DIRECT ELECTROPORATION OF ADHERENT CELLS
BY HYDROGEL-BASED MICROELECTRODES
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ABSTRACT
We have developed a technique to fabricate Au microelectrodes on hydrogel substrates such as polyacrylamide, agarose, collagen etc. Electropolymerized poly (3,4-ethylenedioxythiophene) (PEDOT) serves as the anchor for bonding the Au electrodes to the hydrogels. The prepared soft hydrogel-based electrode can be directly laminated to adherent cells for in-situ electroporation. Owing to the molecular permeability of the hydrogels, continuous post-cultivation with the laminated electrode substrate was also possible. The electroporation-assisted uptakes of dye molecules and genes were demonstrated for normal human dermal fibroblast cells and human iPS cells.

KEYWORDS: Electroporation, Hydrogel, Adherent Cell, GFP

INTRODUCTION
Electroporation is a convenient and versatile technique to deliver biological molecules such as DNA, proteins and drugs into living cells. A short, high electric-field pulse causes temporal formation of transmembrane pores on the cellular membrane and uptake of molecules and genes. The process is simple and biochemically “safe”, which is a crucial advantage for clinical applications of genetically engineered cells, including induced pluripotent stem (iPS) cells. However, while traditional cuvette-based electroporation is convenient for cell suspensions, previous trypsinization of adherent cells is required. Such extra treatments of trypsinization and re-seeding are invasive to cells and decrease efficiency of cell processing.

We report here the development of a technique to prepare microelectrodes on hydrogel substrates that are soft enough for direct lamination to the cultured adherent cells. Owing to the molecular permeability of the hydrogels containing ~80% H2O, continuous post-cultivation with the laminated electrode substrates is also possible. Previously, we reported the fabrication of microelectrodes of a conducting polymer on hydrogels [1, 2]. However, due to its unignorable high resistance, it does not work well as an electrode for electroporation that requires shorter, higher voltage pulses. Therefore, we have newly developed here a method to make metal electrodes on hydrogel.

EXPERIMENTAL
As shown in Figure 1a, the process starts with the preparation of an Au microelectrode by photolithographically patterning of a sputtered Au film (0.3μm-thick) on a glass plate. Importantly, the adhesion between Au and glass is weak, which is an advantage for subsequent transfer of the Au pattern to hydrogels. As illustrated in Fig. 1b and c, after forming or laminating hydrogels onto the Au microelectrode substrate, poly(3,4-ethylenedioxythiophene) (PEDOT) was electropolymerized in order to bond the Au film to the hydrogel matrix. The PEDOT polymerization of charge density 150 mC/cm² was conducted by applying 1.0 V vs. Ag/AgCl to the Au film in an aqueous solution containing 50 mM EDOT and 100 mM LiClO4. The thickness of the growing PEDOT depends on the kind of hydrogel used. For reference, the thickness of the PEDOT layer (300 mC/cm²) grown in agarose was ca. 5 μm [1]. Since the conductivity of the PEDOT grown in hydrogel is a few orders of magnitude lower than that of the Au electrode, it functions simply as an anchor for bonding the Au electrode to the hydrogel. The Au microelectrodes bonded to the hydrogel by PEDOT were peeled from the glass substrate (Figure 1d), and the bare surface of Au was modified by another electropolymerization of PEDOT in order to decrease the surface impedance.

Figure 1: Schematic fabrication process of Au microelectrodes on hydrogel substrates
RESULTS AND DISCUSSION

Figure 2a shows the Au microelectrode attached to the surface of a polyacrylamide (PAAm) substrate (2 mm thick). The electrode pattern prepared is of an annular interdigital form; the width and spacing of electrodes are 100μm and 500μm, respectively (0.3 cm² total electrode area), which is optimized by Hung et al. for effective electric field generation to the gap of electrodes. Thanks to the bonding effect of PEDOT, the Au electrode was successfully transferred with almost 100 % probability to every kind of natural/synthetic hydrogels tested including collagen, agarose, glucomannan, polyacrylamide, and poly(2-hydroxyethyl methacrylate). The interdigital area of electrode was modified by second polymerization of PEDOT, visible as the black areas in Figure 2b. It is widely known that the modification of the electrode with conducting polymer is effective to decrease the interface impedance, which is advantageous for low-invasive cell stimulation. The double-layer charging capacity of the electrode / electrolyte interface was more than 100 times increased by PEDOT, as calculated from the AC impedance values.

Figure 2. Photographs of the Au microelectrode attached to a PAAm substrate (a) before and (b) after the second polymerization of PEDOT.

The prepared electrodes were used for electroporation of Normal Human Dermal Fibroblasts-Neonatal (NHDF-Neo) that was cultured in FBM medium supplemented with FGM-2 SingleQuots. Although the direct lamination of the electrode to the cells on the culture dish was fairly successful, partial cell detachment of cells was occasionally observed due to the fluid flow that occurred during the lamination process. Therefore, some of experiments were conducted for the NHDF-Neo monolayers transferred onto fibrin gel sheets [3], in which cells were held by the gel, and were thus sufficiently stable for the lamination. A 10 μl pulse medium containing 1.0 mg ml⁻¹ proidium iodide (PI.) was interposed during the lamination, and then 30 electric pulses (applied voltage V_{app} = 60 V; pulse width t_p = 0.1 ms; pulse frequency f_p = 1 Hz) were applied by the electroporator. The longer pulse width over 1 ms caused degradation of PEDOT. PI is an impermeable nuclear stain dye that can be used as a probe to visualize the membrane poration. For evaluation of viability, the cells were treated with 2 μM solution of Calcein-AM, and incubated for 1 h while keeping the laminating condition, followed by the imaging with a conventional fluorescent microscopy.

Figure 3a shows the results of the electroporation with a bare Au/PAAm electrode for a NHDF monolayer transferred to a fibrin sheet. The photograph (a1) shows gas-bubble evolution caused by electrolysis at the polarized bare Au electrode. The occurrence of electroporation can be examined by the fluorescent image of the PI-stained nuclei (a2). However, many of cells do not show green fluorescence of Calcein (a3), indicating their death probably due to the drastic pH change caused by the electrolysis. On the other hand, as shown in Figure 3b, a PEDOT-modified Au/PAAm electrode was effective for electroporation (PI uptake) without significant damage to the cells; most of cells at the electrode gap show both PI and Calcein fluorescence. In fact, no significant gas evolution was observed during the electric pulse application owing to the low-impedance, high-capacity properties of the PEDOT that prevent electrode polarization. Next we applied the PAAm-based electrode to gene delivery into NHDF cells using a pulse medium containing 1.0 mg ml⁻¹ pAcGFP1-C1 plasmid encoding a derivative of green fluorescent protein from Aequorea coerulescens. After 24 hrs incubation, the expression of AcGFP1 was recognized (Figure 3c). Due to the difficulty in uptake of the larger plasmid into a cell than the fluorescent dye, the GFP expression was observed for only part of cells. Further optimization of the pulse voltage, pulse width, and concentration of plasmid should increase the transfection efficiency.
CONCLUSION

Here we have realized Au microelectrodes supported by hydrogel substrates, and demonstrated in-situ electroporation of the adherent cells, by direct lamination of the soft, moist, molecular permeable gel-based electrodes. The PEDOT modification was effective to prevent electrolysis during the electroporation. This in-situ electroporation system is applicable to the normal, standard cultivation protocol. In addition, post-cultivation while keeping the electrode lamination is possible. These advantages apply not only to cultured cells, but also to in-vivo electroporation.

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REFERENCES


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