MECHANICAL ENHANCED HYDROGEL FIBER ENCAPSULATING CELLS FOR LONG-TERM TRANSPLANTATION

Fumisato Ozawa[1], Jun Sawayama[1], and Shoji Takeuchi[1]
[1]Institute of Industrial Science, The University of Tokyo, Japan

ABSTRACT

This paper describes a core-shell hydrogel microfiber consisted of alginate and tetra-arm poly(ethylene glycol) (tetra-PEG) encapsulating rat insulinoma cells using a microfluidic process with a coaxial device and rapid gelation induced by diffusion of two different cross-linkers of barium ion and dithiothreitol (DTT) in the laminar flow (Fig. 1). Microfluidic techniques are able to encapsulate the cells in 3D configuration such as beads and fibers. For the medical treatment, an excellent method was reported that the retrievable graft of rat primary islet cell fiber for the treatment of diabetic mice [1]. However, the microfiber constructed of alginate hydrogel becomes weak of the strength, this issue is important for long-term transplantation. We here took an approach to improve the material, and developed composite hydrogel microfibers encapsulating cells with high mechanical strength. Using the double-network hydrogel consisted of barium-alginate and tetra-PEG as microfibers shell, the microfibers were able to encapsulate cells and maintained their shape without broken for 14 days intraperitoneal transplantation.

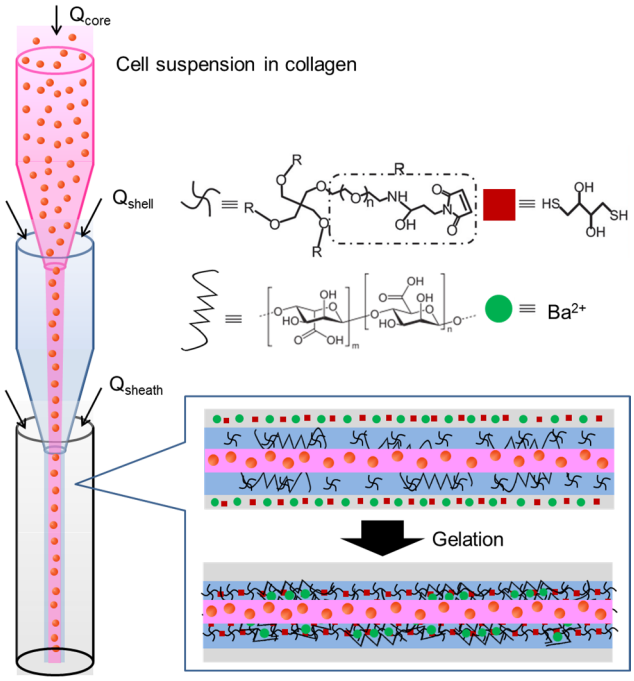

Figure 1. Schematic illustration of the process to fabricate core-shell alginate/tetra-PEG hydrogel fiber encapsulating cells using a coaxial microfluidic device and rapid gelation between alginate and pentaerythritol tetra{[3-(3-maleimido-1-oxopropyl)amino]propyl}-polyoxyethylene (tetra-PEG-MA) crosslinking with Ba^{2+} and dithiothreitol (DTT) in the laminar flow.

INTRODUCTION

Back Ground

Microencapsulation of drugs and cells with hydrogel is widely used in biomedical applications such as drug screening, drug delivery and cell therapy. Among them, alginate hydrogel fibers encapsulating cells are useful materials for retrievable graft. However, for long-term transplantation, the fibers are lowering of the strength because alginate hydrogels are gradually dissolved *in vivo*. Thus the development of stable and strength hydrogel fiber has great demand in the field of bio-encapsulation, especially for islet encapsulation to treat diabetic mellitus.

Description of the New Method

In order to achieve the fabrication of stable and strength hydrogel fiber for long-term transplantation, we combined the microfluidic fabrication of core-shell alginate hydrogel fiber [1] and tetra-PEG hydrogel fiber [2]. After generation of the laminar flow in a co-axial microfluidic device, composite hydrogel fiber with core-shell geometry was formed by reacting alginate and tetra-PEG-MA with Ba^{2+} and DTT, respectively. We demonstrated the encapsulation of rat insulinoma cells into the core of the fiber and investigated their physicochemical property. Furthermore, we assessed the *in vivo* stability of the fiber after 14 days transplantation.

EXPERIMENTAL RESULTS

Preparation of alginate/tetra-PEG hydrogel fiber

For preparation of alginate/tetra-PEG hydrogel fiber, each solution (the core solution was the rat insulinoma cell (INS-1) suspension in 0.24 % collagen type-IA at the concentration of 4×10^8 cells/ml, the shell solution was mixture of 1.5 % alginate sodium and 10 % tetra-PEG, and the sheath solution was 20 mM $BaCl_2$, 0.1 % DTT.) was introduced into the co-axial microfluidic device at the flow rate 50 µl/min (core), 350 µl/min (shell) and 3.6 ml/min (sheath), respectively. Figure 2 shows alginate/tetra-PEG hydrogel fiber encapsulating cells. The encapsulated cells were viable after cultivation for 4 days.

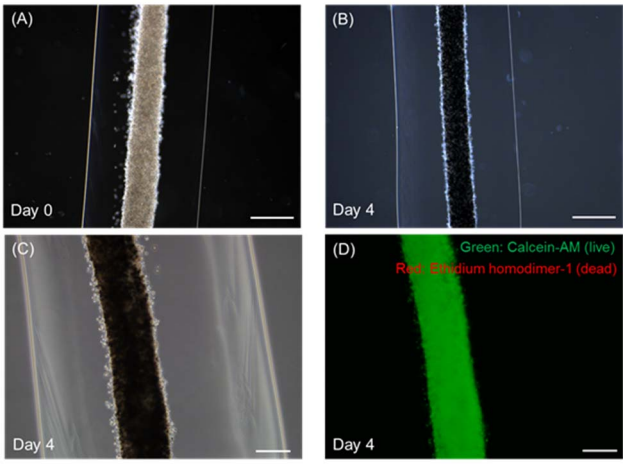

Figure 2. Fabrication of core-shell alginate/tetra-PEG hydrogel fibers. The micrograph of alginate/tetra-PEG hydrogel fibers encapsulating rat insulinoma cells. (Day 0 (A) and day 4 (B), Scale bar: 500 µm) The optical (C) and fluorescent (D) images of encapsulated the cells stained with live/dead reagent after 4 days. (Scale bar: 200 µm)

Mechanical properties of the hydrogel fibers

To evaluate the mechanical properties of the hydrogel fiber, we prepared alginate and alginate/tetra-PEG hydrogel fibers and measured the respective mechanical strength and strain via an elongation test. Figure 3 describes the mechanical measurements of alginate/ tetra-PEG hydrogel fiber compared with conventional alginate hydrogel fiber. The alginate/tetra-PEG hydrogel fiber had higher stretchability than the alginate one.

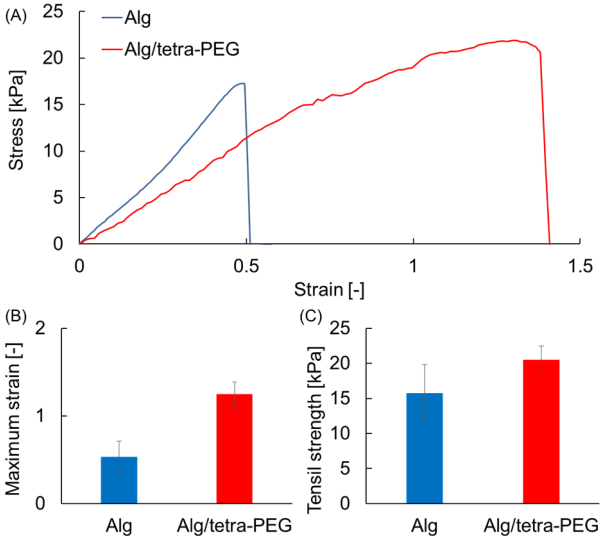

Figure 3. Mechanical property of alginate/tetra-PEG hydrogel fiber. Stress-Strain curve (A), maximum strain (B) and tensile strength (C) of the alginate and alginate/tetra-PEG hydrogel fibers.

Semipermeability of alginate/tetra-PEG hydrogel fiber

To confirm the permeability of alginate/tetra-PEG hydrogel fiber, the fibers were washed in saline and transferred into a 0.05% FITC-labeled dextran/saline solution, followed by incubation at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for predetermined time. Figure 4 showed the semipermeability of alginate/tetra-PEG hydrogel fiber that the fiber was able to be permeated under 70 kDa size of FITC-dextran but not to be permeated over 150 kDa size of one. This result indicates that small molecules less than 70 kDa including glucose, insulin and oxygen permeate through the hydrogel membrane, in contrast large molecules over 150 kDa and micron-sized cells are difficult to infiltrate into the hydrogel fibers.

CONTACT

* F. Ozawa, tel: +81-3-5452-6650; f-ozawa@iis.u-tokyo.ac.jp

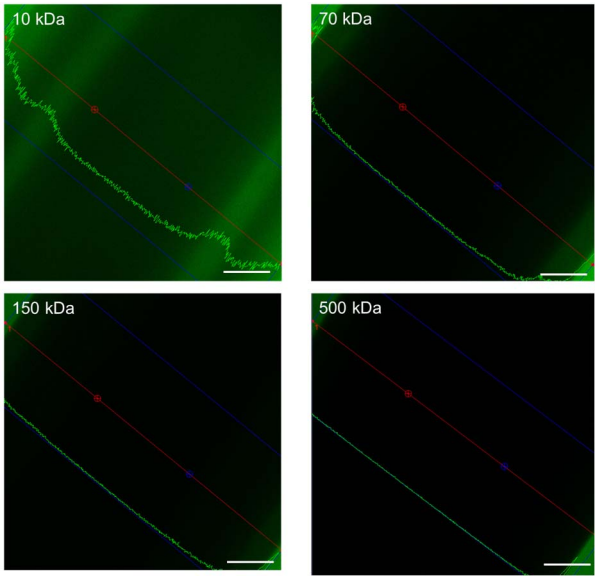

Figure 4. Semipermeability of alginate/tetra-PEG hydrogel fibers. The fluorescent images show the different molecular weight FITC-dextran permeate from the solution into the fiber. (Scale bar: 200 µm)

Transplantation of alginate/tetra-PEG hydrogel fiber

To evaluate *in vivo* stability of alginate/tetra-PEG hydrogel fiber, the fiber was transplanted into intraperitoneal space of immunocompetent C57BL/6 mice. After 14 days transplantation, the alginate/tetra-PEG hydrogel fibers maintained their shape without broken.

CONCLUSION

In this study, we developed the mechanical enhanced alginate/tetra-PEG hydrogel fiber for long-term transplantation. This hydrogel fiber was able to encapsulate rat insulinoma cells and maintain cell viability. In addition, the fiber had stretchability, semipermeabilty, and *in vivo* stability. Now, we investigate to prepare alginate/tetra-PEG hydrogel fibers encapsulating rat islets or hiPSC derived islet cells and transplanted into diabetic mice to control their blood glucose concentrations.

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