A miniaturised method for measuring inhibition of bacterial growth

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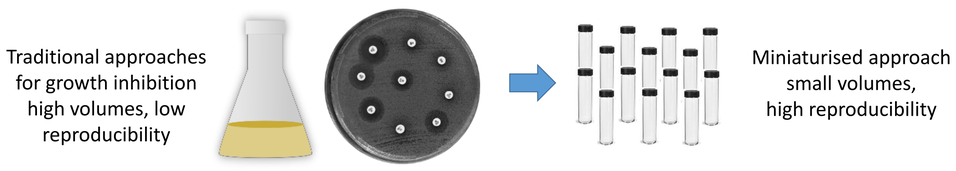
INTRODUCTION

Quantification of microbial growth inhibition is used for the assessment of antimicrobial effects of chemicals [1], plant extracts [2] and novel materials [3]. These tests rely on measurements of growth of microorganisms in either solid or liquid media and effectively the estimation of the growth inhibition. Common tests include agar plates and diffusion assays or monitoring growth in culturing tubes with liquid media for the estimation of the minimal inhibitory concentrations and these methods have been extensively reviewed and compared [4]. Monitoring microbial growth in liquid media which is performed in large culturing volumes and vessels (i.e. culturing flasks) and monitoring microbial cell density by absorbance at 600 nm. Miniaturising growth monitoring tests would allow feasibility, reproducibility and increase of the test performance itself.



EXPERIMENTAL DESIGN

In this study we used *Escherichia coli* and tested how bacterial growth is impacted by different parameters such as the volume of culturing media and the cell concentration at inoculation. Bacteria were cultured in 90% LB media 10% sterile phosphate buffer saline (PBS) at 30°C under agitation (200 rpm) in a shaking culture incubator. For each experiment bacteria were pelleted by centrifugation at 3,000 *g* at room temperature for 5 minutes and the supernatant containing media and debris was discarded. The cells were re-suspended in sterile PBS and pelleted again. A cell inoculum in sterile PBS was prepared at a concentration of 80,000,000 cells per ml (corresponding to a 0.1 absorbance at 600 nm). This cell suspension was further diluted in sterile PBS to the required concentration for the cell inoculum. For culturing and exposures (described below) we used 1 volume of sterile PBS (10%) and 9 volumes of LB media (90%) and following incubation for 20 hours at 30°C under agitation (200 rpm) in a shaking culture incubator, absorbance was measured at 600 nm using a microplate reader.

RESULTS AND DISCUSSION

Miniaturising bacterial culturing requires optimizing the number of cells at inoculation and defining the linear time range for growth during culture. Initially, different amounts of cells were used for inoculation and growth was estimated after incubation for 20 hours at 30°C under agitation (200 rpm) via absorbance measurements at 600 nm (Fig. 1A). Having chosen the inoculation concentration, kinetic growth curve were performed to define the linear time for measuring growth (Fig. 1B).

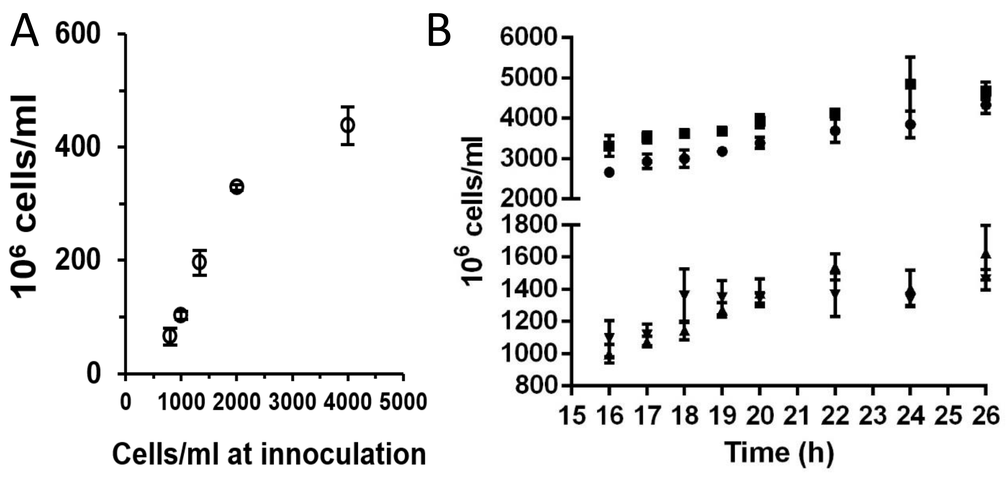


Figure 1. Defining the parameters of miniaturising bacterial culturing and optimisation of (A) the number of cells at inoculation and (B) the time incubation period for linear growth for different flasks (●50 ml, ■25 ml) and miniaturised vials (▲10 ml, ▼1 ml).

Having established the inoculation concentration and the linear time of growth culturing for miniaturised vessels, we established an exposure protocol in 1 volume sterile PBS (10%) for 1 to 4 hours at 30°C under agitation (200 rpm) followed by addition of 9 volumes LB media (90%) and incubation for 20 hours at 30°C under agitation (200 rpm) (Fig. 2A). Additionally, this approach proved to be a stable and reproducible system, which we tested with independent biological and technical replicates which had a coefficient of variance less than 10% (Fig. 2B).

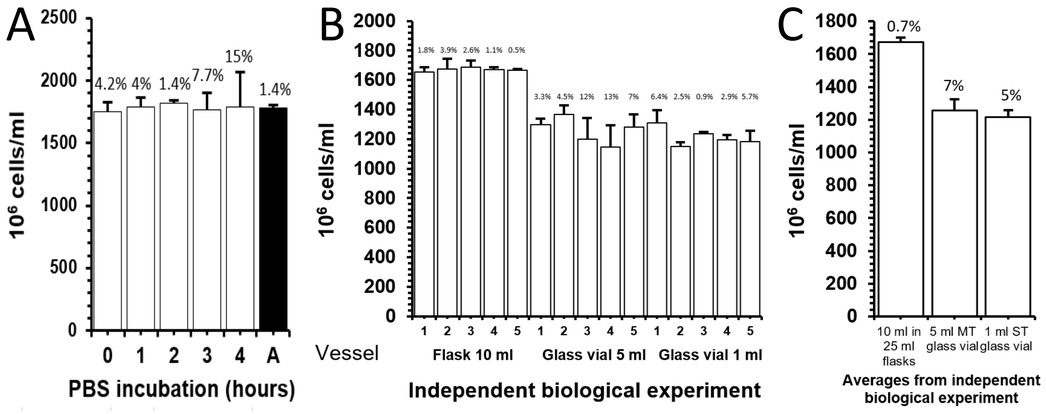


Fig. 2. The miniaturised assay was tested for the effect of (A) incubation time in PBS and (B) reproducibility.

The established optimised miniaturised approach was used to assess the antibacterial effect of in-house produced [5] silver nanoparticles (AgNPs) on coated on a biopolymer surface (Fig. 3).

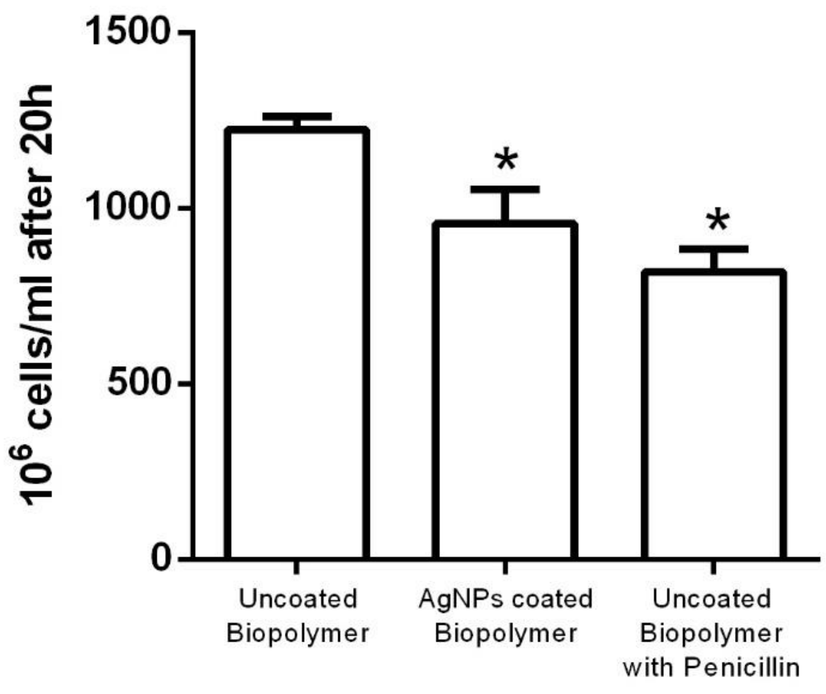


Figure 3. Quantification of bacterial growth inhibition upon exposure to silver nanoparticles coated on a biopolymer surface.

In conclusion, a miniaturised method for fast, reproducible culturing was developed. This approach was used for the estimation of the antimicrobial effect of nanomaterials on coated biopolymer surfaces. Future steps of this work would be focused on the optimisation of this approach further for cryopreservation of cells with the aim to develop a commercial test for assessment of antimicrobial effects.

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