Multifunctional nanoparticle platforms for pathogen diagnostic applications

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ABSTRACT

We have developed a series of vancomycin-modified nanoparticles for the affinity magnetic capture of multiple species of Gram-positive and Gram-negative bacteria. The orientation/architecture of the vancomycin moiety on the nanoparticle surface is important in determining how effectively the nanoparticles can bind to the surface of bacteria, which in turn affects the ability for the nanoparticles to magnetically confine the bacteria. With the proper surface orientation, the vancomycin-modified nanoparticles can effectively confine at least eight different species of bacteria. We have also prepared single-domain antibody-modified nanoparticles for the selective magnetic capture of *S. aureus* cells. In parallel we are developing hybrid nanoparticle detection platforms that utilize hybrid magnetic/luminescent nanoparticles comprised organic fluorescent dyes and superparamagnetic nanoparticles, where the hybrid nanoparticles can be exploited for simultaneous magnetic confinement and optical detection of a single bacteria species from a given sample.

Keywords: magnetic nanoparticle, vancomycin, antibody, bacteria, luminescence

1 INTRODUCTION

Infectious diseases and bioterrorism are of tremendous global concern and developing strategies to quickly identify the presence and identity of potential threats to public health presents a difficult challenge because detection strategies have to be fast, inexpensive and appropriate for point-of-care based applications.[1] The miniaturization of detection scaffolds onto microfluidic devices is an increasingly popular strategy employed in potential point-of-care-based diagnostics.[2-4] However, there is a large disconnect between clinical sample volumes (5-10 mL) and the volumes appropriate for incorporation into microfluidic devices (>100 µL). This disconnect can be quite important when considering water, food or even blood sample analysis, where pathogen concentrations as low as 10 bacteria/mL can be quite dangerous. In this circumstance sampling just 50-100 µL could easily result in a false negative. Hence, to bridge the gap between clinical sample volumes and volumes appropriate for introduction into microfluidic devices, we are interested in developing superparamagnetic nanoparticles capable of effectively interacting with Gram-positive and Gram-negative bacteria. Because the nanoparticles are superparamagnetic they can impart magnetic character to the bacteria following binding. As a result these nanoparticles can be valuable in sample preparations for analysis of bacteria on microfluidic device platforms, where it is possible to magnetically pre-concentrate labeled bacteria from large volumes to volumes more appropriate for analysis on the microfluidic platform.[5, 6]

There are a variety of substrates that can mediate interactions between nanoparticles and receptors on bacteria surfaces and our group is focused on employing small molecule probes and antibodies. One small molecule probe that is particularly useful in mediating interactions between nanoparticles and bacteria is a molecule named vancomycin. Vancomycin is a glycopeptide antibiotic that is capable of interacting with a large range of different bacteria species, so a vancomycin-modified nanoparticle should be able to magnetically confine many different types of bacteria.[7] Interestingly, because vancomycin is significantly less complicated than large antibodies, its surface orientation/architecture can be easily controlled (Figure 1).[8] This allows for the systematic investigation of how the orientation of the probe molecule effects the binding/magnetic confinement of a series of bacteria. Because vancomycin-modified nanoparticles can confine a broad range of bacteria, these nanoparticles are potentially useful for point-of-care applications where the confined bacteria could be identified based on a DNA fingerprint within a microarray.

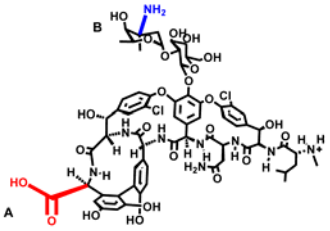

Figure 1. The structure of vancomycin and the functional groups employed to anchor the molecule to the surface of NP-1 (A) and NP-2 (B) with different orientations/architectures.

Though it is interesting to magnetically confine many bacteria with a single nanoparticle probe, there is also value in developing nanoparticle probes that can selectively bind

to and magnetically isolate a single bacterial species away from other species of bacteria. In this case, an antibody is much more appropriate for mediating the interaction between the nanoparticle and the bacteria. Herein we report how both small molecule probes and antibodies anchored to the surface of nanoparticles can mediate the magnetic confinement of several different species of bacteria. Furthermore, we propose how hybrid nanoparticle architectures can potentially provide a luminescence output signal that could be used to identify what bacteria has been isolated.

2 RESULTS AND DISCUSSION

Amine and carboxylic acid-modified silica encapsulated iron oxide nanoparticles were modified with vancomycin in two different orientations through two different architectures giving rise to NP-1 and NP-2 (Figure 2). Following incubation of these nanoparticles with a variety of Gram-positive and Gram-negative bacteria (100-300 cfu/mL), it was determined that NP-1 provides a significantly better magnetic confinement than that mediated by NP-2 (Figure 2).

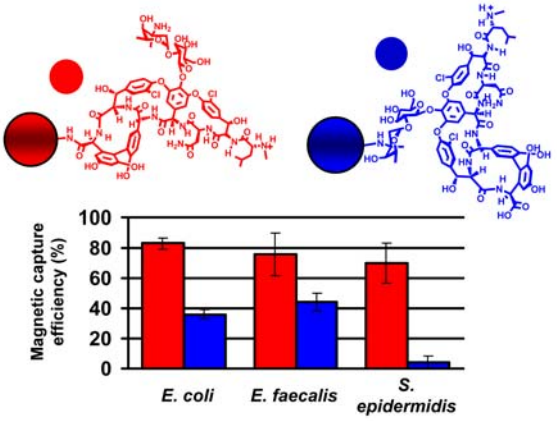

Figure 2. Bar graph representations of the magnetic confinement of *E. coli*, *E. faecalis* and *S. epidermidis* by NP-1 (red) and NP-2 (blue). Note that NP-1 captures all bacteria species investigated more effectively than NP-2.

Microagglutination assays suggest that the binding affinity of NP-1 is at least 8-10 times greater than that of NP-2 for *S. aureus*, explaining why the magnetic confinement efficiency for NP-1 is superior to that for NP-2. NP-1 was then incorporated into a magnetic capture assay for several different bacteria species (variety of 9 different Gram-positive and Gram-negative bacteria) and we were pleased to see that the nanoparticles were able to effectively confine each of the bacteria with the exception of *E. faecalis*, a vancomycin-resistant strain of *enterococci* (Figure 3). As mentioned above, the ability to magnetically confine several species of bacteria with a single nanoparticle is interesting within the context of a point-of-care device that could potentially identify the confined bacteria by means of a DNA fingerprint in a microarray. Within such a microfluidic device the isolated bacteria could be ruptured to liberate the genomic DNA,[3] the DNA could be amplified with PCR,[2] and the amplicons could be used to speciate the isolated bacteria based on a fluorescent signal in a DNA microarray (Figure 3).[4] A multiplexed approach such as this has the potential to identify several different harmful bacteria species from large volume food, water or even blood samples, though nonspecific absorption of blood-based proteins would significantly complicate the specific targeting of the bacteria by the functionalized nanoparticles.

Bacteria species capture efficiency with NP-1

	E. faecalis	*S. Epidermidis*	*S. agalactiae*	*S. aureus*	*E. faecium (VRE)*	*E. coli*	*P. aeruginosa*	*K. oxytoca*	*S. maltophilia*
NP-1	76%	70%	88%	71%	7%	83%	66%	45%	60%

Gram-positive Gram-negative

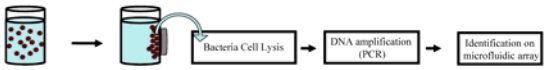

Figure 3. A table highlighting the variety of Gram-positive and Gram-negative species of bacteria that can be isolated with NP-1 and a schematic representation of how the isolated bacteria could be detected in a series of microfluidic devices.

Alternatively there are instances where the selective labeling and isolation of a single bacteria species, perhaps in the presence of several other species of bacteria, will be required. Antibodies are ideal for this type of application because they can selectively interact with unique surface antigens that will be specific to each species of bacteria. To carry out a selective magnetic confinement, we have anchored a single-domain antibody[9-11] to the surface of silica-encapsulated iron oxide nanoparticles in order to mediate selective interactions between *S. aureus* and the nanoparticles (NP-3, Figure 4). The sdAbs have been engineered to target protein A, a prevalent protein on the surface of *S. aureus* cells. The selectivity of the magnetic confinement mediated by these sdAb-modified nanoparticles is highlighted in Figure 4. In a competition assay NP-3 can be mixed with an equal number of *S. aureus* and *Salmonella* cells and the nanoparticles will selectively target and magnetically isolate the *S. aureus* cells (Figure 4). Because of the excellent confinement selectivity, it is of interest to expand the utility of these nanoparticles in order to develop multifunctional nanoparticle probes that can allow for the simultaneous magnetic confinement and identification of a given pathogen. A potential candidate for such an approach is a

nanoparticle with both a magnetic component (allows for the magnetic isolation of a given bacteria) and a luminescent component (endows the bacteria with a luminescent label).

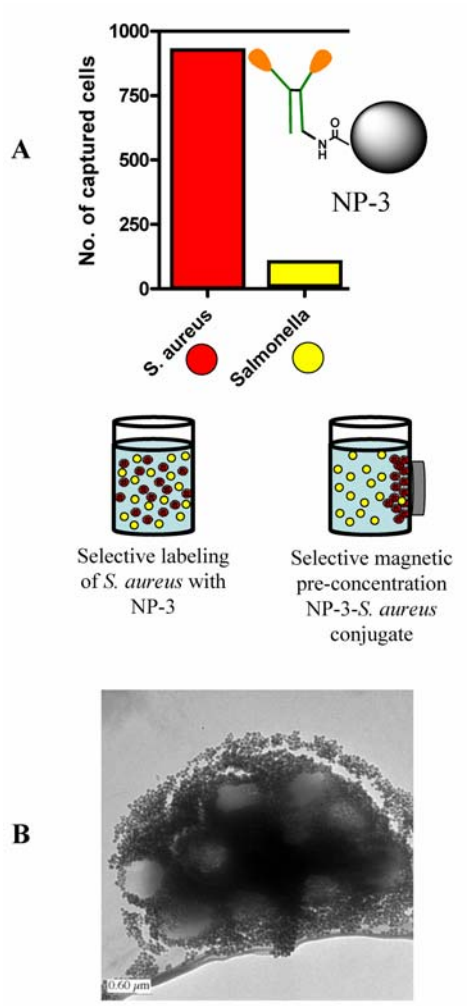

Figure 4. Bar graph and cartoon representations of the magnetic confinement of *S. aureus* (red) and *Salmonella* (yellow) by NP-3 (A) and a TEM image of the NP-3/*S.aureus* conjugates (B).

The development of multifunctional nanoparticles with both luminescent and magnetic components is an interesting challenge. To address these challenges we have designed nanoparticles containing an iron oxide component to provide magnetic character and an organic fluorphore as the luminescent marker. To ensure that the resulting nanoparticles retain strong magnetic responses to the application of an external magnetic field, it is important that significant masses of non-magnetic material not be incorporated into the hybrid nanoparticles. Fortunately organic dyes do not have densities significantly different that that of the silica matrix itself, so the magnetic properties of hybrid nanoparticles comprised of iron oxide nanoparticles and organic dyes should be similar to those of NP-1, NP-2 and NP-3.[12] In order to maximize the luminescent properties of these nanoparticles the silica matrix was doped with increasing concentrations of organic dyes such as tetramethylrhodamine (TMR) (Figure 5). Interestingly, significantly increasing the concentration of dye into the nanoparticle shells does not necessarily increase the fluorescence intensity (Figure 5). In fact when more than 100 TMR molecules are incorporated into the nanoparticle shell, there appears to be significant internal quenching, resulting in a decrease in fluorescence intensity. Despite the fact that only ~100 TMR molecules can be incorporated into a dye-doped magnetic nanoparticle, upwards of 3000 nanoparticles can be accommodated on a typical bacteria. This translates to ~300000 dyes per bacteria, which should provide a signal sufficient for detection/identification of the labeled bacteria with a fluorescence microscope or using flow cytometry.

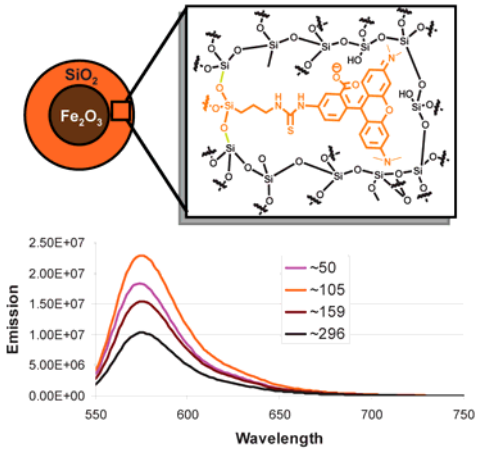

Figure 5. A pictorial representation of the covalent incorporation of TMR into the silica matrix encapsulating an iron oxide nanoparticle and the effect of incorporating more TMR into the silica shell on the fluorescence intensity for samples with the same number of nanoparticles. Note that loadings of TMR<100 within the silica shell results in a decrease in the fluorescence intensity.

3 CONCLUSIONS

We have demonstrated that vancomycin-modified nanoparticles are very useful for the magnetic confinement of a broad range of Gram-positive and Gram-negative different bacteria. In addition, with a small molecule probe such as vancomycin, we have illustrated that controlling the orientation/architecture of a molecule on the surface of a nanoparticle can drastically affect its ability to effectively interact with target bacteria. We have also highlighted the

power of single-domain antibody-modified nanoparticles for the selective isolation of *S. aureus* cells in the presence of *Salmonella*. Interestingly, the development of hybrid nanoparticles comprised of both magnetic and luminescent components could be employed to both magnetically confine and luminescently label *S. aureus* cells and allow one to utilize a simple technique such as fluorescence microscopy or flow cytometry to establish if this potentially harmful bacteria was present in a food or water sample. The combination of hybrid nanoparticles and controlled surface modification of nanoparticles should result in the fast and efficient isolation and detection of pathogenic species from biological samples.

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