Preparation of Funnel-Like Collagen Porous Scaffolds and Application for Cartilage Tissue Engineering

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Introduction: Porous scaffolds have been widely used in tissue engineering to provide a three-dimensional structure for cell adhesion, proliferation, differentiation, and secretion of extracellular matrices to guide new tissue formation and regeneration. Many methods such as particle leaching, freeze-drying, phase separation, gas foaming, electrospinning, fiber bonding, and rapid prototyping have been developed to control the porous structures. However, scaffolds prepared by these methods have various problems concerning the opening structure of surface pores and interconnectivity of inner bulk pores. In this study, by using embossing ice particulates as a template, a novel method was developed to fabricate a new type of funnel-like collagen sponges that have large open surface pores and highly interconnected inner bulk pores. The funnel-like collagen sponges were used for three-dimensional of bovine chondrocytes for cartilage tissue engineering.

Methods: Micron-sized water droplets were formed by spraying pure water onto a Teflon film wrapped on a copper plate. The size of water droplets was controlled by spraying times. Embossing ice particulates of different dimensions were prepared after freezing the water droplets. The ice particulate templates were placed at a designated temperature for 1 hour to allow the templates to reach the designated temperature at which the collagen aqueous solution was applied and frozen. Four temperatures (-1, -3, -5, and -10°C) were chosen to investigate their effect on the porous structure of the collagen sponges. Aqueous solution of porcine type I collagen (1.0 wt %) was eluted onto the embossing ice particulates and kept at the designated temperatures for 1 hour to gradually freeze. The frozen constructs were freeze-dried. After cross-linking with glutaraldehyde vapor and washing with water, the new type of collagen sponges was prepared. The microstructure of the collagen sponges was observed by a scanning electron microscope. For cell culture use, the scaffolds were punched into discs with a diameter of 12 mm and sterilized with 70% ethanol. Bovine chondrocytes were isolated from articular cartilage derived from the knees of an 8-week-old male calf. P1 chondrocytes were seeded into the open surface layers of the funnel-like collagen sponges (6.0×10^7 cells/mL, 100 µL/sponge) and cultured in DMEM serum medium with shaking at 60 rpm in a 5% CO_2 atmosphere at 37°C. The medium was changed twice per week. After *in vitro* culture for 1 wk, the cell/scaffold constructs were subcutaneously implanted into the dorsa of athymic nude mice. After implantation for 3 wk, the mice were scarified and the implants were harvested for DNA contents, sGAG amount, gene expression and histological analyses.

Results: The new type of collagen sponges prepared with the ice particulate template was referred as to funnel-like collagen sponges because their unique porous structure was somewhat like that of a Büchner funnel. The funnel-like collagen sponge had a hierarchical structure of large open pores on the top surface and interconnected small pores in the inner bulk body. The shape, size, and density of the surface large pores were determined by the ice particulates that were used as templates while the interconnected small pores were determined by the freezing temperature. Six funnel-like collagen sponges prepared with ice particulates of three different sizes (180 µm, 400 µm, and 720 µm) at a freezing temperature of -3ºC, and with 400 µm ice particulates at four different freezing temperatures (-1ºC, -3ºC, -5ºC, and -10ºC) were used for chondrocytes culture. The funnel-like porous structure of the new collagen sponges facilitated cell seeding, cell penetration, and distribution throughout the scaffold. Scaffolds that were prepared with 400 µm ice particulate templates and a freezing temperature of -3ºC resulted in the best cell distribution, ECM production, and chondrogenesis. Although the funnel-like collagen sponges prepared with 400 µm ice particulate template and a freezing temperature of -1ºC and 720 µm ice particulate and a freezing temperature of -3ºC, showed even cell distribution, the cell seeding efficiencies and sGAG amount per cell were low. However, the scaffolds prepared with 400 µm ice particulate templates and a freezing temperature of -5ºC or -10ºC showed a limited effect on the improvement of cell distribution and chondrogenesis. Control collagen sponges without ice particulates failed to support the formation of homogenous cartilage-like tissue. These results indicate that funnel-like collagen sponges were superior to control collagen sponges and that scaffolds prepared with 400 µm ice particulate templates at -3ºC were optimal for cartilage tissue engineering.

Conclusions: Funnel-like collagen sponges were fabricated with embossing ice particulate templates and used for culturing bovine chondrocytes for cartilage tissue engineering. By controlling the size of the ice particulates and the temperature of freezing, different pore structures of funnel-like collagen sponges were prepared. The funnel-like collagen sponges prepared with a 400 µm ice particulate template and a freezing temperature of -3ºC improved three-dimensional cell distribution, and promoted ECM production and cartilage-like tissue regeneration. The funnel-like collagen sponges will be useful for cartilage tissue engineering.

References: Ko Y. Kawazoe N. Tateishi T. Chen G. J Bioact Compat Polym. 2010; 25:360-373.
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