Feasibility of a novel image guided injection tool for non-surgical implantation of orthotopic tumors

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1 Background

- Orthotopic tumor models are becoming more widely adopted in preclinical cancer research as these models more faithfully recapitulate the original tumor microenviroment and exhibit greater clinic translatability.
- Traditionally, orthotopic tumors are instantiated either via open surgery or blind free-hand injection. The former requires specific surgical skill, is time consuming, low-throughput, and highly invasive, while the latter may be prone to error, technician variability, and unintended animal harm.
- Ultrasound-guided percutaneous injections offer a minimally invasive approach to target an organ of interest with high accuracy and repeatability.

GOAL: Evaluate the feasibility of a newly-designed image guided injection platform by measuring hit-rate in the prostate, pancreas, liver, and left ventricle of the heart.

2 Imaging Methods

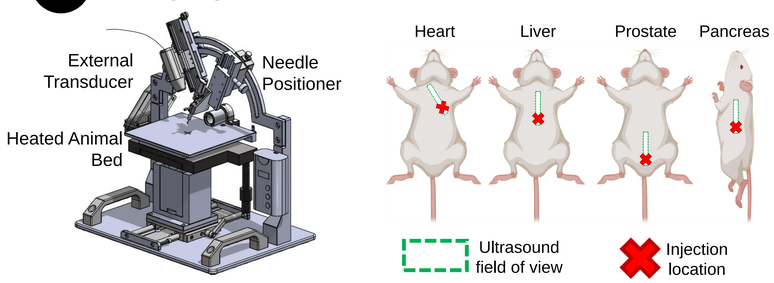

Figure 1: *Schematic of the image-guided injection system (left) and positioning of the animal for different organ injections (right). Transducer was connected to Vega imaging engine (Revvity), and ultrasound stream visualized in SonoEQ software (Revvity). In the positioning diagrams, the green box indicates the approximate location of the ultrasound transducer, and the red 'X' indicates the entry point of the needle.*

3 Study Design

Male athymic nude mice (n=20) were split evenly into two groups:
- Group 1 received intracardiac injections (left ventricle).
- Group 2 mice received injections in the pancreas, prostate, and liver.

Injection solutions were prepared as follows:
- Group 1 – 250 µL IVISBrite HepG2 Red F-luc cells (Revvity, 2.5e5 cells/mL in PBS), 50 µL luciferin (15 mg/mL), 30 µL VesselVue ultrasound contrast agent (Revvity)
- Group 2 – 250 µL IVISBrite HepG2 Red F-luc cells (2.5e5 cells/mL in 50% Matrigel, 50% PBS), 50 µL luciferin (15 mg/mL), 5 µL VesselVue ultrasound contrast agent

For each injection, animals were positioned on the heated platform and translated such that the target site was in the line-of-sight of the ultrasound beam and the needle (Figure 1). Using the ultrasound livestream as a visual aid, the needle was guided to each target organ.
- Group 1 – 100 µL injection volume
- Group 2 – 20 µL injection volume

Hit-rate verification:
- Group 1 – In addition to the enhancement on the ultrasound stream, the animals were euthanized and the injected organs dissected, placed in a six-well plate, and imaged *ex vivo* using an IVIS Kinetic (Revvity).
- Group 2 – The mice were imaged *in vivo* using an IVIS Kinetic to verify that the bioluminescent signal was detected throughout the mouse.

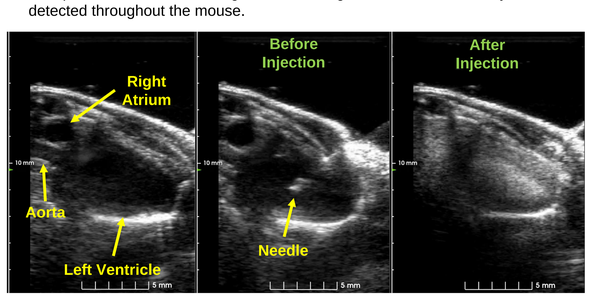

Figure 2: *Representative ultrasound images pre- and post-injection for Group 1 (cardiac).* Left: *image of the left ventricle before the needle has been inserted.* Center: *image of the needle at the injection site.* Right: *image acquired post-injection show clear enhancement from the microbubbles, confirming that cells were delivered into circulation.*

4 Results: Representative Ultrasound Images

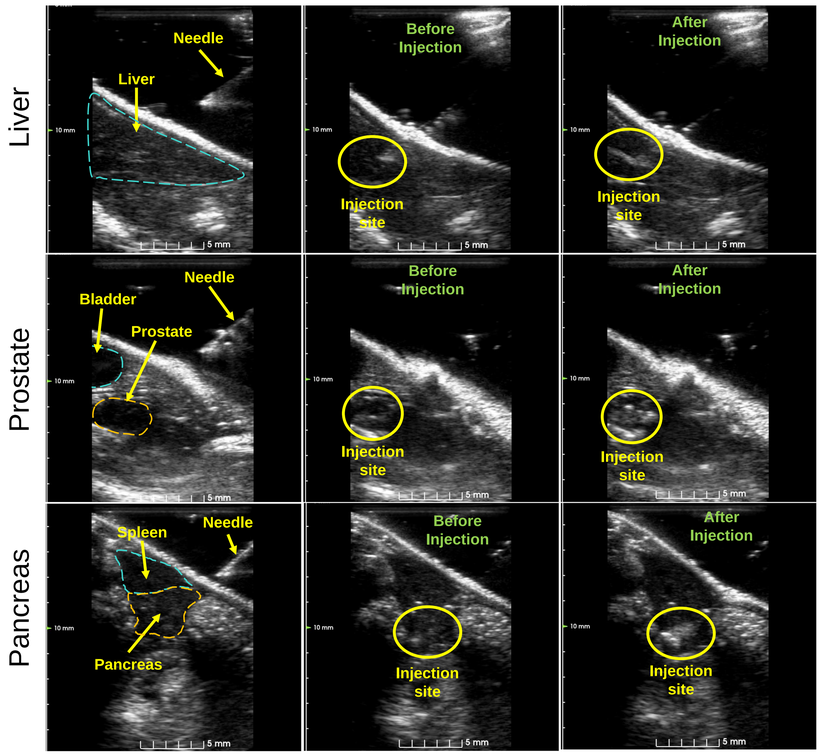

Figure 3: *Representative ultrasound images pre- and post-injection.* Left column: *images of the target organs before the needle has been inserted into the tissue.* Center column: *images of the needle at the injection site.* Right column: *images acquired post-injection show clear enhancement from the microbubbles, confirming that cells were delivered to the target organs.*

5 Results: Intra-Cardiac Injections

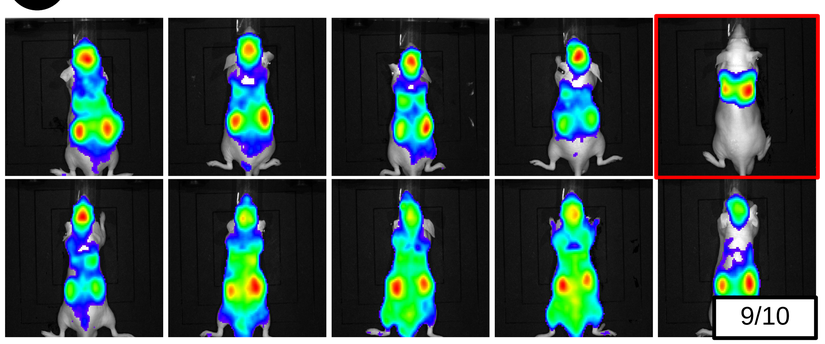

Figure 4: *IVIS images collected post-injection for Group 1. Nine out of ten animals show full-body signal with hot spots at the brain and kidneys. One animal (top row, right) showed signal only in the lungs and was considered a failure. It should be noted that bright-red blood was seen in the hub of the needle after this injection, and microbubbles were detected in the liver and kidneys using ultrasound imaging. This suggests that the injection was indeed in the left ventricle, but it remains unclear why the cells were only deposited in the lungs. One hypothesis is that the cells were clumping, which may affect their distribution or the right ventricle was pierced causing dissemination directly to the lung.*

6 Results: Liver, Prostate, & Pancreas Injections

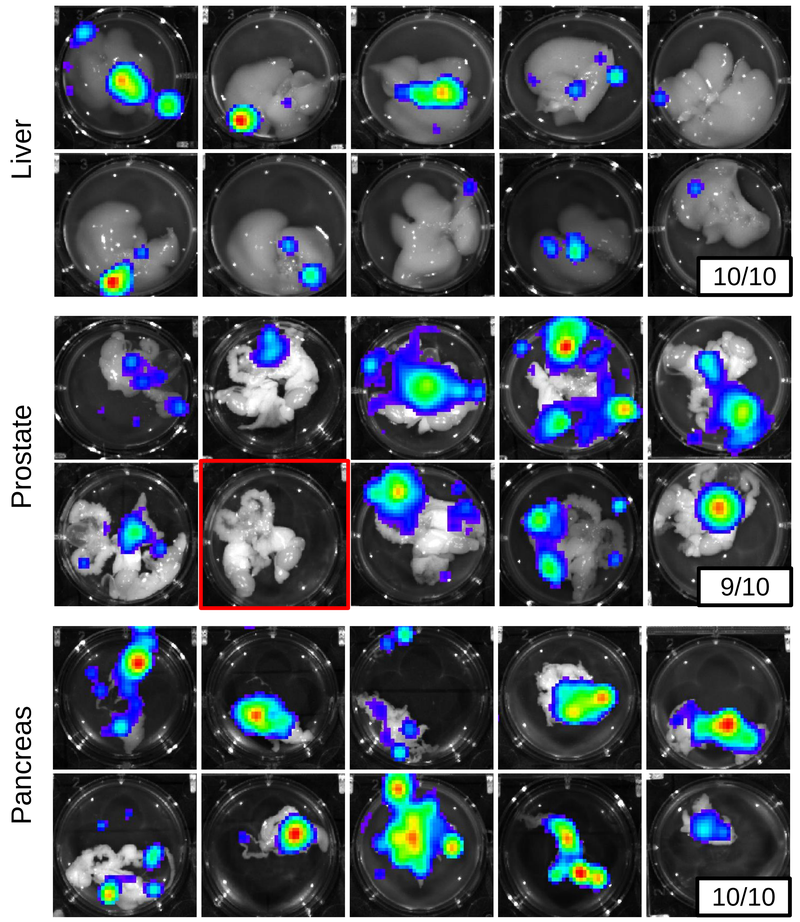

Figure 5: *Ex vivo IVIS images collected post-injection for Group 2 (all displayed on logarithmic scale with 3x3 smoothing). Clear BLI signal was seen in 10/10 liver images, 9/10 prostate images, and 10/10 pancreas images. In some cases, the liver images were relatively dim, and repeat images were collected in various orientations to ensure that the signals were genuine. It was noted during these injections that the density of the liver tissue sometimes resulted in cells leaking along the needle path rather than remaining at the site of injection. Additionally, the liver is a highly vascular organ and the cells could be seeing going into a large blood vessel and being carried away from the injection site. Both of these factors may have contributed to the brightness of these data.*

7 Conclusions

- Device achieved a 95% hit rate across 4 different anatomical targets
  - Time to perform 3 injections in the same mouse = 36 minutes
  - Time to perform a cardiac injection = 6 min
- Future studies will explore design improvements to further increase speed of organ localization and injection, and test additional injection site accuracy (including: kidney, spleen, bladder, ovary, thymus, and others).

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