Light-Addressed Electrodeposition of Polysaccharide Chitosan Membranes on a Photoconductive Substrate

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Abstract: This paper describes a light-addressed electrolytic system used to electrodeposit polysaccharide chitosan membranes on a photoconductive substrate using a digital micromirror device (DMD). In this system, a patterned-light illumination is projected onto a photoconductive substrate serving as a photo-cathode to electrolytically produce hydroxide ions, which leads to an increased pH gradient. The high pH generated at the cathode can cause a local gelation of chitosan through sol-gel transition. Controlling the illumination pattern on the DMD allowed for the light-addressed electrodeposition of chitosan membranes with different shapes and sizes, as well as multiplexed micropatterning. Experiments were performed to assess the effect of illumination time of the light pattern and the effect of the applied current density on the dimensional resolution of chitosan membrane formation. The spatial resolution in our proposed method was demonstrated to be about 40 μm by patterning with a variety of line-widths and gap distances. We anticipate that photo-electro-chemically-induced deposition of chitosan-based hydrogels may offer interesting opportunities for the integration of biological systems into miniaturized devices.

Keywords: light-addressed electrodeposition; chitosan; digital micromirror device

Introduction

Chitosan is a naturally derived polymer obtained from the deacetylation of chitin, which is an abundant, biocompatible and biodegradable biomaterial. Due to its pH dependent solubility, porous structure, and dense amine groups, chitosan films are well-suited for use as a matrix for the attachment of biomolecules, such as proteins, enzymes, antibodies, and DNA [1-3]. Recently, electrodeposition has emerged as a promising technique for depositing pH-responsive chitosan at specific addresses and in specific shapes on preformed metal thin-film electrode surfaces to generate hydroxide ions (OH^-) by electrolysis on the electrode cathodes [4-7]. This technique allows for improved spatial and temporal control of the chitosan film location and thickness. The electrical co-deposition of chitosan with biomolecules, such as proteins, enzymes, antibodies, and DNA has been successfully demonstrated for biosensors [8-11]. However, gel patterns involving locations, shapes and

dimensions are completely subject to the pre-defined configurations of microelectrodes. Changing these patterns requires the re-design and re-fabrication of the photo-masks, microelectrodes and sometimes the chip structures, thus hampering the development of biological applications which require dynamic and multiplexed micropatterning of chitosan membranes for the attachment of various biomolecules on the same device.

Our previous work [12] proposed a novel light-addressable electrolytic system to electrodeposit calcium alginate hydrogels using a digital micromirror device (DMD). Here, we propose a light-addressed electrolytic system to electrodeposit polysaccharide chitosan membranes on a photoconductive substrate using a DMD. The DMD provides spatio-temporal illumination pattern switching, which is used to achieve flexible electrode patterning for the dynamic and multiplexed electrodeposition of chitosan membranes. Changing the pattern does not require the re-design and re-fabrication of the photo-masks. By controlling the

illumination pattern on the DMD, a light-addressed electrodeposition of chitosan membranes with different shapes and sizes, as well as multiplexed micropatterning, was performed.

Principle

Figure 1 shows a light-addressed electrolytic system used to electrodeposit polysaccharide chitosan membranes on a photoconductive substrate using a DMD. A photoconductive substrate consisting of 0.2 μm of heavily doped hydrogenated amorphous silicon (n+ a-Si:H) and 1 μm of undoped a-Si:H on a 700- μm indium tin oxide (ITO) glass substrate, serves as a light-addressable photo-cathode. A polydimethylsiloxane (PDMS) spacer measuring 0.5 mm in height was bonded to the photoconductive substrate, and an ITO cover glass serving as the anode was used to seal the spacer to form a microchamber. The deposition solution, which contains soluble chitosan, was introduced into the microchamber. A cathode voltage was applied to the photoconductive substrate via the ITO layer, and ITO cover glass served as the anode. Figure 1(b) shows the PDMS spacer bonded to the photoconductive substrate, using an ITO cover glass/anode to seal the microchamber.

When a DC voltage is applied concurrently, light illumination on a photoconductive substrate generates a conducting point that acts as a photo-cathode to electrolytically produce hydroxide ions (OH^-), which can lead to an increased pH gradient (Eq. (1)). The high pH generated at the cathode can cause local gelation of chitosan through sol-gel transition (Eq. (2)):

Chitosan is an amino-polysaccharide (Chit-NH_3^+) that under moderately acidic conditions ($\text{pH} < 6$) is a soluble, cationic polyelectrolyte. When the pH is raised above about 6.3, the amino groups become deprotonated and this polysaccharide can form an insoluble hydrogel network (Chit-NH_2).

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Figure 1. (a) Schematic diagram of a light-addressed electrolytic system for the electrodeposition of polysaccharide chitosan membranes on a photoconductive substrate using a DMD. (b) The PDMS spacer bonded to the photoconductive substrate, using an ITO cover glass as the anode to seal the microchamber (green dye was introduced into the microchamber for visibility purposes).

Experiment Setup

Figure 2 shows an experimental setup for a light-addressed electrolytic system to electrodeposit polysaccharide chitosan membranes on a photoconductive substrate. To observe the illumination pattern and fluorescent images, a standard upright BX51 fluorescence microscope (Olympus, Tokyo, Japan) was used with a charge coupled device (CCD) camera (INFINITY2-1, Lumenera, Ontario, Canada). A DMD-based light-addressing projection optical system was set up under the microscope stage. This setup uses a modified commercial MP525P DMD projector (BENQ, Hsinchu, Taiwan) using a mercury lamp unit as the light source. We modified the commercial DMD projector by simply removing the projection lens and the color filter wheel [12]. The structured light patterns of the DMD were controlled by a computer (C1). The continuous light illuminated the DMD uniformly through the built-in condensing lens within the DMD projector and then spatially projected it onto the photoconductive substrate through a focus lens (L1 and L2), a 50/50 beam splitter, and an objective lens with 10 $\times$ magnification. A cathode voltage was applied to the photoconductive substrate via

membranes on a photoconductive substrate using a DMD as shown in Fig. 4.

Figure 4. By controlling the illumination pattern on the DMD, the logo of National Taiwan Ocean University was patterned in the form of fluorescently labeled chitosan membranes on a photoconductive substrate using a DMD.

Characteristics of Light-Addressed Electrodeposition of Chitosan Membranes

Figure 5 shows the effect of the illumination time of the light pattern and the applied current density on the dimensional resolution (D/D_0) of chitosan membrane formation for the deposition solution of 1% w/v of chitosan at pH = 5.5. The ratio of D/D_0 is defined as the dimensional resolution, where D_0 and D respectively denote an illumination pattern of circular shapes with $D_0 = 400 \mu\text{m}$ in diameter and the corresponding diameter (D) of the chitosan membrane produced by the illumination pattern. The data indicate the average results of five experiments, and the error bar shows the standard deviation under the same operating conditions.

The D/D_0 ratio increases with the applied current density for illumination times lasting 2.5, 5, and 8 min. For a fixed applied current density, the D/D_0 ratio increases with illumination time. These results indicate that an increase in the illumination time or in the applied current density for electrolysis results in an increase in the number of OH^- ions produced at the photo-cathode. The OH^- ions diffuse and then react with the chitosan solution to produce chitosan membranes of larger sizes (D). These results demonstrate that chitosan membranes of varying sizes can be controllably electrodeposited by changing either the illumination time or the applied

current densities. The applied current density and the illumination time can also influence the height of chitosan formation [13].

Figure 5. The effect of the illumination time of the light pattern and the applied current density on the dimensional resolution (D/D_0) for a deposition solution (1% w/v of chitosan; pH = 5.5) with applied current densities of 4 and 6 $\text{A}\cdot\text{m}^{-2}$. (D_0 and D respectively denote the illumination pattern of circular shapes with $D_0 = 400 \mu\text{m}$ in diameter and the corresponding diameter (D) of the chitosan membranes produced by the illumination pattern).

Multiplexed Micropatterning of Chitosan Membranes

We also demonstrate the capability of multiplexed micropatterning to form a specific arrangement of multiple concentric rings of chitosan membranes by performing sequential light-addressed electrodepositions, as shown in Fig. 6. Two different deposition solutions mixed with two different fluorescent colors were sequentially electrodeposited using two different illumination light patterns. First we introduced the deposition solution of soluble chitosan (1% w/v, pH = 5.5) mixed with Rhodamine B (Sigma-Aldrich) into the microchamber. The light-addressed electrodeposition was performed using an illumination pattern to produce the chitosan membranes with a red fluorescence. DI water was introduced to flush the remaining un-solidified chitosan. We then repeated the electrodeposition to form chitosan membranes with a green fluorescent indicator (FITC) adjacent to the former using another illumination pattern. The rightmost image in Fig. 6(a) shows the fluorescence micrograph of multiple concentric rings of chitosan membranes in a specific arrangement. We also performed multiplexed micropatterning to form a 3 by 3 grid of chitosan membranes with overlap at the intersection points as shown in Fig. 6(b). The lower rightmost image in Fig. 6(b) shows a fluorescence micrograph of an assembled 3 by 3 grid of chitosan membranes.

Figure 6. Demonstration of the capability for multiplexed micropatterning of chitosan membranes created by light-addressed electrodeposition using two different illumination light patterns in sequence. (a) Multiple concentric rings of chitosan membranes in a specific arrangement. The rightmost image shows the fluorescence micrograph of multiple concentric rings of chitosan membranes. (b) Multiplexed micropatterning to form 3 by 3 grids of chitosan membranes overlapping at the intersection points. The rightmost image shows the fluorescence micrograph of a 3 by 3 grid of chitosan membranes.

Conclusion

This study proposes a novel electrolytic system for the electrodeposition of polysaccharide chitosan membranes at desired locations on a photoconductive electrode substrate. Light illumination spatially patterned by a DMD produces a photo-cathode that causes the gelation of chitosan membranes (i.e., causes pH to increase to > 6.3). The flexible patternability of the photo-electrodes allows for the generation of desired chitosan membrane patterns at a specific location, providing a great advantage over the electroaddressing of chitosan membranes using conventional metal microelectrodes. We demonstrate the ability to perform micropatterning of chitosan membranes with different shapes and sizes and with multiplexed micropatterning. The spatial resolution in our proposed method was demonstrated at about $40\ \mu\text{m}$. We anticipate that photo-electro-chemically-induced deposition of chitosan-based hydrogels may offer interesting opportunities for the integration of biological systems into miniaturized devices.

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