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A MICROPHYSIOLOGICAL MODEL OF HUMAN BONE TISSUE

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INTRODUCTION

The identification and development of therapeutics for bone disease currently rely on simple 2D *in vitro* assays, and subsequent complex *in vivo* animal models. While these systems have been invaluable to the life sciences, simple *in vitro* culture can be too far removed from the *in vivo* condition, and while animal models are substantially more complex; with the often significant differences between humans and animals questioning the validity of these systems (1). To bridge the chasm of complexity between these two models, microphysiological systems (MPS), or organ on chip platforms, have been developed to mimic key features of human organsat the microscale. MPSs are a promising technology for *in vitro* pre-clinical analysis of therapeutics and basic study of biological functions. The objective of this study was to develop a human bone tissue with supporting vasculature for incorporation into a MPS. To this end we developed cell-laden hydrogels to recreate the cellular, extracellular and vascular components of human bone tissue forming a MPS that recapitulates bone physiology.

MATERIALS AND METHODS

Screening: Bone Marrow Stem Cells (MSCs) (Lonza, USA) were cultured in rat tail type 1 collagen gels (Corning, Germany) for two weeks. Design of experiments (DOE) methods were used to evaluate the effects of 6 culture parameters on osteocytogenic differentiation; collagen matrix stiffness (4-8mg/ml), supplementation (unsupplemented / osteogenic supplementation); nano-hydroxyapatite (nHA) concentration (0.27-2.7mg/ml), oxygen tension (5-20%), cell density (0.1-0.5x10^6 /ml), and retinoic acid concentration (5-10µM). Differentiation was evaluated with sclerostin ELISA, cell morphology and ALP expression at day 14.

Optimisation: For optimisation experiments, matrix stiffness and nHA content were further evaluated. To enhance matrix stiffness, collagen gels were reinforced with Alginate (Pronova, Norway) to produce stiffer interpenetrating network (IPN) gels. MSC culture in the optimised stiffness gels was evaluated for osteocytogenesis with mineralisation, sclerostin ELISA, cell morphology, and expression of osteocyte specific markers PDPN and DMP-1.

Vascularisation: GFP-Human umbilical chord endothelial cells (HUVECS) and MSCs were co-cultured (1:1 to 13:1) within microfluidic devices (Fig. 1A). On day 7 fluorescent 10kDa Dextran was injected into devices to evaluate perfusability.

RESULTS

A screening DOE revealed collagen matrix stiffness and nHA concentration produced the largest effects when evaluated for osteocytogenesis after 2 weeks (data not shown). In the proceeding optimisation experiment, MSCs in the least stiff collagen only gels produced a highly mineralised matrix (Fig 1B), significantly higher amounts of sclerostin (Fig 1C), and were positive for osteocyte markers E11 and DMP-1. Incorporation of alginate seemed to abrogate sclerostin production, but produced a highly dendritic osteocyte-like morphology (Fig 1D and E). HUVEC:MSC co-cultures at 13:1 produced open perfusable vasculature within the tissue chips (Fig 1F).

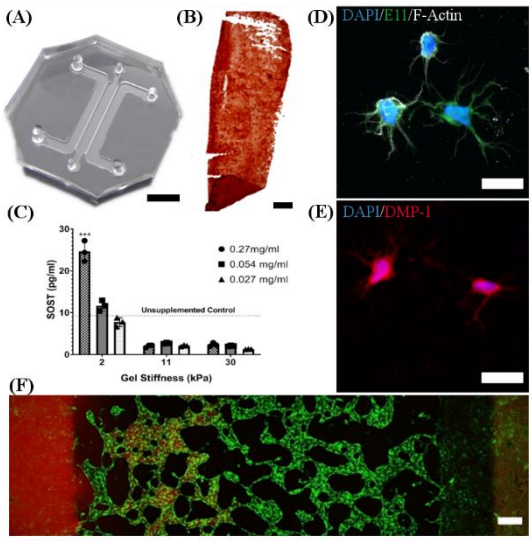

Figure 1 MSCs display an osteocyte-like signature in developed hydrogels. (A) Image of MPS for human bone analogue culture. Scale = 3mm (B) MSCs mineralize collagen nHA matrix after 21 days. Scale = 500µm (C) Less stiff, collagen only gels increase sclerostin expression $p<0.0001$. Scale = 20µm (D-E) MSCs express DMP-1 and E11 after 21 days in culture. (F) perfusable vascular after 7 days in culture on MPS. Scale = 200µm

DISCUSSION

We found that DOE methods could be used to identify hydrogel and culture conditions that promote differentiation of MSCs along an osteogenic pathway, with MSCs encapsulated within soft collagen-nHa gels exhibiting an osteocyte-like signature. Complex 3D models of human bone models are currently lacking and this report describes a method of generating the key features of human bone and vasculature for future incorporation in a MPS. Such organ-on-chip platforms will transform research into the (patho)physiology of bone and accelerate the development of new treatments for damaged and diseased tissue

REFERENCES

Ledford *et al.*, Nature News 447:526-528, 2011