Nano Contrast Agents in Photoacoustic Imaging: In Vitro and Ex Vivo Insights

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Introduction

DNA nanosensors are structures designed to monitor chemical processes within the body with high precision, enabling real-time tracking of chemical reactions in the body at the nanoscale level. DNA analogs, such as phosphorothioate DNA (PS-DNA) are also used due to features such as enhanced stability, specificity, and sensitivity [1].

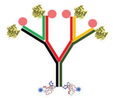


Figure 1. A fluorescent nanosensor with a DNA scaffold developed to track the release of acetylcholine [2].

Photoacoustic imaging (PAI) is a non-invasive, emerging imaging modality with applications in biomedical research [3]. Existing PAI platforms enable imaging at the molecular level and high-resolution visualization of various biological systems like the nervous system [3].

Contrast agents like indocyanine green or ICG improve the visualization of structures or chemical processes [4] acquired through imaging modalities such as PAI.

Photoacoustic imaging (PAI) is a non-invasive, fast-growing imaging modality with applications in biomedical and clinical research [5]. Existing PAI platforms with high sensitivity enable imaging at the molecular level and high-resolution visualization of various biological systems like the nervous system [5].

Intrathecal injections through the cisterna magna is a minimally invasive injection method, enabling cerebrospinal fluid (CSF)-facilitated delivery and diffusion of substances such as contrast agents to the brain via the subarachnoid space.

Figure 2. The rat cisterna magna is located at the base of the skull just under the occipital crest.

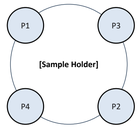
Objective

The objective is to validate the use of PS-DNA-ICG in brain imaging to enhance contrast. This will be done by comparing the diffusion of ICG-functionalized PS-DNA (PS-DNA-ICG) through the brain with an intrathecal injection method to the diffusion of unmodified ICG.

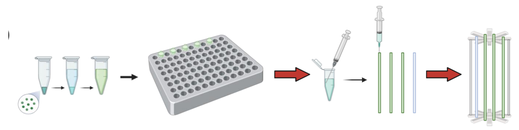
This lays the groundwork for developing ICG-conjugated DNA nanosensors for photoacoustic imaging.

Methods

Part 1: In Vivo ICG Study



Sample Holder	P1	P2	P3	P4
Scan 1 - ICG Filter	Control (H_2O)	200 µM	100 µM	50 µM
Scan 2 - ICG Filter	Control (H_2O)	25 µM	12.5 µM	6.25 µM



Part 2: Ex Vivo ICG and PS-DNA-ICG Studies

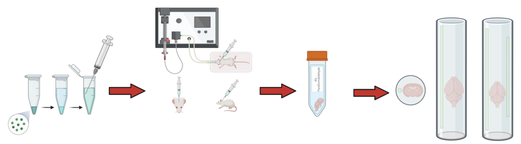
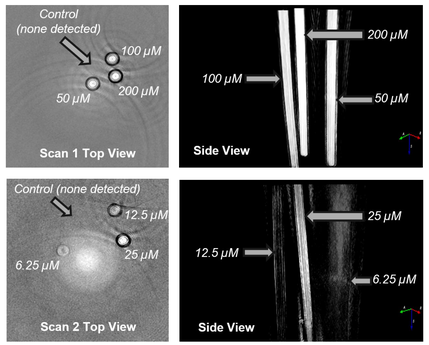


Image Analysis

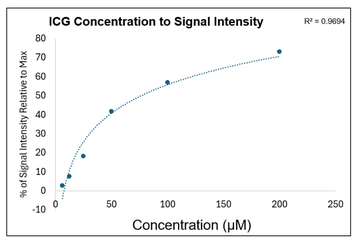
- Photoacoustic signal acquisition and processing
- Image reconstruction
- Segmentation for signal intensity quantification
- Maximum intensity projection for 3D image visualization

Results

Part 1: In Vivo ICG Study

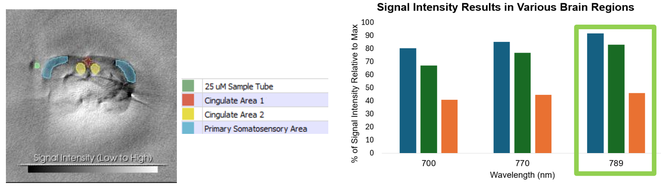


Photoacoustic reconstruction and 3D image

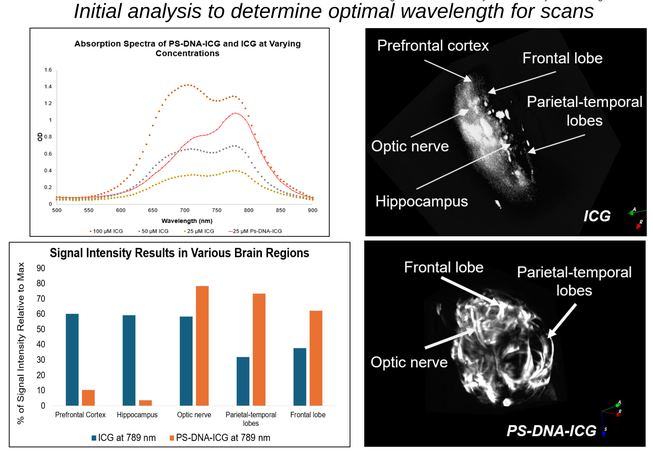


Quantification of signal intensity in vitro

Part 2: Ex Vivo ICG and PS-DNA-ICG Studies



Initial analysis to determine optimal wavelength for scans



Comparison of diffusion between ICG and PS-DNA-ICG ex vivo

Conclusion & Future Work

Diffusion of ICG in the brain extended to deeper tissue layers, while PS-DNA-ICG diffusion predominantly reached the brain's outer regions and borders, particularly enhancing the contrast of structures such as the optic nerve and the boundaries of the frontal and parietal-temporal lobes.

Ideal Contrast Agent For Improving Structure Visibility in the Brain

- Visualization of deeper structures: ICG
- Visualization of fissures, border, etc.: PS-DNA-ICG

Possibilities that could explain difference between diffusion/signal

- ICG, being a smaller molecule, penetrated deeper into the tissue but with reduced contrast due to its widespread distribution
- Differences in size between ICG and PS-DNA-ICG may influence their clearance from the brain and affected their diffusion patterns.
- The attachment of PS-DNA to ICG possibly enhanced the stability of the molecule, resulting in improved retention and reduced degradation within brain tissue.

Future Work

Conducting real-time in vivo studies and clearance studies in vivo to track contrast agent distribution and elimination over time will be crucial steps for the continued development of ICG-based DNA contrast agents in photoacoustic imaging that would set a solid foundation for the development of ICG-conjugated DNA nanosensors.

References

[1] R. Saran, Z. Huang, and J. Liu, “Phosphorothioate nucleic acids for probing metal binding, biosensing and nanotechnology,” Coordination Chemistry Reviews, vol. 428, p. 213624, Feb. 2021, doi: 10.1016/j.ccr.2020.213624.

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Acknowledgments

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