Monocytes Hybridized with PAMAM Dendrimer-Doxorubicin Conjugates for Anticancer Drug Delivery

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Statement of Purpose: Current strategies in cancer therapies include drug-targeting systems to alleviate negative side effects of chemotherapeutic agents.[1] The goals are to decrease systemic drug concentrations to minimize toxicity to healthy tissues and to concentrate drug molecules at the target site. This research draws from our biological toolbox with aims to create a functional cellular therapy in conjunction with multifunctional biomaterials. A known trait of blood monocytes is their ability to respond to inflammation and chemotactic gradients to partake in repair functions. Specifically, monocytes can extravasate leaky endothelium of irregular vasculature found near tumors. Monocytes are attracted to hypoxic areas and chemotactic gradients of tumors and have the inherent ability to penetrate tumor spheroids.[2] This natural tumor-targeting ability offers a potential therapeutic role by monocytes and tumor progression. Doxorubicin (DOX) is a widely used chemotherapeutic and intercalates with DNA to prevent cell proliferation.[3] Free DOX has cardiotoxic side effects, so the goal is to protect healthy cells from DOX exposure via conjugation to PAMAM dendrimers.[4] The objective of this project was to demonstrate the feasibility immobilizing DOX to the surface of monocytes and examine whether the modified monocytes would retain the migration ability for improved drug penetration in the tumor.

Methods: *Synthesis of DOX-G4.5-PEG:* PAMAM G4.5 dendrimer was first activated using the NHS/EDC chemistry and then coupled to PEG diamine (MW= ~ 3350 g mol^{-1}). DOX was then coupled to G4.5-PEG via hydrazone linkage following a reported method.[5]
Hybridizing monocytes with DOX-G4.5-PEG: RAW264.7 monocytes were hybridized with DOX-G4.5-PEG following the procedure described in our previous work.[6] Hybrid vehicles were imaged with a Zeiss fluorescence microscope.
Spheroid model setup and migration studies: Spheroids using FaDu cell line transfected with yellow fluorescence protein (YFP) plasmid were formed with the hanging drop method.[7] After spheroid formation at day 5, 5×10^3 CellTracker™ Red labeled monocytes were incubated for 24 hours with YFP expressing spheroids. Spheroids were collected and either imaged live using confocal microscopy or snap frozen in freezing medium then cryosectioned before imaging.

Results: Monocyte surfaces contain sialic acid residues which can be modified by $NaIO_4$ into amine-reactive aldehydes for binding to amine groups found on dendrimer conjugates. Once materials are affixed to monocytes $NaCBH_3$ is introduced to reduce the transient Schiff linkage into a stable secondary amide bond. Cell surface modification and hybridization has been confirmed with our previous work using PEG-G4.5-5'AAF conjugates.[6] Our recent studies confirmed that the same surface modification strategy could be used to couple DOX-G4.5-PEG to the surface of monocyte as shown in Figure 1. Using YFP-expressing FaDu cells for a spheroid model, CellTracker™ labeled monocytes (red) were shown to penetrate the *in vitro* spheroids (green).

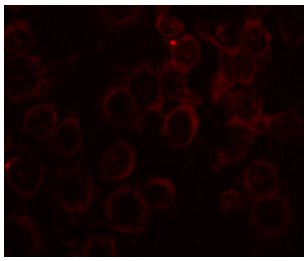

Figure 1. *RAW264.7 monocytes hybridized withDOX- G4.5-PEG (red) conjugates via surface modification (630×).*

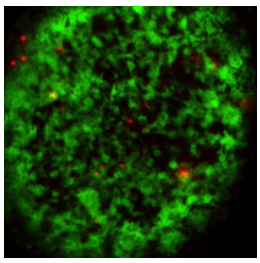

Figure 2. *Monocytes stained with CellTracker Red penetrated into the YFP FaDu spheroid (green) after 24 h-incubation (200×).*

Conclusions: RAW264.7 monocytes showed similar hybridization with PEG-G4.5-DOX as shown in our previous work using PEG-G4.5-AAF. CellTracker labeled monocytes exhibited penetration within tumor spheroids. Cell viability and migration ability will be further examined. In addition, controlled release of DOX from the hybrid vehicle as well as drug penetration and distribution in spheroids will be studied.

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