbit chondrocytes on a novel chitosan-based hyaluronic acid hybrid polymer fiber. The objectives of the current study were to evaluate the chondrocyte adhesion, proliferation, and the synthesis of the ECMs in the chitosan-based hyaluronic acid hybrid polymer fiber and to show the superiority of this novel fiber as a scaffold biomaterial for cartilage tissue engineering.

2. Materials and methods

2.1. Polymer fibers

Polymer fibers were developed by the wet spinning method as described by Tamura et al. [15] with the following modification. Fig. 1 shows the process of

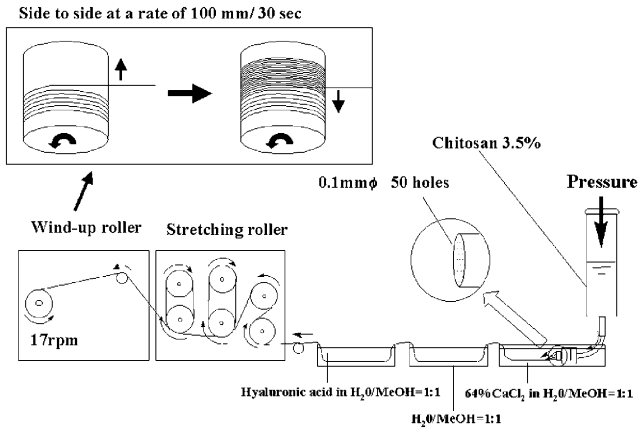

Fig. 1. The original roller system.

developing the fibers using an original apparatus [15]. Chitosan is a commercial material purchased from Kimitu Chemical Co. Inc., (Tokyo, Japan). Hyaluronic acid produced by lactic acid bacteria, with a viscosity average molecular weight of 2,400,000, was gifted from DENKI KAGAKU KOUGYO Co. Ltd. (Tokyo, Japan). The degree of deacetylation of the chitosan was 81%, and viscosity average molecular weight was 600,000. To prepare the polymer fiber 7 g of chitosan powder was dissolved in 200 ml of 2% aqueous acetic acid solution to give 3.5% of polymer concentration. Dope of chitosan was spun into a calcium coagulant bath (64% $CaCl_2$ dissolved in 50% aqueous methanol solution) through a stainless steel spinnlet (0.1 mm diameter, 50 holes) at a winding speed of 4.4 m/min at room temperature. Then, 50% aqueous methanol solution was used as a second coagulation bath and 0.04 or 0.07% hyaluronic acid dissolved in 50% aqueous methanol solution was a third coagulation bath. Using an original roller system (Okada Co. Inc., Sapporo, Japan), the resulting fibers were stretched and treated with 0.8% sodium hydroxide (NaOH) dissolved in 90% aqueous methanol solution to neutralize the acidity of the fibers. The fibers wound in the roller were washed with methanol and dried at room temperature. The diameter of each fiber was 0.03 mm.In the current study, chitosan polymer fiber (chitosan group) and chitosan-based hyaluronic acid hybrid polymer fiber (chitosan coated with hyaluronic acid 0.04%, HA0.04% group; chitosan coated hyaluronic acid 0.07%, HA0.07% group) were originally developed. For further investigations of the chondrocyte culture system, we automatically made a fiber sheet using the original apparatus (Fig. 1). Coagulated fibers were passed through a cross feeding guide and wound onto a stainless roller (120 mm diameter and 120 mm wide) at the rate of 17 rpm. The cross feeding guide set forward in the roller was moved from side to side at a rate of 100 mm/30 s. The cross-feed length and rotation count were 100 mm and 40 times,