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Blood oxygenation using microbubble suspensions

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Abstract Microbubbles have been used in a variety of fields and have unique properties, for example shrinking collapse, long lifetime, efficient gas solubility, a negatively charged surface, and the ability to produce free radicals. In medicine, microbubbles have been used mainly as diagnostic aids to scan various organs of the body, and they have recently been investigated for use in drug and gene delivery. However, there have been no reports of blood oxygenation by use of oxygen microbubble fluids without shell reagents. In this study, we demonstrated that nano or microbubbles can achieve oxygen supersaturation of fluids, and may be sufficiently small and safe for infusion into blood vessels. Although Po_2 increases in fluids resulting from use of microbubbles were inhibited by polar solvents, normal saline solution (NSS) was little affected. Thus, NSS is suitable for production of oxygen-rich fluid. In addition, oxygen microbubble NSS effectively improved hypoxic conditions in blood. Thus, use of oxygen microbubble (nanobubble) fluids is a potentially effective novel method for oxygenation of hypoxic tissues, for infection control, and for anticancer treatment.

Keywords Oxygenation · Microbubble · Nanobubble · Microbubble fluid · Fluid oxygenation

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Introduction

Microbubbles are miniature gas bubbles, <50 μm in diameter, in liquids, which mostly contain oxygen or air (Kurup and Naik 2010; Qin et al. 2009). Microbubbles have been used in a variety of ways: in soil fermentation and hydroponic plant growth, in aquaculture, for environmental improvement of water and sewage treatment, and in engineering production. Microbubbles have several unique properties (Takahashi et al. 2003, 2005, 2007). They remain relatively stable in water for a long time (they have a long lifetime), or rise very slowly, gradual shrink, and finally collapse (i.e., shrinking collapse), whereas macrobubbles increase in size, rise rapidly, and burst at the water surface. The internal pressure of microbubbles is also much higher than that of the local environment, which accelerates dissolution of the gas into the liquid (i.e., efficient gas solubility). They also have a negatively charged surface, and thus will not merge to form larger bubbles, and the ability to produce free radicals, for example –OH.

The most beneficial property of microbubbles is the highly efficient gas solubility. The mechanism of supersaturation of oxygen gas in water is expressed by the Young–Laplace equation (Takahashi et al. 2007):

$$P = P_l + 2\sigma/r$$

where P is the gas pressure, P_l is the liquid pressure, σ is the surface tension of the liquid, and r is the bubble radius. Inertial gas pressure is higher for smaller bubbles, so the inertial pressure of shrinking microbubbles increases with decreasing size. According to Henry's law, the amount of dissolved gas around the shrinking bubble increases with increasing gas pressure. DP is defined as the inertial increase in gas pressure relative to environmental pressure:

$$DP = 2\sigma/r = 4\sigma/2r = 4\sigma/D$$

where D is the bubble diameter.

For microbubbles 10 μm in diameter, the surface tension of water is 72.8 mN/m at 20 °C and DP is approximately 0.3 atm. However, when the microbubble diameter becomes 1 μm, DP increases tenfold (to approx. 3 atm).

Hypoxia, a condition of inadequate oxygen supply to tissues or the body, can lead to severe tissue damage and can be life threatening. Tissue hypoxia can develop if there is a decrease in cardiac output (ischemic hypoxia), hemoglobin concentration (anemic hypoxia), or oxygen saturation (hypoxic hypoxia), or an increase in the metabolic demands of the body (Abdelsalam and Cheifetz 2010). Hypoxia also induces physiological, cellular, and biochemical responses, which can effect pharmacological metabolism (Taylor and Moncada 2010; Ward et al. 2011, Donovan et al. 2010). Thus hypoxia is the most crucial issue in the treatment of a variety of diseases.

In medicine, microbubbles have been used as diagnostic aids to scan various organs of the body (Badea et al. 2009; Dijkmans et al. 2004; Lapotko 2011) and they have been studied for use in drug delivery and gene delivery (Dijkmans et al. 2004; Lapotko et al. 2011; Juffermans et al. 2004). There have recently been reports on use of microbubbles as local drug-delivery systems, using ultrasound with special shell reagents (Xu et al. 2011; Ferrara et al. 2009). However, biocompatibility of the shell reagents and ultrasound damage to the body are major problems that remain to be solved, as also is microbubble stability (Juffermans et al. 2006). Reports have also focused on special shell reagents for packing oxygen gas into microbubbles to extend the duration of oxygen delivery for injections (Cavalli et al. 2009; Swanson et al. 2010). In clinical applications of oxygen delivery to hypoxic patients, intravenous drip infusion is preferable because a large amount can be administered at once or over a long time.

Commonly used methods for generating microbubble suspensions are mechanical agitation, sonication, and pressurized gas–liquid mixing (microchannel emulsification); these usually result in the formation of microbubbles with wide size distributions. Gas–liquid mixing systems are preferable for generation of large amounts of microbubble suspensions at once, and bubble sizes are smaller. However, blood cannot be used directly in the machine because of blood cell destruction.

Therefore, methods for preparing fluids containing oxygen microbubbles (oxygen microbubble fluids) from conventional clinical fluids by use of gas–liquid mixing systems will be useful. There are no reports of blood oxygenation by use of oxygen microbubble fluids without shell reagents. This study was performed to determine:

- whether microbubbles could be used to efficiently increase the level of dissolved oxygen in liquids;
- how dissolved molecules such as electrolytes (e.g., NaCl) or glucose affect oxygenation of liquids; and
- whether oxygen microbubble fluid can improve oxygenation of hypoxic blood.

We found that normal saline solution (NSS) containing oxygen microbubbles (OMNSS) improves hypoxic conditions in blood.

Methods

Liquid solutions and blood samples

Sodium chloride and glucose (Wako Pure Chemical Industries, Osaka, Japan) were dissolved in ultrapure water as 1–10 % and 5–20 % solutions, respectively.

Approximately 100 ml blood was collected in a blood bag for autologous blood transfusion (Terumo CPDA blood bag, 200 ml; Terumo, Tokyo, Japan), from the ear vein of a healthy swine under general anesthesia, and stored in a refrigerator at 4 °C for 4 weeks. Each blood sample was taken from the blood bag by use of a disposable syringe (Terumo syringe, 2.5 ml; Terumo, Tokyo, Japan) to avoid contamination with air.

NSS (Otsuka Pharmaceutical, Tokyo, Japan) containing oxygen microbubbles was mixed with blood in dilution ratios of 10, 20, 30, 50 %. The samples were then gently shaken for 3 min before use in experiments.

These experiments were approved as animal experiment "no. 22-5" by the Ethics Review Committee for Animal Experimentation of Okayama University of Science.

Generation of microbubbles

Fine microbubbles of oxygen gas were generated by use of a micro-nanobubble aerator (AS-MA II; Asupu, Shizuoka, Japan), with hydrodynamic function, for 15 min, during which time oxygen gas was supplied at 1 l/min (Fig. 1). In the apparatus, water or another liquid (150 ml), introduced by a pump, spirals up along a wall and down to an outlet along the center of the apparatus. The centrifugal force

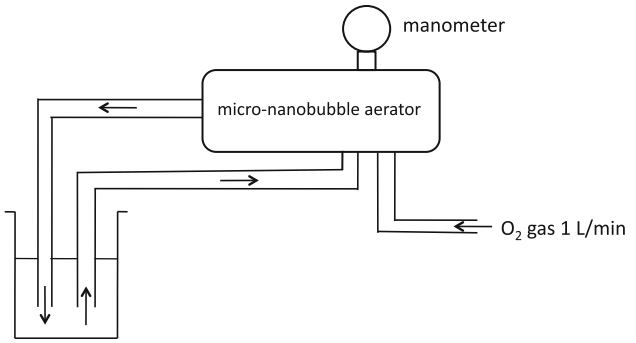

Fig. 1 Experimental setup. Relatively fine microbubbles of oxygen gas were generated by use of a micro-nanobubble aerator, at a peak pressure of 1–1.5 MPa, through which water or another liquid (150 ml) was circulating, for 15 min, and to which oxygen gas was supplied at 1 l/min. Fine microbubbles of oxygen gas were generated after brief sonication

caused by the circulation automatically introduces oxygen gas from the gas inlet and a vortex of oxygen gas is formed along the central axis. The body of the oxygen gas is separated into fine bubbles at the outlet of the apparatus by the strong shearing force of the dispersed water/liquid and circulation power. After generating water/liquid with oxygen gas microbubbles, the fluid was exposed to an ultrasonic bath (AUC-1L; As One, Osaka, Japan) for a few seconds to eliminate relatively large microbubbles.

For comparison purposes, macrobubbles were generated by use of an aquarium air stone (Round Air Stone S-2S; Daiko, Nagoya, Japan), to which oxygen gas was supplied at 1 l/min.

Morphological analysis of oxygen microbubbles generated in liquid

After brief sonication each 10 μm of the ultrapure water containing oxygen microbubbles was immediately mounted on a dual chamber slide (TC10^{TM} System Sample Slides; Bio-Rad, Hercules, CA, USA). The oxygen microbubbles were then captured by microscopy (BZ-8100; Keyence, Tokyo, Japan), morphologically analyzed, and the bubble sizes measured.

Evaluation of dissolved oxygen in liquid or blood samples

After application of brief sonication, each fluid containing oxygen microbubbles was left for 30 s. The dissolved oxygen partial pressure (Po_2) in each liquid and blood sample mixed gently with NSS containing oxygen microbubbles for 3 min was then immediately measured, as an index of oxygenation, by use of a blood gas analyzer (ABL510; Radiometer, Copenhagen, Denmark). Each sample was taken by use of a disposable syringe to avoid contamination with air at room temperature (25 °C).

Statistical analysis

Results from the experiments are shown as the mean ± standard error of the mean (SEM). Statistical analysis was performed using StatMate III software (ATMS, Tokyo, Japan). Means were compared by use of unpaired *t* tests, or by analysis of variance (ANOVA) and Tukey's post-hoc test. In all analyses, $P < 0.05$ was taken as indicative of statistical significance.

Results

Generation of microbubbles

To confirm the generation of fine microbubbles by the micro-nanobubble aerator, ultrapure water containing fine oxygen microbubbles was morphologically analyzed by microscopy.

Figure 2a shows the distribution of oxygen microbubbles in ultrapure water after circulation through the micro-nanobubble aerator—96.2 % of the generated oxygen bubbles were less than 50 μm in diameter (microbubble). Most (35.7 %) microbubbles were 20–30 μm in diameter, and a significant amount (26.2 %) of relatively fine microbubbles less than 20 μm in diameter was also present.

Figure 2b shows the fine oxygen microbubbles generated after brief sonication. Bubbles were less than 10 μm in diameter and inhomogeneous (ranging from <1 to 6 μm in diameter). Some fine microbubbles were seen to shrink and collapse during morphological analysis.

Evaluation of dissolved oxygen in liquids containing macrobubbles and microbubbles

To examine the potency of microbubbles for dissolving oxygen gas in liquid, the Po_2 in ultrapure water was compared between macrobubbles ($f > 1$ mm) and microbubbles by blood gas analysis. Figure 3 shows the Po_2 values in ultrapure water. Means were compared by ANOVA and Tukey's post-hoc test. On average, oxygen gas was dissolved with Po_2 of 170 ± 5.6 mmHg (mean ± SEM) in the control ($P < 0.01$ vs. macro and microbubbles), 776.8 ± 19.3 mmHg in ultrapure water exposed to oxygen macrobubbles ($P < 0.01$ vs. control and microbubbles), and 1,003.2 ± 25.5 mmHg in ultrapure water containing oxygen microbubbles ($P < 0.01$ vs. control and macrobubbles). These results suggest that oxygen microbubbles could significantly increase the Po_2 values in ultrapure water compared with macrobubbles.

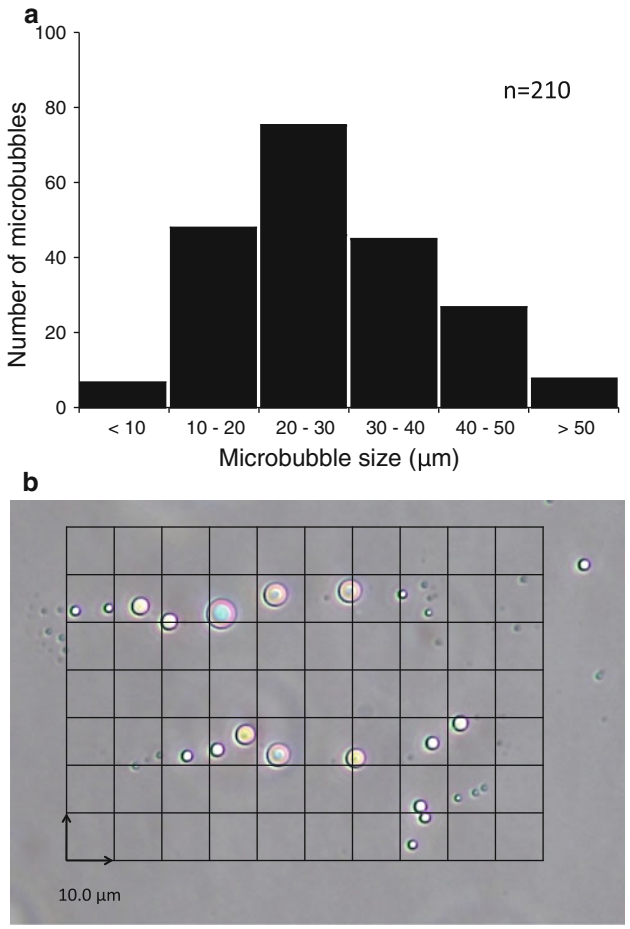

Fig. 2 Morphological analysis of generated oxygen microbubbles. **a** The distribution of the microbubbles generated by the micro-nanobubble aerator. **b** Microbubbles were inhomogeneous in size. After brief sonication, relatively large microbubbles disappeared and fine microbubbles remained. The bubbles were <6 μm in diameter and nanobubbles were also present

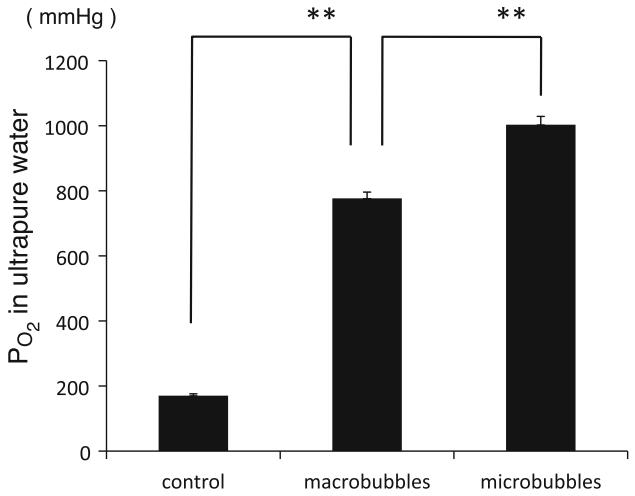

Fig. 3 Comparison of Po_2 increase in ultrapure water between oxygen macrobubbles and oxygen microbubbles. Oxygen macrobubbles were generated in ultrapure water (150 ml) by use of porous ceramic with 1 l/min oxygen gas supply for 15 min. Oxygen microbubbles were generated by use of a micro-nanobubble aerator. The Po_2 in ultrapure water was determined by blood gas analysis. Results are shown as mean ± standard error of the mean from five separate experiments, each performed in duplicate. $^{**}P < 0.01$

Effects of solvents on the Po_2 increase by microbubbles

To examine the effect of solvent on the Po_2 increase after microbubble treatment, the Po_2 in ultrapure water containing a 1, 5, or 10 % NaCl, an electrolyte, or a 5, 10, or 20 % glucose, were measured by blood gas analysis. Means were compared by ANOVA and Tukey's post-hoc test.

Figure 4a shows the effects of NaCl on Po_2 increase by use of oxygen microbubbles. On average, Po_2 values were 1,003.2 ± 25.5 mmHg in the control ($P < 0.01$ vs. 5 % and 10 % NaCl solution), 985.6 ± 27.1 mmHg in 1 % NaCl solution ($P < 0.01$ vs. 5 % and 10 % NaCl solution), 829.5 ± 18.6 mmHg in 5 % NaCl solution ($P < 0.01$ vs. control and 1 % NaCl solution), and 745.8 ± 11.4 mmHg in 10 % NaCl solution ($P < 0.01$ vs. control and 1 % NaCl solution). These results suggested that the oxygenation by microbubbles decreased with increasing concentration of the NaCl solution. Above, less than 1 % NaCl solution was enough to increase the Po_2 in the liquid by use of oxygen microbubbles compared with untreated ultrapure water.

Figure 4b shows the effects of glucose on the Po_2 increase by use of oxygen microbubbles. On average, Po_2 values were 1,003.2 ± 25.5 mmHg in the control ($P < 0.05$ vs. 5 % glucose solution, $P < 0.01$ vs. 10 and 20 % glucose solution), 866.3 ± 38.6 mmHg in 5 % glucose solution ($P < 0.05$ vs. control), 828.3 ± 17.8 mmHg in 10 % glucose solution ($P < 0.01$ vs. control), and 763.8 ± 29.8 mmHg in 20 % glucose solution ($P < 0.01$ vs. control). The results suggested that oxygenation by microbubbles decreased with increasing concentration of glucose. Even 5 % glucose solution significantly inhibited the Po_2 increase by oxygen microbubbles.

Blood oxygenation by use of oxygen microbubble fluid

To examine the potency of blood oxygenation by fluid containing oxygen microbubbles, OMNSS was mixed with swine venous blood in different ratios. After mixing for 3 min at dilution ratios of 10–50 %, the Po_2 in blood samples was measured by blood gas analysis.

Figure 5a shows Po_2 values in blood diluted with 10, 20, 30, or 50 % OMNSS or NSS. Means were compared by use of unpaired *t*-tests. The average Po_2 in control blood (without dilution) was 64.6 ± 1.3 mmHg. Average Po_2 values were 81.9 ± 3.3 mmHg and 72.4 ± 1.5 mmHg in blood diluted with 10 % OMNSS/NSS ($P < 0.05$), 89.5 ± 4.2 mmHg and 78.9 ± 2.5 mmHg in blood diluted

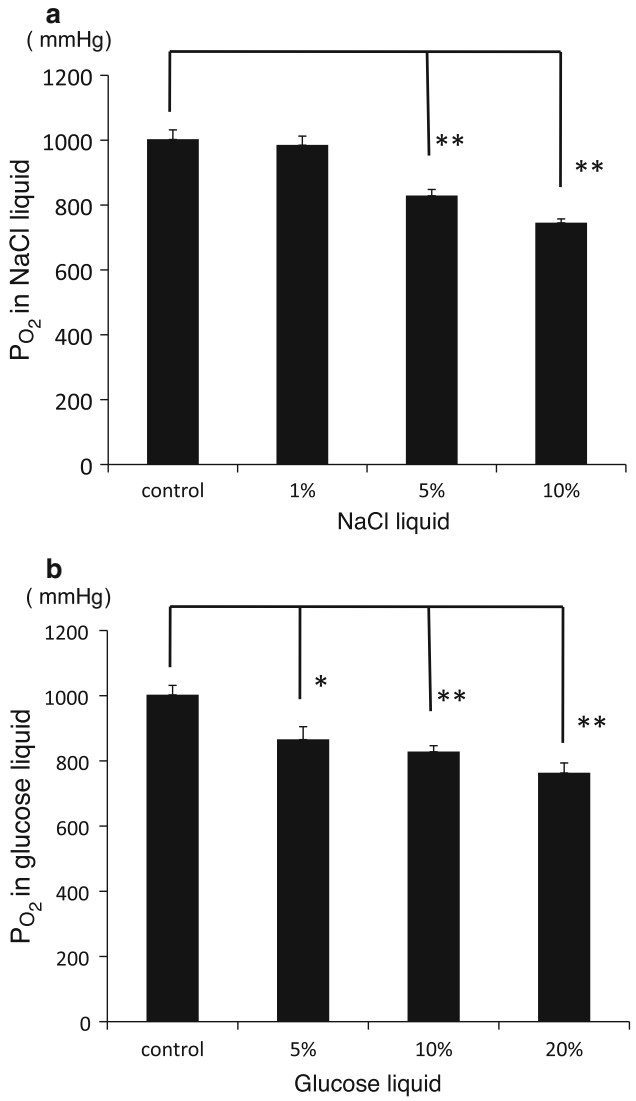

Fig. 4 Effects of solutes on the Po_2 increase in fluid resulting from use of oxygen microbubbles. **a** NaCl. **b** Glucose. Results are mean ± standard error of the mean from five separate experiments, each performed in duplicate. *$P < 0.05$; **$P < 0.01$

with 20 % OMNSS/NSS ($P < 0.05$), 110.4 ± 8.9 mmHg and 82.1 ± 4.1 mmHg in blood diluted with 30 % OMNSS/NSS ($P < 0.05$), and 166.6 ± 25.3 mmHg and 106.0 ± 13.1 mmHg in blood diluted with 50 % OMNSS/NSS ($P < 0.05$), respectively. These results suggest that OMNSS results in a significantly greater Po_2 increase in blood than NSS, and the difference in Po_2 value between OMNSS and NSS becomes greater with increasing dilution ratio in the blood.

Figure 5b shows Po_2 values at 10 % dilution with NSS with and without oxygen microbubbles in blood. Means were compared by ANOVA and Tukey's post-hoc test. On average, the Po_2 values were 64.6 ± 1.4 mmHg in control venous blood ($P < 0.05$ vs. 10 % OMNSS), 72.4 ± 1.5

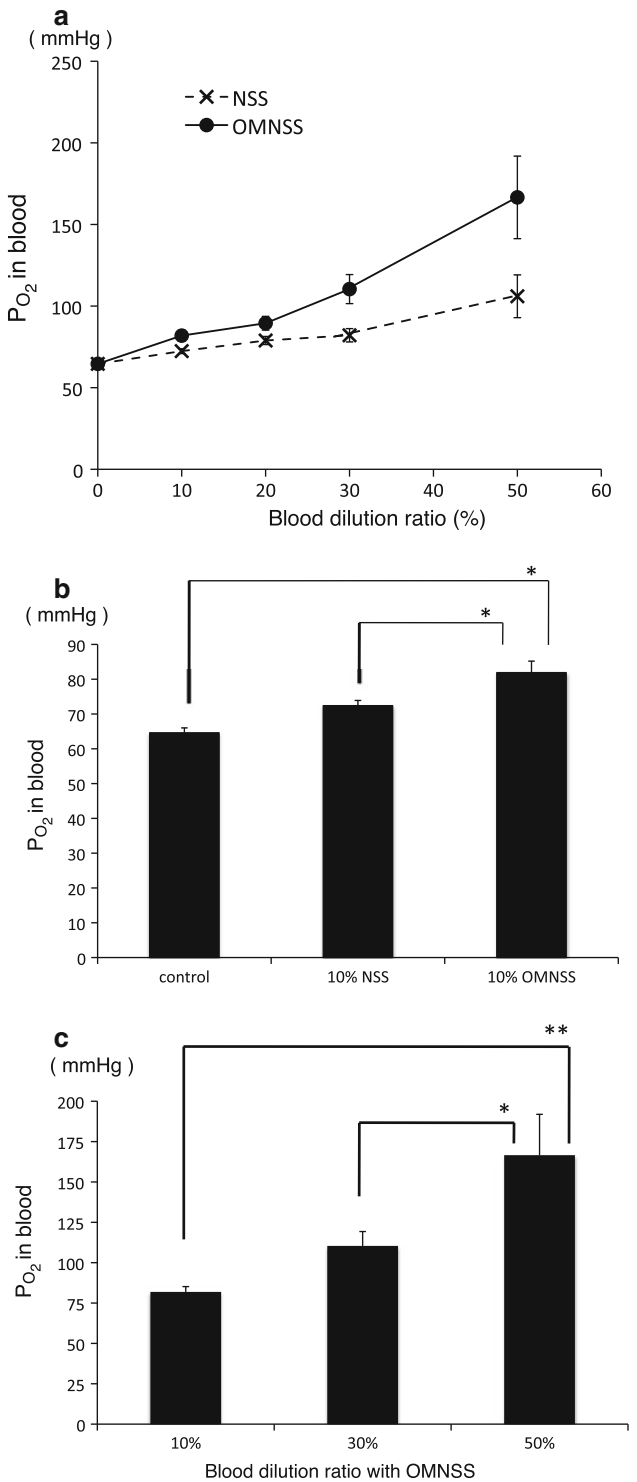

Fig. 5 Po_2 increases in blood by use of oxygen microbubble NSS (OMNSS). Swine venous blood was diluted with OMNSS at 10, 20, 30, or 50 %, and mixed gently for 3 min. The Po_2 in blood was determined by blood gas analysis. **a** Comparison of Po_2 increases in blood between NSS and OMNSS. **b** Comparison of Po_2 increases in blood at 10 % dilution with NSS or OMNSS. **c** Po_2 increases in blood at each dilution ratio with OMNSS. Results are shown as mean ± standard error of the mean from five separate experiments, each performed in duplicate. *$P < 0.05$; **$P < 0.01$

mmHg in blood diluted 10 % with NSS ($P < 0.05$ vs. 10 % OMNSS), and 81.9 ± 3.3 mmHg in blood diluted 10 % with OMNSS ($P < 0.05$ vs. control and 10 % diluted blood with NSS). The results suggested that OMNSS significantly improved the Po_2 in hypoxic blood.

Figure 5c shows Po_2 values in blood diluted with 10, 30, or 50 % OMNSS. Means were compared by ANOVA and Tukey's post-hoc test. The average Po_2 values were 81.9 ± 3.3 mmHg in blood diluted with 10 % OMNSS ($P < 0.01$ vs. 50 %), 110.4 ± 8.9 mmHg in blood diluted with 30 % OMNSS ($P < 0.05$ vs. 50 %), and 166.6 ± 25.3 mmHg in blood diluted with 50 % OMNSS ($P < 0.01$ vs. 10 %, $P < 0.05$ vs. 30 %). These results suggested that Po_2 in hypoxic blood was significantly increased by increasing the mixing volume of OMNSS. In addition, blood diluted with 50 % OMNSS was significantly more oxygenated than 10 % diluted blood ($P < 0.05$).

Discussion

Microbubbles are gas bubbles <50 μm diameter in liquids; they have a variety of unique properties (Kurup and Naik 2010; Qin et al. 2009). In particular, their efficient and high oxygen gas solubility is beneficial for oxygenation of hypoxic tissues (Bitterman 2009; Abdelsalam and Cheifetz 2010; Raoof et al. 2010; Guo and DiPietro 2010).

In our experiments, most of the oxygen microbubbles generated after application of brief sonication were <10 μm in diameter (Fig. 2b), and were categorized as fine microbubbles (Takahashi et al. 2003, 2005, 2007). These fine microbubbles were thought to be captured as the result of the gradual shrinking of microbubbles that finally collapse. Fine oxygen microbubbles are expected to have a variety of useful properties in medicine (Barbosa et al. 2009; Betit 2009; Kulikovsky et al. 2009). Microbubbles <10 μm in diameter are thought to be clinically safe; the filter pore sizes for cardiopulmonary bypass machines are usually in the range 28–40 μm, and oxygen microbubbles 10 μm in diameter are therefore negligibly small (Barak and Katz 2005).

The most beneficial property of microbubbles is the highly efficient gas solubility, which is expressed as Po_2 increases in liquids including blood. The Po_2 values in water containing oxygen microbubbles were significantly higher than the theoretical Po_2 value (760 mmHg). According to the Young–Laplace equation, the shrinking oxygen microbubble increases the inertial pressure and oxygen gas dissolves to a much greater extent water by Henry's law. The smaller the bubble size, the higher the Po_2 value in water. In our experiments, Po_2 values in water were approximately 30 % greater than full oxygen saturation (Fig. 3). Nanobubbles must increase the Po_2 value in water to a much greater extent than microbubbles.

Next, we examined how solutes in the fluid, for example NaCl and glucose, which are commonly used in daily clinical practice, affect the increase in Po_2 by microbubbles. NaCl and glucose both inhibited the Po_2 increase by microbubbles, in accordance with their concentration (Fig. 4a). As oxygen gas has low polarity and tends to dissolve in low-polarity liquids, our results indicating that Po_2 in water was reduced by NaCl or glucose, which are polar in solution, are credible. However, NSS used in daily clinical practice is of very low concentration (only 0.9 %) and had little effect on the Po_2 values compared with the control. Therefore, NSS containing microbubbles can be a clinically useful fluid for tissue oxygenation. The Po_2 values in 5 % glucose solution were significantly (~15 %) reduced compared with the control. Twenty percent glucose solution, which is used in intravenous hyperalimentation, had almost the same Po_2 as the theoretical maximum Po_2 value in water (Fig. 4b). Thus, fluids containing glucose are not suitable for tissue oxygenation using this method.

OMNSS increased the Po_2 values in blood in accordance with the mixing volume (ratio) (Fig. 5a). At 10 % dilution in blood, OMNSS resulted in significantly higher Po_2 than NSS (Fig. 5b). The oxygen volume in blood is expressed as follows (Scholz et al. 2010):

$$\text{Total } Co_2 = (a \cdot Po_2 + b \cdot Hb \cdot \text{Sat}) \cdot V$$

where a is the solubility coefficient of oxygen in blood (0.003 ml/l mmHg), b is the oxygen-carrying capacity of hemoglobin (1.34 ml/gHb), Hb is the concentration of hemoglobin in blood (15 g/dl), V is blood volume (5 l), and Sat is the saturation of hemoglobin.

In addition, Sat in blood under conditions of pH 7.4 at 37 °C is expressed as follows (Dash and Bassingthwaighte 2010):

$$\begin{aligned} Sat &= Ko_2 \cdot Po_2^n / (1 + Ko_2 \cdot Po_2^n) \\ &= (Po_2/P_{50})^n \{1 + (Po_2/P_{50})^n\} \end{aligned}$$

where Ko_2 is the Hill coefficient and n is the Hill exponent ($n = 2.7$). They are related by $Ko_2 = (P_{50})^{-n}$ where P_{50} is the level of Po_2 at which Hb is 50 % saturated by O_2 ($P_{50} = 26.6$ mmHg) (Goutelle et al. 2008).

In this study, the mean Po_2 of the control blood (64.6 mmHg) was improved from 81.6 to 166.6 mmHg by dilution with OMNSS. Given these data at 37 °C, the saturation value of hemoglobin was calculated to be 91.3 % at $Po_2 = 64.6$ mmHg, 95.2 % at $Po_2 = 81.6$ mmHg, and 99.3 % at $Po_2 = 166.6$ mmHg. The total Co_2 was calculated as 927 ml for control blood, 970.8 ml for blood diluted with 10 % OMNSS, and 1004.3 for blood diluted with 50 % OMNSS. The value of total Co_2 for blood diluted with 10 % OMNSS was 43.8 ml higher than that of the control blood; this oxygen excess corresponds to

87.6 % of the oxygen consumption of the brain per minute in adults. In addition, the value of total Co_2 in blood diluted with 50 % OMNSS was 121 ml higher than that of the control blood; this oxygen excess corresponds to 48.4 % of the oxygen consumption of the whole body per minute in adults. Therefore, the oxygen microbubble fluid described here can be locally injected into hypoxic tissues but is not sufficient for general infusion. For general infusion, it will be necessary to achieve an approximately tenfold higher density of oxygen microbubbles. This will require more innovative methods for production of finer microbubbles (nanobubbles) or shell reagents for bubbles.

In summary, finer micro-nanobubbles can be used to achieve oxygen supersaturation in fluids, and may be small and safe enough for infusion into blood vessels. Although increases in Po_2 in fluids by use of oxygen microbubbles were inhibited by polar solvents, NSS had little effect. Thus, NSS will be suitable for production of oxygen-rich fluid. In addition, OMNSS effectively improved hypoxic conditions in blood. Use of oxygen micro/nanobubble fluids is an effective novel method for oxygenation of hypoxic tissues caused by ischemia and general hypoxia, for infection control caused by anaerobic bacteria, and for anticancer treatment. However, it has been suggested that microbubbles also cause tissue damage and that oxygen itself can be toxic (Barak and Katz 2005; Dennery 2010; Wang et al. 2010; Allen et al. 2009). Further studies of fine micro/nanobubbles are required to increase the Po_2 under hypoxic conditions and to assess the effects on tissues and the whole body.

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References

Abdelsalam M, Cheifetz IM (2010) Goal-directed therapy for severely hypoxic patients with acute respiratory distress syndrome: permissive hypoxemia. Respir Care 55(11):1483–1490

Allen BW, Demchenko IT, Piantadosi CA (2009) Two faces of nitric oxide: implications for cellular mechanisms of oxygen toxicity. J Appl Physiol 106(2):662–667

Badea R, Seicean A, Diaconu B, Stan-Iuga R, Sparchez Z, Tantau M, Socaciu M (2009) Contrast-enhanced ultrasound of the pancreas: a method beyond its potential or a new diagnostic standard? J Gastrointestin Liver Dis 18(2):237–242

Barak M, Katz Y (2005) Microbubbles: pathophysiology and clinical implications. Chest 128(4):2918–2932

Barbosa FT, Juca MJ, Castro AA, Duarte JL, Barbosa LT (2009) Artificial oxygen carriers as a possible alternative to red cells in clinical practice. Sao Paulo Med J 127(2):97–100

Betit P (2009) Extracorporeal membrane oxygenation: Quo Vadis? Respir Care 54(7):948–957

Bitterman H (2009) Bench-to-bedside review: oxygen as a drug. Crit Care 13(1):205

Cavalli R, Bisazza A, Giustetto P, Civra A, Lembo D, Trotta G, Guiot C, Trotta M (2009) Preparation and characterization of dextran nanobubbles for oxygen delivery. Int J Pharm 381(2):160–165

Dash RK, Bassingthwaighte JB (2010) Erratum to: blood HbO_2 and $HbCO_2$ dissociation curves at varied O_2, CO_2, pH, 2,3-DPG and temperature levels. Ann Biomed Eng 38(4):1683–1701

Dennery PA (2010) Oxygen administration in the care of neonates: a double-edged sword. Chin Med J 123(20):2938–2942

Dijkmans PA, Juffermans LJ, Musters RJ, van Wamel A, ten Cate FJ, van Gilst W, Visser CA, de Jong N, Kamp O (2004) Microbubbles and ultrasound: from diagnosis to therapy. Eur J Echocardiogr 5(4):245–256

Donovan L, Welford SM, Haaga J, LaManna J, Strohl KP (2010) Hypoxia: implications for pharmaceutical developments. Sleep Breath 14(4):291–298

Ferrara KW, Borden MA, Zhang H (2009) Lipid-shelled vehicles: engineering for ultrasound molecular imaging and drug delivery. Acc Chem Res 42(7):881–892

Goutelle S, Maurin M, Rougier F, Barbaut X, Bourguignon L, Ducher M, Maire P (2008) The Hill equation: a review of its capabilities in pharmacological modeling. Fundam Clin Pharmacol 22(6):633–648

Guo S, DiPietro LA (2010) Factors affecting wound healing. J Dent Res 89(3):219–229

Juffermans LJ, Dijkmans PA, Musters RJ, van Wamel A, Bouakaz A, ten Cate FJ, Deelman L, Visser CA, de Jong N, Kamp O (2004) Local drug and gene delivery through microbubbles and ultrasound: a safe and efficient alternative for viral vectors? Neth Heart J 12(9):394–399

Juffermans LJ, Dijkmans PA, Musters RJ, Visser CA, Kamp O (2006) Transient permeabilization of cell membranes by ultrasound-exposed microbubbles is related to formation of hydrogen peroxide. Am J Physiol Heart Circ Physiol 291(4):H1595–H1601

Kulikovsky M, Gil T, Mattanes I, Karmeli R, Har-Shai Y (2009) Hyperbaric oxygen therapy for non-healing wounds. Isr Med Assoc J 11(8):480–485

Kurup N, Naik P (2010) Microbubbles, A novel delivery system. J Pharmaceut Res Health Care 2(3):228–234

Lapotko D (2011) Plasmonic nanobubbles as tunable cellular probes for cancer theranostics. Cancers 3(1):802–840

Qin S, Caskey CF, Ferrara KW (2009) Ultrasound contrast microbubbles in imaging and therapy: physical principles and engineering. Phys Med Biol 54(6):R27–R57

Raoof S, Goulet K, Esan A, Hess DR, Sessler CN (2010) Severe hypoxemic respiratory failure: part2-nonventilatory strategies. Chest 137(6):1437–1448

Scholz AW, Eberle B, Heussel CP, David M, Schmittner MD, Quintel M, Schreiber LM, Weiler N (2010) Ventilation-perfusion ratio in Perflubron during partial liquid ventilation. Anesth Analg 110(6):1661–1668

Swanson EJ, Mohan V, Kheir J, Borden MA (2010) Phospholipid-stabilized microbubble foam for injectable oxygen delivery. Langmuir 26(20):15726–15729

Takahashi M (2005) The ς potential of microbubbles in aqueous solutions, Electrical property of the gas-water interface. J Phys Chem B 109(46):21858–21864

Takahashi M, Kawamura T, Yamamoto Y, Ohnari H, Himuro S, Shakutsui H (2003) Effect of shrinking microbubble on gas hydrate formation. J Phys Chem B 107(10):2171–2173

Takahashi M, Chiba K, Li P (2007) Free-radical generation from collapsing microbubbles in the absence of a dynamic stimulus. J Phys Chem B 111(6):1343–1347

Taylor CT, Moncada S (2010) Nitric oxide, cytochrome C oxidase, and the cellular response to hypoxia. Arterioscler Thromb Vasc Biol 30(4):643–647

Wang C, Zhang X, Liu F, Paule MG, Slikker W Jr (2010) Anesthetic-induced oxidative stress and potential protection. Sci World J 10:1473–1482

Ward DS, Karan SB, Pandit JJ (2011) Hypoxia: developments in basic science, physiology and clinical studies. Anaesthesia 66(Suppl 2):19–26

Xu Q, Nakajima M, Liu Z, Shiina T (2011) Biosurfactants for microbubble preparation and application. Int J Mol Sci 12(1):462–475