Functionalized Gold Nanorods: Dual Confocal Receptor-Specific Contrast and Thermal Therapy Agents

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Statement of Purpose: Targeted nanoparticles have the potential to lower the cost and radically localize disease diagnostics and therapy. The goal of this research was to engineer novel receptor-targeted gold nanorods for use as potential dual use biological optical contrast and heat therapeutic agents. In vitro binding assays and localized thermal ablation were performed on a receptor-engineered colon cancer cell line in a confocal microscope system.

Methods: A colon cancer cell line was genetically engineered to express both a green fluorescent protein (GFP) and the δ-opioid receptor (δOR). Control cells not expressing the receptor were engineered to express red fluorescent protein (RFP) and could be grown in co-cultures with the GFP/δOR cells. Europium-binding assays were performed with DTPA ligand on GFP/δOR cells. Gold nanorods were synthesized through aqueous redox chemistry in the presence of surfactants ($HAuCl_4$, CTAB, Sigma, St. Louis, MO). Rods were functionally coated with synthetically-fabricated deltorphin, another ligand with strong affinity to the δ-opioid receptor, through a polyethylene glycol (PEG)-thiol linker. Cells were incubated with the deltorphin nanorods for two hours, placed in a closed temperature-controlled chamber (Bioptechs, Butler, PA), and imaged in a three-excitation confocal system, where GFP, RFP, and gold nanorods, which are known to give two-photon excited luminescence (Wang H et al. PNAS 2005;102,44:15752), could all be imaged. Binding assays were performed by comparing the integrated gold nanorod signal in GFP and RFP cell cultures. Thermal ablation was performed on gold-coated cells by turning up the power of the Ti:sapphire laser; as a control, uncoated cells in the same field were radiated at the same conditions. Cells were stained with trypan blue in death assay.

Results/Discussion: Eu binding assays showed strong specific binding of the DTPA ligand to GFP/δOR cells, shown in Figure 1.

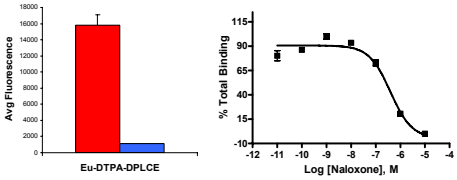


Figure 1

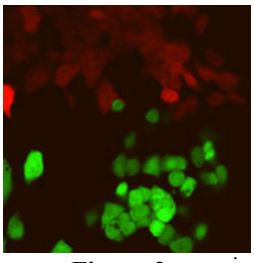
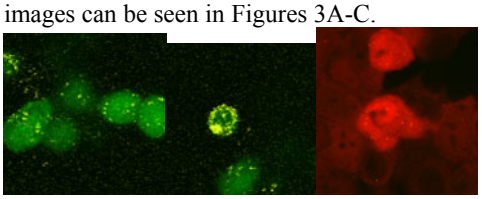


Figure 2

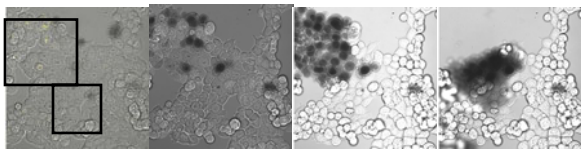
GFP and RFP cells were able to be grown in co-cultures and imaged in the confocal microscope (Figure 3), though some cells expressed neither color and therefore could not be differentiated. Gold nanorods provided nonlinear optical (NLO) signal (yellow) and could also be imaged in the confocal micro-scope. Consistently, deltorphin-coated gold nanorods preferentially bound to GFP cells rather than RFP cells. Representative images can be seen in Figures 3A-C.



(A) (B) (C)

Figure 3

Finally, gold nanorods were used successfully as thermal therapy agents on cells. Thermal damage was successfully performed on a group of cells and a single cell. The group of cells coated with gold were irradiated with 4 pulses at 100% power. Uncoated control cells in the adjacent field were irradiated at the same conditions. A time series can be seen in Figure 4, where trypan blue was flowed into the cell chamber to mark dead cells.

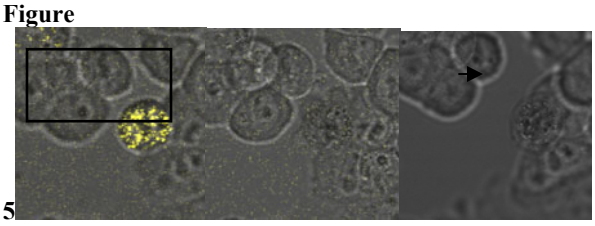


(A) (B) (C) (D)

Figure 4

(A) shows locations of ablations with the coated cells in the upper left. Post ablation frames are shown with (B) 10 minutes after ablation, (C) 23 minutes after ablation, and (D) after more trypan was flowed in and the ablated cells peeled off the coverslip. Notice no cell death occurred in the control spot. A single cell was also ablated. In this experiment, two adjacent cells, one coated and one uncoated, were irradiated with one pulse at 100% power, seen in Figure 5.

Figure



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(A) (B) (C)

(A) shows the area ablated, (B) is an image immediately after ablation, where the gold signal is lost probably due to melting, and clear morphological changes have occurred in the coated cell on the right, probably due to boiling. (C) shows the cell as it peeled off the coverslip when trypan was flowed into the system.

Conclusions: This study shows that receptor targeted gold nanorods can be used as both contrast agents and heat therapeutic agents in a confocal system. Combined with current confocal endoscopic technology, these results hold much promise for subcellular *in vivo* diagnostics and heat therapy.