Supplementary Data

Indocyanine green loaded hyaluronan-derived nanoparticles for fluorescence-enhanced surgical imaging of pancreatic cancer

Bowen Qi[1], Ayrianne J. Crawford[2], Nicholas E. Wojtynek[1], Megan B. Holmes[1], Joshua J. Souchek[1], Graca Almeida-Porada[3], Quan P. Ly[4], Michael A. Hollingsworth[2,6], Aaron M. Mohs[1,5,6,*]

[1]Department of Pharmaceutical Sciences, [2]Eppley Institute for Research in Cancer and Allied Diseases, [4]Department of Surgery, [5]Department of Biochemistry and Molecular Biology, and the [6]Fred and Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, Nebraska 68198, United States; and the [3]Wake Forest Institute for Regenerative Medicine, Wake Forest University Health Sciences, Winston-Salem, North Carolina 27157, United States

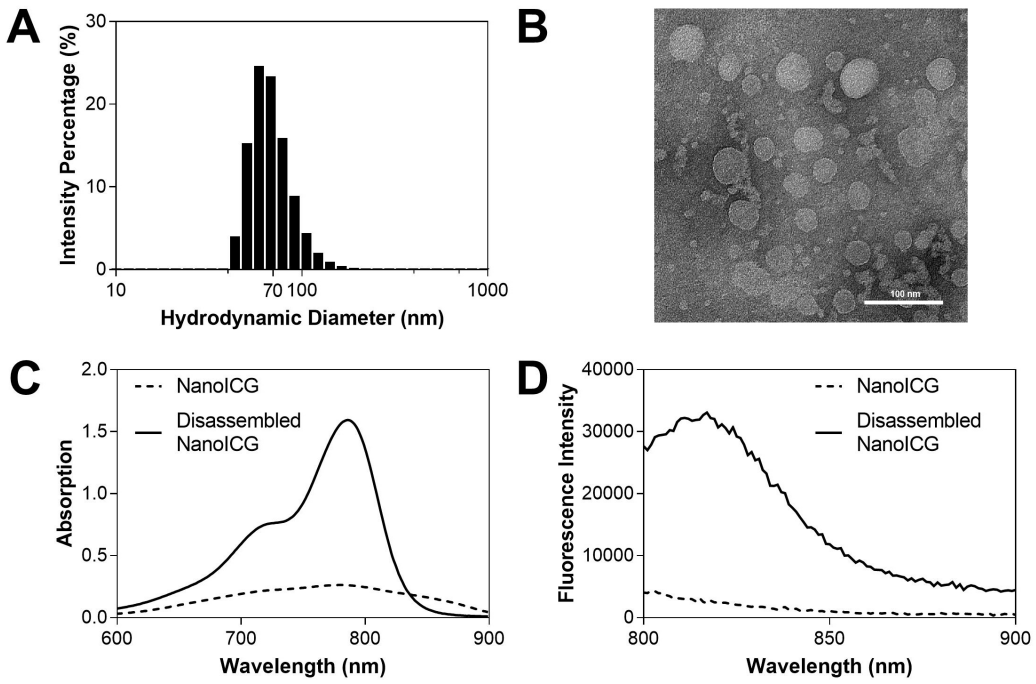
Characterization of NanoICG

The mean hydrodynamic diameter of NanoICG was 71 ± 14 nm with the average polydispersity index of 0.24 ± 0.02 (n = 6). The average zeta-potential of NanoICG was (-21.4 ± 0.3) mV. According to TEM image (Supplementary Figure B), the average diameter of spherically shaped NanoICG is around 40 nm. Absorption spectra for intact NanoICG was indicative of ICG being tightly packed within amphiphilic HA (Supplementary Figure C). In comparison, dissolved ICG and disassembled NanoICG (in 50/50 DMSO/H_2O), has strong NIR absorbance around 785 nm, 6-fold higher than NanoICG at the same wavelength. Correspondingly, fluorescence emission is minimized (5-fold lower) due to packing of ICG and is indicative of ICG entrapment in the self-assembled NP (Supplementary Figure D), which is consistent with previous reports by us and others.[1,2] The quenched fluorescence of NanoICG becomes activated in serum and tissue interaction due to nonspecific interaction with protein hydrophobic domains.[3]

Reference

1. Hill TK, Abdulahad A, Kelkar SS, et al. Indocyanine green-loaded nanoparticles for image-guided tumor surgery. *Bioconjug Chem*. 2015;26(2):294-303. doi:10.1021/bc5005679.
2. Saxena V, Sadoqi M. of Indocyanine Green in Polymeric Nanoparticulate Systems. *Microscope*. 2004;74(1):29-38. doi:10.1016/j.jphotobiol.2004.01.002.
3. Hill T, Kelkar S, Wojtynek N, Souchek J. Near Infrared Fluorescent Nanoparticles Derived from Hyaluronic Acid Improve Tumor Contrast for Image-Guided Surgery. *Theranostics*. 2016;6(13):2314-2328. doi:10.7150/thno.16514.

Supplementary figure:



Supplementary Figure: Physical characterization of NanoICG (A) Percentage intensity of distribution for hydrodynamic diameter of NanoICG; (B) TEM image of NanoICG with negative staining, scale bar represents 100 nm; (C) UV absorbance scanning spectra of intact NanoICG and disassembled NanoICG from 600 nm to 900 nm, as determined by UV-visible spectrophotometer; (D) Fluorescence activation of due to NanoICG disassembly as determined by spectrofluorometer with excitation wavelength of 775 nm and emission wavelength ranges from 790 nm to 900 nm.