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Exploring the potential of magnetic antimicrobial agents for water disinfection

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ABSTRACT

Industrial and urban activities yield large amounts of contaminated groundwater, which present a major health issue worldwide. Infectious diseases are the most common health risk associated with drinking-water and wastewater remediation is a major concern of our modern society. The field of wastewater treatment is being revolutionized by new nanoscale water disinfection devices which outperform most currently available technologies. In particular, iron oxide magnetic nanoparticles (MNPs) have been widely used in environmental applications due to their unique physical–chemical properties. In this work, poly(ethylene) glycol (PEG)-coated MNPs have been functionalized with $(RW)_3$, an antimicrobial peptide, to yield a novel magnetic-responsive support with antimicrobial activity against *Escherichia coli* K-12 DSM498 and *Bacillus subtilis* 168. The magnetic-responsive antimicrobial device showed to be able to successfully disinfect the surrounding solution. Using a rapid high-throughput screening platform, the minimal inhibitory concentration (MIC) was determined to be 500 μM for both strains with a visible bactericidal effect.

1. Introduction

According to the guidelines for Drinking-water Quality (World Health Organization, 2011) infectious diseases caused by pathogenic bacteria, parasites and viruses are the most common health risk associated with drinking-water. The high concentration of various contaminants present in the water affect the health of millions of people (Cundy et al., 2008), with many developing countries facing drinking water problems (Tiwari et al., 2008). The treatment and disinfection of water is a considerable priority of our current society (Tiwari et al., 2008; Xu et al., 2012). Nowadays, wastewater disinfection methods rely on three different methodologies to achieve the final high grade standards for reusable drinking water. These include temperature treatment, chemical (e.g. addition of chemical disinfectants such as chlorine, ozone, poly (quaternary ammonium) salts, antibiotics) and physical (e.g.

Abbreviations: mnps, iron oxide magnetic particles; MIC, minimal inhibitory concentration; AMPs, antimicrobial peptides; PEG, poly(ethylene glycol); MNP_PEG, iron oxide nanoparticles coated with poly(ethylene glycol); $MNP\_PEG\_NH_2\_(RW)_3$, iron oxide nanoparticles coated with poly(ethylene glycol) functionalized with the peptide RWRWRW; OD_{600nm}, optical density at 600 nm; HTS, high-throughput screening.

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flocculation and filtration) methods (Blin et al., 2011). However, temperature-based treatments can denaturate other sensitive bioproducts used for disinfection (Blin et al., 2011) and physical methods require very controlled conditions or are prone to membrane fouling (Srinivasan et al., 2013). Regarding chemical methods, they can present several and distinct drawbacks depending on the selected method. For example, the use of chlorines may lead to the formation of harmful by-products (e.g. carcinogens, toxic derivates) (Blin et al., 2011; Hossain et al., 2014; Tiwari et al., 2008), while mostly used antibiotics present limited antimicrobial activity and their extensive use can lead to the development of multidrug-resistance pathogens (Blin et al., 2011). Therefore, there is intensive research on the development of water insoluble and reusable antimicrobial agents that can overcome these drawbacks.

Nanotechnology has been explored as a promising approach for wastewater disinfection (Hossain et al., 2014) due to the unparalleled properties of the materials at the nano-scale. Iron oxide magnetic nanoparticles (MNPs) are the most widely used nanoparticles for environmental applications, specifically for disinfection of polluted water or underground environments (Tang and Lo, 2013). MNPs are generally recognized as safe, being already used in the biotechnological and biomedical fields. They are inexpensive to produce, compatible with aqueous solutions, amenable to versatile surface coatings and easily manipulated by a magnetic field (Xu et al., 2012), which allows facile removal from solution and prevents environmental dissemination. Their current use in wastewater treatment has been thoroughly reviewed elsewhere (Cundy et al., 2008; Tiwari et al., 2008; Xu et al., 2012) and includes them as prime candidates for metal contaminants removal (by adsorption) or reduction; as photocatalysts; as agents for co-precipitation; for the detection of nutrients, algae, bacteria, parasites, virus, or antibiotics; and separation of pollutants.

The former examples are based mostly on the natural chemical properties of MNPs. However, these can be improved if the nanoparticles are functionalized with a molecule that allows interaction with bacteria components and/or exhibits antimicrobial activity. For example, Ping et al. have described the functionalization of silver nanoparticles with amoxicillin, an β-lactam antibiotic, and reported their increased antimicrobial activity when compared with their sole activity (Li et al., 2005). Another application of magnetic platforms is the functionalization of magnetic carriers for the detection and clearance of bacterial lipopolysccharides (Liu et al., 2014). However, the extensive exposure of microorganisms to antimicrobial drugs, such as antibiotics, antivirals and antifungals, has led to the development of multidrug-resistant strains, which are the source of many serious infectious diseases (Gao et al., 2011a) and may be a major contaminant of drinking water. Therefore, there is a large incentive to discover new classes of antimicrobial agents, which are less prone to develop pathogen resistance and which present advantages when compared with traditional methods for water disinfection.

Antimicrobial peptides (AMPs) are capable of eliminating a broad spectrum of microorganisms, including bacteria, parasites, fungi and viruses (Onaizi and Leong, 2011). Currently, they are mainly used in the biomedical field, with the aim of inhibiting biofilm formation on biomedical devices (Forbes et al., 2013), but can also find applications in the environmental field for the disinfection of aqueous solutions (Blin et al., 2011). Cationic synthetic peptides with arginine–-tryptophan (RW) tandem repeats have been recognized as effective microbicidals (Chen et al., 2011), with an optimal antimicrobial activity against several pathogens (e.g. *Staphylococcus aureus*, *Staphylococcus epidermidis*) when using three repeats (RWRWRW, $(RW)_3$) (Strøm et al. 2003).

However, the direct use of AMPs for water disinfection would be an extremely expensive alternative to traditional methods. Therefore, immobilizing the AMPs to a solid support presents a better alternative as they have shown increased antimicrobial activity and selectivity along with high local charge density (Engler et al., 2012). However, most of the nanomaterials have been employed mainly for biomedical applications (Engler et al., 2012; Gao et al., 2011a; Hequet et al., 2011; Kazemzadeh-Narbat et al., 2013; Liu et al., 2013; Qi et al., 2011) with a very few examples addressing AMPs or immobilized AMPs for environmental applications (Blin et al., 2011).

This manuscript reports the functionalization of MNPs with the AMP $(RW)_3$ and demonstrates their applicability for solution disinfection. For this purpose we have used a high-throughput screening (HTS) platform for the rapid evaluation of MNPs antimicrobial activity and determination of the minimal inhibitory concentration (MIC) against two strains: Gram-negative *Escherichia coli* K-12 DSM 498, by taking into account that *E. coli* is a model organism used as a biological indicator of water contamination (Anderson et al., 2006; Edberg et al., 2000) and also with Gram-positive *Bacillus subtilis* 168 BGSC1A1.

2. Experimental (materials and methods)

2.1. Materials

All chemicals were >96% pure. Di-sodium hydrogen phosphate 2-hydrate, ethanol absolute PA, N,N-Dimethylformamide (DMF), sodium chloride, sodium di-hydrogen phosphate 1-hydrate and sodium hydroxide were purchased from Panreac. Iron (II) chloride tetrahydrate, iron (III) chloride hexahydrate, phenol and potassium cyanide were purchased from Sigma. (3-Aminopropyl) triethoxysilane (APTES), N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) were obtained from Aldrich. Ammonium hydroxide solution, ninhydrin and Poly(ethylene glycol) 10,000 (PEG) were acquired from Fluka. Pyridine was purchased from Carl Roth. Glycine was obtained from Acros Organics. Nitrogen gas was provided by Air Liquide. Luria broth (LB) was purchased from Nzytech. Agar powder was acquired from Himedia. $(RW)_3$ peptide with a purity of 95% was synthesized by Genecust. *Escherichia coli* K-12 DSM498 culture was kindly provided by Dr. Pedro Vidinha (FCT-UNL) and *Bacillus subtilis* 168 BGSC1A1 was kindly provided by Prof. Isabel Sá Nogueira (FCT-UNL).