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Light triggered self-assembled nanobombs of polydopamine cores and polystyrene beads for intracellular delivery

Tesi in

Transport Phenomena

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Alla mia famiglia

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Summary

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Riassunto

L'immunoterapia che utilizza cellule modificate con recettori chimerici dell'antigene (car) è un approccio promettente per il trattamento del cancro, che richiede la produzione di prodotti a base di cellule car. Una parte essenziale del processo di produzione delle car è la trasfezione delle cellule derivate dal paziente con il costrutto car e con composti che eliminano le vie di soppressione immunitaria per aumentare il loro potenziale terapeutico. Questo progetto mira a migliorare la consegna intracellulare di composti di editing genico nelle cellule mediante "nanobombe" innescate dalla luce durante un processo di fotoporazione cellulare. Queste sono nanostrutture costituite da un nucleo fototermico circondato da nanoproiettili. In particolare, il progetto esplorerà diversi modi per sintetizzare le nanobombe, con l'obiettivo di massimizzare l'efficienza di trasfezione e la stabilità colloidale a lungo termine utilizzando un promettente materiale fototermico, ossia la polidopamina, e non particelle a base metallica, come oro e ossido di ferro. Lo scopo è la produzione di un sistema più biocompatibile e biodegradabile. La temperatura di reazione e le condizioni del sistema per la produzione di polidopamina sono state analizzate per osservarne l'impatto sulla crescita delle particelle (PDA NPs), misurando le dimensioni idrodinamiche e il potenziale zeta (ZP) attraverso lo strumento di Dynamic Light Scattering (DLS). È stata confermata la riproducibilità della sintesi della PDA, ottenendo un protocollo modificato con un tempo di reazione ottimizzato. È stata osservata la stabilità di queste particelle nel tempo. Sono stati studiati diversi tipi di strategie per fissare le particelle. La strategia migliore includeva l'utilizzo di un reagente reticolante, la glutaraldeide, usata per funzionalizzare le perline di polistirene usate come nanoproiettili. È stato possibile scegliere una strategia tra quelle testate, analizzando prima le dimensioni e la stabilità delle particelle assemblate tramite DLS e poi la morfologia

tramite la microscopia confocale e le immagini al SEM. La determinazione della soglia di nanobolle di vapore (VNB) delle NPs di PDA è la parte finale di questa tesi. Dopo aver irradiato le particelle con impulsi laser di breve intensità, le VNBs possono essere visualizzate e contate utilizzando la microscopia in campo oscuro. Questo è decisivo per considerare le particelle adatte a essere utilizzate nel processo di fotoporazione cellulare futuro.

Abstract

Immunotherapy using chimeric antigen receptor (car)-modified cells is a promising approach to treat cancer, which requires the manufacturing of car-cell products. An essential part of the manufacturing process is the transfection of patient-derived cells with the car construct and with compounds to knock-out immune suppressive pathways to enhance their therapeutic potential. This project is aimed at enhancing the intracellular delivery of gene-editing compounds in cells by light-triggered ‘nanobombs’, in photoporation process. These are assembled nanostructure with a core surrounded by nanoprojectiles. In particular, the project will explore different ways to synthesize nanobombs, aimed at maximizing transfection efficiency and long-term colloidal stability by using a promising phototermal material, i.e. polydopamine and not metal based particles, such as gold and iron oxide. The purpose is the production of a more biocompatible and biodegradable system. The reaction temperature and the system conditions to produce polydopamine have been detected to observe their impact on the growth particles (PDA NPs), measuring the hydrodynamic size and zeta potential (ZP) through the use of Dynamic Light Scattering (DLS) instrument. The PDA synthesis reproducibility was confirmed, obtaining an adapted protocol and the particles stability over the time has been detected. Several types of strategies to attach the particles are investigated. The best strategy included the use of a crosslinking reagent, glutaraldehyde, used to functionalize the polystyrene beads, used as nanoprojectiles. It was possible to choose the successful method, detecting the size and the stability of obtained particles using the DLS instrument and then the morphology using Confocal Microscopy and SEM images. The

determination of the vapor nanobubbles (VNB) threshold of PDA NPs is the final part on this dissertation. After irradiating the NPs with short intense laser pulses, the VNBs can be visualized and counted using dark field microscopy. This is crucial in considering particles suitable for use in the future cell photoporation process.

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