A Pharmaceutical Approach of Transcutaneous Oxygen Therapies

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1 Introduction

Oxygen is an essential fuel and also a poison for living organisms. On the one hand, it can violently oxidize vital macromolecules (e.g., proteins and nucleic acids), and on the other hand, some of these macromolecules are specially designed to capture, transport, and use oxygen in many cascades of chemical reactions. These paradoxal properties have led, through adaptive evolution, organisms to develop strategies to precisely control and adapt the absorption of oxygen from the environment. Mammals mainly use their lungs through breathing and have developed their interfacial surface to meet their body's requirements in oxygen. The second interfacial surface of body in direct contact with environmental oxygen is human skin. Very naturally, an intimate relation emerged between the skin and oxygen homeostasis. And although the skin cannot anymore be considered as a "breathing organ" in humans, it has deep implications in oxygen supply management.

Nowadays, medical attention has been driven toward body supply in oxygen due to its wide impact on various physiopathological processes such as wound healing, skin aging, and immune reactions. Beyond hyperbaric oxygen therapy, innovative formulation technologies are being developed to enable oxygen delivery in a handy and efficient manner.

2 General Aspects on Oxygen and Skin

2.1 Physicochemistry of Oxygen

Atomic oxygen is a highly electronegative chemical element (symbol "O"; atomic number, 8) that forms compounds with most elements in often highly exothermic reactions. It is the most abundant element on earth surface. Furthermore, it represents 62 % (m/m) of the human body. Under normal conditions of temperature and pressure, atomic oxygen binds to form a diatomic colorless, odorless gas called oxygen (O_2). Oxygen is a small molecule with a molecular weight of 31.999 g/mol that exhibits a pressure and reverse-temperature-dependent solubility in water (i.e., 8.3 mg/L at 25 °C under atmospheric pressure).

2.2 Oxygen Physiology

Oxygen acts toward the human body at the same time (i) as a major metabolic substrate for cellular chemistry and (ii) as a signal in multiple metabolic pathways with a remnant effect after return to normal oxygen levels (Ladizinsky and Roe 2010).

Many essential physiological reactions require the presence of oxygen. Most importantly, oxygen is the terminal electron acceptor in mitochondrial respiration and it also plays a central role in many other metabolic processes. For instance, oxygen is required (i) for the production of nitric oxide by NO synthases, (ii) for the hydroxylation of the collagen, and (iii) for cholesterol synthesis.

Furthermore, oxygen levels are monitored at different points within the human body (e.g., the carotid, liver, and kidney) to induce homeostatic response to hypoxia (i.e., differential vasoconstriction/dilatation, erythropoietin, and red blood cell production). In case of acute environmental hypoxia, concomitant pulmonary vasoconstriction and systemic vasodilatation occur to ensure correct oxygenation of vital organs. Surprisingly, unlike the rest of systemic vasculature, skin vasculature undergoes vasoconstriction in response to acute environmental hypoxia. If hypoxia is prolonged, a hypoxia-inducible transcription factor (HIF)-mediated response progressively restores the cutaneous blood flow in the skin, leading to liver and kidney hypoxia and subsequent erythropoietin synthesis to enhance blood's oxygen-carrying capacity. Thus, the skin is the central actor of a bimodal response to acute/chronic environmental hypoxia in mammals (Boutin et al. 2008; Semenza 2008).

Eventually, oxygen supply is intimately intricate in skin aging processes as a cause (i.e., production of reactive oxygen species or ROS) and a potential treatment for a variety of skin discomforts; therefore, the development of oxygen therapies is a major interest of cosmetic companies with important economic and sanitary outcomes (Asadamongkol and Zhang 2014).

2.3 Oxygen Supply

Oxygen supply to human body's organs and tissues is mainly ensured by pulmonary absorption. In the lungs, gaseous oxygen needs to become dissolved in a fluid state to cross alveolar membrane. Once dissolved, oxygen diffuses within the body from areas of high concentration to areas of low concentration. Hemoglobin within red blood cells acts as a reservoir ensuring that plasmatic dissolved oxygen level is sufficiently maintained to ensure delivery to organs and tissues via capillary perfusion (Ladizinsky and Roe 2010).

Although transcutaneous absorption of oxygen is now known to occur in human, the ratio of the absorption surface between the skin and lungs (1–2 m^2 and 70 m^2, respectively) limits its impact as a source of systemic oxygenation. Overall, apart in premature infants, atmospheric oxygen transported through the human skin contributes in a negligible way (i.e., 2 %) to body oxygenation (Roe et al. 2010). As dermal vasculature does not cross its basal membrane, the epidermis can be considered as a physiologically hypoxic tissue where anaerobic glycolysis is likely to be important to sustain its high metabolic activity due to constant renewal (Boutin et al. 2008; Straseski et al. 2009). Moreover, due to its highly organized structure, skin barrier function further limits the extent of transcutaneous absorption of oxygen. However, considering local skin oxygen supply, transcutaneous absorption is (i) a physiologically relevant oxygen source down to the superficial dermis and (ii) the major source of oxygen for the outermost layers of unperfused epidermis (Ladizinsky and Roe 2010).

In human, skin's partial pressure in oxygen levels (pO_2) is highly correlated to the physiopathological state of the skin (Reading et al. 2013). For instance, wound repair outcome may be estimated regarding local oxygen tension. Non-hypoxic wounds (i.e., pO_2 >30 mmHg) will heal rapidly with no complications such as infection oppositely to hypoxic wounds (i.e., pO_2 between 13 and 30 mmHg). When pO_2 drops under 13 mmHg, basal metabolic activity cannot be sustained which leads to gangrenization (Roe et al. 2010).

2.4 Oxygen Skin Permeability

Due to its small molecular size, permeation of oxygen through the skin is likely to occur massively through porous structures such as skin appendages (e.g., eccrine glands). Permeation of oxygen through nonporous regions of the skin could also follow the transcellular pathway via transmembrane proteins such as aquaporins that exhibit gas transport capacities (Roe et al. 2010; Wang and Tajkhorshid 2007). Data is scarce on oxygen skin permeability but suggests it relies mostly on passive physicochemical-mediated transport and not active cellular mechanisms (Roe et al. 2010). Oxygen delivery from a saturated aqueous vehicle was assessed *ex vivo* using porcine skin. Exogenous (i.e., via the *stratum corneum*) and endogenous (i.e., via the dermis) administrations were simulated and revealed a higher permeation for endogenous delivery and a better skin penetration supposedly due to reservoir effect of *stratum corneum* lipid matrix with exogenous administration. However, some studies suggest tape stripping of the epidermis leads to enhanced oxygen penetration into the skin from an aqueous formulation (Ladizinsky and Roe 2010; Atrux-Tallau et al. 2009). Therefore, it is not clear if skin stratified structure and more specifically the *stratum corneum* can be considered as a major barrier to transcutaneous penetration of oxygen. Overall, oxygen absorption in the epidermis from the environment can be described using a Fickian model where a pO_2 gradient governs oxygen penetration. This theoretically enables local therapeutic approaches based on topical application of oxygen-loaded formulations (Reading et al. 2013).

3 Compounding Strategies for Oxygen

3.1 Gaseous Formulations of Oxygen

Oxygen therapy in the context of wound healing should be able to deliver sufficient oxygen to reach pO_2 found in healthy well-perfused tissues (i.e., >30 mmHg). Hyperbaric oxygen therapy is known to have significant impact on wound healing processes as long as blood perfusion is effective in the targeted tissue. Concerning the skin, hyperbaric oxygen therapy raises subcutaneous oxygen tension for several hours after return to normobaric conditions (Ladizinsky and Roe 2010). However, gaseous formulations of oxygen require that gaseous oxygen overcomes the gas/liquid phase boundary before it becomes bioavailable (Roe et al. 2010). Furthermore, high-pressure gaseous oxygen administration is limited as it will mechanically cause microvascular occlusion at administration site (Reading et al. 2013).

3.2 Liquid Formulations of Oxygen

3.2.1 Aqueous Formulations

As oxygen is bioavailable if dissolved in a fluid state, liquid formulations of oxygen might provide better biopharmaceutical capacity to deliver oxygen to the skin. Dissolved oxygen applied topically shows (i) faster rate and (ii) deeper depth of penetration in human skin than gaseous oxygen formulations applied topically (Roe et al. 2010).

Water shows higher penetration capacities through the skin than molecular oxygen and is capable of retaining up to 50 mg of oxygen per liter using simple enriching techniques. Therefore, water may be an interesting and inexpensive vehicle for a liquid formulation of oxygen. Furthermore, an aqueous-based formulation is likely to be supplemented with a large variety of compounds meant to support wound healing process in addition to oxygen (Reading et al. 2013).

Recent reports have shown the absorption of oxygen through the plantar surface of the foot and palmar surface of the hand, when immersed in water that contains high levels of dissolved oxygen. Moreover, age and patient's condition (e.g., diabetes) influence oxygen absorption and/or retention (Reading et al. 2013).

3.2.2 Fluorocarbons

Fluorocarbons are inert synthetic linear of cyclic hydrocarbons ranging from C8 to C10, wherein all or part of the hydrogen atoms have been replaced by fluorine or bromine. They exhibit low molecular weights (i.e., 450–500 Da) and are immiscible with both hydrophobic and aqueous solutions. They are used in industry as a refrigerant and propellant in aerosol canisters but also show a high capacity to dissolve and release oxygen and carbon dioxide. With their oxygen-carrying capacity being 50 times greater than that of water, fluorocarbons feature a better liquid vehicle than water for oxygen transport through the skin (Isaacs et al. 2011). Although nearly twice as dense, most have a similar kinematic viscosity as water.

The solubilization and release of oxygen by fluorocarbon are a physical entirely passive process, unlike the bonding and release of oxygen from hemoglobin in the blood. The amount of dissolved gas is (i) proportional to the partial pressure of the gas in the liquid which is in equilibrium, (ii) inversely proportional to the molecular weight of the fluorocarbons, and (iii) directly related to the number of fluorine atoms present. Physicochemical properties of selected fluorocarbons are given in Table 1 (Kaisers et al. 2003).

Linear fluorocarbons are better oxygen carriers. They are biologically and chemically inert and non-metabolized, and, being volatile, they are excreted through the lungs within a week. However, they accumulate in the reticuloendothelial system where their long-term effects are not yet established (Krafft 2001).

A major drawback consists in their immiscibility to water. Therefore, they must be administered either (i) as an emulsion, to be carried in the vasculature, or (ii) as a dispersion of fine particles of 0.1–0.2 mm in suspension in isotonic saline electrolyte. To obtain stable emulsions at ambient temperature, emulsifiers are necessary. Emulsifiers commonly used, such as egg yolk

Table 1 Physicochemical properties of selected PFCs

	FC-77	FC-75	FC-3280	Rimar 101	Perfluorodecalin	Perflubron
Chemical formula	50/50 mix of two isomers of $C_8F_{16}O$	40/40/20 mix of two isomers of $C_8F_{16}O$ and C_8F_{18}	C_8F_{18}	$C_8F_{16}o$	$C_{10}F_{18}$	$C_8F_{17}Br$
Molecular weight (Daltons)	Approx. 416	Approx. 420	438	416	462	499
Boiling point (°C)	97	102	102	101	142	143
Density at 25 °C (g ml^{-1})	1.78	1.78	1.76	1.77	1.95	1.93
Kinematic viscosity at 25 °C (Pa.s)	0.80	0.82	0.80	0.82	2.90	1.1
Vapor pressure at 37 °C (mmHg)	85	63	Approx. 51	64	14	11
Surface tension at 25 °C (dyne. cm^{-1})	15	15	15	15	15	18
Oxygen solubility at 25 °C (ml gas per 100 ml PFC)	50	52	Approx. 48	52	49	53
Carbon dioxide solubility at 25 °C (ml gas per 100 ml PFC)	198	160	Approx. 176	160	140	210

phospholipids, provide stable emulsions and can be sterilized without degradation. The ability to transport oxygen of these emulsions is dependent on the concentration of fluorocarbon. The emulsions that contain 45–60 % of fluorocarbon (w/v) seem optimal to transport oxygen, but their high viscosity limits the concentrations used.

3.3 Colloidal Formulations

3.3.1 Micro- and Nanosponges

Nanosponge and microsponge delivery systems were originally developed for topical delivery of drugs. This controlled release technology for topical agents consist of nano- or microporous beads loaded with active agent obtained by cross-linking of cyclodextrin. The average diameter of a nanosponge is below one micrometer, whereas for microsponges it ranges from 10 to 25 μm. These vehicles are designed to deliver efficiently a pharmaceutical active ingredient at minimum dose and to enhance stability. Nanosponge formulations might be potential gas delivery systems showing the ability to store and to release oxygen slowly over time. Furthermore, nanosponges exhibit high loading capacity for other gases (e.g., carbon dioxide and methylcyclopropene) (Cavalli et al. 2010; Patel and Oswal 2012).

3.3.2 Micro- and Nanobubbles

Microbubbles and nanobubbles are spherical gas-filled low-density structures with a mean diameter of 1–8 μm and >1 μm, respectively. They comprise a spherical cavity containing gas (e.g., oxygen) and gas-loading agents such as fluorocarbons. They are stabilized by a lipidic or polymeric coating. When introduced in an oxygen-deprived environment, oxygen-containing fluorocarbon microbubble will deflate, liberating their oxygen content. Subsequently, if the environment is reoxygenated, microbubbles will reload oxygen and recover a spherical shape. This property has important medical applications, for instance, in the development of synthetic blood substitutes. Furthermore, micro- and nanobubbles respond to ultrasonic stimulation by liberating their gaseous content, enabling to develop ultrasonic-driven targeting therapeutic strategies (Unger et al. 2004). For instance, gene transfection in reconstructed human skin was achieved by ultrasonic-enhanced microbubble delivery (Yang et al. 2005), and micro-/nanobubbles are presented as ultrasound-targeting oxygen delivery systems as adjuvant to antibiotics in anaerobic infections. Furthermore, many medical conditions, such as diabetes, burns, bedsores, and wounds, are possible fields of application of oxygen-filled micro-/nanobubbles besides other oxygenation approaches (Cavalli et al. 2009).

4 Clinical Applications

4.1 Interest of Oxygen Therapies

Numerous diseases are caused by tissular ischemia or hypoxia. Under some conditions, the HIF-mediated homeostatic response is not sufficient, and the use of hyperbaric oxygen therapy is required. Hyperbaric oxygen therapy has been shown to enhance bone, muscle, and skin healing, particularly in conditions of ischemia and low oxygen tension. In the skin, it is proved that hyperbaric oxygen has positive treatment effect on promoting wound healing of, e.g., chronic ulcers and diabetic foot (Thackham et al. 2008; Eskes et al. 2010). Moreover, hyperbaric oxygen increases levels of growth factors, such as vascular endothelial growth factor, and stimulates vasculogenic stem cell mobilization from the bone marrow in response to oxidative stress.

The goal of an oxygen therapy for wound care is to transfer sufficient oxygen to interstitial tissues to maintain a concentration above 30 mmHg found in healthy, well-perfused tissues. Oxygen concentration is paramount to the proliferation and differentiation of a variety of cell types. Various mechanisms have been proposed to explain positive effects of oxygen therapies on injured tissues. Indeed, high levels of oxygen stimulate the proliferation of fibroblasts and endothelial cells, differentiation and migration of keratinocytes, and angiogenesis and decrease edema in the periwound skin, through a vasoconstrictive effect. The functioning of several enzymes needed for the correct synthesis of collagen requires oxygen as a cofactor (Zgonis et al. 2005). Furthermore, hyperoxia facilitates the elimination of anaerobic bacteria by leukocytes through activation of the oxygen-dependent peroxidase system and promotes the production of ROS that attack bacterial structures (Fife et al. 2002).

4.2 Drawback of Oxygen Therapies

The use of oxygen therapy in hypoxic conditions is promising; however, abusive use poses risks, notably oxidative stress. This is especially true in the context of widespread oxygen therapies for cosmetic and esthetic means.

One potential danger is that ROS produced from oxygen are highly reactive and therefore exhibit potent toxicity, which often occurs under hyperbaric conditions. When oxygen exposure overpowers the tolerance of tissues, further exposure may eventually result in toxicity or oxidative injury. Oxygen toxicity occurs after extended periods of hyperbaric oxygen exposure and/or at high pressure.

It has also been reported that hyperbaric oxygen therapy increases the production of ROS within the tissue (Matsunami et al. 2009), thereby mobilizing cellular antioxidant responses (Godman et al. 2010). ROS can oxidize proteins and lipids and

Table 2 Contrasting hyperbaric oxygen therapy with topical oxygen delivery modalities for wound care

Systemic hyperbaric oxygenation	Topical delivery of oxygen
Systematically oxygenated blood at 2–3 atm	Topically oxygenated wound tissue at 1 atm
Requires specialized facilities and personnel	Portable device
Relatively expensive	Inexpensive
Relies on vascular system to deliver oxygen in wounds	Delivers oxygen to superficial wounded tissues severed from circulation
Poor vascularity of wound tissue limits oxygen diffusion	Oxygenation is independent of vascular bed
Risk of multiorgan oxygen toxicity	Limited and localized risk of toxicity

react with DNA to cause single-strand breaks and base modifications. This process imparts a number of molecular changes to skin tissue and an overwhelming of cellular protective responses, ultimately leading to increased levels of cell death (Pustisek and Situm 2012).

4.3 Topical Oxygen Delivery Strategies

As we have seen, different formulations exist that enable oxygen loading and dermal application in chronic wounds and to address the lack of oxygen due to low perfusion in certain skin diseases. As oxygen was found to better penetrate skin layers from exogenous supply than from endogenous supply, local topical delivery of oxygen appears a rational approach to enhance pO_2 in the skin (Atrux-Tallau et al. 2009).

Gas formulations are readily developed and available such as hyperbaric oxygen therapy; however, they remain inconvenient in terms of administration (Table 2). Liquid formulations in aqueous media or enhanced loading capacity media such as fluorocarbons appear handier, but drawbacks in compounding and delivery control have led to develop more complex formulations with targeting and controlled release characteristics. Colloidal formulations able to entrap oxygen show promising aptitudes and best meet these criteria. Other strategies such as incorporation of a vasodilating agent (e.g., nicotinate esters) may be interestingly combined to oxygen delivery systems to improve skin oxygen content by exogenous and endogenous supply (Krzic et al. 2001).

5 Conclusion

The perceived health and physiologic functioning of the skin depend on adequate oxygen availability, both oxygen present in the body and oxygen contributed by the external environment. The capacity of the skin to absorb oxygen from air has often been overlooked but can account for up to 2 % of the total oxygen consumed by the body and is especially important to the epidermis.

Formulations have been developed to overcome skin hypoxia. Complex colloidal formulations are not yet used at their full potential in the skin care area, however in rapid progress. Nevertheless, vigilance is required about frequent and or abusive use as high amounts of oxygen can cause oxidative stress resulting in the exact opposite of therapeutically expected effect.

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