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| **Optimising the loading of Nitric Oxide into Perfluorocarbon Droplet Emulsions** |
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| **Background:** Perfluorocarbon-core droplets (PDs) are promising nanocarriers for antimicrobial interventions, particularly in the treatment of biofilm-associated infections. Nitric oxide (NO) is a potent signaling molecule known to induce biofilm dispersal; however, it has an extremely short half-life, making it difficult to deliver to a biofilm in therapeutically relevant amounts. The high gas solubility of PFCs is of key interest. The liquid perfluorocarbon (PFC) core of NDs is stabilized by a lipid shell and can be loaded with NO. PFC NDs are responsive to ultrasound waves and can be converted into gas-filled microbubbles through acoustic droplet vaporization (ADV). The aim of this study is to optimize the loading of perfluorocarbon droplet emulsions with NO and quantify its passive release profile. Subsequently, formulations will be tested on biofilms to assess antibiofilm activity. |
| **Methods:** To manufacture PDs, a lipid solution and perfluoro-n-pentane (PFP, Strem Chemicals, UK) were emulsified by two-stage sonication, using a Model 120 Sonic Dismembrator (Fisher Scientific, UK). Lipids were dispersed in phosphate-buffered saline (PBS) in the first stage of sonication. 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and polyoxyethylene (40) stearate (PEG40S) were combined in a 9:1 molar ratio as the lipid shell constituents. Prior to second sonication, PFP was added in a 1:20 volumetric ratio and vortexed for 5 s to create precursor droplets. The second sonication stage utilized a pulsed regime to allow the sample to cool between pulses. To load the PD emulsion with nitric oxide (NO), the headspace of 500 μl samples was flushed with ~1000 ppm NO + room air. NO was generated by a NOxLab Model D (Fourth State Medicine, UK). Excess NO2 was converted to NO using a catalytic converter (CG-2, M&C TechGroup, Germany). Samples of PDs, lipid-only suspension and PBS were equilibrated with NO for 5 min, and sealed within a 50 ml syringe. The headspace volume was subsequently reduced by 50%, and the sample cooled to 5 °C for 1 hr. Quantification of NO was assessed using a NO assay kit (Abcam , UK), measuring the total nitrate/nitrite concentration produced from breakdown of NO. Samples were diluted 1:100 in PBS prior to assaying. Sample absorbances and nitrate standard curve were measured at 540 nm on a plate reader (FLUOstar Omega, BMG Labtech Ltd., UK). Particle diameter and concentration was assessed using a Multisizer 4e (Beckman Coulter, USA) |
| **Results:** Concentrations of NO in the lipid-only and PD samples were 1.425 ± 0.05 μM and 1.834 ± 0.08 μM, respectively. Using an unpaired t-test with Welch’s correction, a significant difference (P<0.05) was found between the lipid-only and PD samples. NO-loaded PDs had a median diameter of 0.61 ± 0.02 µm with a concentration of 7.612 e10 ± 2.38 e10 particles/ml (n=3). |
| **Conclusions:** The NO-loading capacity of PFC droplet emulsions was assessed by colorimetric assay and found to be higher than that of PFC-free formulations. Further testing of alternative loading procedures, including under oxygen-free conditions, will be assessed in the future. Additionally, alternative NO-quantification methods, such as those utilising chemiluminescence and electrochemical sensors, will be explored. |