

Low temperature, low pressure nanoimprinting of chitosan as a biomaterial for bionanotechnology applications

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Micro- and nanoscale structures of chitosan were fabricated by nanoimprinting lithography and biochemically functionalized for bionanodevice applications. Chitosan solutions were prepared and a nanoimprinting process was developed for it, where chitosan solution is used as a functional resist for nanoimprinting lithography. A low temperature (90 °C) and low pressure (5–25 psi) nanoimprinting with polydimethylsiloxane mold could achieve not only microscale structures but also nanoscale features such as nanowire and nanodots down to 150 nm dimensions. The nanoimprinted structures were chemically modified and used for the immobilization of protein molecules. © 2007 American Institute of Physics. [DOI: 10.1063/1.2709914]

Chitosan is a β -1,4-linked polysaccharide obtained by a partial deacetylation of chitin, which is easily obtained from shrimp or crab shells. Chitin is a polysaccharide consisting of (1 \rightarrow 4)-linked *N*-acetyl- β -D-glucosamine with a similar chemical structure to cellulose. The boundary between chitosan and chitin is normally set at approximately 60% degree of acetylation, based on chitosan's solubility in acids. Chitosan has been used in a variety of applications such as wound healing treatment,¹ cosmetics,² artificial skin,³ pharmaceutical additive,⁴ metal capture agent in water treatment,⁵ etc. Recently, its usage has been further extended in other bioengineering such as controlled drug release^{6,7} and biochemical functionalization of surfaces as an antibacterial agent⁸ and cell adhesion promoter.⁹ These wide varieties of applications of chitosan are due not only to its excellent biocompatibility, biodegradability, and economic efficiency but also to distinct chemical structures with high percentage of primary amino groups ($-\text{NH}_2$) for easy binding with biomolecules such as DNAs and proteins.¹⁰ Many physical forms and corresponding approaches have been used for the manufacturing of chitosan-based products and devices. Its physical phases include microfiber by interfacial polyelectrolyte complexation,¹¹ electrowet spinning,¹² membrane or thin film by spin casting,^{13,14} or porous sponge by hydrogel formation from regeneration reactions followed by subsequent freeze-drying.¹⁵

Although there already exist some fabrication methods for chitosan structures, relatively little work has been reported in the micro-nanoscale patterning of chitosan except for electrodeposition on metal electrodes.¹⁶ The patterning of chitosan at the microscale and nanoscale will enable the integration of chitosan as a useful biomaterial in micro-nanodevices (eg. biodetection sensors, biomimetic sensors, and actuators). Electrodeposition has been a popular technique for the microscale desposition of chitosan due to chitosan's distinctive pH-dependent properties.¹⁷ In other studies, spin casting of chitosan solution in acetic acid on metal

patterned substrates and localized phenol reaction cascade by electrochemical oxidation along metal patterns created a localized transformation of chitosan film.¹⁴ Also, microcontact printing method has been applied to create a microscale grating pattern of chitosan for localized cell adhesion.¹⁸ Recently, an imprinting technique with the aid of plasticizer mixture has been developed for the low temperature/low pressure submicron-scale patterning of chitosan thin films.¹⁹ Although the aforementioned patterning methods provide their own advantages, there are various limitations. Electrodeposition techniques always require a metallic layer underneath chitosan, which can sometimes be incompatible with some sensing applications as well as the acidic solution itself. Furthermore it does not provide a smooth surface morphology. Microcontact printing methods can only create very thin layers of chitosan. Usage of plasticizer in imprinting lithography for lowering the viscosity of chitosan solution may interfere with the chemical stability and biofunctionality of chitosan molecules and also involves additional complexity of fabrication protocol.

In this letter, we introduce single-step nanoimprinting lithography of chitosan at the micro- and nanoscale without the introduction of additional chemistry such as plasticizers. Conventional nanoimprinting lithography requires high temperature/pressure (hot embossing method) or UV irradiation (step and flash approach). In our study, we take advantage of unique fluidic properties and low temperature solidification properties of chitosan solutions to achieve a low temperature/pressure nanoimprinting process. We first show the control of the fluidic viscosity of chitosan solution by adjusting the concentration of chitosan in the solution of acetic acid (HAc)+de-ionized water (DI H₂O). Then, the imprinting results of chitosan patterning are explained with surface morphology issues. Finally, a biochemical modification and protein immobilization on imprinted chitosan structures are demonstrated.

Medium molecular weight (approximately 250 kDa) chitosan with an average deacetylation of 80% was used in the chitosan solutions. It was dissolved in HAc:DI H₂O=1:2

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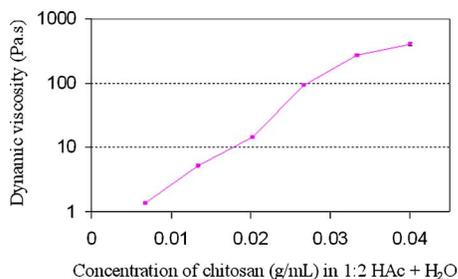


FIG. 1. (Color online) Estimation of dynamic viscosity of chitosan solution for different concentrations at room temperature.

solution. To form a hydrogel, the solvent was first stirred and heated to 40 °C. Once the solvent temperature stabilized, the chitosan powder was added slowly while the solution was continuously mixed. Vacuum filtering of the solutions was then performed utilizing membrane filters from 0.22 to 5 μm pore size to filter out remaining microcontaminates including dust particles. The dynamic viscosity of prepared chitosan solution at room temperature was estimated by using a concentric viscometer. Chitosan solutions were poured onto a chamber where a cylindrical bar was rotated at constant speeds through equilibrium between viscous friction by chitosan solution and weight connected through a pulley system. The viscosity of solution was calculated by measuring rotation periods of the cylindrical bar. For simplicity of the analysis, we have assumed that the chitosan solution is a Newtonian fluid and that the friction force from the bottom surface of viscometer is negligible. The viscosity of the chitosan solutions was found to be a very strong function of the concentration of chitosan, as shown in Fig. 1. It spans from 1.393 Pa s for 0.0068 g/ml solution to 376.57 Pa s for 0.0467 g/ml solution.

Polydimethylsiloxane (PDMS) was used as a material for the nanoimprinting mold. Molds with microscale patterns were made using photolithographically defined *G*-line photoresist patterns on a silicon wafer. Molds with nanoscale patterns were prepared by using nanoscale chrome patterns fabricated by electron-beam lithography of polymethyl methacrylate (PMMA) and metal lift-off process. A small volume of chitosan solution was dispensed at the center of the substrate. Then the PDMS mold was placed on top of the drop of chitosan solution. A moderate heating temperature (90 °C) and pressure (5–25 psi) were applied onto the PDMS mold for 30 min. The usage of solvent-based chitosan solution as a nanoimprint resin required paths for the efficient evaporation of solvent during the heating step. PDMS molds, as opposed to silicon or glass-based hard molds, absorbs the solvents during the hot embossing process. After cooling down to room temperature, the PDMS mold was carefully taken off from the substrate. The atomic force microscopy (AFM) images of fabricated microscale and nanoscale features are shown in Fig. 2. Microstructures such as microwell (3.5 μm diameter and 220 nm depth), micropost (2.2 μm width and 350 nm height), and checkerboard (2.2 μm width and 280 nm height) are shown in Figs. 2(a)–2(c). Also, nanoscale features such as nanowire (150 nm width; 500 nm pitch) or nanodot (150 nm width; 400 nm pitch) were successfully patterned, as shown in Figs. 2(d) and 2(e). No adhesion of the chitosan layer on the PDMS mold was noticeable after the nanoimprinting process. PDMS provides a very sound surface chemistry for

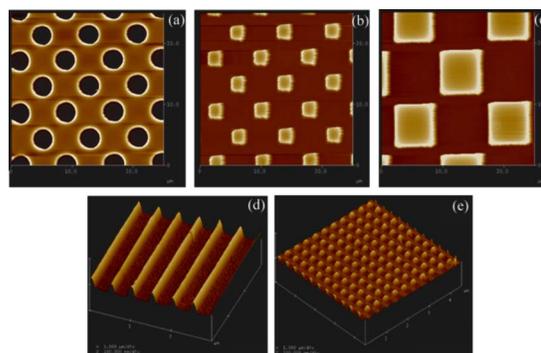


FIG. 2. (Color online) Atomic Force Microscopy (AFM) images of microscale [(a) microwell, (b) micropost, and (c) checkerboard] and nanoscale [(d) nanowire and (e) nanodot] structures of chitosan fabricated by nanoimprinting lithography.

easy and clean demolding performance with no sticking problems without any additional surface treatment. However, three-dimensional morphology shows a raised height around the feature edges for microstructures with dimensions $>2.2 \mu\text{m}$. This “rabbit-ear” effect becomes more significant for larger features, as shown in Fig. 3. We speculate that this effect is due to (1) wetting of chitosan solution along the surface of microscale trenches of the mold, (2) incomplete filling of trenches during the imprinting process, and (3) pulling of imprinted chitosan features by molds during the demolding step. In nanoscale features, the surface-to-volume ratio in the trenches of the mold becomes much larger, which in effect helps better fill the nanoscale trenches with the chitosan solutions. Also, the height of the nanoscale trench is much smaller ($\sim 30 \text{ nm}$) than that of microscale trench ($\sim 1 \mu\text{m}$). This gives little contact area along the trench’s sidewalls for pulling of the nanoimprinted structure during the demolding step.

Chitosan contains a large quantity of primary amino groups ($-\text{NH}_2$) readily available for chemical modification by different biochemical groups such as peptide,²⁰ sugar,²¹ ether,²² etc. In order to use these micro-nanostructures of chitosan for bionanodevice applications, they have to be modified with the desired chemical groups. Most buffers for the chemical modification are water-based solutions. Chitosan is a type of hydrogel, which shows a great degree of swelling in aqueous environments. Therefore the mechanical and chemical robustness of the nanoimprinted chitosan struc-

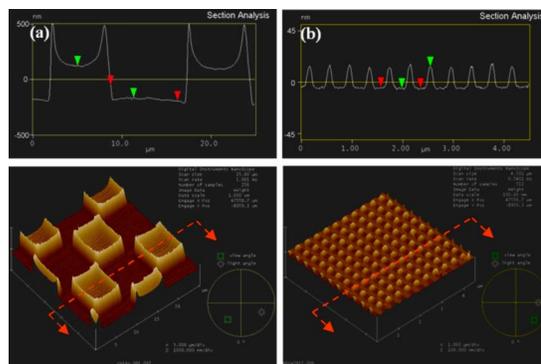


FIG. 3. (Color online) “Rabbit-ear” effects for nanoimprinting of chitosan. (a) This effect is significant for checkerboard with $6.7 \times 6.7 \mu\text{m}^2$ squares (b) This effect cannot be found for nanoscale features as array of nanodots with 150 nm width and 400 nm pitch.

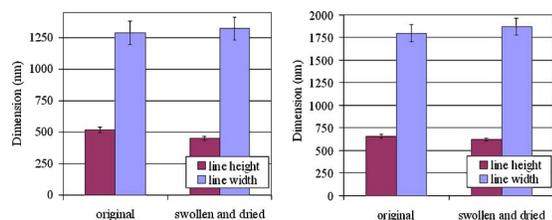


FIG. 4. (Color online) Effect of swelling in de-ionized H_2O on the dimensions of sub- $2 \mu m$ imprinted chitosan imprinted with (a) 0.013 g/ml solution and (b) 0.030 g/ml solution.

tures should be verified for further chemical modification. Figure 4 shows the effect of hydration on the dimensions of imprinted chitosan by DI H_2O . An array of sub- $2 \mu m$ lines was scanning by AFM tapping mode and then immersed in the DI H_2O for 1 h at room temperature. Then, after gentle drying with N_2 , its dimension was measured again by AFM. Chitosan structures imprinted with 0.013 g/ml solution show a slight decrease in the mean height (519.8 to 451.1 nm) after swelling and subsequent drying. However, the change in width is negligible. Also, the structures imprinted with 0.030 g/ml solution do not show any noticeable change both in their widths and heights. Thus, the swelling by chemical modification during chemical modification process appears not to cause any structural deformation or damage on the nanoimprinted features of chitosan. It is speculated that the decrease of mean height for 0.013 g/ml solution stems from either the compression of structures by the N_2 blow dry or slight dissolution of chitosan in the aqueous environment.

We verified that the nanoimprinted chitosan patterns contain abundant primary amino groups available for further chemical modification by using 5- (and 6-) carboxyfluorescein, succinimidyl ester (NHS fluorescein). NHS ester reacts with primary amino groups in a pH of 7–9 environment to form stable amide bonds. After the nanoimprinted chitosan pattern was immersed in the solution of NHS fluorescein for 30 min at room temperature, it showed a bright green fluorescent image from the structures of chitosan, as shown in Figs. 5(a) and 5(b). Here, the bright illumination plays as an indicator of abundant primary amino groups in the imprinted chitosan structure for further chemical modification. Finally, we tested the immobilization of protein molecules on chemically modified chitosan structures. The nanoimprinted chito-

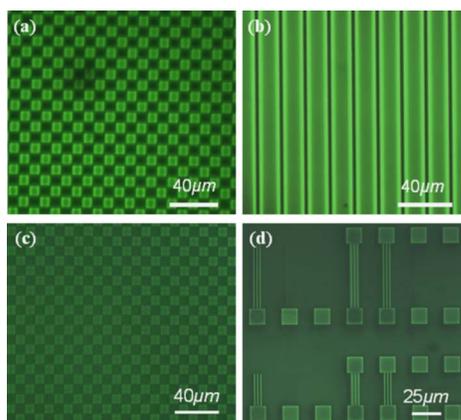


FIG. 5. (Color online) Fluorescence image of imprinted chitosan microstructures [(a) and (b)] chemical modification of chitosan by NHS-fluorescein. [(c) and (d)] FITC-streptavidin immobilized on biotinylated chitosan microstructures [(c) checkerboard and (d) microbeam].

san structures were first modified with sulfosuccinimido-biotin (sulfo-NHS-biotin) for the biotinylation of primary amino groups. Then the fluorescein (FITC) conjugated streptavidin molecules were immobilized on the biotinylated chitosan structures. The fluorescence images of FITC-streptavidin immobilized on the nanoimprinted and biotinylated chitosan structures are shown in Figs. 5(b) and 5(c). This result indicates that nanoimprinted chitosan structures can serve as a useful platform for biomolecule immobilization and detection.

Nanoimprinting lithography of chitosan imprinting resist provides an extremely simple and inexpensive fabrication method for micro- and nanoscale structures of chitosan. The imprinted chitosan can be chemically modified due to abundant primary amino groups available for the chemical reaction. Optical detection of streptavidin immobilized on the biotin-modified chitosan structures has been demonstrated. We anticipate that nanoimprinted chitosan patterns will serve as versatile structures for numerous bionanotechnology applications.

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