IMMOBILIZATION AND CULTURING OF MAMMALIAN CELLS WITH BIO-COMPATIBLE ELECTRODEPOSITION OF CALCIUM ALGINATE GEL IN MICROFLUIDIC DEVICES

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ABSTRACT

This paper reports a unique approach to immobilize mammalian cell populations (mouse myeloma NS0) through *in situ* gelation of calcium alginate triggered by an electrical signal under physiologically relevant conditions. This is the first experimental observation to evaluate the electrically triggered assembly of calcium alginate gel for entrapping and culturing mammalian cells. Subsequent cell viabilities immediately after electrodeposition and after three days of culturing are studied. Our cell assembly strategy is applicable to fragile mammalian cells and can be used for *in vitro* study of dynamic cellular processes and cell-based assays under a microfluidic environment.

KEYWORDS: Calcium alginate, Electrodeposition, Mammalian cell, Cell culture, Microfluidics

INTRODUCTION

Integrating living cells into microfabricated systems is important for the study of cellular processes and to permit cell-based assays. In many applications it is necessary to immobilize the viable cells, capturing them *in situ* and maintaining them in a specific location in a bioMEMS device. This paper reports a unique approach to immobilize cell populations (mouse myeloma NS0) through *in situ* gelation of calcium alginate triggered by an electrical signal under physiologically relevant conditions. Electrodeposition of alginate on conductive surfaces has been reported before [1]. This work extends previous *E. coli* cell assembly work [2, 3] by fine-tuning the deposition pH condition, enabling spatially selective, electroaddressable scaffolds for entrapping pH sensitive cell populations *in situ* at pre-patterned surfaces in a bioMEMS device. Compared with other work in the field, the advantages of this method include the electroaddressing capability. In this paper, we demonstrate a viable approach to assemble and culture mammalian cells using calcium-responsive alginate hydrogels electrochemically deposited in a microfluidic device and visualized at sidewall electrodes. Here the alginate sol-gel transition is caused by the release of Ca^{2+} ions triggered by the reactions between suspended $CaCO_3$ particles and electrochemically generated protons from the anode surface. The pH of the hydrogel during deposition can be maintained in a physiological conditions by appropriately adjusting the amount of buffering $CaCO_3$ particles in the deposition solution. The viability study of the assembled cells after three days culturing suggest that our cell assembly strategy can be used for immobilization and culturing of fragile types of cells.

EXPERIMENTAL

A schematic view of the microfluidic device [4] in use is shown in Figure 1. Multiple parallel electrodes and leads were defined by angled thermal evaporation of chromium and gold onto a glass slide using a bent shadow mask. Two glass slides with such patterned sidewall electrodes were placed side by side with a separation of ~1 mm and sandwiched between two pieces of cured PDMS to form a fluidic channel with height and width both 1 mm. With built-in electrodes defined on the sidewall of the fluidic channel, the side view provides resolution of the entrapped cells perpendicular to the electrode.

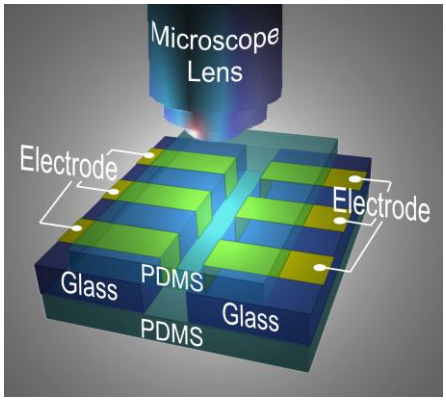

Figure 1. Schematic diagram of the microfluidic device integrated with sidewall electrodes in the channel. The sidewall electrodes are defined onto the top and the side surfaces of a glass slide by applying a shadow mask with slits during metal evaporation. Two patterned glass slides are placed side by side and sandwiched between two thin layers of solid PDMS.

1% and 2% (w/v) sodium alginate solutions were prepared by dissolving sodium alginate powder (Sigma Aldrich, extracted from brown algae, medium viscosity) in distilled water, followed by ultrasonication (10 min) and stirring (10 hr). Sodium alginate deposition solutions with different $CaCO_3$ concentration (w/v, 0.25%, and 0.5%) were prepared by suspending different amounts of $CaCO_3$ powder (Sigma Aldrich) into sodium alginate solutions with constant stirring.

The mouse myeloma NS0 cells were initially cultured in a Gibco CD Hybridoma chemically defined medium (Invitrogen) supplemented with 1x MEM non-essential amino acids (Invitrogen) to a density of ~10^6 mL^{-1}. They were gently mixed with the alginate-$CaCO_3$ deposition solution by pipetting before electrodeposition. After deposition, the deposition solution in the microfluidic channel was replaced with 1x sterile Dulbecco's phosphate-buffered saline (DPBS) or pure culture media before imaging.

RESULTS AND DISCUSSION

The mechanism of the electro-induced calcium alginate deposition and associated ions concentration distribution are depicted in Figure 2. The channel is initially filled with deposition solution which normally contains 1% alginate and 0.25% $CaCO_3$ particles. The deposition solution is in direct contact with cathode and anode electrodes which are positioned opposite to each other with a separation of about 1 mm (channel width). The anode and cathode are connected with the "+" and "-" terminals of a source meter with a typical current source of 4 A/m^2. The mechanism can be explained in three steps illustrated in Figures 2 b, c, and d. First, the electrical signal triggers proton generation by the electrolysis of water and these protons move along the concentration gradient (diffusion) and the electric field line (electrophoretic migration) to form a pH gradient at the anode surface (Figure 2b). Second, the protons encounter and react with the suspended $CaCO_3$ particles in alginate solution and result in the release of calcium ions (Figure 2c). This reaction leads to a partial dissolution of insoluble $CaCO_3$ particles. Depending on the proton concentration, the reaction results in partial or complete dissolution of $CaCO_3$ particles. Third, the localization of calcium cations in the vicinity of the negatively charged carboxylate groups on the G blocks (guluronic acid) of alginate leads to alginate macromolecular chain crosslinking (Figure 2d).

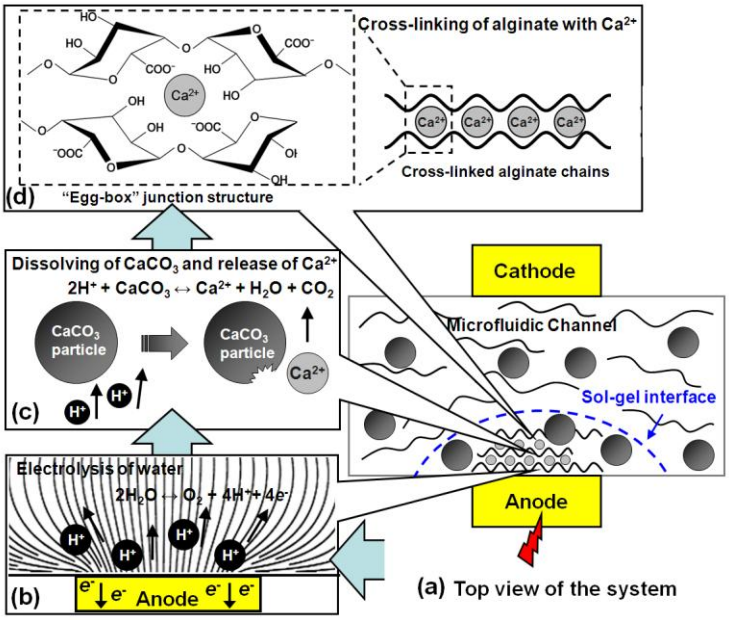

Figure 2. Schematic diagram illustrating the electrochemical reactions and subsequent chain crosslinking associated with the electrodeposition of a calcium alginate hydrogel. (a) Top view of the deposition system. (b) Electrolysis of water reaction ($2H_2O \leftrightarrow O_2 + 4H^+$) at the anode surface for proton generation. (c) Dissolution of $CaCO_3$ and release of Ca^{2+} ($H^+ + CaCO_3 \leftrightarrow Ca^{2+} + H_2O + CO_2$).(d) The crosslinking of alginate molecular chains by Ca^{2+} ions ($Ca^{2+} + 2Alg\text{-}COO^- \leftrightarrow Alg\text{-}COO^-\text{-}Ca^{2+}\text{-}^-OOC\text{-}Alg$).

We first examined the possibility of using this approach to immobilize fragile mammalian cells while maintaining their viability. The channel was initially filled with deposition solution which contains NS0 cells (~10^6 cells/mL), sodium alginate (1%, w/v) and uniformly dispersed $CaCO_3$ particles (0.2-0.5%, w/v). The cell assembly was accomplished by applying a constant anodic current (4 A/m^2) with the cathode grounded for 120 sec. The channel was then drained and flooded with culture media. A live/dead viability fluorescence kit was then used to assess the viability of the assembled cells. A previous study suggested that the pH level of the gel during the deposition can be tuned by adjusting the $CaCO_3$ concentration [5]. Figure 3 shows the viability of the assembled mouse B cells in the gel by varying the $CaCO_3$ concentration from 0.2% to 0.5% in the deposition solution. Here the presence of abundant $CaCO_3$ particles buffers the pH, preventing an overly acidic environment from developing. By adjusting the $CaCO_3$ concentration, assembly of fragile, pH-sensitive cell types with decent viability is possible.

We then evaluated the applicability of our assembly approach for cell culturing. A fluorescence micrograph of the assembled cell/gel structure following the same deposition procedure is shown in Figure 4a, with live cells in green and dead cells in red. The same procedure was followed to produce a cell/gel structure and cultured for three days in a cell incubator (37 ℃) with culturing media flowing through the channel at a volumetric flow rate of 6 μL/min. Figure 4b shows a fluorescent micrograph of the cells after culturing. An increase in cell population suggests the electrodeposited gel can serve as a scaffold for immobilization and culturing of NS0 cells.

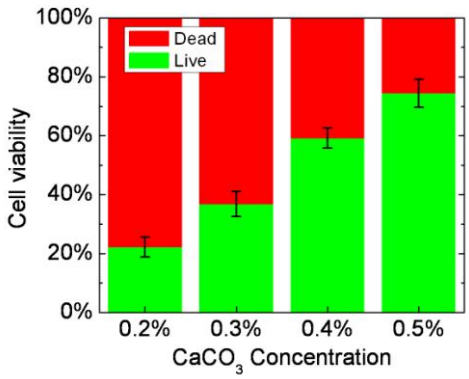

Figure 3. $CaCO_3$ concentration dependent viability assessment of assembled NS0 cells in the electrodeposited calcium alginate hydrogel.

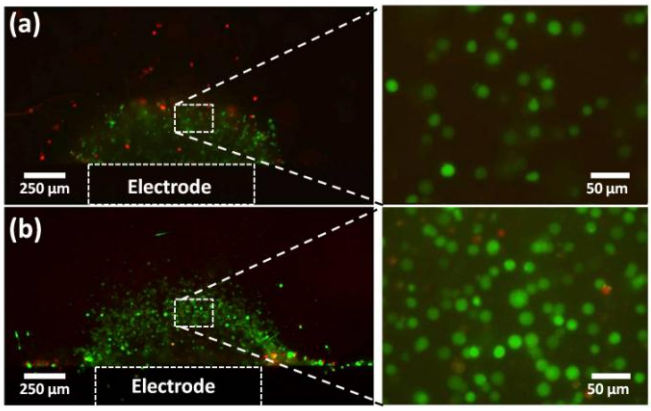

Figure 4. Fluorescent micrographs of NS0 cells entrapped in the electrodeposited calcium alginate gel. (a) before and (b) after culturing(3 days).The two insets show a magnified view of the assembled cells entrapped in the gel before and after culturing.

CONCLUSION

In conclusion, the stimuli-responsive hydrogel polymer alginate enables the electrical signal guided assembly of live mammalian cells on electrode surfaces in bioMEMS devices under physiological pH conditions. The observation of a decent viability of the assembled mammalian cells and an increase in cell population after culturing suggests the electrodeposited calcium alginate hydrogel an excellent scaffold for mammalian cell culture. We envision an impact spanning the spectrum from physiological and functional *in vitro* cellular behavior studies to applications such as cell-based sensors, biomedical devices and drug candidate screening systems.

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