Microcontainers for improved treatment of *Pseudomonas aeruginosa* biofilm infections

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Learning objectives:

1. Explain the idea behind using microcontainers for improved treatment of biofilm
2. Describe which types of coatings that can be used for interaction with biofilm
3. Evaluate the impact of coated and antibiotic loaded microcontainers on PAO1 grown in planktonic or biofilm mode

**INTRODUCTION**

Lung infections with *Pseudomonas aeruginosa* are difficult to eradicate as it forms biofilm that protects the bacteria from the host immune response and hinders antibiotics to reach the site of infection [1]. Inhaled powdered antibiotics have the limitation that they distribute in all areas of the lungs and a certain degree of dilution takes place hence, a more targeted delivery is needed. Micrometer sized polymeric containers (microcontainers) have been developed for oral delivery of therapeutics and studies have shown that the microcontainers are engulfed by the intestinal mucus [2]. We hypothesize that the microcontainers can be utilized in the treatment of biofilms as they are suggested to penetrate the biofilm and release the antibiotics in a more controlled manner at the target site compared to conventional inhaled antibiotics. Therefore, the purpose of this work was to load microcontainers with antibiotic, to coat with three different coatings and lastly test the microcontainers on *P. aeruginosa* grown planktonically or as biofilm.

**METHODS**

Ciprofloxacin hydrochloride (CIP) was loaded into SU-8 microcontainers and sealed by spraying a lid of Eudragit®S100, chitosan or polyethylene glycol (PEG), which are known to be pH sensitive, mucoadhesive or mucus penetrating, respectively. Drug release was studied using a µDiss profiler in phosphate buffer at pH 7.4. The impact of drug-loaded microcontainers on planktonic growth inhibition of *P. aeruginosa* PAO1 was studied with OD analysis on a plate reader. The impact of microcontainers on biofilm-associated PAO1 was investigated in a flow cell system [2]. 3D-images were acquired by confocal scanning laser microscopy and Comstat analysis was used for quantification of live/dead bacteria.

**RESULTS**

Microcontainers were successfully loaded and coated (Fig. 1). A retarded release of CIP was seen for all coatings, and 50 % of the drug was released within 6-14.5 h. It was shown that all coated and non-coated microcontainers were able to inhibit planktonic growth of PAO1. In addition, test of empty microcontainers showed no toxicity, as they did not inhibit growth. Bacterial biofilms were successfully grown in a flow cell system and results showed that the non-coated microcontainers were able to kill the bacteria only in the locations around the microcontainers (Fig. 2). Further studies with coatings and unconfined drug will be performed.

**CONCLUSION**

Lids of Eudragit®S100, Chitosan or PEG resulted in retarded release of CIP from microcontainers. The coated microcontainers were shown to inhibit bacterial planktonic growth and non-coated microcontainers were shown to kill bacteria in biofilm in near proximity of the microcontainers.
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REFERENCES


PRESENTER BIOGRAPHY

The presenting author, Stine Egebro Hansen, has a M.Sc. in Pharmacy from the University of Copenhagen where she among other things worked with oromucosal drug delivery combining the use of permeation enhancers and matrix-assisted laser absorption ionization to determine the spatial localization of drug and enhancer in tissue sections. Stine Egebro Hansen is now enrolled in a PhD programme at DTU Health Tech at the Technical University of Denmark.

FIGURES

Fig. 1: Scanning electron microscopy (SEM) images of a microcontainer (A) empty, (B) loaded with ciprofloxacin hydrochloride, (C) coated with Eudragit® S100, (D) coated with polyethylene glycol and (E) coated with chitosan.

Fig. 2: Confocal scanning laser microscopy images showing (A) an early biofilm development of PAO1 where clusters start to form, and (B) the microcontainer in relation to the dead cells (stained by propidium iodide).