Repeated Phase-Change Induced Ultrasound and Photoacoustic Contrast Enhancement from Perfluorohexane Nanodroplets Trafficking in Tumor-Draining Lymphatics Over 24 Hours

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Background, Motivation, and Objective

Tumor-draining lymphatics play a critical role in cancer: the extent of lymphatic metastasis is highly prognostic, and tumor-draining lymph nodes are a key control point of the adaptive immune response modulated during immunotherapy. Perfluorocarbon nanodroplets (PFCnDs) are sub-micrometer phase-change ultrasound and photoacoustic (US/PA) imaging contrast agents that should permit size-dependent interstitial flow into lymphatic vessels. Successful PFCnD trafficking through tumor-draining lymphatics would enable numerous US/PA-guided diagnostic and therapeutic cancer applications.

Statement of Contribution/Methods

Regrettably, standard PFCnDs (≤5 carbon core) offer only a single phase-change event *in vivo*. The persistent microbubbles produced are too large to traffic through lymphatics, and a single imaging time point cannot account for variable lymphatic drainage. Instead, we leverage the enhanced stability, repeatable phase-change potential, and dual-contrast of optically-triggered perfluorohexane nanodroplets (PFHnDs; 6 carbon core) to image a single injection longitudinally while maintaining the favorable size-dependent trafficking of PFCnDs. PFHnDs were synthesized with a PFH core, 8:1:1 molar ratio of DSPE-mPEG2k:DSPC:Cy5 PE, and near-infrared IR-1048 dye for optical triggering. A subcutaneous peritumoral injection of PFHnDs (Z-Avg: 207 nm, PDI: 0.124) was performed in a 4T1 syngeneic model of metastatic breast cancer. US/PA imaging was executed using a CL15-7 transducer and Vantage 256 system integrated with a pulsed 1064-nm Nd:YAG laser. Resultant post-laser differential 8 kHz plane-wave pulse-inverted US data were fit to a decaying exponential function, indicative of unique recondensation from optically triggered PFHnDs, to create a mask for corresponding PA data.

Results/Discussion

Masked PA data highlight regions containing PFHnDs with high specificity hours after injection. Repeated US/PA imaging was performed on a single extravascular bolus *in vivo* over 24 hours, an impossible feat with standard PFCnDs. PFHnDs drained from the caudal primary tumor through afferent lymphatics to the ipsilateral subiliac node (Fig. 1B). By 24 hours, more PFHnDs reached the node, with some even exiting through efferent lymphatics (Fig. 1C). Ongoing studies seek to optimize delivery and further characterize PFHnD lymphatic kinetics.

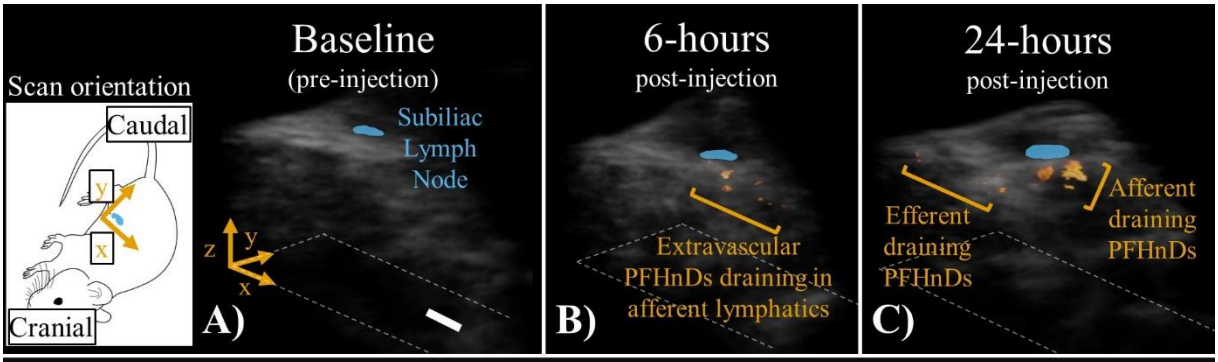

Fig. 1. Volumetric renderings of PFHnD-enhanced US/PA images at baseline (**A**) as well as at 6-hours (**B**) and 24-hours (**C**) post-injection within the same mouse. Mouse contains a 4T1 primary tumor in the ipsilateral fourth mammary fat pad. Regions of coincident differential pulse-inversion US and PA contrast *(red-orange/yellow)* highlight PFHnDs trafficking through regional lymphatics near the tumor-draining subiliac lymph node *(light blue)*. PFHnDs can be seen flowing cranially from afferent vessels towards the node (**B**) and even through efferent vessels by 24-hours post injection (**C**). Scale bar in lower-right region of (**A**) is approximately 2.5 mm.