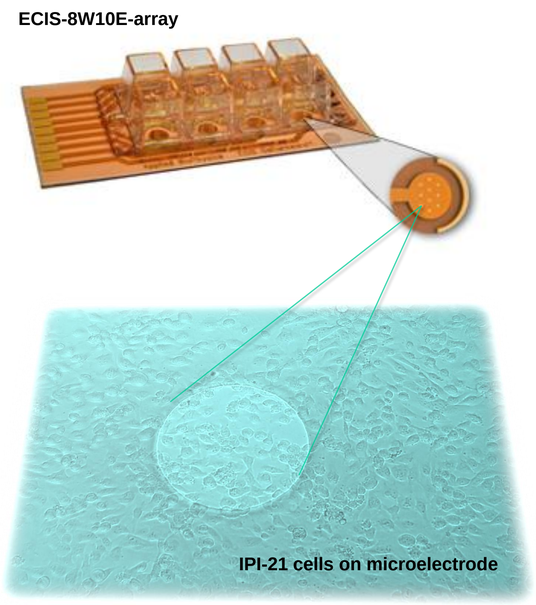
Titer dependent cytotoxicity of viral vectors measured in real time via ECIS-technology

Jakob Müller [a], Christian Thirion [b], Michael W. Pfaffl [a]

[a] Physiology Unit, Technische Universität München, Research Center for Nutrition and Food Science, Weihenstephaner Berg 3, 85350 Freising – Weihenstephan, Germany
[b] Friedrich-Baur-Institute, Klinikum der LMU, Department of Neurology, Marchioninistrasse 17, 81377 Munich, Germany

Introduction



Recombinant viral vectors are widespread tools for transfer of genetic material in various modern biotechnological applications like functional studies, vaccine development, gene therapy or RNA interference. However the practical handling often bears crucial problems. An accurate and reproducible titer assignment represents the basic step for most downstream applications of viral vectors not only considering precise MOI adjustment. As necessary scaffold for the studies described in this work we developed a qPCR based approach for viral particle measurement[1].

Proximate a fundamental vector originated problem concerning physiological effects is that the appliance of viral vectors can be attended by toxic effects on the individual cell culture model or tissue. To determine the individually critical viral dose we utilize Electric Cell-substrate Impedance Sensing (ECIS) to reveal toxic effects leading to cell death. It was first introduced in cell culture by Giaever and Keese[2] and can be utilized for examination of cell growth[3], cell motility[4], cell barrier function[5], in vitro toxicology[6] and is even applied in cancer research[7]. With ECIS technology the impedance change of a current flow through the cell culture medium in an array plate is measured in a non-invasive manner. The device visualizes effects on cellular level like cell attachment, cell-cell contacts or proliferation. Here we describe the potential of this online measurement technique in an in vitro model using the porcine ileal ephithelial cell line IPI-2I in combination with an adenoviral transfection vector (Ad5 derivate). With this approach we can show a clear dose-depending toxic effect. The amount of applied virus correlates with the level of cell death. So this assay offers the possibility to discriminate the minimal non toxic dose of the individual transfection method.

Viral titer assessment

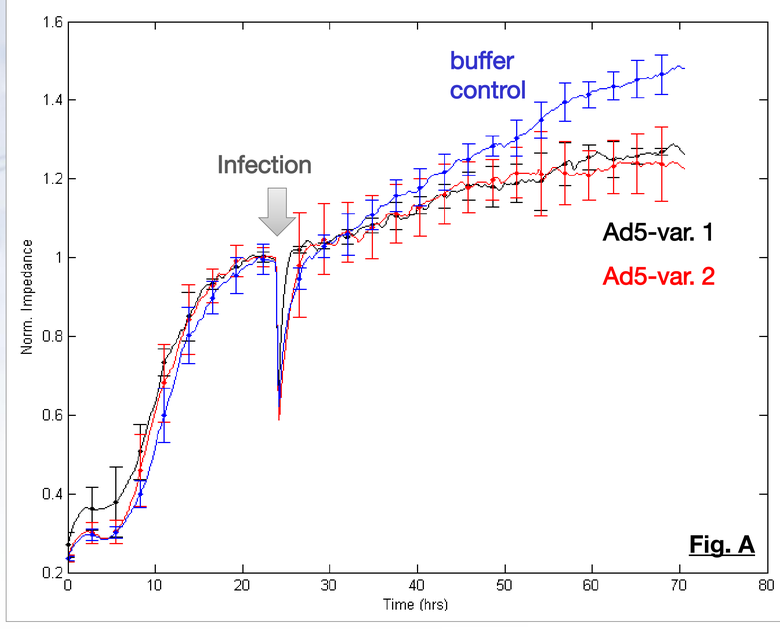


Fig. A

qPCR based virus titration verified in ECIS-device

Absolute quantification of adenovirus 5 (Ad5) genomes (Fig. B)

Capsid packed (intact) viral genomes (yellow & red curves) are extracted selectively and quantified on the Ad5-pTP-gene against a yeast tRNA stabilized pAd/PL-DEST (Invitrogen) Plasmidstandard (blue curves).

Evaluation of titration in ECIS 8W10E-arrays (Fig A)

Equal amounts of two different Ad5 vectors (black & red graphs) acertained by this qPCR-based titration method cause the same rate of cytotoxicity compared to the viral buffer solution (blue graph)

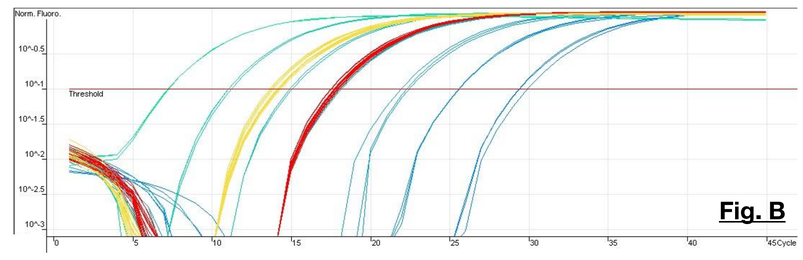


Fig. B

Cytotoxicity assay

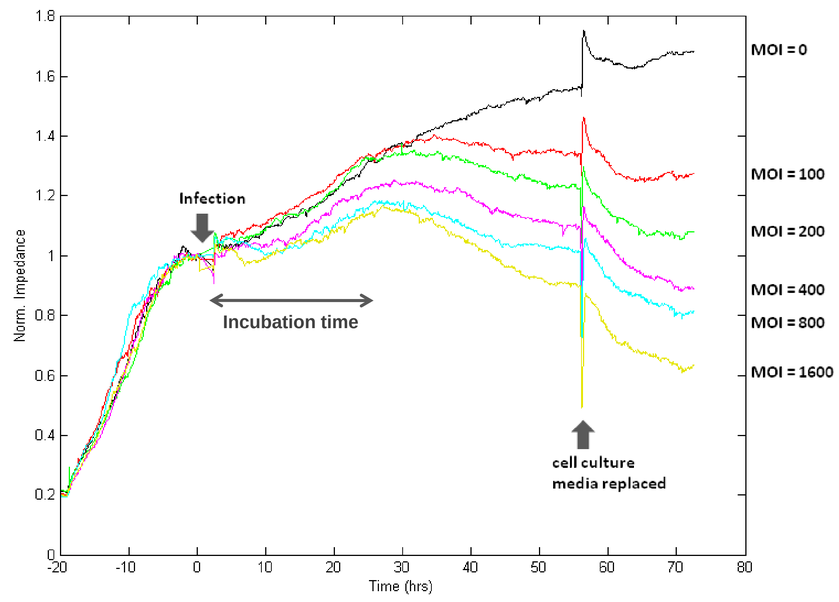
Solely measuring the impact of the infection

ECIS Cytotoxicity Assay : IPI-21 cell line infected with Ad5-U6Stop

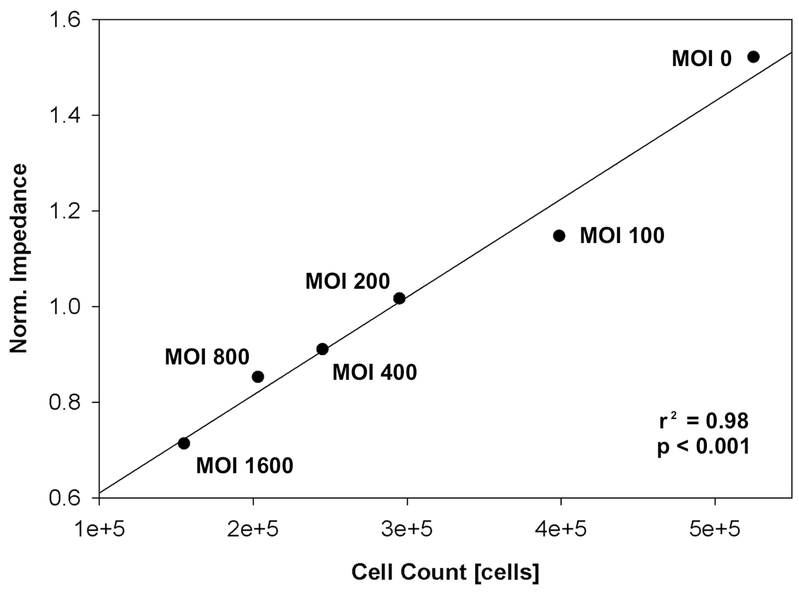
The porcine ileal epithelial cell line IPI-21 (1x10^5/well in 300µl DMEM) (HPACC, Sailsbury, GB) is treated with a MOI (multiplicity of infection) Gradient (0, 100, 200, 400, 800, 1600) of Adenovirus 5 and its reaction is monitored in real time (over 92h) in 8W10E-arrays using the ECIS 1600 device (30kHz, 60sek interval) (Applied Biophysics, Troy, US).

Here Ad5-U6Stop (SIRION-Biotech, Martinsried, DE) is used which has no further biotechnological tool function as viral vector. It serves exclusively as an infection control. The infection is performed after complete settlement of the dense seeded cells.

ECIS measurements (each curve grouped from biological duplicates) reveal that after a certain incubation time the toxic effect of the viral infection results in signal decrease corellated to the amount of inserted virus. The effect insists after the cell culture media is replaced.



Conclusion



Cytotoxicity results in cell death

What shows the ECIS-impedance signal?

The decrease in signal could stand for a loss of adhesion of the cells on the array surface, induced by the viral attack.

To clarify we conducted paralell cell enummerations what revealed that the signal shows loss of cells and that means cell death. The blot to the left shows a linear correlation between inserted virus (MOI), measured impedance and remaining cells at 72h post infection.

The study shows that already the application of viral vectors as biotechnological tools carries along a crude influence on the physiology of the individual cell culture model and that even at relatively low viral loads. That should not be neglected when questions of e.g. gene expression in viral mediated knockdown models are in concern.

Acknowledgements

The project was funded by the *Vereinigung zur Förderung der Milchwissenschaftlichen Forschung an der Technischen Universität München e.V.*. The know-how for viral vectors production came from SIRION-BIOTECH (Am Klopferspitz 19, D-82152 Martinsried). Technical support for the ECIS-device was provided by IBIDI GmbH (Am Klopferspitz 19, D-82152 Martinsried).

References

1 Müller, J., Thirion, C., and Pfaffl, M. W., Biosens Bioelectron 26 (5), 2000 (2011).
2 Giaever, I. and Keese, C. R., Proc Natl Acad Sci U S A 81 (12), 3761 (1984).
3 Mitra, P., Keese, C. R., and Giaever, I., Biotechniques 11 (4), 504 (1991).
4 Lo, C. M., Keese, C. R., and Giaever, I., Exp Cell Res 204 (1), 102 (1993).
5 Tiruppathi, C. et al., Proc Natl Acad Sci U S A 89 (17), 7919 (1992).
6 Ceriotti, L. et al., Biosens Bioelectron 22 (12), 3057 (2007).
7 Lovelady, D. C. et al., Phys Rev E Stat Nonlin Soft Matter Phys 76 (4 Pt 1), 041908 (2007).