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● *Original Contribution*

INTERSTITIAL MATRIX PREVENTS THERAPEUTIC ULTRASOUND FROM CAUSING INERTIAL CAVITATION IN TUMESCENT SUBCUTANEOUS TISSUE

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Abstract—We search for cavitation in tumescent subcutaneous tissue of a live pig under application of pulsed, 1-MHz ultrasound at 8 W cm^{-2} spatial peak and pulse-averaged intensity. We find no evidence of broadband acoustic emission indicative of inertial cavitation. These acoustic parameters are representative of those used in external-ultrasound-assisted lipoplasty and in physical therapy and our null result brings into question the role of cavitation in those applications. A comparison of broadband acoustic emission from a suspension of ultrasound contrast agent in bulk water with a suspension injected subcutaneously indicates that the interstitial matrix suppresses cavitation and provides an additional mechanism behind the apparent lack of *in-vivo* cavitation to supplement the *absence of nuclei* explanation offered in the literature. We also find a short-lived cavitation signal in normal, non-tumesced tissue that disappears after the first pulse, consistent with cavitation nuclei depletion *in vivo*. **(E-mail: koulakis@physics.ucla.edu)**

Key Words: **Cavitation, Tumescent injection, Ultrasound, Nuclei depletion, Ultrasound-assisted lipoplasty, Therapeutic ultrasound.**

INTRODUCTION

Ultrasound is a technology with remarkable potential for a wealth of biomedical applications (Goertz and Hynynen 2016; Mitragotri 2005). Generally operating at intensities of ~1–10 W cm^{-2}, *therapeutic* ultrasound has been evaluated for applications such as thrombolysis (Braaten et al. 1997; Datta et al. 2006), sonophoresis (Polat et al. 2010), sonoporation (Tomizawa et al. 2013), lipoplasty (Cook 1997) and accelerated wound healing (Cullum et al. 2010; Hart 1998). At much higher intensities, >1000 W cm^{-2}, high-intensity-focus-ultrasound (HIFU) (Kennedy et al. 2003) is used as a non-invasive means of lithotripsy, histotripsy and tissue ablation.

Whether ultrasound is considered safe for diagnostic purposes and effective for other applications depends critically on whether cavitation occurs *in vivo* (Holland et al. 1996). Cavitation no doubt exists at the high intensities of focused ultrasound and could exist—if proper cavitation nuclei are present—at much lower intensities (Holland and Apfel 1989; Holland et al. 1992). This report focuses on intensities in the 1–10 W cm^{-2} therapeutic regime that are strong enough to cause cavitation in bulk liquid, but do not reliably cause cavitation in tissue. The apparent lack of cavitation nuclei *in vivo* has the dual effect of making diagnostic ultrasound safe at higher intensities (Church 2002), but also hinders the use of cavitation for therapeutic applications. Controlling the location of cavitation inception, growth and sustentation is a problem that has been mostly solved at high intensities (Hockham et al. 2010; Xu et al. 2004), but its difficulty at lower intensities has slowed the adoption of therapeutic ultrasound technology. The development of ultrasound contrast agents (Keller et al. 1989) and phase-shift nanodroplets (Rapoport 2012) has provided a convenient method of introducing cavitation nuclei into the body to address the inception problem.

Cavitation, for our purposes, is defined as the expansion, compression and dynamics of gas pockets in liquid or tissue in response to sound pressure oscillations. It can be *stable*, characterized by relatively small amplitude

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oscillations that result in an emission spectrum of harmonics and half-odd-integer harmonics (Lauterborn 1976) (ultra-harmonics). Or cavitation can be *inertial*, characterized by high-amplitude, chaotic oscillations resulting in violent collapse and broadband sound emission (Frohly et al. 2000; Hauptmann et al. 2012). A specified ultrasound frequency will resonantly excite optimally sized bubbles (Leighton 1994; Minnaert 1933) (3.7 μm radius at 1 MHz in water), minimizing the cavitation threshold for that pair, while the ultrasound intensity determines the range of bubble size that can be driven to cavitate (Holland and Apfel 1989). Therefore, whether ultrasound of a given frequency and intensity induces cavitation is determined by the size distribution of gas pockets (cavitation nuclei) present in the medium.

Time-averaged (during the sound period) bubble dynamics in sonicated free liquid can be very rich (Lauterborn and Kurz 2010; Neppiras and Coakley 1976; Plesset and Prosperetti 1977), displaying phenomena such as streaking (translational motion), rectified-diffusion (Hsieh and Plesset 1961; Louisnard and Gomez 2003), coalescence (Crum 1975) and fission from surface mode instabilities (Fransecutto and Nabergoj 1978) or asymmetric collapse (Brennen 2002). In particular, rectified diffusion is the process whereby an oscillating bubble will grow because more gas diffuses inward during the bubble's expansion than diffuses outward during the compression thanks to the difference in the bubble's surface area. This process has been the basis for some to argue (Crum and Hansen 1982; ter Haar et al. 1982) that low levels of ultrasound induce bubble growth in tissue. Others have pointed out that there is little range in the relevant parameter space for rectified diffusion to occur without almost immediate inertial cavitation (Church 1988).

Decades of searching for cavitation *in vivo* (Frizzell et al. 1983; Holland et al. 1996; ter Haar et al. 1982) have found that, aside from sensitive areas of the body such as the lungs and intestines, cavitation exists, but only at very high amplitudes that far surpass those required in free liquid. Nightingale et al. (2015) reviewed studies with positive cavitation results and put the threshold for cavitation at 1 MHz to be greater than 5 MPa peak rarefactional pressure. Cavitation is not a robust, reliably occurring phenomenon at typical diagnostic or therapeutic levels. One hypothesis to explain this is that the body is completely free of cavitation nuclei, but the phenomenon of decompression sickness ("the bends") provides a counter example (Blatteau et al. 2006; Papadopoulou et al. 2013; Tikuisis 1986). A more refined hypothesis is that there are no cavitation nuclei of the appropriate size.

Routinely used in tumescent anesthesia and liposuction (Klein 1987), tumescent injections infuse large volumes of physiologic saline into adipose and subcutaneous tissue, causing it to expand and become firm. Anesthetics, vasoconstrictors (Klein 1990), antibiotics (Klein 2017; Silberg 2013) or other additives are routinely mixed into the tumescent solution for specific effects. The tumescent technique eliminates the need for systemic anesthesia, reduces overall blood loss and shortens recovery time (Klein 1993). Ultrasound is often applied either internally (Zocchi 1992) or externally (Cook 1997; D'Andrea et al. 2008; Mendes 2000; Rosenberg and Cabrera 2000; Silberg 1998), and is hypothesized to exert fat loosening or emulsifying effects through cavitation or other means (Coleman et al. 2009; Gasperoni and Salgarello 2000; Rohrich et al. 2000). Internal ultrasound-assisted lipoplasty employs probes that vibrate underneath the skin at ~ 35 kHz to disrupt adipose tissue mechanically; whereas external ultrasound-assisted lipoplasty (EUAL) attempts to create similar acoustic conditions with a ~ 1-MHz transducer applied on the skin surface. Both methods have been demonstrated to cause cavitation in bulk water (Weninger et al. 1999, 2000), but the existence of cavitation in tumescent tissue has not been verified in either case. As the ultrasound intensities of EUAL ($2 - 3$ W cm^{-2}) are ~ $100\times$ lower than cavitation thresholds of non-tumesced tissue (Center for Devices and Radiological Health 2008; Nightingale et al. 2015), the role of cavitation in EUAL is in question.

In this report, we investigate whether a tumescent injection of physiologic (0.9%) saline (Fig. 1) can provide sufficient nuclei to seed inertial cavitation *in vivo* and thereby lower cavitation thresholds to EUAL levels. In addition to providing nuclei, the expansion of tissue under tumesced conditions might lower the cavitation threshold in and of itself, thanks to the much larger water fraction. Indeed, a close inspection of Fig. 1b suggests a $3 - 4\times$ volumetric expansion ratio, saline fraction of 65%–75% and a cavitation threshold much closer to that in bulk fluid. After tumescent injection into healthy, live pigs, we search for inertial cavitation by applying therapeutic ultrasound and listening for broadband acoustic scattering that is indicative of the chaotic motion of bubbles undergoing inertial cavitation (Frohly et al. 2000; Hauptmann et al. 2012). Injections of ultrasound contrast agent provide a positive control. We also test a freshly prepared suspension of powdered cefazolin, an antibiotic routinely added to tumescent solutions (Silberg 2013), to determine whether motes in the powder provide a source of nuclei.

MATERIALS AND METHODS

Figure 2 is a block diagram of the cavitation excitation and detection system. The drive signal is made by chopping a continuous-wave signal (output of DS345 function generator, Stanford Research Systems, Sunnyvale, CA, USA) with the help of a Stanford Research Systems DG535 pulse generator and high-isolation TTL switch (ZASWA-2-50 DR + , Mini-Circuits, Brooklyn, NY, USA). It is