ACOUSTICALLY-STIMULATED DRUG CARRIERS FOR TARGETED DRUG RELEASE AND BONE FRACTURE REPAIR

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Background: Impaired fracture healing has a significant physical and mental impact on patients, in addition to a financial burden for healthcare services. The overall aim of our work is to develop acoustically-stimulated microbubbles (MBs) and nanodroplets (NDs) as targeted drug delivery systems for bone repair, in order to overcome pharmacokinetic limitations which, have halted progress of therapeutic agents. In this study, we tested the hypothesis that MB and ND preparations are non-toxic to human cells. We also determined the bioactivity of NDs loaded with an anabolic activator of the Wnt signaling pathway, which is involved in osteogenic differentiation - 6-bromoindirubin-3'-oxime (BIO).

Methods: MB suspensions were prepared with a 9:1 molar ratio of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) to polyoxyethylene(40) stearate (PEG40S). Lipid films were hydrated in PBS and sonicated to form air-containing MBs, or ND emulsions of perfluoropentane (PFP) with and without BIO. Primary, patient derived, bone marrow stromal cells were used to assess MB and ND cytotoxicity with exposure to varying concentrations up to 3×10^7 MBs/ml and 1×10^9 NDs/ml, for up to 72 hours. Alamar Blue®, Picogreen and Live/Dead assays were carried out as an indicator of cell viability. Bioactivity of BIO was assessed by exposure of BIO-containing NDs to 3T3 cell lines transfected with luciferase under the control of a Wnt-responsive promoter.

Results: DSPC:PEG40S MBs induced a dose-dependent decrease in metabolic activity at 72 hours exposure, with a significant reduction at 3×10^7 MBs/ml (63% ± 6%, $p<0.0001$). However, with exposures up to 24 hours, both MBs and NDs did not exhibit any inhibitory effect on cell metabolism. Increasing concentrations of free BIO induced Wnt expression with a peak at 5 µM. BIO-loaded NDs activated Wnt signaling to 40% of the maximal value with free BIO. Unloaded NDs had no inhibitory effects on Wnt-signaling induced by free BIO.

Conclusions: Microbubbles do not inhibit cell metabolism at concentrations up to 3×10^7 MBs/ml, while nanodroplets have no inhibitory effect on cell metabolism up to 1×10^9 NDs/ml, over 24 hours. These results validate the use of MBs and NDs in future studies as drug delivery agents. BIO-loaded NDs have displayed reduced activity compared to free drug, demonstrating their suitability as a drug delivery agent, maintaining drug activity prior to controlled release upon ultrasound stimulation. Future work will investigate ultrasound activated BIO release.