Molecular Afterglow Imaging for Cancer Detection, Drug Screening and Imaging Guided Therapy

Optical agents with long-lasting luminescence after removal of light excitation are promising for ultrasensitive *in vivo* imaging due to minimized tissue background. However, such imaging agents are rare and generally limited to inorganic nanoparticles. In this study, we introduce a new generation of purely organic agents based on semiconducting polymer nanoparticles (SPNs) that can store photon energy via chemical defects and gradually emit near-infrared (NIR) afterglow luminescence at 780 nm after light irradiation. The *in vivo* afterglow of SPNs is more than two orders of magnitude brighter than that of concentration-matched inorganic agents, and its signal to background ratio (SBR) is more than two orders of magnitude higher than NIR fluorescence. Such a high sensitivity couple with the ideal biodistribution allow SPNs to rapidly detect xenograft tumors with the size as small as 1 mm^3 and tiny peritoneal metastatic tumors that are almost invisible to naked eye. Moreover, the temperature-correlated afterglow signal of SPNs permits real-time temperature monitoring during photothermal cancer therapy in living mice. The structural versatility of SPNs enables a smart activatable afterglow probe with activated signal towards biothiols, permitting excitation-free real-time imaging of drug-induced hepatotoxicity. In addition, in vitro afterglow sensors can be developed by integrating with the quencher-tagged aptamers, allowing for multiplex detection of cancer exosomes. This study provides the basis for an entirely new class of optical reporters for molecular imaging and in vitro diagnostics at a sensitivity level not achievable with conventional NIR fluorescence imaging.