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| **Permeabilisation of Bone Cells Using Ultrasound Stimulated Microbubbles for Bone Fracture Repair** |
| Sam Sloan1, Oliver Pattinson1, Simon Tilley2, Janos Kanczler1, Dario Carugo3, Nicholas D Evans1 |
| 1 Faculty of Engineering and Physical Sciences, University of Southampton, Southampton, UK; 2 Southampton General Hospital, Southampton, UK; 3 Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Science, University of Oxford, UK |
| **Background:** Non-union or delayed-union fracture is a debilitating condition with a high healthcare burden, compromising the quality of life of affected individuals. Present therapies of this condition are invasive, may not be effective, and have associated risks of infection. Ultrasound simulated microbubbles delivered through the vasculature offers a promising method for non-invasive, targeted, drug delivery. These methods allow for a transfer of molecules across the cell membrane in a process driven by oscillation (or cavitation) of microbubbles nearby cells, under ultrasound stimulation. We hypothesize that human bone marrow stromal cells (HBMSC) and MG63 cells exposed to microbubbles under ultrasound stimulation enhances cell permeability and will allow for the transfer of molecules across the cell membrane.  |
| **Methods:** Microbubbles were made by thin film hydration of DSPC and PEG40s at a 9:1 molar ratio and sonication at the air/liquid interface. MG63 cell line and HBMSC collected from patients undergoing hip replacement surgery, were cultured in a 35 mm diameter Ibidi dish. Both cell types were exposed to ultrasound stimulation in the presence of microbubbles. Before stimulation, propidium iodide (PI) (molecular weight ≈ 660) was added to both cultures to identify permeabilised cells; fluorescently labelled dextrans (molecular weight ≈ 4500) were added to the MG63 culture to model the transport of larger bioactive molecules. Fluorescence microscopy was used to detect transport of PI and dextrans into the cells. The peak acoustic pressure was varied from 0-0.5 MPa with frequency, pulse repetition frequency and duty cycle held constant at 900 kHz, 1 kHz and 30% respectively. |
| **Results:** A significant increase in the number of permeable HBMSCs was observed for acoustic pressures greater than 0.2 MPa. An average of 17.2% ± 16.2, 21.9% ± 20.9, and 21.3% ± 20.3 (p < 0.002) cells were permeabilised at acoustic pressures of 0.3, 0.4, and 0.5 MPa, compared to 1% ± 1 and 0% for the ultrasound and microbubble only controls respectively. Similarly, MG63 cells also saw a significant increase in uptake of both PI and dextrans at 0.5 MPa. An average 10.2% ± 9.5 (p < 0.005) and 8.9% ± 6.2 (p <0.05) increase was seen in PI and dextran uptake respectively, compared to the ultrasound and microbubble only controls (0.7% ± 2.1 and 1.8% ± 2.1 respectively for PI, and 2.5% ± 2 and 1.6% ± 2 respectively for dextrans). Furthermore, no significant difference in uptake was observed between PI and dextrans at any acoustic pressure in MG63 cells. |
| **Conclusions:** To conclude, successful application of ultrasound stimulated microbubbles is demonstrated to induce permeabilisation of bone cells, and uptake of model drugs is increased with increasing acoustic pressure *in vitro*. Furthermore, uptake of relativelylarge molecules has been demonstrated, further validating the potential of ultrasound stimulated microbubbles as a non-invasive, localized drug delivery strategy for bone repair. |