Hormone-responsive, patient-derived models of the uterine wall in a microfluidic array

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Abstract theme: Bioengineered Models

Introduction

Despite their prevalence and their profound impact on patient's lives, chronic gynaecological disorders (such as endometriosis and adenomyosis) remain under-researched. Both to advance our understanding of the cellular behaviours underlying these conditions and to provide new tools for screening of potential therapies, advanced *in vitro* models are required. 3D patient-derived cultures that mimic the uterine wall, comprising both endometrial and myometrial cell types, are an attractive and unexplored route. Here, we describe an organ-on-a-chip approach to creating such cultures, demonstrating multiplexed, hormone-responsive uterine models within a microfluidic array.

Materials and Methods

Endometrial (obtained from pipelle biopsies) and myometrial (from patients undergoing elective C-sections) tissue samples were enzymatically dissociated to produce epithelial, stromal and myometrial cell fractions [1]. These cell suspensions were sequentially seeded into a microfluidic device with multiple, individually addressable channels, each containing a 5x5 microwell array. The devices were fabricated in PDMS and functionalised to create a non-adherent surface as previously published [2]. The resultant 3D cultures were maintained *in vitro* for up to 15 days and were subjected to hormone stimulation with β-estradiol (E2, 10 nM), medroxyprogesterone acetate (MPA, 100 nM) and cAMP (500 µM). Cellular organisation was monitored by live-cell imaging using fluorescent tracers and by end-point immunocytochemistry (pan-cytokeratin for epithelial cells, calponin for myocytes (SMCs), vimentin for stromal cells), with model functionality assessed by measuring IGFBP-1 and osteopontin secretion (by ELISA) following hormone stimulation.

Results and Discussion

A series of cell injection protocols, sequentially seeding the three cellular fractions over 2-4 days, were investigated. Regardless of protocol, epithelial cells ultimately localised to the outer periphery of the 3D cultures. Optimum results – where self-organisation resulted in robust 3D cultures with an outer epithelial layer encircling an inner myometrial core, thus mimicking the architecture of the uterine wall – were obtained when the myometrial cells were seeded last and when 5% (v/v) Matrigel® was incorporated into the culture media. Consistent cellular organisation was obtained when directly comparing patient-derived cultures produced from different endometrial biopsies (n=3, all biopsies from secretory phase tissue). Exposure of these cultures to E2, MPA and cAMP resulted in an increase in the secretion of both osteopontin and IGFBP-1, indicating stromal decidualisation [3].

Conclusions

These functional, 3D multicellular cultures show promise for personalised modelling of gynaecological disorders, enabling screening of patient-derived cultures in a medium-throughput format.

References

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Acknowledgements

CB was supported by an Organ-on-a-Chip Technologies Network Pump Priming Project grant, CJH/DKH by a Wellbeing of Women project grant RG 2137 and DKH by a MRC CTRF (MR/VOO7238/1).