Generation of alginate gel particles with AuNPs layers by polydimethylsiloxan template

Zhi-Xiao Guo, Meng Zhang, Li-Bo Zhao, Shi-Shang Guo,^{a)} and Xing-Zhong Zhao^{a)}

Key Laboratory of Artificial Micro- and Nano-structures of Ministry of Education and School of Physics and Technology, Wuhan University, Wuhan 430072, People's Republic of China

(Received 4 December 2010; accepted 15 May 2011; published online 28 June 2011)

The authors report a feasible and simple microfluidic approach for synthesizing anisotropic gel particles based on template method. By filling arrays of microwells with alginate hydrogel and synthesizing gold nanoparticles (AuNPs) on the gel surface, anisotropic alginate gel particles with single side gold nanoparticles layers were produced in microwells on the polydimethylsiloxan template. AuNPs and the anisotropic feature were characterized using scanning electron microscopy and x-ray photoelectron spectrum analyses. The anisotropic particles made of biocompatible gels could be released from the template and collected with uniform sizes, which might have a powerful potential in biological detection and sensing. © 2011 American Institute of Physics. [doi:10.1063/1.3602119]

I. INTRODUCTION

Recently, gold nanoparticles (AuNPs) have been widely investigated to study the physical and chemical properties.¹ According to their special optical, catalytic, and electrical natures, gold nanoparticles could be used in labeling, delivery, heating, and sensing, and have a great advantage for applications in biochemistry, cell biology, and medicine.² There have been numerous reports on utilization of AuNPs in tumor-targeting drug delivery,^{3,4} biomolecular detection,^{5,6} and medical diagnostics.⁷

Alginate has been used as stabilizer,^{8,9} but there were few report on biopolymer used as immobilization matrix for the metal ions such as Au³⁺.^{1,10} The biopolymer matrix for the synthesis of gold nanoparticles shows great potential in biomedical applications. Alginate acid is a kind of biopolymer, which containing 1,4-linked α -D-mannuronic and L-guluronic acid residues arranged randomly along the chain.¹ Based on its low cost and naturally occurring property, alginate gel has been widely used in food industry, biology, chemistry, and medicine. For example, this hydrogel might function as capsule for living organisms or drugs. Alginate capsules produced by traditional methods have large bulk with significant size distribution. Taking the great advantage of microfluidic technology, different approaches have been designed to shrink the particles to microscale.^{11–13}

Previously, we selected a template approach to fabricate anisotropic alginate microparticles with AuNPs layers.¹⁴ Chloroauric acid (HAuCl₄) is usually reduced by physical or chemical method to produce AuNPs in aqueous solution. Here we choose L-ascorbic acid as the deoxidizer for its nontoxic character. Compared to other reduction methods, it is more convenient and environment-friendly since special conditions such as UV light source, or poisonous chemicals are not needed. By using such a method, the microparticles are produced with uniform size. Gel

^{a)}Authors to whom correspondence should be addressed. Electronic addresses: xzzhao@whu.edu.cn and gssyhx@whu.edu.cn.

FIG. 1. Schematic diagram of the process for generating alginate gel microparticles with single AuNPs layers.

particles with one side modified AuNPs and another side encapsulated certain drug agents may have great potential application in cell therapy.^{15,16}

II. EXPERIMENT

A. Microfluidic device fabrication

The PDMS (RTV615, momentive performance materials) template was fabricated using soft-lithography technique.¹⁷ SU8-2050 (Microchem.) positive relief structure was made on silicon wafer. PDMS polymer was cast from this mold in a ratio of 5:1 after being cured at 80 °C for 2 h.

B. Materials

Sodium alginate, calcium chloride (CaCl_2), chloroauric acid, L-ascorbic acid, and ethanol were bought from China National Medicines (Co. Ltd., China). De-ionized (DI) water was prepared with a Millipore water purification system (Milli-Q Advantage, Millipore, Worcester, MA). Sodium alginate at 1% (w/w) was prepared by magnetic stirring. CaCl_2 powders were dissolved in DI water to prepare 1% (w/w) CaCl_2 solution. 50 mM L-ascorbic acid solution and 1 mM chloroauric acid solution were also prepared by DI water.

C. Instruments

Scanning electronic microscope (SEM) images were accumulated by placing the samples on a silicon wafer in an emission scanning electron microscope (Philips XL30-FEG Sirion, Eindhoven, Netherlands). X-ray photoelectron spectroscopy (XPS) measurement was performed with a Kratos XSAM 800 spectrophotometer. Gel formation was recorded using an inverted microscope (IX71, Olympus, Japan) equipped with a charge coupled device (DP71, Olympus, Japan) camera.

D. Particle fabrication

Figure 1 shows the schematic diagram of the synthesis process. After 5 min oxygen plasma treatment, the PDMS surface became hydrophilic¹⁸ so that the PDMS microwells were easily filled with alginate solution at 1% w/w [Fig. 1(a)], a vacuum air pump was used to remove the trapped air. All the microwells were fully filled and without any trapped bubbles. The superfluous alginate was scraped off from the PDMS template by a glass slide, but each well was still filled with certain volume of solution. This filled microwell plate was immediately immersed into 1% calcium chloride solution. The Ca^{2+} ions diffused into the gel solution and cross-linked the polymer chains of alginate into a gel state through electrostatic interactions.¹⁹ After alginate gel solidification, the PDMS template was removed from the solution. The PDMS template was then immersed into chloroaurate acid solution (HAuCl_4 , 1.0 mM) after rinsed by DI water twice. The HAuCl_4 solution had diffused into the surfaces of the gel particles [Figs. 1(b) and 2(a)] after 15 min. After the template being washed twice by deionized water, L-ascorbic acid solution (50 mM) was added to deoxygenated the Au^{3+} ions [Fig. 1(c) and 2(b)]. The HAuCl_4 and L-ascorbic acid diffused in alginate gel reacted to generate AuNPs in the front surface of the gel [Figs. 2(c) and 2(d)]. Excess

FIG. 2. The diffusion of reagents and reaction in alginate gel. (a) HAuCl_4 diffused in the front surface of the gel. (b) Soon after, L-ascorbic acid diffused in the gel. (c) L-ascorbic acid reacted with HAuCl_4 . (d) Excessive reagents were removed by DI water.

reagents were washed away by DI water. Freestanding alginate gel microparticles could be released from the PDMS microwells by shrinking the gels using ethanol [Fig. 1(d)].

III. RESULTS AND DISCUSSION

A. Particle preparation

Figure 3 shows a sequence of images demonstrating the anisotropic gels formation. Figure 3(a) shows a microscopy picture of a PDMS template with a rectangular pattern of square wells (200 μm in side length and 80 μm in height spaced by 200 μm); these wells were filled with alginate solution that was gelled by using 1% (w/w) calcium chloride solution. The rectangular gel particles were covered with thin AuNPs layers after 15 min treatment with HAuCl_4 and 2 min with L-ascorbic acid solution [Fig. 3(b)]. Proportion of oxidant and reducer was set as 1:50 to control the size of nanoparticles.²⁰ Reaction time would be long enough for the formation of plenty gold nanoparticles, but the HAuCl_4 solution would diffuse in the whole particles with over reaction. Gels released from the PDMS template and suspended in water for observation [Fig. 3(c)] after being immersed in ethanol. The cross-sectional area and size distribution of the gel particles (CV value) were 30109 μm^2 and 8.89%. Reproducibility of gel particles was also determined in this way. The relative standard deviation was 7.02% for three successive assays.

B. Gold nanoparticle characterization

To further analyze the distribution of gold nanoparticles on the gel surface, SEM and XPS studies have been performed. It could be observed from the SEM images that gold nanoparticles spread cover the alginate surface [Fig. 4(a)]. The front side as well as the back side of the particles were both shown in the SEM picture. As expected, gold nanoparticles were found in the front side

FIG. 3. Process of generating anisotropic alginate gels. (a) PDMS template with solid gels in it. (b) Gel particles with AuNPs layers in the template. (c) Gel particles released from the microwells. The scale bars in (a) and (b) are 200 μm , and scale bar in (c) is 500 μm .

FIG. 4. SEM images of dried microparticles' surfaces with AuNPs layers. (a) Image of a single gel put the right way up. (b) The front side of one gel was at the bottom of the picture, and the back side of another gel was at the top side of the picture. The scale bars in the inserted pictures are, respectively, 50 μm in (a) and 100 μm in (b).

of the gel particles, but there were no gold nanoparticles observed in the back side of the gels [Fig. 4(b)]. The average diameter and CV value of the gold nanoparticles were, relatively, 32.9 nm and 21%.

Single side AuNPs alginate gel particle was demonstrated as Fig. 5 described. The Au 4f region of XPS spectrum showed two peaks for Au 4f_{5/2} and Au 4f_{7/2} transitions, which indicated the presence of metallic gold in the sample. There were no distinct peaks in XPS spectra of back side of the gels in the Au 4f region. The experimental data indicated that there were AuNPs in the front side of the anisotropic.

FIG. 5. XPS spectra of front side of the gels.

IV. CONCLUSION

In conclusion, we have demonstrated a simple method for the fabrication of microgels with AuNPs layers. The anisotropy of the gels was determined by characterizing with SEM and XPS analysis. This chip-based fabrication method has several advantages over conventional techniques: (1) The fabrication apparatus is simple, reactants are nontoxic, and the resulting particles have uniform sizes; (2) The fabrication process does not require specific conditions, such as high temperatures or UV exposure, and sensitive biological materials like proteins could be easily incorporated into the resulting gels. Considering the good biocompatibility, degradability, and anisotropy properties, gels with AuNPs layers may have potential biomedical applications.

ACKNOWLEDGMENTS

Z.X.G. and M.Z. contributed equally to this work. This work was partially supported by the China National Funds for Distinguished Young Scientists (Grant No. 50125309), National Natural Science Foundation of China (Grant Nos. 10904117 and 10804087) and National Science Fund for Talent Training in Basic Science (Grant No. J0830310).

- ¹ S. Saha, A. Pal, S. Kundu, S. Basu, and T. Pal, *Langmuir* **26**, 2885 (2010).
- ² R. A. Sperling P. Rivera gil, F. Zhang, M. Zanella and W. J. Parak, *Chem. Soc. Rev.* **37**, 1896 (2008).
- ³ G. F. Paciotti, D. G. I. Kingston, and L. Tamarkin, *Drug Dev. Res.* **67**, 47 (2006).
- ⁴ J. M. Bergen, H. A. Von Recum, T. T. Goodman, A. P. Massey, and S. H. Pun, *Macromol. Biosci.* **6**, 506 (2006).
- ⁵ Y. Liu, D. Zhang, E. C. Alcolija, and S. Chakrabartty, *Nanoscale Res. Lett.* **5**, 533 (2010).
- ⁶ W. J. Wang, C. L. Chen, M. X. Qian, and X. S. Zhao, *Anal. Biochem.* **373**, 213 (2008).
- ⁷ R. Wilson, *Chem. Soc. Rev.* **37**, 2028 (2008).
- ⁸ T. A. Nguyen, V. P. Dang, N. D. Nguyen, D. D. Bui, and Q. H. Nguyen, *Radiat. Phys. Chem.* **79**, 405 (2010).
- ⁹ S. Y. Gao, Y. G. Shi, S. X. Zhang, K. Jiang, S. X. Yang, Z. D. Li, and E. Takayama-Muromachi, *J. Phys. Chem. C* **112**, 10398 (2008).
- ¹⁰ E. Torres, Y. N. Mata, A. L. Blazquez, J. A. Munoz, F. Gonzalez, and A. Ballester, *Langmuir* **21**, 7951 (2005).
- ¹¹ K. Liu, H. J. Ding, J. Liu, Y. Chen, and X. Z. Zhao, *Langmuir* **22**, 9453 (2006).
- ¹² H. Zhang, E. Tumarkin, R. Peerani, Z. Nie, R. M. A. Sullan, G. C. Walker, and E. Kumacheva, *J. Am. Chem. Soc.* **128**, 12205 (2006).
- ¹³ S. Sugiura, T. Oda, Y. Aoyagi, R. Matsuo, T. Enomoto, K. Matsumoto, T. Nakamura, M. Satake, A. Ochiai, N. Ohkohchi, and M. Nakajima, *Biomed. Microdevices* **9**, 91 (2007).
- ¹⁴ L. B. Zhao, S. Z. Li, H. Hu, Z. X. Guo, F. Guo, N. G. Zhang, X. H. Ji, W. Liu, K. Liu, S. S. Guo, and X. Z. Zhao, *Microfluid. Nanofluid.* **10**, 453 (2011).
- ¹⁵ H. J. Bai, H. L. Gou, J. J. Xu, and H. Y. Chen, *Langmuir* **26**, 2924 (2010).

- ¹⁶D. G. Woo, M. S. Shim, J. S. Park, H. N. Yang, D. R. Lee, and K. H. Park, [Biomaterials](#) **30**, 5631 (2009).
- ¹⁷J. C. M. David, C. Duffy, O. J. A. Schueller, and G. M. Whitesides, [Anal. Chem.](#) **70**, 4974 (1998).
- ¹⁸B. Kim, E. T. K. Peterson, and I. Papautsky, Proceedings of the 26th Annual International Conference IEEE EMBS, 2004 (unpublished), Vol. 26, p. 5013.
- ¹⁹C. Qiu, M. Chen, H. Yan, and H. K. Wu, [Adv. Mater.](#) **19**, 1603 (2007).
- ²⁰K. Philippot and B. Chaudret, [C. R. Chim.](#) **6**, 1019 (2003).