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Preparation of collagen porous scaffolds with a gradient pore size structure using ice particulates

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ABSTRACT

Direct comparison using scaffolds with a gradient pore size structure is important to investigate the effect of pore size on tissue regeneration. Collagen porous scaffolds with a pore size gradient were prepared by using pre-prepared ice particulates as a porogen material. The ice particulates had diameters of 150–250, 250–355, 355–425 and 425–500 μm. The gradient collagen scaffolds had well-interconnected pore structures with compactly packed spherical pores. The gradient pore scaffolds were used for culture of bovine articular chondrocytes, directly examining the effect of pore size on cartilage regeneration. Chondrocytes adhered and showed a homogenous distribution throughout the scaffolds. *In vivo* implantation results indicated that the micropores in the scaffolds prepared with ice particulates in the range of 150–250 μm showed the best effect on cartilage regeneration.

1. Introduction

Porous scaffolds have been widely used in tissue engineering to control cell functions and to guide new tissue formation [1–3]. Scaffolds serve as a temporary template to provide biological and physical cues to support cell adhesion, to promote proliferation and to induce differentiation of stem cells or progenitor cells into specific lineage [4]. The pore structure of scaffolds is an important factor affecting tissue regeneration efficiency. Although a number of porous scaffolds have been developed from various types of biomaterials, controlling the pore structure remains a key factor to create ideal porous scaffolds for tissue engineering [5–8]. Many reports show the effects of the pore size of a porous scaffold on tissue regeneration [9,10]. Some of the reported results do not agree with each other. Thus far, studies on the effects of pore size on tissue regeneration have primarily compared individual scaffolds with different pore sizes after they are separately cultured. Variations in the separate cultures may cause some unpredicted factor to influence the results. The use of a gradient pore structure may provide a useful and practical tool to compare the effect of different pore sizes under the same culture conditions. In this study, collagen porous scaffolds with a gradient pore size structure were prepared by using pre-prepared ice particulates as a porogen material. The scaffolds were used to culture articular chondrocytes, comparing the effect of the pore size on cartilaginous matrix production and cartilage regeneration.

2. Materials and methods

2.1. Scaffold preparation and characterization

The scaffolds were made by using pre-prepared ice particulates as a porogen material. At first, an aqueous collagen solution (2% (w/v)) of porcine type I collagen (Nitta Gelatin, Osaka, Japan) in a mixture of ethanol and acetic acid (10:90 v/v, pH 3.0) was prepared. The ice particulates were prepared by spraying Milli Q water into liquid nitrogen using a sprayer. The ice particulates were sieved to obtain ice particulates having diameters of 150–250, 250–355, 355–425 and 425–500 μm. The spherical shape and size of ice particulates were confirmed by observation under a phase-contrast microscope. Then, the aqueous collagen solution was mixed with the ice particulates in a 50:50 (v/w) ratio at a −4 °C low-temperature chamber. Each of the four mixtures of collagen solution and ice particulates of different diameters was poured into a silicone frame. The four mixtures in their frames were stacked together with ice particulate sizes increasing from bottom to top (Fig. 1a). Finally, the entire set was frozen at −80 °C for 6 h and freeze-dried for 3 days in a Wizard 2.0 freeze dryer (VirTis, Gardiner, NY). The freeze-dried constructs were cross-linked for 4 h with glutaraldehyde vapor that was saturated with

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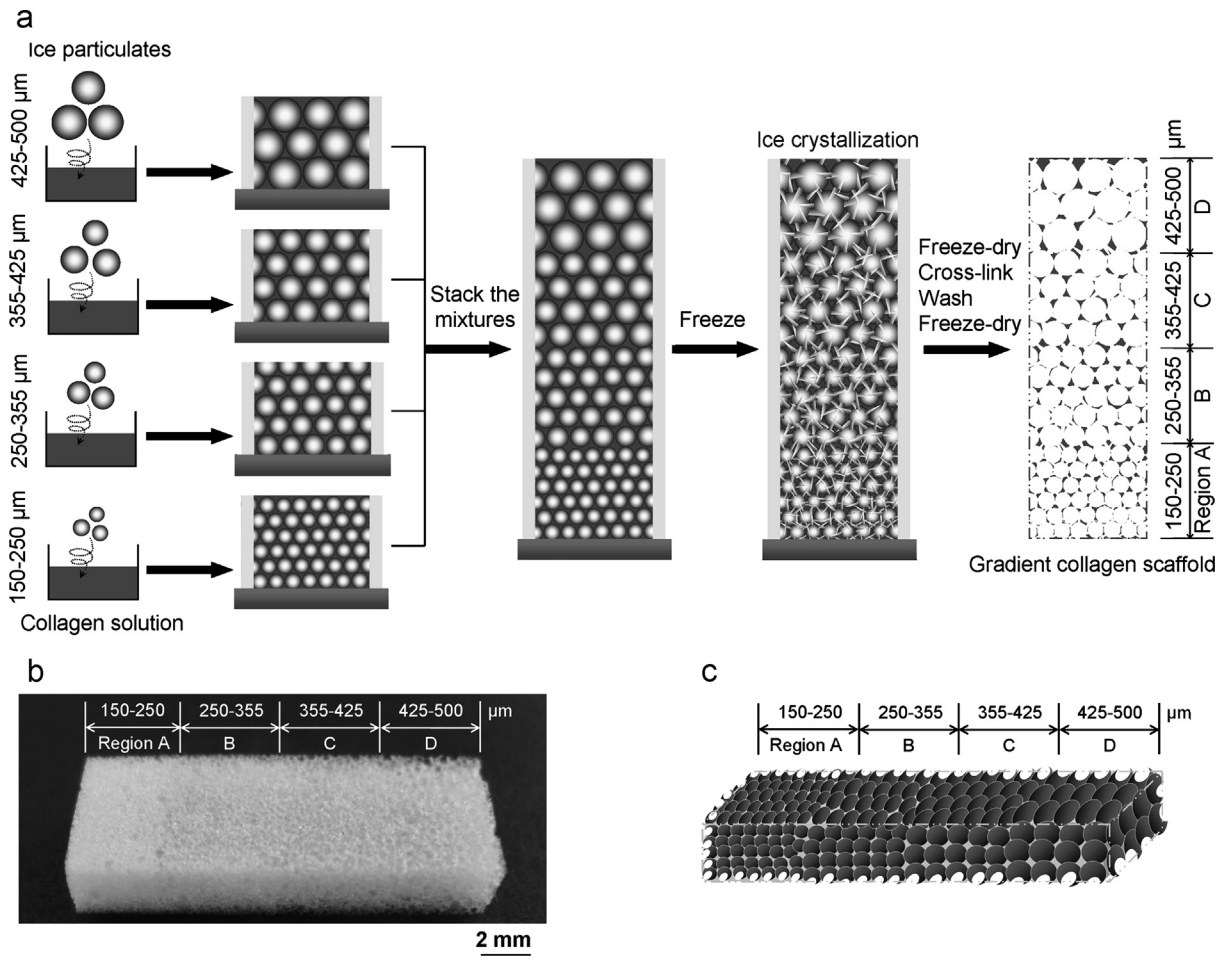

Fig. 1. The preparation scheme for gradient collagen scaffolds, using ice particulates as a porogen material (a). A photograph (b) and an illustration of the pore structure (c) of the gradient collagen scaffolds.

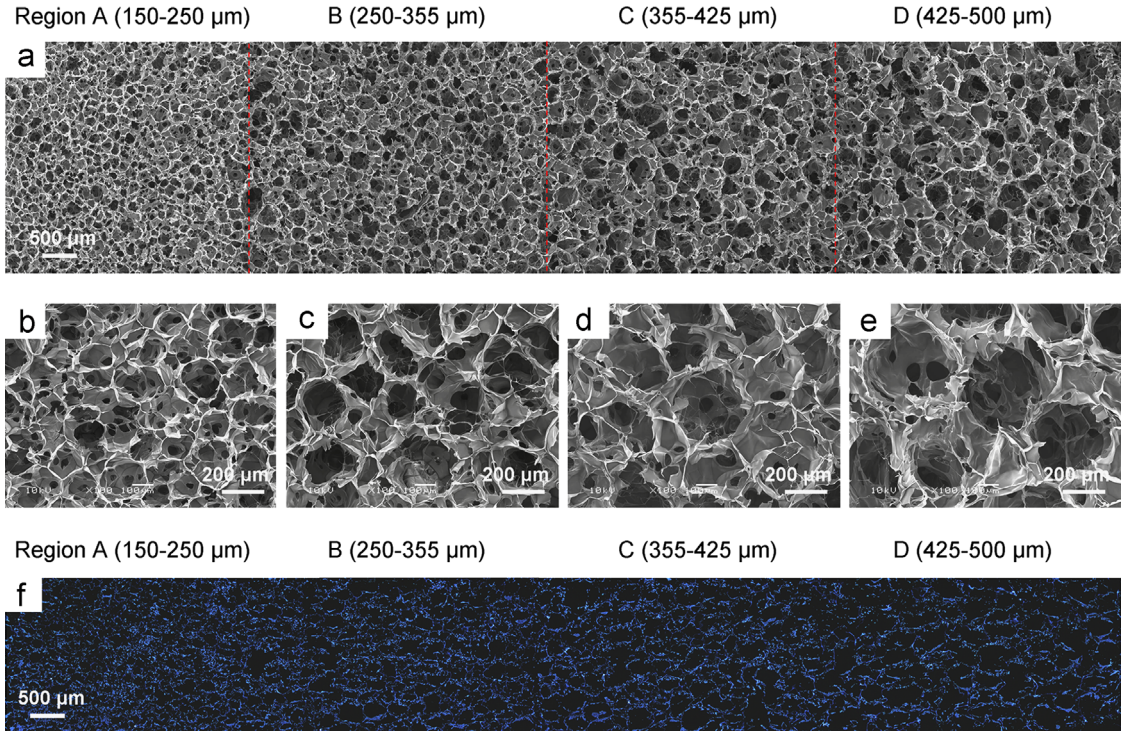

Fig. 2. SEM photomicrographs of the entire scaffold cross-section (a) and highly magnified photomicrographs of the four different regions, prepared with ice particulates having a diameter range of 150–250 (b), 250–355 (c), 355–425 (d) and 425–500 μm (e). Cell distribution in a gradient collagen scaffold (f).