Development of a physiologically relevant osteocytic network in vitro to model bone (patho)physiology

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Short Summary:
Drug development is a long, flawed process costing €2.3Billion per drug over 15 years. 90% of drugs fail in clinical studies while 10% of drugs fail even after regulatory approval, costing ~€2Billion annually. A key determinant in this failed process is the lack of adequate models to test drug efficacy. Current human cell culture models are rudimentary and pre-clinical animal models do not accurately replicate the human response. Moreover, the current drug development system does not yield mechanistic insight on drug efficacy and thus the vast majority of drugs are simply discarded.

Every 3 seconds a person suffers an osteoporosis-related bone fracture globally, resulting in significant morbidity and mortality. Osteoporosis (OP) arises when there is an imbalance between bone resorption and formation resulting in a net bone loss. Current therapeutics target receptors known to inhibit resorption but are associated with side effects. Although enhanced resorption is a major contributor to OP, reports indicate that bone loss also occurs due to a decrease in bone forming capacity and this hypothesis is strengthened by reduced defect and fracture healing rates in osteoporotic bone. This therefore demonstrates that **effective therapies to treat osteoporosis or enhance bone defect repair need to be multitargeted.** This lack of effective treatments has resulted in "a crisis in the treatment of osteoporosis [1]". Given that the burden of osteoporotic fractures on healthcare is ~€36billion within the EU, there is a **significant clinical need** and commercial opportunity for an effective **multi-targeted therapy to enhance bone healing and regeneration in osteoporosis**.

Osteoporosis arises when there is an imbalance in the bone remodelling cycle. Remodelling is performed by organised packets of osteoblasts and osteoclasts called bone multicellular units (BMUs). The key determinant of whether osteoclasts form and contribute to remodelling is the ratio between RANKL, a cytokine which stimulates osteoclastogenesis, and OPG, its inhibitor [2]. **A major producer of RANKL/OPG, is the osteocyte, which is a dendritic lineage committed and non-dividing cell type which forms an interconnected network within mineralised bone**. Therefore, the osteocyte is largely believed to be the master coordinator of bone physiology. Research into metabolic bone disorders is primarily performed *in vivo*. However, in the last decade there has been increased interest in generating *in vitro* models that can reduce or replace our reliance on animal testing and generate more relevant human data. With recent advances in biomaterials and tissue engineering the feasibility of laboratory-based alternatives is growing; however, **to date there are limited models of human bone containing functional osteocytes and no established *in vitro* models of dendritic osteocyte networks.**

Direct Laser Writing (DLW) is a manufacturing technology that is able to 3D print structures on a nanoscale utilising two photon polymerisation (2PP) [3]. A stl file, created through a CAD modelling system creates a pathway to be followed by a laser. Inside a transparent material, two photons are absorbed to polymerise the material in a precise location by illuminating a photoresist. Absorption of these photons creates an excited state within the material, which polymerises within this specific voxel. Templates for cell culture which control cellular morphology, such as dendritic neurons, have been designed utilising this approach [4].

Therefore, the goal of this project is to utilize DLW to develop a microphysiological model of the osteocytic network in bone that can be used to better understand the (patho)physiology of bone. This project will require the establishment of close collaborations with Cornell University (Prof. Karl Lewis).

Research Question:
Can Direct Laser Writing be used to form functional anatomically relevant osteocyte networks *in vitro*.

Research Objectives (RO):
(RO1): *Fabrication of physiologically relevant osteocytic network templates using Direct Laser Writing.*
Timeline; Location: Summer 1, Weeks 1-3; Prof. David Hoey Lab, Trinity College Dublin.
Methods: Computer aided design (CAD) software will be utilised to design anatomically accurate templates of the osteocyte lacunar-canalicular network of bone. The printing of these templates will be optimised via DLW (nanoscribe) using two different materials (IP-L 780 and IP-DIP). Print accuracy will be verified, and shrinkage of material quantified. The surface of the print will then be coated with a layer of nano needle hydroxyapatite to better represent the composition of mineralised bone [5].
(RO2): *Analysis of osteocyte phenotype cultured in physiologically relevant networks.*
Timeline; Location: Summer 1, Weeks 4-6; Prof. David Hoey Lab, Trinity College Dublin.
Methods: In conjunction with the Hoey Lab team, the MLO-Y4 osteocyte cell line will be seeded onto templates manufactured in RO1 at various densities. Cell viability will be assayed via a Live/Dead assay, cellular morphology will be analysed by fluorescent microscopy following actin staining (phalloidin), and secreted RANKL/OPG ratio will be determined by ELISA at 24, 48, and 72hrs post seeding.

Impact: This research proposal represents an innovative strategy with the potential to develop physiologically relevant osteocytic networks *in vitro* to model bone (patho)physiology, which could be used to better test drug efficacy and reduce animal testing. Moreover, it will provide an interdisciplinary, international, training platform using cutting edge techniques enabling me to develop as a Biomedical Engineer.

References:

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