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Lethal photosensitisation of bacteria using silica-TBO nanoconjugates

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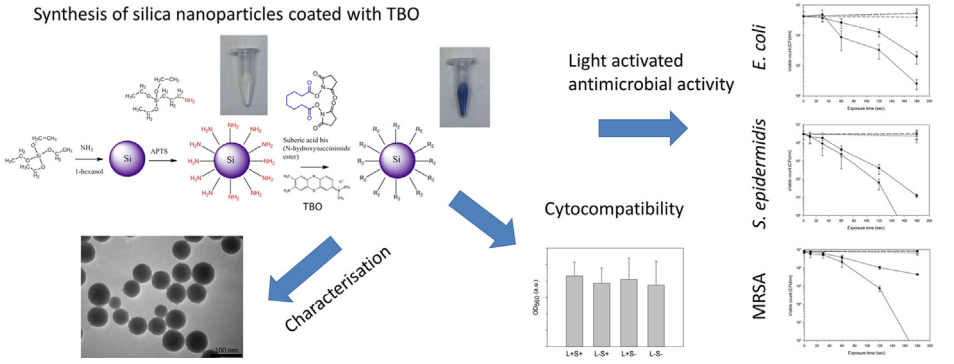
HIGHLIGHTS

- Toluidine Blue O (TBO) was covalently bound to silica nanoparticles.
- TBO release is pH dependent.
- Bacteria can be inactivated after exposure to laser light when mixed with the nanoconjugates.
- *E. coli* is more resistant than *S. epidermidis* and MRSA to these light activated nanoparticles.

GRAPHICAL ABSTRACT



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ABSTRACT

Pathogenic microorganisms are gradually becoming resistant to antibiotics, thereby novel antimicrobial technologies are urgently needed. Photodynamic therapy (PDT) is a process that employs the energy of photons to generate reactive oxygen species through a class a chemicals known as photosensitisers. PDT has shown antimicrobial activity as the oxygen reactive species can inactivate microorganisms, at the same time, the doses required to provide antimicrobial actions are not lethal to mammalian cells.

We covalently bound Toluidine blue O (TBO), a very common and safe photosensitiser, to silica nanoparticles. The conjugates exhibited antimicrobial activity against MRSA, *S. epidermidis* and *E. coli* when irradiated with laser light at 630 nm. Using a light source with a power of 500 mW the bacterial reduction exhibited a dose-response behaviour and it was 2 $\log_{10}$ for *S. epidermidis* and *E. coli* after 2 and 3 min, respectively. No antimicrobial activity was exhibited by the unconjugated nanoparticles or by the laser light alone. The release of TBO from the nanoparticles was pH dependent with higher amounts of photosensitisers detected at pH = 4 than pH = 7 consistent with the formation of amide bonds between nanoparticles and TBO.

The light activated nanoparticles developed in this work offer a platform for the controlled delivery of TBO through a pH responsive mechanism for antimicrobial applications.

1. Introduction

Non-antibiotic based antimicrobial therapies are an urgent need in the management of infections in light of the rising number of microorganisms exhibiting resistance to one of more antibiotics [1]. Skin and soft-tissue infections (SSTIs) caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are very frequent and their

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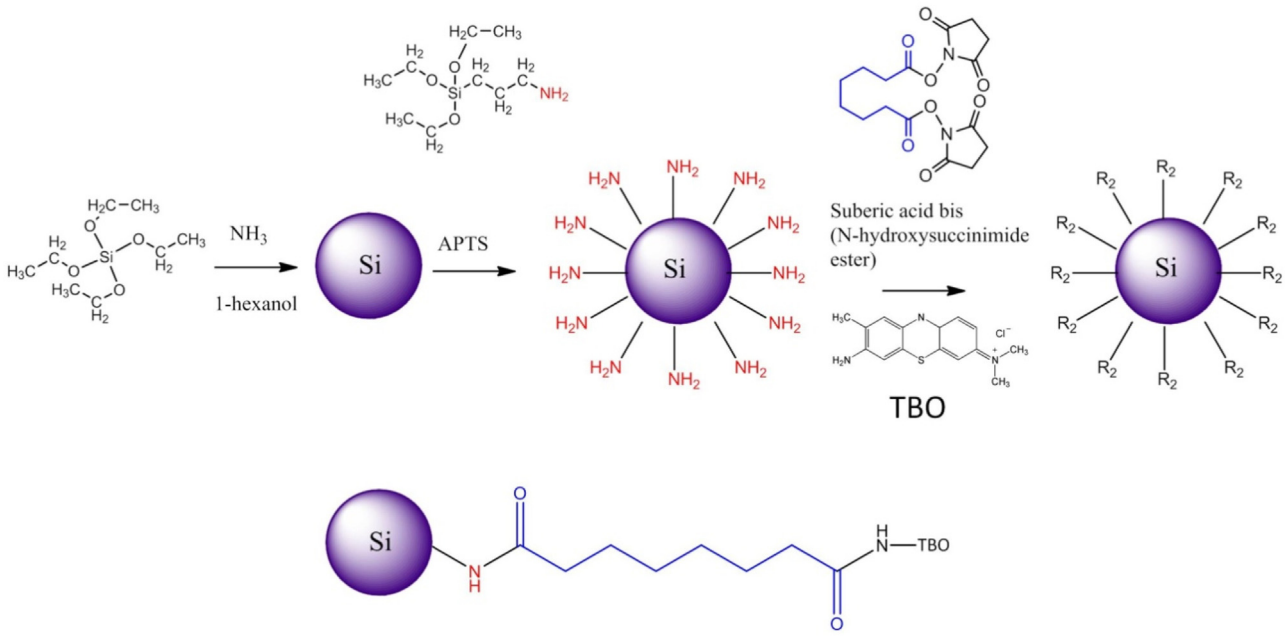

Fig. 1. Reaction scheme of the preparation of silica nanoparticles and TBO conjugation.

rate has increased 20 folds in the last decade [2]; it has been estimated that every MRSA infection costs an extra 9000 £ to the NHS [3]. Despite the media focus on MRSA, numerous other pathogenic species have developed resistance, i.e. *Vancomycin-Resistant Enterococci* (*VRE*). SSTI incidence is 24.6 per 1000 person everyyear while among hospitalized patients the incidence is 7% to 10% [4]. The symptoms range from mild conditions, such as pyoderma, to serious life-threatening infections, such as necrotizing fasciitis. Microbial resistance can be originated by the cell altering the target of the drug without losing functionality (i.e. MRSA) through DNA mutations [5,6].

The prevention and treatment of infections can be through antibiotic drugs or no antibiotic based techniques; cold gas plasma, metal nanoparticles and Photodynamic therapy (PDT) are examples of the technologies that do not employ antibiotics to inactivate microorganisms. Photodynamic therapy is a process based on the adsorption of photons by a photosensitiser (PS) and its transfer through a FRET (Förster resonance energy transfer) process (radiationless transfer of energy to neighbouring compound) to either oxygen (type 2 reaction) or other substrates (type 1 reaction). The energy adsored by the PS induces its transition from the ground state to an excited states (singlet or triplet), from these unstable states the PS returns to the ground state transferring energy to oxygen molecules, forming reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) and other radicals (superoxide ($O_2^{\bullet}$) and $OH^{\bullet}$). ROS are then responsible for the oxidation of the substrate molecules [7]. Such potential cytotoxic activity of PDT has found applications in cancer treatments and as antimicrobial technique, so called antimicrobial PDT (aPDT); this has been possible because of the existence of biologically safe PS (i.e. TBO, MB and indocyanine green) whose lethal dose towards pathogenic microorganisms is smaller than for mammalian cells [8,9]. Another important benefit of aPDT is its efficacy against antibiotic resistant cells, broad spectrum of action and inability to induce further resistance in sensitive cells [10,11]. All these virtues are due to the multi targets lethality mechanism of aPDT as ROS are unspecific in their interaction. The main applications of aPDT have been in dentistry [12], and dermatological set-ups [13], however, more recently, light activated materials have been developed as self-cleaning/self-sterilising surfaces with possible applications in catheters and open surfaces [14–21].

As the threat posed by bacteria resistant to one or more antibiotics is a growing concern [22–25], the need for more effective antimicrobial techniques not based on antibiotic drugs i.e. aPDT is a pressing need.

Nanotechnology has been applied to aPDT in order to enhance the antimicrobial outcome [7,26], for example PS have been encapsulated in nanoparticles to guide their penetration inside cells or they have been conjugated to nanocarriers to drive their accumulation close to the cell wall. Among the nanocarriers employed as drug delivery systems, silica has been widely used because of the ease of production and biological compatibility. Silica nanoparticles based treatments have been developed in cancer [27], and antimicrobial applications [28,29]. Moreover, silica nanoparticles loaded with antimicrobial compounds have been shown capable of reversing antibiotic resistance [29–32].

In this work, TBO, a very common photosensitiser, has been bound to silica nanoparticles previously functionalised to exhibit amino groups. The physical-chemical properties of the nanoconjugates have been characterised and the antimicrobial activity against examples of Gram+ (*S. epidermidis* and MRSA) and Gram- (*E. coli*) pathogens determined. Furthermore, the biocompatibility of the nanoconjugates towards fibroblast cells has been assessed and the TBO release has been shown to be pH dependent.

2. Materials and methods

2.1. Chemicals

Toluidine Blue O (TBO), tetraethyl-orthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTS), 2-(4-morpholino)-ethane sulfonic acid (MES), suberic acid bis-(N-hydroxy-succinimide ester), Triton X-100, NaOH, Sodium dodecyl sulfate (SDS), MTT (3-(4,5-dimethythiazol-2yl)-2,5-diphenyltetrazolium bromide), Brain Heart Infusion (BHI) broth and Agar were purchased from Sigma-Aldrich. Ammonium hydroxide (29.6%), cyclohexane, *n*-hexanol, *iso*-propyl alcohol and methanol were purchased from Fisher Scientific.

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