Progress Report:

Low Intensity Pulsed Ultrasound (LIPUS) for the Treatment of Spinal Disc Degeneration

The objective of our research project is to develop a LIPUS exposimetry system specific to targeting degenerated disc in a rat tail model, and to apply and quantify the effects of LIPUS on disc healing response *in vivo*. Additionally, we plan MR experiments and anatomic simulations to define limitations and applicability of this approach.

With respect to Aims 1-2, ultrasound exposimetry systems have been devised and fabricated specific to delivering LIPUS to damaged caudal discs in the rat tail (Fig. 1). The three configurations developed consisted of 2.5 cm diameter spherically focused and planar PZT4 transducers (1.0 MHz, f=1; 1.6 MHz, f=3.8; 1.0 MHz – planar), integrated within a water-filled 3D printed plastic housing with a mylar window w/ cross-hairs, designed as an acoustic standoff to place the focus within the targeted rat tail disc. Each applicators/apparatus were evaluated with comparative beam plot measurements with/without insertion of sectioned rat tails *ex vivo (Fig*. 2ab). The column or cone height was empirically adjusted for each transducer to optimize beam uniformity at the point of placement of the disc to be exposed. We have also designed and 3D printed specific holders and platforms to position the animals. Intensity measurements and radiation force observations with and without the tail sections (Fig. 1c, 2c) demonstrate the focused and planar systems can deliver LIPUS (at 40-50% of mylar surface intensity) through the skin and connective tissue, to the center of the narrow ~1.5 mm x 5 mm rat tail disc, while reducing exposure to significant portions of the adjacent bone and negligible temperature elevation.

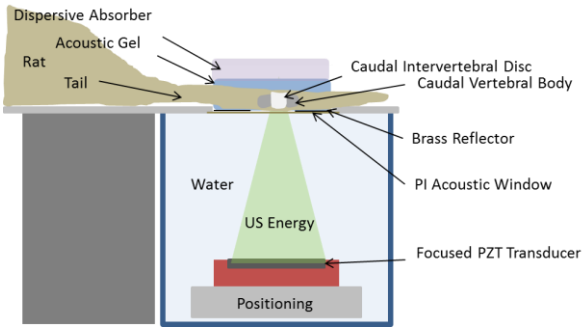
We are now in the process of performing the in vivo rat tail studies. These *In vivo* studies using the disc damage model are performed following standard procedures approved by UCSF IACUC, with stab incisions applied within 16 Sprague-Dawley rats to generate damage/inflammation in tail discs. Five daily p-FUS exposures (I_{SPTA}120 mW cm-2) are to be applied to stab discs and compared to stab only and normal controls. As described in the proposal, histology and microarray gene analysis are to be performed at 5 days and 28 days after injury, also assessing changes to cartilage, fibrous tissue, and bone.

In addition, we have coordinated a clinical study under Dr Viola Rieke to perform MR scans of human volunteers to investigate placement and acoustic windows for using the Insightec table array and the conformal bone array. Only the treatment planning software will be used for these studies, without sonication. We have established the logistics and workflow to work with the Willed Body Program at UCSF to get cadavers for some preliminary studies. Once the sonication strategies are worked out in volunteer(s), we will apply this to a cadaver and use a short sonication and heating as a surrogate to demonstrate that delivering energy to the disc is possible.

Ancillary studies, although not specified in the original proposal, have also been initiated to allow us to gather preliminary data of the ultrasound exposimetry on cell culture of human intervertebral discs. The goal so to provide us with additional preliminary data to help support more extensive NIH funding for this project. Figure 3 shows the custom multi-layer absorber we are designing to remove standing waves and heating from the cell culture plates. The cell culture plates can be placed directly on top of the ultrasound devices as currently designed.

Publications and Presentations: An abstract has been submitted, and presentation of our results at the upcoming Focused Ultrasound meeting is planned. Further, an abstract is being submitted to the SPIE Bios meeting in February 2017, which will also be an extended conference proceeding.

Pending NIH Grant: We are currently submitting an R21 to the NIH to support further in vivo and include in vitro studies.



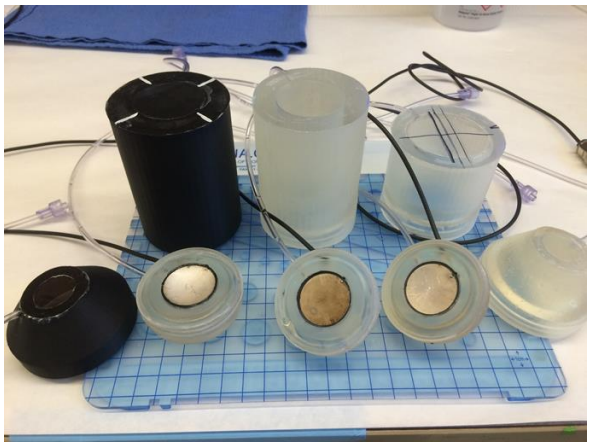


Fig. 1. (a) Conceptual plan and schema of the p-FUS exposimetry system, designed for LIPUS delivery at calibrated intensity exposures to damaged rat-tail IVDs *in vivo*; (b) selection of focused and planar ultrasound exposimetry systems designed for LIPUS to the rat tail disc, with transducer assembly and conical or cylindrical stand-offs to ensure known uniform field; (c) spherically focused 1.65 MHz, 2.5 cm OD, f=3.8 with integrated housing and mylar membrane to position focus at rat tail placement; (d) sectioned rat tail with sonication energy penetrating the disc, visualized as transmitted radiation force on water surface.

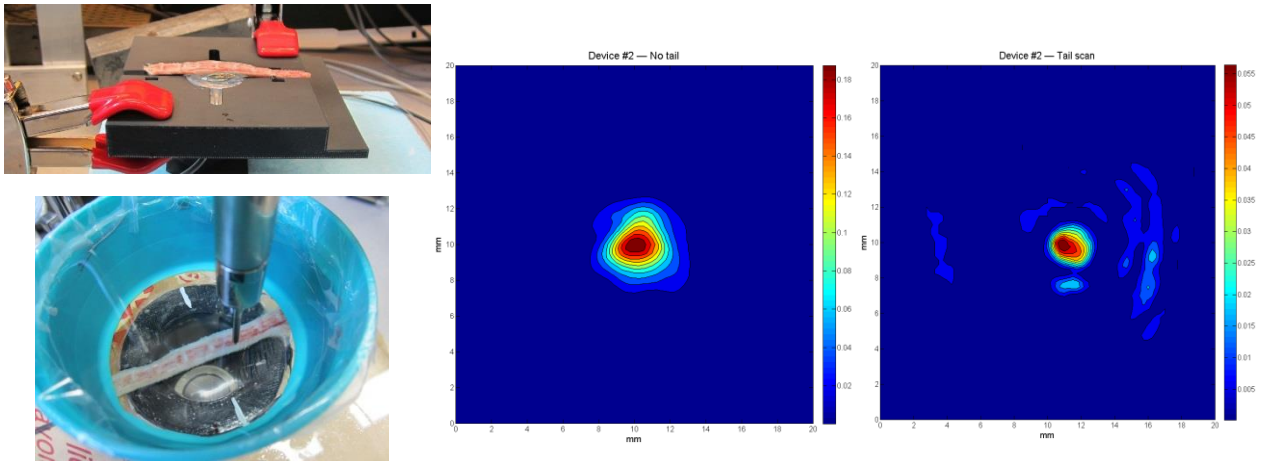


Fig. 2. (a) Example positioning platform and p-FUS system for disc exposure with sectioned rat tail; (b) hydrophone scanning setup without absorbers; and beam plots of (c) no tail and (d) tail demonstrating penetration through the IVD as targeted with a focused transducer.

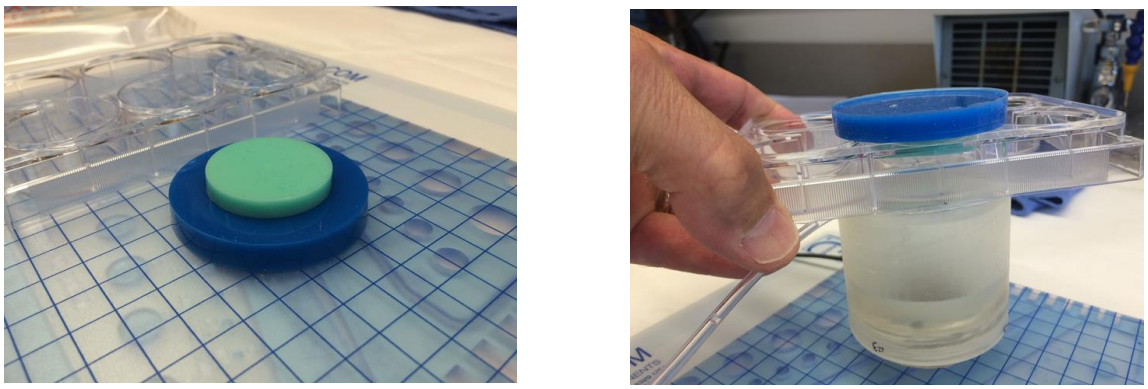


Fig. 3. Exploratory development of cell culture system designed for studying LIPUS with cultured human disc cells. (a) a multilayer acoustic absorber that can be sterilized and placed above the cell media will be used to ensure negligible standing waves and heating during LIPUS; (b) the cell culture plates can be placed on our ultrasound applicator within an incubator as shown. This represents ancillary studies to gather preliminary data to help support NIH funding.