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Magnetic targeting of surface-modified superparamagnetic iron oxide nanoparticles yields antibacterial efficacy against biofilms of gentamicin-resistant staphylococci

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ABSTRACT

Biofilms on biomaterial implants are hard to eradicate with antibiotics due to the protection offered by the biofilm mode of growth, especially when caused by antibiotic-resistant strains. Superparamagnetic iron oxide nanoparticles (SPIONs) are widely used in various biomedical applications, such as targeted drug delivery and magnetic resonance imaging. Here, we evaluate the hypothesis that SPIONs can be effective in the treatment of biomaterial-associated infection. SPIONs can be targeted to the infection site using an external magnetic field, causing deep penetration in a biofilm and possibly effectiveness against antibiotic-resistant strains. We report that carboxyl-grafted SPIONs, magnetically concentrated in a biofilm, cause an approximately 8-fold higher percentage of dead staphylococci than does gentamicin for a gentamicin-resistant strain in a developing biofilm. Moreover, magnetically concentrated carboxyl-grafted SPIONs cause bacterial killing in an established biofilm. Thus magnetic targeting of SPIONs constitutes a promising alternative for the treatment of costly and recalcitrant biomaterial-associated infections by antibiotic-resistant strains.

1. Introduction

The average life expectancy in the Western world is steadily increasing and is currently well over 70 years, both for men and women. With aging, the natural ability of the human body to restore function after trauma or wear is decreasing, while frequently also (oncological) intervention surgery, such as after total laryngectomy for the removal of a laryngeal tumour, yields loss of function as an unwanted side-effect. Biomaterial implants are indispensable in modern medicine for the restoration of function and allow large numbers of patients to maintain a high quality of life as they grow old. Infection of biomaterial implants or devices constitutes their major cause of failure and can develop many years after implantation [1]. Biomaterial-associated infection (BAI) can develop from perioperative bacterial contamination of implant surfaces during implantation, immediately post-surgery during hospitalization or by haematogenous spreading of bacteria from infections elsewhere in the body [2]. In general, *Staphylococcus epidermidis* and *Staphylococcus aureus* are the most frequently isolated pathogens from infected biomaterial implant surfaces. Almost 50% of infections associated with catheters, artificial joints and heart valves are caused by *S. epidermidis* [3], whereas *S. aureus* is detected in approximately 23% of infections associated with prosthetic joints [3].

The bacteria involved in BAI often protect themselves against antibiotics and the host immune system by producing a matrix of exopolymeric substances (Fig. 1A) that embeds the organisms and is impenetrable for most antibiotics and immune cells. Metals such as silver, copper, gold, titanium and zinc have been used as antibacterial agents for centuries, but their efficacy has been surpassed by modern antibiotics and their use has diminished. Since there is growing concern that the era of antibiotics may well come to an end over the coming decades and more and more multiple-antibiotic-resistant strains are arising, alternative strategies are badly needed especially against antibiotic-resistant strains [4]. Application of metals in their nanoparticulate form is currently

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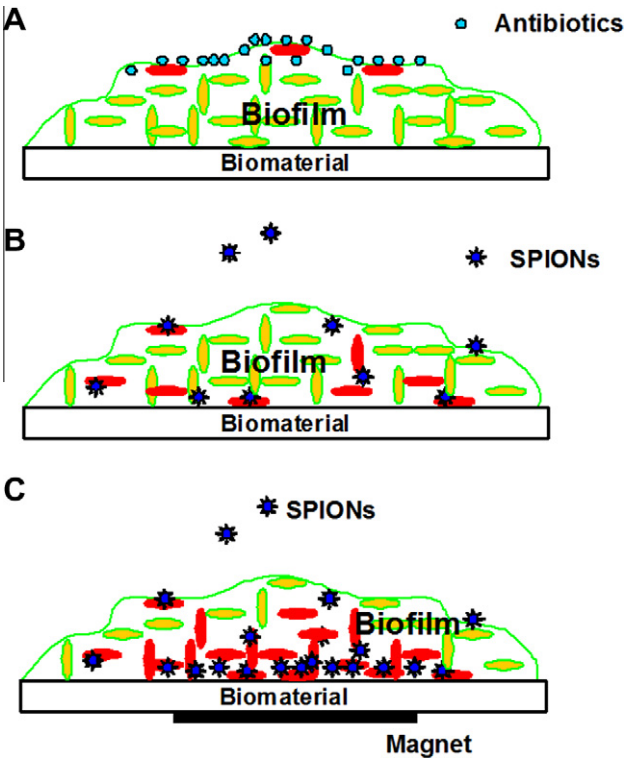

Fig. 1. The biofilm mode of bacterial growth on a biomaterial surface prevents the penetration of antibiotics into the biofilm (A), while it may allow penetration of SPIONs (B). An external magnetic field can facilitate deep penetration of SPIONs into the biofilm, and magnetic concentration in a region can enhance antibacterial efficacy (C). Red colour represents dead bacteria and green colour represents live bacteria.

being considered to resolve bacterial infections, but has attracted scientific attention only over the past decade [5]. Nanoparticles are less than 100 nm in diameter and, as a result, properties such as surface area, chemical reactivity and biological activity alter dramatically. The antibacterial efficacy of metal nanoparticles has been suggested to be due to their high surface area to volume ratio rather than to the sole effect of metal ion release [6]. A high surface area to volume ratio is generally accompanied by increased production of reactive oxygen species (ROS), including free radicals [7,8]. These characteristics allow nanoparticles to interact closely with microbial membranes, damage their structure and inactivate bacteria [5]. Metal oxide nanoparticles are of particular interest as antibacterial agents, as they can be prepared with extremely high surface areas and unusual crystalline morphologies with a high number of edges and corners, and other potentially reactive sites [9].

Superparamagnetic iron oxide nanoparticles (SPIONs) are a special class of metal oxide nanoparticles with unique magnetic properties and superior biocompatibility. Consequently, SPIONs have a wide history of application in the field of biomedical engineering, including their use as contrast agents for magnetic resonance imaging [10,11] and magnetic fluid hyperthermia [12], carriers for targeted drug delivery [13,14], magnetic separation of immune cells [15], proteins or other biomolecules [16] and tissue engineering applications [17,18]. Recently, Taylor et al. [19] proposed the use of superparamagnetic nanoparticles to prevent orthopaedic implant infection, showing that SPIONs in a concentration range of 0.01–2 mg ml^{-1} were able to kill up to 25% of *S. epidermidis* in a 48 h old biofilm [19]. Although the shape and size of SPIONs can contribute significantly to their antibacterial activity [20], specific surface chemical functionalities of SPIONs have also been suggested to be crucial to their interaction with bacterial cell membranes [21].

Here, we evaluate the hypothesis that SPIONs can be effective in the treatment of BAI (Fig. 1B), because they can be targeted to the infection site using an external magnetic field, causing deep penetration in a biofilm (Fig. 1C) and possibly effectiveness against antibiotic-resistant strains. First, we demonstrate the effects of surface-functionalized SPIONs (see Fig. 2) on soft tissue cells and on the viability of staphylococcal biofilms for a gentamicin-susceptible and -resistant strain, as compared with gentamicin, a frequently used antibiotic for treatment and prevention of prosthetic, orthopaedic joint infections. Next we demonstrate that magnetic targeting of SPIONs yields antibacterial efficacy against a developing and established biofilm of gentamicin-resistant staphylococci.

2. Materials and methods

2.1. Materials

$FeCl_2.4H_2O$, $FeCl_3.6H_2O$, diethylene glycol, 3-aminopropyltriethoxysilane (APTES), *o*-(2-aminoethyl)-*o*′-methylpolyethylene glycol (PEG-NH_2, MW = 750), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, tetramethylammonium hydroxide, polyethylene glycol 8000 and *N*,*N*-dimethylformamide (DMF) were purchased from Sigma-Aldrich, Germany. Carboxyethylsilanetriol (CES) was provided by Wacker-Chemie GmbH, Burghausen, Germany.

2.2. Synthesis of SPIONs and grafting of surface functional groups

In order to obtain nanoparticles with a narrow size distribution, the polyol method was employed [22]. Briefly, 5 ml of an aqueous solution of $FeCl_2.4H_2O$ (0.045 M) and $FeCl_3.6H_2O$ (0.0375 M) were added to 250 ml of diethyleneglycol. The mixture was heated to 170 °C and maintained at this temperature for 15 min before addition of the base (i.e. solid NaOH until a final concentration of 0.375 M). Subsequently, temperature was maintained at 170 °C for a period of 1 h before cooling to 60 °C. The synthesized SPIONs were collected with a neodymium magnet and washed with 100 ml of a HNO_3 (1 M) solution.

Carboxyethylsilanetriol (CES) was grafted on the surfaces of SPIONs, as described elsewhere [23]. Briefly, 100 ml of nanoparticle solution (0.3 M iron) was added to 100 ml DMF and 45 ml of 0.15 M CES was slowly added before adding 25 ml water followed by 15 ml of NaOH solution (1 M) at room temperature and under homogenization (about 8000–24,000 rpm). The solution was heated to 100 °C for 24 h under continuous stirring. The SPIONs were precipitated by addition of acetone/ether (50/50) mixture and collected with a neodymium magnet. The precipitate was washed with acetone several times and finally dispersed in water. Excess of silane derivative and other chemicals were removed by dialysis using a dialysis bag (Spectrum Laboratories, Inc; MWCO = 10,000) for 48 h in water.

Aminopropyltriethoxysilane (APTES) was grafted onto SPIONs by first dissolving 15 ml of APTES in 50 ml methanol and subsequent dropwise addition to a suspension of nanoparticle solution (20 ml, [Fe] = 0.3 M) at room temperature. After stirring for 24 h at room temperature, 20 ml of glycerol was added to the mixture and subsequently methanol and water were removed by rotary evaporation. Next, 50 ml of acetone was added and, after mixing, the nanoparticles were separated by magnetic decantation. After removing the supernatant, SPIONs were washed several times with acetone. Afterwards, the magnetic nanoparticles were dispersed in 40 ml of water and purified by dialysis using a dialysis bag (MWCO = 10,000) for 48 h in water.

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