ELECTRIC MODULATION OF POLYMER GEL CONTRACTION
AND ANIMAL CELL PROLIFERATION

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ABSTRACT

Modulation of gel morphology of synthetic polymer and biopolymer upon low potential application was investigated. Morphology of polymethacrylic acid (PMAA)-Ca\(^{2+}\) chelate microgels has been electrically modulated on a conducting polymer film-coated electrode. The electrode induced a drastic change of ion flux in the vicinity of the conducting polymer film without any water electrolysis, which caused the PMAA-Ca\(^{2+}\) microgels to change rapidly their shape. Electric effects on animal cells cultured on an electrode were also investigated in this line of study. Morphology and proliferation of HeLa cell was dependent on electrode potential during culture. The modulation mechanism is interpreted in relation to the structural change in cytoskeletal gel composed of F-actin.

INTRODUCTION

Polymer gels are widely employed in biomedical fields since these polymers are relatively inexpensive and simple to use. Their use is, however, mostly limited to passive purpose, which may include the uses as a matrix for immobilizing enzymes and living cells.

In contrast to such passive uses, an increasing interest has arisen in developing polymer gels as active elements. Stimuli-responsive motions of polymer gels are attracting an intensive attention in a wide area. An expected application concerns with the development of an actuator system modeled on muscular system\(^1-3\). It may also be exemplified by the modulation of biopolymer gel structures. The morphology of living cells may be regulated by such a modulation, because cells contain gel phase biopolymers.

This paper primarily concerns with the electric modulation of synthetic polymer gel contraction and the designing of an actuator system modeled on the neuromuscular system. Although electrically controlled contraction of polymer gels has been reported by several researchers, these systems need high voltage application in the range from several Volt to several tens of Volt to induce gel contraction, because these electrically induced gel contraction is based on electrophoresis and electro-osmosis\(^4-9\). High voltage application may result in water electrolysis. In order to avoid water electrolysis, gel contraction should be preformed by low voltage application.
On the other hand, tissue contraction by a small electric stimulation is carried out in the neuromuscular system which may suggest the most sophisticated actuator system. In this system, an impulse, small membrane potential change through a neuron, is transduced to molecular signal, a release of neurotransmitters. The release induces a Ca$^{2+}$ flux from the sarcoplasmic reticulum in the muscular cell. This ion flux promotes the contraction and relaxation of myofibril, which is regarded as a gel actuator element. This mechanism suggests that polymer gel morphology may be modulated by controlling ion flux upon low potential application.

We have taken notice of an electrochemical incorporation and release of ions onto conducting polymers to modulate contraction of ion responsive polymer gel by low potential application. An electrochemical incorporation and release of ions onto a polyallyl film-coated electrode were reported by our group$^{10}$. Furthermore, the drastic pH change due to OH$^{-}$ transfer in the vicinity of a polypyrrole film by small shift of potential was also described in a previous paper$^{11}$. Based on these findings, a polypyrrole electrode was coupled with a pH responsive mecanochemical polymer gel. In this study, polymethacrylic acid (PMAA)-Ca$^{2+}$ chelate microgel was employed to achieve rapid modulation of gel morphology by an electrically induced pH change.

In the further study, the electric modulation has successfully been extended to control morphology of living cells. This modulation is expected to be promising to control cellular morphology and cell function such as proliferation.

Electrical techniques have been attracting strong interest in cell manipulation, since it has been shown that cells suspended in a non-electrolytic solution are easily fused upon high d.c. voltage application. However, few studies on cellular behavior under low d.c. potential have been reported to date. In our previous publications we reported the electrochemical effects on erythrocytes as an animal cell model. We employed an optically transparent electrode (OTE) for d.c. potential application to induce the changes in morphology and membrane fluidity of the cells. By taking advantage of the transparency of an indium trioxide (In$\text{O}_3$) sputtered OTE, we were able to observe directly the lysis of erythrocytes at a potential of ca. 1.5V vs. Ag/AgCl$^{12}$.

In this paper, HeLa cells, a human cancer cell, was cultured on an In$\text{O}_3$ OTE to know changes in cellular morphology and proliferation rate under low potential application. These electrochemical effects are discussed in relation to the change of cytoskeleton, a biopolymer gel.

**EXPERIMENTAL**

**Preparation of PMAA-Ca$^{2+}$ Microgels**

An aqueous solution containing 25% polymethacrylic acid (PMAA) sodium salt (molecular weight of 15000), calcium chloride and sodium chloride were purchased and used without further purification. Pyrrole was used after vacuum distillation. All reagent solutions were prepared with ion exchanged and distilled water.

PMAA-Ca$^{2+}$ microgel suspension was prepared by mixing of 1% PMAA Na aqueous solution with 0.1M CaCl$2$ aqueous solution in a volume ratio of 1 : 1. When the two solutions were mixed with a mixer, the solution was turbid due to the formation of microgels. Spherical shape of PMAA-Ca$^{2+}$ chelate microgels in this suspension was observed by a microscope. The average diameter of gels was 3μm.

**Preparation of a Polypyrrole Film-coated OTE**

A Pt thin layer was prepared on a glass plate (1cm × 3cm) by an