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Magnetic nano-nets for capture of microbes in solution based on physical contact

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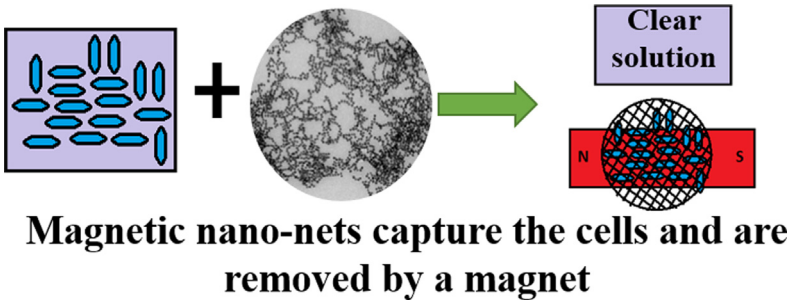
GRAPHICAL ABSTRACT



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ABSTRACT

Self-assembly of Au nanoparticles with Fe ions is used to develop magnetic nano-nets similar to fishing nets for capture and removal of microbes in aqueous medium. The nano-nets have a high aspect ratio, span microns in length with openings of 80–300 nm. This allows them to sample the liquid medium even at low volume fraction and also entrap the microbes in the solution. The nets and the trapped microbes can be effectively pulled from the solution by using an off the shelf magnet. Since the capture is based on physical contact, the nano-nets overcome the ability of the microbes to develop resistance to the cytotoxic effects of chemical compounds and nanomaterials. Using the nano-nets an absolute inactivation of 0.9 is achieved in 5 min. in a non-deaerated solution with *Escherichia coli* (*E. coli*). Further the removal of the nano-nets along with the captured microbes also predominantly eliminates the nanomaterial from the aqueous medium for future use.

1. Introduction

Effective neutralization of microbes in water either by capture or by cytotoxic agents is of critical importance to prevent diseases. This has gained further urgency due to the spread of bacterial resistance to antibiotics [1–4] and increasing scarcity of access to clean water [5–7]. Nanoparticles of Ag, Fe, Au and Fe-oxides are antimicrobial due to generation of oxidative stresses, disruption of cell membranes and cellular processes, and have been extensively researched for this purpose [8–18]. The use of these nanoparticles has also been shown to enhance the effect of antibiotics on bacteria through synergy [19–21]. A rising challenge now is the reported development of microbial resistance to the cytotoxic effects of these nanoparticles [22]. Therefore, nanomaterials that interact and capture the microbes with efficacy by employing alternative strategies are required. To this effect, here we present the use of magnetic nano-nets that are made of Au nanoparticle chains cemented together with Fe-oxides for effective capture of microbes. Unlike 0-Dimensional nanoparticles, these nano-nets

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have high aspect ratio geometry, which spans microns in size with openings in the range of 80–300 nm. The combined magnetic characteristics of these nets and their ability to sample a greater volume leads to capture of the microbes with great efficacy and also their collection using an off the shelf magnet. Further due to the cytotoxic effects of the constituting materials (Fe oxides) the captured microbes in these nets are also neutralized. These nano-nets effectively combine the physical capture of the microbes due to their geometry with the microbicidal properties of their constituting materials. The removal of these nano-nets from the solution along with the captured microbes also clears the residual water of the nanomaterial which may have adverse effects on its subsequent use [23,24].

The dimensionality of nanomaterial (and aspect ratio) is a basic physical parameter that critically affects their interaction volume in composites and fluids. For example, the percolation threshold of 1-D materials such as Carbon nanotubes (CNT's) is orders of magnitude smaller than that of spherical nanoparticles [25,26]. The effect of aspect ratio is also seen in thermal conductivity of fluids and composites with nanomaterials as fillers [27,28]. Similar to the effects seen in these processes and parallel to fishing nets used for capture of aquatic organisms, the developed nano-nets function as an effective means for both physical capture and cytotoxic effects induced neutralization of the microbes. The neutralization of microbes using nanomaterials can be considered a two-step process. First there has to be a direct contact (or close proximity) between the microbe and the nanomaterial in the fluid environment. Success in this step is required for both physical capture of the microbe and also to induce cytotoxic effect on the microbe. The second step is the nanomaterials inducing the cytotoxic effects on the microbe as a result of the direct contact or close proximity. The effect of having a net like nanomaterial is two fold; first due to its high aspect ratio even a small volume fraction of the nets is able to sample a large volume in water, similar to the percolation effects in composites [25,26]. This greatly increases the probability of contact between the microbe and the nets, satisfying the first step for capture of the microbe. Second as the material is organised into a large network, the interactions with the microbes are at a greater scale than compared with those of single nanoparticles. We show that using these magnetic nano-nets *E. coli* in the concentration of 1.6×10^6 colony forming units (CFU) can be removed from solutions in less than 5 min with more than 90% efficiency. Further the capturing ability of these nano-nets based on their volume fraction in solution and the number density of the target species is characterized using 1 μm Poly(methyl methacrylate) (PMMA) microspheres and also *E. coli*. By using Au nanoparticles, the minimum size of particles that can be effectively captured by the nano-nets is found to be 100 nm. Also we show their effectiveness in capturing microbes with different physiology by using a mixture of *E. coli* (a prokaryotic gram negative bacteria) and *Saccharomyces cerevisiae* (*S. cerevisiae*, bakers yeast, a eukaryotic microbe) in water and also toward *Bacillus subtilis* (a prokaryotic gram positive bacteria).

2. Experimental

2.1. Materials

E. coli strain ATCC 47046, *Saccharomyces cerevisiae* (yeast) BY4741 are purchased from ATCC. *Bacillus subtilis* (Strain 168) was kindly supplied by Prof. Trevor Charles group at University of Waterloo. Au nanoparticles (10 nm with concentration 5.7×10^{12} particles/ml; 50 nm with concentration 4.5×10^{10} particles/ml and 100 nm with concentration 5.6×10^9 particles/ml) were purchased from Ted Pella (unconjugated gold colloid manufactured by BBI International). Yeast Extract-Pepton-Dextrose (YPD) broth and Nutrient broth No. 1 were purchased from Sigma-Aldrich. $FeCl_2{\cdot}4H_2O$ (>99% purity), $FeCl_3{\cdot}6H_2O$ (>99% purity) and $NaBH_4$ (>98% purity) were also purchased from Sigma-Aldrich and used as such. 1 μm sized PMMA microspheres were purchased from Sigma-Aldrich (10% by volume). All the glass vials, caps, pipette tips and Millipore water were autoclaved to sterilize the equipment for elimination of any possible contaminants.

2.2. Material characterization

Ultraviolet–visible (UV–Vis) spectroscopy was carried out by using MINI-D2T deuterium tungsten light source and USB4000 Miniature fiber optic spectrometer from Ocean Optics. Field emission scanning electron microscopy (FESEM) was used to evaluate morphologies of the samples and study them. Images were taken by using ULTRA PLUS and Leo 1530 from Carl Zeiss. Transmission electron microscopy (TEM) images were obtained with a LEO 912ab transmission electron microscope. X-ray diffraction (XRD) patterns were obtained from the samples deposited on a silicon substrate with a native oxide layer using a glancing incidence X-ray diffraction (GIXRD) using a PANalytical X'Pert Pro MRD diffractometer with Cu Kα radiation (λ = 1.54 Å) at an incidence angle of 0.4°. X-ray photoelectron spectroscopy (XPS) was used to study the oxidation state and compositions of the iron oxide-gold nanoparticle composite. The instrument used for this purpose was Thermo-VG Scientific ESCALab 250 Microprobe equipped with a monochromatic Al K-alpha X-ray source (1486.6 eV). Zeta Sizer Nano ZS90 from Malvern Instruments was used for measuring both zeta potential and size distribution of our samples. Optical microscope DMI 3000 B from Leica equipped with a Hamamatsu CCD was used for optical imaging.

2.3. Assembly and formation of nano-nets

270 μL of a solution containing both 1 mg/mL $FeCl_2$ and 2.718 mg/mL $FeCl_3$ dissolved in Millipore water was added to a glass vial which contains 4.0 mL of the stock Au nanoparticles. The final solution was put on a shaker for eight hours for assembling of Au nanoparticles. As the 10 nm Au nanoparticles interact with the Fe^{2+} and Fe^{3+} ions and self-assemble into chains, the solution mixture becomes blue in color. 600 μL of 10 mg/mL $NaBH_4$ was added to the Au-Fe nanoparticle chains to reduce Fe^{2+} and Fe^{3+} ions to Fe^0. The Fe on contact with air is oxidized immediately (within 15–30 min) to Fe_2O_3. The composite in the vial is now magnetic and the gold chains can now be pulled with the help of a handheld magnet. Magnets are ¾ in. ceramic disks sold by Hillman. 4–8 pieces of magnet stacked on top of each other are used to pull the chains on to the sides of the vial which can be easily removed later.

2.4. Bacterial concentration determination

Nutrient agar plates were prepared by dissolving 2.8 g of nutrient agar in 1 L of water and autoclaving the solution at 121 °C for fifteen minutes. The solution was cooled until it reached 40–50 °C and then poured into petri-dishes. CFUs were determined by plating the solution before and after extraction of the bacteria (or as required) on the Agar plates and after being incubated overnight at bacteria growing conditions (37 °C and no light exposure). The CFU was calculated using the formula: CFU/ml = (no. of colonies × dilution factor)/volume of culture on plate. The microspheres and the microbes were also counted using Brightline haemocytometer made by Hausser Scientific and following the accompanying instructions.