GelMA Hydrogels: Towards the Development of an In Vitro Model of the Human Endometrium

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The human endometrium is a complex, multicellular tissue and is the site of embryo implantation. Dysregulated endometrial function can lead to maternal-fetal complications and a variety of reproductive pathologies such as endometriosis preeclampsia and infertility. The mechanisms involved in disrupted endometrial function and perturbed endometrial-embryo communication remain largely unknown due to a lack of appropriate of study models and 3D in vitro culture systems that support multiple endometrial cell types. Our aim was to develop novel biomaterials capable of supporting the *in vitro* growth of multiple endometrial cell types. Here, we investigate how photocrosslinked GelMA hydrogels can be tailored to the culture of both human endometrial stromal cells (ESCs) and endometrial epithelial organoids (EEOs), which have been shown to recapitulate key features of *in vivo* endometrial glands. We produced GelMA hydrogels with a range of stiffness degrees by altering the degree of substitution during the synthesis of GelMA and the concentration of GelMA used in hydrogel fabrication. Endometrial biopsies were collected from consenting patients undergoing gynaecological surgery. Endometrial stromal and epithelial gland clumps were isolated from four patient biopsies. ESCs were expanded and passaged in 2D, at passage 2 cells were used experimentally and encapsulated in GelMA hydrogels at a seeding density of 10,000 cells per 5µl gel droplet. ESCs were grown for up to 15 days in DMEM/F12 supplemented with 10% FBS. ESCs were tested in comparison to a commercially available collagen hydrogel control. Epithelial gland clumps were immediately encapsulated in GelMA hydrogels upon isolation and allowed to grow and form organoids over 10 days in expansion medium. Matrigel was used as the control hydrogel for all EEO experiments. To encapsulate primary human endometrial cells in photocrosslinked GelMA hydrogels, we developed a simple protocol employing a cytocompatible photoinitiator (LAP) and a short 3-minute UV exposure time (365 nm). ESCs in both soft and stiff GelMA hydrogels maintained high levels of viability after a 7-day culture period. In soft GelMA hydrogels ESCs adopted an elongated morphology whereas in stiff GelMA hydrogels ESCs adopted a rounded morphology. An increase in cell number, detected by the MTS assay, was seen over a long-term culture period of 15 days in soft GelMA hydrogels indicative of cell proliferation. ESCs retained their functional ability to adopt a differentiated phenotype and secrete prolactin in response to 4 days treatment with cAMP and MPA. EEOs only form in an appropriate 3D matrix. Soft GelMA hydrogels failed to support organoid formation whereas stiff GelMA hydrogels enabled EEOs to form. The efficiency of organoid formation was enhanced by addition of the basement membrane protein, laminin. 10% DS100 GelMA supplemented with laminin at a ratio of 1-in-10 was determined as the optimal formulation for EO formation. This optimised hydrogel formulation was then tested and confirmed to support an ESC-EEO co-culture. In conclusion, endometrial cells can quickly and simply be encapsulated in GelMA hydrogels and maintain high cell viability. The mechanical and biochemical properties of GelMA hydrogels can be tuned to suit the culture of different cell types. Engineering a platform to study the human endometrium in vitro is the first step towards understanding implantation failure and recurrent pregnancy loss and will pave the way for new treatments.