Enhancing Vascular Imaging using NIR-II Fluorescent Nanoprobes

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Introduction

Fluorescence imaging has emerged as a valuable tool for clinical angiographic and cardiovascular imaging, allowing for visualization and quantification of biological processes. Recent advancements in the development and synthesis of contrast agents have significantly improved the ability to visualize these processes. Among the range of fluorescence imaging windows, near-infrared (NIR) imaging has shown great promise as a non-invasive modality for angiographic and cardiovascular imaging. NIR-II (1000-2000 nm) imaging offers several advantages, including use of non-ionizing radiation and reduced light scattering, enabling deeper tissue penetration and improved image quality.

Indocyanine green dye (ICG), a well-established fluorescent agent used in clinical settings, exhibits emission in the NIR-II wavelengths. However, ICG has limitations concerning targeting specificity and tends to aggregate at high concentrations, compromising its imaging performance. To overcome these challenges, we developed a biocompatible DNA-based platform for conjugation with ICG dyes and targeting moieties. By harnessing the inherent fluorescence and safety of ICG and enhancing its imaging performance through these modifications, the DNA-ICG platform offers a more effective imaging tool for angiographic and cardiovascular studies.

Objective

The primary objective of this pilot study is to evaluate the efficacy of the DNA-ICG platform for contrast-enhanced NIR-II fluorescence imaging in a mouse model. Specifically, we aim to investigate and characterize the biodistribution pattern of the contrast agent and assess the level of contrast enhancement achieved over a 24-hour period.

Methods

The nude (NU/J) mouse model was selected for this study and subjected to anesthesia using isoflurane. A total of four mice were included in the experimental groups: one mouse received free ICG dye, and three mice were treated with different variants of the DNA-ICG nanoparticle platform. Each mouse received a tail vein injection (100 µL) of the respective contrast agent, each corresponding to 200 µM concentration of ICG.

NIR-II fluorescent images were captured using the IR Vivo imaging system developed by Photon etc. (Montréal, Canada). The imaging system utilized an 808 nm laser to excite the DNA-ICG probe, and the resulting fluorescence emission was recorded at NIR-II wavelengths (>1250 nm). Multiple imaging sessions were conducted at predetermined time intervals, including 1 hour, 3 hours, 7 hours, and 24 hours following the administration of the contrast agent.

Results

The corrected total fluorescence of anatomical structures was compared among the four mouse subjects in this study. Analysis of the time series imaging data demonstrated the distribution pattern of the contrast agent within the circulatory system. Initially, the contrast agent traveled throughout the vascular network before accumulating in the heart and liver. Eventually, the agent was excreted through the urinary and gastrointestinal systems.

Throughout the experiment, various organs were observed, including the heart, liver, spleen, caecum, and intestines. Notably, vascular structures in the tail, spinal column, and head remained visible for hours after the administration of the contrast agent.

Comparing the different experimental groups, mice treated with DNA-ICG probe variants exhibited higher brightness and longer circulation time in comparison to those treated with free ICG dye. This observation indicates the improved imaging performance and prolonged retention of the NIR-II fluorescent nanoprobes provided by the DNA-ICG platform.

Conclusion

Our pilot study demonstrates the effectiveness of the DNA-ICG platform for contrast-enhanced NIR-II imaging in a mouse model. The DNA-ICG nanoparticle variants of the contrast agent exhibited prolonged circulation, allowing for enhanced visualization of various anatomical structures. The biodistribution analysis revealed the accumulation of the contrast agent in vital organs such as the heart and liver, followed by excretion.

The DNA-ICG platform holds promise as an effective imaging tool for angiographic and cardiovascular studies. By leveraging the inherent fluorescence and safety of indocyanine green (ICG) and overcoming its limitations through conjugation with DNA nanoparticles and biocompatible targeting moieties, the DNA-ICG platform offers improved imaging performance and enhanced contrast enhancement.

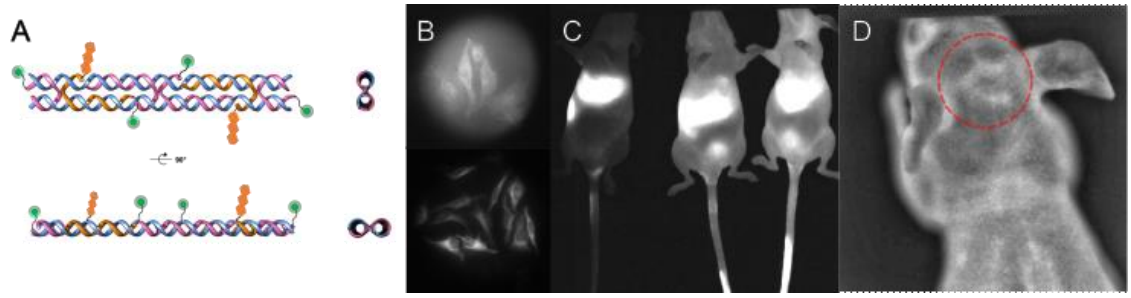

Figure 1: The DNA-ICG platform deployed in living systems. (**A**) Schematic showing the spatial configuration of dyes (green) and targeting moieties (orange) to the DNA scaffold, (**B**) Top: HeLa cells stained with the DNA-ICG platform lacking targeting moiety, Bottom: HeLa cells stained with DNA-ICG conjugated with targeting moiety, (**C**) Left: mouse injected with free ICG dye, Center and Right: mice injected with DNA-ICG variants, (**D**) Mouse treated with DNA-ICG after three hours; the circle of Willis (circled) visible beneath the skull.