

## Laser-Directed Deposition of Mannan-Functionalized Silver Nanostructures

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Mannan is a polymannose isolated from the cell wall of *Saccaromyces cerevisiae* and has strong binding affinity to mannose receptors on antigen presenting cells (APCs), such as dendritic cells and macrophages. Mannan-functionalized nanomaterials or nanostructures are thus of high interests in studying immune responses towards fungi. In this work, we investigated the fabrication of mannan-coated silver nanostructures using a laser-deposition technique. Specifically, two different starting materials were applied for laser-deposition. One is a mixture solution of AgNO<sub>3</sub> and mannan, and the other one is the suspension of silver nanoparticles synthesized with mannan as the sole reducing and capping agent. Using 405 nm diode laser in a confocal microscope, we successfully fabricated mannan-covered micropatterns by laser-induced photoreduction of silver ions or aggregation of mannan-capped AgNPs. The results show that both starting materials can be applied to deposit micro- or nanoscaled structures that are covered with mannan, which was confirmed by fluorescence microscopy. These silver nanostructure-supported mannan patterns are promising candidates to mimic the fungal membrane and beneficial for immune cell studies.

**Keywords:** Laser-deposition, Silver Nanostructures, Mannan, Photoreduction, Silver Nanoparticles.

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### 1. INTRODUCTION

Nanofabrication techniques are widely used in producing functional micro-/nanostructures for a variety of contemporary applications, such as photonics, sensing, electronics, energy, and biomedicine [1]. Among numerous fabrication techniques, laser-directed deposition has emerged recently as a convenient and efficient way to create metal [2] or polymer [3] nanostructures in either 2D or 3D configurations. Compared to other nanofabrication techniques, such as scanning beam lithography or nano-printing, laser-deposition is more time and labor efficient, lower cost, and more flexible for various substrates. Although the resolution of laser-deposition is limited by optical diffraction, such a limit can be overcome by using two-photon absorption [3,4]. Laser-deposition of noble metal nanostructures are particularly interesting due to their unique chemical, physical, and optical properties [5]. Especially, superior biocompatibility and versatile surface chemistry further make noble metal nanostructures a promising candidate for biological applications with complex functionalities and outstanding performance [6].

Mannan is a biodegradable and biocompatible polysaccharide that potentially targets antigen-presenting cells (APCs), such as dendritic cells and macrophages, which express mannose receptors (MRs) on their surfaces. The strong binding affinity of mannan to MRs enables targeted antigen delivery and specific bioimaging using mannan-functionalized polymeric [7] or magnetic [8] nanoparticles. While previous studies mainly focused on free-standing nanoparticles functionalized with mannan, nano- or microscaled spatiotemporal domains consisting of or covered by mannan are also highly valuable for the investigation of immune re-

sponses of APCs, as these engineered patterns of mannan would mimic the biochemical features of fungal membranes. A convenient and flexible nanofabrication technique is thus highly desirable in making programmable nanostructures functionalized with mannan.

In this study, we deposited silver nanostructures that are coated with mannan on glass substrates using the laser-deposition technique. Two different strategies were employed to create mannan-coated silver nanostructures. One uses a mixture solution of AgNO<sub>3</sub> and mannan as the starting material, the other one uses silver nanoparticles (AgNPs) synthesized using mannan as the reducing and capping agent. The coating of mannan on silver nanostructures was verified by fluorescence microscopy using a mannan-specific dye as the fluorescence label. The investigation of spatiotemporal recognition of anti-fungal receptor using these patterned nanostructures is thus anticipated.

### 2. EXPERIMENTAL

#### 2.1 Synthesis of Mannan-Capped AgNPs

Silver nitrate, mannan, anthrone, and concentrated sulfuric acid were purchased from Sigma-Aldrich. In a typical synthesis, 15 mg of mannan was added into 50 mL of aqueous AgNO<sub>3</sub> solution (0.2 mM). The solution was then kept boiling for 4 h. The nanoparticles were then centrifuged and washed with D.I. water three times and stored in dark at 4°C.

#### 2.2 Laser Deposition

Two different starting materials were applied for laser-deposition. The mixture solution of AgNO<sub>3</sub> (2 mM) and mannan (3 wt%) or aqueous suspension of

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mannan-capped AgNPs was dropped onto a glass cover slip which was placed under a Leica TCS SP5 confocal laser scanning microscope. A diode laser with a wavelength of 405 nm was employed to deposit silver nanostructures from the solution.

### 2.3 Sulfuric Acid/Anthrone Assay

1 mL of freshly-prepared anthrone solution in concentrated  $H_2SO_4$  was added into 0.5 mL of mannan-capped AgNP solutions. The solution was then heated to 100°C and stirred for 10 min. After cooling to room temperature, the UV-vis spectra of the resulting color product were recorded.

### 2.4 Characterization

Scanning Electron Microscopy (SEM) characterization was performed on a Tescan SEM using an accelerating voltage of 20 kV. Fluorescence microscopy was performed on a Leica TCS SP5 confocal laser scanning microscope. Absorption spectra were collected by a UV-vis spectrometer (Lambda 1050, Perkinelmer).

## 3. RESULTS AND DISCUSSION

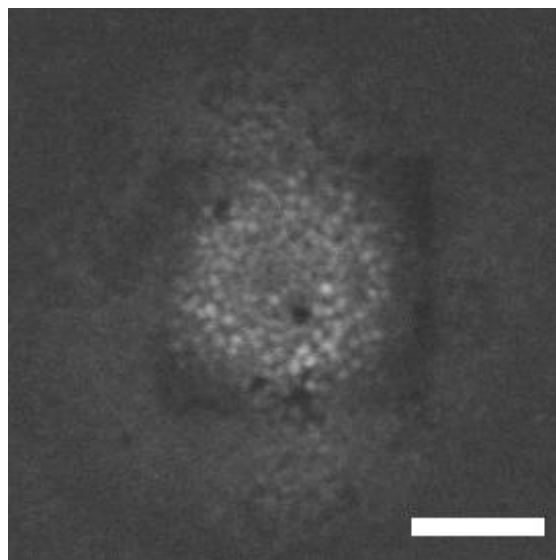
### 3.1 Laser-Deposition Using $Ag^+$ as the Precursor

A commonly used method to deposit metallic nanostructures with laser is to use photo-reduction of metal ions with or without reducing agents [9]. Metal nanostructures are formed at the focal plane of laser and deposited at the solution-substrate interfaces. Figure 1a shows a typical SEM image of mannan-coated silver nanostructures deposited using a mixture solution of  $AgNO_3$  and mannan. The laser power was 7 mW and deposition time was 500 ms. Previously, we have shown that the size of deposited structure using sodium citrate as the reducing agent is dependent on experimental conditions including laser power and deposition time [10]. Although mannan is a much weaker reducing agent as compared to citrate, it is still efficient enough to reduce silver ions upon exposure to laser. It is anticipated that the spatial features of mannan-coated silver nanostructures can also be manipulated by varying corresponding deposition parameters.

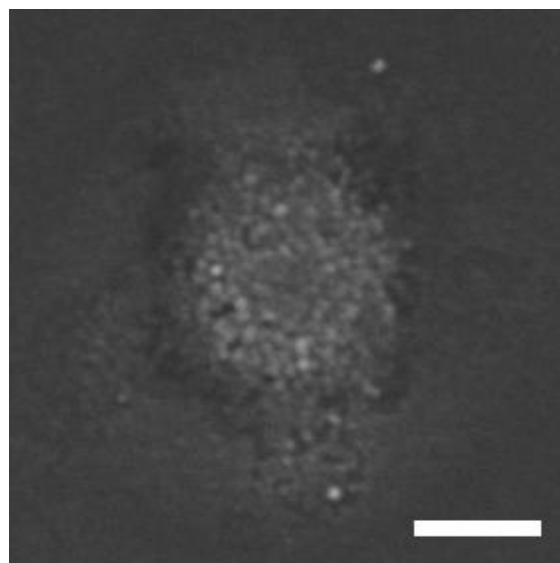
### 3.2 Laser-Deposition Using AgNPs as the Precursor

Another strategy to deposit metal nanostructures with laser usually employs metallic nanoparticles as the starting material [11]. In this study, AgNPs were synthesized using mannan as the sole reducing and capping agent. As shown in Figure 2a, an absorption peak at 420 nm, corresponding to a typical surface plasmon resonance (SPR) of AgNPs, indicates the successful synthesis of stable mannan-capped AgNPs. Figure 1b shows an SEM image of deposited silver nanostructures using AgNPs synthesized with mannan as the sole reducing and capping agent. Although this deposition strategy involves an extra synthetic procedure, which increases the complexity of the whole deposition process, a potential advantage of using mannan-functionalized AgNPs is a relatively uniform and con-

trollable coating of mannan on the surface of silver nanostructures. On the other hand, since the deposition of silver nanostructures using  $AgNO_3$  can be achieved without any reducing agent, the coating of mannan on those structures may not be uniform. More detailed study is necessary to evaluate the coating efficiencies of mannan on silver nanostructures using these two different starting materials.



a

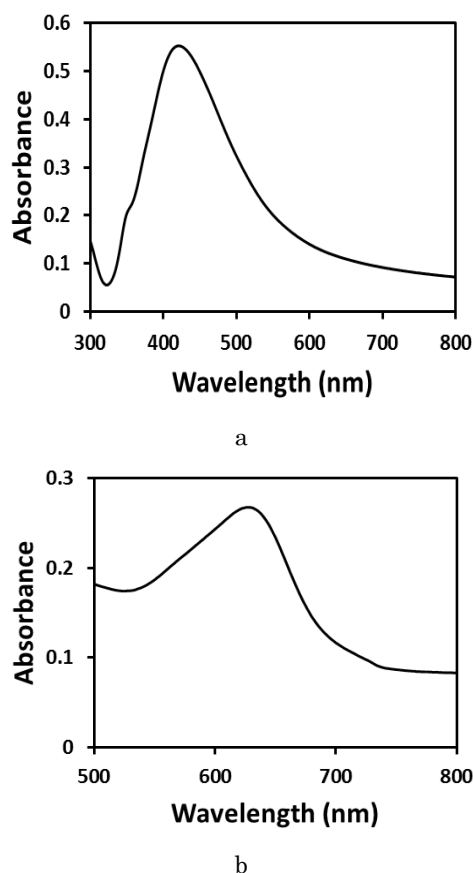


b

**Fig. 1** – SEM images of mannan-coated silver nanostructures deposited with (a)  $AgNO_3$  and mannan solution; and (b) AgNPs synthesized using mannan as the reducing and capping agent. Scale bar = 1  $\mu m$ .

The ultimate goal of this study is to develop a laser-deposition technique that can be used to create micro- or nanoscaled mannan patterns with arbitrary spatial features. Therefore, it is critical to confirm the presence of mannan on silver nanostructure surfaces. In this study, the presence of mannan on AgNP surfaces before laser-deposition was testified by the sulfuric acid/anthrone assay, a widely used colorimetric assay for

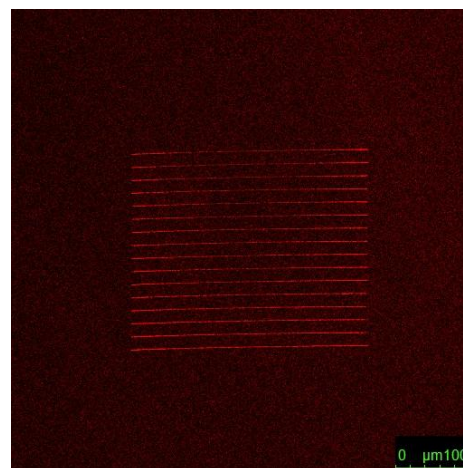
the determination of carbohydrate concentrations [12]. As shown in Fig. 2b, an absorption peak with the maximum at 620 nm indicates the presence of mannan on AgNP surfaces.



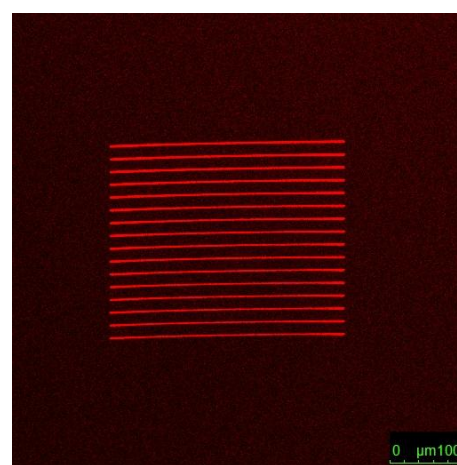
**Fig. 2** – UV-vis spectra of (a) mannan-capped AgNPs and (b) color product of mannan-capped AgNPs after the sulfuric acid/anthrone assay.

### 3.3 Fluorescence Microscopy of Mannan-coated Silver microstructures

To verify the successful coating of mannan on deposited silver nanostructures using two different starting materials mentioned above, fluorescence microscopy was employed to image dye-labelled silver nanostructures. In purpose of better visualization, microscaled silver wires were deposited using 405 nm laser. Glass substrates with deposited silver nanostructures were rinsed with D.I. water and ethanol repeatedly, followed by soaking in D.I. water for overnight. After air drying, the substrates were soaked in ConA-AF674 solution overnight at 4°C. The substrates were then rinsed with D.I. water and ethanol repeatedly, and soaked in D.I. water at 4°C for 72 h before characterization. Figure 3 shows fluorescence images of ConA-AF674-labelled silver nanostructures. Both structures show strong fluorescence signals, indicating that the surface of silver nanostructures was successfully covered by mannan. Detailed studies, such as the quantitative analysis of mannan coverage on silver nanostructure surfaces, will be carried out in future.



a



b

**Fig. 3** – Fluorescence micrographs of mannan-coated silver microwires labeled with ConA-AF647. The silver wires were deposited using (a) solution of AgNO<sub>3</sub> and mannan; and (b) mannan-capped AgNPs.

## 4. CONCLUSION

We investigated the fabrication of mannan-coated silver nanostructures onto glass substrates using a laser-directed deposition technique. Using a confocal microscope, we successfully fabricated mannan-covered micropatterns by laser-induced photoreduction of silver ions or aggregation of mannan-capped AgNPs. The results show that both starting materials can be applied to deposit micro to nanoscaled structures that are covered with mannan, which was confirmed by fluorescence microscopy. These silver nanostructure-supported mannan patterns can be further manipulated to mimic the fungal membrane, thereby enabling the investigation of their receptor's nanodomain structure on antigen-presenting cell surfaces.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. B.D. Gates, Q. Xu, M. Stewart, D. Ryan, C.G. Willson, G.M. Whitesides, *Chem. Rev.* **105**, 1171 (2005).
2. H. Shin, H. Lee, J. Sung, M. Lee, *Appl. Phys. Lett.* **92**, 233107 (2008).
3. W.H. Zhou, S.M. Kuebler, K.L. Braun, T.Y. Yu, J.K. Cammack, C.K. Ober, J.W. Perry, S.R. Marder, *Science* **296**, 1106 (2002).
4. K. Kaneko, H.B. Sun, X.M. Duan, S. Kawata, *Appl. Phys. Lett.* **83**, 1426 (2003).
5. A. Moores, F. Goettmann, *New J. Chem.* **30**, 1121 (2006).
6. P.K. Jain, X. Huang, I.H. El-Sayed, M.A. El-Sayed, *Plasmonics* **2**, 107 (2007).
7. M. Gou, M. Dai, X.Y. Li, L. Yang, M. Huang, Y. Wang, B. Kan, Y. Lu, Y. Wei, Z. Qian, *Colloids Surf. B: Biointerfaces* **64**, 135 (2008).
8. H. Vu-Quang, M. Muthiah, Y.K. Kim, C.S. Cho, R. Namgung, W.J. Kim, J. H. Rhee, S.H. Kang, S.Y. Jun, Y.J. Choi, Y.Y. Jeong, I.K. Park, *Carbohydr. Polym.* **88**, 780 (2012).
9. E.J. Bjerneld, K.V.G.K. Murty, J. Prikulis, M. Kall, *Chemphyschem.* **3**, 116 (2002).
10. K. Jiang, K. Spindler, A.O. Pinchuk, *Proc. of SPIE* **9163**, 916314 (2014).
11. B.B. Xu, R. Zhang, H. Wang, X.Q. Liu, L. Wang, Z.C. Ma, Q.D. Chen, X.Z. Xiao, B. Han, H.B. Sun, *Nanoscale* **4**, 6955 (2012).
12. L.H. Koehler, *Anal. Chem.* **24**, 1576 (1952).