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● *Original Contribution*

CHARACTERIZATION OF BIOEFFECTS ON ENDOTHELIAL CELLS UNDER ACOUSTIC DROPLET VAPORIZATION

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Abstract—Gas embolotherapy is achieved by locally vaporizing microdroplets through acoustic droplet vaporization, which results in bubbles that are large enough to occlude blood flow directed to tumors. Endothelial cells, lining blood vessels, can be affected by these vaporization events, resulting in cell injury and cell death. An idealized monolayer of endothelial cells was subjected to acoustic droplet vaporization using a 3.5-MHz transducer and dodecafluoropentane droplets. Treatments included insonation pressures that varied from 2 to 8 MPa (rarefactional) and pulse lengths that varied from 4 to 16 input cycles. The bubble cloud generated was directly dependent on pressure, but not on pulse length. Cellular damage increased with increasing bubble cloud size, but was limited to the bubble cloud area. These results suggest that vaporization near the endothelium may impact the vessel wall, an effect that could be either deleterious or beneficial depending on the intended overall therapeutic application. (E-mail: joebull@umich.edu) **© 2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Gas embolotherapy, Acoustic droplet vaporization, Endothelial cells, Bioeffects.**

INTRODUCTION

Acoustic droplet vaporization (ADV) is capable of transforming superheated microdroplets into bubbles that are up to 150 times larger in volume than their original size (Bull 2005, 2007; Kripfgans et al. 2000; Wong and Bull 2011). This dramatic change in size will provide enough volume for occlusion of small blood vessels supplying tumors and has the potential to be applied as a therapy through tissue starvation. This rapid volume change could also lead to significant cell injury because of the generation of high pressures and shear stresses during bubble conversion and expansion inside blood vessels (Kripfgans et al. 2000, 2004; Qamar et al. 2010, 2012; Ye and Bull 2004, 2006). Bubble expansion resulting from ADV inside tubes has been previously investigated, but it was not until recently that the effects of the liquid consumption phase were investigated (Qamar et al. 2010, 2012). Experimental evidence that the droplet interface begins to expand before the phase change is complete was obtained through ultra-high-speed imaging and was the motivation for new theoretical (Qamar et al. 2010) and computational (Qamar et al. 2012) studies. These studies described the evolution of a droplet undergoing ADV inside a rigid tube, from the liquid consumption phase to bubble expansion. The results revealed the existence of a critical droplet size below which the bubble evolution is highly oscillatory, whereas a damped evolution is observed otherwise. Three bubble growth regimes with particularly high pressures in the early stage of bubble evolution were also observed. With these new studies, shear stresses were found to be dependent on the initial droplet size, but were significantly lowered (by five orders of magnitude) compared with those reported in earlier studies, which considered the phase transition to occur before the expansion (Ye and Bull 2004, 2006). Nonetheless, calculated pressures were still far above those found physiologically. More recently, a few studies focused on the phase transition stage of ADV providing a possible explanation with respect to the mechanism (Li et al. 2014; Shpak et al. 2013a, 2013b, 2014). These studies provided extensive experimental data along with numerical models that described three distinct regimes present in droplet vaporization (Shpak et al. 2013a) and how the ultrasound wave is distorted and refocused inside the droplet (Li et al. 2014; Shpak et al. 2014). This refocusing event

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caused by higher harmonics was found to be responsible for the generation of large local negative pressures. These local pressures (a nearly sixfold increase from incident pressure) inside the droplet would then be able to induce a cavitation-like event (*i.e.*, nucleation site) leading to the phase change and subsequent bubble expansion, but could also be the source of local cell injury.

Droplet concentration, droplet size, relative location inside the blood vessel and the selection of acoustic parameters could determine the range of bioeffects associated with ADV and, consequently, their clinical relevance and potential application. The high probability of these events resulting from droplet concentration in conjunction with high pressures and shear stresses generated during vaporization and bubble expansion could translate to a high risk of affecting the endothelium, for example. As thousands of these microdroplets of various sizes will circulate the bloodstream spanning the entire cross section of a blood vessel, it will be possible for vaporization events to occur at or near the vessel wall (Fig. 1). The size of the ultrasound (US) beam relative to the diameter of the blood vessel under treatment will likely determine the extent of damage, making the walls of smaller vessels more susceptible to ADV events. These events will be important not only as the bubbles expand, but also after they have reached their final size, providing cavitation nuclei (Hilgenfeldt et al. 1998; Khismatullin 2004; Minnaert 1933; Plesset and Prosperetti 1977) capable of inducing a number of bioeffects on tissue.

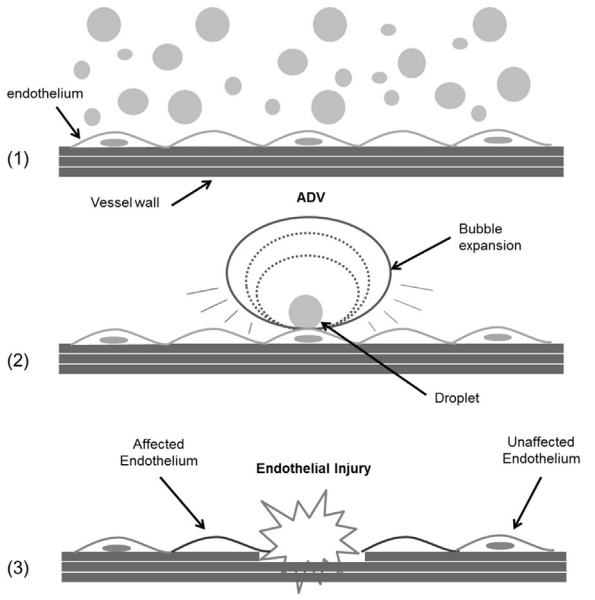

Fig. 1. Droplets of various sizes flow inside the blood vessel (1) until an ultrasound beam triggers ADV, which may occur near or at the vessel wall, (2) impacting endothelial cells and ultimately important vessel functions (3). Note that the droplet in (2) is the source of the final bubble pointed to by the *arrow*. ADV = acoustic droplet vaporization.

However, if under control, these effects could aid in a number of applications that can be synergistic with vessel occlusion, such as cellular permeability and local occlusion through thrombosis.

Another important consideration preceding vaporization is the ability of the droplets to extra-vasate in some regions of the vascular tree into the interstitial space. This, of course, will be dependent on their size and the relative permeability of the endothelium across the vasculature. For example, endothelium of large arteries and that of the brain are much less permeable than the endothelium of capillaries and post-capillary venules (Ryan 1988). In addition, tumor vasculature is abnormally more permeable than normal vasculature (Jain and Stylianopoulos 2010; Siemann 2011). Particles greater than 3 nm will not able to passively cross endothelial junctions in continuous endothelium; however particles as large as a few micrometers are able to cross the discontinuous walls of tumor vasculature (Jain and Stylianopoulos 2010; Mehta and Malik 2006). This distinctive characteristic of the vascular endothelium, along with an appropriate selection of droplet sizes, may influence the accumulation of droplets, an outcome that could be beneficial for the local delivery of drug-loaded droplets to tumors.

In this work, ADV events close to an idealized endothelial monolayer are investigated using a 3.5-MHz transducer and a suspension of perfluorocarbon droplets. Other parameters like rarefactional pressure and pulse length are varied, whereas droplet concentration is held constant. Resulting bubble clouds are recorded to observe localization of the damage, and fluorescence microscopy is used to quantify the bioeffects from a cellular standpoint. With this *in vitro* study we intend to provide the first insights into bioeffects of ADV on endothelial cells, perhaps corresponding to a worst case scenario, in which the cells are in direct contact with the cell surface in the absence of flow. It is of particular interest to characterize the direct effects of ADV while finding those acoustic parameters that would allow us to perform significant ADV with minimal damage to the endothelium, as well as understanding the underlying mechanism of cellular injury.

METHODS

Cell culture

Primary human umbilical vein endothelial cells were cultured and supplemented with endothelial cell growth medium-2 (Lonza Clonetics, Walkersville, MD, USA). These cells were incubated at 37°C in a humidified environment and 5% CO_2. Cells were grown in culture flasks for one passage and then transferred to OptiCell culture chambers (Nalgene Nunc International, Rochester, NY, USA) previously coated with fibronectin (Catalog No. 354008, BD Biosciences, San Diego, CA,