Polymeric Nanoparticle-Based Enzymatically Activatable Near-Infrared Nanoprobes for Optical Detection of Cancer

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Statement of Purpose: The survival rate of cancer patients depends on early detection, which saves thousands of lives each year because it increases the chances for successful treatment. Near-infrared (NIR) fluorescent nanoprobes have emerged as an alternative for the detection, monitoring, and image-guided resection of tumors because they offer advantages such as increased imaging depth due to reduced light scattering, minimal autofluorescence from living tissues, lower cost, portability, potentially high spatial resolution, and avoidance of the use of ionizing radiation. Proteolytic enzymes, or proteases, participate in the degradation of the extracellular matrix during the processes of tumor growth and metastasis. Overexpression of proteolytic enzymes has been observed in a number of cancers (1). Tumor lysosomal proteases can be used as triggers of enzymatically activated nanoprobes (EANPs) to develop NIR signal that can provide sufficient contrast between normal and cancerous tissue, thereby enabling specific imaging of tumors. In this study, EANPs were synthesized as cancer-specific contrast agents for optical imaging and characterized in vitro.

Methods: EANPs (Figure 1) were prepared from blends of amphiphilic block copolymers poly (lactic acid)-b-poly(ethylene glycol) and poly(lactic-co-glycolic acid)-b-poly(L-lysine) via nanoprecipitation. These polymers were synthesized via ring opening polymerization and carbodiimide chemistry, respectively, and they were characterized by ^{1}H-NMR and FTIR. The poly (L-lysine) was used as an anchor for the NIR fluorescent molecule AlexaFluor-750 (AF750). The AF750-labeled EANPs were characterized by electron microscopy and their size by dynamic light scattering.

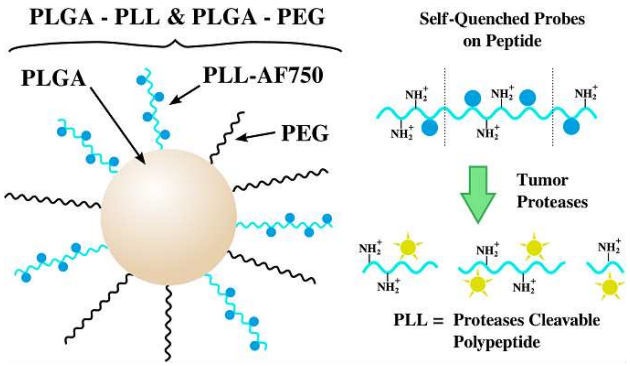

Figure 1. Design and Envisioned Function of Proposed EANPs

Due to the close proximity among AF750 fluorescent molecules, the NIR fluorescence is quenched by self-absorption. When the EANPs reach the tumor, proteolytic enzymes are expected to cleave the polypeptide poly (L-lysine), providing mobility and space between the fluorophores. As a result of this mechanism, cleaved peptide fragments will fluoresce. To demonstrate this concept in vitro, trypsin from porcine pancreas was used as a model protease for activation of the fluorescence of the AF750-labeled EANPs via fluorescence spectroscopy. EANPs were exposed to trypsin in the presence or absence of different concentrations of the protease inhibitor N_{α}-tosyl-L-lysine chloromethyl ketone (TLCK). The biocompatibility and contrast potential of EANPs were determined in cancerous and noncancerous cells with the MTT assay and microscopy in the highly invasive, protease over-expressing MDA-MB-231 cells. The potential of the EANPs as contrast agents for NIR fluorescence imaging and the depth dependence of the minimally resolvable concentration were studied in tissue phantoms.

Results: The diameter of spherical EANPs was in the range of 70–150 nm. Fluorescence activation of the AF750-labeled EANPs by treatment with trypsin resulted in a 15-fold optical signal enhancement within 120 minutes. The health of MDA-MB-231 breast cancer cells was confirmed during and after exposure to EANP suspensions via microscopy and MTT assay, even at high concentrations (1 mg/mL). Enhanced fluorescence was observed on the MDA-MB-231 cells exposed to EANPs (Figure 2). Fluorescence decreased with increasing concentrations of TLCK protease inhibitor. Imaging studies in tissue phantoms confirmed the ability of a simple imaging system based on a laser source and cooled CCD camera to image dilute suspensions of the nanoprobe at depths of up to 4 mm, as well as a 13-fold contrast-to-background ratio for enzymatically activated EANPs compared to un-activated EANPs at the same concentration.

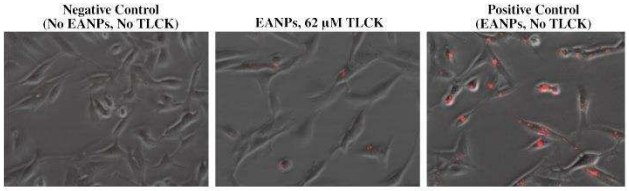

Figure 2. Enzymatic Activation of Fluorescence in Cells

Conclusions: Nanoprecipitation of copolymer blends containing poly (L-lysine) was utilized as a method for preparation of highly functional nanoprobes with potential as contrast agents for selective fluorescence based imaging of tumors. Future studies will investigate the potential of these contrast agents for in vivo imaging in animal models of cancer.

References: (1) (Beliveau R. Can J Physiol Pharmacol 1999; 77:465-480).