Innovative Photodynamic Strategies for Antimicrobial Treatments: Biosafety and Effectiveness in a Cnidarian Model

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Abstract Thiophene-based materials (TMs) have emerged as promising candidates in the field of photodynamic therapy (PDT) as photosensitizers agents owing to their remarkable electron transport properties, which facilitate efficient energy transfer processes crucial for PDT. In detail, TMs exhibit favourable optical characteristics, making them suitable candidates for the absorption and conversion of light energy into reactive oxygen species (ROS), thereby inducing cytotoxic effects in targeted cells. Recent studies have explored natural carriers, including proteins and phages, for enhanced cell uptake and permeation of photosensitizers, thereby enabling the induction of apoptosis across various cell lines. Despite the remarkable potential of this approach for PDT purposes, clinical translation necessitates *in vivo* models to validate these innovative tools. Here, we investigated the nanosafety and *in vivo* efficacy of these phototheranostic agents using the tissue-like animal model *Hydra vulgaris*. The transparency, softness, structural simplicity, and ethical neutrality of *Hydra* collectively render it an exemplary model for such inquiries. These features facilitate rapid screening of cytotoxicity and the effectiveness for photodinamic purposes.

1 Introduction

Prior to the advent of antibiotics, traditional phage therapy relied on natural lytic phages to counteract bacterial infections. However, the limited lytic spectrum of phages (often species- or strain-specific) and the potential emergence of phage-resistant bacterial mutants limits its utilization for therapy purposes[1]. In response to these challenges, photodynamic therapy (PDT) has emerged as promising antimicrobial treatment against drug-resistant bacteria[2]. PDT is based on the utilization of a photosensitizing compounds (sensitizers) that accumulates within bacterial cells, and upon light activation, generates reactive oxygen species (ROS) that cause damage to membranes, proteins, and DNA/RNA, ultimately leading to bacterial death.
Oligothiophene-bioconjugates have also demonstrated excellent photo-transduction properties and hold a great potential for photodynamic purposes[3]. To overcome several issues related to oligothiophene delivery in nanomedicine (e.g. intra- and inter- batch variability, no cell-specific targeting), novel strategies were recently proposed using viruses as starting material to conjugate on their capsid oligothiophene based sensitizing agents. These novel delivery nanotools have been suggested as versatile platform for targeted antimicrobial PDT[4].
To advance the development and clinical use of these novel nanoplatforms it is crucial to establish models for assessing nanosafety and evaluating PDT performance *in vivo*. In this context, invertebrates provide a promising alternative, bridging the gap between cell cultures and vertebrate models.
In this study, we investigated the nanosafety and *in vivo* effectiveness of M13 phage functionalized in the capsid with the photosensitizing agent ECB04. By using *Hydra vulgaris* polyp as model system we demonstrated biosafety of the ECB04 sensitizer in absence of photostimulation. Figure 1 shows that the continuous incubation up to 72h of functionalized M13-ECB04 at different concentrations (5 nM and 50 nM) does not impact *Hydra* morphology (both body column and tentacles), suggesting no dark toxicity (Figure 1).

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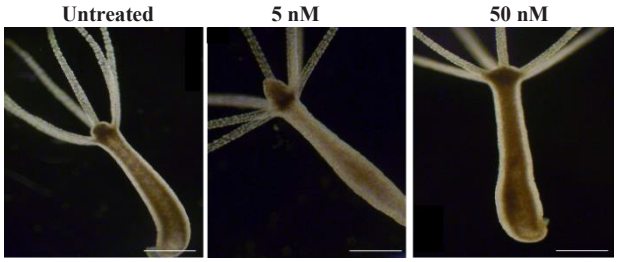

Figure 1. Biosafety of M13-ECB04 in Hydra *vulgaris*. Polyps were continuously incubated with M13-ECB04 at the indicated concentrations (related to the bacteriophage) and up to 72 h. Scale bars: 200 µm. (n=10).

In vivo photodynamic treatment with 50 nM M13-ECB04 for 30 min highlighted a significant cell disassembly and lysis around the tentacles (Figure 2A) already after 15 min of irradiation (0.4 W m^{-2}). Toluidine blue staining of nematocysts, specific cnidarian cells involved in prey capture, shows an alteration of the number and their aberrant distribution after treatment and irradiation (Figure 2B). These data suggest a potential accumulation of M13-ECB04 within storage vacuoles, as observed when polyps were incubated with other nanoparticles (i.e. quantum rods and gold nanoparticles)[5,6].

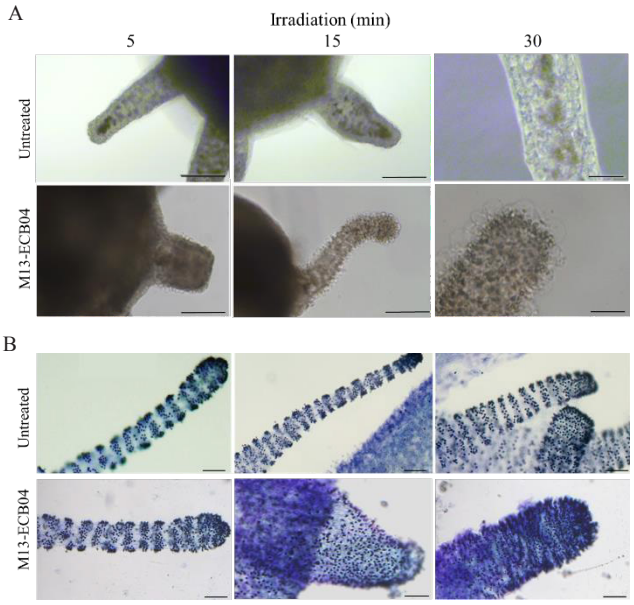

Figure 2. *In vivo* photodynamic treatment with M13-ECB04. A) Polyps were treated with 50 nM of M13-ECB04 for 30 m and irradiated (light power density of 0.04 mW cm^{-2}) at different durations. The images show tentacles and body tissue of treated polyps after 5, 15 and 30 min of irradiation. Cell blebbing and damages of tentacle cells increase progressively as the irradiation time increases. Scale bars: 200 µm (5' and 15' irradiation) and 50 µm (30' irradiation). B) Toluidine-blue staining of tentacles showing the nematocytes organized in battery cells. The organization and the number of nematocytes are depleted in animals treated with M13- ECB04 and progressively increase with the irradiation time. Scale bars: 200 µm.

2 Conclusion

The possibility to use a hydrid phage-based system for photodynamic therapy represents an innovative approach for selectiv delivering of numerous copies of the sensitizer to a specific bacterial target. In our study, we demonstrated the biocompatibility and the absence of dark toxicity of the highly photoactive M13-ECB04 *in vivo* using the *Hydra vulgaris* model system as well as significant cell disassembly and lysis around the tentacles after photostimulation. These findings highlight the potential of M13-EBC04 as a targeted antimicrobial PDT agent, with minimal dark toxicity and significant bactericidal effects upon light activation. Although the final target is a novel device to threat human multiresistant bacterial infections, it is crucial to minimize vertebrate experimentation and restrict their use to the clinical trials. In this direction, experimental validation of new nanomaterials designed for photodynamic therapy in a simple invertebrate organism will contribute to reduce vertebrate experimentation.

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