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Cell-selective encapsulation in hydrogel sheaths via biospecific identification and biochemical cross-linking

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ABSTRACT

Selective encapsulation of a particular cell population from heterogeneous cell populations has potential applications such as studies in cell-to-cell communication, regenerative medicine, and cell therapies. However, there are no versatile methods for realizing this. Here we report a method based on biospecific identification of the target cells through antigen–antibody reaction and subsequent enzymatic hydrogel sheath formation on the cell surfaces by horseradish peroxidase (HRP). Human hepatoma cell line HepG2 cells were selectively encapsulated in alginate-based hydrogel sheath from the mixture with mouse embryo fibroblast-like cell line 10T1/2 fibroblasts using anti-human CD326 antibody conjugated with HRP. The viability of the encapsulated cells was 93%. The cells released at 6 days of the encapsulation by degrading the sheath using alginate lyase grew almost the same as those free from encapsulation. The versatility of the method was confirmed using another antibody, cells, and hydrogel sheath material: Only human vein endothelial cells were encapsulated in gelatin-based hydrogel sheath from the mixture with 10T1/2 fibroblasts using anti-human CD31 antibody conjugated with HRP. The cell-selective encapsulation was also achieved by a system using a primary antibody with a secondary antibody conjugated with HRP.

1. Introduction

Mammalian cell-encapsulation in semipermeable hydrogel membranes has been studied and advanced since the 1980s for a variety of applications such as cell therapy, fundamental researches in cell biology, and tissue engineering [1–3]. To date, while a number of encapsulation methods have been developed, there are no versatile methods for encapsulation of a particular cell population from heterogeneous cell populations. Cell-selective encapsulation would be useful in applications such as studies of cell behavior caused by direct cell–cell contact, studies of cell responses to mechanical stimuli from the surrounding environment in the presence of other type of cells, and cell therapies requiring encapsulation of only therapeutically effective cells. A common feature of existing methods is that hydrogel membranes can form regardless of the presence of cells. For example, Ca-alginate microparticles can be obtained from solutions with or without cells, by dropping an alginate solution into a gelation bath containing Ca^{2+} [4].

Here, we propose the first method capable of achieving cell-selective encapsulation in a hydrogel sheath. This cell-selective encapsulation is accomplished through two established technologies: cell identification via an antigen–antibody reaction and hydrogel formation through a horseradish peroxidase (HRP)-catalyzed reaction. As shown in Fig. 1a, first, the cells in a particular population are identified from those in heterogeneous populations by antibodies conjugated with HRP. Then, the cells containing both the identified and non-identified ones are soaked in a solution containing H_2O_2 and polymer possessing the moieties cross-linkable through the enzymatic reaction. The hydrogel sheath formation only on the surface of the identified cells is induced by the HRP immobilized with the antibodies. Today, cell identification based on antigen–antibody reactions is an essential technology in a variety of fields, such as histology, cell biology, and molecular biology. Cells expressing specific antigens can be identified using this technology. The HRP-catalyzed cross-linking of polymer molecules in aqueous solution, resulting in hydrogelation, has attracted increasing attention for applications in drug delivery and tissue

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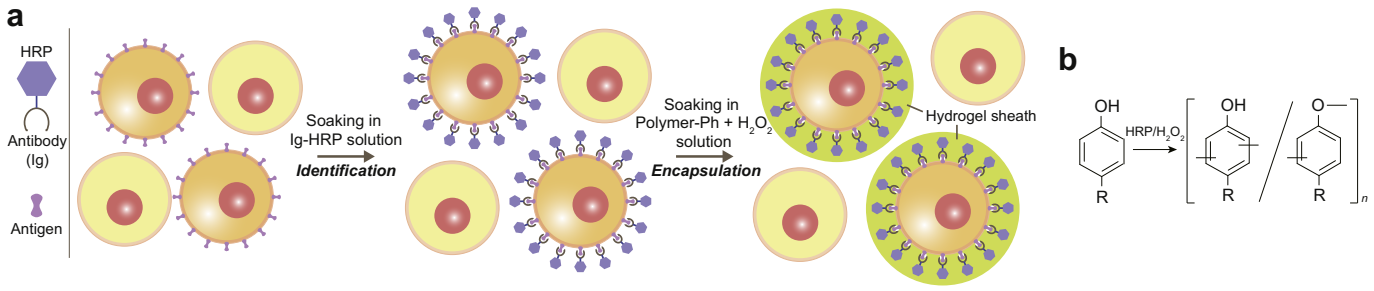

Fig. 1. Schematics of a) cell-selective hydrogel sheath formation on a target cell surface via cell identification through an antigen–antibody reaction, followed by HRP-catalyzed cross-linking of Ph moieties in polymer molecules, and b) HRP-catalyzed cross-linking of Ph moieties.

engineering because of its biocompatible reaction conditions [5–8]. HRP catalyzes the oxidative coupling of phenol derivatives, resulting in polyphenols linked by C–O and C–C groups, via the consumption of H_2O_2 (Fig. 1b). An advantage of HRP-catalyzed hydrogel sheath formation is the abundance of available materials because all water-soluble polymers that possess moieties that are cross-linkable through the enzymatic reaction are potential candidates. To date, hydrogels have been prepared from HRP-containing aqueous solutions of a variety of polymers, unmodified natural polysaccharide [9], derivatives of polysaccharides [5,10–13] and proteins [14], and synthetic polymers [15,16]. The abundance of available materials enables the preparation of hydrogel sheaths with specific properties that are customized for individual applications. For example, sheaths with a less cell adhesiveness and non-degradable with proteolytic enzymes, such as obtained from alginate and polyvinyl alcohol-derivatives [13,16], would be useful in cell therapies for isolating cells from the host immune system. Sheaths with a higher cell adhesiveness and degradable with proteolytic enzymes, such as obtained from gelatin derivative [14,17], would be useful for constructing tissues in vitro by accumulating individual cells [18,19]. The effectiveness of on-cell surface cross-linking of polymers using HRP immobilized on the cell surface, but not cell-selective encapsulation, was recently reported [20].

2. Materials and methods

2.1. Cell culture

Human hepatoma cell line HepG2 cells and mouse embryo fibroblast-like cell line 10T1/2 cells (the Riken Cell Bank, Ibaragi, Japan) were cultured in Dulbecco's modified Eagle's medium (DMEM, Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal bovine serum (FBS). Human umbilical vein endothelial cells expressing green fluorescence protein (GFP-Huve cells, Takara Bio, Shiga, Japan) were cultured in MCDB107 medium (Cell Science & Technology Institute, Miyagi, Japan) containing 10% FBS, 10 ng/mL human epidermal growth factor (Sigma, MO, USA) and 10 ng/mL human recombinant fibroblast growth factor-2 (ReproCELL, Kanagawa, Japan).

2.2. IgCD326- and IgCD31-HRP preparation

Allophycocyanin (APC)-labeled anti-human CD326 antibody from mouse (IgCD326, Miltenyi Biotec, CA, USA) and anti-human CD31 antibody from mouse (IgCD31, Diaclone, Besançon Cedex, France) were conjugated with HRP using commercially available HRP possessing succinimidyl moieties (Peroxidase Labeling kit $-NH_2$, Dojindo, Kumamoto, Japan) according to the protocol given by the supplier. The resultant specimens were denoted as IgCD326-HRP and IgCD31-HRP, respectively.

2.3. Alg-fPh and gelatin-rPh synthesis

Derivatives of alginate and gelatin possessing Ph moieties were synthesized by conjugating sodium alginate (I-1G, MW 70,000, 61% in guluronic acid residues, Kimica, Tokyo, Japan) or gelatin (type A from porcine skin, 300 Bloom, Sigma) with tyramine hydrochloride (Tokyo Chemical Industry, Tokyo, Japan) using 1-ethyl-3-(3-dimethylaminopropyl carbodiimide) (Peptide Institute, Osaka, Japan) and *N*-hydroxysulfosuccinimide (NHS, Wako Pure Chemical Industry, Osaka, Japan) based on previously reported method [13,14]. The contents of Ph moieties in the resultant alginate and gelatin derivatives determined by measuring the absorbance of 0.1% (w/w) aqueous solution at 275 nm was 1.0×10^{-4} mol-Ph/g and 1.6×10^{-4} mol-Ph/g, respectively. The alginate and gelatin derivatives were then conjugated with 4-aminofluorescein (Sigma) and NHS-rhodamine (Thermo Fisher Scientific, MA, USA) for obtaining alginate derivative possessing Ph and fluorescein moieties (Alg-fPh) and gelatin derivative possessing Ph and rhodamine moieties (gelatin-rPh) based on previously reported method [20].

2.4. Floating cell encapsulation using primary antibody

HepG2 cells were stained with Cell Tracker™ Orange (Life Technologies, MD, USA) according to the protocol given by the supplier. The cells were mixed with 10T1/2 cells at 2.5×10^5 cells each, and then soaked in 200 μL FBS-free DMEM containing IgCD326-HRP at 0.55 μg-antibody/mL for 30 min. After rinsing with PBS twice, the cells were soaked in 200 μL PBS containing 1.0% Alg-fPh and 0.1 mM H_2O_2 for 10 min. For evaluating the cytotoxicity of the encapsulation method, the viabilities of the cells at each encapsulation step was determined by counting viable cells using a hemocytometer and the trypan-blue exclusion dye. In addition, the encapsulated cells prepared from HepG2 cells alone were soaked in the medium containing 0.2 mg/mL alginate lyase (from *Flavobacterium multivorum*, Sigma) for removing the hydrogel sheath and then seeded on a 96-well cell culture dish at 5×10^3 viable cells/well for evaluating the effect of encapsulation on growth of them. The growth of the cells was evaluated based on mitochondrial activity increase ratio per well during 1–3 days of culture using a colorimetric assay kit (Cell Counting Kit-8; Dojindo, Kumamoto, Japan) according to the protocol given by the supplier. Formation of hydrogel sheaths was determined using a fluorescence microscope (BZ-9000, Keyence, Tokyo, Japan) and a flow cytometer (BD Accuri™ C6, BD Bioscience, CA) after several rinses with PBS.

Apart from above experiment, GFP-Huve cells and 10T1/2 cells were mixed at 2.5×10^5 cells each, and then soaked in 200 μL FBS-free medium containing IgCD31-HRP at 5 μg-antibody/mL for 30 min. After rinsing several times with FBS-free medium, the cells were soaked in 200 μL PBS containing 5% gelatin-rPh and 0.1 mM H_2O_2 for 10 min for evaluating the versatility of the cell-selective encapsulation method.

2.5. Effect of factors on hydrogel sheath formation

The effects of concentrations of IgCD326-HRP (0.11, 0.22 and 0.55 μg-antibody/mL) and Alg-fPh (0.1, 0.5 and 1.0% (w/v)), and the times of soaking in IgCD326-HRP solution (5, 10, and 30 min) and Alg-fPh solution (5, 10, and 30 min) on the hydrogel sheath formation were investigated by measuring the fluorescence intensity attributed to the existence of Alg-fPh on individual cells using a flow cytometer. For determining the effect of individual parameters, one of the values were changed from the following condition, 0.55 μg-antibody/mL IgCD326-HRP solution, 30 min of soaking in IgCD326-HRP solution, 1.0% (w/v) Alg-fPh, and 10 min of soaking in Alg-fPh solution. The concentration of H_2O_2 in Alg-fPh solution was fixed at 0.1 mM.

2.6. Floating cell encapsulation using primary and secondary antibodies

The HepG2 cells stained with Cell Tracker™ Orange and non-stained 10T1/2 cells were soaked in 200 μL FBS-free DMEM containing 1/20 vol of purchased IgCD326 solution for 30 min and rinsed several times with FBS-free DMEM. Then, the cells were soaked in FBS-free DMEM containing 1/10 vol of purchased HRP-labeled anti-mouse IgG + IgM (H + L) from goat (Ig-HRP, Kirkegaard & Perry Lab, Gaithersburg, MD, USA) for 30 min and rinsed several times with PBS. Subsequently, the cells were soaked in PBS containing 1.0% Alg-fPh and 0.1 mM H_2O_2 for 10 min.

2.7. Adhering cell encapsulation

The HepG2 cells stained using Cell Tracker™ Orange and non-stained 10T1/2 cells were seeded in a well of 24-well cell culture dish at 0.5×10^5 cells/well each. After overnight culture for allowing adhesion and spreading of the cells, they were washed several times using PBS and then soaked in 200 μL PBS containing IgCD326-HRP at 0.55 μg-antibody/mL for 30 min. Subsequently, the cells were rinsed with PBS and then soaked in 100 μL PBS containing 1.0% Alg-fPh and 0.1 mM H_2O_2 for 10 min.