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| **Optimising Nanodroplets for Use in Oxygen Delivery for Bone Repair** |
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| **Background:** Perfluorocarbon nanodroplet emulsions have been investigated for use in oxygen delivery in a wide range of applications from cancer treatment to red blood cell substitutes. They consist of a perfluorocarbon core stabilised by a phospholipid shell and are capable of dissolving large amounts of oxygen (approx. 20 times that of water). Oxygen has been shown to affect the metabolism, proliferation and differentiation of osteoblasts and osteoclasts, with both cells being heavily involved in bone repair. Perfluorocarbon nanodroplets offer a potential method for delivery of oxygen to hypoxic tissues associated with non-union fractures. Although sonication is the most common laboratory process for making nanodroplets with perfluorocarbons heavier than perfluorobutane. Sonication is a stochastic process if the conditions are not controlled, and this can result in reduced repeatability. In this work, the sonication process was optimised to allow for improved control over its output and increase overall reproducibility. |
| **Methods:** To make nanodroplets, films of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and polyoxyethylene (40) stearate (PEG40s) at a molar ratio of 9:1 made by evaporation were rehydrated in PBS at 4 mg/ml. Perfluoropentane (PFP) was then added, and nanodroplets formed using a pulsed sonication (2 s on, 15 s off) on ice. Sonication time (15-1200 s) and amplitude (20-100%), perfluoropentane concentration (0-10 %vol) and the sonicator tip height (2-12 mm from the bottom of the tube) were varied and size of the resultant nanodroplets was then measured using nanoparticle tracking analysis (NTA). When interaction with MC3T3E1 cells was investigated, dilutions of 1 %vol/vol and less were used. Cell metabolism was investigated using an alamar blue assay and proliferation was investigated using DAPI staining. |
| **Results:** An increase in nanodroplet diameter as a function of PFP concentration was observed (P<0.0001). However, there was no significant difference in nanodroplet diameter when PFP concentrations below 5 %vol were employed and in the absence of PFP. Interestingly, increasing the tip height within the Eppendorf tube strengthened the correlation between the PFP concentration and nanodroplet diameter. At 5 %vol PFP and a 12 mm tip height, a median size of 156.4 nm and standard deviation of 91.73 nm were obtained. These were found to be the optimal conditions as the nanodroplet satisfied the desired dimensional range both when measured using NTA and light microscopy. These conditions were used to investigate the effect of sonication amplitude and total sonication time. Greater sonication amplitudes led to smaller nanodroplets with 20% and 40% amplitude failing to mix the PFP-lipid solution. A sonication time of 90 s was found to produce the smallest nanodroplets. A nanodroplet concentration of 1 %vol/vol decreased MC3T3E1 proliferation while a concentration of 0.1% was found to have the opposite effect. All concentrations of ≤ 1 %vol/vol were found to increase cell metabolism. |
| **Conclusions:** All variables investigated had a significant effect on nanodroplet size, the most significant of which was sonication amplitude. For the application of nanodroplet as an oxygen delivery mechanism to bone, 5 %vol PFP with a 12 mm tip height, a sonication amplitude of 100% and sonication time of 90 s was considered to be optimal due to the small diameter, small size dispersity and improved reproducibility. |