Development of a Novel Perfused Bioreactor with Ultrasonic Mechanical Stimulation for Bone Tissue Engineering
Supacharn Tangviriyasirikul¹, Katsuko Furukawa², and Anathathios Mantalaris,¹*
¹Department of Chemical Engineering, Imperial College London, SW7 2AZ, UK ²Department of Bioengineering, School of Engineering, University of Tokyo (*a.mantalaris@imperial.ac.uk)

Introduction
Bioreactors provide a dynamic cultivation system within a controlled environment that enables controlled expansion and differentiation of cell populations. Furthermore, bioreactors allow for automated bioprocessing enabling standardization which is required in ensuring product quality and reproducibility, a must for clinical applications [1]. Finally, bioreactors can be designed to generate a “functional” culture environment. Specifically, because bone is able to sense and adapt to mechanical stimulation by modulating its mass, geometry, and structure, various types of mechanical stimulation (compressive loading, plated displacement, pneumatic-driven, hydrostatic pressure, etc.) have been considered as a powerful technique to enhance bone formation over the past two decades.

Materials and Methods
Mesenchymal stem cells have been shown to differentiate toward the osteogenic lineage by mechanical stimulation. Moreover, the use of cytokines can be replaced by the presence of low intensity ultrasonic stimulation. We have demonstrated previously [2] the generation of 3D mineralized megamolecular cellular constructs from embryonic stem cells (ESCs) encapsulated within hydrogels that have mechanical properties with a Young’s modulus 15-fold higher than PLGA scaffolds. Furthermore, ART-FTIR confirmed the presence of hydroxyapatite while SEM-EDS confirmed a ratio of Ca/P of 1.21 in the “bone-like” constructs.

Results
Herein, we describe a novel “functional” perfusion bioreactor that provides mechanical stimulation that is automatable. Perfusion and recycle ratio are controlled through micro-peristaltic pumps capable of perfusion rates of 0.0003-19 ml/min. The propereties of the ultrasound transducer are maximum 120mW/cm² intensity, maximum 3MHz frequency, 70V peak to peak amplitude, and 0.5cm² surface areas. Encapsulated ESCs were differentiated towards the osteogenic lineage and the influence of perfusion, oxygen tension, and mechanical stimulation was evaluated.

References
[1] Placzek MR; Chung IM; Macedo HM; Ismail S; Mortera Blanco T; Lim M; Cha JM; Fauzi I; Kang Y; Yeo DC; et al. (06 Mar 2009). Stem cell bioprocessing: fundamentals and principles. J R Soc Interface. 6:209-232.

Acknowledgments
Dr. Sakis Mantalaris
Dr. Katsuko Furukawa