# The Use of High Intensity Focused Ultrasound for Targeted Tissue Decellularization in Vivo

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**Statement of Purpose:** One of the main challenges in biofabrication of tissues and organs is the development of biomimetic scaffolds that replicate the complex architecture of native tissue, including 3-D vasculature. Tissue decellularization can be used for obtaining such scaffolds, however, current techniques require the use of chemical and biological agents, often in combination with physical force, which can result in chemical alteration to the matrix. Moreover, such decellularization can only be performed *ex vivo* and the engineered tissue or organ would require cultuvation *in vitro* and further surgical transplantation. Recently developed boiling histotripsy (BH) technology uses millisecond-long pulses of focused ultrasound waves with high amplitude shock fronts to selectively fractionate tissue into subcellular debris without thermal effect [1–3]. This technology has shown great potential to decellularize unwanted tissue *in vivo* inside the body, through the skin, while leaving collageneous structures such as vessels at least grossly intact [4, 5]. Here we report the results of pilot acute studies on transcutaneous volumetric BH ablation of porcine liver and kidney tissues demonstrating potential capabilities of such technology for noninvasive tissue decellularization toward organ biofabrication *in vivo*.

**Methods:** BH treatments were performed on anesthetized pigs (37–40 kg, n=4) placed on the surgical table in either lateral (for kidney treatment) or supine (for liver treatment) position. A 1.5 MHz spherically focused transducer (Figure 1) was integrated with an ultrasound imaging probe for treatment guidance; sonication protocol included repetitive focused ultrasound pulses of 1–10 ms durations, 1–2% duty factor, 5–30 pulses per focal point representing an increase in the treatment dose, and the focal points spaced 1–1.5 mm apart in a rectangular grid with 5–15 mm linear dimensions. BH lesions or volumes of tissue decellularization were generated in liver and in both kidneys. Necropsy was performed after BH treatments and the samples of the treated tissues were collected for gross and histologic assessment.

**Results:** Volumetric BH lesions were successfully produced in both porcine kidneys and livers. At necropsy no gross evidence of collateral damage was observed within the beam path and no animals had gross hematuria thus demonstrating the safety of the BH exposures for the intervening tissues. In livers, complete homogenization of hepatocytes was obtained in the central region of the lesions while at the lesion periphery the treatment effect was less demarcated most probable because of the respiration-induced motion of the target site. Contrary to the mechanical homogenization of hepatocytes, the collagen-rich intralobular septa remained mostly intact with evidence of intact vasculature (Figures 2 and 3).

Figure 1. Focused ultrasound transducer of 1.5 MHz frequency used in the study.

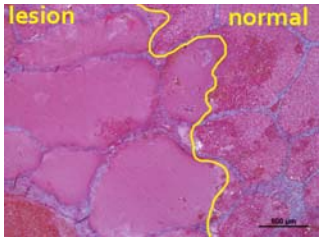


Figure 2. Histology of lesion in liver showing intact intralobular septa surrounding homogenized hepatocytes.

In kidneys, volumetric lesions containing cortex, medulla, and renal sinus tissues were created. In the cortex, all doses of BH exposures resulted in completely fractionated lesions sharply demarcated from histologically normal untreated tissue (Figure 3). In the medulla, higher dose exposures were required before damage to the vessels was observed, as confirmed by the appearance of blood within the tubules. Within the wall of the collecting system, focal petechial hemorrhage was visualized only at the higher dose exposures without disruption of the wall.

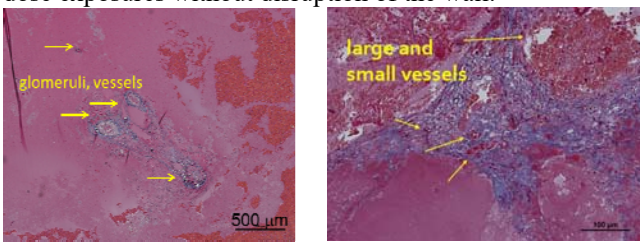


Figure 3. Histology of kidney (left), showing vessels inside the lesion, and liver (right) showing large and small vessels within the intralobular septa.

**Conclusions:** This study showed the feasibility of using the BH method to transcutaneously induce mechanical homogenization of kidney and liver tissue *in vivo* in a large animal model. Different susceptibilities of different tissue types to BH treatments were observed showing that at certain treatment conditions decellularized tissue volumes can be obtained while sparring major collageneous connective structures inside the volume. With further exploration of the BH pulsing parameters, this treatment modality could be optimized for rapid non-invasive organ decellularization to produce biomimetic scaffolds ready for recellularization with autologus cells *in vivo*. Work supported by NIH EB007643, RFBR 16-02-00653, and K01 EB 015745.

**References:**

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