Materials Letters 137 (2014) 479–482

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Synthesis and anti-bacterial activity of AuNRs–PS–MNPs

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ARTICLE INFO

Article history:
Received 14 July 2014
Accepted 13 September 2014
Available online 22 September 2014

Keywords:
Biomaterials
Nanocomposites
Photothermal properties
Recyclable
Magnetic retrieval

ABSTRACT

Novel, recyclable anti-bacterial Janus particles (AuNRs–PS–MNPs) were produced successfully by combination of a confined convective assembly method and modified layer-by-layer technology. The Janus particles, one hemisphere of polystyrene coated by gold nanorods (AuNRs) and the other was coated by magnetic nanoparticles (MNPs), have both the properties of AuNRs and MNPs, therefore AuNRs–PS–MNPs could be collected by a magnet and produce heat under NIR irradiation. The survival rate of *Escherichia coli* (*E. coli*) and *Staphylococcus epidermidis* (*S. epidermidis*) treated with AuNRs–PS–MNPs and NIR were investigated using live/dead staining techniques, which confirmed the novel Janus particles worked as an efficient antibacterial agent.

1. Introduction

Globally, pathogenic bacteria infection is becoming one of the greatest challenges today which causes serious infectious diseases [1]. Furthermore, the emerging drug resistance in the microorganisms motivates researchers to find new, highly efficient antimicrobial agents, urgently. Metal nanoparticles exhibit excellent antibacterial properties than their bulk phase. Among them, gold nanoparticles have been studied in vast research due to their unique chemical and photophysical properties. Especially, gold nanorod can be an efficient agent in photothermal therapy due to its peculiar photothermal properties. Compared with other structures of gold nanoparticles, gold nanorods could absorb and scatter light from visible to near infrared region by adjusting the aspect ratio [2]. In addition, the absorbed light energy could be converted by the gold crystal lattice into heat energy which ultimately transfers to the surroundings [3]. Making photothermally effective nanoparticle along with magnetic property in a single nanocomposite provides efficient photothermal therapy with cell separation. For this purpose, silica coated iron oxide nanoparticles (SiO_2/Fe_2O_3) have been utilized which have shown the properties of biocompatibility and magnetism [4].

Owing to the asymmetric structure, Janus particles have been found in extensive applications, which however make it difficult to be prepared [5]. In recent years, several methods have been achieved, such as Pickering emulsion, phase separation, and microfluidics [6–8]. In this report, we propose a new method based on a combination of the confined convective assembly technique [9] and modified layer-by-layer method [10] to produce unique Janus particles. The one hemisphere of polystyrene nanoparticle composed of gold nanorods and the other hemisphere with iron nanoparticles (AuNRs–PS–MNPs), as a recyclable anti-bacterial agent. The anti-bacterial activity of AuNRs-PS-MNPs after laser irradiation against Gram negative and Gram positive bacteria have been studied using live/dead staining techniques.

2. Experiments

Gold nanorods were synthesized using the modified seed-mediated method [11]. SiO_2/Fe_2O_3 (MNPs) were prepared by Stober and sol–gel processes [4], and modified by amine using the method which was described in the literature [12]. To check the zeta potential values of the modified MNPs, we employed the Malvern Zeta-Sizer 3000HS instrument. Monolayer polystyrene arrays on a glass plate were fabricated by polystyrene particles (PS), Cat no. 3495A Duke scientific, using the confined convective assembly method, with substrate moving speed of 35 μm/s and PS concentration of 0.7 wt% [9]. PS arrays became hydrophilic by oxygen plasma treatment, and AuNRs dispersion was immediately deposited on the PS arrayed surface. After drying at room temperature for 24 h, the resulting AuNRs–PS was cured in an oven with temperature of 110 °C for 15 min and followed by sonication to detach particles in DI water. The amine surface

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http://dx.doi.org/10.1016/j.matlet.2014.09.059
0167-577X/

ligand modified SiO_2/Fe_2O_3 was added into the AuNRs–PS particle dispersion and shaken overnight. Finally the mixture was washed with DI water. To analyze the morphologies and composition, field emission scanning electron microscopy (FE-SEM, JEOL Corp., JSM6700F) connected with an energy dispersive X-ray spectroscopy was used.

The photothermal efficiency of AuNRs–PS–MNPs was recorded using a thermocouple (K type, Omega) connected with a data acquisition system (34970, Agilent, CA, USA), when irradiated by a red light from a DPSS laser (Dream laser system, Japan). By an external magnet, the Janus particles were separated from the solution and then redispersed in new water to check the photothermal efficiency again.

An optimum confluence of *E. coli* (KACC 10005) and *S. epidermidis* (KACC 13234) was cultured in Luria-Bertani broth (Miller, AMERSCO, USA). AuNRs–PS–MNPs were added into the bacterial suspension irradiated by laser. Janus particles were separated using a permanent magnet, and the bacterial suspension was stained by Live/Dead® BacLight™ Bacterial Viability Kit (Invitrogen), as per the manufacturer's protocol. The stained *E. coli* cells were analyzed by laser scanning fluorescence microscopy (Nikon Eclipse TE 2000-U) and FE-SEM.

3. Results and discussion

Gold nanorods synthesized using the modified seed mediated method were coated by a cationic surfactant, and showed localized surface plasmon resonance peak (LSPR) of 670 nm with longitudinal mode. SiO_2/Fe_2O_3 nanoparticles prepared by the Stoeber method of modified sol–gel processes had the zeta potential value of −38.2 mV and amine surface ligand immobilization induced a positive value of +31 mV, confirming the deposition of amino group on the surface of MNPs.

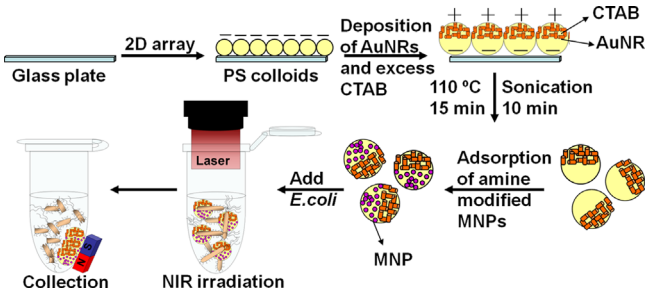

Fig. 1. Diagram of synthesis and application process of AuNRs–PS–MNPs.

Fig. 1 demonstrates the processes of preparing AuNRs–PS–MNPs. Monolayer PS arrays were fabricated on the glass plate using the confined convective assembly method [9]. For confined convective assembly, the density and thickness of arrays could be controlled by the concentration of samples, water evaporation rate and moving rates of the substrate. By adjusting PS's concentration and rising rate, ordered monolayer of PS colloids was produced (in Fig. 2a). Fig. 2b shows that after drying the dropped AuNRs dispersion, most of the AuNRs were deposited on the hemisphere opposite to the glass plate. The AuNRs–PS was sintered in a hot oven with temperature of 110 °C for the purpose of fixing AuNRs on PS. As at 110 °C, which is a little higher than the glass transition temperature of PS, the molecular chains of PS could move and intertwine AuNRs without neck formation between PS particles [13]. Even after being sonicated in deionized water, AuNRs were not exfoliated from PS (data not shown). For AuNRs–PS particles, AuNRs were positively charged, while PS was negatively charged [14]. Therefore when positively charged MNPs were added, MNPs headed for and attached on bared PS hemisphere. Even in the case where there were voids between AuNRs, MNPs did not fill the voids on the upper hemisphere. This was because the preabsorbed positively charged ligand, cetyltrimethylammonium bromide (CTAB) of AuNRs repulsed MNPs; in Au rod dispersion, excess CTAB exists. Initially, the particle size of the PS was measured as 495 nm from the randomly selected 200 particles using FE-SEM. After conjugation with 2.5 aspect ratio (20 nm × 49 nm) AuNR and 20 nm sized MNPs the circumference of the Janus particle was measured to be 550 nm. Figs. 2c and 2d show that AuNRs–PS–MNPs have one hemisphere coated by AuNRs and the another one coated by MNPs, respectively. The EDS of spectrum 1 results in Fig. 2e demonstrates the combination of Au, Fe and Si and C in the AuNRs–PS–MNPs, which further confirms the conjugation between these three different nanomaterials. EDS has the spatial resolution of micrometer scale, hence the AuNRs in back face could also be detected.

Our purpose is producing a novel recyclable anti-bacterial material. AuNRs could quickly transmit heat into the surrounding when are irradiated by light with the wavelength similar with longitudinal LSPR of AuNRs. Compared with other types of gold nanoparticles, AuNRs have the largest absorption and scattering cross sections which will radiate more heat after absorbing light [15]. The absorbed light energy first transformed into hot electrons in the lattice of the particle and then cooled by transmitting its heat to the surroundings in a picosecond time scale [3]. So, AuNRs could be an excellent photothermal agent. As reported previously, the maximum absorption of gold nanorods at the near-infrared range allows more heat energy scattering which could cause the damage of the cell membranes [13]. The combined MNPs could be separated from solution

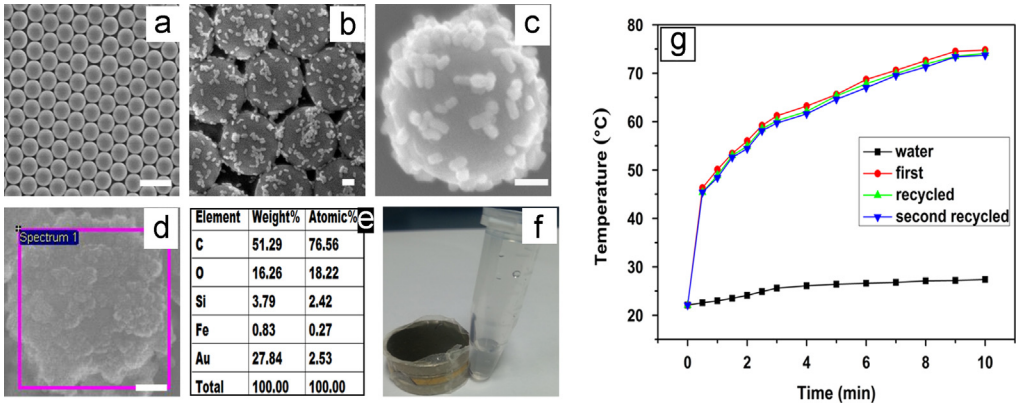

Fig. 2. FE-SEM images of (a) monolayer PS arrays, (b) AuNRs deposited on PS, (c,d) AuNRs–PS–MNPs, (e) elemental composition data of AuNRs–PS–MNPs using EDS, (f) collection of AuNRs–PS–MNP by an external magnet and (g) time dependent temperature increase graphs of laser irradiated AuNRs–PS–MNPs. Scale bar in (a) equals 1 μm. Scale bars in (b–d) equal 100 nm.

carrying AuNRs–PS–MNPs by an external magnet. Fig. 2g shows the time dependent temperature increase curve of the repeated using of AuNRs–PS–MNPs exposure to laser (power of 200 mW). The temperature rose to more than 45 °C for 30 s, reached almost 70 °C in 7 min, and finally increased to 75 °C. After the first laser irradiation, the redispersed AuNRs–PS–MNPs were collected by a magnet for showing repeated photothermal effects in recyclable manner (Fig. 2f).

Further we have studied the anti-bacterial properties of AuNRs–PS–MNPs. Here we chose model organisms *E. coli* and *S. epidermidis* as the target bacteria. The viability of bacteria was surveyed using live/dead staining techniques. Live/Dead® BacLight™ Bacterial Viability Kit contains two nucleic stains, SYTO9 and propidium iodide (PI) to quantify live and dead microbes. SYTO9 can permeate cell membrane and stains DNA in green fluorescence color for live bacteria, while PI labels dead bacteria with destroyed membrane into red fluorescent color. Hence live and dead bacteria could be distinguished legibly by fluorescent color difference using a fluorescence microscope. Figs. 3a and 3c are the fluorescence microscopy images of the control bacteria samples of *E. coli* and *S. epidermidis*, respectively with only NIR exposure showing most of the cells to be alive, while Figs. 3b and

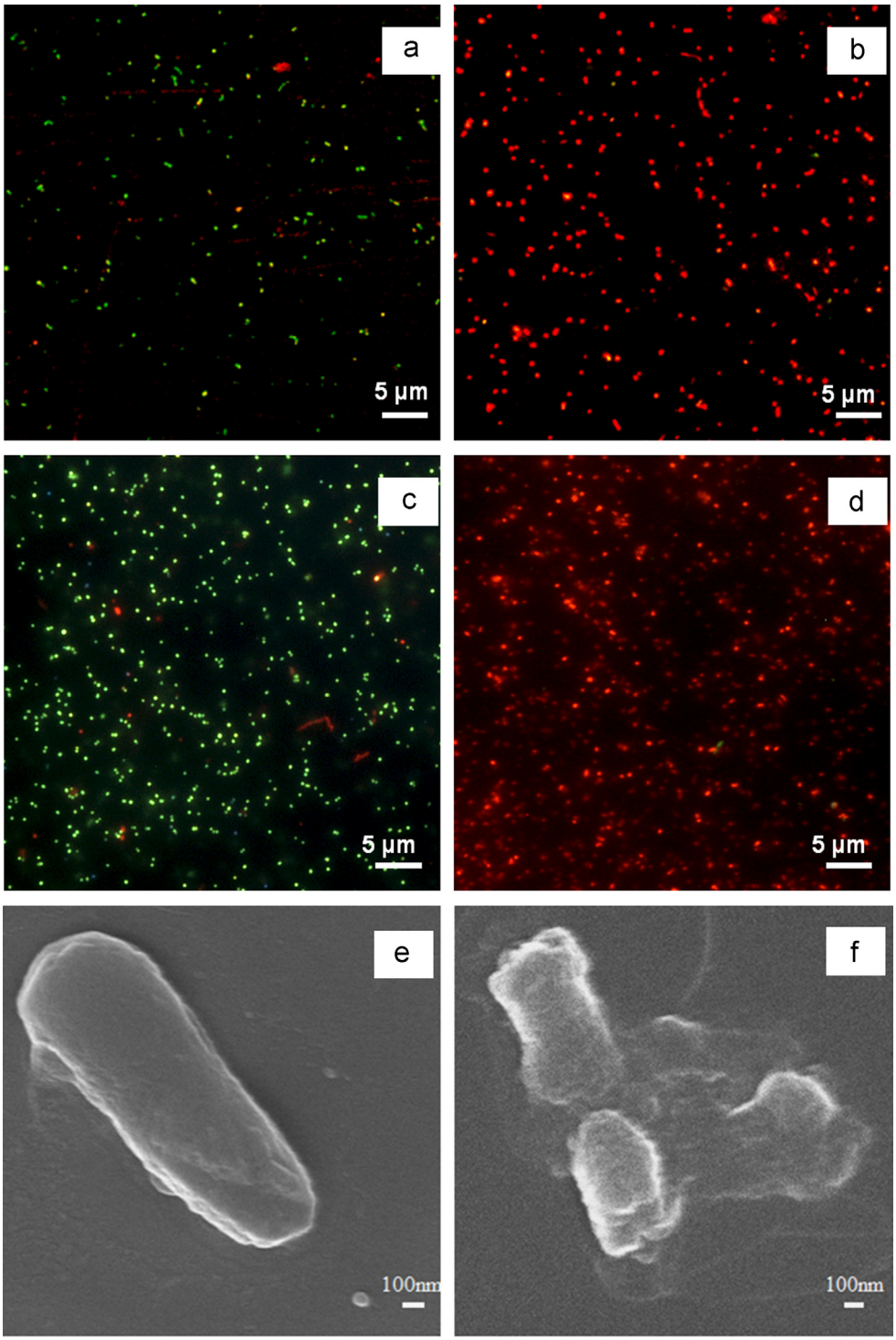

Fig. 3. Fluorescence microscopy images of *E. coli* cells (a), *S. epidermidis* (c) with only NIR exposure, and treated by AuNRs–PS–MNPs and NIR irradiation, respectively (b), (d). Scale bar equals 5 μm. FE-SEM images of *E. coli* (e) controlled with intact membrane and (f) dead due to destroyed membrane.

3d are the fluorescence microscopy images of the experimental samples of two bacteria which were treated with NIR irradiation and AuNRs–PS–MNPs respectively, exhibiting a majority of dead cells. The mortality rate of experimental *E. coli*, compared with the control (8.6%), was remarkably increased to 95.3%. And for *S. epidermidis*, compared with control 10.1% the cell count was ended with 98.9% as superior cell lysis. Figs. 3e and 3f represent the FE-SEM images of *E. coli* in live and dead conditions, respectively. The electron micrograph clearly demonstrates that the control *E. coli* own an intact and smooth surface. In contrast, the treated *E. coli* exhibits massive cell membrane' wrinkling and irreparable disruption resulted in leak of cellular components. In view of experimental data, the photothermal property of AuNRs could be the key role for the destruction of *E. coli* cells; however the clear mechanisms of bacteria lysis have not been understood yet. However, here the used nano-particles were unfunctionalized or devoid of such functional group for tagging with surface of bacteria hence the mechanism is only by external heat produced by the hyperthermic effect caused by photothermal interaction of gold core and input laser. Based on the work by An et al., we claim that the photothermal radiative property is the key factor for the eradication of bacteria [16]. We believe our new AuNRs–PS–MNPs have the ability to be used as effective anti-bacterial agents. Although this is an initial attempt to control only bacteria, further this system needs to be optimized to eradicate other types of microorganisms as well. And Au material cytotoxic effects caused by CTAB must be addressed using specific surface modification technique such as non-toxic coating layer introduction over the Au surface.

4. Conclusion

In conclusion, Janus shaped AuNRs–PS–MNPs were successfully synthesized using combination of the confined convective assembly technique and the modified layer-by-layer method. The resulting AuNRs–PS–MNPs particles could be rapidly collected by a magnet and redispersed for recyclable photothermal activity. Antibacterial ability of AuNRs–PS–MNPs was measured by live/dead staining techniques. More than 95% of pathogenic bacteria were severely destructed after treating with our Janus particles combination with NIR irradiation. Consider all, AuNRs–PS–MNPs can be applied as a recyclable, rapid, direct and high effective antibacterial agent. Furthermore, using our methods, different kinds of nanocomposited Janus particles with various properties could be synthesized.

Acknowledgment

This research was supported by National Research Foundation of Korea, K20704000003TA050000310 Global Research Laboratory Program, and supported by 2013 Research Fund of Myongji University. Authors appreciate Dr. Georgia C. Papaefthymiou for proofreading and comments.

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