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Evolution of bubbles from gas micronuclei formed on the luminal aspect of ovine large blood vessels

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ABSTRACT

It has been shown that tiny gas nanobubbles form spontaneously on a smooth hydrophobic surface submerged in water. These nanobubbles were shown to be the source of gas micronuclei from which bubbles evolved during decompression of silicon wafers. We suggest that the hydrophobic inner surface of blood vessels may be a site of nanobubble production. Sections from the right and left atria, pulmonary artery and vein, aorta, and superior vena cava of sheep ($n = 6$) were gently stretched on microscope slides and exposed to 1013 kPa for 18 h. Hydrophobicity was checked in the six blood vessels by advancing contact angle with a drop of saline of $71 \pm 19^\circ$, with a maximum of about $110 \pm 7^\circ$ (mean ± SD). Tiny bubbles ~30 μm in diameter rose vertically from the blood vessels and grew on the surface of the saline, where they were photographed. All of the blood vessels produced bubbles over a period of 80 min. The number of bubbles produced from a square cm was: in the aorta, 20.5; left atrium, 27.3; pulmonary artery, 17.9; pulmonary vein, 24.3; right atrium, 29.5; superior vena cava, 36.4. More than half of the bubbles were present for less than 2 min, but some remained on the saline-air interface for as long as 18 min. Nucleation was evident in both the venous (superior vena cava, pulmonary artery, right atrium) and arterial (aorta, pulmonary vein, left atrium) blood vessels. This newly suggested mechanism of nucleation may be the main mechanism underlying bubble formation on decompression.

1. Introduction

Using atomic force microscopy, it has been shown, mainly in the last decade, that tiny, flat gas nanobubbles measuring 5–100 nm form spontaneously when a smooth hydrophobic surface is submerged in water containing dissolved gas (Tyrrell and Attard, 2001; Yang et al., 2007). A number of theories have been proposed in explanation of the formation and stability of these nanobubbles (Seddon et al., 2011; Weijs et al., 2012). In ultrasound irradiation, rectified diffusion increased the volume of the nanobubbles (Brotchie and Zhang, 2011), suggesting that they might expand in a state of gas supersaturation. In our previous studies (Arieli and Marmur, 2011, 2013), these nanobubbles were shown to be the source of gas micronuclei from which bubbles evolved during decompression on smooth hydrophobic, but not hydrophilic, silicon wafers.

Hills (1992) showed that the inner surface of blood cavities such as the umbilical vein, right ventricle, pulmonary vein, and left ventricle are hydrophobic. He also demonstrated an oligolamellar lining of phospholipids on the luminal aspect of many blood vessels in the sheep, in venules and capillaries of the cerebral cortex and the aortic endothelium. These surfaces may be the site where nanobubbles and gas micronuclei form spontaneously. In a study of the effect of solutions on nanobubble production, Mazumder and Bhushan (2011) showed that saline, alkalinity and roughness each increased the density of nanobubbles compared with a smooth surface and pure water. We therefore suggest that the hydrophobic inner surface of blood vessels, which has a certain measure of roughness and in the living animal is bathed by alkaline and saline plasma, may be the site of nanobubble production and thus also of gas micronuclei and bubbles on decompression. For the experimental model, we chose large blood vessels from the sheep, some of which were shown by Hills (1992) to be hydrophobic.

2. Methods

2.1. Tissue preparation

The complete heart and lungs from six slaughtered sheep were obtained at the abattoir, and on removal intact from the thoracic cavity were immediately immersed in a cooler filled with saline. In the laboratory, under saline and without any exposure to air, samples (area $6 \pm 2\,cm^2$, mean ± SD) from the right and left atria,

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pulmonary artery and vein, aorta and superior vena cava, were gently stretched on microscope slides using metal clips and with the luminal aspect exposed. The six slides were placed without exposure to air on the bottom of a Pyrex bowl (diameter 26 cm, height 5 cm) under 3 cm saline.

In preliminary tests, samples of the blood vessels were exposed to high pressure and decompressed. We initially looked for bubbles adhering to the surface of the blood vessels similar to our findings in silicon wafers (Arieli and Marmur, 2011, 2013), but no such bubbles were observed. Tiny bubbles were seen to rise vertically from the vessels' luminal surface; some at least remained for a time at the liquid–air interface, and these were the bubbles on which we concentrated. Because of their diminutive size, it was difficult to focus the camera on the surface of the saline. Small pieces of Parafilm, which floated on the saline surface, were tied loosely to some of the clips to make it easier to focus the camera.

2.2. Protocol

The bowl containing the samples was transferred to a 150-l hyperbaric chamber (Roberto Galeazzi, La Spezia, Italy), and was placed on double-walled metal plates with circulating water at 12 °C for tissue preservation. The pressure was raised to 1013 kPa (90 msw) for overnight equilibration (18 ± 2 h) and decompressed at a rate of 100 kPa/min. The bowl was carefully placed on a nearby table for photography. We immediately started photographing each sample in turn (Canon EOS 500d with macro lens 100 mm F/2.8 EF USM), continuing for up to 80 min from decompression. The 80-min limit was chosen because it seemed that at that point in time the rate of bubble production began to drop. The time interval between repeated photography of the same blood vessel was 105 ± 5 s.

Usually, tiny bubbles <0.1 mm in diameter rose directly upwards from the luminal surface of the blood vessel to the saline–air interface. These could remain for a time on the surface, and the camera was therefore focused there (Fig. 1). The floating Parafilm used for focusing can also be seen in the figure. Evidently, some bubbles rose to the surface and burst between two consecutive photographs, and so could not be counted. Recent bubbles are small, whereas the larger bubbles appeared earlier and expanded thereafter. It was very rare for bubbles to adhere to the tissue and expand there (Fig. 2), whereas this had been a common occurrence with the silicon wafers (Arieli and Marmur, 2011, 2013).

Many more bubbles were formed along the cut edges of the tissue and the metal clips which held the tissue on the slide. The area covered by these bubbles expanded as they accumulated, and they

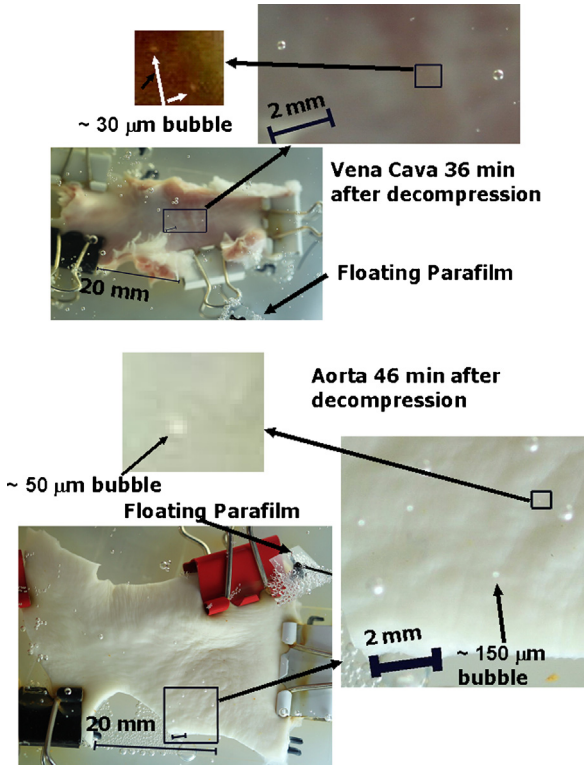

Fig. 1. Two photographs of blood vessels (aorta and superior vena cava) taken for the analysis of bubble production. Floating Parafilm was used for focusing on the surface of the saline and not of the tissue. Different sizes of bubbles represent different growth times. The sections which have been enlarged twice present the first appearance of visible bubbles (contrast was enhanced on the second enlargement).

could have masked the free surface of the tissue (Fig. 3). The upper surface of the saline with its bubbles was therefore carefully suctioned every 20 min using a vacuum device. In two control runs with the same setup but no tissues, no bubbles rose from the glass slides and no bubbles were created by the suction procedure. After three such procedures, the saline level was too low to allow for a fourth. Observation of bubble formation with the passage of time did not show any effect of suction on bubble formation at 20, 40 and 60 min after decompression.

At the end of the 80 min of photography, the slides with the tissues were removed and photographed against graph paper for later scaling. For the estimation of hydrophobicity, we measured both its determinants: advancing contact angle (drop stabilised

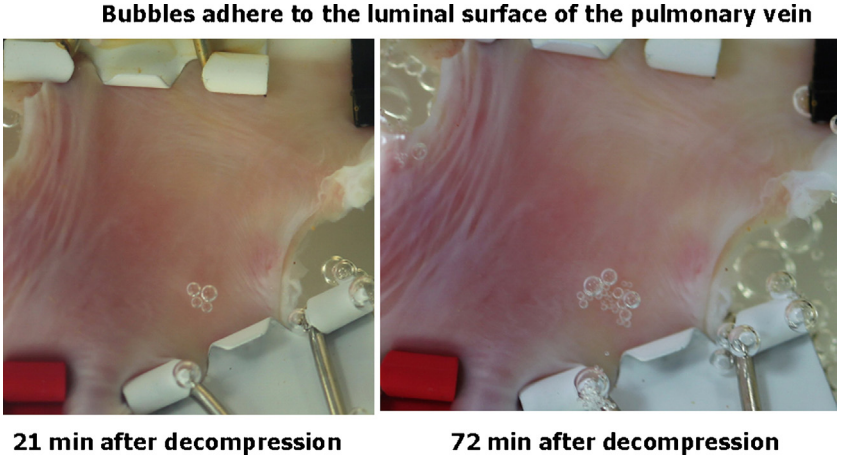

Fig. 2. Bubbles adhering to the luminal surface of the pulmonary vein (sheep 5) at two points in time after decompression.