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Vancomycin-modified LaB_6@SiO_2/Fe_3O_4 composite nanoparticles for near-infrared photothermal ablation of bacteria

Bo-Hung Lai, Dong-Hwang Chen *

Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan, ROC

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ABSTRACT

LaB_6 nanoparticles possess excellent near-infrared (NIR) photothermal conversion properties. Vancomycin can interact strongly with a broad range of Gram-positive and Gram-negative bacteria. Fe_3O_4 nanoparticles could be used as the carrier for magnetic separation. In this work, vancomycin and Fe_3O_4 nanoparticles were successfully bound onto the surface of LaB_6 nanoparticles with a silica coating and carboxyl functionalization to fabricate vancomycin-modified LaB_6@SiO_2/Fe_3O_4 (Van-LaB_6@SiO_2/Fe_3O_4) composite nanoparticles as a novel nanomaterial for the NIR photothermal ablation of bacteria. From the analyses of absorption spectra, transmission electron microscopy images and X-ray diffraction patterns, the formation of Van-LaB_6@SiO_2/Fe_3O_4 composite nanoparticles was confirmed. The resulting Van-LaB_6@SiO_2/Fe_3O_4 composite nanoparticles possessed nearly superparamagnetic properties, retained the excellent NIR photothermal conversion property of LaB_6 nanoparticles and could capture the bacteria *Staphylococcus aureus* and *Escherichia coli* efficiently. Owing to these capabilities, they were demonstrated to be quite efficient for the magnetic separation and NIR photothermal ablation of *S. aureus* and *E. coli*. Furthermore, the magnetic property made the Van-LaB_6@SiO_2/Fe_3O_4 composite nanoparticles useful for the magnetic assembling of bacteria, which could further enhance the photothermal ablation efficiency.

1. Introduction

Infectious bacterial diseases continue to be a leading cause of death and disability. In particular, infections resulting from Gram-positive bacteria (i.e. staphylococci and streptococci) remain a leading cause of morbidity and mortality in humans [1,2]. Vancomycin is a commonly used glycopeptide antibiotic, whose action primarily results in the inhibition of cell wall synthesis. Specifically, vancomycin exerts its antibacterial activity by forming hydrogen bounds with the terminal D-alanyl-D-alanine (D-Ala-D-Ala) moieties of the *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) peptide subunits [3,4]. This binding prevents the incorporation of NAM/NAG peptide subunits into the major structural component of Gram-positive cell walls, the peptidoglycan matrix, and thus results in inhibition of cell wall synthesis and ultimately bacterial cell death. Furthermore, according to the studies of Gu et al. [5] and Kell et al. [6], Gram-negative bacteria also could be captured by vancomycin due to either unspecific binding between receptors on the pathogen surface and the glycosides on the vancomycin moiety or breaks/deformities in the outer membrane of Gram-negative bacteria, exposing D-Ala-D-Ala groups on the interior bacteria surface. In addition, vancomycin-modified magnetic nanoparticles have also been demonstrated to be useful as affinity probes to trap vancomycin-resistant Gram-positive or negative bacteria selectively [6–9].

Photothermal therapy is an attractive therapeutic technique that uses photosensitizers to generate heat from light absorption which then kills cancer cells [10,11]. Plasmonic nanomaterials with high optical absorption in the near-infrared (NIR) region are usually used as the photosensitizers because they not only give this technique spatial selectivity but also avoid the nonspecific heating of healthy cells and allow deeper penetration into tissues [12]. Typical plasmonic nanomaterials used for NIR photothermal therapy include gold nanorods [13–15], gold nanoshells [16,17], gold nanocages [18], single-walled [19–21] or multi-walled [22] carbon nanotubes, graphene or reduced graphene oxide [23] and germanium [24]. Among them, gold-based nanomaterials received most attention.

The utilization of NIR photothermal therapy in the treatment of bacterial infections has also been attempted. For example, vancomycin-bound Fe_3O_4@Au nanoeggs [25], Fe_3O_4-Au_{rod} necklace-like probes [26] and popcorn-shaped gold nanoparticles [27] were demonstrated as photothermal agents for the selective killing of bacteria. It is noteworthy that lanthanum hexaboride (LaB_6) is also a metal-like plasmonic material. It exhibited a strong NIR absorption via surface plasmon resonance after grinding to nanoscale [28–30]. Recently, we demonstrated that LaB_6 nanoparticles

* Corresponding author. Tel.: +886 6 2757575x62680; fax: +886 6 2344496.
E-mail address: chendh@mail.ncku.edu.tw (D.-H. Chen).

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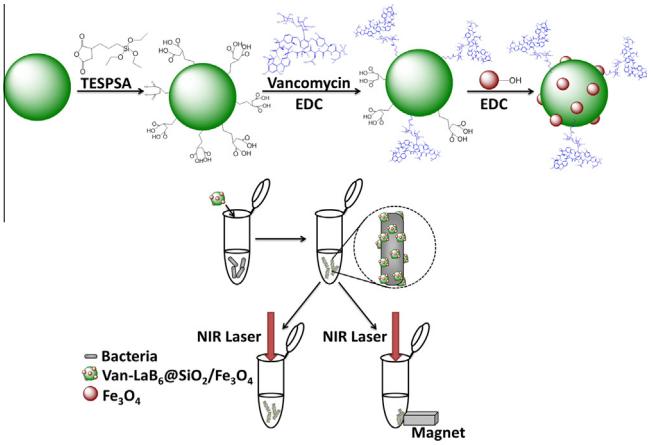

Fig. 1. Fabrication of Van-LaB_6@SiO_2/Fe_3O_4 nanoparticles for the targeted magnetic separation and NIR photothermal ablation of bacteria.

possessed an excellent NIR photothermal conversion property comparable and even slightly superior to gold-based nanomaterials [31]. Because of the relatively low price, LaB_6 nanoparticles might be used as an alternative to gold-based nanomaterials for NIR photothermal therapy.

Magnetic nanocarriers have been widely used in bioseparation, enzyme immobilization, drug delivery, hyperthermia and magnetic resonance imaging [32–34]. The combination of LaB_6 nanoparticles with magnetic nanoparticles makes the resulting composite nanoparticles magnetically recoverable and efficient in the separation of targets. Furthermore, the composite nanoparticles could be agglomerated via magnetic assembly. The NIR irradiation-induced heating on the agglomerated nanoparticles should be more efficient than on the dispersion solution of nanoparticles. Thus, the combination with magnetic nanoparticles might thereby decrease the time required to effectively inhibit the bacterial cell growth or to kill the bacteria [25]. Accordingly, in this work, vancomycin and Fe_3O_4 nanoparticles were bound onto the surface of silica-coated LaB_6 (LaB_6@SiO_2) nanoparticles successively to develop vancomycin-modified LaB_6@SiO_2/Fe_3O_4 (Van-LaB_6@SiO_2/Fe_3O_4) composite nanoparticles for the targeted magnetic separation and thermal ablation of bacteria. The silica coating of the LaB_6 nanoparticles was done to improve the stability and biocompatibility.

The fabrication of Van-LaB_6@SiO_2/Fe_3O_4 composite nanoparticles is shown in Fig. 1. First, the surface of LaB_6@SiO_2 nanoparticles was modified with 3-(triethoxysilyl)propylsuccinic anhydride (TESPSA) to introduce the carboxyl group to yield the carboxylated LaB_6@SiO_2 (COOH-LaB_6@SiO_2) nanoparticles [35]. Next, the COOH-LaB_6@SiO_2 nanoparticles were bound with vancomycin via carbodiimide (i.e. *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC)) activation to yield the vancomycin-bound LaB_6@SiO_2 (Van-LaB_6@SiO_2) nanoparticles. Finally, Van-LaB_6@SiO_2/Fe_3O_4 composite nanoparticles were obtained by further binding the Van-LaB_6@SiO_2 nanoparticles with Fe_3O_4 nanoparticles via carbodiimide activation. To demonstrate the capability of Van-LaB_6@SiO_2/Fe_3O_4 composite nanoparticles for the targeted magnetic separation and thermal ablation of bacteria, two bacteria (the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli*) were used as the models.

2. Experimental and methods

2.1. Chemicals

Lanthanum hexaboride powders (LaB_6, primary particle size: ~1–2 μm) were obtained from Wako Pure Chemical Ind., Ltd. Ammonium (28 wt.%) and 2-propanol were supplied by J.T. Baker. The grinding beads yttrium-stabilized zirconia (95% ZrO_2, 5% Y_2O_3, density: 6060 kg m^{-3}) with a diameter of 50 μm were obtained from Toray Ind., Inc. Ferric chlorides 6-hydrate, ferrous chloride tetrahydrate, tetraethylorthosilicate (TEOS), EDC, vancomycin, 2-(*N*-morpholino)ethanesulfonic acid (MES), sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, agar and yeast extract were supplied by Sigma–Aldrich Co. Tryptone was obtained from Becton, Dickinson and Co. TESPSA was purchased from Gelest, Inc. The bacteria *S. aureus* and *E. coli* were obtained from the Bioresource Collection and Research Center in Taiwan.

2.2. Preparation of Van-LaB_6@SiO_2 nanoparticles

LaB_6 nanoparticles were prepared according to our previous work [31]. For the silica coating, LaB_6 suspended in 2-propanol (20 ml, 0.4 mg ml^{-1}) was mixed with ammonia (28%, 0.5 ml) and sonicated in an ice bath for 10 min. Subsequently, TEOS (0.5 ml) was added and the mixture was sonicated for 3 h and then stirred for another 21 h. The resulting LaB_6@SiO_2 nanoparticles were collected by centrifugation, washed twice with ethanol and then redispersed into ethanol (5 ml). Next, LaB_6@SiO_2 nanoparticles were modified with carboxyl groups. The LaB_6@SiO_2 suspension was mixed with TESPSA (0.2 ml) and then stirred at 40 °C for 24 h. After centrifugation, the resulting COOH-LaB_6@SiO_2 nanoparticles ware dried in a vacuum oven. For comparison, in the absence of LaB_6@SiO_2 nanoparticles, the above silica coating process was conducted to yield the pure SiO_2 nanoparticles.

For the binding of vancomycin, the solution of COOH-LaB_6@SiO_2 (1 ml, 10 mg ml^{-1} in 0.1 M MES buffer at pH 5) was mixed with the solution of vancomycin (5 ml, 1 mg ml^{-1} in 0.1 M MES buffer at pH 5) and EDC (5 ml, 2 mg ml^{-1} in 0.1 M MES buffer at pH 5). The mixture was sonicated for 3 h. The resulting Van-LaB_6@SiO_2 nanoparticles were washed with water by centrifugation and redispersed in MES buffer (0.1 M, pH 5).

2.3. Preparation of Van-LaB_6@SiO_2/Fe_3O_4 composite nanoparticles

Fe_3O_4 nanoparticles were prepared by the co-precipitation of Fe^{2+} and Fe^{3+} ions with ammonia solution and following hydrothermal treatment according to our previous work [36]. The ferric and ferrous chlorides (molar ratio 2:1) were dissolved in water (40 ml) at a concentration of 0.3 M iron ions. Chemical precipitation was achieved at 25 °C under vigorous stirring by adding NH_4-OH solution (10 ml, 28%). During the reaction process, the pH was maintained at ~10. The precipitates were heated at 80 °C for 30 min. The resulting Fe_3O_4 nanoparticles were washed several times with water and ethanol, and then dried in a vacuum oven at 70 °C.

For the preparation of Van-LaB_6@SiO_2/Fe_3O_4 composite nanoparticles, the Van-LaB_6@SiO_2 nanoparticles (10 mg ml^{-1}) in 1 ml of MES buffer (0.1 M, pH 5) was mixed with EDC (2 mg ml^{-1}) via sonication for 10 min. Subsequently, Fe_3O_4 nanoparticles (4 mg ml^{-1}) were added to the mixture and then sonicated for 1 h. The resulting Van-LaB_6@SiO_2/Fe_3O_4 composite nanoparticles were washed several times with water and then dried in a vacuum oven.

2.4. Characterization

Transmission electron microscopy (TEM) analysis was carried out using a Hitachi Model H-7500 at 80 kV. The sample was obtained by placing a drop of colloid solution onto a Formvar-covered copper grid and evaporated in air at room temperature. X-ray diffraction (XRD) measurement was performed on a Rigaku D/max III.V X-ray diffractometer using Cu K_α radiation ($\lambda = 0.1542$ nm).