

Chemically Modified Chitosan for Real-time Direct Laser Writing of Microstructures

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In this paper, chemically modified chitosan is presented as an environmentally friendly, optically transparent, elastic and durable material for the fabrication of various microstructures in real time. The brittle chitosan layer was chemically modified to become elastic and stretchable, while the photosensitivity of the layer was achieved by anthocyanin food dye E163. The biocompatibility test confirmed that the chitosan layer chemically modified in this way is non-toxic. Various microstructures were produced on the layer by direct laser writing at wavelength of 488 nm, with a maximum output power of 100 mW, using a homemade laser writing system. Concave or convex microlenses (single and arrays), microchannels, diffraction gratings and other complex structures. are rapidly produced. The fabricated microstructures can be used directly, without additional chemical processing and any waste. The resulting structures are suitable for numerous applications including lab-on-a-chip, medical lasers, optical sensors, light field cameras, security technologies and biomedicine.

Key Words: chitosan, anthocyanin food dye E163, microstructures, direct laser writing, biocompatibility

1. INTRODUCTION

Chitosan is a partially deacetylated derivative of chitin-natural polymer found in the exoskeleton of crustaceans and insects. Chitosan is a linear polysaccharide composed of β -(1-4)-linked d-glucosamine and N-acetyl-d-glucosamine (Figure 1). It is available in a range of molecular weights and degrees of deacetylation [1, 2].

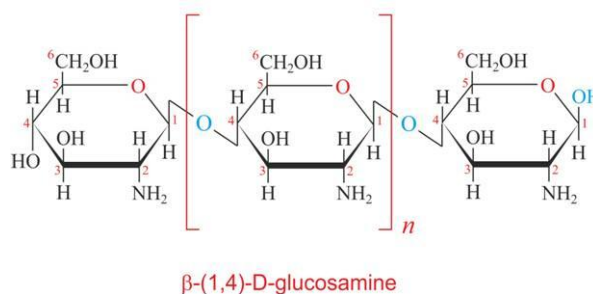


Figure 1 – Structure of chitosan

Chitosan is insoluble in pure water and organic solvents, but dissolves in diluted acidic aqueous solutions where the solubility depends on pH and deacetylation degree. Chitosan as non-toxic, antibacterial, biocompatible and biodegradable was initially used in

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pharmacy and biomedicine. However, the need for new natural materials has expanded the applications range of chitosan to bioengineering, agriculture, dentistry, food industry, cosmetics, water filtration and many other fields [3-5].

The microstructures such as: concave/convex individual microlenses, closely packed square and hexagonal arrays of microlenses, microchannels, diffraction gratings and other complex structures have found applications in lab on a chip, medical laser, wavefront sensors, light-field cameras, security technologies, and biomedical systems [6-11].

Microstructures are usually fabricated on polymers, photosensitive glass, composites, and other materials using techniques such as direct laser writing (DLW), photolithography, hot embossing, and droplet-based processing. In many cases these techniques are complex and use of toxic chemicals [12-16].

Similar to our previous work with gelatin [12], chitosan was also chemically modified and photosensitized using non-toxic compounds (food-grade quality). In this way, the easily and inexpensively prepared chitosan layer retaining its biocompatibility and was used for the rapid, one-step fabrication of microoptical structures by direct laser writing (DLW) at a wavelength of 488 nm.

The fabricated microstructures can be used directly without any additional chemical treatment and without any waste material. Also, they can be replicated by contact copying into dental composite, PDMS and other related materials [17, 18]. These microstructures are suitable for a wide range of applications, including optical sensors, security devices, compound-eye-inspired optical systems, lab-on-a-chip, and many other photonic and biomedical structures [19, 20].

2. EXPERIMENTAL PROCEDURE

2.1. Chitosan layer preparation

All chemicals used for chitosan modification are well known, readily available, inexpensive and non-toxic.

First, a 2% chitosan solution was prepared by dissolving 2 g of low molecular weight chitosan and 85% deacetylated chitosan (Sigma-Aldrich) in 100 ml of 1% aqueous acetic acid solution (Sigma-Aldrich). The chitosan solution was stirred on a magnetic stirrer at 1000 rpm and 50°C for several hours until it became homogeneous. An aqueous solution of excipients designated as ES was prepared and used to modify chitosan. ES consisted of food plasticizers, humectants and preservatives (0.2 ml glycerol, 0.3 g sucrose, 8 mg glucose, 2 µl polysorbate 80, 6 mg citric acid and 2 mg sodium benzoate), all expressed per 1 ml of solution.

The photosensitivity of chitosan was achieved by adding anthocyanin food dye E163. With constant stirring, 2 ml ES and 1 ml of E163 dye were added to 10 ml of 2% chitosan solution. After complete dissolution, the resulting modified chitosan solution was centrifuged (Cole Parmer 17250-10 at 3400 rpm/min) to remove any remaining impurities.

The chitosan layer was prepared by gravity settling method [21] by pouring 1 ml of chitosan solution onto a microscope slide surrounded by a Plexiglas frame. The layer was dried in the dark at room temperature and 50% to 60% humidity. As a result of the modification and photosensitization, the mechanical and optical properties of the chitosan layer were changed.

2.2. Layer thickness

The thickness of the chitosan layer was measured with a digital micrometer, with an accuracy of 10 µm, at six different locations, and the average value was taken as the result.

2.3. Direct laser writing microstructures

Microstructures were fabricated on the chitosan layer using a home-made laser writing device [22] with a blue laser (iBEAM SMART, manufactured by Toptica) operating at wavelength of 488 nm and a maximum power of 100 mW. The laser beam was focused onto the chitosan layer using a microscope objective (Mitutoyo, 50×0.55NA), as schematically shown in Figure 2.

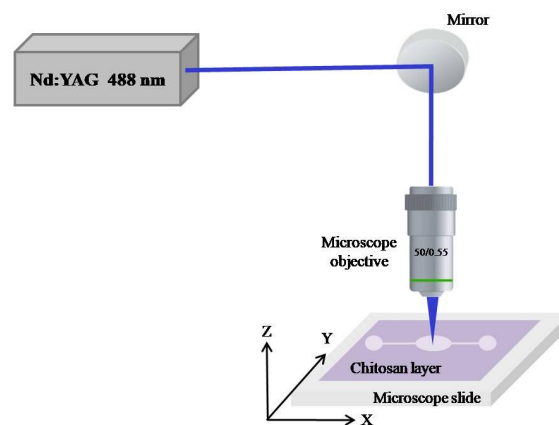


Figure 2 – Experimental setup for microstructure fabrication

A color CMOS camera (Thorlabs CS505CU5 Megapixel) was used to position the chitosan layer at the desired location relative to the focal point. A precision XY linear translation stage (Ludl BioPoint2), with a step resolution of 50 nm and a position repeatability of 2 µm, was used to move the sample layer relative to the laser beam, while a G-code-enabled Arduino microcontroller controlled the stage motion to ensure accurate layer positioning [23]. Synchronization of the laser beam and the coordinate table was achieved using

appropriate software. By controlling the laser power, exposure time, and laser writing speed, different microstructures can be formed.

2.4. *In vitro* cytotoxicity of chitosan sample towards HaCaT cells

Cytotoxicity of chitosan layer was determined on spontaneously immortalized keratinocyte cell line (commonly referred as HaCaT) using crystal violet assay, as described previously [24].

3. RESULTS AND DISCUSSIONS

The layer thickness was changed by varying the amount and chemical composition of the chitosan solution. For a given volume of solution, a chitosan layer thickness of 100 μm was measured.

The microlenses array is recorded lens by lens using the program which determines the microlens array parameters from the image file. By controlling the laser power, exposure time and distance among neighbor microlenses we were able to create concave or convex microlenses (individual or closely packed arrays).

The concave dip was formed on the chitosan layer by its controlled and local melting. Following the laser beam profile, the surface tension forces form a lens, without any waste.

An image of digit „-2“ taken by a digital camera through the optical microscope and 3×3 concave microlens array is shown in Figure 3. The lens diameter of 50 μm was obtained with 35 mW of laser power and 200 ms exposure time.

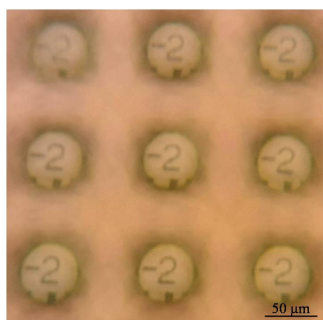


Figure 3 – Optical image of the digit „-2“ reproduced by the 3×3 concave microlens on chitosan layer in transmission mode

Good quality positive microlenses were produced by making an arrangement of 8 polygonally positioned spots. The spherical surface in the polygon center acts as a convex (positive) microlens. The radius of curvature, as well as the corresponding focal length of microlenses can be controlled by the diameter of a polygon.

Figure 4 presents the optical characterization of the fabricated convex microlens array. Figure 4a

shows the reflection image of a 5×5 array of convex microlenses fabricated on the chitosan layer. Each microlens was produced by laser irradiation using a power of 50 mW and an exposure time of 200 ms, resulting in a microlens diameter of approximately 25 μm . Figure 4b demonstrates the imaging performance of the microlens array by reproducing the digit „-2“, captured through an optical microscope with a digital camera. The fabricated convex microlenses exhibited excellent uniformity and high fabrication repeatability.

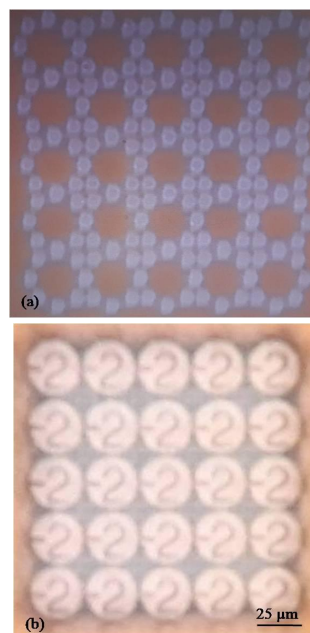


Figure 4 – (a) Reflection optical micrograph of a 5×5 array of convex microlenses fabricated on the chitosan layer. (b) Optical image of the digit „-2“ reproduced by the 5×5 convex microlens array in transmission mode.

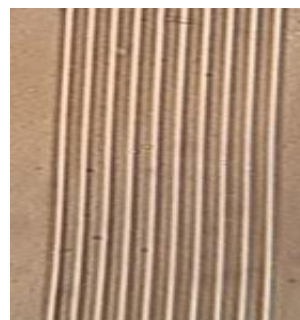


Figure 5 – Diffraction gratings recorded on chitosan layer

Diffraction gratings were recorded line by line using a program that determines the line parameters (number, length and width) from the corresponding image file. By writing a series of gratings the spatial resolution of 100 lp/mm was found, for the laser power of 35 mW, exposure time of 200 ms, and line width of

0.01 mm. The image of diffraction grating taken by a digital camera through the optical microscope is shown in Figure 5.

By precisely controlling the movement of the laser beam on the surface of a chitosan layer, microstructures of various shapes, including very complex geometries, can be fabricated on it. Based on our previous research [12], we assume that by adjusting the laser power, exposure time, writing speed, and layer thickness, microstructures with widths and depths of up to several hundred micrometers can be produced on chitosan layers. The exposure time can be shortened by increasing the laser power. In most cases, no post-processing is required, which allows the microstructures to be used immediately after fabrication. An optical microscope image of a complex microchannel structure fabricated on a chitosan layer is shown in Figure 6.

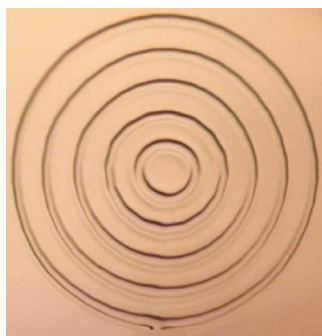


Figure 6 – Microchannel recorded on chitosan layer

Preliminary studies have shown that the cross-section of the microchannel is approximately semi-circular.

Relative growth rate of HaCaT cells in the presence of different concentrations of tested sample compared to untreated control is presented in Figure 7.

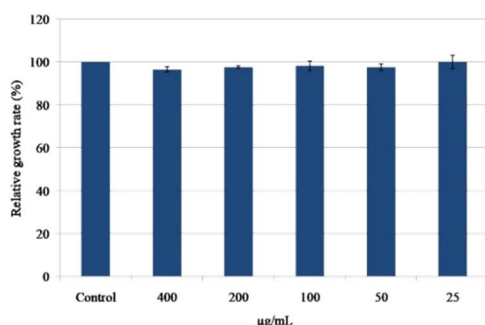


Figure 7 – Relative growth rate of HaCaT cells in the presence of different concentrations of chitosan

Tested sample was evaluated as non-toxic to the HaCaT cell line with respective IC₅₀ values of > 400 mg/mL, a concentration which is considered as the limit of toxicity [25].

4. CONCLUSION

Chitosan, an eco-friendly and biocompatible biopolymer, was chemically modified to obtain an elastic, non-toxic material suitable for the rapid, single-step fabrication of a wide range of microoptical structures by direct laser writing (DLW) at wavelength of 488 nm. Various microstructures, including individual and array of convex and concave microlenses, microchannels, diffraction gratings, and other complex microoptical elements, can be fabricated using inexpensive laser sources. The resulting microstructures on the chitosan layer are suitable for numerous applications, including optical sensors, medical devices, imaging systems, security technology, and bioinspired optical structures. The fabricated microlenses exhibited good optical and imaging performance, while the elasticity of the modified chitosan layer makes it suitable for adaptive optics applications.

Future work will focus on detailed profilometric and microstructural characterization, as well as on improving the physicochemical properties of chitosan layer.

5. ACKNOWLEDGEMENT

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REZIME

HEMIJSKI MODIFIKOVAN HITOZAN ZA DIREKTAN LASERSKI ZAPIS MIKROSTRUKTURA U REALNOM VREMENU

U ovom radu, hemijski modifikovani hitozan je predstavljen kao ekološki prihvatljiv, optički transparentan, elastičan i izdržljiv materijal za izradu različitih mikrostrukture u realnom vremenu. Krhki sloj hitozana je hemijski modifikovan da bi postao elastičan i rastegljiv, dok je fotoosetljivost sloja postignuta antocijaninskom prehrambenom bojom E163. Test biokompatibilnosti je potvrdio da je sloj hitozana hemijski modifikovan na ovaj način netoksičan. Različite mikrostrukture su proizvedene na sloju direktnim laserskim zapisom na talasnoj dužini od 488 nm, sa maksimalnom izlaznom snagom od 100 mW, korišćenjem „domaćeg“ sistema za pisanje. Konkavna ili konveksna mikrosočiva (pojedinačna i nizovi), mikrokanali, difrakcione rešetke i druge složene strukture se brzo proizvode. Dobijene mikrostrukture se koriste direktno, bez dodatne hemijske obrade i bilo kakvog otpada. Formirane strukture su pogodne za brojne primene, uključujući „laboratorije na čipu“ (lab-on-a-chip), medicinske lasere, optičke senzore, kamere svetlosnog polja, tehnologije zaštite i biomedicinu.

Ključne reči: hitozan, antocijanin prehrambena boja E163, mikrostrukture, direktan laserski zapis, biokompatibilnost