Review of Oxygenation with Nanobubbles: Possible Treatment for Hypoxic COVID-19 Patients
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ABSTRACT: The coronavirus disease (COVID-19) pandemic, which has spread around the world, caused the death of many affected patients, partly because of the lack of oxygen arising from impaired respiration or blood circulation. Thus, maintaining an appropriate level of oxygen in the patients’ blood by devising alternatives to ventilator systems is a top priority goal for clinicians. The present review highlights the ever-increasing application of nanobubbles (NBs), miniature gaseous vesicles, for the oxygenation of hypoxic patients. Oxygen-containing NBs can exert a range of beneficial physiologic and pharmacologic effects that include tissue oxygenation, as well as tissue repair mechanisms, antiinflammatory properties, and antibacterial activity. In this review, we provide a comprehensive survey of the application of oxygen-containing NBs, with a primary focus on the development of intravenous platforms. The multimodal functions of oxygen-carrying NBs, including antimicrobial, antiinflammatory, drug carrying, and the promotion of wound healing are discussed, including the benefits and challenges of using NBs as a treatment for patients with acute hypoxemic respiratory failure, particularly due to COVID-19.

KEYWORDS: nanobubbles, COVID-19, oxygenation, antimicrobial activity, hypoxia, antiviral activity

1. INTRODUCTION
At the present time, humans are faced with an ongoing pandemic outbreak of a new coronavirus responsible for a respiratory illness caused by “severe acute respiratory syndrome coronavirus 2” (SARS-CoV-2), known as COVID-19. The SARS-CoV-2 virus directly infects the alveoli in the lungs, where oxygen is transferred from the air spaces into the blood vessels; therefore, most infected patients have low blood oxygen saturation pressure (SpO2) of <90% and impaired blood microcirculation, resulting in a condition called hypoxia. In general, hypoxia, or low tissue oxygen tension, can be caused by various pathologies ranging from inflammation to cancer. Normal oxygen pressure depends on the tissue type, although for normal tissues, the physiological range is between 4 and 8% of the atmospheric oxygen level. Within the cells, this is even lower, ranging from 1.3 to 2.5%, while in most tissues, cellular hypoxia occurs when it is reduced below 2%. On the other hand, this virus produces a phenomenon called “silent hypoxia”, wherein patients can function relatively normally but are measured with much lower oxygen levels than anticipated (~50–80% saturation, while the normal saturation level is 95% or higher). In this situation, their lungs do not fully oxygenate the blood, although these patients claim to feel almost well, to the extent that doctors debate whether to intubate them or not. Therefore, deficient oxygen levels are often found in COVID-19 patients and result in subsequent adverse clinical outcomes if left untreated.

Because the sustained hypoxia caused by COVID-19 can cause major health problems, like compromised cellular aerobic metabolism, increased levels of DNA breaks, accumulation of DNA replication errors, instability and mutagenesis of the genome, brain damage, and cardiac cell death and arrest, the administration of oxygen therapy is required. More than 75% of COVID-19 hospitalized patients require supplemental oxygen therapy. At the first stage, pure oxygen breathing through nasal tubes or a facemask is usually employed. In more critical cases, increasing the supply of oxygen using mechanical lung ventilation, noninvasive positive-pressure ventilation, or other invasive methods, such as extracorporeal membranous oxygenation (ECMO), becomes necessary. Because of the lack of ventilation equipment in...
many health care centers, the development of alternative techniques is an urgent critical care challenge in the supply of medical equipment. In this regard, some cutting-edge technologies such as hyperbaric oxygen therapy, high-flow nasal cannula,13 and the intravenous (IV) delivery of liquid oxygen carriers, such as ultrafine oxygen nanobubbles (ONBs), have been considered in this field.

Nanobubbles (NBs) are spherical stable gas-containing vesicles suspended in an aqueous solution, which were directly observed in 2000 for the first time by atomic force microscopy.14 Later in 2004, NBs were investigated as ultrasound (US) contrast agents.15 What makes NBs superior to all other scalable gas-transfer platforms is their ability to remain suspended in liquid in view of their negative surface potential, meaning they neither disappear nor coalesce with each other and continue to transfer gases throughout the liquid volume until they collapse.16 Their elevated properties, for example, nanosized, high surface-to-volume ratio, high inner pressure, prolonged stability, electrostatic charge properties, acoustic properties, and biocompatibility, have led researchers to investigate this platform for biomedical applications.17 Interestingly, NBs exhibit particularly exogenous stimulus-responsive characteristics, responding to environmental or external triggers, namely, US waves, mechanical forces, light stimuli, temperature changes, electric and magnetic fields, or endogenous stimuli like pH, redox, or multimodal stimuli.18 In particular, US-responsive NBs have been suggested as favorable carriers for organ/cell oxygenation.

Intravascular oxygen delivery using ONBs is an emerging technology to deal with hypoxic cells and tissues that has been investigated in cancer therapy and diseases with an impairment in microcirculation systems.19 Remarkably, the possibility of utilizing these platforms in clinical practice no longer appears to be beyond reach. Stable ONBs have been prepared with some advantages, such as high gas solubility and long-lived retention of oxygen, which could significantly increase the oxygen concentration in aqueous fluids without the formation of any macrobubbles.20 These ONBs could act as a blood oxygenation platform that only needs a limited volume of IV fluid injection without gas bubble formation, thus preserving the function of the organs.

COVID-19 can also weaken the immune systems of patients, thereby allowing the development of superimposed infectious diseases caused by other viruses, bacteria, or fungi. In observational studies, ~8% of COVID-19 hospitalized patients experience a bacterial or fungal coinfection.21 Additionally,
hypoxia could hasten bacterial growth and promote microbial resistance to drugs and remedies by preventing the delivery of sufficient oxygen to the tissues needed to overcome bacterial infections. The emergence of NBs and their burgeoning biomedical applications for antimicrobial, antiinflammatory, and wound-healing effects has been a significant development. Therefore, the NBs could carry out a simultaneous multifunctional role in the treatment of COVID-19 patients. These functions include increased oxygenation to cells, down-regulation of hypoxia-inducible factor-α protein (HIF-1α), increased adenosine triphosphate (ATP) production and mitochondrial function, prevention of microbial infections, stimulation of wound healing, and the delivery and release of antiviral, antiinfective, and antiinflammatory drugs. Last but not least, this virus could turn into the plague of the century because it may not be controlled unless widely available, safe, and effective vaccines can be rapidly developed and deployed. It should be noted that the virus has already begun to undergo mutations and resurface in other animal hosts, which are circulating globally; therefore, additional treatment methods are urgently required until it is completely stamped out. To combat the COVID-19 pandemic, there is an urgent need to develop improved scientific capabilities, knowledge, and facilities. In recent decades, advances in nanoscience and nanotechnology have addressed many human medical needs by allowing the design of appropriate nanomaterials that can be used for diagnosis, treatment, and biomedical engineering. Nanomedicines could help to overcome the pandemic challenge by treating COVID-19 infection and alleviating the symptoms. On the basis of the World Health Organization (WHO) report, 20% of all COVID-19 patients need oxygen therapy; therefore, the enormous global demand for alternatives to ventilators is a top priority. This need prompted us to review the recent advances on oxygen delivery using Nb as a multipurpose therapeutic approach for treating COVID-19 patients.

1.1. Scope of the Review. The present review provides a summary of the use of intravenous O\textsubscript{2} (IVO\textsubscript{2}) therapy mediated by NBs. Representative methods of measuring the oxygen concentration in diverse tissues and the oxygen requirements for the critical organs that could be infected by the virus, such as lungs, heart, kidneys, liver, blood, and retina, are summarized. The pathophysiology of COVID-19 is briefly discussed for a better understanding the role of ONBs in tissue oxygenation in COVID-19 patients. In addition, a general description of NBs with a focus on the physicochemical features of ONBs, their structure, synthetic procedures, and gas release mechanisms is provided. Next, the potential role, performance, and benefits of NBs for the oxygenation of different tissues through IVO\textsubscript{2} or other administration routes are summarized. The majority of this section discusses the oxygenation of hypoxic cells within malignant tumors because it has been estimated that at least 50–60% of advanced solid tumors encompass regions of hypoxic tissue. This section also considers micro/nanobubbles (MNBs), a phrase referring to a solution containing both microbubbles (MBs) and NBs, as ideal oxygen carriers.

Under in vivo conditions, the oxygen level of tissue is controlled through the HIF-1α transcription factor and the expression of vascular endothelial growth factor (VEGF). The HIF-1α subunit is a principal regulator of the hypoxia response and cellular oxygen homeostasis. HIF-1α responds to oxygen deficiency because of the sensitivity of its molecular structure to the presence of oxygen. Under hypoxic conditions, HIF-1α is not hydroxylated, resulting in its stabilization and its ability to trigger gene expression. While under normoxic conditions, in the presence of normal levels of oxygen in tissues and blood, HIF-1α becomes hydroxylated, followed by polyubiquitination by a complex involving Von Hippel Lindau (VHL), which leads to its degradation in the proteasomes. Under hypoxic conditions, the expression of HIF-1α is elevated, thus inducing the expression of several genes (importantly VEGF) regulated by hypoxia-regulated elements (HREs) to maintain cell survival under low-oxygen conditions by stimulating the formation of new blood vessels in a process known as angiogenesis (Figure 1). Accordingly, a quick decrease in HIF-1α is a direct and rapid physiological consequence of an increase in the partial pressure of oxygen (pO\textsubscript{2}). Consequently, supplying sufficient oxygen to hypoxic tissues could promote the degradation of HIF-1α. On the other hand, there is also a distinct relationship between HIF-1α and inflammation, wherein the down-regulation and inhibition of HIF-1α plays a role in reducing proinflammatory cytokines and preventing lung injury.

COVID-19 patients could run the risk of developing inflammation and further infections (nearly 8% of hospitalized patients) if they have chronic wounds (CWs) caused by diabetes or other chronic diseases or postsurgical wounds after cardiovascular surgery or child birth, and some patients were ventilated noninvasively. In this regard, the multipurpose role of NBs as oxygen carriers, as well as antimicrobial and wound-healing agents, could provide additional benefits, which are also summarized in detail. Finally, the benefits and challenges facing the application of NBs in COVID-19 patients are discussed.

2. BRIEF HISTORY OF IVO\textsubscript{2} THERAPY

In some medical conditions, the blood shows an impaired capacity to transfer oxygen, while, on the other hand, the direct injection of oxygen gas into the bloodstream is limited by the occurrence of hemolysis. Therefore, diverse techniques have been developed to increase in vivo oxygen levels. Oxygen therapy is a valuable therapeutic method that helps to maintain cell metabolism and preserve organ function in various clinical situations, especially in acute respiratory diseases, where the restoration of normal tissue oxygenation is needed. One of the major challenges is to overcome hypoxia and hypoxemia using a technology that reduces harm while supplying adequate oxygen in a cost-effective, biocompatible, and user-friendly manner. IVO\textsubscript{2} is an alternative to invasive methods of alveolar gas exchange (such as ECMO), providing direct oxygen delivery through IV administration of a physiologic oxygen solution at concentrations exceeding normal atmospheric pressure to hypoxemic patients. Historically, the first application of IVO\textsubscript{2} using simple gaseous oxygen was introduced by Nysten in the early 19th century. In 1902, the first oxygen administration to a living human via the IV route was performed by Martani. From then until recently, most in vivo and in vitro studies have concentrated on the deployment of IV gaseous oxygen, although this approach has a risk of pulmonary embolism. To address these issues, scientists took advantage of Henry’s law, which expresses that the solubility of dissolved gas in a liquid is directly proportional to the partial pressure of the gas above the liquid. This enabled the design of oxygen-saturated physiological solutions, like normal saline (NSS) or lactated ringers solutions, for injection,
which dates back to 1987.\textsuperscript{58} Using this platform, oxygen can be dissolved under high pressure, and it remains in solution at that partial pressure even with a decrease in the external pressure to the atmospheric level and can circulate within the human body for several hours.\textsuperscript{59} Additionally, by injection of an oxygen-saturated solution into patients, deoxygenated hemoglobin (Hb) can bind to free \( \text{O}_2 \) molecules, thus instigating an increase in \( \text{SpO}_2 \) without any dependence on continuous oxygen delivery in vivo.\textsuperscript{53} The best clinical route involves an IV infusion because of its ability to be delivered in large volumes at one time or spread out over long periods.

The advent of oxygen-filled MBs and NB-based hyperbaric oxygen carriers has led to innovative approaches for the oxygenation of hypoxic tissues.\textsuperscript{60} Oxygen microbubbles (OMBs) have a core-shell structure and can serve as a new route for oxygen delivery to hypoxic tissues. In this regard, the design of stable MBs has focused on varying the shell and surfactant components, as well as reducing surface tension for the preparation of intravascular oxygen-delivery systems.\textsuperscript{61,62}

In one early approach to tissue oxygenation, an aqueous suspension of OMBs was stabilized by dodecafluoropentane (DDFP; \( \text{bp} = 29 \, ^\circ\text{C} \)), which is a volatile perfluorocarbon (PFC), and it was shown to increase the survival of erythrocyte-depleted rats and pigs. The pigs were subjected to potentially lethal hemorrhagic shock with severe right-to-left shunts and treated with a 0.002–0.014 mL/kg body weight of 2\% emulsified DDFP.\textsuperscript{63,64} It was found that lipid-based OMBs showed advantages such as remarkable stability\textsuperscript{64} and extended shelf life,\textsuperscript{65} and they could supply enough oxygen to maintain life for 15 min without any breathing in asphyxiated animals.\textsuperscript{66} However, these OMBs suffered from some limitations such as (a) limited intravascular dwell time due to their large size (>1 \( \mu\text{m} \)), (b) the need for repeated administration caused by an increase in serum viscosity, which could be dangerous to patients, and (c) the risk of long-lasting toxicity to blood and tissue.\textsuperscript{57} Consequently, more stable ONBs that do not undergo dissolution or aggregation would be more clinically useful for curing tissue hypoxia. Several research efforts that have been made in pursuit of this goal are evidence of the efficiency of these nanomaterials.

### 3. ORGAN OXYGEN DEMAND: REQUIRED PARTIAL PRESSURE OF OXYGEN IN TISSUES

Tissue oxygenation is one of the most pivotal procedures in the human body. This process is governed by blood flow, arterial oxygen tension, oxygen-carrying capacity, Hb dissociation conditions, and local oxygen consumption.\textsuperscript{68} It starts with the appropriate uptake of atmospheric oxygen in the lungs, followed by diffusion to the blood, absorption by red blood cells, and then transfer of the oxygenated blood to diverse organs by heart pumping.\textsuperscript{69} The transfer of oxygen to its destination in parenchymal cells occurs once the blood reaches microcirculation through tiny microvessels. Notably, microcirculation is the final and most important step in the regulation of tissue oxygenation.\textsuperscript{70}

Typically, the source of oxygen is environmental air, which contains 21\% oxygen. The \( \text{PO}_2 \) is approximately 160 mmHg at sea level, wherein the atmospheric pressure is 760 mmHg. Notwithstanding, the available \( \text{PO}_2 \) for diffusion within the alveoli of the lung is not the same. Therefore, for calculation of the alveolar oxygen partial pressure, which is estimated to be 99.7 mmHg at sea level without any supplemental oxygenation, the following alveolar gas equation is applied:\textsuperscript{71}

\[
\text{pA}_2 \text{O}_2 = (\text{P}_{\text{atm}} - \text{P}_\text{H,O})\text{F}_\text{O}_2 - \mu_2 \text{CO}_2/RQ
\]

where \( \text{pA}_2 \text{O}_2 \) is the alveoli partial pressure of oxygen, \( \text{P}_{\text{atm}} \) the atmospheric pressure (760 mmHg at sea level), \( \text{F}_\text{H,O} \) the partial pressure of water at the patient’s body temperature (\( \approx 47 \) mmHg at \( 37 \, ^\circ\text{C} \)), \( \text{F}_\text{O}_2 \) the fraction of inspired oxygen (21\% or 0.21 at sea level without support, while each liter of supplemental oxygen increases this value by \( \approx 4\% \) or 0.04), \( \mu_2 \text{CO}_2 \) the alveoli carbon dioxide partial pressure (\( \approx 40–45 \) mmHg in normal physiological conditions), and RQ the respiratory quotient, depending on the diet and metabolic condition of the individual (0.82 for a typical human diet).

\( \text{pA}_2 \text{O}_2 \) is the motive force for oxygen diffusion across the alveolar membranes, through pulmonary capillary walls, and into the arteriolar blood flow for circulation in the whole body. Gas diffusion from alveoli to capillaries is driven by the gradient in \( \text{pO}_2 \) between alveoli (\( \text{pA}_2 \text{O}_2 \)) and the surrounding capillaries, which are measured through the following A-a gradient formula:

\[
\text{A-a oxygen gradient} = \text{pA}_2 \text{O}_2 - \text{pO}_2
\]

where \( \text{pA}_2 \text{O}_2 \) is quantified using an arterial blood gas.

A larger gradient demonstrates that oxygen transfer into the capillary is inhibited by the pathological state. This phenomenon could affect the available \( \text{pO}_2 \) throughout the body.

Body organs and tissues vary in their oxygen dependency, but the physiological range of oxygen is 4–8\%, equal to \( \text{pO}_2 \) of 40–60 mmHg, substantially less than the atmospheric level of 21\%. This amount is even lower within cells, ranging from 1.3 to 2.5\%. By a decrease of the extracellular \( \text{pO}_2 \) levels to 2\% (10 mmHg) or less, hypoxia occurs in vitro. Below \( \sim 1\% \) \( \text{pO}_2 \), cellular ATP levels descend and apoptosis can be triggered.\textsuperscript{72}

Specific tissue oxygenation is associated with systemic oxygen delivery to the organs and tissues per minute (DO\(_2\)) and systemic arterial blood pressure. \( \text{DO}_2 \) (mL/min) can be calculated as\textsuperscript{73}

\[
\text{DO}_2 = \text{CO} \times C_2 \text{O}_2
\]

where \( \text{CO} \) is the cardiac output or SBF (L/min or L/min/m\(^2\)) and \( C_2 \text{O}_2 \) the sum of the quantity of oxygen bound to Hb and oxygen dissolved in the plasma in arterial blood (mL/100 mL blood).

The oxygen content of arterial blood (mL of \( \text{O}_2/\text{DL} \) blood) can be calculated as

\[
C_4 \text{O}_2 = S_4 \text{O}_2 \times \text{Hgb} \times 1.34 + p_4 \text{O}_2 \times 0.0031
\]

where \( S_4 \text{O}_2 \) is the arterial oxygen saturation, \( \text{Hgb} \) is the Hb concentration (g/\text{DL}), Constant\(^1 = 1.34 \) is the amount of oxygen attached per gram of Hb (mL) at 1 atm, \( p_4 \text{O}_2 \) is the arterial oxygen partial pressure, and Constant\(^2 = 0.0033 \) multiplied by \( p_4 \text{O}_2 \) equals the amount of oxygen dissolved in plasma at 1 atm.

The oxygen concentration in the organs varies depending on the metabolic activity of each organ. For instance, the oxygen concentration of the human brain is between 0.5 and 8\% with \( \text{pO}_2 \) of 4.1–60 mmHg. The heart has a value of 5–14\%, and the lung alveoli are supplied with oxygen levels of 13–14\% and \( \text{pO}_2 \) of 110 mmHg. Other organs, such as the liver and kidneys, show a value of 4–14\%, and by contrast, in the skin, oxygen only reaches 1\% and \( \text{pO}_2 \) of 5–11 mmHg. Arterial blood (13.1%, 100 mmHg) needs more oxygen compared to venous
blood (5.3%, 40 mmHg) (Table 1). Oxygen demand can also be measured per 100 g weight of an organ to indicate oxygen consumption; heart usage is highest, followed by the kidneys, then the brain, and then the liver.74

### Table 1. Oxygen Concentration in Diverse Human Organs

| organs/tissue          | oxygen level (concentration %) | partial pressure of oxygen (mmHg) | ref |
|------------------------|--------------------------------|----------------------------------|-----|
| brain                  | 0.55–8                         | 4.1–60                           | 75  |
| lung alveoli           | 13–14.4                        | 110                              | 76, 77 |
| heart                  | 5–14                           | 78, 79                           |
| liver                  | 4–14                           | 79, 80                           |
| kidneys                | 4–14                           | 80–82                            |
| superficial skin       | 1.1                            | 5–11                             | 83  |
| intestines             | 7.9                            | 53.0–71.0                        | 84  |
| arterial blood         | 13.1                           | 100                              | 77  |
| venous blood           | 5.3                            | 40                               | 76  |
| eye (retina, corpus vitreous) | 1–5                         | 85                              |

### 4. PATHOPHYSIOLOGY, HYPOXIA, AND LUNG FAILURE IN COVID-19 PATIENTS

The mechanism by which the SARS-CoV-2 virus gains entry into the host cells is through S spike protein attachment to angiotensin-converting enzyme 2 (ACE2), acting as a receptor on the epithelial cell surface for internalization assisted by transmembrane protease serine 2 (TMPRSS2). ACE2 and TMPRSS2 are both expressed in host target cells, especially alveolar epithelial type II cells.26 The interaction of the virus with ACE2 could increase inflammatory signaling and elevate angiotensin II effects in predisposed patients.27 After invasion of the virus to the lung cells, myocytes, and endothelial cells of the vascular system, other important inflammatory changes, including degeneration, edema, and necrotic changes, may occur. These changes mostly correlate to proinflammatory cytokines like interleukin IL-6, IL-1, and TNF-α, including degeneration, edema, and necrotic changes, may occur. These changes mostly correlate to proinflammatory cytokines like interleukin IL-6, IL-1, and TNF-α, macrophage inflammatory protein 1α, monocyte chemoattractant protein 1, and elevated expression of programmed cell death 1.28

Importantly, infection with the SARS-CoV-2 virus also triggers hypoxemia as the obvious symptom of COVID-19 pneumonia, which causes the accumulation of oxygen-free radicals and lactic acid, intracellular pH changes, electrolyte changes, and further cellular damage. Diverse stages of acute respiratory distress syndrome (ARDS) can cause hypoxic conditions like the impairment of oxygenation, i.e., a ratio of pO₂/FiO₂ of 300 mmHg or less. Also, clinical signs such as fever, cough, and a respiratory rate of more than 30 breaths/min are the main signs of COVID-19 pneumonia in adults, evidenced by either impaired pO₂ relative to F O₂ or SpO₂ < 93%.29

Compensatory mechanisms to maintain oxygen delivery, for instance, elevated respiratory efforts, hypoxic vasoconstriction, and CO₂ are thought to ultimately alleviate efficacy with rising COVID-19 severity. Therefore, hypoxia becomes life-threatening with a further decline in functional lung capacity and regularly necessitates intensive care support. Mechanisms contributing to COVID-19 severity encompass increased dead space ventilation secondary to endothelial inflammation and microthrombin elevated diffusion barrier secondary to alveolitis and pulmonary edema. This is accompanied by right-to-left shunt formation secondary to atelectasis, which permits to increased edema and fibrosis in the long term; these mechanisms concertedly decline the gas exchange capacity.29

Additionally, as mentioned above, silent hypoxia is a scarce phenomenon in COVID-19 in comparison with other causes of respiratory failure, characterized by critically low pO₂ but just gentle respiratory discomfort and dyspnea.30 Pathophysiologically, hypoxemia plays an utterly moderate role in the sensation of breathlessness,31 but the response to low pO₂ (<60 mmHg) raises the respiratory drive, defined as the intensity of the neural stimulus to breathe through regulation of the respiratory rate and depth (tidal volume, Vt). Hence, tachypnea and high Vt (but not necessarily dyspnea) are signs of hypoxia, particularly in COVID-19 pneumonia.30

This hypoxia has been explained by several hypotheses, including SARS-CoV-2 influence on the oxygen receptor chemosensitivity,32 the loss of hypoxic vasoconstrictive mechanisms,33 and reduction of the diffusion capacity.34 Additionally, in COVID-19 patients, lung perfusion (Q) or ventilation (Vf; dead space ventilation or right-to-left shunt formation) are affected by many pathophysiological processes, which could cause a V/Q mismatch. Altered lung mechanics due to progressive lung edema produce sustained pulmonary inflammation, alveolar collapse, atelectasis, and fibrosis, which further impair global lung function and lead to progressive tissue hypoxia. Nevertheless, advanced testing is necessary to confirm these hypotheses with solid proof.

### 5. NBS: FORMATION AND STABILITY

NBSs are stable gas-containing vesicles in an aqueous solution that usually have a spherical shape with diameters <1000 nm; however, when prepared by hydrodynamic or acoustic cavitation, they are mainly in the range of 100 nm.35,36 Most NBSs have a core–shell structure, where the core is the largest part of the NB containing the selected gas and the shell could have different compositions and structures. The formation and stabilization of echogenic NBSs are governed by the pressure difference between the inside of the NBS and the liquid outside the NBSs (internal and external pressure), called the Laplace pressure. This excess pressure is equal to 2σ/R, where σ is the surface tension and R is the bubble radius.37 Furthermore, the surface tension is attributed to the interface between the electrically charged gas and liquid. Negatively charged hydroxide anions (OH−) are the main factor that prevents bubble coalescence by creating repulsive forces; hence, the random Brownian motion can overcome the buoyancy.38 Therefore, different formulations were tested to reduce the surface tension and improve the NB stability with a focus on nanofluidic systems.39 The attention paid to these nanomaterials and their applications has increased dramatically since their first introduction in 1994.40 This resurgence of interest for the application of NBSs in many fields of science and engineering has been driven not only by their high surface area and reactivity, compared to macrobubbles and MBs, but also by their higher cellular uptake and stability against coalescence, collapse, or bursting, allowing them to survive in liquids for several weeks.39,41 Because of their inherent advantageous physicochemical features and facile production methods, as well as their ability to disrupt the blood–brain barrier (BBB) discovered in recent years,42 NBSs have received widespread attention especially in the biomedical field.18 Their biomedical applications include nanomotor-based machinery,43,44 drug delivery,45,46 gene delivery,105 cancer immuno-
therapy and chemotherapy, wound-healing and tissue regeneration.\cite{107,108}

It is worth noting that the stability and reactivity of NBs depend on the types of gases contained within the cavities. With the further insight gained in this field, it has been observed that NBs in pure water are generally negatively charged because of the excess OH\(^{-}\) groups at the interface. The average size depends on the gas solubility in water, while the \(\zeta\) potential depends on the capacity of the gas to generate OH\(^{-}\) ions at the water/gas interface.\cite{109} It is proposed that H\(^{+}\) ions are more hydrated and hence prefer to remain in the bulk aqueous side, while the less hydrated and more polarized anions prefer to attach to the NB surface.\cite{110} The \(\zeta\) potential or surface charge density of NBs can also depend on other factors, such as the pH, viscosity, density and concentration of the bulk solution, temperature, chemical surfactants, flow rates, and so on.\cite{111} Additionally, the occurrence of aggregation or coalescence within the NBs will hamper the performance of injected NBs. In this regard, an understanding of these physicochemical factors and their optimization is essential in the design of newly engineered applications of NBs with desirable performance. In this regard, some of the physicochemical behaviors of NBs are described below.

The composition of the shell and surfactant play a pivotal role in the stability of the NBs.\cite{112} Surfactants reduce the external Laplace pressure. Therefore, the appropriate amount of surfactant in the NB shell can provide more stability.\cite{113} Poly(ethylene glycol) (PEG) is one of the frequently used surfactants in NB preparation, which also increases biocompatibility.\cite{114,115} Additionally, the shell composition is vital to determination of the rigidity, permeability to diffusion, renal clearance, and echogenic response.\cite{116} Diverse types of lipids, proteins, nanoparticles, or polymers have been used as shell components, although the lipid monolayers are tremendously cohesive and biocompatible and have low surface tension. Hence, they are used to produce stable NBs that can expand and contract as well as allow gas diffusion across the shell (Figure 2).\cite{117}

It has been found that the bubbles are more unstable when their \(\zeta\) potentials are low, which triggers bubble coalescence. Therefore, the formation of NBs in acidic environments is complex because lowering the solution pH lowers the concentration of OH\(^{-}\) ions and decreases the \(\zeta\) potential.\cite{118} The negative \(\zeta\) potential is increased with increasing solution pH because a large number of hydrogen bonds are formed around the NBs to prevent gas diffusion and enhance the stability of the NBs.\cite{119} Furthermore, a decrease in the solution temperature is accompanied by lower mobility of the ions, which allows more absorption of OH\(^{-}\) ions onto the NB surface, thereby increasing the negativity of the \(\zeta\) potential.\cite{120} Besides increasing the pH, the addition of a chemical surfactant is another promising strategy for the generation of more OH\(^{-}\) ions to stabilize the NBs.\cite{112} Overall, the most stable NBs are the smaller-sized bubbles that remain suspended for longer times and have higher \(\zeta\) potentials to decrease the possibility of bubble coalescence.\cite{121}

6. ONBS

ONBs have recently garnered much attention because of their high gas solubility and long lifetime in liquids, which can provide a high oxygen concentration in solution.\cite{122} This unique feature allows blood oxygenation using only a small volume of IV injection fluid without any unwanted formation of gas bubbles. When ONBs dissolve in the water, there is a rapid rise in temperature to about 2800 K and a rise in the internal pressure to 4.5 GPa, at the very moment of bubble dissolution.\cite{123} Recently, the physicochemical factors that affect the stability of ONBs have been examined and are summarized in Figure 3. An investigation into the impact of temperature on the size distribution of ONBs in water confirmed the reduction in the ONB size with increasing temperature from 255 nm (at 6 °C) to 147 nm (at 40 °C), which could be due to a decrease in the water surface tension with increasing temperature.\cite{124} The pH of the water could affect the \(\zeta\) potential of uncoated ONBs in aqueous media, which decreases (becomes more negative) with increasing pH. Meegoda’s group confirmed that the \(\zeta\) potential at pH 10 (~27 mV) was higher than that at pH 4, which was only ~4 mV. Additionally, the \(\zeta\) potential was negatively charged within the range of ~34 to ~45 mV at pH 6.2–6.4, which governs their stability, cellular uptake, and resistance against coalescence.\cite{125} They also reported that the pH influenced the dimensions of the ONBs because the NB size at pH 10 (~80 nm) was much smaller than that at pH 4 (~350 nm). The Nirnmalak research group revealed that the bubble size was unaffected by pH changes when the pH was above 4, but at pH below 4, the bubble diameter enlarged notably as the pH decreased further.\cite{126} Besides, over 1 week, the size increased gradually to 200 nm at pH 7 and 300 nm at pH 10, and they lost an effective charge of about 5 mV/week.\cite{127} Overall, studies have indicated that the \(\zeta\) potential

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{structure_of_nbs.png}
\caption{Structure of NBs showing diverse types of shells.}
\end{figure}
and size depend on the pH. The \( \zeta \) potential increases with increasing pH, while the dimensions decrease. It should be noted that the above-mentioned results can vary depending on the different methods of ONB production.

As opposed to free oxygen gas, the oxygen from ONBs steadily diffuses into the surrounding water and lasts for more than 70 days when the diameter is <200 nm.\(^{121}\) The higher oxygen pressure provides higher pO\(_2\) values in water enhanced by the smaller bubble size compared to macro/microbubbles.\(^{97}\) Also, pressurized ONB suspensions cause an increase in the oxygen tension of the surrounding aqueous solution up to >1000 mmHg, as well as an improvement in the oxy-Hb saturation in a dose-dependent manner.\(^{126}\)

ONB water/saline solutions can be prepared using medical oxygen gas by several methods such as the deployment of microwaves,\(^{125}\) temperature changes,\(^{128}\) laser ablation, or prolonged contact with ceramic nanofiltration membranes,\(^{123}\) without the need for any additional biological or chemical reagents. The NB dispersion can be sterilized after preparation, using thermal sterilization in an autoclave, filtration through microbiological filters, or ultrasonication in a sonotrode or ultrasonic bath.\(^{119}\) In addition, the lipid-shell ONBs can be synthesized using sonication, solvent exchange, microfluidic devices,\(^{130}\) or vigorous shaking.\(^{131}\) Importantly, freeze-dried polymeric ONBs can be stored in powder form and simply reconstituted in water before their use, thus avoiding storage problems.\(^{131}\) To overcome the low stability caused by ONB deformation, which could occur if the freeze-drying process is performed in the presence of gas within the core, a great deal of surface-active reagent could be added to the NB suspension before freezing. Therefore, the ONBs could be protected from the impacts of freezing and thawing, which could prohibit the collapse of ONBs.\(^{122}\) In the case of ONBs that have shells made of either polymeric or lipid scaffolds, the shells are thicker, thus providing a stronger barrier against dissolution of the entrapped oxygen and improving oxygen delivery under hypoxic conditions.\(^{133}\)

It has been found that NBs require a critical level of hydrophobicity to maintain their stability. NBs generally have monolayer shells with a hydrophobic gas core and a hydrophilic or amphiphilic shell arrangement.\(^{134}\) The shell type is an essential factor. For instance, amphiphilic phospholipids and surfactants can self-assemble around a gaseous core to constitute a hydrophilic–hydrophobic monolayer, wherein the water is faced with hydrophilic heads, while the hydrophobic acyl tails face the gas.\(^{135}\) In the case of oxygen-filled NBs, the ONBs would be slightly hydrophobic because of the nonpolarity of oxygen. Also, when the gas core contains a mixture of PFC and oxygen, hydrophobic ONBs are formed because the PFC is hydrophobic.\(^{136}\) Amphiphilic and hydrophobic biomolecules can also participate in the shell structure, while hydrophobic agents and drugs can load inside the ONB cores.\(^{137}\)

The development of noncentrifuge-based rotor/stator devices in order to subject NSS (0.9% sodium chloride, pH 5.6) to Taylor-Couette-Poiseuille flow can create powerful multidimensional mechanical shear forces, which trigger cavitation phenomena within a fluid. Under elevated oxygen pressure (1 atm) at 4 °C, ONB-enriched solutions termed RNS60 could be prepared for various biological applications.\(^{137}\) Generally, RNS60 contains water, sodium chloride, and oxygen with a concentration of 55 ± 5 ppm, without any active pharmaceutical ingredients. In the case of NB solutions, the oxygen concentration can be 2–6 times higher than the normal solubility of oxygen in pure water. This feature makes it possible to deliver oxygen in a quantitative controlled manner through the cardiovascular system rather than the lungs, using an IV infusion of homogeneous ONBs for direct control of SpO\(_2\).\(^{138}\) In Table 2, a summary of recent ONB platforms for organ/cell oxygenation is summarized based on the types of ONB shells, the organs oxygenated, the level of the oxygenated and release mechanism, and cellular uptake and cytotoxicity, which are discussed in detail in section 7.

6.1. Oxygen Release Mechanism and Measurement. When ONBs are used as IVO\(_2\), for the oxygenation of human blood, they do not trigger the complement system or cause any hemolysis and could elevate the blood oxygenation up to 95%, with no side effects or damage to red blood cells.\(^{67}\) It is proven that endocytosis processes are involved in ONB cellular uptake.\(^{146}\) Additionally, the ability of ONBs to act as US contrast enhancement agents allows the guidance and real-time monitoring of oxygen delivery.\(^{40}\) When needed, additional oxygen release from ONBs can be gently provided by US administration via cavitation phenomena or a diffusion mechanism. In the case of US-triggered release, high-intensity US waves generate high- and low-pressure zones within the propagating wave, resulting from resonance and rupture of the bubbles, which causes the release of the core gas (Figure 4a). In the second mechanism, because most of the NB shells and especially the lipid shells are permeable to oxygen, gas diffusion quickly occurs along the concentration gradient through a spontaneous process (Figure 4b).\(^{60}\)

Different techniques have been employed for monitoring systemic, tissue, and cellular oxygenation. In systemic oxygenation, the measurements are divided into four categories of (a) blood and gas sample analysis, such as a polarographic electrode (Clark) and a fast oxygen sensor, (b) oxygen saturation measurement through spectrophotometric methods (UV/IR) and pulse oximetry using spectrophotometry (SpO\(_2\)), (c) systemic oxygen delivery (calculation of CO), and (d)
| type of ONB                       | organ/cell oxygenated                  | safety/biodistribution                                                                 | level of oxygenation          | oxygen release mechanism             | dual role | ref  |
|---------------------------------|----------------------------------------|----------------------------------------------------------------------------------------|-------------------------------|--------------------------------------|-----------|------|
| chitosan/polymeric              | JEG-3 cell                             | no hemolytic activity on human blood; no cytotoxicity on JEG-3 cells                   | from 0.4 to 4.6 mg/L           | US-mediated                          |           | 139  |
| dextran/polymeric               | vero cell                              | no hemolytic activity tested on in vitro red blood cells; no toxicity at 5 and 10 mg (48 h); cellular uptake internalized in the cell and showing diffuse cytoplasmic distribution without cytotoxicity (1 h) | from 0.4 to 5.5 mg/L           | US-mediated                          |           | 140  |
|                                 | in vitro, R28 and ARPE-19 cells; in vivo, rat model of hypoxia/reperfusion in the eye | cell viability: >90% (0.2–1.5% w/v) toward R28 and ARPE-19 (48 h)                     | in vitro, 25–20 mmHg; inner retina, 5–20 mmHg; outer retina, 15–80 mmHg; choroid, ~45 mmHg |           |           | 141  |
| OMNB (dextran, albumin, and lipid) | blood                                 | no hemolytic activity on human blood; no cytotoxicity on blood; no toxicity on JEG-3 cells; no hemolytic activity on JEG-3 cells                        | in solution, 10.53 ± 9.2 mmHg in blood, 179.0 ± 23.6 mmHg; *, the average pO₂ increases 284.6% |           |           | 126  |
| CMC/polymeric                   | MB49 and HeLa cell in mice             | no toxicity events (mortality, convulsions, lethargy, or coma) in heart, liver, kidney, spleen, and lung; no significant growth or necrosis in the muscle (28 days) | first day, 50–120 mmHg; 3 days, 85 mmHg; *, pO₂ increases 140% inside the tumor | diffusion through acidic pH          |           | 142  |
|                                 | PC3 cell; MB49 cell                    | cellular and nuclear uptake; uptake in single cells in ex vivo tumor tissue; in the PC3 cell cytoplasm and nucleus; ex vivo in MB49 | 0 h, from ~50 to 100 mmHg; 48 h, 60 mmHg | optical imaging agent; drug carrier |           | 143  |
|                                 | MB49 cell                              | in vitro cell viability: 100–50% from 0.02 to 20 mg/mL (LD50: 20 mg/mL); uptake by MB49 cell; ex vivo mouse bladder | in vitro; R28 and ARPE-19 (48 h) | imaging; US-guided drug carrier |           | 144  |
| lipid–polymer shell (DPPG, DSPC, and DSPE-PEG) | C6 glioma cancer cells | no toxicity (0.6–40 μg/mL); cellular uptake, presented in the cytoplasm | from 4.0 to 8 mg/L |           |           | 131  |
|                                 | MDA-MB-231 cells                       | cell viability of 60% (416 mg/mL)                                                     | 1 min: 1.4–4.9 mg/mL           | diffusion                            |           | 135  |
|                                 | PBMC cell; spleen, liver, lung, and kidney | cell viability: <95% (1–50 μg/mL); biodistribution of spleen, liver, lung, and kidney until 24 h | 5–20% O₂ increase to 4 mg/L at 25 °C and 6 mg/L at 41 °C | drug carrier; anti-inflammatory |           | 145  |
|                                 | MDA-MB-231 cell; HeLa cells            | no cytotoxicity; cell viability of 79.6% with MDA-MB-231; cell viability of 67.5% with HeLa cells | 0 h, from ~50 to 100 mmHg; 48 h, 60 mmHg | diffusion                          |           | 146  |
|                                 | ONB solution                           | no cytotoxicity on EBC-1, MDA-MB-231, and BEAS-2B cells                                | in vitro; R28 and ARPE-19 (48 h) | diffusion                            |           | 147  |
|                                 | EBC-1 cell; MDA-MB-231 cells           | cell viability of 60% (416 mg/mL)                                                     | 1 min: 1.4–4.9 mg/mL           | diffusion                            |           | 135  |
| dextran-shell-SPIONS/polymer-NPs | TUBO cells                            | cellular uptake: internalized by TUBO cells, localized in cellular cytoplasm          | increase to 4 mg/L at 25 °C and 6 mg/L at 41 °C | diffusion                            |           | 148  |
|                                 | hBMEC cell                             | no hemolytic activity on red blood cells; cellular uptake, localized in the cytoplasm | from 4 to 24 mmHg; *, increase in the intratumoral pO₂ up to ~30 mmHg | theranostic hyperthermic agent     |           | 149  |
|                                 | CNE2 cells                             | no cytotoxicity, ~90% at 4 mg/mL; cellular uptake of heart, liver, spleen, lung, and kidneys | from 4 to 24 mmHg; *, increase in the intratumoral pO₂ up to ~30 mmHg | oxygen release mediated by pH pH-responsive |           | 150  |
| phospholipid-dextran/polymeric bilayer | HepG2 cell                           | no toxicity (~100%; 1 Nm) in vitro; in vivo biodistribution of heart, liver, lungs, spleen, and kidneys; no pathological abnormality on the mice’s major organs (1 week) | in solution, 7 mg/L (5 nM); in vivo, 20 to 50% (SO₂) | hyperoxic inducer |           | 151  |
| biogenic NB                      | murine primary osteoclast cell; PBMC cell | in vitro; R28 and ARPE-19 (48 h) | in solution, 8.3–18.0 mg/L in medium, 9–17.2 mg/L in cell, 2–20% O₂ | sonodynamic agent |           | 152  |
| UFN salce solution              | murine primary osteoclast cell; PBMC cell | in vitro; R28 and ARPE-19 (48 h) | in solution, 8.3–18.0 mg/L in medium, 9–17.2 mg/L in cell, 2–20% O₂ | sonodynamic agent |           | 153  |
| OMNB–poly (vinyl alcohol)       | lung                                   | preserved rat’s life for over 40 min, blood pressure of 79.5 ± 14.7 mmHg; in vivo content of 45 mg/L versus saline content of 6 | preserved rat’s life for over 40 min, blood pressure of 79.5 ± 14.7 mmHg; in vivo content of 45 mg/L versus saline content of 6 |           |           | 154  |
downstream marker determination. Tissue oxygenation is based on the measurement of pO₂ in the microvasculature and cells through phosphorescence-based methods and oxygen pressure electrodes, while measurement of mitochondrial pO₂ is used for cellular monitoring.71

In a typical procedure for determining oxygen release from ONB solution, an appropriate amount of formulation is injected into a hypoxic saline solution with an oxygen concentration of about 0.4 mg/L. The oxygen concentration is monitored at 25 °C for a period for the detection of gas release. To evaluate oxygen release in the presence of US, ONB formulation is injected into a small plastic container and dipped into a thermostatic water bath or the US probe is inserted into the solution to generate US propagation followed by monitoring of the oxygen concentration through the transducer.139 Also, Winkler’s method is used for evaluation of the oxygen in ONB solution by Noguchi et al.152 Both electrochemical or fluorescence quenching methods are also employed to evaluate the amount of oxygen released from the MBs.158

An OxyLite oxygen probe, as a localized sensor, is the frequently used system for determining the oxygen release content in cells by installing the oxygen probe into the organs144 and tumor,141,153,155 followed by measurement of pO₂. Direct measurement of the intratumoral oxygen level via a microelectrode is also investigated through insertion of the electrode into the center of the tumors under the guidance of US imaging.150 A new method based on a spectrophotometric change between oxy- and deoxy-Hb is also examined wherein Hb was utilized as an oxygen indicator to detect the oxygen content through spectrophotometry quantitatively.154 In addition, the oxy- and deoxy-Hb levels in subcutaneous tumors could monitor through a photoacoustic imaging featuring a hybrid US transducer.151 The other commercial instruments that have been used for oxygen release measurements from ONBs are oximeter (Portamess 913 OXY, JPB-607A),131,139 blood gas analyzer (ABL510),126 real-time oxygen detector with a fluorogenic response detector151 and dissolved oxygen meter.135

7. OXYGENATION WITH NBS

In COVID-19 patients, both the microcirculation and oxygen diffusion to alveoli are disrupted. Pathologically in patients, the alveolar collapse produces a difference between the alveolar and arterial partial pressure (pAO₂ and paO₂) and leads to arterial hypoxemia.159 On the other hand, it has been reported that ONBs can affect many cellular signaling or metabolic pathways and can stimulate oxidative metabolism in a manner similar to that of normally functioning mitochondria. For instance, treatment with RNS60-containing ONBs could benefit neuroinflammatory or neurodegenerative disorders by suppressing proinflammatory activation of microglia,160 reducing T-mediated neuronal death,161 promoting plasticity in hippocampal neurons,162 and ameliorating Parkinson’s disease.163 This approach has been tested in an animal model of multiple sclerosis caused by experimental allergic encephalomyelitis with promising results.164,165 In another laboratory model involving high-frequency stimulation of the giant squid synapse, acute superfusion with artificial seawater containing ONBs increased the neurotransmitter release amplitude and restored synaptic transmission.166 An investigation in Xenopus laevis oocytes showed that treatment with a RNS60-based ringers solution induced the membrane potential hyper-

| Table 2. continued |
| --- |
| type of ONB | organ/cell oxygenated |
| surfactant-stabilized ONB | B10C-3 cells |
| oxygenated artificial Cyt | spinal cord |
polarization, affected the voltage-dependent electrical membrane properties, and also increased ATP production and mitochondrial function. After RNS60 was applied to \textit{X. laevis} oocytes, the increased intracellular ATP was blocked both by 500 nM rotenone (an inhibitor of the mitochondrial respiratory chain) and by 2.5 mg/mL oligomycin A (an inhibitor of ATP synthase).

A report in 2016 evaluated the effectiveness of an ONB RNS60 solution on nerve transmission at neuromuscular junctions and muscle function in the murine phrenic nerve diaphragm. It was suggested that increased neurotransmission could be beneficial in muscle fatigue. In this report, no effects on the isometric muscle force were seen using an ONB solution with a total O2 content of \(\sim 55 \pm 5\) ppm, although muscle fatigue was reduced. Recovery of the maximal isometric force in the diaphragm after fatigue produced by 20 Hz phrenic nerve stimulation was significantly improved. The authors hypothesized that increased cytosolic calcium influx, as well as more ATP could explain improvement in the neurotransmitter release. A recent report showed that RNS60 could increase mitochondrial biogenesis in dopaminergic neurons. Another study looked at the therapeutic effects of RNS60 in the SOD1 mouse model of amyotrophic lateral sclerosis and showed that glia were protected and the peripheral nerve function was rescued. Because these effects were blocked by inhibitors of mitochondrial respiration, it was proposed that ONBs acted by stimulating mitochondrial metabolism and increasing the cellular ATP levels and therefore could beneficially oxygenate the hypoxic cells in COVID-19 patients. In a continuation, diverse types of pathways for organs and tissues oxygenation with ONB platforms are discussed in detail as promising alternatives for COVID-19 patients who are nonresponsive to traditional oxygen therapy systems.

### 7.1. Oxygenation by IVO2

In an early study, Cavalli’s group described the US-mediated oxygen delivery using chitosan (CS) ONBs, with a preliminary evaluation on human chorionic cancer cells (JEG-3). They prepared the two different NB formulations described in Table 3. The main difference was the inclusion of perfluoropentane (PFP) in formulation B to increase the oxygen loading due to the high solubility of oxygen in PFC.

| component                      | A (% w/v) | B (% w/v) |
|--------------------------------|-----------|-----------|
| sodium chloride                | 0.61      |           |
| palmitic acid                  | 0.02      | 0.02      |
| Epikuron 200                   |           | 0.05      |
| chitosan (medium MW)           | 0.20      | 0.16      |
| perfluoropentane               |           | 7.03      |
| B-glycerophosphate             | 0.34      |           |
| NaOH                           | 0.01      |           |
| water                          | 95.36     | 88.94     |
| ethanol                        | 3.46      | 3.80      |

In their study, 3 mL of each formulation (A and B) with 30 mg/L of oxygen concentration was injected into 20 mL of a hypoxic (O2 concentration 0.4 mg/L) saline solution (0.9% mg/mL). The oxygen release results at 25 °C over 60 min revealed that, although both formulations increased the oxygen concentration, formulation B transferred more oxygen (4.6 mg/L) compared to formulation A (2.7 mg/L) (Figure 5). The results after the application of US show that this platform could supply oxygen to JEG-3 cell lines cultured in hypoxic media, leading to the inhibition of HIF-1\(\alpha\) expression with no cytotoxic effect. The amount of oxygen released depended on both the time duration and frequency of the US waves. They also designed a NB-based nanoplatform with PFP as the core, dextran as the shell, poly(vinylpyrrolidone) (PVP) as the stabilizing agent, and Rhodamine B as the fluorescent marker. The stable fluorescent NBs had a diameter of \(\sim 500\) nm, could be internalized by Vero cells after 1 h, and exhibited diffuse cytoplasmic distributions without cytotoxic effects after 48 h at

![Figure 4. Oxygen release mechanism from NBs: (A) ONB disruption through US; (B) oxygen diffusion across the concentration gradient. During US-triggered oxygen release, a cavitation phenomenon is generated by high-intensity US waves, which produce high- and low-pressure zones along the propagating wave due to the bubbles resonating and rupturing, thereby releasing the core gas. In the second mechanism, gas diffusion easily occurs along the concentration gradient by a spontaneous process.](https://doi.org/10.1021/acsanm.1c01907)
Under severe hypoxic conditions (\(O_2\) concentration 0.4 mg/L), oxygen was released in greater amounts compared to those under moderate hypoxic conditions (\(O_2\) concentration 4 mg/L).

Matsuki and colleagues studied the implementation of MNBs as an IV infusion into blood vessels. NSS was found to be best for the preparation of an oxygen-rich infusion solution containing MNBs. The pO2 values found at a 10% dilution of NSS with or without OMNBs in blood confirmed the improvement of blood oxygenation under hypoxic conditions (Figure 6A), which depended on the dilution ratio (Figure 6B). However, no in vitro/in vivo investigation was done.

Tumor hypoxia is an important phenomenon that limits the success of oxygen-consuming cancer treatments. It can lead to low patient survival rates, tumor resistance to cytotoxic therapies, and increased metastasis. \(^{174,175}\) pO2 in the healthy subcutaneous tissue is commonly between 40 and 60 mmHg, but many cancers show pO2 values between 2 and 18 mmHg. \(^{176}\) This hypoxia is caused by mechanical forces generated during tumor growth that are able to compress blood vessels, thus reducing the perfusion rates. \(^{177}\) The process of angiogenesis is designed to restore adequate tissue oxygenation through VEGF production and new vessel formation. \(^{178}\) Moreover, radiation treatment, \(^{179,180}\) chemotherapy, or other oxygen-based remedies for cancer treatment such as photodynamic therapy (PDT) \(^{181,182}\) are only highly efficient when a sufficiently high local concentration of oxygen is present in the tumor tissue; consequently, all of them could benefit from the administration of oxygen-delivery therapies. \(^{183−186}\) Several reports have suggested that ONBs could be used to improve the treatment of many cancers. In one interesting report, Bhandari and co-workers designed oxygen nanocarriers composed of \(~50\) nm sodium carboxymethylcellulose (CMC) polymeric shells loaded with oxygen to form \(100−200\) nm NBs (Figure 7A,B). These could be triggered to release oxygen by sonication to induce epigenetic changes, reversing the 5-methylcytosine (5mC) DNA hypomethylation status in hypoxic cancer cells in culture, and also inhibited tumor growth. \(^{142}\) Oxygen release to the hypoxic tumor cells via the diffusion mechanism occurred through disintegration of the NB shells in the acidic microenvironment of the tumor cells, which increased the 5mC levels in a dose-dependent manner in vitro (Figure 7C). Additionally, hypermethylation has been detected in the promoter DNA region of BRCA1, a human tumor suppressor gene responsible for repairing DNA damage, after ONB treatment. Several other hypoxia-associated and tumor suppressor genes, like MAT2A and PDK-1, could be reprogrammed. Further, the intratumoral injection of ONBs into severely hypoxic MB49 and HeLa tumors growing in mice in vivo led to a significantly lower degree of hypoxia and a reduction in HIF-1\(\alpha\) expression. In vivo biodistribution confirmed no adverse toxicity effects (mortality, lethargy, convulsions, or coma) in the heart, liver, kidneys, spleen, and lung, along with no considerable growth or necrosis in the muscle for over 28 days after ONB injection. In another study, synthesis of the ONB distribution and diffusion coefficient within the cell was performed, both in vitro in live cells and ex vivo in MB49 mouse bladder tumor tissues, which could be tracked and quantified in single cells and tissues using hyperspectral dark-field microscopy (HSDFM) based on light scattering. \(^{143}\) Cellular uptake results showed \(~88\)% higher efficacy for 400 nm ONBs compared to 800 nm ONBs, while the intranuclear uptake efficiency was
-56% higher for the 400 nm ONBs after 3 h as a result of better uptake of the smaller NBs through endocytosis.

Song’s group compared different lipids, 1,2-dipalmitoyl-sn-glycero-3-phospho-(1’-rac-glycerol) (DPPG), [1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE)]−PEG, to form bilayer shell-conjugated chlorin e6 (Ce6) photosensitizer ONBs, which were synthesized by an emulsion/solvent evaporation technique with internal phase separation. They had an average size of 263.2 ± 10.3 nm and were tested as oxygen-containing carriers for PDT. The tumor-targeting efficiency and biodistribution of ONBs on glioma-bearing nude mice showed significant tumor-targeting capability, long retention time within the tumor, and an accumulation 70 times higher than normal tissues. In this condition, the fluorescence signal gained its maximum amount after 3 h of ONB injection and was maintained for 24 h with a negligible decrease. In addition, the cellular uptake investigation proved the presence of both free Ce6 and ONB in the cytoplasm after endocytosis.

In another effort, phospholipid (DSPC) and PEGylated lipid-shelled ONBs (DSPE-PEG-2000-amine/DSPE-PEG-2000-biotin) with a molar ratio of 85:8:7 were synthesized via sonication at 190 W in a pulsed mode for 5 min. Their ability to reverse hypoxic conditions within a tailor-made chamber containing MDA-MB-231 breast cancer cells was shown by the increased degradation of HIF-1α after the introduction of ONBs with a cell viability of 60%. In this synthetic procedure, the hydrophilic heads of phospholipids faced outward toward the Dulbecco’s phosphate-buffered saline (DPBS) solvent, and the hydrophobic tails surrounded the oxygen core gas, thereby forming a monolayer shell with a thickness of ∼5−10 nm. A total size of the ONBs was around 200−400 nm, at a high concentration of 4.2 × 10¹¹ NBs/mL (Figure 8A). The same group in 2019 investigated the efficiency of the above-mentioned phospholipid-PEG-ONBs for oxygen release after sonication while preventing hemolysis, which is a major obstacle faced by the direct exposure of blood to oxygen pressure. The results revealed that the prepared carrier did not cause any hemolysis even at 20% v/v (less than 2% hemolysis between 1 and 200 μL/mL), while allowing oxygen release via the diffusion mechanism. The toxicity experiments on NIH-3T3, MDA-MB-231, CHO, and HADScs cells showed great biocompatibility in a wide concentration range (1−100 μL/mL). Additionally, it has been confirmed that the ONBs produce echoes at higher US frequencies, so they could also be used for US imaging (Figure 8B).
Khan et al. synthesized another phospholipid-shell ONB with a mean size of 261 ± 41 nm via the sonication method designed to down-regulate HIF-1α and reduce inflammation. Interestingly, the designed platform could reduce the release of cytokines and therefore control the inflammation and lead to increasing survival rates of animals. The biodistribution of Cy5.5-labeled ONBs (ONB/Cy5.5) via an in vivo imaging system observed the presence of ONBs in lung, liver, and kidney tissues 10 min postinjection. From 30 min to 3 h, high amounts of ONBs in the liver as well as spleen, lung, and kidney tissues were detected. ONBs were visible in the body from 6 to 24 h, after which they appeared to be eliminated from the body. ONBs could be detected in the body 24 h after IV injection and 72 h following intraperitoneal injection.

They also used the phospholipid shell composition to load the anticancer drug doxorubicin (DOX). They assessed the efficiency of DOX-loaded ONBs to enhance the DOX activity in hypoxic media, suppress the HIF-1α activity, enhance the oxygen levels, and increase reactive oxygen species (ROS) generation in HeLa cells or MDA-MB-231 breast cancer cells. The cell viabilities of MDA-MB-231 and HeLa cells with ONBs were 79.6% and 67.5%, respectively. Also, FITC-ONBs had higher fluorescence intensity, confirming ONBs delivery to the cells.

Recently, Iijima and co-workers prepared water containing very small ONBs (2–3 nm) at a concentration of 2 × 10^18 particles/mL without using any chemical compounds. The goal was to overcome resistance to ionizing radiation in EBC-1 and MDA-MB-231 cancer cell lines, and a clear inhibition of the HIF-1α expression was detected. A novel setup called ΣPM-S allowed the mixing of oxygen with water in a pressurized tank at 0.4 MPa (Figure 9A). After pumping out the oxygenated water from the small hole (diameter, 0.3–0.6 mm) in the nozzle (Figure 9B), single-nanometer-sized NBs are formed. They showed no adverse effects on the viability of breast cancer, human lung cancer, or noncancerous bronchial cells under normoxic conditions.

Another report by Cavalli et al. depicted the use of lipid-shell NBs as theranostic hyperthermic agents for tissue oxygenation. In this work, magnetic lipid ONBs increased tumor oxygenation via direct intracellular oxygen transfer. The carrier was synthesized through the combination of a dextran shell and a PPF core, and then the superparamagnetic iron oxide nanoparticles (SPIONs; 2 mg/mL) were attached to the surface of the ONBs. Synthesized ONBs are stable over 2 months. The 380 nm size nanocarriers could be internalized by TUBO tumor cells and increased the tissue temperature upon exposure to radio-frequency magnetic fields but could only localize in the cellular cytoplasm compartment (Figure 10).

The in vitro oxygen release kinetics of these magnetic ONBs showed a biphasic profile; oxygen was quickly released in the first 4 h, and oxygen concentrations were elevated to 4 and 6 mg/L at 25 and 41 °C, respectively, following by an almost constant and moderate oxygen release 24 h postinjection. In 2020, they used the same approach for the synthesis of SPIONs on the surface of dextran-shell NBs as multipurpose oxygen and chemotherapy carriers to target brain tumors. The nanocarriers could cross the BBB to access the central nervous system. The synthesized magnetic NBs were saturated with oxygen (35 mg/L concentration) and could deliver their cargoes to specific sites, as shown by monitoring with either magnetic resonance imaging or US sonography. In vitro cytotoxicity assay and confocal microscopy images revealed the biocompatibility of magnetic ONBs through human brain microvascular endothelial cells (hBMECs) that localized in the cytoplasm compartment of the cells.

In a recent report, pH-responsive ONBs (325.4 ± 12.5 nm, −13.2 ± 2.1 mV) were synthesized using emulsion/solvent evaporation with an internal phase separation process. The ONBs contained DPPC, DPPI, and DSPE–PEG phospholipids and acetylated dextran (AC-DEX), which formed polymer bilayer shells. They were used for spontaneous oxygenation in response to the slight pH drop within the tumor microenvironment (Figure 11A). The shell acted as a powerful barrier against gas dissolution in the circulating blood and produced a ~6-fold increase of the intratumoral oxygen level in CNE2 tumor-bearing mice after IV administration. In vivo outcomes illustrated that the ONBs can noticeably increase the intratumoral pO2 up to ~30 mmHg. The pH-responsive feature allowed a sudden burst release of oxygen in the mildly acidic tumor environment (Figure 11B). In particular, the dissolved oxygen concentration in the acidic solution reduced at a steadier rate compared to the normal solution because of the rapid acid-catalyzed hydrolysis of the AC-DEX shell (Figure 11C). Moreover, the appearance of NBs incubated in the acidic solution became clearer (Figure 11D).

The growth inhibition effects of water containing ONBs (<200 nm) on tumor growth in 4T1 breast cancer-bearing mice over a 14-day treatment period were investigated by Mahjour et al. A significant decrease in HIF-1α and VEGF gene expression was detected in ONB water-treated mice in comparison to the controls. This was ascribed to the elevated available oxygen in the tumor environment, followed by decreased hypoxia, and the induction of p53-dependent apoptosis that inhibited tumor growth.

In 2020, biogenic gas vesicles (GVs), naturally shaped gas-filled protein-shelled compartments modified on the surface by a layer of liposome were tested as NBs to alleviate tumor hypoxia. Different from conventional synthetic NBs, GVs are known to be the first biomolecular acoustic reporters with the ability to edit genes. GVs are naturally formed by cyanobacteria or archaea microorganisms. The GV walls
exclude water while allowing gas to freely penetrate into and out of the shells; therefore, only a negligible oxygen pressure gradient is required, imparting greater stability to the GVs.\textsuperscript{190} Using lipid-GVs(O\textsubscript{2}) with a size of $\sim310-340$ nm, a considerable improvement in the oxygen concentration within hypoxic HepG2 cells (Figure 12A,B), as well as subcutaneous tumors (Figure 12C,D) was observed, with the GVs being stable in solution over 6 months and showing low toxicity both in vitro and in vivo. The biodistribution of the indocyanine green-functionalized lipid-GVs in vivo revealed considerable fluorescence in the liver, lungs, spleen, and kidneys within 2 h and declined over time. No pathological abnormality on the mice’s major organs (heart, liver, spleen, lungs, and kidneys) was observed 7 days after injection. The PDT procedure produced apoptosis and necrosis in the tumor cells in vitro and tumor growth inhibition in vivo. These multifunctional lipid-GVs could be candidates for clinical treatment. Very recently, the first direct evidence of intact NBs existing within the extravascular space in close proximity to the tumor cells within a tumor was provided.\textsuperscript{191} The local oxygen concentration strongly affects bone metabolism; therefore, the effect of ONBs in saline IV injected in a mouse model of glucocorticoid-induced osteoporosis (GIO) was investigated, wherein the ONBs prevented bone loss by inhibiting osteoclastogenesis and affecting RANK-TRAF6-c-Fos-NFATc1 and RANK-p38 MAPK signaling. In vivo results proved improvement in the oxygen content from 2% to 20%. Notably, this work reported the hyperoxia condition.\textsuperscript{152} In order to investigate the potential efficacy of NBs for the treatment of COVID-19 patients, recently, Pan and co-workers suggested the administration of ONBs by IV injection as an alternative to mechanical ventilation to safely maintain the blood oxygen pressure at normal levels in critical COVID-19

Figure 10. (A) In vitro oxygen release from magnetic ONBs at different temperatures (i.e., 25, 37, and 41 °C). (B) ONB internalization by the TUBO cell line. Cell nuclei after DAPI staining (in blue; ONBs, in green). Magnification 63×. Reproduced with permission from ref 148. Copyright 2019 Frontiers Media SA.

Figure 11. (A) pH-responsive ONBs preparation. (B) pH-triggered oxygen release. (C) In vitro oxygen release kinetics of the ONBs incubated at pH 6.5 and 7.4 (*, $p < 0.05$). (D) Optical images of the ONBs stored in a neutral solution (pH 7.4) and an acidic solution (pH 6.5) for 3 h. Reproduced with permission from ref 150. Copyright 2019 American Chemical Society.
patients. Their theoretical data analysis indicates that the IV delivery of 400 mL of a ONB physiological saline solution containing 40 mg of O2/L could improve pO2 from 0.133 kPa (typical for critical patients) to a normal level of 13.3 kPa (normal individuals).

7.2. Oxygenation Delivered by Other Routes. Among the diverse routes available for oxygen delivery in the human body, intravesical therapy has been proposed as an effective way to treat bladder cancer, by delivering ONBs directly into the bladder via the urethra. In this context, in 2018, mitomycin-C (MMC)-loaded ONBs with a CMC shell (200 nm, 0.02−20 mg/mL) were synthesized, injected through a catheter into the bladder of a mouse, and steered to the tumor using a US beam. The MMC-loaded ONBs increase the localization of ONBs at the MB49 tumor site and allow a decrease of the MMC dose by 50%. Moreover, sonoporation increases penetration of the ONBs into the tumor vasculature and allows reoxygenation of the tumor. The pO2 values for saline- and ONB-treated MB49 cells in vitro were measured as 50 and 100 mmHg, while beam conditions led to pO2 110 mmHg. The reversal of chronic hypoxia by the ONBs increases the activity of MMC and leads to significantly lower expression of HIF-1α and VEGF in the tumor samples.

The idea of using an oxygen-rich liquid in liquid ventilation using OMNBs was proposed in 2015 by Kakiuchi and co-workers. A low-pressure, time-cycling total liquid ventilation system was constructed for rats. It contained a MNB generator, which allowed the lungs to be filled with MNBs in saline, and a subsequent gas exchange was performed with the volume of the solution. The MNB solution containing 500 nm bubbles has an oxygen content of 45.1 ± 0.1 mg/L and, when administered to anesthetized rats, preserves life for over 40 min, with a blood pressure of 79.5 ± 14.7 mmHg and a dissolved oxygen content that is nearly 8 times higher than that provided by NSS (6 mg/L).

Another pathway that has been explored for oxygen delivery is the administration of stabilized ONB foam via the stomach. In such a scenario, the saturated solution of ONBs could be administered via gavage, which could be superior in terms of patient acceptability/compliance, cost, convenience, and reduced risk of infection. Additionally, oral administration of ONB suspensions is under active investigation as a possible means of relieving tissue hypoxia, with the advantages of low risk of infection, patient acceptability, minimum risks of embolism associated with IV injection, and low cost. Stride’s group investigated the possibility of reducing tumor hypoxia in a mouse xenograft tumor model of human pancreatic cancer (BxPc-3 cells in male SCID mice) using an oral delivery of a single dose of a 100 μL suspension of surfactant-stabilized ONBs, with a mean diameter of ∼340 nm. In vivo average pO2 measurements exhibited an increase from 0.1 to 0.9 kPa after 30 min. The mRNA and protein expressions of HIF-1α are reduced by 75% and 25%, respectively, accompanied by a considerable decline in VEGF and an increase in ARD1A.

Another possible route is the administration of NBs by direct injection into the cerebrospinal fluid (CSF) to provide oxygen to the spinal cord tissues and possibly ameliorate ischemic injury to the spinal cord while also suppressing inflammatory responses. CSF-pO2 is known to be reduced by 75% and 25%, respectively, accompanied by a considerable decline in VEGF and an increase in ARD1A.

Figure 12. Lipid-GVs(O2)-mediated oxygen delivery: (A) Representative images of cells cultured with 200 μL of PBS(O2) or lipid-GVs(O2) (1 nM) in hypoxic conditions for 1 h. (B) Quantification of hypoxic staining. (C) Photoacoustic images of the tumor oxygen levels (oxy-Hb and deoxy-Hb levels) from in vivo tumor-bearing mice at different time points by the tail vein injection of 200 μL of saline, PBS(O2), and 5 nM lipid-GVs(O2). Red pixels: oxy-Hb. Blue pixels: deoxy-Hb. (D) Quantification of the tumor oxygen levels (*, p < 0.05 vs control). Reproduced with permission from ref 151. Copyright 2020 Elsevier.
nonoxygenated group 75 min after reperfusion compared to that in the oxygenated group (Figure 13).157

Very recently, the use of dextran-based ONBs for intravitreal delivery of oxygen to rescue the inner retina from ischemic damage has been demonstrated and led to the treatment of central retinal artery occlusion.141 The synthesized NBs showed 119.6 ± 44.9 nm size distribution with a ζ potential of −35.54 ± 10.54 mV and a concentration of 5.26 × 10⁸ NBs/mL with a stability for >4 months in amber vials at 5 ± 3 °C. The oxygen-delivery efficiency of this nontoxic platform in retinal precursor cell lines and in a rat model of hypoxia/reperfusion in the eye allowed significant recovery of the ganglion and inner retinal cell layers, while electroretinography displayed normal retinal function. The oxygen release profile of ONBs was determined by monitoring the oxygen release rate in artificial aqueous humor (Figure 14a), vitreous humor (Figure 14b), and porcine serum (Figure 14c) at 35 °C in a nitrogen environment, confirming oxygen diffusion into the inner retina. The pO₂ profile were measured in vitreous (25–20 mmHg), inner retina (5–20 mmHg), outer retina (15–40 mmHg), and choroid (~45 mmHg), while the hypoxic eye showed much lower pO₂ values for all tissues. In addition, mapping through HSDFM showed significant cellular uptake of ONBs by the ARPE-19 and R28 cell lines.

Figure 13. (A) Intragroup comparison of the differences between the baseline, after 15 min of spinal cord ischemia, and after oxygenated CSF replacement. (B) Intergroup comparisons of pO₂ at time points throughout the experiment. Reproduced with permission from ref 157. Copyright 2020 Elsevier.

Figure 14. Oxygen release profile of dextran-ONBs in (a) simulated aqueous humor, (b) simulated vitreous humor, and (c) porcine serum: oxygen-saturated porcine at 35 °C. Reproduced with permission from ref 141. Copyright 2021 American Chemical Society

8. MULTIPURPOSE ROLE OF ONBS: ANTIMICROBIAL, ANTIINFLAMMATORY, AND WOUND-HEALING PROPERTIES

Hospitalized COVID-19 patients often have weakened immune systems, which puts them at particular risk for other microbial infectious diseases, inflammation, and nonhealing CWs.193 It has been observed that ~8% of COVID-19 hospitalized patients experience a bacterial or fungal coinfection.21 On the one hand, CWs are generally characterized by persistent tissue hypoxia and chronic inflammation; on the other hand, clinical reports have proven the increased risk of surgical wound infections in patients who have received supplemental oxygen treatment.194,195 In cases where COVID-19 patients require urgent surgical operations due to wounds, postsurgical infections, or inflammation, the importance of using antimicrobial agents with the simultaneous ability to transfer oxygen has been highlighted. Consequently, providing additional oxygen to the tissues in COVID-19 patients could be a prominent pathway to conquer microbial infections while at the same time relieving hypoxia.
The role of oxygen therapy for the treatment of microbial infections as well as the healing of CWs is well-known.\textsuperscript{196,197} Using oxygen therapy as a part of hydrotherapy could be an inexpensive technology for the oxygenation of burns and CWs. This effect is attributed to it is ability to improve keratinocyte differentiation and reepithelization during the proliferative phase of wound healing. It could kill bacteria during the inflammatory phase, as well as assist myofibroblast differentiation and collagen cross-linking during the maturation phase.\textsuperscript{24,198,199} Hyperoxia, the excess supply of oxygen to tissues and organs, has also been shown to inhibit the growth of some fungi and bacteria and to potentiate the antifungal effects of amphoterocin B.\textsuperscript{197}

Recently, the growth in nanotechnology has triggered the design and synthesis of innovative nanomaterials with intrinsic antibacterial activity to conquer drug-resistant bacterial strains. For instance, a wide range of metal nanoparticles (e.g., silver, copper, cobalt, titanium, and zinc),\textsuperscript{200} pseudoenzymes,\textsuperscript{201} natural-based nanoparticles (like CS),\textsuperscript{202} metal–organic frameworks\textsuperscript{203} and carbon-based nanomaterials\textsuperscript{204} have been tested to treat bacterial infections. Nevertheless, some of these antibacterial agents only affect a few species of bacteria, but antimicrobial NBs have garnered attention in dentistry, especially for endodontics,\textsuperscript{205} antibiotic release, and the disruption of biofilm formation in a broad spectrum of strains.\textsuperscript{206,207} The combination of NBs with antimicrobial agents could increase the drug concentration and penetration into the infected site. On the other hand, NBs that respond to external physical stimuli such as US or light could promote antibiotic delivery at the site of infection through sonophoresis or photouncaging.\textsuperscript{209} Additionally, it has been proven that the mechanical impact of an expansion and subsequent implosion of NBs could result in a local but efficient destruction of dense cell clusters in Gram-positive and Gram-negative bacterial biofilms.\textsuperscript{209} As an example, the malaria parasite could be detected and mechanically destroyed in nanoseconds in a drug-free manner using a one-step theranostic process via hemozoin-generated gas-filled NBs.\textsuperscript{210}

Additionally, the generation of ROS by several types of nanomaterials to kill microorganisms is oxygen-dependent; therefore, supplying additional oxygen to the tissues could improve the activity against bacterial infections.\textsuperscript{211} As mentioned, hypoxia and inflammation are interrelated and can show bidirectional influences on each other; therefore, the inhibition and down-regulation of HIF-1\textalpha also play a vital role in decreasing inflammation. As a result, the utilization of ONBs for local oxygen delivery to hypoxic microenvironments such as wounded tissues and Gram-positive or Gram-negative bacterial biofilms highlights the multipurpose role of this platform for treating COVID-19 patients. For instance, the antiinflammatory ability of ONBs was investigated by measuring the downstream signal transduction of TNF-\alpha (a cytokine involved in systemic inflammation) in rat aortic endothelial cells.\textsuperscript{212} Moreover, the use of ONBs decreased oxalate-induced macrophage chemoattractant protein 1, thus inhibiting the migration of macrophages to inflamed areas, prevented osteopontin overexpression, and reduced renal tubular cell injury in rats.\textsuperscript{212} Also, ONBs showed a remarkable ability to deliver the immunosuppressive drug mycophenolic acid, thereby controlling inflammation, while enhancing the survival rate and reducing the release of cytokines (i.e., IL-2, IL-6, and TNF-\alpha).\textsuperscript{213}

Sayadi and co-workers prepared saline infused with OMNBs, using a generator system, for burn wound healing measured 2 weeks after burn infliction. The OMNBs-treated burn wounds showed better healing and began to proliferate and remodel at earlier time points than saline control-treated animals. In addition, spatial frequency domain imaging measurements confirmed the increase in tissue oxygenation.\textsuperscript{213}

The exposure of adipose tissue to MBNs for 15–30 min also showed a statistically significant decrease in oxygen-free radicals, which was attributed to lower hypoxia compared to that of the controls.\textsuperscript{19} The same group investigated the ability of ONBs to enhance pancreatic islet cell survival postharvest. In order to carry out islet cell transplantation to treat type 1 diabetes, the cells should be protected from hypoxia during harvest. They used a model of hypoxia in Sprague–Dawley rat pancreas tissue.\textsuperscript{214,215} The average measured size of the MBNs was found to be 167.3 ± 13.0 nm, with a concentration of 1.46 × 10^8 ± 2.49 × 10^7 MBNs/mL created via a shear–stress-based generation system.
The dual role of the NBs for simultaneous oxygenation, along with antibacterial activity against a particularly important drug-resistant bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA), was demonstrated in 2019. The well-known advantages of NBs accompanied by the antimicrobial features of the CS natural polysaccharide were investigated to develop advanced antimicrobial oxygen-based therapies. In this regard, Banche and co-workers reported the antimicrobial effects of CS-shell, PFP-core ONBs against bacteria. These carriers show prominent long-term effects to promote wound healing and inhibit the growth of both staphylococcal and streptococcal bacterial species. In another example of the multipurpose role of ONBs, the Akhavan research group devised an efficient approach for the preparation of reduced graphene oxide (rGO)/copper peroxide (CuO_2_) nanoshuttles that also allowed the controlled release of ONBs and simultaneously inhibited bacterial respiration. The oxygen carrier comprised a solid source of oxygen CuO_2_ and rGO as a carrier with a photothermal-sensitizing ability, to provide the needed oxygen in situ. Subsequently, laser irradiation was used to transfer the energy into localized heat, which damaged cell membranes and increased the release rate of the ONBs. The results show the remarkable antibacterial effects of the nanoshuttles on Gram-positive *S. aureus* compared to Gram-negative *Escherichia coli* bacteria, particularly in the company of rGO. As can be seen in Figure 15, noticeable wrinkles and disruptions in the bacterial cell membranes are obvious after 6 h of incubation, while with an increase in the incubation time to 24 h, more pronounced disruption to the cell membranes is created. The use of laser irradiation for 10 min, followed by 24 h incubation in darkness, caused the most damage and the complete loss of cellular integrity.

**Figure 16. Diverse routes of oxygen delivery through ONBs.**

9. CONCLUSION AND FUTURE OUTLOOK

There is an urgent global need to combat the present COVID-19 pandemic, as shown by the fact that many of the most severely affected patients around the world have died from ARDS. Because the availability of effective vaccines and drugs is still restricted and because of the fact that the coronavirus has already mutated and these variants are circulating globally, improved noninvasive alternatives to mechanical ventilation could prove to be vital in preventing a large number of deaths. Moreover, using a simpler method of blood oxygenation would be a convenient strategy for many clinical applications and diseases. According to the WHO report, Chinese data suggest that, although most of the COVID-19 patients have only a mild (40%) or moderate illness (40%), nevertheless almost 15% of them develop a severe illness that requires oxygen therapy. Moreover, 5% will become seriously ill in an intensive care treatment unit, and these patients especially require mechanical ventilation.

This review has highlighted the use of gaseous NBs (that can also be smart or stimulus-responsive) as a possibility for a cure for patients with acute hypoxic respiratory failure. Oxygen-saturated solutions of NBs have emerged as a possibility to treat diverse types of hypoxic cells and tissues, after IV delivery or by other routes of administration, without any cytotoxic effects. Furthermore, preliminary laboratory data suggest that IVO_2_ could play a vital role in the treatment of patients with hypoxic respiratory failure. The small sizes of the ONBs increase the probability of extravasation from blood vessels into the surrounding tissues via a US-targeted site-specific or simple diffusion release mechanism in a minimally invasive manner. A possible mechanism of oxygen delivery by ONBs involves passing into the bloodstream based on the administration route, circulating around the body and potentially absorbing oxygen during their passage through the lung capillary bed, and, subsequently, releasing oxygen in...
the areas of hypoxia. In addition, studies on the use of ONBs in liquid ventilators suggested that this could be an alternative to traditional mechanical ventilators. Some other routes for the administering of NBs, such as into the stomach, bladder, or CSF, have been suggested (Figure 16).

It has been proven that ONBs could overcome hypoxic conditions in cells, degrade HIF-1α, and promote tissue oxygen levels. For COVID-19 patients who are nonresponsive to conventional oxygen therapy, oxygenation with ONBs could be an appropriate alternative to improve endothelial barrier disruption, dysfunctional alveolar—capillary oxygen transmission, and impaired oxygen diffusion capacity. This platform could also improve the oxygenation to the blood as well as organs and tissues by regulating the ratio of $p_{\text{O}_2}$ to $F_{\text{O}_2}$. As discussed, COVID-19 produces changes in the intracellular pH; therefore, pH-stimulus-responsive carriers could efficiently oxygenate hypoxic tissues. Additionally, treatment with ONBs, e.g., RNS60 solutions, could conquer hypoxia by stimulating mitochondrial metabolism and increasing the cellular ATP levels. Also, it could improve the oxygen supply and blood circulation at the same time to overcome these crucial complications in these patients. Although 50% of the mechanically ventilated patients could experience hyperoxemia, which is an increase in $p_{\text{O}_2}$ to a level greater than 120 mmHg (16 kPa), in the case of oxygenation with NBs, there has been only one report about hyperoxemia that is attributed to the effect of the ONB solution on osteoclastogenesis in mouse bone marrow cells. However, further detailed investigation is required to prevent the risk of oxygen toxicity.

The simultaneous multifunctional role of these nanoplatforms not only could allow the transfer of oxygen but also could have a positive effect on COVID-19 patients with weakened immune systems who are known to be at risk of further infectious diseases. ONBs could oxygenate cells, while preventing microbial infections as well as expediting wound healing. Because hypoxia increases microbial resistance to antibiotics and treatments, the provision of extra oxygen to the tissues could help to conquer bacterial infections. Besides, the efficiency of NBs as a potential treatment for eradicating the persistent SARS-CoV-2 in hospital wastewater has been examined with promising results. Moreover, ONBs can also act as US contrast agents, allowing simultaneous treatment and imaging as a multifunctional theranostic approach. Moreover, US could trigger the release of antimicrobial or antiinflammatory drugs from the NBs, thereby increasing the drug concentration and drug penetration into the site of infection or inflammation. Therefore, it is conceivable that ONBs may be used for oxygenation while delivering clinically approved drugs for the treatment of COVID-19, such as remdesivir, corticosteroid dexamethasone, hydroxychloroquine, azithromycin, or baricitinib. Furthermore, inhibitors of proinflammatory cytokines (TNF-α, IL-1, or IL-6) like Tocilizumab could be delivered as well. These drugs could be loaded into the ONBs either by encapsulation in the core or by coating onto the outer shell using covalent or noncovalent attachment depending on their hydrophobicity/hydrophilicity and structure. Additionally, targeted ONBs could be enhanced by incorporating specific molecular ligands onto the surface of the NBs with the ability to bind directly to the damaged lung tissue. This approach could expand the application of already established drugs to reduce the mortality of this devastating disease.

Some concerns have been expressed about the use of $\text{IVO}_2$ because of the possibility of producing gaseous emboli originating from oxygen release from the solution or the possibility of oxygen toxicity. However, recent studies have proven that $\text{IVO}_2$ delivered by NBs is a noninvasive technique that could be safe enough to be included in the arsenal for hypoxic respiratory failure. Another concern is related to premature oxygen release during the vascular injection of ONBs, which could be overcome by the development of various chemical or biological formulations to decline the surface tension and to improve the stability of ONBs in the human body. Free ONBs that are not coated and stabilized by any component in the shell structure dissolve quickly in the circulation, while the use of surfactants, lipids, proteins, and their combinations can avoid disproportionation, coalescence, and undesired oxygen release. The designed shell makes a layer around the oxygen to protect from endogenous scavengers and increase stability, and it reduces the diffusion rate into the surrounding media. It has been confirmed that PEG-based shells could prolong the circulation time because the diffusion around the rigid protein shells is limited. Additionally, improved in vivo stability and longevity of ONBs has been obtained using low-solubility PFC as a hydrophobic gas with a high molecular weight in combination with oxygen gas, thus providing a longer circulation time. On the other hand, the strategy of using a solid source of oxygen or oxygen-generating molecules like peroxides is an alternative route for controlling the release mechanism. Overall, many of the reports that are summarized in this review have benefited from these techniques by enhancing the in vivo stability and designing oxygen carriers that could specifically release oxygen in the desired site.

The comparison results revealed that most of the ONB systems for in vivo oxygenation to cells are based on the incorporation of oxygen at the synthesis stage by cavitation or sonication compared to oxygen-generating molecules that generate oxygen in the body. One of the reasons could be attributed to the concern about the fate of the remaining components within the body and the energy input that is needed for oxidation of these materials. We believe that, for COVID-19 patients in which where sustained oxygen delivery is necessary for physiological needs over a prolonged period of time, ONBs are better candidates because they could generate more oxygen for a longer period in hypoxic cells. However, further research is needed to confirm these recommendations with more evidence. Because NBs can be internalized to cells through endocytosis, in the case of ONBs made from lipids or proteins, the body could metabolize them through natural cellular processes. For polymeric shell design, biodegradable polymers such as dextran, cellulose, or CS are preferred given their ability to undergo biodegradation.

After the completion of successful clinical trials, this method could be rapidly used for COVID-19 patients suffering from breathing or respiratory problems or from low oxygen levels. Countless lives could be saved in every country because of its easy implementation and exceptionally low cost, considering the wide availability of IV protocols and equipment. NBs could also be administered in emergency situations in an ambulance by direct injection into the bloodstream, enabling suffocating patients extra time during transportation to the hospital.

During our literature survey, we have not found any specific description of hydrogen-loaded NBs used as a supplement to oxygen therapy, although their antioxidant activity has been
investigated earlier. Moreover, long-lived hydrogen NBs have been shown to have antiinflammatory activity in diverse cells by regulating cellular metabolism. It is important to point out that inhalation of a mixture of hydrogen/oxygen gas (66.6%/33.3%) has been recommended by Chinese medical authorities to treat COVID-19 patients. In view of the fact that the production of excess mucus in COVID-19 patients reduces the absorption of oxygen, a mixture of oxygen and hydrogen could further expand the bronchioles and the alveoli of the lungs and optimize the absorption of oxygen. The catalytic role of the hydrogen NBs could accelerate the binding of Hb to oxygen and instigate the release of carbon dioxide from Hb. Thus, it appears that a broader utilization of a saline solution containing hydrogen/oxygen-loaded NBs, which could be produced through the electrolysis of H2O or by a droplet reaction, could save many lives by reducing the incidence of severe pneumonia and could help to reduce this fatal risk in a future inexpensive treatment for COVID-19.

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### Notes

The authors declare no competing financial interest.

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### ABBREVIATION

AC-DEX, acetaldehyde dextran  
ACE2, angiotensin-converting enzyme 2  
ARD1A, arrest-defective protein 1 homologue A  
ARDS, acute respiratory distress syndrome  
ARPE-19, retinal pigment epithelia cell line  
ATP, adenosine triphosphate  
BBB, blood–brain barrier  
BEAS 2B, noncancerous human bronchial cells  
BRCA1, breast cancer type 1 susceptibility protein  
BxPc-3, human pancreatic tumor cells  
C, arterial oxygen content  
CHO, Chinese hamster ovary cells  
CMC, carboxymethylcellulose  
CNE2, human nasopharyngeal carcinoma cells  
CO, cardiac output  
CS, chitosan  
CSF, cerebrospinal fluid  
DDPF, dodecylurea ureapentane  
DO2, systemic oxygen delivery to the organs per minute  
DON, doxorubicin  
DPBS, Dulbecco’s phosphate-buffered saline  
DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine  
DPPG, 1,2-dipalmitoyl-sn-glycero-3-phospho-(1′-rac-glycerol)  
DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine  
DSPE-PEG-2000-amine, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]  
DSPE-PEG-2000-biotin, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000]  
EBC-1, lung cancer cell line  
ECMO, extracorporeal membranous oxygenation  
GIO, glucocorticoid-induced osteoporosis  
HADSCs, human adipose-derived stem cells  
Hb, hemoglobin  
hBMECs, human brain microvascular endothelial cells  
HeLa, cervical cancer cell  
HepG2, human liver cancer cell line  
HIF-1α, hypoxia-inducible factor-1α protein  
HREs, hypoxia-regulated elements  
HSDFM, hyperspectral dark-field microscopy  
IV, intravenous  
IVO, intravenous O2  
JEG-3, human choric cancer cells  
MAT2A, methionine adenosyltransferase 2A  
MB49, mouse urothelial carcinoma cell line  
MBs, microbubbles  
5mC, 5-methylcytosine  
MCP-1, monocyte-chemoattractant protein-1  
MDA-MB-231, human breast cancer cell line  
MMC, mitomycin-C  
MNPs, micro/nanobubbles  
NBs, nanobubbles  
NIH-3T3, mouse embryonic fibroblasts  
NPs, nanoparticles  
NSS, normal saline solution  
OMBs, oxygen microbubbles  
ONBs, oxygen nanobubbles  
PBMcs, peripheral blood mononuclear cells  
PC3, human prostate cancer cell lines  
PDK-1, phosphoinositide-dependent kinase-1  
PEG, poly(ethylene glycol)  
PFC, perfluorocarbon  
PFP, perfluoropentane  
PVP, poly(N,N-dimethylacrylamide)  
pO2, oxygen partial pressure  
R28, retinal cell line  
rGO, reduced graphene oxide  
RNS60, isotonic saline containing oxygen nanobubbles  
SPIONs, superparamagnetic iron oxide nanoparticles  
SpO2, blood oxygen saturation pressure  
4T1, breast cancer cell line  
TMPRSS2, transmembrane protease serine 2  
TNF-α, tumor necrosis factor
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