***In vitro* and *in vivo* monitoring of oxygen release from ultrasonophorated chitosan-shelled oxygen-loaded nanobubbles.**

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Introduction. Ultrasonophorated 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, two water formulations of ultrasonophorated chitosan-shelled OLNs made with two alternative fluorocarbons (decafluoropentane, DFP: boiling point: 51°C; and perfluoropentane, PFP: boiling point: 30°C) were tested *in vitro* and *in vivo* for efficiency in O_2 delivery through skin membranes.
Methods. OLNs were characterized for physical-chemical properties by optical microscopy and light scattering. *In vitro* O_2 delivery from OLNs through skin membranes was monitored after ultrasound (US) treatment (f=2.5MHz; P=5W; t= 15 sec) up to 135 min by using a home-made apparatus consisting of two cylindrical chambers sealed by pig skin, with the lower chamber being connected to a US transducer and filled with OLNs (or alternatively O_2-free nanobubbles (OFNs) and O_2-saturated solution (OSS) as controls), and the upper chamber connected to a Hach-Lange LDO oxymeter and filled with 0.9 % NaCl hypoxic solution. *In vivo* O_2 release from OLNs after US treatment (f=1 MHz; P=5W; t= 15 sec) was measured by monitoring up to 45-60 min O_2 transcutaneous tension ($TcPO_2$) of small portions of shaved mouse skin through TINA TCM30 oxymeter. All procedures were done in accordance with the EU guidelines and with the approval of the University of Torino animal care committee.
Results. Both OLN formulations displayed spherical morphology, with diameters of ~750 nm (PFP: 726,55 ± 123,07 nm; DFP: 745,20 ± 117,89 nm) and cationic surfaces. *In vitro*, US-treated OLNs delivered through pig membranes higher and more time-sustained amounts of O_2 than OFNs and OSS, with DFP-OLNs being more effective than PFP-OLNs. *In vivo*, all mice displayed higher oxygenation levels in a time-sustained manner after topical administration of ultrasonophorated DFP-OLNs.
Conclusion. Ultrasonophorated chitosan-shelled/DFP-containing OLNs effectively crossed the skin barrier and increased $TcPO_2$ levels of previously hypoxic tissues, confirming that topical administration of exogenous O_2, properly encapsulated in nanobubble formulations, might be a new suitable and efficient approach to treat hypoxia-associated pathologies of superficial tissues.
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