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Novel antibody/gold nanoparticle/magnetic nanoparticle nanocomposites for immunomagnetic separation and rapid colorimetric detection of *Staphylococcus aureus* in milk

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ABSTRACT

We demonstrated the new antibody/gold nanoparticle/magnetic nanoparticle nanocomposites (antibody/AuNP/MNPs) and their application in the detection of the foodborne pathogen, *Staphylococcus aureus* (*S. aureus*), in milk. The nanocomposites were synthesized by coating the MNPs with bovine serum albumin (BSA) then adsorbing the AuNPs and anti-*S. aureus* antibodies on their surface. Using the completed immunomagnetic nanostructures, *S. aureus* inoculated in the milk sample was captured and isolated from the medium using the permanent magnet. The nanoparticle-bound cells as well as the unbound cells in the supernatant were enumerated via surface plating to evaluate the target binding capacity of the nanocomposites. The capture efficiencies of the antibody/AuNP/MNPs were 96% and 78% for *S. aureus* in PBS and the milk sample respectively, which were significantly higher than those of the antibody-coupled MNPs without any AuNP. The captured cells were also applied to the selective filtration system to produce color signals that were used for the detection of the target pathogen. During the filtration, the cells bound to the antibody/AuNP/MNPs remained on the surface of the membrane filter while unbound nanoparticles passed through the uniform pores of the membrane. After the gold enhancement, the cells-particles complex resting on the membrane surface rendered a visible color, and the signal intensity became higher as the target cell concentration increased. The detection limits of this colorimetric sensor were 1.5×10^3 and 1.5×10^5 CFU for *S. aureus* in PBS and the milk sample respectively. This sensing mechanism also had the high specificity for *S. aureus* over the other pathogens such as *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. The assay required only 40 min to obtain the results. With the use of the appropriate antibodies, our immunomagnetic nanocomposites-based detection strategy can provide an easy, convenient, and rapid sensing method for a wide range of pathogens.

1. Introduction

A rapid detection of bacteria is extremely critical for an effective prevention and treatment of many foodborne illnesses, microbial infections and pathogenic diseases. The complications caused by the intake of contaminated foods are particularly gaining a world-wide attention as the number of food-related health issues has shown a steady increase in recent years. In the United States alone, the foodborne diseases lead to nearly 76 million cases of illnesses and 5000 deaths each year (World Health Organization, 2007). According to the Centers for Disease Control and Prevention (2012), *Staphylococcus aureus* (*S. aureus*) is one of the top five pathogens that contribute to the most foodborne illnesses in America. Since the majority of *S. aureus*-related food poisoning is caused via the contamination of milk and cheese (Centers for Disease Control and Prevention, 2006), a rapid and accurate detection of *S. aureus* in these food products is vital for an effective prevention of severe health problems.

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The most commonly used tools for determining the presence and concentration of pathogens are the colony counting, the polymerase chain reaction (PCR), and the enzyme-linked immunosorbent assay (ELISA) (Jay et al., 2005; Tenover, 1989). In order to analyze pathogens in a complex medium such as food, these methods usually have to be preceded by the immunomagnetic separation (IMS) technique that utilizes the antibody-coated magnetic beads (Cudjoe et al., 1995; Mine, 1997; Wright et al., 1994). Small magnetic particles (MP) with the sizes ranging from few hundred nanometers (Gu et al., 2006; Varshney et al., 2005) to several microns (Baldrich and Muñoz, 2008; Jung et al., 2012; Liébana et al., 2009) have been especially useful for the rapid IMS due to their large surface to volume ratio that induces an efficient interaction with the target even in a dilute sample containing various background materials. These particles are generally coated with the layers of polymers like dextran, polyacrylic acid, and silica (Kaittanis et al., 2010; Qiu et al., 2009) to prevent the oxidation of the iron oxide core and the formation of clusters (Wu et al., 2008). With the polymer coatings, the magnetic nanoparticles (MNPs) can have a high dispersion stability in an aqueous solution (Gupta and Gupta, 2005), becoming an ideal platform for the separation and detection of foodborne bacteria.

After capturing and isolating the pathogens using the IMS, the cells-MPs complexes can be applied to a variety of detection methods such as mid-infrared (mid-IR) spectroscopy (Ravindranath et al., 2009), surface-enhanced Raman scattering (SERS) immunoassay (Wang et al., 2011), flow cytometry (Seo et al., 1998), and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Schlosser et al., 2007). Although these techniques can detect various microbial species with ultrahigh sensitivity, they usually require time-consuming and labor-intensive processes as well as expensive equipments (Lazcka et al., 2007) that make them unsuitable for the rapid, easy and low-cost sensing of foodborne bacteria. As an alternative for the complicated detection methods, the antibody-labeled gold nanoparticles (AuNPs) have been used as an immunological reporter for an easy visual sensing of the biomolecules (Jian and Huang, 2011; Kim et al., 2012; Li et al., 2009). Since AuNPs possess high absorption coefficients and scattering cross-sections (Kim et al., 2012), their aggregation upon binding with the target entities produced a clear color signal that enabled a rapid colorimetric detection, but the test samples had to be purified and concentrated prior to the immunological reaction to achieve a specific and sensitive response with low noise (Li et al., 2009). As a result, a suitable combination of AuNPs with a practical target separation technique such as the IMS is highly desirable to realize the fast and simple biosensor systems for foodborne pathogens.

In this paper, we demonstrate the effective capture and the rapid colorimetric detection of *S. aureus* in the milk samples using the novel antibody/AuNP/MNP nanocomposites that maintain both the optical properties of the AuNPs and the magnetic properties of the MNPs. The target binding efficiency of the immunomagnetic nanostructure was evaluated via surface plating and enumeration of the captured cells as well as the unbound cells. The detection sensitivity of the colorimetric sensor, which combines our nanocomposites with the selective filtration system, was also tested using various concentrations of the target pathogen, and the limit of detection was determined after the gold enhancement of the nanoparticles remaining on the surface of the membrane filter. In addition, the sensor's selectivity was examined by applying various non-target bacteria to the system.

2. Materials and methods

2.1. Reagents and materials

Carboxylated MNPs with a mean diameter of 100 nm were purchased from Chemicell Inc. (Berlin, Germany). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) were purchased from Biacore Inc. (Piscataway, NJ, USA). Bovine serum albumin (BSA), sodium bicarbonate ($NaHCO_3$), potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), glycerol, boric acid, borax, hydroxylamine, hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 3H_2O$), sodium citrate, citric acid, and glycine were obtained from Sigma (St. Louis, MO, USA). 10 nm diameter AuNP colloid was purchased from BB International (Cardiff, UK), mouse monoclonal anti-*S. aureus* antibody from Abcam Inc. (Cambridge, MA, USA), 10 mM phosphate buffer saline (PBS) containing 140 mM NaCl at pH 7.4 from Gibco (Grand Island, NY, USA), and Tween-20 from Duchefa Biochemie BV (Haarlem, Netherlands). Cellulose acetate (CA) membrane filters with a pore size of 0.8 μm was purchased from Advantec (Tokyo, Japan) and the Dynal magnet for the magnetic particle separation was purchased from Invitrogen (Grand Island, NY, USA). The deionized water (DW) used in all experiments was purified with a Milli-Q system (Millipore, Brussels, Belgium). The pasteurized milk in 500 mL cartons was purchased from a local supermarket.

2.2. Preparation of microbial samples

S. aureus (ATCC 25923), *Escherichia coli (E. coli*, ATCC 25922), *Listeria monocytogenes* (*L. monocytogenes*, ATCC 19111) and *Salmonella enterica* (*S. enterica*, ATCC 14028) were obtained from the American Type Culture Collection (Manassas, VA, USA). *S. aureus* and *E. coli* were cultured in trypticase soy broth medium, while *L. monocytogenes* and *S. enterica* were cultured in brain-heart infusion (BHI) broth and nutrient broth medium (Difco Laboratories, USA) respectively at 37 °C with 180 rpm shake for 18–24 h. The cultured bacteria were then serially diluted and surface plated on the agar dish containing the appropriate culture medium. After a 24-h incubation at 37 °C, concentrations of the bacteria were initially estimated with the spectrometer and subsequently confirmed by the colony counting. Lastly, the cell cultures were re-suspended in 15% glycerol-PBS and stored at −80 °C before use.

2.3. Preparation of BSA/MNPs, antibody/AuNP/MNP nanocomposites and antibody/MNP conjugates

The synthesis procedures for the BSA-coated MNPs (BSA/MNPs), the antibody/AuNP/MNP nanocomposites and the MNPs directly coupled to the antibodies (antibody/MNP conjugates) are schematically illustrated in Fig. 1. The surface of the carboxylated MNPs was first coated with BSA (Fig. 1(a)) by adding 50 μL of 25 mg/mL MNP solution to a mixture containing 2.5 μL of 0.4 M EDC and 50 μL of 0.1 M NHS in 450 μL of 10 mM phosphate buffer at pH 7.4 (PB) with 1% BSA (w/v). The solution was then incubated for 30 min at room temperature (RT) on a 30 rpm rotary shaker to promote the covalent bonding between the MNPs and the BSA. The incubation of the mixture was followed by the addition of 50 μL of 100 mM glycine in PB which blocks the bare carboxylic groups on the MNP surface and reduces nonspecific interactions with the other molecules. After an additional 30-min RT incubation on a 30 rpm rotary shaker, the solution was centrifuged for 20 min at 6000 rpm and 4 °C. The supernatant was discarded, and the pellet was re-suspended in 0.2 mL of the borate buffer (10 mM, pH 8.5). Finally, the centrifugation and re-suspension process was repeated once to complete the synthesis of the BSA/MNPs. In the meantime, 0.5 mL of 5X AuNP colloid was mixed with the BSA/MNP suspension in the borate buffer, followed by a 30-min incubation at RT on a 30 rpm rotary shaker. During the incubation, the AuNPs were effectively attached to the MNP surface due to their non-covalent electrostatic interactions with the BSA (Burt et al., 2004).