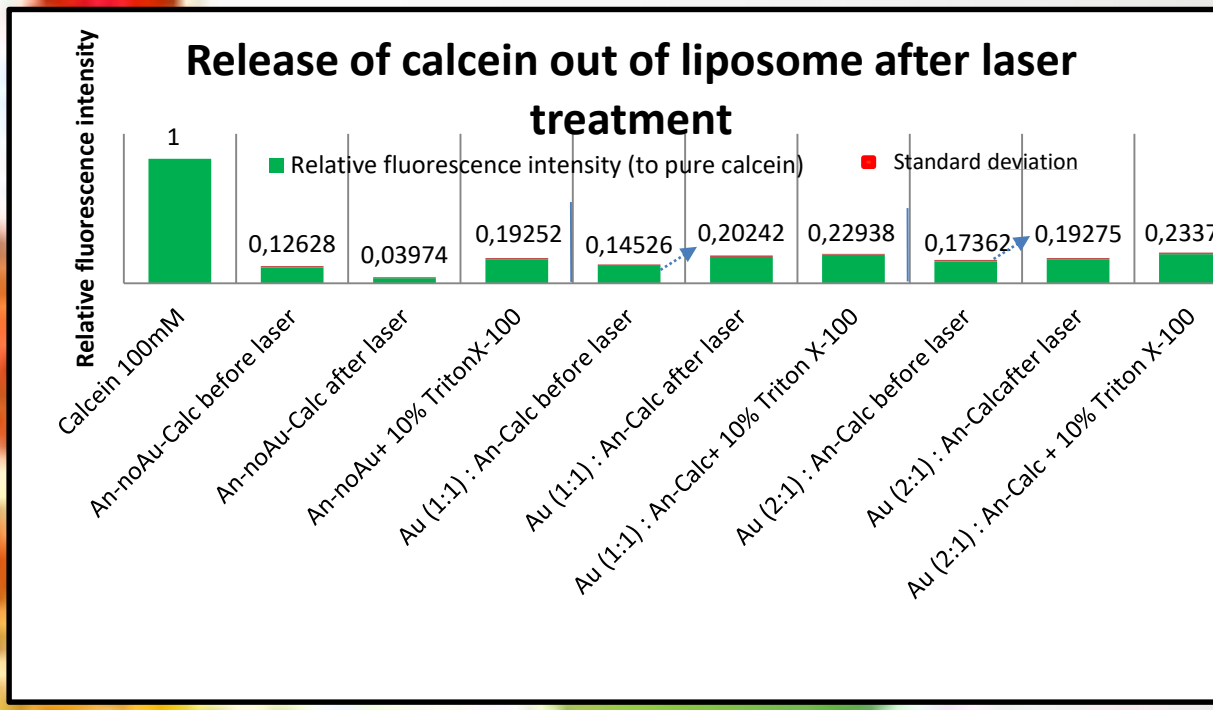
Combining nanocarriers for antibiotic delivery with gold nanoparticles for light-triggered release and biofilm disruption



Rosanna Alessandra Catapano

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Rosann A.Catapano

UNIVERSITÀ DEGLI STUDI DI SALERNO

Facoltà di Ingegneria
Dipartimento di Ingegneria Industriale
Corso di Laurea in Ingegneria Chimica

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Tesi in
Fenomeni di trasporto

Relatori:
Prof. Ing. Anna Angela Barba
Prof. Ing. Gaetano Lamberti
Prof. Kevin Braeckmans
Prof. Stefaan De Smedt
Correlatore
Ph.D. Eline Teirlinck

Candidata:
Rosanna Alessandra Catapano
matricola 0622200245

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To me, mom, dad and Daniele

Questo testo è stato stampato in proprio, in Times New Roman

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Summary

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Riassunto

In questo lavoro di tesi è stata valutata la capacità di rilascio del contenuto liposomiale tramite l'utilizzo di un nuovo tipo di effetto foto-termico. I liposomi, resi sensibili alla luce tramite funzionalizzazione con AuNPs (nanoparticelle di oro), sono stati irradiati con un laser a luce pulsata ed è stato valutato il rilascio del contenuto. Sono stati preparati liposomi con carica negativa, incapsulando una soluzione di calceina ed è stato realizzato un test per verificare il rilascio del contenuto dai liposomi. Le varie formulazioni sono state caratterizzate in termini di dimensioni, valutando il potenziale-zeta con il Dynamic Light Scattering, l'assorbanza, UV-VIS, tramite spettroscopia UV-VIS e la concentrazione tramite FSPT, monitoraggio a fluorescenza per singola particella. Al fine di rendere i liposomi reattivi alla luce, sono stati funzionalizzati con nanoparticelle di oro con carica positiva. Diversi rapporti Au:liposoma sono stati sintetizzati, e la loro stabilità è stata esaminata con l'ausilio del DLS. I sistemi finali sono stati trattati con un laser pulsato di 561 nm in condizioni tali da creare nanobolle di vapore attorno ai AuNPs. Il rilascio di calceina dai liposomi attivato dalle VNB è stato valutato utilizzando la microscopia a fluorescenza. Al fine di confermare visivamente la formazione di VNBs, sono state acquisite delle immagini durante l'irradiazione dell'impulso laser usando la microscopia in campo oscuro.

In seguito è stato valutato l'effetto sinergico dei liposomi contenenti antibiotico e nanobolle di vapore nel trattamento delle infezioni batteriche causate da biofilm. Sono stati preparati liposomi caricati di tobramicina e sono stati caratterizzati tramite Dynamic Light Scattering.

Le AuNPs sono introdotte nei biofilm di *Burkholderia multivorans* e di *Pseudomonas aeruginosa* e dopo irradiazione del laser pulsato,

sono state create nanobolle di vapore. Infine, i biofilm sono trattati con i liposomi carichi di tobramicinia per 24 ore. Per determinare l'efficacia del trattamento è stata utilizzata la tecnica del conteggio su piastra.

Abstract

The ability of a new type of photo-thermal effect as trigger for liposomal content release has been investigated. Light sensitive liposomes have been produced by functionalizing them with AuNPs (gold nanoparticles), and after pulsed laser irradiation the release of the content have been evaluated. Negatively charged liposomes have been created, encapsulating a solution of calcein, and the calcein release assay has been used to test content release out of the liposomes. The formulations has been characterized in terms of size and zeta-potential with Dynamic Light Scattering, UV-VIS absorbance via UV-VIS spectroscopy and concentration via fluorescence Single Particle Tracking, FSPT. In order to make the liposomes reactive to light, they have been functionalized with the positively charged gold nanoparticle. Different Au:liposome ratios have been synthesized whose colloidal stability and examined with the aid of DLS. The final systems has been treated with a pulsed laser of 561 nm in such conditions that vapor nanobubbles are created around the AuNPs. The release of calcein from the liposomes, activated by VNB, has been evaluated using , fluorescence microscopy. In order to visually confirm the formation of VNBs, pictures have been taken during the laser pulse irradiation using dark field microscopy.

After, the synergistic effect antibiotic-loaded liposomes and vapor nanobubbles in the treatment of bacterial biofilm infections has been evaluated. Therefore, tobramycin-loaded liposomes have been prepared and characterized via Dynamic Light Scattering.

AuNPs have been introduced inside *Burkholderia multivorans* and *Pseudomonas aeruginosa* biofilms and after pulsed laser irradiation, vapour nanobubbles have been created. Finally, the biofilms have been treated with tobramycin-loaded liposomes for 24 hours. The

plate counting technique has been finally used to determine the effectiveness of treatment.

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