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Improving antibiotic treatment of *Pseudomonas aeruginosa* biofilms using N-acetylcysteine-functionalized microcontainers

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LEARNING OBJECTIVES

1. Describe the rational of using microcontainers for improved delivery of antibiotics to biofilms
2. Characterize microcontainers functionalized with N-acetylcysteine and chitosan
3. Evaluate the effect of the functionalized microcontainers towards *Pseudomonas aeruginosa* biofilm

INTRODUCTION

Treatment of biofilm-related infections often fails as antibiotics cannot be dosed in concentrations that are sufficient to eradicate a bacterial biofilm. We have recently found that by using microcontainers functionalized with chitosan, we can eradicate *P. aeruginosa* biofilms with substantially lower antibiotic concentration [1]. N-acetylcysteine (NAC) is a mucolytic agent that promotes disruption of mature biofilms [2]. This study aims to characterize NAC/chitosan-functionalized microcontainers and investigate whether this combination could provide disruption of the mucin-containing biofilm matrix, thereby improving bacterial susceptibility towards antibiotics and ultimately biofilm eradication.

METHODS

Ciprofloxacin hydrochloride (CIP) was loaded into microcontainers, and a lid consisting of a NAC:chitosan ratio of 8:1 w/w was applied. Scanning electron microscopy (SEM) was used to assess the quality of the functionalization and the coating thickness was determined by optical profilometry. Drug release from the microcontainers was investigated in FAB minimal medium [1]. Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) was used to determine the effect of NAC on mucin. *P. aeruginosa* biofilms were grown (72 h) using centrifugal microfluidics [3] and treated (24 h) with NAC/chitosan-functionalized microcontainers or CIP in flow. The 3D-images of the biofilm were acquired with confocal microscopy and COMSTAT software was used for quantification.

RESULTS

We confirmed that CIP was loaded into the microcontainers and that chitosan as well as NAC/chitosan coating was achieved (Figure 1A). We found that NAC accelerated the release of CIP from the microcontainers as 35.3±2.8%, 4.5±7.7% and 98.0±4.2% was released within 1 h in the absence of coating, with chitosan or NAC/chitosan coating, respectively (Figure 1B). Addition of chitosan to the QCM-D sensor coated with mucin showed a Δfrequency=6.10 and Δdissipation=0.66 demonstrating that chitosan was absorbed onto the mucins and that swelling occurred. Addition of NAC with chitosan led to Δfrequency=13.60 and Δdissipation=1.93, indicating a softer, less viscous mucin layer, proving the mucolytic effect of NAC in the presence of chitosan. Additionally, treatment of the *P. aeruginosa* biofilm with CIP-containing microcontainers functionalized with NAC/chitosan or CIP in flow led to a killing of 68.3% and 59.2% bacteria, respectively, indicating the effectiveness of the functionalized microcontainer (Figure 2).

CONCLUSION

Microcontainers were functionalized with NAC/chitosan and we observed that NAC significantly increased CIP release rate. QCM-D revealed the mucolytic effect of NAC and we found that functionalized microcontainers showed improved eradication of *P. aeruginosa* biofilm. The current work illustrates the relevancy of utilizing functionalized microcontainers for improved biofilm eradication.

ACKNOWLEDGEMENTS

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FIGURES

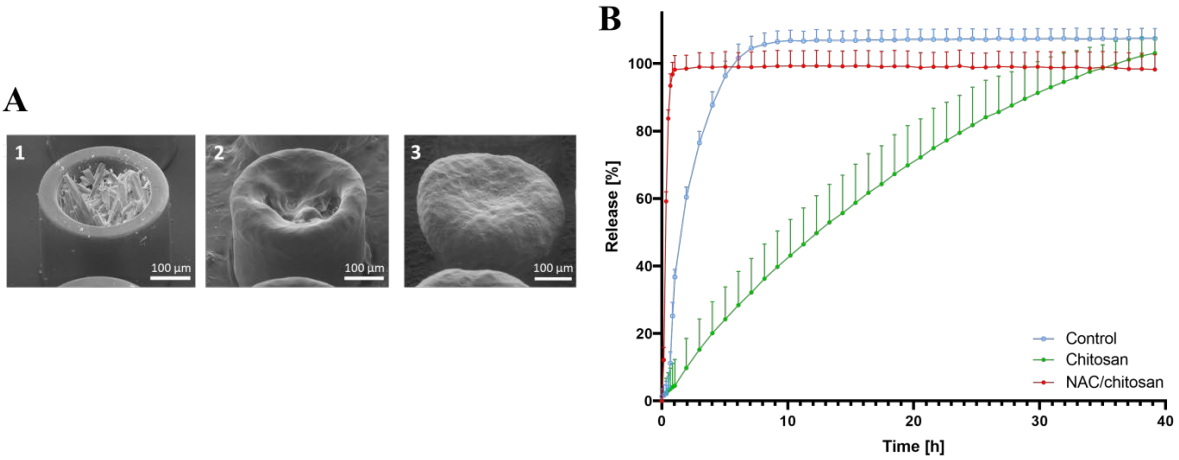

Figure 1: A) Scanning electron microscopy (SEM) images of a microcontainer loaded with ciprofloxacin hydrochloride (CIP) (1) and coated with chitosan (2) or NAC/chitosan (3). B) *In vitro* cumulative release of CIP from microcontainers in the absence of coating, with chitosan or NAC/chitosan coating in FAB minimal medium. Data are presented as mean+SD (n=3).

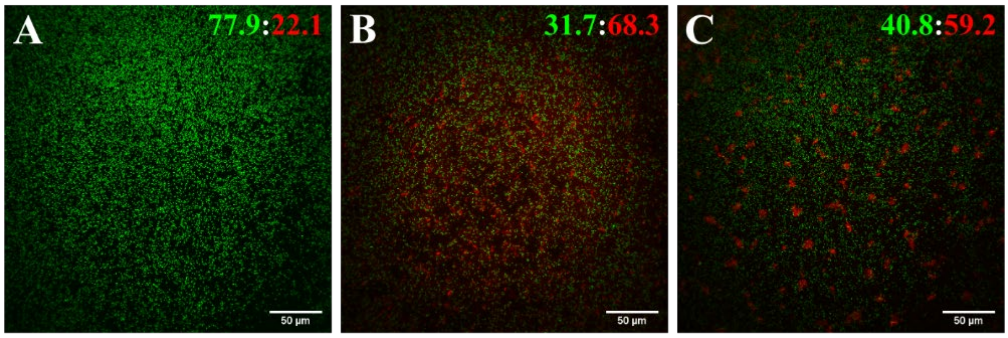

Figure 2: Confocal laser scanning microscopy images of *Pseudomonas aeruginosa* biofilms. A) Biofilm prior to treatment after 72 h growth. B) 24 h-post treatment with NAC/chitosan-functionalized microcontainers containing ciprofloxacin hydrochloride (CIP). C) 24 h-post treatment of CIP in flow. Green represents live bacteria. Red represents dead bacteria.