

Poster: alternative testing methods for toxicity to reproduction

Towards an *in vitro* model for xenobiotic passage at the materno-fetal interface based on permanent cell lines

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Currently, studies on transplacental passage are largely performed in or *ex vivo*. However, species differences are known and in humans, studies can only be performed using at term placentae with limited relevance for embryo-/ early fetotoxicity and teratogenicity. Liu et al. (1997) and others have suggested syncytia-forming human BeWO choriocarcinoma cultures grown on permeable filter inserts (Transwells) as models for transport in the developing placenta. It was the aim of this study to investigate the experimental conditions for producing reproducible and stable monolayer cultures with a controlled paracellular permeability.

BeWO cells obtained from DSMZ were seeded on uncoated polyester or collagen-coated Transwell filter inserts (Corning) at densities from 25,000-200,000 cells/cm². Medium (Ham's F12+10% FCS) was exchanged and transepithelial electrical resistance (TEER) was measured daily to assess paracellular permeability for up to 10 days. For induction of syncytia, forskolin (10-100 µM or di-butyryl-cAMP (1 mM, db-cAMP) was added. The effect of DMSO was tested at concentrations between 0.1 and 3%.

Uncoated polyester membranes with a diameter of 12 mm were found superior to smaller or collagen-coated filters with regard to monolayer homogeneity and development of TEER. Seeding densities between 100,000 and 200,000/cm² resulted in TEER values 50-70 Ohm x cm² above background within 3-4 days, while up to one week was required after seeding of 75,000 or 50,000/cm² to reach a similar level of paracellular permeability. The maximum of TEER was usually observed 1-2 days after the cell layer reached confluency and was concurrent with a reduction of cross-sectional area per cell. Cells remained mono-layered thereafter for at least 2 further days.

Addition of 30 or 100 µM forskolin or 1 mM db-cAMP induced syncytia formation of BeWO cells grown on plastic as well as on filter support. This was confirmed by trypsinisation of cells and subsequent counting with cell size classification. However, no syncytia formation could be observed at seeding densities above 50,000/cm² and for confluent cell layers in general. Importantly, addition of forskolin to BeWO cultures grown on Transwells reduced cell-cell contacts and prevented development of low-permeability monolayers. In contrast, the solvent DMSO (1%) promoted TEER development.

Therefore, a strategy based on seeding of pre-formed syncytia onto filter inserts was evaluated. Treatment of cells seeded at ~25,000/cm² on culture dishes with 30 µM forskolin for 6 days produced the highest yield of syncytia with 38% of all particles. After careful resuspension, these adhered well to Transwell filter inserts, but permeability of the resulting monolayers remained high with stagnant TEER values below 20 Ohm x cm². Seeding of a second layer of forskolin-induced cells or addition of 1% DMSO did not improve the result.

Induction of syncytia formation in BeWO cells grown on filter inserts by forskolin or db-cAMP was not compatible with formation of monolayers with controlled paracellular permeability as indicated by the absence of an increase in TEER. Similarly, pre-formed BeWO cell syncytia failed to form low-permeability monolayers upon single or repeated seeding onto Transwells. Surprisingly, DMSO stimulated TEER development, but only in the absence of forskolin (pre)treatment.

References

Liu, F., Soares, M. J., Audus, K. L. (1997). Permeability properties of monolayers of the human trophoblast cell line BeWo. *Am. J. Physiol.* 273, C1596-1604.

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