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Research paper

Formation of stable cell–cell contact without a solid/gel scaffold: Non-invasive manipulation by laser under depletion interaction with a polymer

Shu Hashimoto, Aoi Yoshida, Taeko Ohta, Hiroaki Taniguchi, Koichiro Sadakane *, Kenichi Yoshikawa *

Faculty of Life and Medical Sciences, Doshisha University, Kyotanabe 610-0394, Japan

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ABSTRACT

We report a novel method for constructing a stable three-dimensional cellular assembly in the absence of a solid or gel scaffold. A targeted cell was transferred to another cell, and the two were kept in contact for a few minutes by optical manipulation in an aqueous medium containing a hydrophilic polymer. Interestingly, this cell–cell adhesion was maintained even after elimination of the polymer. We discuss the mechanism of the formation of stable multi-cellular adhesion in terms of spontaneous rearrangement of the components embedded in the pair of facing membranes.

1. Introduction

The fabrication of three-dimensional (3-D) cellular assembly systems with multiple cells through the desired positioning of individual cells has become increasingly important with recent developments regarding the outcome of cell differentiation as well as the discovery of dedifferentiation techniques using induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) [1–4]. To achieve this end, a chemically controlled gel has frequently been applied as a scaffold to achieve a 3-D cellular assembly [5–8]. Additionally, various other approaches such as magnetic liposomes [9], polymeric aqueous two-phase systems [10], printing of cells with polymers [11] and well-controlled micro-arrays of a solid substrate [12] have been proposed. Unfortunately, artificial scaffolds and/or artificial chemical substances are thus far indispensable for the assembly of cells, and such xenobiotics may have non-negligible effects on the structure and function of the assembled cells. As a noninvasive method of cell manipulation in bulk aqueous medium under remote control, laser tweezers [13] have been applied for the transport of targeted cells in aqueous medium [14,15]. Although the use of laser tweezers is a well-known method for transferring a targeted cell, it has been difficult to sustain stable cell–cell contact after the laser is switched off. Living mammalian cells are usually suspended in an aqueous solution and tend to dissociate due to repulsive interactions between the facing membrane surfaces [16]. Under these circumstances, the current methodologies for cellular assembly are mostly restricted to the adoption of solid or gel substrates to anchor and assemble cells. Here, we report our experimental observations on the formation of a stable 3-D cell-assembly in bulk medium using laser tweezers without any solid/gel scaffolds, through the use of a crowding effect with macromolecules [17–19].

2. Materials and methods

NAMRU mouse mammary gland epithelial cells (NMuMG) [20] were cultured in Dulbecco's modified Eagle's medium (DMEM) (Wako Pure Chem. Ind., Japan) supplemented with 10% fetal bovine serum (FBS) (Cell Culture Biosci., Nichirei Biosci. Inc., Japan), 40 μg/ml streptomycin, and 40 units/ml penicillin (Life Tech. Corp., USA). The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Sub-confluent cells were harvested with trypsin (0.25% Trypsin–EDTA (1X)) (Life Tech. Corp.) and cryopreserved with CELLBANKER1 (Nippon Zenyaku Kogyo, Japan). For preparation of the crowding polymer, we used polyethylene glycol (PEG) (50,000; molecular biology-grade) (Wako Pure Chem. Ind.). We prepared 10–40 mg/ml of polymer solution with PEG (50 k) using DMEM. Microscopic images were obtained using a Nikon TE-300 inverted microscope equipped with a CCD camera (WAT-120N) (Watec Co. Ltd., Japan), and an optical laser manipulation system (Millennia IR) (Spectra-Physics. Corp., Japan) with a Nd:YAG laser

* Corresponding authors at: Laboratory of Life Physics, Faculty of Life and Medical Sciences, Doshisha University, 1-3 Tatara Miyakotani, Kyotanabe, Kyoto 6100321, Japan.

E-mail addresses: ksadakan@mail.doshisha.ac.jp (K. Sadakane), keyoshik@mail.doshisha.ac.jp (K. Yoshikawa).

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(cw; 1064 nm) operating between 380 and 780 mW. The optically trapped cell was transferred using an auto-stage (Sigmakoki Co. Ltd., Japan). All experiments were carried out at room temperature (23 °C).

3. Results

Fig. 1 illustrates the process by which cell–cell contact is induced through the use of laser tweezers, where (A) and (B) indi-

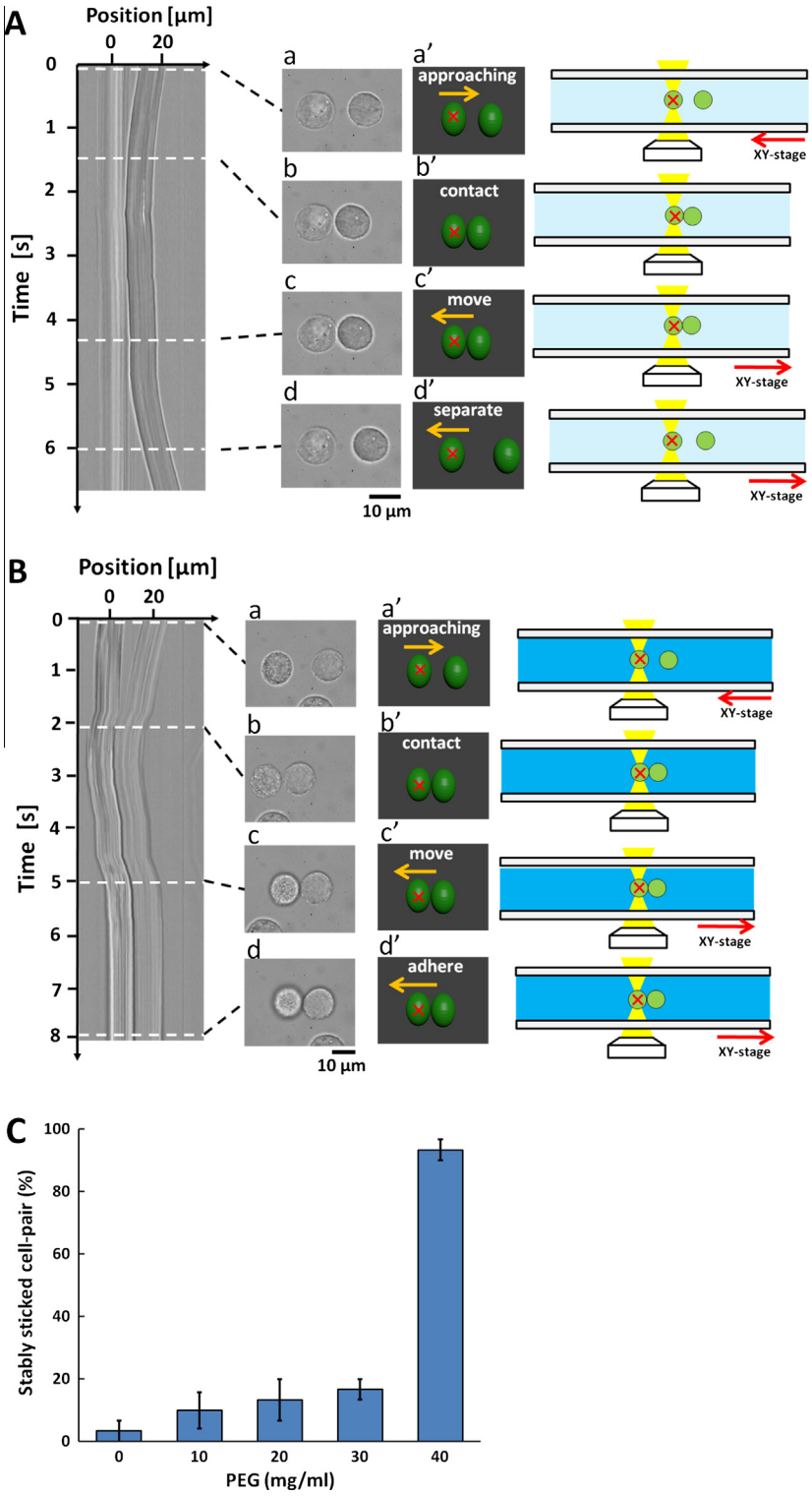

Fig. 1. Laser manipulation of a pair of epithelial cells (NMuMG); (A) in the absence and (B) in the presence of PEG (40 mg/ml): Left, spatio-temporal diagram illustrating the process of manipulation; second-left, photomicrographs of a pair of cells; third-left, schematics indicating the position of the laser focus as marked by 'x'; right, schematic illustration. (a) and (b) Transport of a targeted cell to make contact with another cell, (b) and (c) Continuation of cell–cell contact under laser tweezing, and (c) and (d) Transport of a grasped cell to the left at a speed of ca. 10 μm/s. For the schematics in a′–d′, the direction of transport of the trapped cell with respect to the cellular environment is shown, indicating the motion of cells with respect to the stage. In (A), the cell-pair separates due to viscous friction as a result of transport. In (B), the cells maintain stable contact throughout transport. (C) The probability of stable cell–cell contact being maintained through optical transport for ca. 5 μm, i.e., the percentage of experimental runs to obtain the result exemplified in (B) where the result in (A) was counted as a failure.