In vitro cell-based models

A) Immortalized cell lines

Established or immortalized animal and human cells, such as Vero, Madin Darby canine kidney (MDCK) or human cancer-derived cells, are the main cell lines used for viral drug and vaccine development and production. Immortalized cells are used to produce viruses and/or vaccines to test immunogenicity (virus neutralization assay) as well as drug toxicity and efficacy. The main advantages of using cell lines are: (i) growth in suspension in culture media under controlled conditions; (ii) long-term culture, (iii) cost-effective, and (iv) easier to scale-up at high cell concentrations, etc.

Also, designer cells for viral vaccine production are available. Designer cells are characterized by the insertion of synthetic organelles that modifies the cell's genetic material to code for entirely new proteins. The engineered cells can then produce novel proteins without any changes in its routine functions (Genzel, 2015). The following table summarizes some examples of designer cells commonly used in the field of vaccine development, including PER.C6, AGE1.CR, QOR/2E11, EB66, PBS-1, SogE, MFF-8C1 cells.

Table 1: Designer Cells Used in Vaccine Development

Cell line name	Organism type/Tissues	Uses	References
PER.C6®	Human/Retina	Adenovirus production and titration	www.thermofisher.com
AGE1.CR®	Bird (*Cairina moschata*)/Fetal eye, retina	Vaccine production	www.probiogen.de/virus-production-cell-lines.html DOI=10.1002/biot.201400388
QOR/2E11	Bird (*Colinus virginianus*) /Fibroblast	Vaccine production	DOI=10.1186/1753-6561-5-S8-P52
EB66®	Bird (*Anas platyrhynchos*)	Production of therapeutic monoclonal antibodies	web.archive.org/web/20180223230832/www.valneva.com/en/technologies/35
PBS-1	Bird (*Gallus gallus)*/Fibroblast	Vaccine production	DOI=10.1016/j.vaccine.2008.04.048
SogE	Bird (*Coturnix japonica)*/ Fibrosarcoma	Vaccine production	DOI=10.1099/0022-1317-83-8-1987
MFF-8C1	Fish (*Siniperca chuatsi*)/Fibroblast	Vaccine production	DOI=10.1007/s10616-013-9642-7
HEK293 & variants	Human Kidney (epithelial)	Wide range of protein and vector production	https://www.atcc.org/products/crl-1573

This document was produced with the support of EC H2020 Project 951668: TRANSVAC-DS, and is a project coordinated by the European Vaccine Initiative (EVI).
UniversitätsKlinikum Heidelberg • Voßstraße 2, Geb. 4040 • 69115 Heidelberg, Germany
• e-mail: transvacinfo@euvaccine.eu

Viral neutralisation assays are pivotal for the study of viral vaccine efficacy and the evaluation of neutralising antibodies. However, the study of highly pathogenic microorganisms requires biocontainment facilities with a biosafety level (BSL)-3 or higher, resulting in important bioethical and biosafety concerns. The generation of pseudoviruses allows to bypass this issue. Pseudoviruses contain the backbone of a poorly pathogenic virus or a non-replicative virus and can be pseudotyped to present the surface protein responsible for receptor recognition and cell fusion from highly pathogenic viruses (such as Ebola virus, Marburg virus, SARS-CoV and MERS-CoV, and more recently SARS-CoV-2, among many others). Furthermore, these pseudoviruses can incorporate reporter genes (mostly GFP, luciferase or β-galactosidase) easing the readout on the immortalized target cell lines used in the assay, which are specific for each virus (Li et al., 2018). The main pseudoviral backbones used for this purpose are from enveloped viruses (VSV, HIV-1/SIV, MuLV, etc.).

A prime example of a relevant immortalized cell line used for vaccine research with pseudoviruses are TZM-bl cells. This cell line has been widely used for HIV-1 studies. TZM-bl-based neutralisation assays were developed by George M. Shaw *et al.* and optimised by Montefiori *et al*. TZM-bl are HeLa cells that express CD4 and CCR5, making them susceptible to HIV-1 infection (Montefiori, 2009). These cells were stably transfected with a luciferase-encoding vector under the transcriptional control of an HIV long-terminal repeat sequence. Beyond the HIV-1 field, HIV-1 pseudoviruses could be pseudotyped to express the receptors of other viruses, like the SARS-CoV-2 spike, to evaluate the effect of neutralising antibodies (using hACE2-expressing TZM-bl cells).

B) Primary cells

Primary cells represent more closely the architecture of the tissue of origin. They are taken directly from the tissue and processed to establish them under optimized culture conditions. Because they are derived from tissue and not modified, their properties are similar to the *in vivo* state and exhibit more physiological conditions. For this reason, they provide excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging, signalling studies, etc.), and the effects of drugs and toxic compounds on the cells. However, primary cells have a limited lifespan and will stop dividing (or become senescent) after a certain number of cell divisions, hence their culture and maintenance can be more difficult than a continuous/immortalized cell line. The variabilities induced in primary cells acquired from donors and during subculture practices are major challenges faced by the researchers who study cell signalling pathways. Researchers prefer to screen cells for sensitivity to common stimuli before embarking on signalling studies. Pre-screened primary cells save valuable resources as they are stimulated for activation of major signalling pathways.

Primary cultures are initially heterogeneous (formed by a mixture of cell types present in the tissue) and can be maintained *in vitro* only for a limited period. Primary cells may be manipulated for indefinite subculture through an *in vitro* process called transformation. Transformation can occur spontaneously or can be chemically or virally induced. When a primary culture undergoes genetic transformation (provided with appropriate fresh medium and space), they divide indefinitely and become an immortalized secondary cell line (see section 3.2.1), which may lead to significant alterations compared to the physiology of the primary cell.

The most popular types of primary cells used in research are epithelial cells, fibroblasts, keratinocytes, melanocytes, endothelial cells, muscle cells, hematopoietic and mesenchymal stem cells as well as immune cells derived from peripheral blood mononuclear cells (PBMCs). The Monocyte Activation Test is a good example of how primary cells have helped replacing animal models in vaccine research and testing. Previously, the rabbit pyrogen test was the gold-standard technique to evaluate the pyrogenic effect of novel drugs and vaccines, but the European Pharmacopeia are campaigning to substitute it for the MAT.

Bibliography

Genzel Y. Designing cell lines for viral vaccine production: Where do we stand? Biotechnol J. 2015 May;10(5):728-40. doi: 10.1002/biot.201400388. Epub 2015 Apr 22. PMID: 25903999.

Li Q, Liu Q, Huang W, Li X, Wang Y. Current status on the development of pseudoviruses for enveloped viruses. Rev Med Virol. 2018 Jan;28(1):e1963. doi: 10.1002/rmv.1963. Epub 2017 Dec 7. PMID: 29218769; PMCID: PMC7169153.

Montefiori DC. Measuring HIV neutralization in a luciferase reporter gene assay. Methods Mol Biol. 2009;485:395-405. doi: 10.1007/978-1-59745-170-3_26. PMID: 19020839.

This document was produced with the support of EC H2020 Project 951668: TRANSVAC-DS, and is a project coordinated by the European Vaccine Initiative (EVI).
UniversitätsKlinikum Heidelberg • Voßstraße 2, Geb. 4040 • 69115 Heidelberg, Germany
• e-mail: transvacinfo@euvaccine.eu