Persistent Luminscence Material for Lamp-Free Photodynamic Therapy to control Lung Infection Bacteria

Light4Lungs

Partners involved

IQS: Institut Químic de Sarrià

Luciano Dibona-Villanueva, Maria Méndez, Roger Bresolí-Obach, Santi Gené, Eugenia Martínez, Emilio Palomares, Chiara Treghini, Giacomo Insero, Giovanni Romano, Santi Nonell, Light4Lungs group

Objectives

Antimicrobial photodynamic therapy has been highlighted as a solid alternative to antibiotics to control bacterial infections due to several advantages including reduction of resistant microbial loads, specific delivery of photosensitisers, and localised light stimulation. On this last topic, the availability of light sets a challenge when the area of irradiation is not easily accessible with external light sources. Several approaches have been considered in PDT to address this problem such as interstitial optical fibers and light-emitting catheters, nevertheless, these solutions are often restrained to endoscopically accessible sites or needle insertion.

Acknowledging these constraints, our research aims to pioneer a novel therapeutic scheme using the potential of persistent luminescent particles as light-delivery sources. This innovative approach proposes a non-invasive, inhalable source of light delivery for antimicrobial PDT, specifically developed to combat light-sensitive bacteria located in the lungs. By circumventing the limitations of traditional light delivery methods, this pioneering scheme holds promise in revolutionizing the treatment landscape of lung infection bacterial treatments, offering a solid alternative against antibiotics and other therapies.

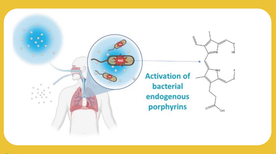

Figure 1

L4L therapeutic scheme and the use of PersL particles to photoinactivate ung-infection bacteria.

Methodology

ZrO_2 material doped with Ti4+ was synthesized via the sol-gel method and functionalized by PEGylation reaction to coat the particles. Physico-chemical properties were analysed using several techniques such as PXRD, SEM, TEM, DLS, and flow cytometry. Photophysical properties were determined by several spectroscopic techniques such as fluorescence spectroscopy, time-resolved fluorescence, actinometry, and integration sphere measurements. Finally, a dynamic flow model was developed to simulate external particle activation and evaluate the effect of the delivered light in the generation of singlet oxygen and photoinactivation of bacteria.

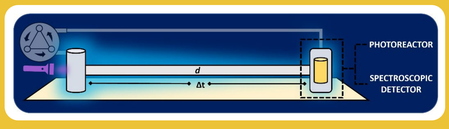

Figure 2

Dynamic flow model to evaluate PersL triggered photochemical and photobiological events.

Results

ZrO_2:Ti^{4+}@PEG particles exhibited a pure crystalline monoclinic phase (m-ZrO_2) with a dispersed spherical shape with a size of 300 nm that shows notable stability in water suspensions. The number of particles per volume was found to be 52×10^9 particles/mL.

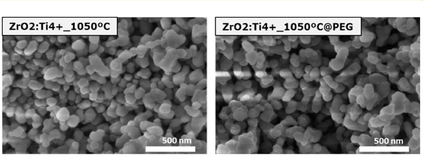

Figure 3

SEM images of ZrO_2:Ti^{4+}@PEG particles.

The particles displayed broad blue-light emission spectra **with peaked at 470 nm and an excitation maximum of 280 nm.** Luminescence decay kinetics can be divided into both prompt luminescence (PromL), in which >90% of the photons are emitted, and a **slow decay component as persistent luminescence (PersL) which lasts for several seconds.** Luminescence efficiency was calculated as 9-13% through quantum yield determination.

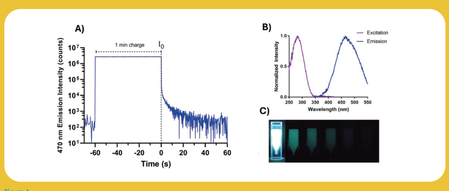

Figure 4

Light emission properties of ZrO_2:Ti^{4+}@PEG particles. A) Light emission events while charging and decharging the particles. B) Excitation and Emission spectra and C) Timelapse pictures depicting persistent luminescence of the particles

The material described in this study is a potential candidate to be the persistent luminescent material basis for the breathable light formulation. In addition to synthesis and photophysical characterization, we have studied the capability of this material to (i) excite a photosensitiser and generate singlet oxygen, (ii) trigger a biologically relevant photooxidation reaction and (iii) the particle's capability to induce photoinactivation on bacteria in vitro.

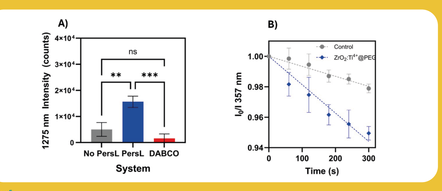

Figure 5

Evidence of PersL triggered $^1O_2$ generation by porphyrins using the dynamic flow model. A) Evaluation of $^1O_2$ generation without PersL (grey), in presence of PersL (blue) and negative control in presence of a $^1O_2$ quencher (red). B) Evaluation of PersL photodegradation of tryptophan. Instrument control (grey) and PersL induced degradation (blue).

Figure 5 shows that **the PersL can remotely trigger photosensitisation in using a porphyrin to generate $^1O_2$** as opening evidence that the particles can be used as a light delivery source for PDT.

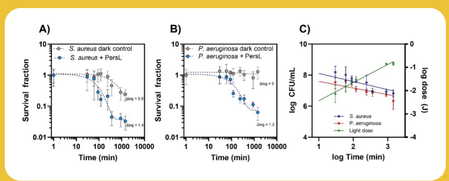

Figure 6

ZrO_2:Ti^{4+}@PEG PersL induced photoinactivation of S. aureus ATCC 6583 and P. aeruginosa ATCC 27853 planktonic d-PBS suspensions supplemented with 50mM KI and 10 mM 5-ALA. A) Survival fraction of S. aureus irradiated by PersL (blue dots) and dark control (grey dots). B) Survival fraction of P. aeruginosa irradiated by PersL (blue dots) and dark control (grey dots) at different times and C) Dependence of light dose (green line) delivered by the particles and photoinactivation of bacteria (S. aureus blue line and P. aeruginosa red line)

Figure 6A and 6B shows the photoinactivation of S. aureus and P. aeruginosa bacteria suspension under continuous irradiation of ZrO_2:Ti^{4+}@PEG PersL. In the case of S. aureus, a 24-hour exposure to PersL resulted in a photoinactivation of 1.4 logs. The latter accounts for any toxicity from d-PBS, KI, 5-ALA, mechanical stress from stirring, oxygen consumption or any other non-photodynamic effects. Thereby, yielding an effective photoinactivation extent of 0.6 logs. In P. aeruginosa, the photoinactivation reaches a reduction of 1.2 logs without evident dark toxicity. In both experiments, exposure to PersL inhibited bacterial growth in a dose-dependent manner, as illustrated in Figure 6C, demonstrating a clear dependence between the light dose delivered to bacteria (positive slope, green line) and the photoinactivation effect (negative slopes, blue line for S. aureus and red line for P. aeruginosa). **This result provides solid evidence that the photoinactivation of bacteria is indeed related to the PersL emitted by the particles.**

Results

A novel persistent luminescent material has been synthesized and characterized. As verified by several methods developed in this work, ZrO_2:Ti^{4+}@PEG particles exhibit an array of convenient properties such as water stability, blue-light emission, and a persistent luminescence capable of promoting remote photosensitisation and photoinactivation of bacteria.

The advantageous properties shown in this study firmly stablish the material as a promising candidate for non-invasive light delivery in antimicrobial treatments, potentially improving their effectiveness. Our findings open avenues for future research into unconventional light-source antimicrobial PDT applications.

IQS UNIVERSITAT RAMON LLULL

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 863102