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Developing highly porous collagen scaffolds by using alginate microsphere porogens for stem cell cultures

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ABSTRACT

A highly porous scaffold with tunable pore sizes is desirable and beneficial to facilitate the migration, spreading, and proliferation of cells in tissue engineering and regenerative medicine applications. In this study, porous collagen scaffolds with various pore sizes were prepared using alginate microspheres as porogen materials. The collagen scaffolds had high water content (up to 98%), various porosities and degradation behaviors, and well connected pore structures. A maximum pore size of 700 μm was obtained; in contrary, the intact porous structures of the scaffolds could not be maintained using Al_2O_3 beads or polystyrene bead porogens. The stress of the collagen scaffolds ranged from 0.29 to 0.43 kPa. *In vitro* studies indicated that seeded rat mesenchymal stem cells (rMSCs) were able to attach, spread, and proliferate on the scaffold surfaces. Furthermore, the human adipose-derived stem cells (hADSCs) proliferated preferentially across the largest pore areas after 14 days of culturing.

1. Introduction

Tissue engineering holds great promise as an alternative strategy for repairing and regrowing damaged tissues or organs. Most tissue engineering strategies rely on three-dimensional porous scaffolds to mimic the natural extracellular matrix as templates on which cells can attach, multiply, migrate, and function. The material, pore size, and pore geometry of the scaffolds play vital roles in directing cell adhesion, proliferation, and differentiation as well as tissue formation and function [1]. High scaffold porosity is often necessary to allow cell distribution and interconnection throughout the engineered tissues. Many researchers have reported optimum pore size ranges and scaffold materials used for different kinds of cells growth or tissues regeneration, for example, vascular smooth muscle cells bind preferentially to poly-L-lactic acid scaffolds with pore sizes ranging from 60 to 150 μm. Collagen scaffolds pore sizes with 40–150 μm is the preferential pore size for fibroblasts, 150–250 μm for cartilage regeneration and 380–405 μm pore size showed better cell growth for chondrocytes an osteoblasts [2,3]. In vascularized tissues, cells located within an estimated 150–200 μm of the nearest capillary to survive and function optimally; furthermore, fibrovascular tissues require pore sizes greater than 500 μm for rapid vascularization and survival of the transplanted cells [4].

Several methods have been developed to create highly porous scaffolds for tissue engineering, including lyophilization, gas forming, and porogen leaching [5]. Lyophilization involves rapid cooling to produce thermodynamic instability in a 3D scaffold, resulting in phase separation and pore formation. Among the methods, porogen leaching is more straightforward, providing easy manipulation and control of pore size and porosity. Chen et al. reported that porous collagen scaffolds with various pore sizes and strong interconnectivity can be prepared using ice particulates as porogen materials [6]. However, this procedure must be limited to a relatively low temperature and short manipulation period to prevent the preprepared ice particulate porogens from dissolving. In this study, porous collagen scaffolds with adjustable pore structures were prepared using alginate microsphere porogens through freeze-drying. The effects of porogens on the preparation process, scaffold pore structures, and culture of stem cells were investigated.

2. Materials and methods

2.1. Fabrication of alginate microspheres porogen and porous collagen scaffold

Alginate microspheres were produced according to our previous study [7]. 1 mL of collagen solution (7 mg/mL) was mixed with the porogens (Al_2O_3 beads, polystyrene beads, or alginate microspheres), and the mixtures were placed in a freeze dryer (Labconco, USA) at −20 °C for 8 h and freeze-dried for 1 day. The freeze-dried

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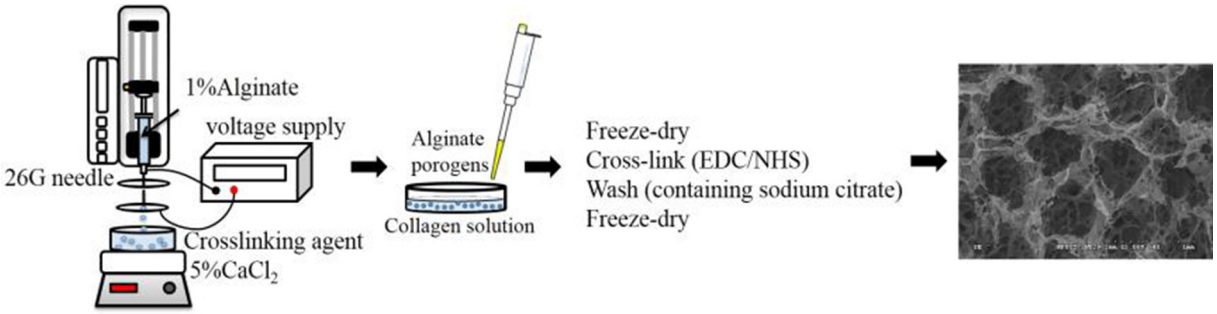

Fig. 1. Schematic representation of collagen scaffold preparations.

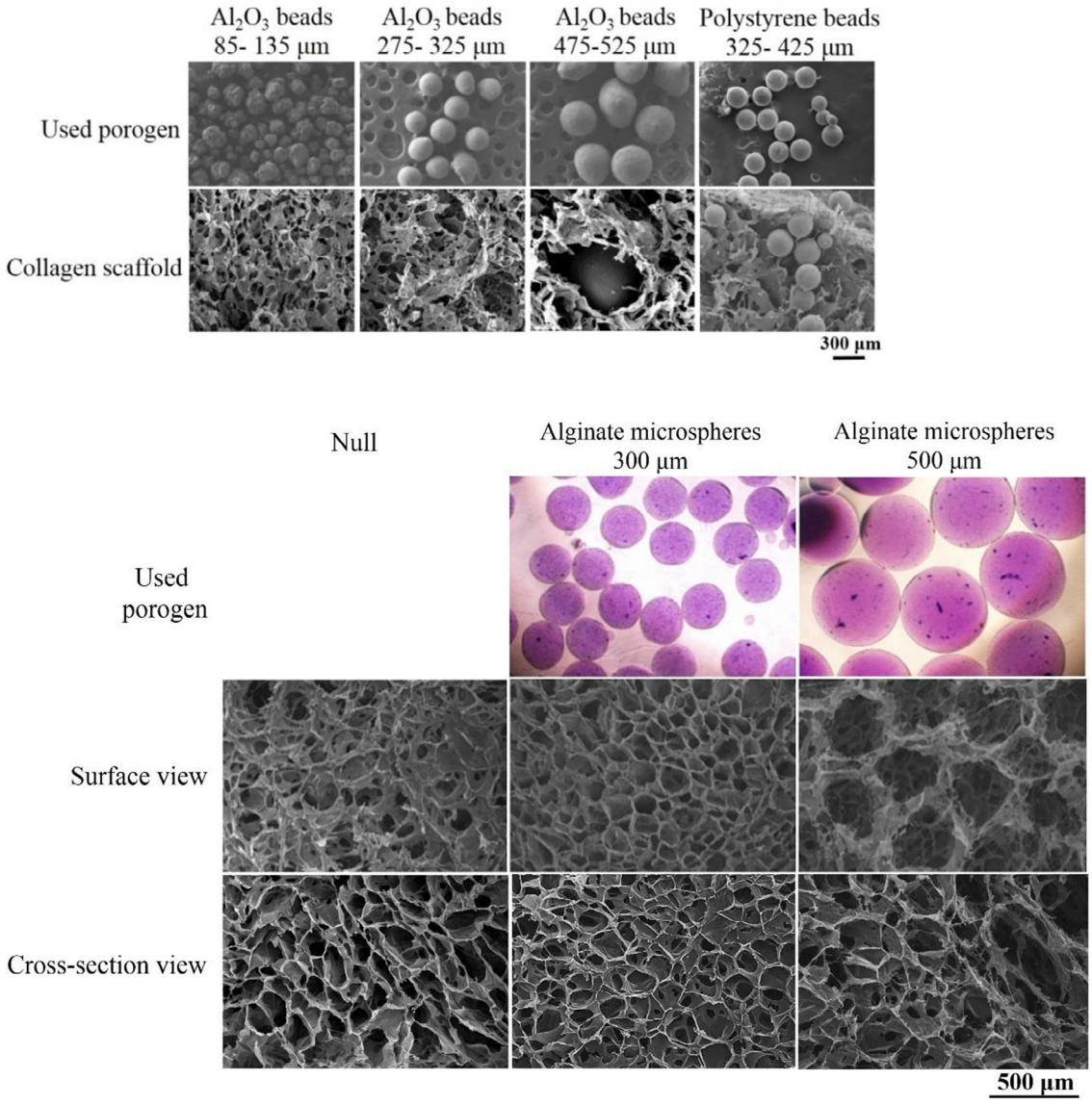

Fig. 2. Porogens and produced collagen scaffolds used in the study.

Table 1
The mechanical properties of collagen scaffolds.

	Force, gf	Elongation, mm	Stress, kPa	Strain
Null	30.4 ± 4.2	10.4 ± 0.35	0.40 ± 0.16	26.6 ± 8.4
Large alginate microspheres	20.7 ± 2.1	11.7 ± 1.5	0.29 ± 0.13	28.7 ± 8.2
Small alginate microspheres	32.7 ± 5.1	11.8 ± 1.2	0.39 ± 0.16	25.9 ± 5.7
Mixed alginate microspheres	36.0 ± 4.8	13.6 ± 1.3	0.43 ± 0.26	31.5 ± 7.9