HepaRG progenitor cells commit to earlier hepatic differentiation and outperform metabolic function of control when cultured on nanopattern substrates

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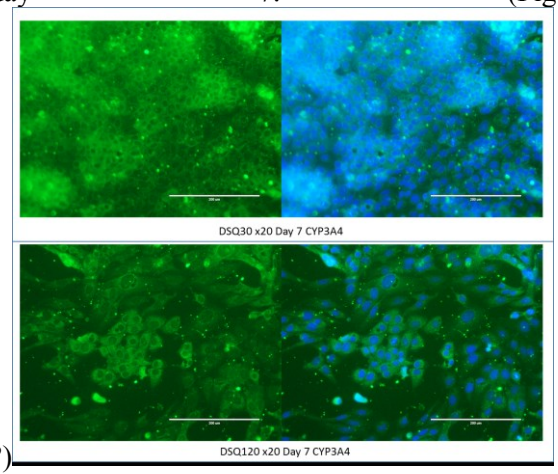
INTRODUCTION: Nanotopography has been increasingly used in the assessment of cellular adhesion, proliferation and function, with stem cells typically used for their plasticity[1]. We previously demonstrated significantly earlier differentiation of HepaRG progenitor cells on prototype 2D-nanopatterned polymer substrates NPS1 and NPS2. Here, we show earlier lineage commitment and better functionality on nanopattern surfaces compared to Corning industry standard.

METHODS: HepaRG101 progenitor cells [Biopredic Inc: BPI] were seeded (>95% viable; 30000 cm^2) and cultured for ≤28 days [BPI Growth Medium] on prototype 2D-nanopatterned polymer substrates (NPS: 2 nanopatterns [each well 0.32cm^2]; including 1x planar control; fabricated using high-resolution electron beam lithography); or standard Corning plastic culture dishes (SCPs). Phenotype and metabolic competence (ATP; Prestoblue assays) of cells were assessed in parallel with phenotypic profiling (immunocytoflourecence staining) using liver-specific/ maturation-differentiation markers (Transferrin; Albumin; HNF4a; Sox9 and CYP3A4). End point and qRTPCR were used to validate and quantify maturation-differentiation markers.

RESULTS: We previously identified nano-topographies NPS1 & NPS2 which show significantly enhanced liver-like morphological features (granular hepatocytes; with round nuclei), which appeared earlier (d6 *vs* d14 on SCPs), as well as d6 planar or SCP controls. Following on from this study, we can now confirm fully functional cells on day 7 expressing higher viability (PB), metabolic function (Immuno staining CYP3A4), and tight junction protein 1 which confirms polarity of functional hepatocytes. Using end point PCR and qRTPCR we also confirm presence of CYP3A4, and reciprocal Sox9/AFP to HNF4a which correlates to previous staining and further validates a mature culture on day 7. (Figure ?)



DISCUSSION & CONCLUSIONS: Previously we reported HepaRG progenitor cells grown on NPS1 and 2 showed early commitment to a hepatocyte lineage. However, we now report that these cells are functional and capable of higher metabolic activity ~7 to 10 days earlier than those grown on industry standard Corning. Quantification of genes of interest using qRTPCR confirms HepaRG progenitors grown on NPS1 and NPS2 out perform those grown on Corning by statistically significant fold increases thus showing this platform to be a time saving and efficient model to test drug toxicity

REFERENCES: [1] Dalby, Gadegaard et al.,, Nature Mater. 2007 Dec;6(12):997-1003.