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Effect of immunomagnetic bead size on recovery of foodborne pathogenic bacteria

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ABSTRACT

Immunomagnetic separation (IMS) as a culture-free enrichment sample preparation technique has gained increasing popularity in the development of rapid detection methods for foodborne pathogens. While the use of magnetic nanoparticles in IMS is on the rise due to substantially larger surface area compared to conventional magnetic microparticles, the effects of immunomagnetic bead (IMB) size on pathogen cell recovery are not fully understood. In this study we used IMBs of different sizes (100, 500, and 1000 nm diameters) to capture *Salmonella* Enteritidis, a common foodborne pathogen, from buffer solutions as well as food matrices (chicken carcass rinse and liquid egg white). The IMS recovery and non-specific binding rate were compared. The recoveries of *Salmonella* cells in buffers was highest using the 100 nm IMBs (88–96%), followed by the 500 nm (31–89%) and 1000 nm (4.1–61%) IMBs, demonstrating a significant size effect. The non-specific binding rates of *E. coli* also increased as IMB size decreased. A 2–72% reduction in *Salmonella* recovery was observed in chicken carcass rinse and liquid egg white samples compared to in buffers, and this reduction was more significant using 500 and 1000 nm IMBs. However, lower IMS recoveries (10–56%) was found in 100 nm IMBs two months after preparation. Overall, magnetic nanoparticles yielded superior IMS efficiency to micrometer size IMBs and were less subjective to interference from food matrices. Nevertheless, their long term stability remains an obstacle towards successful use in IMS.

1. Introduction

Sample preparation is a critical step in food pathogen detection. The aims of sample preparation include elimination of interference from food matrix components and background flora, and concentration of target pathogens to detectable amounts (Stevens and Jaykus, 2004). In standard microbiological testing (USDA-FSIS, 2017a), culturing in a non-selective medium followed by selective media serves to enrich target pathogen while repressing competitive microorganisms, which typically outnumber target initially. Despite their high specificity and sensitivity, traditional detection methods often fail to meet the need to monitor the rapid spread of a foodborne outbreak, primarily due to the lengthy culture steps. Numerous rapid detection techniques based upon real-time polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), lateral flow, colorimetric and other assays have been proposed and validated in various food matrices by independent organizations, and several methods have been adopted as screening tests in conjunction with standard microbiological methods (USDA-FSIS, 2017b). In these assays, sample preparation is usually streamlined to a single enrichment step in non-selective media. Though elimination of selective culture shortens the sample preparation step, growth for 16–24 h remains the time limiting step, considering that rapid detection techniques require only 1–3 h to results. Consequently, improvements in rapidity of pathogen tests depend on more aggressive reduction of sample preparation time. Moreover, some rapid techniques, such as PCR, are highly sensitive to interference from components of the food matrix or growth media (Schrader et al., 2012). Thus, next-generation sample preparation should also provide selective purification of targeted species prior to rapid detection.

In such a context, immunomagnetic separation (IMS) has become widely recognized as a sample preparation technique for rapid pathogen detection (Olsvik et al., 1994). The IMS technique utilizes antibody functionalized superparamagnetic beads (i.e., immunomagnetic beads or IMBs) to selectively capture target analytes from multi-component samples. Through simple manipulation of the external magnetic field, IMBs with bound bacteria can be isolated from the matrix, or re-dispersed in washing and elution buffers for purification and subsequent analyses. Negative effects of selective media on the target pathogen, such as cell death and plasmid loss during enrichment (Hill et al., 1985), can also be circumvented (Olsvik et al., 1991).

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Compared to traditional culture-free enrichment approaches such as centrifugation and filtration, IMS offers superior robustness and simplicity. Thus far, automated and medium throughput IMS systems have become commercially available for versatile applications in biomedical and pharmaceutical research, as well as environmental and food analysis applications (Chen et al., 2014; Lau et al., 2012).

Central to the IMS technique are the IMBs - treated flakes of magnetic oxides (most commonly γ-Fe_2O_3 and Fe_3O_4) that are stabilized by surface chemical groups or in a polymer phase (Lu et al., 2007; Olsvik et al., 1994). The effectiveness and efficiency of IMS partly rely on the IMBs, including the concentration and corresponding epitope of the coating antibody (Lund et al., 1988). Sample volume, initial bacterial concentration, and mode of bacteria-IMB interaction also play important roles in IMS (Chen et al., 2014; Lund et al., 1988). Commercially available IMBs sized between 1 and 4.5 μm in diameter are commonly adopted in sample preparation of rapid pathogen detection methods (Chandler et al., 2001; Fu et al., 2005; Kapperud et al., 1993; Liu et al., 2001; Mansfield and Forsythe, 2001; Rijpens et al., 1999; Skjerve et al., 1990; Wang et al., 2007). The size effect of micrometer size IMBs on IMS has been noted by Tu et al. In their studies it was found larger magnetic beads (2.8 μm) were more effective in capturing bacteria than smaller beads (1 μm) of the same density using IMS-time resolved fluorescence (Tu et al., 2009). With advances in nanomaterials and nanotechnology, direct use of magnetic nanoparticles (e.g. 80–300 nm diameter) in isolation and purification of small molecular targets and macromolecules (proteins and nucleic acids) has been on the rise (Schlosser et al., 2007). Early studies have indicated that the large specific surface area of nanoparticles led to higher scavenging efficiencies for removing toxic compounds from the environment (Mandel and Hutter, 2012). While nanoparticles have yielded higher recovery of relatively small molecular targets, similar advantages in the case of cellular targets, such as bacteria, cannot be assumed due to the much larger dimensions relative to the IMBs. A previous study showed that magnetic nanoparticles provided equivalent or lower detection limits compared to their micrometer-sized counterparts in the case of IMS of *Salmonella* captured by 300 nm and 2.8 μm IMBs followed by detection using an electrochemical immunosensor (Brandão et al., 2015). Otherwise, magnetic nanoparticles have most commonly been prepared in individual laboratories for IMS and adapted to various detection techniques (Yang et al., 2007; Zhao et al., 2009), but comparison of IMB size on target recovery has not been adequately reported.

Salmonella is responsible for the largest number of foodborne outbreaks in the United States, and *S.* Enteritidis is an important serotype commonly found in egg and egg products and causes the most *Salmonella* cases (Marder, 2017). As of April 2017, 60 rapid detection methods have been validated for *Salmonella* detection from various food matrices (USDA-FSIS), but most methods still require several hours of enrichment in culture media.

Thus, the main objective of this study was to investigate the potential size-related effects of nanoparticle-based IMBs on recovery of targeted pathogen cells, using *Salmonella* Enteritidis as a representative model. The second objective was to investigate size-related matrix effects in food samples. Lastly, stability of different IMBs and its impact on pathogen recovery was also examined.

2. Materials and methods

2.1. Materials

N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), *N*-Hydroxysuccinimide (NHS), skim milk powder, Tween® 20, and 4′,6-diamidino-2-phenylindole (DAPI) were obtained from Sigma-Aldrich (St. Louis, MO). Carboxylic acid functionalized 100, 500, and 1000 nm Super Mag beads and activation buffer for ligand conjugation (25 mM MES with 0.01% Tween® 20, pH 6.0) were purchased from Ocean Nanotech (San Diego, CA). Phosphate buffered saline (10 ×) and anti-*Salmonella* antibody (PA1-85849) was purchased from ThermoFisher Scientific (Waltham, MA). Tryptic soy broth (TSB) and tryptic soy agar (TSA), Brilliant Green sulfa agar (BGS), and buffered peptone water (BPW) were obtained from BD (Franklin Lakes, NJ). Raw whole chicken and liquid egg white products were obtained from a local grocery store.

2.2. Functionalization of magnetic particles

Carboxylic acid functionalized superparamagnetic beads were modified with anti-*Salmonella* antibody according to the manufacturer's instructions with minor modifications. Briefly, 100 μL of magnetic beads (10 mg/mL) were mixed with 100 μL of activation buffer and 50 μL of freshly prepared EDC/NHS solution (10 mg/mL each in activation buffer), and reacted at room temperature (RT) for 15 min with continuous mixing. The activated magnetic beads were rinsed twice by placing the microcentrifuge tubes in a magnetic separator (MagneSphere®, Promega, Madison, WI) for 5 min, removing supernatant, and resuspending collected beads in activation buffer through vortexing (5 min) and sonication (20 s) (Model 5510, Branson Ultrasonics, Danbury, CT). Subsequently, 100 μL of anti-*Salmonella* antibody (1 mg/mL in activation buffer) was added and allowed to react at RT for 2.5 h with continuous mixing. Unconjugated carboxyl groups were deactivated by adding 500 μL of 100 mM Tris-HCl (pH 7.4) and incubating at RT for 30 min. The resultant IMBs were collected, rinsed three times with PBS with 0.01% Tween 20, and resuspended in 500 μL of this buffer for storage up to 2 weeks at 4 °C. The final IMB concentration was equivalent to 2 mg/mL of original particle weight.

2.3. Bacterial culture

S. Enteritidis and *E. coli* strains were isolated from chicken carcass rinse samples and stored at − 80 °C. Before each experiment, 10 mL of TSB medium was inoculated by a loopful of bacterial colonies maintained on TSA slants, and incubated at 37 °C for 16 h. The bacterial culture (1 mL) was harvested, rinsed once with PBS with 0.05% Tween 20 (PBST), and serially diluted in PBST to yield 10^{-1} and 10^{-5} dilutions, which were selected to mimic high and low contamination levels in actual samples. The inocula were estimated by drop plating 3–6 replicates at each dilution onto TSA or BGS plates and incubating at RT overnight. The CFUs were counted under a dissecting microscope.

2.4. IMS protocol

Prior to IMS, 20 μL aliquots of IMB suspensions (40 μg of nanoparticles each) were blocked with 40 μL of filtered skim milk solution (1% in PBS) at RT for 1 h to minimize non-specific binding (NSB) with matrix components and background flora. For IMS, 500 μL of bacterial cells (10^4–10^8 CFU/mL) in PBST were mixed with 60 μL of blocked IMBs, and incubated at RT for 30 min while shaking at 1000 rpm. The IMB/bacteria complexes were collected on a magnetic separator, and rinsed twice with 1 mL of PBST before being resuspended in 500 μL PBST. This suspension was then serially diluted 10-fold, and 5 μL of each dilution were drop plated onto TSA plates in triplicate or sextuplicate and incubated at RT overnight for enumeration of captured pathogen cells. The use of drop plating was to limit the number of agar plates involved in each experiment, and the results obtained by drop plating was first confirmed in a preliminary experiment to yield similar results to spread plating (Supplementary Table S1).

The IMBs functionalized with anti-*Salmonella* antibodies were mixed with *S.* Enteritidis for estimating the recoveries of the IMS targets. *E. coli* was used as a negative control to assess the degree of NSB in IMS. All experiments were carried out in triplicate.

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