Multifunctional Rare-Earth Doped Nanoparticles in Encapsulated Albumin Nanocarriers for Tumor Targeting

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Statement of Purpose: The conventional use of visible-light, excitable fluorophores is limited by poor tissue penetration and autofluorescence, while many inorganic nanoparticles such as quantum dots cause localized toxicity. We propose a novel design of multifunctional nanobiomaterials for tumor tissue targeting and imaging. The design is based on upconversion fluorescence generated by the near-infrared excitation of Yb,Er co-doped $NaYF_4$ rare earth nanoparticles (RE-NPs) encapsulated with albumin nanocarriers. The encapsulation renders RE-NPs dispersible in aqueous solution, provides functional groups for conjugating disease targeting agents, and reduces localized cytotoxicity of NPs. Human serum albumin nanocarriers are biocompatible with many cell types over a large range or concentrations, taken up readily by cells, non-immunogenic, bind reversibly to hydrophobic substances and have numerous functional entities available for conjugating ligands and other moieties. Here we report on the use of albumin nanocarriers to encapsulate RE-NPs, their feasibility for imaging cancer both *in vitro* and in a murine model, as well as their potential to track spatio-temporal responses to localized drug therapies *in vivo*.

Methods: The size and shape of the albumin encapsulated RE-NPs (RE-ANCs) were characterized through dynamic light scattering and scanning electron microscopy. The presence and location of the Yb,Er co-doped $NaYF_4$ nanoparticles within the RE-ANCs was determined through thermogravimetric analysis and energy dispersive X-ray spectroscopy. RE-ANCs were functionalized with the tumor targeting peptide cyclic RGD, tested in the U87 and A172 human glioma cell lines and imaged using confocal microscopy. Proof-of-concept drug tracking was investigated by allowing RE-ANCs to bind to temozolomide, an alkylating agent commonly used for the treatment of glioblastoma multiforme, and imaged in a murine mouse model for upconversion fluorescence.

Results: We have developed a new approach for fabricating water dispersible, biologically targeted RE-NPs by encapsulation in autofluorescent human serum albumin nanocarriers. The size of the nanoparticles can be tuned between 75-275 nm, while exhibiting narrow polydispersity and high stablity in aqueous solution for over 30 days. The ANC-encapsulation significantly reduced the cytotoxicity of the RE-NPs *in vitro* while providing surface amine groups for chemically conjugating targeting ligands. RE-ANCs modified with cyclic RGD rapidly targeted $\alpha_V\beta_3$ integrin receptors overexpressed on the U87 glioblastoma cells with minimal targeting of the low integrin expressing A172 cells. Temozolomide readily adsorbed onto pre-formed nanoparticles with >90% loading efficiency. Drug-loaded particles were able to be visualized under near infrared light after injection into a healthy mouse.

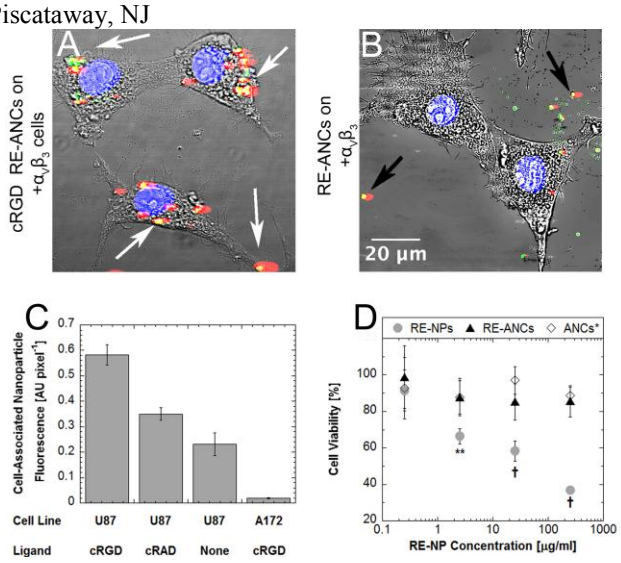

Figure 1 - Confocal images of human glioblastoma cells incubated with: (A) cyclic RGD functionalized RE-ANCs and (B) unmodified albumin encapsulated ceramic nanoparticles. Images reveal that the highly $\alpha_V\beta_3$ integrin expressing U87 cells readily internalize RE-ANCs conjugated with cRGD, as shown by the white arrows, compared to (B) unfunctionalized particles, where little cell-particle association is seen as indicated by the black arrows. Green – autofluorescent albumin nanocarriers; red – RE-NPs; yellow – co-localization of signal; blue – cell nucleus stain. (C) Quantification of co-localized fluorescence signal per pixel area around cells confirms targeting of cyclic RGD conjugated RE-ANCs to specific, integrin expressing cells seen in selected images. ±SE. (D) Cell viability assay of U87 cells incubated with samples for 24 h reveals that albumin coating enhances the biocompatibility of ceramic nanoparticles at elevated concentrations. *ANC concentrations were normalized to protein content in RE-ANCs. ±SD. †, $p<0.001$; ** $p<0.01$, RE-NPs compared to RE-ANCs (*t* test).

Conclusions: The nanomaterials developed and presented here exhibit size tunability, cellular internalization and imaging of cancerous cells *in vitro*. The albumin nanocarrier encapsulation of RE-NPs provides significant reduction of RE-NP cytotoxicity, an aqueous-dispersible coating, and surface groups capable of chemical conjugation for the purpose of cellular targeting. Modification of these nanocarriers by encapsulating chemotherapeutic drugs and by immobilizing targeting ligands allows for the creation of multifunctional nanoparticles for both imaging and drug delivery applications. Our results indicate that RE-ANPs are suitable for imaging cancer *in vitro* and have the potential for combined imaging and targeted drug delivery *in vivo*.

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