ASSEMBLING BACKPACKING BACTERIA FOR DIAGNOSTICS AND THERAPEUTICS

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ABSTRACT

Bacteria-based biohybrid devices are being increasingly investigated for diagnostics and therapeutic applications as they combine the benefits of bacteria with those of the devices they are attached to. We demonstrate the assembly of 'backpacking bacteria' – bacteria bound to nanostructures such as curved nanoshapes and nanowires utilizing a dual binding scheme for attaching bacteria to the nanostructures viz. antibody-antigen binding and neutravidin-biotin binding. This dual binding scheme results in efficient attachment of bacteria to cargo as compared to controls. Bacteria in these biohybrid conjugates retain their motility upon release in solution. We envision utilizing backpacking bacteria in diagnostic and therapeutic applications.

KEYWORDS: Bacteria, nanostructures, nanowires, antibody-antigen, neutravidin-biotin, motility, microrobotics

INTRODUCTION

Bacteria are attractive in constructing biohybrid devices for use in diagnostic and therapeutic applications on account of their small sizes (of the order of microns); their ability to respond to stimuli (chemicals, magnetic field, light); their ability to convert chemical energy into motion/motility and their ability to grow naturally in regions in the body that are not easily accessible externally (e.g. skin, mucus, hair, urinogenital tract) [1]. Advances in nano/microfabrication technologies have facilitated creation of nanostructures of varying geometries such as beads, wires and other geometries that are of relevance in diagnostic and therapeutic applications [2, 3]. In this work, we present a method to interface motile bacteria with nanostructures to create biohybrid 'backpacking bacteria' (Figure 1). Specifically, we demonstrate a dual binding method to attach motile bacteria to nanoshapes and nanowires. We investigate antibody-antigen binding and neutravidin-biotin binding for efficient attachment. The biohybrid devices combine the advantages of bacteria (such as motility and stimuli responsiveness) with the nanostructures (such as magnetic responsiveness and absorption at specific wavelengths).

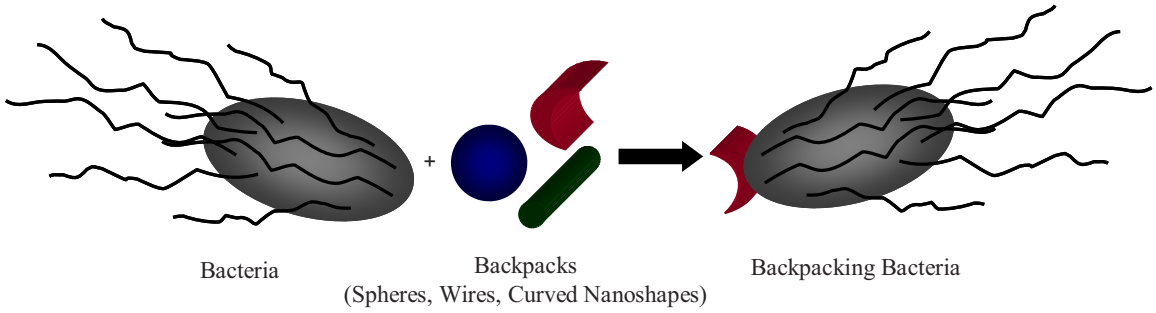

Figure 1. Schematic of backpacking bacteria for diagnostics and therapeutics.

THEORY

In our studies we attach the bacteria *Escherichia coli* (*E. coli*) to gold coated nickel/tin curved nanoshapes (Figure 2a) and gold nanowires (Figure 2b).

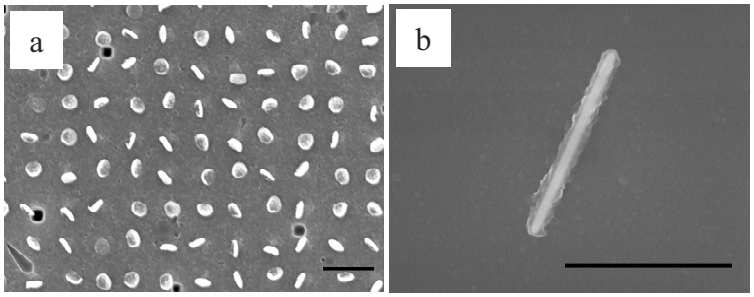

Figure 2. Scanning electron microscope images of (a) etched gold/nickel/tin curved nanoshapes and (b) gold nanowires used in this study. Scale bar in panels a and b = 1 μm.

To attach bacteria to the gold coated nanoshapes, we use two antibody-antigen binding interactions serially. First, the gold coated nanoshapes are functionalized with an antibody (goat and rabbit antibody; antibody 1) using thiol chemistry and protein G (Figure 3a) [1]. Next, the surface of the bacteria is coated with a second antibody (rabbit anti *E. coli*, antibody 2; Figure 3b) that is polyclonal and consequently binds multiple epitopes on the surface of the bacteria (e.g. lipopolysaccharide, flagella). Antibody 1 binds antibody 2 and thus multiple binding sites on the surface of a bacterium are converted to a single binding interaction between the antibody on the nanoshape and the antibody coating the bacterium thereby assembling backpacking bacteria (Figure 3c). This method is more efficient than coating the nanoshapes directly with antibody 2 as it would require correct orientation of the bacteria and nanoshape for attachment.

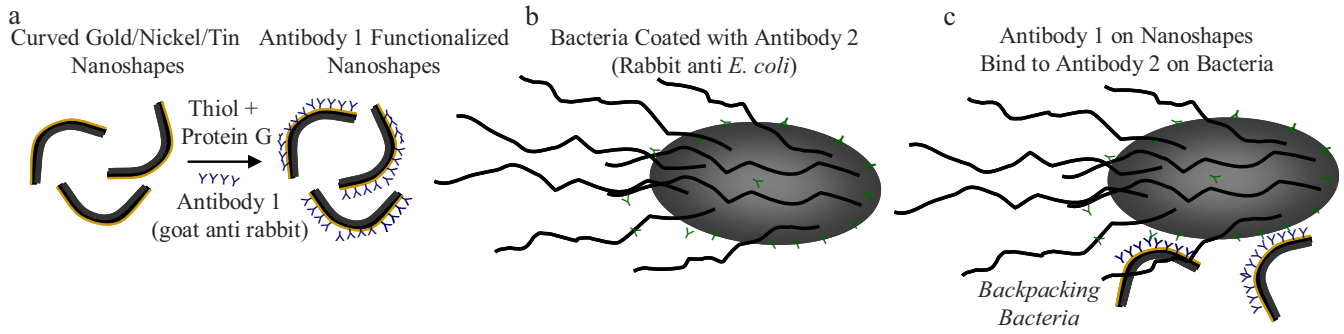

Figure 3. Two antibody scheme for assembling backpacking bacteria on curved nanoshapes. (a)Curved gold/nickel/tin nanoshapes are coated with Antibody 1 (goat anti rabbit antibody)y using thiol chemistry and protein G. (b) Bacteria are coated with antibody 2 (rabbit anti E. coli). (c) Antibody 1 on nanoshapes binds to Antibody 2 coating bacteria.

To attach bacteria to the nanowires, we use antibody-antigen binding in conjunction with neutravidin-biotin binding. Gold nanowires are functionalized with neutravidin (Figure 4a). Next the surface of the bacteria is coated with a polyclonal biotinylated antibody (biotinylated rabbit anti *E. coli*; Figure 4b). Neutravidin on the nanowires binds to the biotin on the antibody coating the bacterial surface thereby assembling backpacking bacteria (Figure 4c). Similar to the two antibody scheme, this enables multiple binding sites on the bacteria to be converted to a single neutravidin-biotin binding facilitating efficient attachment.

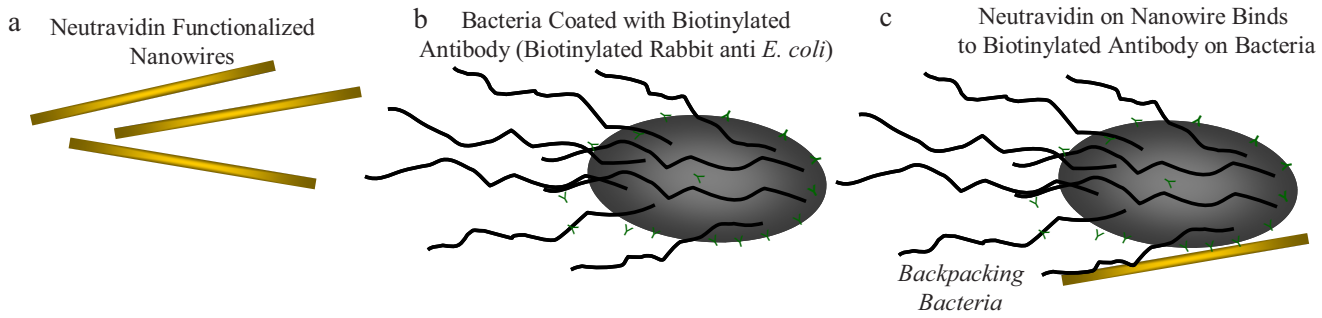

Figure 4. Dual binding scheme for assembling backpacking bacteria on gold nanowires. (a)Neutravidin coated nanowires. (b) Bacteria are coated with biotinylated antibody (biotinylated rabbit anti E. coli). (c) Neutravidin on nanowires binds to biotin on the antibody coating the bacterial surface.

EXPERIMENTAL

The curved gold/nickel/tin nanoshapes, fabricated by tin reflow [2] (350 nm in diameter, etched on a silicon substrate) are first functionalized with goat anti rabbit antibody (Antibody 1, AbD Serotec) using NTA thiol, Ni^{2+}, histidine-tagged protein G as described previously [1]. *Escherichia coli* strain RP437 is cultured overnight in tryptone broth at 30 ^{o}C and 150 rpm. The overnight culture is then subcultured 1:10 in fresh tryptone broth at 30 ^{o}C and 150 rpm for a further 3 hours. The culture conditions facilitate growth of motile bacteria. To coat the surface of the bacteria with rabbit anti *E. coli* (Antibody 2, AbD Serotec), the bacteria are collected by centrifuging at 2000 xg for 5 minutes and resuspending in motility buffer (10 mM PO_4^{3-}, 0.1 mM EDTA, 1 μM methionine and 10 mM lactic acid, pH 7.3) containing Antibody 2 (1 μM Rabbit anti *E. coli*) for 1 hour at 37 ^{o}C. To remove unbound antibody, the cultures are collected at 2000 xg for 5 minutes and resuspended in motility buffer. Antibody 2 functionalized *E. coli* RP437 is then added to the chips containing Antibody 1 coated nanoshapes or nanowires for 1 hour at 37 ^{o}C. To attach bacteria to the gold nanowires (Nanopartz, 1.2 microns long and 30 nm in diameter),

the bacteria attached are first coated with biotinylated antibody (biotinylated rabbit anti *E. coli*, AbD Serotec) as described above. The nanowires are then added to the "biotinylated" bacteria and treated as above.

RESULTS AND DISCUSSION

The two antibody scheme resulted in efficient binding of the bacteria to the gold/nickel/tin nanoshapes (Figures 5a and b) as compared to controls where the binding scheme was not used (Figure 5c). Thus by appropriately choosing a complementary antibody pair, the efficiency of binding of the nanostructures to the bacteria was increased.

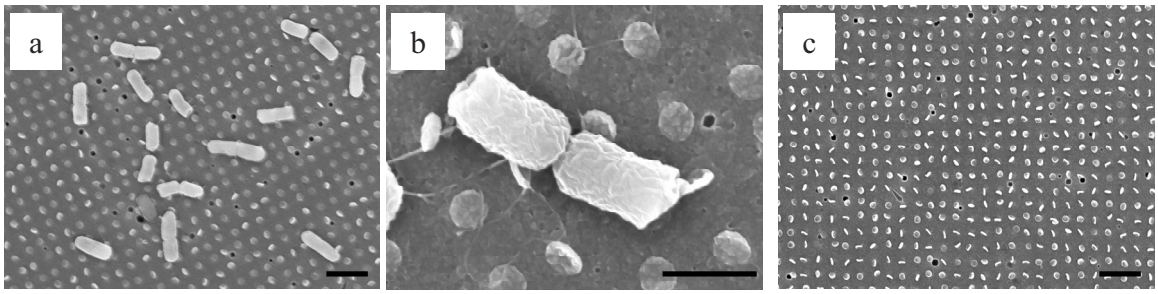

Figure 5. Two antibody scheme results in efficient binding of bacteria to nanoshapes (panels a and b) as compared to controls (panel c) where binding is absent. Scale bar in panels a and c = 2 μm and panel b = 1 μm.

Similarly when antibody-antigen binding was used in conjunction with neutravidin-biotin binding, we observed efficient binding of bacteria to gold nanowires (Figures 6a and b). Direct addition of neutravidin coated nanowires to the bacteria resulted in little to no binding (data not shown).

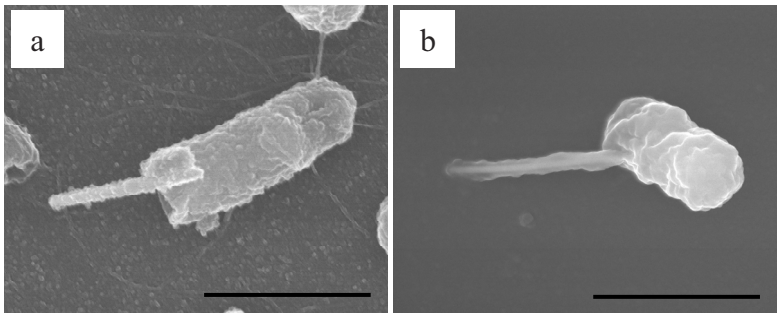

Figure 6. Antibody-antigen followed by neutravidin-biotin binding scheme results in efficient binding of bacteria to nanowires (panels a and b) as compared to controls (data not shown). Scale bar in panels a and b = 1 μm

CONCLUSION

We have presented a robust and flexible dual binding scheme for attaching bacteria to nanostructures. First, we demonstrated attachment of bacteria to curved gold coated nanoshapes using a complementary two antibody method. We also demonstrated attachment of bacteria to nanowires using antibody-antigen binding in conjunction with neutravidin-biotin binding. The binding schemes enable a wide variety of cargo types to be efficiently attached to bacteria thereby increasing the versatility of the resulting biohybrid device as each nanostructure provides its own unique property such as magnetic responsiveness and absorption at specific wavelengths to the biohybrid device. Current investigations are underway to utilize our backpacking bacteria for diagnostic and therapeutic applications.

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