**In vitro and in vivo monitoring of oxygen release from decafluoropentane-containing oxygen-loaded nanobubble gel formulations: a photoacoustic approach**

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**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 OLN 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 OLN 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*, OLN 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 \( \mu \)m).

**Results.** All OLN formulations displayed spherical morphology. Dextran-shelled OLN showed average diameters of \(~500\) nm and anionic surfaces; chitosan-shelled OLN showed average diameters of \(~750\) nm and cationic surfaces. *In vitro*, all OLN delivered higher and more time-sustained amounts of \( O_2 \) than \( O_2 \)-saturated solution (OSS), with DFP-OLN being more effective than PFP-OLN (for both shells). *In vivo*, photoacoustic monitoring revealed that either dextran- or chitosan-shelled DFP-containing OLN 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 OLN 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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