Colloids and Surfaces B: Biointerfaces 151 (2017) 47–57

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

# T4 bacteriophage conjugated magnetic particles for *E. coli* capturing: Influence of bacteriophage loading, temperature and tryptone

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ARTICLE INFO

*Article history:*
Received 4 May 2016
Received in revised form 1 December 2016
Accepted 7 December 2016
Available online 8 December 2016

*Keywords:*
Bacteriophages
Adsorption
Surface modifications
Bacteria capture
Magnetic particles

ABSTRACT

This work demonstrates the use of bacteriophage conjugated magnetic particles ($Fe_3O_4$) for the rapid capturing and isolation of *Escherichia coli*. The investigation of T4 bacteriophage adsorption to silane functionalised $Fe_3O_4$ with amine (—$NH_2$), carboxylic (—COOH) and methyl (—$CH_3$) surface functional groups reveals the domination of net electrostatic and hydrophobic interactions in governing bacteriophage adsorption. The bare $Fe_3O_4$ and $Fe_3O_4$-$NH_2$ with high T4 loading captured 3-fold more *E. coli* (~70% capturing efficiency) compared to the low loading T4 on $Fe_3O_4$-COOH, suggesting the significance of T4 loading in *E. coli* capturing efficiency. Importantly, it is further revealed that *E. coli* capture is highly dependent on the incubation temperature and the presence of tryptone in the media. Effective *E. coli* capturing only occurs at 37 °C in tryptone-containing media with the absence of either conditions resulted in poor bacteria capture. The incubation temperature dictates the capturing ability of $Fe_3O_4$/T4, whereby T4 and *E. coli* need to establish an irreversible binding that occurred at 37 °C. The presence of tryptophan-rich tryptone in the suspending media was also critical, as shown by a 3-fold increase in *E. coli* capture efficiency of $Fe_3O_4$/T4 in tryptone-containing media compared to that in tryptone-free media. This highlights for the first time that successful bacteria capturing requires not only an optimum tailoring of the particle's surface physicochemical properties for favourable bacteriophage loading, but also an in-depth understanding of how factors, such as temperature and solution chemistry influence the subsequent bacteriophage-bacteria interactions.



## 1. Introduction

Foodborne illnesses have become a major concern worldwide, with the majority of foodborne diseases caused by *Salmonella* sp., *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, *Campylobacter jejuni* and *Bacillus cereus* [1]. The conventional methods of detecting pathogens in food rely on cell culturing, which have been the "gold standard" microbiological technique [2–4]. This process consists of pre-enrichment, selective enrichment, plating and conformation and it may take up to 3–7 days to complete, making the process very lengthy [5]. With the need for rapid yet sensitive techniques, many detection methods have been developed, with some success. Several assay techniques, including enzyme-linked immunoassays (ELISA), immunosensors, polymerase chain reaction (PCR) and flow cytometry, have shortened the detection time to less than 2 h [6]. Regardless, due to the complexity of the food matrix, the low level of target bacteria and the diversity of other microflora in food, the applications of such techniques remain very challenging, as they still rely on the sample enrichment steps that in most cases will take days to complete [7,8]. Although lengthy, sample enrichment is critical, as it promotes amplification of target bacteria, revives some injured target bacteria and suppresses the interference from food matrices [8,9]. It is difficult to completely eliminate the reliance of many rapid detection methods on the sample enrichment process. Further advancement in sample enrichment technique is therefore required to fully achieve a rapid detection of bacteria in food.

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http://dx.doi.org/10.1016/j.colsurfb.2016.12.009
0927-7765/

The concept of concentration and isolation of bacteria that is culture-independent has become increasingly popular [10], as it allows isolation and concentration of bacteria from other interferences and reduction of sample volume within a short period of time. The application of magnetic particles as a magnetically controllable material has revolutionised the isolation and separation of many chemical and biological materials especially when the concentration is low [11,12]. When introduced into food samples, magnetic particles are capable of capturing bacteria, separating them from the food components through subsequent application of a magnetic field [10]. The specificity of the bacterial capturing is however, limited, as also shown in other studies with bare and functionalised magnetic particles [13]. The specificity of magnetic particles towards target pathogenic bacteria is an essential parameter as both pathogenic and non-pathogenic bacteria may be present in food [14]. Specific capturing of target pathogenic bacteria may aid downstream pathogen detection and reduce the potential for "false-positives" arising.

The specificity of magnetic particles can be tuned through coupling with a recognition element. One potential example of the recognition element is bacteriophage. Bacteriophages are ubiquitous viruses with a broad or specific range of bacterial host [15–17]. Exploiting their specificity and infectivity towards target bacteria, bacteriophages have come into vogue as a recognition agent in biosensing [18,19], antibacterial surfaces [20] and as a model virus for membrane filtration testing [21]. The integration of bacteriophage and magnetic particles to isolate and capture target bacteria has not been well studied. Earlier studies have reported a very low bacteria recover (less than 20%) of such system [22,23]. After almost two decades, a higher extent of bacteria recovery has only been reported by Chen et al. [24], with 60–70% recovery of *E. coli* K12 ($10^5$ cfu/mL) using T7 bacteriophage conjugated with mixed metal oxide nanoparticles containing 30% cobalt. However, the effectiveness of immobilised bacteriophage in infecting bacteria has been reported to reduce in the presence of environmental interferences, such as human albumin and extracellular polysaccharide [25]. Moreover, the presence of certain cations ($Ca^{2+}$ and $Mg^{2+}$), solutions' pHs and temperatures have also been reported to significantly affect the interaction between bacteria and bacteriophage [26,27]. Yet, thus far there are no studies that investigate the influence of such variables to the performance of bacteriophage based magnetic particles in capturing bacteria. With increasing interest in the use of bacteriophage based magnetic particles to capture bacteria in real food samples, further work is required to better understand and determine the critical parameters required to achieve successful capturing. Thus, the aim of this study is to begin to address that knowledge gap.

Herein, we employed surface functionalisation of magnetic iron oxide nanoparticles ($Fe_3O_4$) via organosilane grafting to provide different surface functional groups and chemical properties, namely amine (—$NH_2$), carboxylic (—COOH) and methyl (—$CH_3$) functional groups. T4 bacteriophages are a non-enveloped, head-tail structure bacteria virus that infects *E. coli*. T4 bacteriophage are widely used as a recognition agent for biosensors [28], a surrogate to model virus removal in membrane filtration system [21] and antimicrobial surfaces [29] due to their well-understood properties [30,31]. In this study, T4 bacteriophages were immobilised onto bare and differently functionalised $Fe_3O_4$. It was found that variation in surface chemistry of the particles influenced the bacteriophage loading. The performance of the T4 bacteriophage conjugated $Fe_3O_4$, with regards to the capture and isolation of *E. coli* was studied. We report a synergistic influence of bacteriophage loading, incubation temperature and the solution chemistry in dictating T4 bacteriophage conjugated $Fe_3O_4$ performance in capturing *E. coli*.

## 2. Materials and method

### 2.1. Reagents and materials

Iron (II,III) oxide nanopowder, 3-aminopropyltriethoxysilane (APTES), succinic anhydride, bovine serum albumin (BSA), octadecyltrimethoxysilane (ODTMS), phosphotungstic acid (PTA) and sodium citrate tribasic dihydrate were sourced from Sigma-Aldrich. Analytical grade methanol, ethanol, glacial acetic acid, sodium chloride, sodium hydroxide pellets, *N,N*-dimethylformamide (DMF), Tris-HCl, glycerol and D-glucose were purchased from Ajax Chemicals. Phosphate buffer saline tablets (8 g/L NaCl, 0.2 g/L KCl, 1.15 g/L $Na_2HPO_4$ and 0.2 g/L $KH_2PO_4$, pH 7.2–7.4), tryptone, yeast extract and agar bacteriological were obtained from Oxoid. Bacteriophage T4 (ATCC 11303-B4) and its host *E. coli* (ATCC 11303) were supplied from the ATCC.

Luria Bertani broth was made by adding 10 g/L tryptone, 5 g/L yeast extract and 10 g/L sodium chloride in Milli-Q water. Tryptone agar was prepared by dissolving 10 g/L tryptone, 8 g/L sodium chloride, 3 g/L D-glucose, 2 g/L sodium citrate tribasic dehydrate and 10 g/L agar bacteriological in Milli-Q water. All media were sterilised by autoclaving at 121 °C.

### 2.2. Surface modification of iron oxide ($Fe_3O_4$) nanoparticles

The amine functionalised $Fe_3O_4$ ($Fe_3O_4$-$NH_2$) was prepared by mixing 0.2 g of iron (II,III) oxide nanopowder with 5 mL anhydrous methanol, 0.25 mL water, 0.5 mL glacial acetic acid, and 30 mL glycerol. The solution was sonicated for 2 min at 20% amplitude (Misonix) and transferred into a three-necked round bottom flask. A mixture of 10% (v/v) of APTES in anhydrous methanol, and was slowly titrated and the solution was heated to 120 °C for 15 h under nitrogen. The particles were subjected to repeated washing after functionalisation in Milli-Q water and ethanol and dried under vacuum. The bare $Fe_3O_4$ was prepared as a control sample by following the same procedure with the absence of APTES.

The amine functionalised $Fe_3O_4$ was further derivatised to introduce carboxylic functional groups. An amount of 0.05 g of $Fe_3O_4$-$NH_2$ was added to 10% (w/v) of succinic anhydride in anhydrous DMF. The mixture was stirred for 3 h under nitrogen. The particles were washed in DMF, Milli-Q water and ethanol successively and dried under vacuum.

The methyl terminated $Fe_3O_4$ was obtained by dispersing 0.05 g bare $Fe_3O_4$ in 5% (v/v) *n*-butylamine in anhydrous methanol and 1 mL of ODTMS under sonication at an amplitude of 20% (Misonix) for 1 h. The suspension was left for 30 min and subjected to serial washing in methanol. The resulted particles were dried under vacuum overnight.

### 2.3. T4 bacteriophage propagation

The propagation of T4 bacteriophage was done by incubating 50 μL of $10^{10}$ plaque forming unit (pfu) per mL in 4 mL of fresh *E. coli* culture for 45 min at 37 °C. The mixture was then added to 200 mL log-phase *E. coli* culture and was incubated at 37 °C in a shaking incubator for 3 h. The solution was centrifuged twice at 5000*g* for 20 min to pellet down the bacteria. The supernatant was then centrifuged twice at 25,000*g* for 2 h at 4 °C to pellet down the bacteriophages from the supernatant. The obtained pellet was re-suspended in 3 mL of 10 mM phosphate buffer pH 6 and stored at 4 °C until ready for use.

### 2.4. T4 bacteriophage adsorption

T4 bacteriophage was allowed to adsorb on to bare and differently functionalised $Fe_3O_4$ by incubating 0.15 mL T4 bacteriophage