Phenotypic and Functional Characterisation of Primary Endometriotic Stromal Cells for *In Vitro* Model Development

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BACKGROUND AND AIMS

Endometriosis is a chronic gynaecological disorder affecting approximately 190 million women of reproductive age. It is characterised by the growth of endometrial-like tissue outside the uterus. Despite its high prevalence, the aetiology and pathophysiology of endometriosis remain poorly understood, contributing to delayed diagnosis and limited treatment options. Although primary endometriotic cell cultures are valuable research models, they often lack comprehensive phenotypic and purity profiling. This study aims to thoroughly characterise primary endometriotic stromal cells that could serve as relevant *in vitro* models and as a basis for establishing a novel immortalised endometriotic cell line.[1]

MATERIALS AND METHODS

Endometriotic lesion tissue samples (n=18) were collected under a standardised protocol at the Department of Gynaecology, University Medical Centre Ljubljana, Slovenia. The lesions included ovarian endometriomas (n=3), peritoneal lesions (n=14), and rectovaginal septum lesions (n=1). Primary endometriotic stromal cells were isolated using two enzymatic digestion protocols: (1) collagenase Type I and DNase I, and (2) collagenase Type I, dispase, and DNase I. All primary cultures were screened for mycoplasma contamination. Cell proliferation and population doubling time were determined by cell counting using a live-cell imaging system (Lionheart FX, Agilent Technologies, Santa Clara, California, USA), while migratory capacity was assessed using the same imaging platform. Further characterisation included analysis of oestrogen-related gene expression using qPCR and immunocytochemical staining for vimentin, cytokeratin, and PAX2. Additionally, the ability of cells to form 3D spheroids was assessed, and their hormonal responsiveness to oestradiol (E2) and medroxyprogesterone acetate (MPA) was evaluated using a live-cell imaging system.

RESULTS

Cultures from four patients failed to proliferate after cell isolation and were excluded from further analysis. The remaining cells maintained >90% viability through passage 5, and all tested negative for mycoplasma. No significant differences in growth kinetics were observed between cells isolated using the two digestion protocols ($p=0.84$; Mann-Whitney test) or between cells obtained during the proliferative and secretory phases of the menstrual cycle ($p=0.43$; Mann-Whitney test). Migratory capacity assessed by wound healing assay showed that most cultures achieved full wound closure within 48–72 hours, and migration kinetics were not affected by the digestion method ($p=0.43$; Mann-Whitney test) or menstrual cycle phase of the donor ($p=0.73$; Mann-Whitney test).

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Both proliferation and migration analyses revealed inter-individual variability, indicating patient-specific cellular behaviour despite similar group averages. Hormonal profiling by qPCR demonstrated significant downregulation of *ESR1* ($p<0.0001$, one-way ANOVA) and a reduced *ESR1/ESR2* ratio ($p<0.005$, one-way ANOVA) in primary cells compared to normal endometrium, confirming retention of disease-specific characteristics *in vitro*. All primary cells exhibited strong vimentin expression and were negative for cytokeratin (antibody recognising cytokeratins 1, 4, 5, 6, 8, 10, 13, 18, and 19), consistent with a stromal phenotype. Additionally, all primary cultures expressed the endometrial marker PAX2, confirming their endometrial origin. Primary cultures formed 3D spheroids within 24 hours, which were stabilised by Day 5, with morphology and roundness varying between patient-derived samples. Endometriotic stromal cells showed heterogeneous responses to E2 and MPA, with reduced E2-induced growth linked to lower *ESR1* expression and variable MPA effects associated with reduced *PGR*, suggesting progesterone resistance.

CONCLUSION

The authors established well-characterised primary endometriotic stromal cell cultures with high viability, migratory capacity, and defined phenotype. These cultures provide a reliable *in vitro* model for endometriosis research and a basis for generating a novel immortalised endometriotic stromal cell line.

Reference

1. Novak Pušić M et al. Phenotypic and functional characterization of primary endometriotic stromal cells for in vitro model development. Abstract 15202. ISGE Congress, 3-6 March, 2026.