Title

Identification of Optimal Conditions for Human Placental Explant Culture and Extracellular Vesicle Release

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P1.15.
IDENTIFICATION OF OPTIMAL CONDITIONS FOR HUMAN PLACENTAL-EXPLANT CULTURE

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Objectives: Recent reports suggest that extracellular-vesicles (EVs) and extracellular-RNAs can mediate intercellular communication, including signaling between the placenta and maternal-tissues. Human placental-explant culture is a widely-used model for studying placental function *in-vitro*. However, systematic studies to determine effect of culture-media and culture-duration on explant viability, function, morphology, and EV/exRNA release have not been performed. Moreover, previously-used media contained undefined components (e.g. FBS, BSA), which carry EVs and exRNAs that can confound studies aimed at determining the roles of these entities in cell-cell communication. Here, we systematically compare functional parameters of placental-explants cultured in different media to identify the optimal timeframe where cell-viability is maintained.
Methods: Placental villi dissected from healthy placentae were cultured in exosome-depleted serum (MCDB153, DMEM/F12, IMDM) and defined (D.KSFM) conditions for 7-days. At serial timepoints, cell-free supernatant was collected to measure cell-metabolism (XTT), cell-death (LDH), and syncytiotrophoblast-function (hCGb). Villous and trophoblast-morphology, were assessed by staining sections of fixed explants with Cytokeratin-7 and Ki67. Size and concentration of EVs released in all culture-conditions were also analyzed.
Results: For both first-trimester and term-placentae, general metabolic activity in all media was stable for 24-48 hours. However, in term-explants, hCGb secretion plateaued after 8 hours, while first-trimester explants continued to secrete hCGb for 72 hours. EVs assessed at 24 hours included a predominant population ~200nm in diameter, with a concentration ~10^{10} particles/ml, and highest in D.KSFM. Overall, the explants from both gestations showed highest viability and functionality in D.KSFM.
Conclusion: Optimal culture-media for placental-explants is D.KSFM. Ideally experiments should be performed during the first 8 hours after dissection for term-explants and 72 hours for first-trimester explants. This protocol will be useful for *in-vitro* modeling of short-term effects of different environmental perturbations (e.g. changes in glucose concentrations, oxygen-tensions) on placental-function and EV-secretion/exRNA-expression, helping model pregnancy complications, such as gestational diabetes and pre-eclampsia.

P1.16.
APPLICATION OF IMPEDANCE SPECTROSCOPY FOR ANALYSIS OF BEWO CLONE B30 HUMAN CHORIOCARCINOMA CELL LINE

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Objectives: BeWo cells are used for the construction of in vitro models of placental barrier. Only the early placenta possesses multiple trophoblast layers whereas third-trimester placenta consists of a single trophoblast layer. BeWo cells do not undergo contact growth inhibition and form multilayer structures. It is important to control the cell state for transport experiments. Impedance spectroscopy was applied for electrical characteristic studying during BeWo cell growth and in response to HIF-1 activator.
Methods: 30,000 cells per insert were seeded into a 96-well Transwell plate (1 μm pore size). After 48 h a potent HIF-1 activator D014-0021 was added at 10 μM concentration. Impedance spectra were acquired with impedance spectroscopy system (Bioclinicum, Russia). For the extraction of electrical parameters, the equivalent electrical circuits were used. Student's t-test was used to calculate the statistical significance.
Results: It was predicted from the mathematical model that medium resistance (R_{med}) and the radius of the impedance hodograph (and hence TEER) will rise linearly with the number of layers. In the case of one layer, the radius can rise due to the formation of tight junctions but the R_{med} should remain stable, but the data shows increasing TEER and R_{med}. There is a statistically significant difference in R_{med} between 48 and 96 h. It can be concluded that after 48 h the BeWo cells form multilayer structures. The addition of D014-0021 leads to a slight increase in TEER after 6 h and a significant decrease in TEER and capacitance after 27 h.
Conclusion: It was shown that formation of multilayer structures can be readily detected with impedance spectroscopy and hence it can be used for quality control of the *in vitro* placental models. It is possible to use impedance spectroscopy for detection of additional parameter changes such as electrical capacitance.
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P1.17.
THE HYALURONAN SYNTHESIS INHIBITOR 4-METHYLUMBELLIFERONE INHIBITS CELL PROLIFERATION AND WOUND HEALING ON IN VITRO ENDOMETRIOSIS MODELS.

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Objectives: The aim of this study was to evaluate the effect of 4-methylumbelliferone (4MU) on cell proliferation, wound healing process and gelatinase activity on human endometrial stromal (t-HESC) or epithelial (ECC-1) cell lines.
Methods: t-HESC and ECC-1 endometrial cell lines were stimulated with different concentrations of 4MU. Cell proliferation was evaluated after 24hs with the cell proliferation reagent WST-1. The area of a scratch closed by the t-HESC was calculated in a wound healing assay, for this purpose photomicrographs were taken at time 0h and time 20hs; and the gelatinase activity in conditioned media of t-HESC was evaluated by zimography. Only $p<0.05$ was considered as statistically significant.
Results: After 24hs of treatment the cell proliferation of ECC-1 was significantly inhibited by 0.5, 1 and 2 mM 4MU ($p<0.01$, $p<0.001$ and $p<0.01$, respectively versus control), whereas cell proliferation of t-HESC was significantly inhibited by 2 and 4 mM 4MU ($p<0.01$ versus control). Already at 0.5 mM 4MU, the migration of t-HESC cells was significantly inhibited and the scratch was closed in a low percentage ($p<0.01$ for 0.5 and 1 mM 4MU versus control, $p<0.001$ for 2 and 4 mM 4MU). Preliminary results of MMP-2 and MMP-9 activities revealed that these gelatinases would not be modulated after 24hs of 4MU treatment.
Conclusion: Endometriosis is a benign gynecological disease affecting 10% of women of reproductive age, characterized by the presence of endometriotic foci outside the uterine cavity. Previous studies from our laboratory demonstrated a strong antiangiogenic activity of 4MU. The present results are, in part, in agreement with those reported by other authors.