Electrochemically Induced Deposition of a Polysaccharide Hydrogel onto a Patterned Surface

Rohan Fernandes,[†,‡] Li-Qun Wu,[†,‡] Tianhong Chen,[†,‡] Hyunmin Yi,[†,§] Gary W. Rubloff,[||,⊥] Reza Ghodssi,[⊥,#] William E. Bentley,[†,§] and Gregory F. Payne*[,†,‡]

Center for Biosystems Research, University of Maryland Biotechnology Institute, 5115 Plant Sciences Building, College Park, Maryland 20742; Department of Chemical and Biochemical Engineering, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250; and Department of Chemical Engineering, Department of Materials and Nuclear Engineering, The Institute for Systems Research, and Department of Electrical and Computer Engineering, University of Maryland at College Park, College Park, Maryland 20742

Received December 20, 2002. In Final Form: February 22, 2003

Hydrogels are increasingly considered for creating three-dimensional structures in miniaturized devices, yet few techniques exist for creating such hydrogel structures. We report a new approach for creating hydrogels using the amine-containing polysaccharide chitosan. Specifically, electrodes are immersed into a slightly acidic chitosan solution and a voltage is applied to promote the proton-consuming hydrogen evolution reaction at the cathode surface. This reaction leads to a high localized pH in the vicinity of the cathode surface, and if this localized pH exceeds about 6.3, then chitosan becomes insoluble and deposits at the cathode surface. As the current density is increased, the region of high pH is expected to extend further from the cathode surface into the bulk solution. Using moderately high current densities (50 A/m^2), we observed that chitosan deposited as a thick hydrogel. Measurements of the water content confirmed that the deposited chitosan was a hydrogel. To suggest the potential utility, we deposited a chitosan hydrogel on a patterned surface to create a channel. Because of chitosan's pH-dependent solubility, this channel could be "disassembled" by mild acid treatment. We envision that electrochemically-induced deposition of chitosan-based hydrogels may offer interesting opportunities for the integration of biological systems into miniaturized devices.

Introduction

Miniaturized systems offer the potential of performing functions rapidly, efficiently, and in a parallel processing format. Polymer-based hydrogels are expected to play an increasing role in miniaturized systems for at least two reasons. First, the three-dimensional structure of hydrogels can be exploited to control flow in microfluidic devices.[1−3] These structures can be static (e.g. channels and barriers) or responsive, depending on the polymeric materials. For instance, stimuli-responsive polymeric hydrogels have been used to create microscale valves and actuators that respond to imposed stimuli.[4−9] A second reason for integrating hydrogels into miniaturized systems is that their aqueous microenvironment is appropriate for sensitive biological systems.[10] Thus, hydrogels are often considered for applications involving proteins and nucleic acids,[11−14] and even intact cells.[15−18] Typically, hydrogels are created in miniaturized systems, as indicated in Scheme 1a. The polymers are generated from synthetic monomers (e.g. acrylates) using standard photoinitiated polymerization chemistries. Polymerization is controlled spatially using a mask to direct where polymerization is initiated.

We report an alternative approach for generating miniaturized hydrogels. Specifically, we exploit the amino-

* Corresponding author. Phone: (301)-405-8389. Fax: (301)-314-9075. E-mail: payne@umbi.umd.edu.

† University of Maryland Biotechnology Institute.

‡ University of Maryland, Baltimore County.

§ Department of Chemical Engineering, University of Maryland at College Park.

|| Department of Materials and Nuclear Engineering, University of Maryland at College Park.

⊥ The Institute for Systems Research, University of Maryland at College Park.

Department of Electrical and Computer Engineering, University of Maryland at College Park.

(1) Sheppard, N. F.; Lesho, M. J.; McNally, P. *Sens. Actuators, B* **1995**, *28*, 95−102.

(2) Seong, G. H.; Zhan, W.; Crooks, R. M. *Anal. Chem.* **2002**, *74*, 3372−3377.

(3) Zhan, W.; Seong, G. H.; Crooks, R. M. *Anal. Chem.* **2002**, *74*, 4647−4652.

(4) Chen, G.; Imanishi, Y.; Ito, Y. *Langmuir* **1998**, *14*, 6610−6612.

(5) Hoffmann, J.; Plotner, M.; Kuckling, D.; Fischer, W.-J. *Sens. Actuators* **1999**, *77*, 139−144.

(6) Beebe, D. J.; Moore, J. S.; Bauer, J. M.; Yu, Q.; Liu, R. H.; Devadoss, C.; Jo, B.-H. *Nature* **2000**, *404*, 588−590.

(7) Beebe, D. J.; Moore, J. S.; Yu, Q.; Liu, R. H.; Kraft, M. L.; Jo, B.-H.; Devadoss, C. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13488−13493.

(8) Liu, R. H.; Yu, Q.; Beebe, D. J. *J. Microelectromech. Syst.* **2002**, *11*, 45−53.

(9) Yu, Q.; Bauer, J. M.; Moore, J. S.; Beebe, D. J. *Appl. Phys. Lett.* **2001**, *78*, 2589−2591.

(10) Ito, Y.; Chen, G.; Guan, Y.; Imanishi, Y. *Langmuir* **1997**, *13*, 2756−2759.

(11) Burns, M. A.; Johnson, B. N.; Brahmasandra, S. N.; Handique, K.; Webster, J. R.; Krishnan, M.; Sammarco, T. S.; Man, P. M.; Jones, D.; Heldsinger, D.; Mastrangelo, C. H.; Burke, D. T. *Science* **1998**, *282*, 484−487.

(12) Sirkar, K.; Pishko, M. V. *Anal. Chem.* **1998**, *70*, 2888−2894.

(13) Revzin, A. F.; Russell, R. J.; Yadavalli, V. K.; Koh, W. G.; Deister, C.; Hile, D. D.; Mellott, M. B.; Pishko, M. V. *Langmuir* **2001**, *17*, 5440−5447.

(14) Revzin, A. F.; Sirkar, K.; Simonian, A.; Pishko, M. V. *Sens. Actuators, B* **2002**, *81*, 359−368.

(15) Stenger, D. A.; Gross, G. W.; Keefer, E. W.; Shaffer, K. M.; Andreadis, J. D.; Ma, W.; Pancrazio, J. J. *Trends Biotechnol.* **2001**, *19*, 304−309.

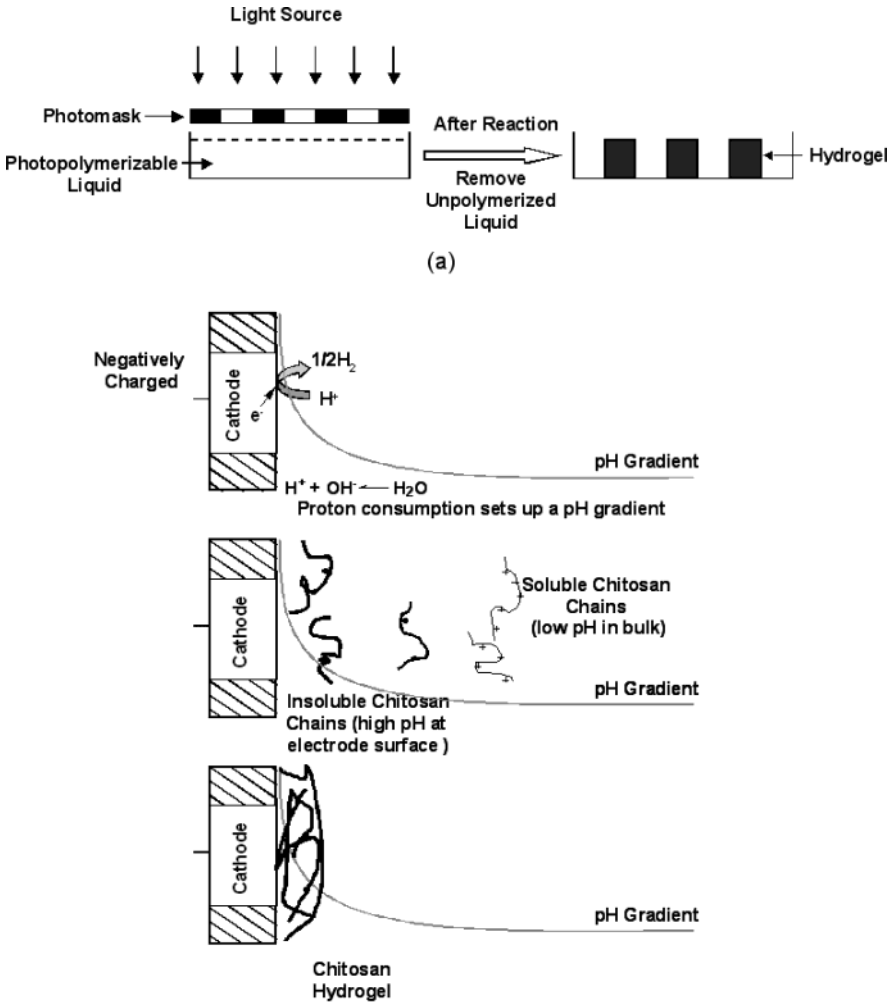
(16) O'Connor, S. M.; Andreadis, J. D.; Shafferf, K. M.; Ma, W.; Pancrazio, J. J.; Stenger, D. A. *Biosens. Bioelectron.* **2000**, *14*, 871−881.

(17) Reininger-Mack, A.; Thielecke, H.; Robitzki, A. A. *Trends Biotechnol.* **2002**, *20*, 56−61.

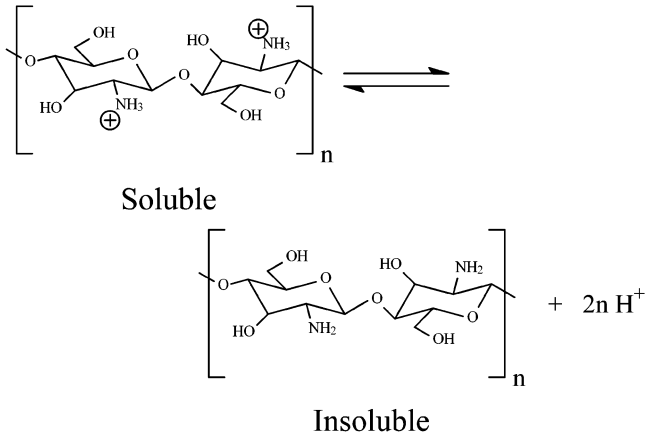
(18) Koh, W.-G.; Revzin, A.; Pishko, M. V. *Langmuir* **2002**, *18*, 2459−2462.

10.1021/la027052h CCC: $25.00
Published on Web 04/15/2003

Scheme 1



polysaccharide chitosan that undergoes pH-dependent hydrogel formation. At low pH (pH < 6), chitosan is protonated and soluble. When the pH is raised above about 6.3, the amino groups become deprotonated and this polysaccharide can form an insoluble hydrogel network.[19,20]



To create hydrogels at the interface between a solid and a chitosan solution, we generate localized regions with high interfacial pHs. Scheme 1b shows that the high localized pH is generated electrochemically at the cathode surface due to the hydrogen evolution reaction. The rate of this electrochemical reaction is proportional to the current density and can be adjusted by the applied voltage.[21] As indicated in Scheme 1b, proton consumption at the cathode surface is partially compensated for by proton generation from the dissociation of water. A pH gradient can be generated adjacent to the cathode surface, depending on the relative rates of hydroxyl ion generation and hydroxyl ion diffusion from the interfacial region. The generation of a pH gradient at the cathode surface is well-established in electrochemical systems and has been used to explain the anomalous codeposition of metals.[22−25]

(19) Varum, K. M.; Ottoy, M. H.; Smidsrod, O. *Carbohyr. Polym.* **1994**, *25*, 65−70.
(20) Sorlier, P.; Denuziere, A.; Viton, C.; Domard, A. *Biomacromolecules* **2001**, *2*, 765−772.
(21) Haripriya, S.; Sangaranarayanan, M. V. *Langmuir* **2002**, *18*, 5572−5578.
(22) Dahms, H.; Croll, I. M. *J. Electochem. Soc.* **1965**, *112*, 771−775.
(23) Higeshi, K.; Fukushima, H.; Urakawa, T.; Adaniya, T.; Matsudo, K. *J. Electochem. Soc.* **1981**, *128*, 2081−2085.
(24) Hessami, S.; Tobias, C. W. *AIChE J.* **1993**, *39*, 149−162.
(25) Paunovic, M.; Schlesinger, M. *Fundamentals of Electrochemical Deposition*; Wiley-Interscience: New York, 1998.

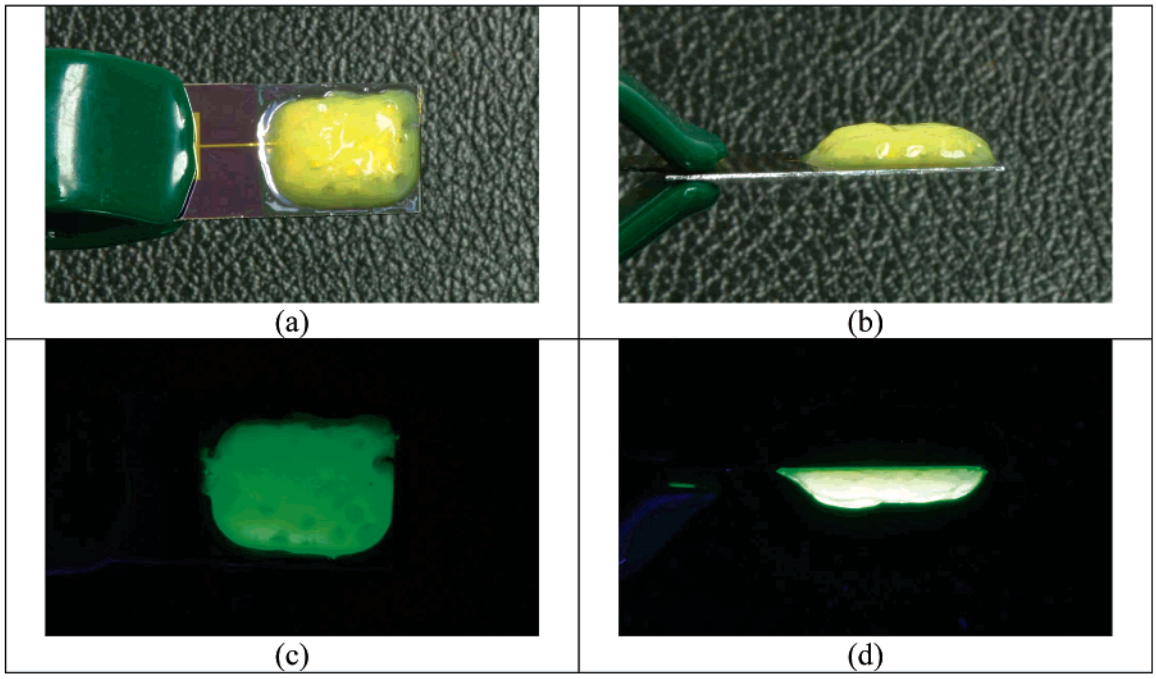


Figure 1. Chitosan hydrogel deposited on a patterned gold cathode: (a) top and (b) side views; (c) top and (d) side views using a UV light source. Deposition was carried out using 1.5% w/w fluorescently labeled chitosan of bulk pH = 5.5 for 20 min at a current density of 50 A/m^2. The thickness (i.e. height) of the deposit was estimated to be 5 mm. The "pores" created by the evolved gas bubbles are evident in these photos.

Scheme 1b illustrates our hypothesis for the creation of miniaturized chitosan hydrogels at the cathode surface. Specifically, the electrodes are immersed in a slightly acidic chitosan solution. A pH gradient is established in the immediate vicinity of the cathode surface when a voltage is applied to the electrodes. A chitosan chain that experiences the localized region of high pH is deprotonated and becomes insoluble when the localized pH exceeds about 6.3. Depending on conditions, the insoluble chitosan chains can form a three-dimensional hydrogel network. This electrochemically induced deposition mechanism allows chitosan hydrogels to be "constructed" in a spatially controlled manner and may offer unique opportunities for use in microfabricated devices.

Materials and Methods

Chitosan from crab shells (85% deacetylation and 370 000 molecular weight, as reported by the manufacturer) was purchased from Sigma-Aldrich Chemicals. Details of how we prepared the solutions and fabricated the patterned surfaces have been described elsewhere.[26,27] Chitosan solutions were prepared by dissolving flakes in HCl, and then NaOH was added to adjust the pH. Fluorescently-labeled chitosan was prepared by reacting NHS-fluorescein (5- (and 6)-carboxyfluorescein succinimidyl ester) with chitosan. The patterned surfaces were fabricated by depositing 150 Å thick chromium and then 2000 Å thick gold layers on 4 in. diameter silicon wafers, which had previously been coated with a 1 μm thick thermal oxide film. Patterning was achieved using standard photolithography materials and methods.

For deposition, the patterned surfaces were immersed in solutions containing 1.5% w/w of either fluorescently-labeled chitosan or unlabeled chitosan. In all cases, the patterned gold surfaces were polarized to serve as negative electrodes (i.e. cathodes). The anode in these experiments was an unpatterned, highly doped silicon wafer. The two electrodes were connected to a dc power supply (Model 6614C, Agilent Technologies) using alligator clips. Deposition was performed for varying times at varying fixed current densities (galvanostatic conditions). After deposition, the electrodes were disconnected from the power supply and removed from the solutions. Photographs of the hydrogel were taken using a digital camera (Canon EOS D-60) with a 90 mm lens. The patterned surfaces were also examined with a fluorescence stereomicroscope (MZFLIII, Leica) using a fluorescence filter set (GFP Plus) with an excitation filter at 480 nm (bandwidth of 40 nm) and an emission barrier filter at 510 nm. Photomicrographs were prepared from the fluorescence microscope using a digital camera (Spot 32, Diagnostic Instruments).

The water content of the hydrogels was determined by weighing the material before and after oven-drying overnight at 45 °C. The pH of the water within the hydrogel was measured by collecting the hydrogel and compressing it to squeeze out the fluid from the hydrogel matrix. The pH of the collected fluid was measured using a pH meter (Basic Accumet, Fisher Scientific).

Results and Discussion

Initial experiments were conducted to demonstrate that chitosan-based hydrogels could be deposited onto the cathode surface. In these experiments, a patterned cathode (1.5 cm^2) and an anode were immersed in a solution containing fluorescently-labeled chitosan (1.5% w/w; pH = 5.5) for 20 min, and a current density of 50 A/m^2 was maintained. After deposition, the electrodes were disconnected from the power supply and removed from the solution. The photograph in Figure 1a shows that a chitosan-based hydrogel is deposited on the cathode surface (the gold region below the hydrogel) while small amounts of chitosan are observed to extend onto the unpatterned regions (the dark silicon oxide region of the wafer). The side view of the deposited hydrogel is shown in Figure 1b. The thickness of the deposited hydrogel was estimated to be 5 mm. Parts c and d of Figure 1 show the top and side views, respectively, of this fluorescently labeled hydrogel illuminated using a UV-light source. The dark spots that are visible in Figure 1c are "pores" that are most probably created by the hydrogen gas bubbles

(26) Wu, L.-Q.; Gadre, A. P.; Yi, H.; Kastantin, M. J.; Rubloff, G. W.; Bentley, W. E.; Payne, G. F.; Ghodssi, R. *Langmuir* **2002**, *18*, 8620−8625.

(27) Wu, L.-Q.; Yi, H.; Li, S.; Rubloff, G. W.; Bentley, W. E.; Ghodssi, R.; Payne, G. F. *Langmuir* **2003**, *19*, 519−524.

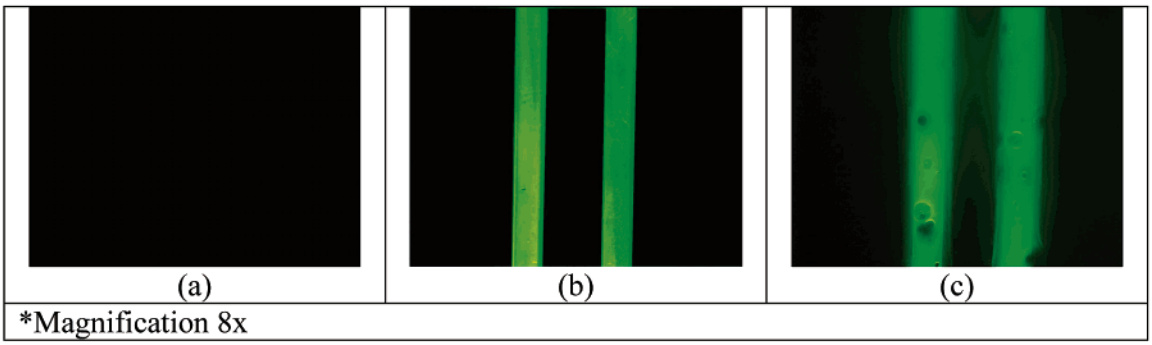


Figure 2. Chitosan deposition is controlled by conditions. Photomicrographs are from a fluorescence stereomicroscope of the cathode. (a) No deposition is observed when a low bulk pH (2) and low current density (2 A/m^2) are used. (b) Thin, well-resolved layers are deposited when a higher pH (5.5) and low current density (2 A/m^2) are used. (c) Thick, less well-resolved hydrogels are deposited when a higher pH (5.5) and high current density (50 A/m^2) are used. Each deposition run was performed using 1.5% w/w fluorescently labeled chitosan for 2 min. Each gold electrode was 1 mm wide and spaced 2 mm apart.

evolved at the cathode surface. As seen from Figure 1d, the hydrogel could be inverted and it remained attached to the electrode surface. The results in Figure 1 provide visible evidence that chitosan-based hydrogels can be deposited at the cathode surface in response to an applied voltage.

The hydrogels could be stored in distilled water for periods of up to 1 week with no noticeable changes in structure. Longer periods of storage were not investigated. When the hydrogels were stored in air, they were observed to dehydrate over the course of 2−3 days.

Separate experiments were also performed in which unlabeled chitosan (1.5% w/w; pH = 5.5) was deposited onto the same electrodes for 1 h at 50 A/m^2. After deposition, the water content of the hydrogel was measured to be 98%, indicating that the deposited material is a hydrogel. In another experiment, the deposited material was collected and compressed to squeeze out the fluid from the hydrogel matrix. We collected this fluid and found the pH to exceed 11 (beyond the range that can be confidently measured by our pH meter). This pH measurement is consistent with Scheme 1b, in which it is proposed that chitosan hydrogels are deposited in response to a high localized pH near the cathode surface.

To test the hypothesis that hydrogel deposition at the cathode surface results from a high localized pH (Scheme 1b), we investigated the effect of two parameters that would be expected to have a significant effect on deposition (i.e. current density and bulk pH). The rate of proton consumption should be directly proportional to current density, and thus the pH gradient is expected to become steeper with increased current density. The bulk pH is proposed to be important because a localized pH must exceed 6.3 if chitosan is to become insoluble and deposit at the surface. Experimentally, a patterned cathode and an anode were immersed in a solution containing fluorescently-labeled chitosan (1.5% w/w) for 2 min. For this study we examined a region of the cathode containing two parallel gold lines (1 mm wide) separated by a 2 mm space. After deposition, the surface was rinsed and viewed using a fluorescence microscope. The view field appears uniformly dark if no deposition of the fluorescently labeled chitosan had occurred.

In experiment a, the bulk solution pH was low (pH = 2) and the current density was also low (2 A/m^2). Both conditions should suppress chitosan deposition. When the electrode was viewed using a fluorescence microscope, Figure 2a shows that no chitosan deposition was observed. It should be noted that deposition could be observed under these conditions if longer deposition times were used.

In experiment b, the bulk solution pH was increased to 5.5 and the current density was maintained low at 2 A/m^2. Under this condition the pH gradient is expected to be shallow although the pH value very close to the cathode surface could exceed a value of 6.3, where chitosan becomes insoluble. Figure 2b shows that a well-resolved layer of fluorescently-labeled chitosan was deposited on the cathode surface.

For experiment c, the bulk solution pH was high (pH = 5.5) and the current density was also high at 50 A/m^2. This condition is expected to yield both a high localized pH and a substantial gradient extending from the cathode surface. Figure 2c shows that a diffuse deposit is observed and pores resulting from the evolution of gas bubbles are clearly visible. A larger number of pores are expected under this condition because the higher current density leads to greater hydrogen gas evolution. The “diffuseness” of the image in Figure 2c is consistent with the deposition of a thicker hydrogel under these conditions.

In summary, the results in Figure 2 support the proposed hypothesis of Scheme 1b that chitosan deposition results from a high localized pH. If conditions do not permit the creation of a region of high localized pH, then no deposition is observed (Figure 2a). If conditions favor a narrow region of high pH immediately adjacent to the cathode surface, then a thin chitosan deposit is observed (Figure 2b). Finally, if conditions favor the creation of an expanded region where the pH exceeds chitosan's solubility limit, then chitosan deposits as a thicker, more diffuse hydrogel (Figure 2c).

A final experiment was performed to suggest a potential use for hydrogel deposition. Specifically, we deposited a “patterned” hydrogel to create a channel and barrier. In this study, a hydrogel was deposited from a solution containing chitosan (1.5% w/w; pH = 5.5) onto a patterned cathode consisting of 1 mm wide gold lines separated by a 7 mm space. The deposition was carried out for 20 min at a fixed current density of 50 A/m^2. Figure 3a is an inclined view of the deposited hydrogel and shows that deposition occurred along the electrode lines. Although some hydrogel was observed to extend past the edge of the gold pattern onto the substrate, no deposition occurred in the unpatterned space midway between the two electrodes. In general, we observed that the lateral resolution of the deposited hydrogel is inversely related to the thickness of the deposit. Figure 3b shows a side view of the deposited hydrogel. The thickness of the deposit along each gold line (i.e. the height of the channel walls) was estimated to be 3.5 mm, and the width of the hydrogel

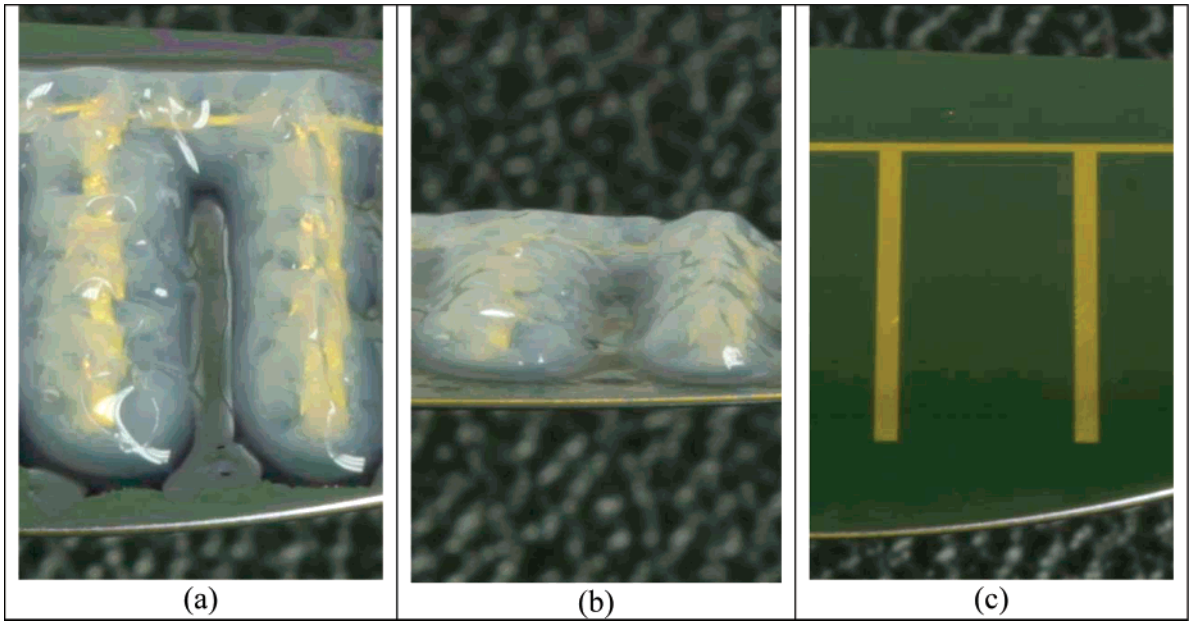


Figure 3. Assembly and disassembly of a chitosan-based hydrogel channel: (a) inclined and (b) side views of the chitosan hydrogel deposited on the patterned cathode. Deposition was performed using 1.5% w/w chitosan of bulk pH = 5.5 for 20 min at a current density of 50 A/m^2. The thickness (i.e. height) and width of the hydrogel deposit were estimated to be 3.5 and 5.5 mm, respectively. (c) Top view of the electrode surface photographed 15 min after the deposited hydrogel was treated with 1 M HCl and rinsed with distilled water. The deposit was completely dissolved. Each gold electrode was 1 mm wide and spaced 7 mm apart.

was estimated to be 5.5 mm. The width of the channel at the base between the hydrogel walls was estimated to be 1.5 mm.

An interesting, and potentially important, feature of chitosan is that its pH-responsive solubility allows chitosan-based structures to be "disassembled" by a mild acid treatment. This disassembly is demonstrated in Figure 3c which shows the cathode surface after the chitosan hydrogel deposit was treated with 1 M HCl for 15 min and then rinsed with distilled water. This treatment completely dissolved the chitosan hydrogel from the surface. Chitosan deposits could also be removed using milder acid treatments if longer times were allowed. The results in Figure 3 demonstrate that chitosan hydrogels can be deposited in a spatially selective manner to create semipermanent structures and that these structures can be disassembled.

Conclusions

We report that chitosan-based hydrogels can be deposited at a cathode surface in response to an applied voltage. Experimental results support the hypothesis that the hydrogel deposits at the cathode surface in response to the localized pH gradient that results from the electrochemical hydrogen evolution reaction. These hydrogels persist in aqueous solution under neutral or basic conditions but can be readily removed from the surface by treatment with mild acid.

We believe these observations are potentially important for three reasons. First, electrochemical deposition may provide an alternative method for generating hydrogels at specific locations within miniaturized devices. There is a growing interest in integrating hydrogels into miniaturized systems (e.g. microelectromechanical systems, MEMS), yet there are few methods available for generating localized hydrogels in such systems. Most studies employ photoinitiated polymerization reactions to generate hydrogels. To our knowledge, this is the first report on the deposition of a hydrogel in response to an electrochemically-generated pH gradient.

The second reason we believe these observations are potentially important is that the deposition procedure is simple and gentle, and could be performed with sensitive biological systems (e.g. cells). Specifically, chitosan is a polysaccharide that has been reported to be biocompatible.[28] Further, chitosan's transition from solubility-to-insolubility occurs at pH values at, or near, physiological conditions, suggesting that chitosan's "smart" properties are well-suited for biological applications.

Finally, chitosan's pH-responsive properties may be useful in devices. For instance, the ability to "disassemble" chitosan hydrogels by mild acid treatment suggests their utility as single-use valves[29] or controlled release systems.[30] Additionally, if the deposited chitosan hydrogels are further cross-linked to prevent them from dissolving, then it might be possible to generate gels with pH-responsive swelling properties. Because chitosan is basic, its pH-responsive swelling properties would complement those properties from acidic gels (i.e. cross-linked chitosan hydrogels would swell at low pH while cross-linked acrylic acid hydrogels swell at high pH).

Acknowledgment. Financial support for this research was provided by the United States Department of Agriculture (2001-35504-10667), Venture Innovations (as a subcontract to NSF Grant DMI-9901868), the National Science Foundation (Grant BES-0114790), and the United States Department of Energy (DE-FG02-01ER63109).

LA027052H

(28) Kumar, M. N. V. R. *React. Funct. Polym.* **2000**, *46*, 1−27.
(29) McDonald, J. C.; Metallo, S. J.; Whitesides, G. M. *Anal. Chem.* **2001**, *73*, 5645−5650.
(30) Santini, J. T.; Cima, M. J.; Langer, R. *Nature* **1999**, *397*, 335−338.