Surface-Modified Monocytes Coupled to PAMAM Dendrimers for Targeted Anticancer Drug Delivery

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Statement of Purpose: In spheroid cancers, tumors often contain areas of hypoxia within their core due to poor vasculature and oxygen usage of outer cells in the spheroid [1]. It has been demonstrated that monocytes have an innate ability to migrate to hypoxic areas, specifically, inside tumor spheroids. The idea is to use the targeting ability of monocytes as a lead for cancer therapeutic systems. This research aims to engineer monocytic cell lines as targeted delivery vehicles through combination with half generation polyamidoamine (PAMAM) dendrimers.

Methods: *Synthesis of AAF-G4.5-PEG:* G4.5 nanoparticles were modified with PEG diamine (MW= ~ 3350 g mol^{-1}) using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and N-hydroxysuccinimide. 5-(aminoacetamido) fluorescein (AAF) was then applied to label G4.5.

Monocyte Treatment: RAW 264.7 monocytes were plated on coverslips at a density of 1.2×10^4 cells/well. At 70% confluence, surface modified groups were incubated with 0.1 mM $NaIO_4$ at 4 °C for 15 min in the dark. 12 µg of DPCs were incubated with monocytes for 0-24 hours with and without surface treatment. To form stable linkages between nanoparticle and the cell surface, the cells were treated for 1-2 hours in 0.1 mM $NaCBH_3$ (pH 7.4-9.0). DAPI staining was used for colocalization of monocyte-dendrimer hybrids. Treatments were imaged with a Leica Confocal microscope at 488 nm excitation and 512 nm peak emission. Image J was used for background subtraction and noise removal as well as intensity profile measurements.

Spheroid Formation: Spheroids were formed with the hanging drop method [2].

Results: Confocal images confirmed hybridization of nanoparticles with monocytes (Figure 1). Control groups exhibited uniform fluorescence distribution. $NaIO_4$ treated groups exhibit preferential fluorescence at the cell surface at short time points with subsequent uniform distribution at longer time points. Groups subject to $NaCBH_3$ exhibit a pronounced fluorescence around cell exterior at all time points. Intensity profiles show increased fluorescence at cell exteriors relative to cell interiors in surface treated groups versus untreated controls.

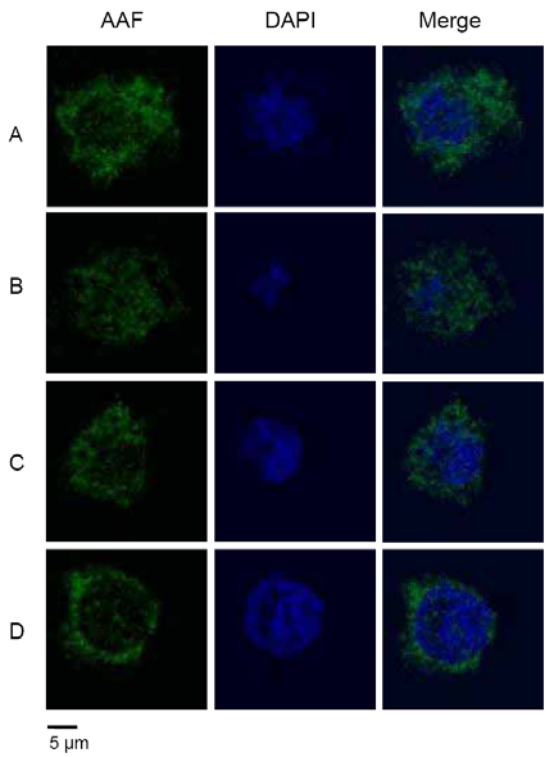

Figure 1. Colocalization assay of AAF-G4.5-PEG (green) with nuclei (blue) by confocal microscopy (630×). (A) Control 1: untreated macrophages incubated with AAF-G4.5-PEG for 1 minute, washed and cultured overnight (24 hours), then fixed and counterstained with DAPI; (B) Control 2: untreated macrophages incubated with AAF-G4.5-PEG overnight (24 hours), then fixed and counterstained with DAPI; (C) Macrophage-T-dendrimer hybrids: sodium periodate-treated macrophages incubated with AAF-G4.5-PEG for 1 minute, cultured overnight (24 hours), then fixed and counterstained with DAPI; (D) Macrophage-S-dendrimer hybrids: sodium periodate-treated macrophages incubated with AAF-G4.5-PEG for 1 minute, treated with sodium cyanoborohydride, cultured overnight (24 hours), then fixed and counterstained with DAPI.

Conclusions: AAF-G4.5-PEG was immobilized to the macrophage cell surface through either a transient Schiff base linkage or a stable amine linkage. The distribution of nanoparticles on the cell surface was found to be dependent on the stability of the linkages connecting nanoparticles to the cell surface. Creation of FaDu spheroids allows for further validation of the model to test for hypoxia, monocyte migration, and drug distribution.

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References:

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