

Variables Controlling Ultrasound Contrast Generation in the Urinary Bladder: A Urinary Reflux Diagnosis

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Abstract—An ultrasound system has been developed to generate contrast microbubbles *in vivo*. Possible uses include diagnosis of reflux in the urinary tract. *In vivo* and *in vitro* experiments were designed to elucidate the microbubble generation process using 1.8 MHz acoustic bursts at 125 ms. In *in vivo* experiments on rabbits, the peak rarefactional pressure threshold for contrast generation, as visualized with a diagnostic ultrasound system, decreased with increasing pCO₂. For the *in vitro* aqueous studies the threshold decreased almost a factor of two for increasing particle concentration (0.2 μm dia. polystyrene) from 10⁸ to 10¹⁰ particles/cc. The thresholds were at least twice as high for more saturated fluids, and CO₂ samples had considerably lower thresholds than respective under-saturations in air. At a fixed pressure amplitude, echogenicity tended to increase with both increasing particle and gas content; this was more pronounced for samples containing CO₂. Even in a restricted-nuclei environment such as the human urinary bladder, generation of vaporous cavitation should be possible; however subsequently, abundant gas is needed to grow vaporous bubbles to persistent and imageable sizes, to assist in the diagnosis of urinary reflux.

I. INTRODUCTION

Urinary reflux, or vesicoureteral reflux, is a condition where urine from the bladder flows improperly towards the kidney due to a dysfunctional valve at the junction of the ureter with the bladder, the ureterovesicular junction (UVJ). This is a common urinary tract abnormality in children. If a bacterial infection exists in the bladder, then urinary reflux can lead to kidney infection. Furthermore, because of the “leak” in the bladder, the kidney can be exposed to high pressures, particularly at the time of micturation. Ultimately continued kidney infection or hypertension can result in acute renal dysfunction and/or failure.

The current standard diagnosis method for urinary reflux is vesicoureteral urogram (VCUG). In this procedure the patient is catheterized via the urethra in order to devoid the bladder of urine and to inject X-ray contrast to fill the bladder. Since often the patient being evaluated is a young child, this procedure can be particularly uncomfortable and traumatic.

Previous experimenters have used ultrasound for diagnosing reflux in human studies. Matsumoto, *et al.* [1] evaluated reflux using color flow imaging, while Kessler and Altman [2] and Hanbury, *et al.* [3] used agitated aqueous solutions to generate contrast bubbles that were injected into the bladder. Atala, *et al.* injected Albunex[®] into porcine bladder, by catheterization, and visualized the microbubbles flowing towards the kidney in a surgically induced reflux. [4] Ivey, *et al.* observed similar results in lower grade reflux induced in a canine model where color flow Doppler visualized flow in the ureters. [5] However, the color flow method was not specific enough to distinguish between different grades of reflux, and the procedures with contrast microbubbles still required catheterization of the patient.

The proposed high pressure ultrasound diagnosis method would minimize or eliminate the need for catheterization and exposure to ionizing radiation. This system will generate ultrasound contrast bubbles transcutaneously, and will keep the contrast persistent in order to make the diagnosis. The *in vivo* rabbit and *in vitro* aqueous experiments, described below, will identify the fluid properties that can be manipulated to improve contrast generation and aid in the eventual transfer of this non-invasive urinary reflux diagnosis technique to humans.

II. METHODS

A 1.8 MHz single-element, spherically focused, transducer (6.35 cm diameter aperture, f1, Etalon Inc., Lebanon, IN) was used to generate acoustic bursts. High amplitude bursts of 125 ms in length from the power transducer were generated using a 300 Watt amplifier (ENI A-300, ENI Inc., Rochester, NY). This transducer was then coupled to a 7.0 MHz endocavitary diagnostic ultrasound probe (Diasonics VST, Diasonics Ultrasound, Milpitas, CA). The focal position of the transducer was identified in the probe image. A video recording was made of scanning performed with the probe.

In Vivo Rabbit Experiments:

Eight rabbits (New Zealand White) were anesthetized with (Ketamine and Rompun). The abdomen of the rabbit was shaved and depilatory creme applied to ensure a clean

and smooth coupling surface. With degassed ultrasound coupling gel coating the abdomen, a stand-off tank was placed over the rabbit's abdominal area (see Figure 1). The tank was a PVC pipe segment of 30 cm diameter. Part of the walls of the pipe were carved to accommodate the shape of the animal, and a plastic bag lined the inside of the tank. The air bubbles between the plastic bag and the coupling gel were squeezed out so that no bubbles were in the treatment or imaging path. Then the tank was filled with water that was recirculated from a reservoir heated at 37°C and continually degassed.

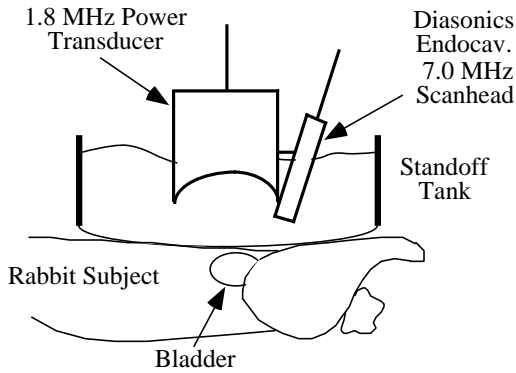


Figure 1. *In vivo* experimental apparatus.

The transducer assembly, described above, was placed in the tank so that the focus was 8 mm from the back wall of the bladder. For each rabbit, the acoustic burst amplitude delivered into the bladder was ramped from low to high. At each amplitude, two 125 ms bursts were applied with 10 seconds delay between consecutive bursts. If no bubbles were visualized, then the amplitude was stepped up. This continued until the threshold for visible contrast generation was achieved.

After completing the contrast generation experiment, urine was drawn from the bladder via abdominal puncture with a syringe and measured using a blood-gas analyzer (ABL300, Radiometer America Inc., Westlake, OH). The rabbit was then euthanized and the bladder was excised. The tissue was stored in formaldehyde for future histological evaluation. All animal experiments performed for this research adhere to the national guidelines for the ethical treatment of animals, in keeping with NIH requirements.

In Vitro Aqueous Cell-Free Media Experiments:

For the aqueous *in vitro* experiments, the same transducer assembly, as described above, was used. A rubber balloon was used as the exposure vessel for these studies. The balloon was an appropriate size such that 50 cc of fluid resulted in the distention of the balloon with little or no over-pressure; this was to simulate a full *in vivo* rabbit bladder. The exposure vessel was immobilized

in a Plexiglas holder during insonification (Figure 2) and placed in a tank of degassed water. The holder was lined with sound absorbing rubber (SOAB, BF Goodrich, Jacksonville, FL) and SOAB was placed beneath the balloon in order to reduce standing wave conditions.

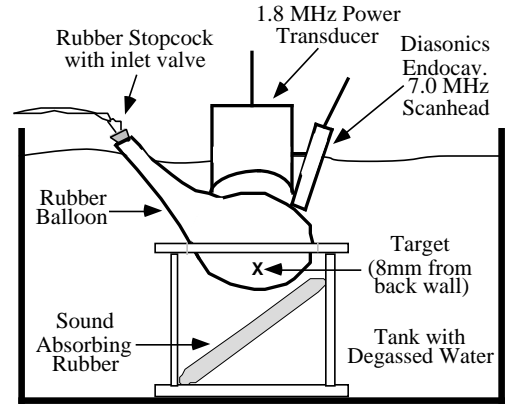


Figure 2. *In vitro* experimental apparatus.

The fluid parameters for testing the dependence of contrast generation on varying gas saturation and particle concentration are shown in Table 1. The shaded boxes indicate the fluid conditions tested. The three groups of experiments performed are signified by a shaded column or row: (1) air-saturated fluids with varying particle concentration, (2) samples with particle concentration of $10^{10}/cc$ at varying gas saturations, and (3) particle free samples at varying gas saturations.

		Particle Conditions			
		filtered DI water	tap water	10^8 particles/cc	10^{10} particles/cc
Gas Conditions	air saturated				
	air under-saturated				
	CO ₂ saturated				
	CO ₂ under-saturated				

Table 1. Fluid properties tested.

All tested fluids were prepared with DI water that was filtered to 0.2 μm and allowed to equilibrate to air saturation for at least 24 hours. To obtain different particle conditions, concentrated solutions of 0.20 μm diameter polystyrene microspheres (Cat. no. 5020A, Duke Scientific Corp., Palo Alto, CA) were diluted into DI water. The variations in air saturation was obtained by degassing DI water and then introducing particles, if needed. CO₂ saturated and under-saturated solutions were prepared by taking degassed water, with or without particles, and bubbling medical grade carbon dioxide (98% pure) into the fluid at atmospheric pressure to a desired gas saturation. The saturation of the fluid with air or carbon

dioxide was measured.

During insonification the focus of the power transducer was positioned 8 mm from the back wall of the exposure vessel and the same pulsing and linear ramping parameters were used, in order to simulate the exposure conditions for the *in vivo* rabbit experiments. Gas analysis of the test fluid was performed before and after the balloon was submersed in the degassed water tank.

The video recordings provided a mechanism for computing echogenicity. All echogenicity values in these experiments were calculated for insonifications at 10.8 MPa peak rarefactional pressure. Echogenicity is a relative measure of the increase in contrast seen in the ultrasound image when an event such as cavitation has occurred. To compute echogenicity, an image of the highest apparent echogenicity was selected from the video recorded images after the burst from the power transducer. A background image, before insonification, was also selected. AVS image processing software (Advanced Visual Systems Inc., Waltham, MA) was used to select identical regions of interest (ROI) within the test vessel from the background and high signal images. The pixel values from the ROIs were log decompressed and subtracted from each other. The sum of the subtraction ROI values gave a quantitative measure of the overall increase in the echogenicity.

III. RESULTS AND DISCUSSION

In Vivo Rabbit Experiments:

In 7 of 8 rabbits a significant amount of cavitation was achieved. The average peak rarefactional pressure for contrast generation in rabbits was 8.9 ± 1.7 MPa. Gas measurement of the urine indicated that there was a correlation between the partial pressure of carbon dioxide (pCO_2) in the urine and the threshold for cavitation, as shown Figure 3 for the 7 rabbits; this dependence was not seen for oxygen content or pH of the urine.

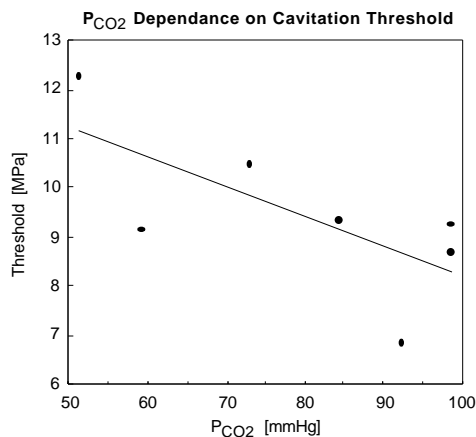


Figure 3. Rabbit thresholds vs. pCO_2 .

From these rabbit studies, there appears to be a significant improvement in the ability to generate contrast with the rabbit urine that has a higher particle content. These rabbit studies indicate an importance of gas content, particularly urine content of CO_2 . The images in Figure 4 show the case of successful transcutaneous generation of contrast at about 8 MPa peak rarefactional pressure. To better delineate the factors for production of copious cavitation bubbles, *in vitro* experiments were performed, as discussed next.

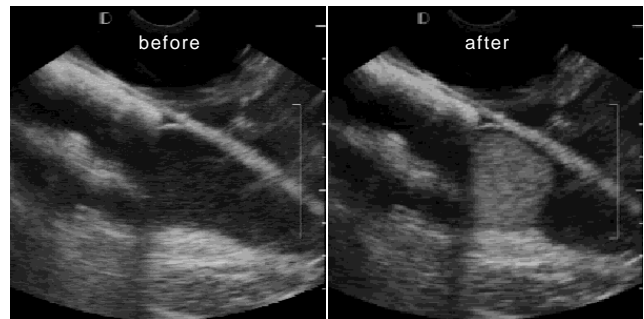


Figure 4. Rabbit bladder contrast generation.

In Vitro Aqueous Cell-Free Media Experiments:

The peak rarefactional pressure threshold for contrast generation, as visualized with a diagnostic ultrasound system, decreased almost a factor of two when particle concentration increased from 10^8 to 10^{10} particles/cc; with the lowest threshold being 5.24 MPa. For samples with 10^{10} particles/cc and gas saturations below 50%, thresholds were at least twice as high as those of more saturated fluids, and samples containing CO_2 had considerably lower thresholds than respective under-saturations in air (see Figure 5); recall that the rabbit results also showed a decreasing threshold with increasing pCO_2 . At a fixed pressure amplitude, echogenicity tended to increase with both increasing particle concentration and gas saturation; this was more pronounced for samples containing CO_2 .

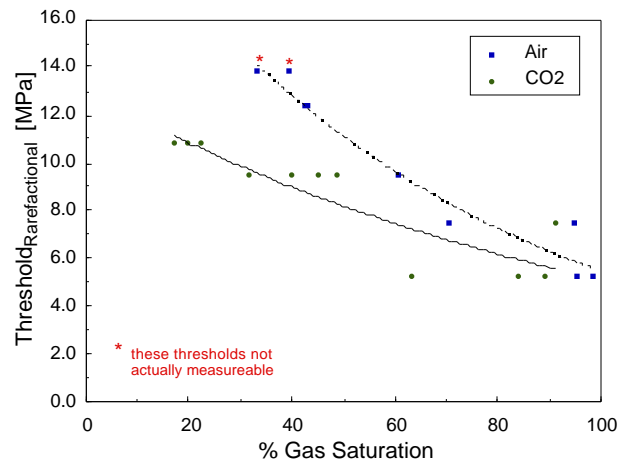


Figure 5. Samples with 10^{10} particles/cc.

However, since it is known that physiological urine is under-saturated [6] and usually highly filtered, then it is more important to look at the effects of gas concentration in filtered water. These bar plots, Figures 6 and 7, are a summary of the three groups of experiments performed. The solid bars are an average of samples with gas, light shading for CO₂ and dark shading for air, at 50% or greater saturation, and the hatched bars are samples averaged with saturation below 50%. When there are sufficient nuclei in the fluid, the threshold decreases and echogenicity increases with increasing gas saturation. Notice that in the case of the more physiological situation of filtered fluids, that the thresholds even in saturated fluids are not significantly different than the under-saturated samples. But note that when comparing the under-saturated air and CO₂ samples, the threshold for CO₂ is statistically lower than that for air, and the echogenicity is also statistically higher than that for air for under-saturated, filtered samples. One can therefore expect that the presence of CO₂ would have an impact on bubble production if not the threshold for initiating cavitation.

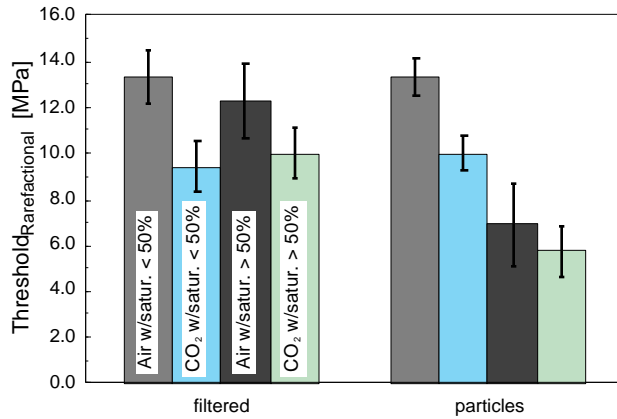


Figure 6. Thresholds for all *in vitro* samples.

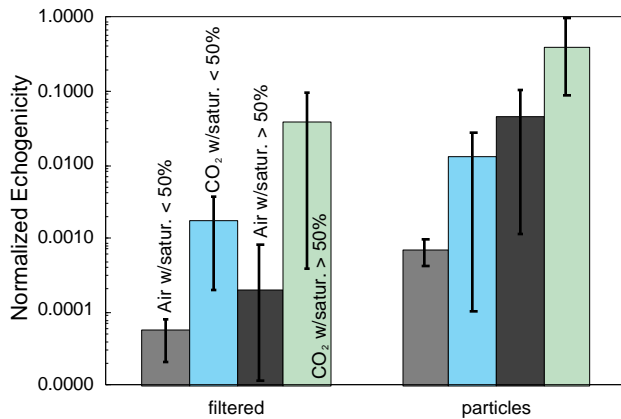


Figure 7. Echogenicity for all *in vitro* samples.

IV. CONCLUSIONS

The *in vitro* results follow the trend of what was qualitatively and quantitatively seen in the rabbit experiments, where higher particle content of the fluid and CO₂ saturation had a profound effect on the ease of cavitation and the generation of abundant contrast. However, since human urine is unlike rabbit urine, notably in particle content, it might be more difficult to cavitate. Manipulation of the urine should be focused on increasing dissolved gas content, particularly increasing pCO₂.

Further work is needed to fully characterize how physiological urine can be manipulated safely in order to generate sufficient contrast within the bladder. Pharmacological and dietary interventions will be investigated as a method for increasing the dissolved CO₂ and particle content in the urine. Ultimately the goal is to be able to use even shorter bursts of high intensity to initiate cavitation, followed by a lower intensity growth and trapping pulse to sustain copious contrast in the bladder in order to effectively and non-invasively diagnose urinary reflux.

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