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Fabrication of large perfusable macroporous cell-laden hydrogel scaffolds using microbial transglutaminase

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ABSTRACT

In this study, we developed a method to fabricate large, perfusable, macroporous, cell-laden hydrogels. This method is suitable for efficient cell seeding, and can maintain sufficient oxygen delivery and mass transfer. We first loaded three types of testing cells (including NIH 3T3, ADSC and Huh7) into gelatin hydrogel filaments, then cross-linked the cell-laden gelatin hydrogel filaments using microbial transglutaminase (mTGase). In situ cross-linking by mTGase was found to be non-cytotoxic and prevented the scattering of the cells after delivery. The gelatin hydrogel constructs kept the carried cells viable; also, the porosity and permeability were adequate for a perfusion system. Cell proliferation was better under perfusion culture than under static culture. When human umbilical vein endothelial cells were seeded into the constructs, we demonstrated that they stably formed an even coverage on the surface of the hydrogel filaments, serving as a preliminary microvasculature network. We concluded that this method provides a viable solution for cell seeding, oxygen delivery, and mass transfer in large three-dimensional (3-D) tissue engineering. Furthermore, it has the potential for being a workhorse in studies involving 3-D cell cultures and tissue engineering.
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1. Introduction

The field of tissue engineering exploits living cells in a variety of ways to restore, maintain or enhance tissues and organs [1,2]. Tissue engineering conjures up visions of organs built from scratch in the laboratory, ready to be transplanted into desperately ill patients. The volume of research in tissue engineering has increased substantially since the 1990s. Nevertheless, there are still many technical challenges, including biological challenges (i.e. cells and their sources) and engineering challenges, to overcome before we can create "off-the-shelf" tissues that represent the translation of scientific discoveries into treatments for millions of patients [3]. Only a few products are clinically applicable currently [1,3–5]. The objective of regenerative medicine is to provide cells with a local environment of artificial extracellular matrix where they can efficiently proliferate and differentiate, and thereby induce the repair of defective tissues. For most tissues or organs to be functional, the constructs should be three-dimensional (3-D) and the sizes should be clinically relevant [6].

The creation of such constructs has been limited by many challenges. Commonly used methods of 3-D cell culture are cell spheroids, microspheres, in situ forming hydrogels and preformed porous scaffolds [7–9]. The spheroids and microspheres lack integral structure. Hydrogels have difficulty in providing sufficient perfusion and mass transfer with increasing size. Preformed porous scaffolds, although widely used in preparing large constructs, have problems in efficient cell seeding and distribution [10]. The retention of cells in the preformed porous scaffolds relies on the ability of the cells to adhere to the surface of the scaffold, and less adhesive cells may be lost during perfusion. Furthermore, cells are exposed to non-physiological shear forces if the perfusion is not well controlled [11,12]. Cells laden in hydrogels can be protected from the hydrodynamic forces if they are perfused over the surfaces.

Efforts have been made to combine the advantages of porous scaffolds and hydrogels by designing methods to produce large porous hydrogel scaffolds that are able to encapsulate viable cells in situ, while at the same time providing plentiful porosity for adequate perfusion. Photolithography was used to fabricate

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cell-encapsulated poly(ethylene glycol) diacrylate (PEGDA) microgels that were further successfully assembled into porous structures by additional photopolymerization or by using a polypeptide cross-linker [13]. However, the risk of damage to DNA by UV light has been a major concern [14]. Furthermore, poor biodegradability of the material and lack of biological cues may set some limitations in clinical applications.

A microbial transglutaminase (mTGase) derived from *Streptoverticillium* species has been used widely in food processing to change the food texture by catalyzing the formation of peptide bonds (between the γ-carboxyamide group of glutamine and the ε-amino group of lysine). Enzymatic cross-linking of gelatin-based materials using this mTGase has been investigated for various applications, such as drug delivery, injectable cell delivery and scaffold fabrication [15–17]. Cells have been reported to tolerate the cross-linking process well, and the activity of the exogenous mTGase may be terminated by cell-secreted proteinases [16].

Here we report a method for fabricating large, perfusable, macroporous and cell-laden hydrogel scaffolds, using gelatin as the matrix and mTGase as the cross-linking agent. Filaments of gelatin gel were cross-linked into a stable and integral construct. The porosity and permeability could be controlled by altering the thickness or the volume fraction of the filaments. The cells tolerated the entire process well, and proliferated better under perfusion culture than under static culture.

2. Materials and methods

2.1. Fabrication of macroporous gelatin hydrogel constructs

The fabrication process is summarized in Fig. 1. Prior to use, the gelatin solution was incubated at 37 °C to obtain a liquid consistency for easier mixing. Gelatin–mTGase hydrogel was prepared by mixing 8% w/v gelatin solution (4 g of gelatin (type A, 175 Bloom; Sigma–Aldrich, St. Louis, MO) dissolved in 50 ml of complete medium, comprising Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Gaithersburg, MD), 10% fetal bovine serum (FBS; Life Technologies) and 100 U ml^{-1} penicillin–streptomycin (Sigma–Aldrich)), cell suspension (1×10^7 cells ml^{-1} in complete medium) and 0.1% w/v mTGase (Activa TG-K (containing 1% mTGase; Ajinomoto, Tokyo, Japan) in DMEM) at 8:1:1 (v:v:v). The final concentration of the mixture was 6.4% w/v gelatin, 1×10^6 ml^{-1} cells and 0.01% mTGase. For experiments without cells, complete medium was used in place of the cell suspension. The mixture was allowed to set at 4 °C for 10 min in a 5 ml syringe to form a hydrogel block and was then extruded through a stainless steel mesh to yield separated hydrogel filaments, which were suspended in 0.03% mTGase in DMEM and collected in a desired mold, usually a 10 ml syringe. After an additional 30 min of incubation at room temperature, the hydrogel filaments were further cross-linked into a construct with adequate interconnected interstices (pores) that was stable at 37 °C and ready for perfusion.

Different volume fractions and thicknesses of the hydrogel filaments were evaluated for the influences on the porosity and permeability of the fabricated constructs. A hydrogel block of 3 ml initial volume in 4.5 ml of suspension solution (0.03% mTGase in DMEM) made a 40% volume fraction, while in 3 ml of suspension solution made a 50% volume fraction. The thickness of the hydrogel filaments were determined by the pore size of the stainless steel mesh. ASTM No. 80 or 60 meshes (American Society for Testing and Materials International E11-95; No. 80: pore size 180 μm No. 60: pore size 250 μm) were used for the experiments. The average measured thicknesses of the hydrogel filaments were 200.1 μm (range 185–219 μm) and 266.8 μm (range 257–282 μm) for the No. 80 and 60 meshes, respectively, and were designated as 200 or 267 μm filaments. A 40% volume fraction and 200 μm filaments were applied in the subsequent experiments if not otherwise mentioned.

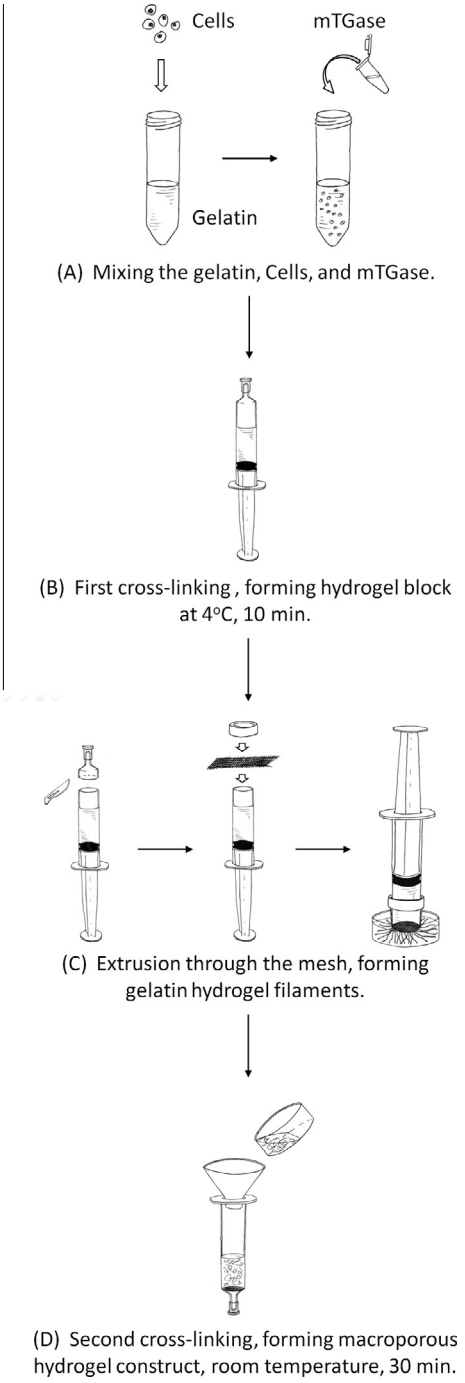

Fig. 1. Fabrication of macroporous gelatin hydrogel constructs.

2.2. Structure and porosity of the constructs

For visualization of the structure and macroporosity of the constructs, the hydrogels were incorporated with tetramethylrhodamine–dextran (MW 2,000 kDa, 0.02 mM; Life Technologies) and the interstices filled with medium containing fluorescein–dextran (MW 2,000 kDa, 0.02 mM; Life Technologies). Six constructs of each group were examined under confocal microscopy, and nine regions of each construct were sampled systematically. Images

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