Abstract

Ultrasound (US) is a widely used diagnostic technique in the medical field, and in recent decades, it has also gained significant interest for therapeutic purposes. In particular, US has been shown to be effective for drug delivery due to its ability to provide better spatial confinement and reduce undesired side effects. In addition to that, phase-shift perfluorocarbon(PFC)-based formulations have been demonstrated to be promising US-sensitive platforms for controlled oxygen delivery to treat tissue hypoxia diseases. This has provided the basis for the synthesis of new oxygen delivery platforms, the oxygen-loaded nanodroplets (OLNDs), characterized by a PFC-based liquid core formulation. These new platforms have shown promising properties, including highly stability, bioinertness and biocompatibility, ease of preparation, and relatively low cost. Additionally, their nanosized dimensions enhance extravasation. The physical mechanism triggering the oxygen release by US is acoustic cavitation. In particular, interacting with the US field, OLNDs first undergo acoustic droplet vaporization (ADV), passing from liquid nanodroplets to gaseous microbubbles, and then acoustic cavitation. Cavitation is actually composed of two separate moments. As the bubbles are formed, they start growing until reaching their resonant sizes around which oscillate (stable cavitation) and release their content in a controlled way. If the acoustic pressure further increases, bubbles undergo a violent collapse (inertial cavitation). However, at present there is no complete characterization at a fundamental level of the different signals produced by US-activated sonocarriers. Therefore, the first aim of this PhD thesis is to obtain a metrological characterization of the cavitation phenomena induced by US through parallel investigation approaches.

In the first part, therefore, three different US-sensitive carriers were used, including two different PFP-based OLNDs using polyvinyl-alcohol (PVA) or chitosan as a polymeric shell, and zinc oxide nanoparticles (ZnO NPs) that behave as cavitation

nuclei for the formation of bubbles inside a liquid solution. A customized setup was developed to monitor the response to US stimuli of the carriers flowing inside a customized channel, through different sensors. A passive cavitation detector (PCD) provided information about cavitation, showing that the occurrence of both stable and inertial cavitation events in the presence of sonosensitive carriers enhanced compared to pure water. The ecographic probe was used for real-time ultrasonic imaging to monitor bubbles formation and oscillation within the channel. From the recorded videos, a novel method was developed to extrapolate quantitative information about stable cavitation activity, finding good correlation with the results obtained through PCD analysis. Finally, a high-speed camera was used for optical imaging to monitor the group behavior of bubbles related to each sample under the excitation of the acoustic field.

In the second part of the study, the focus shifted towards an improvement of the OLNDs performances in order to overcome some limitations due to the US activation source. In particular, acoustic field presents a limited ability to reach deeper zones of the human body due to absorption that US undergoes while travelling across human tissues. A possible solution to this challenge is the alternative use of the magnetic field which is characterized by a more penetrating profile. However, OLNDs first need to be functionalized through magnetic agents (nanoparticles) to become sensitive to the magnetic field for this approach to be effective.
A few studies have been found in the literature that have investigated magnetic OLNDs (MOLNDs), but their focus has mainly been on the use of the droplets as US imaging platforms during hyperthermia treatments performed by the magnetic NPs bound on their surface. This work, on the other hand, aims to obtain a dual-sensitive system capable of releasing oxygen through the use of either ultrasound or magnetic field obtaining comparable results in terms of performance. The mechanism behind the oxygen release mediated by an alternating current (AC) magnetic field is the magnetic droplet vaporization (MDV). Specifically, the nanoparticles interacting with the AC magnetic field are able to produce local heat on the droplet surface, leading to an increase of the temperature up to the boiling point of the PFC-core employed, thus enabling the vaporization and the gas release.
Six different OLNDs, differing in core and coating, were prepared for the study, and Fe_3O_4 NPs were used as magnetic functionalization agents. The optimal NPs concentration was found in order to ensure a good bounding over the droplets surface

and a detailed preparation method was presented. After a physico-chemical characterization of the MOLNDs, providing information about their morphology, shape and ζ potential, also the acoustic response was tested, in order to evaluate the NPs influence on the OLNDs behavior. It was found a shift of the cavitation thresholds, both stable and inertial, towards lower acoustic pressures due to the functionalization. Two different setups were then used to assess that correct occurrence of the functionalization phase, one based on the ecographic imaging while the other on a microfluidic setup, both evaluating the influence of a static magnetic field on the MOLNDs streaming velocity and trajectory. Finally, the oxygen released following US and AC magnetic field was monitored and compared, confirming the effectiveness of the magnetically-mediated approach as valid alternative as therapeutic method. Furthermore, the comparison between the cores and coatings allowed to perform an evaluation of the optimal carrier in terms of functionalization rate and oxygen release control.

Overall, this study provides new insights into the potential of sono-sensitive nanosystems to release oxygen. More information about OLNDs acoustic response is provided, together with a novel method for monitoring the cavitation activity. A standardized method is also proposed to make OLNDs dual-sensitive systems using as activation source also the magnetic field, proving therefore their high potential as promising and versatile oxygen delivery systems.