Optimization and In Vitro Validation of Protease-Activated Quantum Dot Probes

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Statement of Purpose: Protease activity has been demonstrated to be an important prognostic and predictive marker in diseases such as cancer and stroke (1). As such, much attention has been given to the development of diagnostic tools that would allow one to assay their activity in living tissues. Initial attempts to accomplish this goal involved the use of organic fluorophores pairs that utilize Fluorescence Resonance Energy Transfer (FRET) but suffered many drawbacks. This work aims to improve on such efforts by using Quantum Dots (QDs) linked to gold nanoparticles (AuNPs) by protease cleavable peptide sequences to serve as probes for assaying protease activity both in vivo and in vitro. The objectives of this work have been the development, optimization, and in vitro testing of the probe.

Methods: A proteolytically degradable peptide sequence (GGLGPAGGCG) was synthesized using Fmoc solid phase peptide synthesis and verified using MALDI-TOF. Cd/Se Zn/S QDs were synthesized as previously described (2). A Poly(Acrylic Acid)- Oleylamine amphiphilic polymer was used to water solubilize the QDs. These were characterized using transmission electron microscopy, UV-Vis spectrophotometry, fluorometry, and inductively coupled plasma with optical emission spectroscopy.

The above probes were synthesized using linkers with 0, 2, and 4 glycine spacers and QD:AuNPs molar ratios of 1:5, 1:10, and 1:20. To test fluorescence recovery, 0.5 ml of 40 nM probe solution was added to 0.5 ml containing 15 units of Collagenase from Clostridium histolyticum (EC 3.4.24.3, Sigma) with a specific activity of 1900 units/mg. To validate their functionality in vitro, probes were then incubated with NIH 3T3 (fibroblasts used as control for base line protease activity in normal tissue), MCF7 (low invasiveness breast cancer with medium protease activity), and HT1080 (high invasiveness fibrosarcoma) cell lines and imaged using epifluorescence microscopy.

Results: QD synthesis and optimzation presented several key findings. Analysis of the level of quenching of the various QD probes validated electromagnetic shielding and the Forster formalism was used to determine spacing of the AuNP to QD with various length linkers. The analysis of the fluorescence recovery of the probes as seen in Figure 1 allowed one to determine that probes with 4 glycine spacer linkers and QD:AuNP ratio of 1:10 possessed the most linear and sensitive response to proteolytic activity.

Kinetic properties of the fluorescence recovery of the probes were analyzed using Michaelis-Menten kinetics. Collagenase was found to have a Vmax of approximately 6 nM min-1 and the Km was found to be 0.3 µM in accordance with literature(3). Cell studies using time-lapse epifluorescence microscopy demonstrated differential protease activity between all three cell lines with normal 3T3 fibroblasts lowest, followed by relatively non-invasive MCF7 tumor cells and then highly invasive HT1080 tumor cells, as seen in Figure 2. By comparing this recovery to standard curves of known collagenase levels, we found that NIH 3T3, MFC7 and HT1080 cells have a collagenase activity respectively equivalent to 2.1, 5.1, and 7.3 NIH units.

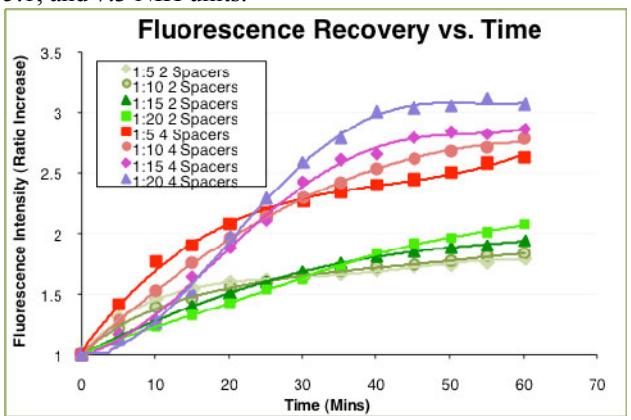

Figure 1. Fluorescence recovery of probes. The top group (longer linkers) show a faster recovery than the bottom group (shorter linkers). Within each group increasing the ratio of AuNP:QD results in slower initial and greater long-term fluorescence recovery.

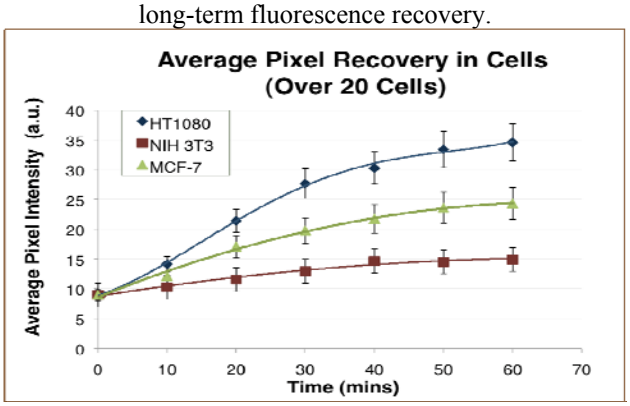

Figure 2. Fluorescence recovery of the three cell lines. Vertical axis represents the average over 20 cells of the average pixel intensity within cell area imaged in a viewfield.

Conclusions: The development of prognostic tools for the detection of proteolytic activity in vivo may play a great role in the prognosis of disease. The initial development and optimization of QD based probes has shown great promise. Demonstration of in vitro functionality has been successfully been completed using three cell lines. Further work will be required to demonstrate in vitro functionality in a 3-d collagen environment as well as in vivo validation.

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References: (1) Egeblad, M. & Werb, Z. Nature Reviews in Cancer. (2002) 2: 161–174. (2) Li J. et al. Journal of the American Chemical Society. (2003) 125: 12567-12575. (3) Vencill, C. et al. Biochemistry. (1985) 24: 3149–3157.