Ultrasound in Med. & Biol., Vol. 42, No. 5, pp. 1145–1166, 2016

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2015.12.011

● *Original Contribution*

ACOUSTIC CLUSTER THERAPY: *IN VITRO* AND *EX VIVO* MEASUREMENT OF ACTIVATED BUBBLE SIZE DISTRIBUTION AND TEMPORAL DYNAMICS

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(*Received* 4 *June* 2015; *revised* 7 *December* 2015; *in final form* 14 *December* 2015)

Abstract—Acoustic cluster technology (ACT) is a two-component, microparticle formulation platform being developed for ultrasound-mediated drug delivery. Sonazoid microbubbles, which have a negative surface charge, are mixed with micron-sized perfluoromethylcyclopentane droplets stabilized with a positively charged surface membrane to form microbubble/microdroplet clusters. On exposure to ultrasound, the oil undergoes a phase change to the gaseous state, generating 20- to 40-μm ACT bubbles. An acoustic transmission technique is used to measure absorption and velocity dispersion of the ACT bubbles. An inversion technique computes bubble size population with temporal resolution of seconds. Bubble populations are measured both *in vitro* and *in vivo* after activation within the cardiac chambers of a dog model, with catheter-based flow through an extracorporeal measurement flow chamber. Volume-weighted mean diameter in arterial blood after activation in the left ventricle was 22 μm, with no bubbles >44 μm in diameter. After intravenous administration, 24.4% of the oil is activated in the cardiac chambers. (E-mail: Andrew.healey@phoenixsolutions.no) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Acoustic cluster therapy, *In vitro* bubble sizing, *Ex vivo* bubble sizing, *In vivo* bubble sizing, Microbubbles, Droplet vaporization, Ultrasound contrast agent, Myocardial perfusion imaging.**

INTRODUCTION

Acoustic cluster technology (ACT) can be considered to belong to the class of technologies based on acoustic droplet vaporization (ADV), a term first used in the literature in 2000 (Kripfgans et al. 2000). Liquid droplets of micron and submicron diameter (also referred to as phase shift emulsions) are provided an energy stimulus (ultrasound for "acoustic" droplet vaporization) to change phase from liquid to gas-forming microbubbles. ADV has been extensively researched for albumin-coated micrometer-sized dodecafluoropentane droplets by the group at the University of Michigan, among others. For a historical review, see Sheeran and Dayton (2012).

Several patents were granted related to ADV in the late 1990s. Apfel (1998) describes dispersions of superheated droplets (droplet liquid has a boiling point below body temperature at atmospheric pressure) of immiscible liquids for infusion, which are vaporizable by ionizing radiation or ultrasound. Applications described include diagnostic contrast agents, drug delivery and embolization. Østensen et al. (1998) describe a similar concept in which micron-sized droplets are mixed with stabilized microbubbles that significantly reduce the ultrasound energy required to vaporize the droplets and allow use of droplets that are not necessarily superheated. Eriksen and Tolleshaug (1999) reported that the efficacy of the concept (described in Østensen et al. 1998) can be substantially enhanced if the emulsion droplets and microbubbles have affinity for each other. These two patents exploit the deposit nature of the vaporized bubbles for perfusion imaging, for bubble sizes sufficient to lodge in the microvasculature. The gold standard for perfusion measurements in tissue is injection of labeled, solid microspheres that are trapped in capillaries because of their size (deposit tracer). The deposition of microspheres will be proportional to flow in tissue if certain conditions are met (Domenech et al. 1969). Vaporized bubbles that are large enough to trap in the capillary bed also behave as a deposit tracer; the number of bubbles deposited in

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tissue is related to tissue perfusion. The gold standard microsphere technique is not suitable for imaging for two main reasons. Solid microspheres do not pass the lung capillaries and must therefore be injected via arterial catheters. The residence time may also be unacceptably long. The use of vaporized bubbles of sufficient size overcomes these two limitations. The liquid droplets have been designed to grow in size when activated by ultrasound after they have passed the pulmonary capillaries and can therefore be administered intravenously. The bubbles so generated contain a gas that dissolves in tissue after deposition and have a residence time of minutes. Thus, the additional backscatter signal provided by the bubbles deposited in tissue is related to tissue perfusion.

Smaller droplets (submicron) will produce smaller bubbles that may not have the same deposit tracer properties. Nanodroplets have the potential to leave the vasculature, for example, by the enhanced permeability and retention effect (Iyer et al. 2006), prior to vaporization and be vaporized in the tissue compartment (Sheeran and Dayton 2012).

Bubbles produced by *in vivo* vaporization of liquid droplets have been proposed for a plethora of applications (for recent reviews, see Lin and Pitt 2013; Rapoport 2012; Sheeran and Dayton 2012) embracing their ultrasound imaging properties including perfusion imaging; embolization; phase aberration correction; thrombolysis; influence on high-intensity focused ultrasound therapy; and drug delivery.

The ACT concept is a two-component microparticle system composed of stabilized microbubbles with a negative surface charge mixed with stabilized perfluoromethylcyclopentane (PFMCP) microdroplets with a positive surface charge (Sontum et al. 2015b). On mixing, small 2- to 8-μm particle clusters are formed by electrostatic attraction. When injected into the bloodstream and exposed to medical ultrasound fields, the microbubble transfers energy to the microdroplet and acts as a vaporization "seed," initiating vaporization of the oil droplet. The presence of the microbubble in the cluster makes the vaporization process occur at much lower acoustic power than it would in its absence (Eriksen and Tolleshaug 1999; Lo et al. 2007; Østensen et al. 1998). The initial vaporization step is rapid (microsecond time scale for ADV droplets without the microbubble present [Doinikov et al. 2014; Shpak et al. 2013, 2014; Wong et al. 2011]) and results in a vapor bubble approximately five times the initial droplet diameter (Kripfgans et al. 2000). Inward diffusion of blood gases and water vapor results in continued bubble growth to reach a maximum diameter. Gas under saturation in blood as a result of O_2 metabolism, surface tension and blood pressure promotes diffusion of gases out of the bubbles and subsequent shrinkage (Van Liew and Burkard 1995a). A low-solubility oil is employed to extend the lifetime of the activated ACT bubble.

The average diameter of lung capillaries has been reported to be approximately 7 μm, with approximately 95% being larger than 4 μm (Hogg 1987). To ensure free-flowing properties and passage through the capillary bed after intravenous administration, medical ultrasound contrast agents are therefore typically <4 μm in mean diameter (Sontum 2008). In dogs, experiments have indicated that bubbles <11 μm are required for lung passage (Butler and Hills 1979). ACT bubbles ≥ 12 μm in diameter will hence be expected to lodge and deposit in the capillary bed, transiently stopping blood flow until they dissolve and become small enough to dislodge, re-condense or dissolve completely. The 20- to 30-μm average diameters imply that they are likely to lodge at the capillary level. Currently, ACT technology is being developed for ultrasound-mediated drug delivery applications as a theranostic agent. For recent reviews of related approaches, see Castle et al. (2013) and Wood and Sehgal (2015).

To enable formulation design and to elucidate attributes and mechanisms, there was an obvious need for experimental methods capable of sizing the activated bubbles *in vitro* and *in vivo*, to track their kinetics and to measure the efficiency of the activation process (*i.e.*, the fraction of injected oil that vaporizes in both the *in vitro* and *in vivo* environments). Measuring bubble size populations in multiphasic media is an important problem in a plethora of applications including industry (nuclear reactors), medicine (decompression sickness), biology and oceanography. A method was required that could size an entire bubble population *in vitro* at controlled concentrations and environmental settings similar to those *in vivo* with a temporal resolution of seconds. The method was also required to operate in whole blood in an extracorporeal flow circuit, to size bubble populations after *in vivo* activation. The activated ACT bubbles are mostly devoid of a stabilizing shell or membrane (except for the remnants of the particle shells in the original cluster). In larger vessels they may be considered as "free" bubbles, which possess relatively "sharp" acoustic resonances in the linear regime. This is due to the increase in Q factor of the bubble system without a shell and the increase in Q factor with bubble size. This allows for use of the acoustic attenuation properties of a bubble population along with a model of bubble resonance to invert the linear attenuation data to recover bubble size. This technique has been used in the sonar literature to size bubbles in the upper ocean (Vagle and Farmer 1998) and is employed in a commercially available bubble-sizing instrument (Wu and Chahine 2010). This quantitative approach also has the advantage of operating well in whole blood and is insensitive to the