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Decompression sickness bubbles: Are gas micronuclei formed on a flat hydrophobic surface?

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ABSTRACT

It is a long-standing hypothesis that the bubbles which evolve as a result of decompression have their origin in stable gas micronuclei lodged in hydrophobic crevices, micelles of surface-active molecules, or tribonucleation. Recent findings supported by atomic force microscopy have indicated that tiny, flat nanobubbles form spontaneously on smooth, hydrophobic surfaces submerged in water. We propose that these nanobubbles may be the gas micronuclei responsible for the bubbles that evolve to cause decompression sickness. To support our hypothesis, we used hydrophilic and monolayer-covered hydrophobic smooth silicon wafers. The experiment was conducted in three main stages. Double distilled water was degassed at the low pressure of 5.60 kPa; hydrophobic and hydrophilic silicon wafers were placed in a bowl of degassed water and left overnight at normobaric pressure. The bowl was then placed in the hyperbaric chamber for 15 h at a pressure of 1013 kPa (=90 m sea water). After decompression, bubbles were observed and photographed. The results showed that bubbles only evolved on the hydrophobic surfaces following decompression. There are numerous hydrophobic surfaces within the living body (e.g., in the large blood vessels), which may thus be the sites where nanobubbles that serve as gas micronuclei for bubble evolution following decompression are formed.

1. Introduction

The main limitation on diving is decompression sickness, which is caused by the evolution of bubbles in tissue supersaturated with inert gases following decompression from high pressure. Decompression sickness is also a hazard in high altitude flight (Webb et al., 2005), space travel, and hyperbaric medical treatment (Cooper et al., 2009). For bubbles to evolve, a critical (minimal) size is required to start the process. Bubbles smaller than this critical size would re-dissolve, due to the high pressure produced by surface tension. A critical bubble in water should contain about 10^4 molecules, but the probability of these appearing simultaneously in one location is extremely low (Hemmingsen, 1975). For example, decompression of clean water or salt solutions in a glass container from supersaturation equivalent to a depth of over one hundred metres did not produce bubbles (Gerth and Hemmingsen, 1980). Thus, as is now widely known, gas micronuclei having a critical radius of curvature must be present before or during decompression for bubbles to evolve in a diver (Hennessy, 1989). It was proposed, for example, that gas micronuclei are formed by tribonucleation, when two solid surfaces in a liquid are separated (Craig, 1996; Hayward, 1967). It was also suggested that stable gas micronuclei exist in crevices on hydrophobic surfaces (Chappell and Payne, 2005; Harvey et al., 1944), or that they are enclosed in micelles of surface-active molecules (Fox and Herzfeld, 1954; Yount et al., 1977). To date, no definite explanation has been offered for the formation of gas micronuclei in the human body, although they have been taken into consideration in recently developed models for the calculation of diving tables (Flook, 2000; Wienke, 1990).

It has recently been shown, using atomic force microscopy, that tiny, flat gas nanobubbles, measuring 5–30 nm, form spontaneously when a smooth hydrophobic surface is submerged in water (Ishida et al., 2000a,b; Meyer et al., 2005; Tyrrell and Attard, 2001; Yang et al., 2007). The mechanism underlying this phenomenon is still controversial, due to the absence of a theory explaining the stability of these nanobubbles. However, the existence of nanobubbles may have important implications for our understanding of the process by which bubbles evolve to cause decompression sickness. There are numerous hydrophobic surfaces in the living body, such as subcutaneous fat, visceral fat, and part of the inner surface of blood cavities: the umbilical vein, right ventricle, pulmonary vein, and left ventricle (Hills, 1992). Hills (1992) also demonstrated an oligolamellar lining of phospholipids on the luminal aspect of many blood vessels: venules and capillaries in the cerebral cortex and the aortic endothelium. These surfaces may be the sites where gas micronuclei form spontaneously, even in the absence of crevices.

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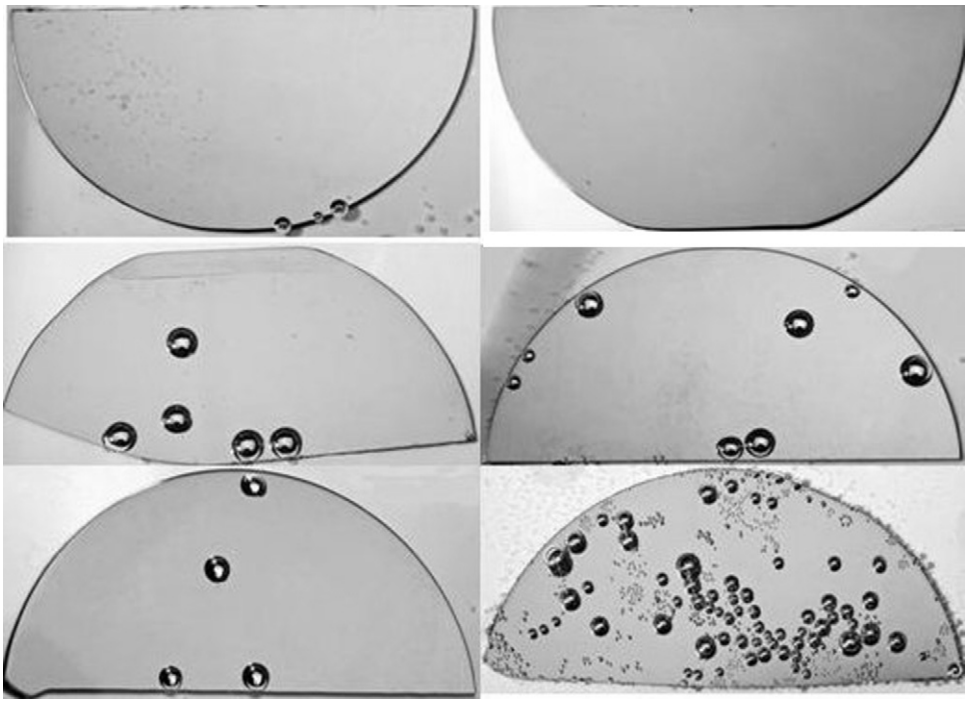

Fig. 1. Two hydrophilic wafers – upper panel, and 4 hydrophobic wafers – middle and lower panels, photographed about 15 min after decompression. Wafer diameter is 5 cm.

The purpose of the present study was to verify this hypothesis, by comparing bubble evolution following decompression on smooth hydrophobic and hydrophilic surfaces submerged in water supersaturated with air. As will be shown, bubbles indeed evolved only on the hydrophobic surfaces. We thus define three types of gas cavity: *nanobubbles* are the tiny, spherical cap-shaped gas cavities formed on the hydrophobic surface, *gas micronuclei* are the nanobubbles that are effective as bubble seeds, and *bubbles* are visible bubbles of any size.

2. Methods

2.1. Wafer preparation

Circular silicon wafers (2 in., 1,0,0, University Wafer, Boston, MA, USA) were cut into half circles. Wafers were cleaned in a Soxhlet with chloroform for 15 min. This was followed by UV radiation, and ozone was applied on each side to destroy organic impurities. Half of the wafers were coated with a monolayer for the production of a hydrophobic surface (Maoz and Sagiv, 1984). The wafers were placed in a Teflon recipient containing a solution of 72 ml hexadecane + 4 ml chloroform + 20 drops trichloro(octadecyl)silane 1 mM. After 15 min in the solution the wafers were removed, rinsed with chloroform, and placed in chloroform for 15 min. This whole procedure was repeated at least four times. For an estimation of hydrophobicity, the advancing and receding contact angles of a drop of water in air at 23 °C were measured using a contact angle measuring instrument (DSA 100 goniometer, Krüss, Hamburg, Germany). The contact angle of the hydrophobic wafers with a drop of water was $108.5 \pm 2.8^{\circ}$.

2.2. Degassing and hyperbaric exposure

A porcelain desiccator plate (diameter 19 cm) was placed in a pyrex bowl (diameter 26 cm, height 5 cm). The bowl was filled with double distilled water (18 MΩ) to a level 3 cm above the plate, and placed for 15 h in a desiccator (Vacuum pump E2M1.5, Edwards, Tewksbury, MA, USA) under low pressure, 5.60 kPa, for washout of dissolved gases and any tiny bubbles. Ambient (room) pressure was restored, and the silicon wafers (8 hydrophobic and 4 hydrophilic) were placed under the water on the porcelain plate with the shiny, almost unimolecular flat surface facing upward. Low pressure was resumed for another 1 h, after which ambient pressure was again restored. The wafers were left under water in the desiccator at ambient pressure and exposed to the surrounding air for 24 h. This time was allowed for the assumed formation of nanobubbles on the hydrophobic surfaces from the dissolved air.

The bowl was then transferred from the desiccator to a 150-l hyperbaric chamber (Roberto Galeazzi, La Spezia, Italy), where it was kept for 15 h at a pressure of 1013 kPa (=90 m sea water). The pressure was then slowly reduced over 9 min to that at the surface (ambient room pressure), where bubble formation was observed and photographed over a period of 3 h. Only bubbles formed on the surface, and not at the edges of the wafer, were considered.

This procedure was repeated in four separate sessions, once a week. A fifth session was conducted, in which the water was kept at low pressure for the entire 16 h without the wafers, which were then placed under the water on the porcelain plate for 24 h at ambient pressure before the high pressure protocol. This was done to ensure that gas micronuclei were not formed on the wafers due to the low pressure.

3. Results

Few bubbles were seen on the hydrophobic wafers when the bowl was removed from the hyperbaric chamber. Once the bowl was outside the chamber tiny bubbles began to appear, small dots about 0.1 mm in diameter. From that point in time, bubbles expanded continuously. More visible tiny bubbles appeared 5 min after the end of decompression, but only on the hydrophobic wafers. About 15 min after decompression, the bubbles that had been observed earlier on the hydrophobic wafers were bigger, and more bubbles were observed (Fig. 1). The size of the bubbles in this and the following figure can be estimated from the diameter of the

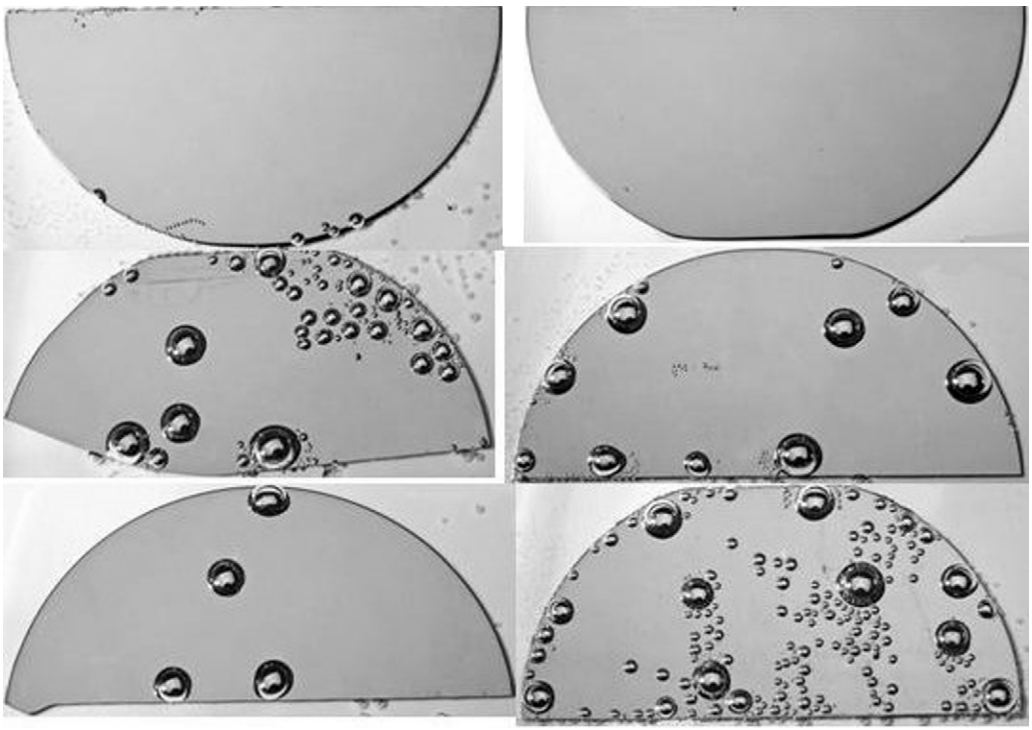

Fig. 2. Two hydrophilic wafers – upper panel, and 4 hydrophobic wafers – middle and lower panels, photographed about 30 min after decompression. The two hydrophobic wafers on the left are those presented on the left in Fig. 1. The two on the right were not presented in Fig. 1.

wafer (5 cm). About 30 min after decompression, additional small bubbles appeared on the hydrophobic wafers (Fig. 2). There were hydrophobic wafers with only a few large bubbles. Others had both large and small bubbles; the former were those observed initially, and the latter those which subsequently appeared. Some of the hydrophobic wafers were densely loaded with bubbles, whereas on others the bubbles were sparser.

Only a few large bubbles were released to the surface of the water; most adhered to the wafers for some considerable time, as long as 3 h after decompression. No bubbles were released to the surface within the first 30 min. Fig. 3 shows the magnification of a 1 cm^2 section of a wafer photographed 35 min after decompression. It can be seen that apart from the large bubbles, there are small and tiny bubbles that became visible later on.

No bubbles at all were seen on the upper face of the hydrophilic wafers. The same results were obtained in each of the sequence of 5 experiments conducted over 5 consecutive weeks, including the fifth experiment in which wafers were placed in the water only at the end of the low pressure session. No statistical analysis was performed, because the results were clear-cut: all of the hydrophobic wafers had bubbles, whereas there were no bubbles on the hydrophilic wafers.

Bubble size increased with time. After about 2 h, bubbles floated to the surface of the water. These were then replaced by large numbers of bubbles, which appeared at exactly the same spot on the wafer. An example is shown in the photograph in Fig. 4, taken 2.5 h after decompression, in which 3 of 4 first generation bubbles were released at about 2 h after decompression.

4. Discussion

Ishida et al. (2000a,b) showed that when a hydrophilic surface was rendered hydrophobic underwater, there were no nanobubbles. They claimed that nanobubbles were already attached to the hydrophobic wafers on immersion, in which case these could not be compared to human body tissue that has never been exposed to air. However, other authors have claimed that nanobubbles are formed underwater from dissolved gas (Borkent et al., 2007; Singh et al., 2006; Switkes and Ruberti, 2004; Tyrrell and Attard, 2001; Yang et al., 2007; Zhang et al., 2006). For example, no nanobub-

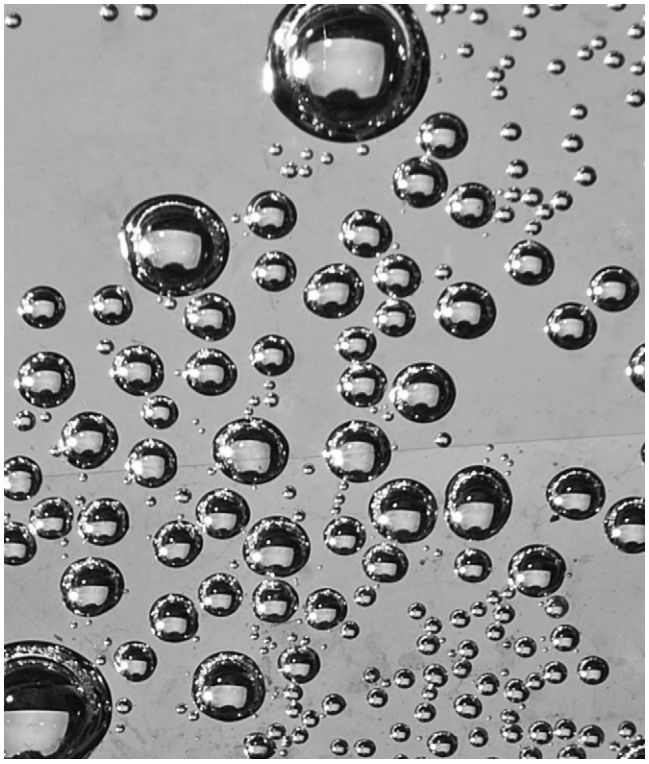

Fig. 3. Example of the sequence in which the bubbles appeared. The large bubbles are those which appeared earliest; the tiny bubbles are the most recent. This is on a 1 cm^2 section of hydrophobic wafer photographed 35 min after decompression.

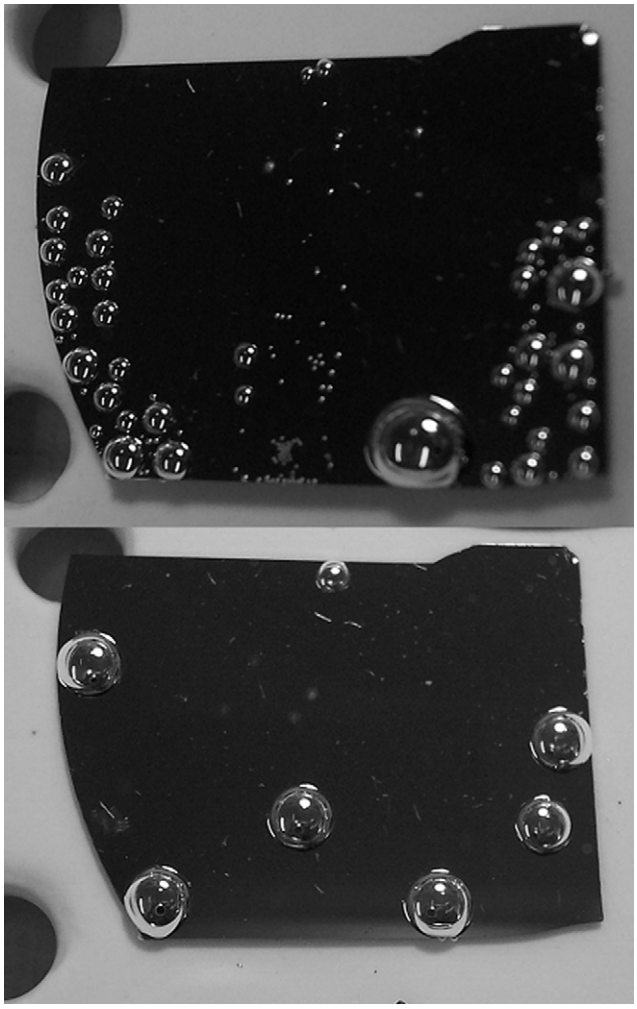

Fig. 4. A wafer is presented before bubbles grew to their final size (lower panel). After all the bubbles but one floated to the surface of the water (about 2 h after decompression), a large number of bubbles appeared on the spot they had previously occupied (upper panel).

bles were observed on a hydrophobic surface covered with ethanol, but nanobubbles did appear after the ethanol was anaerobically replaced with water containing dissolved gas. No nanobubbles appeared when the ethanol was replaced with degassed water. After nanobubbles were swept off the hydrophobic surface, others appeared in under an hour. Several studies showed that nanobubbles were missing when most of the dissolved gas was removed (Borkent et al., 2007; Considine et al., 1999; Meyer et al., 2005; Stevens et al., 2005; Switkes and Ruberti, 2004; Zhang et al., 2006). Degassing using a low pressure of 10 kPa, compared with 5.6 kPa in the present study, was effective in preventing the appearance of nanobubbles (Zhang et al., 2006). When degassing was started in our preliminary tests after the wafers were placed in the water, a profusion of bubbles appeared very soon on all of the hydrophobic surfaces. However, this phenomenon was not observed when the water was degassed for 15 h before the wafers were inserted. Zhang et al. (2006) suggested that gas in supersaturation, left behind after ethanol having a high concentration of dissolved gas was replaced with water, caused the appearance of nanobubbles. Gas in supersaturation could act via a similar mechanism during decompression. We therefore believe that the nanobubbles which appeared on the hydrophobic wafers in our study were formed from dissolved gas during the normobaric and hyperbaric periods that followed degassing, and served as gas micronuclei. Borkent et al. (2007) remarked on the stability of nanobubbles, after they found that surface nanobubbles did not act as nucleation sites for cavitation bubbles following a drastic reduction of the pressure in a liquid. However, our study was conducted in different conditions, in which there was very high supersaturation of gases.

Decompression sickness after diving, high altitude flight, space missions, and hyperbaric medical treatment is caused by bubbles evolving following decompression in tissues supersaturated with an inert gas. Although for the past half century it has generally been accepted that bubbles grow from pre-existing gas micronuclei, their mode of formation and precise nature have remained unclear. It has been hypothesised, for example, that hydrophobic crevices or micelles formed by surface-active molecules can sustain stable gas micronuclei. However, nobody has been able to provide a definitive explanation for the formation of these nucleation sites. We now suggest, on the basis of the present experimental data, that bubbles may evolve spontaneously on smooth hydrophobic surfaces following decompression. This can be explained by the findings of recent studies using atomic force microscopy, in which tiny, flat nanobubbles measuring 5–30 nm were formed spontaneously from dissolved gas on hydrophobic surfaces submerged in water. Thus, some of these nanobubbles would appear to be the long-sought gas micronuclei that result in decompression sickness. The relatively high radius of the curvature of these nanobubbles, which according to Tyrrell and Attard (2001) is ~100 nm, can be above the critical value, thus leading to the evolution of bubbles rather than re-dissolution of the gas. This hypothesis is confirmed by the present experimental data, since bubbles evolved only on the smooth hydrophobic surfaces (monolayer-covered silicon wafers), not on the hydrophilic smooth silicon wafers.

More bubbles are detected in the venous blood of divers following the second dive on the same day (Dunford et al., 2002; Castagna et al., 2009). This correlates well with the finding that when a first generation bubble is released, a large number of active nucleation sites appear on the spot previously occupied by that bubble.

The formation of gas micronuclei on hydrophobic surfaces on the inner lining of large blood cavities such as the ventricles of the heart, the pulmonary vein and the aorta (Hills, 1992), as well as in other tissues, may be the main factor enabling the evolution of bubbles following decompression. Calculations for recently developed diving tables were based on the growth of bubbles from gas micronuclei (Flook, 2000; Wienke, 1990). The present novel concept may help construct better diving tables. Adiposity is a known risk factor in decompression sickness. This was linked mainly to the high solubility of nitrogen in adipose tissue, but can now also be related to the formation of nanobubbles as gas micronuclei. The skin rash commonly observed as a mild form of decompression sickness may be related to nucleation in the subcutaneous adipose tissue.

5. Conclusion

We and others have recently suggested that besides the commonly employed procedures for reducing the inert gas load on decompression, the process of denucleation (gas exchange via an oxygen prebreath and mechanical dislodgement via exercise or vibration) may help reduce the risk of decompression sickness (Arieli et al., 2009, 2002; Castagna et al., 2009; Dujić et al., 2004; Germonpré et al., 2009). Unravelling the nature of gas micronuclei may help us devise denucleation procedures and improved models for the production of safer diving tables.

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References

Arieli, R., Boaron, E., Abramovich, A., 2009. Combined effect of denucleation and denitrogenation on the risk of decompression sickness in rats. J. Appl. Physiol. 106, 1453–1458.

Arieli, Y., Arieli, R., Marx, A., 2002. Hyperbaric oxygen may reduce gas bubbles in decompressed prawns by eliminating gas nuclei. J. Appl. Physiol. 92, 2596–2599.

Borkent, B.M., Dammer, S.M., Schönherr, H., Vancso, G.J., Lohse, D., 2007. Superstability of surface nanobubbles. Phys. Rev. Lett. 98, 204502.

Castagna, O., Gempp, E., Blatteau, J.-E., 2009. Pre-dive normobaric oxygen reduces bubble formation in scuba divers. Eur. J. Appl. Physiol. 106, 167–172.

Chappell, M.A., Payne, S.J., 2005. A crevice bubble growth model for the analysis of decompression sickness. Conf. Proc. IEEE Eng. Med. Biol. Soc. 3, 2240–2243.

Considine, R.F., Hayes, R.A., Horn, R.G., 1999. Forces measured between latex spheres in aqueous electrolyte: non-DLVO behavior and sensitivity to dissolved gas. Langmuir 15, 1657–1659.

Cooper, P.D., Van den Broek, C., Smart, D.R., Nishi, R.Y., Eastman, D., 2009. Hyperbaric chamber attendant safety. I. Doppler analysis of decompression stress in multiplace chamber attendants. Diving Hyperbar. Med. 39, 63–70.

Craig, V.S.J., 1996. Formation of micronuclei responsible for decompression sickness. J. Colloid Interface Sci. 183, 260–268.

Dujić, Z., Duplančic, D., Marinovic-Terzić, I., Baković, D., Ivančev, V., Valic, Z., Eterović, D., Petri, N.M., Wisløff, U., Brubakk, A.O., 2004. Aerobic exercise before diving reduces venous gas bubble formation in humans. J. Physiol. 555, 637–642.

Dunford, R.G., Vann, R.D., Gerth, W.A., Pieper, C.F., Huggins, K., Wacholtz, C., Bennett, P.B., 2002. The incidence of venous gas emboli in recreational diving. Undersea Hyperbar. Med. 29, 247–259.

Flook, V., 2000. The physics and physiology of decompression. Eur. J. Underwater Hyperbar. Med. 1, 8–13.

Fox, F.E., Herzfeld, K.F., 1954. Gas bubbles with organic skin as cavitation nuclei. J. Acoust. Soc. Am. 26, 984–989.

Germonpré, P., Pontier, J.-M., Gempp, E., Blatteau, J.-E., Deneweth, S., Lafère, P., Marroni, A., Balestra, C., 2009. Pre-dive vibration effect on bubble formation after a 30-m dive requiring a decompression stop. Aviat. Space Environ. Med. 80, 1044–1048.

Gerth, W.A., Hemmingsen, E.A., 1980. Heterogeneous nucleation of bubbles at solid surfaces in gas-supersaturated aqueous solutions. J. Colloid Interface Sci. 74, 80–89.

Harvey, E.N., Barnes, D.K., McElroy, W.D., Whiteley, A.H., Pease, D.C., Cooper, K.W., 1944. Bubble formation in animals. I. Physical factors. J. Cell. Comp. Physiol. 24, 1–22.

Hayward, A.T.J., 1967. Tribonucleation of bubbles. Br. J. Appl. Phys. 18, 641–644.

Hemmingsen, E.A., 1975. Cavitation in gas-supersaturated solutions. J. Appl. Phys. 46, 213–218.

Hennessy, T.R., 1989. On the site of origin, evolution and effects of decompression microbubbles. In: Brubakk, A.O., Hemmingsen, B.B., Sundnes, G. (Eds.), Supersaturation and Bubble Formation in Fluids and Organisms: An International Symposium, Kongsvoll. Sweden, 6–10 June, 1988.. Tapir Publishers, Trondheim, Norway, pp. 292–332.

Hills, B.A., 1992. A hydrophobic oligolamellar lining to the vascular lumen in some organs. Undersea Biomed. Res. 19, 107–120.

Ishida, N., Inoue, T., Miyahara, M., Higashitani, K., 2000a. Nano bubbles on a hydrophobic surface in water observed by tapping-mode atomic force microscopy. Langmuir 16, 6377–6380.

Ishida, N., Sakamoto, M., Miyahara, M., Higashitani, K., 2000b. Attraction between hydrophobic surfaces with and without gas phase. Langmuir 16, 5681–5687.

Maoz, R., Sagiv, J., 1984. On the formation and structure of self-assembling monolayers. I. A comparative ATR-wettability study of Langmuir–Blodgett and adsorbed films on flat substrates and glass microbeads. J. Colloid Interface Sci. 100, 465–496.

Meyer, E.E., Lin, Q., Israelachvili, J.N., 2005. Effects of dissolved gas on the hydrophobic attraction between surfactant-coated surfaces. Langmuir 21, 256–259.

Singh, S., Houston, J., van Swol, F., Brinker, C.J., 2006. Superhydrophobicity: drying transition of confined water. Nature 442, 526.

Stevens, H., Considine, R.F., Drummond, C.J., Hayes, R.A., Attard, P., 2005. Effects of degassing on the long-range attractive force between hydrophobic surfaces in water. Langmuir 21, 6399–6405.

Switkes, M., Ruberti, J.W., 2004. Rapid cryofixation/freeze fracture for the study of nanobubbles at solid–liquid interfaces. Appl. Phys. Lett. 84, 4759–4761.

Tyrrell, J.W.G., Attard, P., 2001. Images of nanobubbles on hydrophobic surfaces and their interactions. Phys. Rev. Lett. 87, 176104.

Webb, J.T., Pilmanis, A.A., Balldin, U.I., Fischer, J.R., 2005. Altitude decompression sickness susceptibility: influence of anthropometric and physiologic variables. Aviat. Space Environ. Med. 76, 547–551.

Wienke, B.R., 1990. Reduced gradient bubble model. Int. J. Biomed. Comput. 26, 237–256.

Yang, S., Dammer, S.M., Bremond, N., Zandvliet, H.J.W., Kooij, E.S., Lohse, D., 2007. Characterization of nanobubbles on hydrophobic surfaces in water. Langmuir 23, 7072–7077.

Yount, D.E., Kunkle, T.D., D'Arrigo, J.S., Ingle, F.W., Yeung, C.M., Beckman, E.L., 1977. Stabilization of gas cavitation nuclei by surface-active compounds. Aviat. Space Environ. Med. 48, 185–191.

Zhang, X.H., Li, G., Maeda, N., Hu, J., 2006. Removal of induced nanobubbles from water/graphite interfaces by partial degassing. Langmuir 22, 9238–9243.