In vitro and _in vivo_ monitoring of oxygen release from decafluoropentane-containing oxygen-loaded nanobubble gel formulations: a photoacoustic approach

Mauro Prato[1], Jithin Jose[2], Chiara Magnetto[3], Adriano Troia[3], Roberta Cavalli[4], Caterina Guiot[1]

[1] *Dipartimento di Neuroscienze, Università di Torino, Torino, Italy*
[2] *FujiFilm VisualSonics, Amsterdam, Netherlands*
[3] *Istituto Nazionale di Ricerca Metrologica (INRIM), Torino, Italy*
[4] *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italy*

Introduction. Photoacoustic imaging is a hybrid technique based on detection of acoustic waves generated by absorption of short laser pulses in biological tissues, combining the advantages of excellent contrast achieved in optical techniques with high resolution of ultrasound imaging. Since multi-wavelength photoacoustic imaging can estimate O_2 saturation and total concentration of hemoglobin (Hb), it appears a suitable tool to test the efficacy of new oxygenating drugs in cutaneous hypoxia-associated pathologies, including diabetic foot and bedsores. Oxygen-loaded nanobubbles (OLNs), constituted by a shell of biocompatible/biodegradable polysaccharide and a fluorocarbon inner core, are new non-invasive and low-cost nanotechnological devices aimed at treating hypoxia-related diseases. Here, four gel formulations of OLNs made by two alternative polysaccharides (dextran; chitosan) and two alternative fluorocarbons (decafluoropentane, DFP; and perfluoropentane, PFP) were tested *in vitro* and *in vivo* for effectiveness in O_2 delivery.
Methods. Dextran/PFP, dextran/DFP, chitosan/PFP, and chitosan/DFP OLNs dispersed in the hydroxy-ethylcellulose gel were characterized by optical microscopy and light scattering. *In vitro* O_2 release was measured through a pulse oxymeter. *In vivo*, OLNs were applied topically on mouse hindlimbs and photoacoustic imaging was performed with commercially available Vevo® LAZR system (VisualSonics) featuring a hybrid ultrasound transducer (central frequency: 21 MHz; spatial resolution: 75 μm).
Results. All OLN formulations displayed spherical morphology. Dextran-shelled OLNs showed average diameters of ~500 nm and anionic surfaces; chitosan-shelled OLNs showed average diameters of ~750 nm and cationic surfaces. *In vitro*, all OLNs delivered higher and more time-sustained amounts of O_2 than O_2-saturated solution (OSS), with DFP-OLNs being more effective than PFP-OLNs (for both shells). *In vivo,* photoacoustic monitoring revealed that either dextran- or chitosan-shelled DFP-containing OLNs increased mouse oxyHb levels significantly and constantly for the entire observational period. OLN effects were specifically dependent on O_2 gradual diffusion from OLN core, as they were not achieved when OSS or O_2-Free Nanobubbles (OFNs) were used: as expected, OSS did induce a high but only transient peak of oxyHb, whereas OFNs did not affect oxy/deoxyHb balances.
Conclusions. Collectively, these data show that OLNs do effectively release O_2 both *in vitro* and *in vivo*. Moreover, photoacoustic imaging appears a useful technique to monitor skin oxygenation during topical administration of exogenous O_2, properly encapsulated in nanobubble formulations, to treat hypoxia-associated cutaneous pathologies.
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