Organotypic epithelial raft cultures for investigations of virus growth, pathogenesis and efficacy of antiviral agents

Commonly used acronym: *OERCs*

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SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Humans - lambs
Type of cells/tissues/organs	Keratinocytes derived from neonatal foreskins and epithelial cells from tonsils

DESCRIPTION

Method keywords

Organotypic epithelial raft cultures
Keratinocyte differentiation
Skin mimic
Viral replication
Tumor cell growth

Scientific area keywords

Skin equivalents
3D keratinocytes-tumor cells co-cultures
virus growth
Antiviral/antitumor activitity

Method description

Organotypic epithelial raft cultures accurately reproduce the process of epithelial differentiation *in vitro* and can be prepared from normal keratinocytes, explanted epithelial tissue, or established cell lines. Normal primary human keratinocytes (PHKs) stratify and fully differentiate in a manner similar to the normal squamous epithelial tissues, while transformed cell lines exhibit dysplastic morphologies similar to the (pre)neoplastic lesions seen *in vivo*. This three-dimensional (3D) culture system provides an essential tool for either alone or co-cultured with PHKs, tumor biology and selectivity of antitumor agents can be analyzed. For the preparation of dermal equivalents, a collagen matrix solution is made with rat-tail collagen mixed on ice with 10-fold concentrated Ham's F-12 medium, 10-fold concentrated reconstitution buffer, and 3T3 J2 murine fibroblasts. One milliliter of the collagen matrix solution is poured into 24-well plates. After equilibration of the gel with 1 ml of growth medium overnight at 37°C, 2.5 × 105 PHKs isolated from neonatal foreskins are seeded on the tops of the gels and maintained submerged for 24 to 48h. The collagen rafts are raised and placed onto stainless steel grids at the interface between the air and the liquid culture medium. The raft cultures can be infected with epitheliotropic viruses at different stages of epithelial cell differentiation. The epithelial cells are then allowed to stratify for 10 to 12 days. The cultures can be harvested at 10-12 days post-lifting and analyzed to check epithelial differentiation by histological examination and viral replication. Cultures can also be prepared using primary

epithelial cells derived from tonsillectomies. Tumor cells can be used to prepare the raft cultures, for instance, cell lines transformed with human papillomavirus (HPV) transformed cells (such as HeLa and SiHa) or Merkel cell carcinomas (MCCs), melanoma cell lines, etc. The tumor cells are seeded alone on top of the gels or in combination with PHKs, resulting, respectively, in the production of 3D cultures with dysplastic morphology (tumor cells monoculture) or 3D cultures with patches of dysplastic morphology in the differentiated epithelial cells.

Lab equipment

Cell culture equipped laboratory:
- Laminar airflow ;
- CO2 incubator ;
- Microscope ;
- Water-bath ;
- Centrifuge.

Method status

History of use
Internally validated
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Faithfully reproduces epithelial cell growth.
- Allows multiple applications in virology and tumor biology.
- Permits the study of virus-host cell interactions in stratified epithelia and the investigations of tumor biology in an *ex-vivo* system that closely resembles the *in vivo* situation.
- Usefulness demonstrated by several reports analyzing viruses that target epithelial cells at least during a part of their life cycles epithelial cells as well as in tumor biology.

Challenges

- Tricky method, includes various crucial steps.

- Experienced and trained personnel required.

Modifications

The method can be further modified by the addition of other cell types such as endothelial cells and mononuclear cells.
The method can be adapted for epithelial cells derived from different animal species.

Future & Other applications

The methodology can be adapted for the study of any virus targeting epithelial cells as well as for the study of co-infections. It also allows the growth of patient-derived biopsies and can be adapted for the (co)culture of different tumor cells.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

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