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Publication date: 2013

Document Version Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

Caviglia, C., Carminati, M., Heiskanen, A., Ferrari, G., Sampietro, M., Andresen, T. L., & Emnéus, J. (2013). Real-time impedimetric monitoring of Poly(ethylenimine)s-mediated cytotoxicity during gene transfection. Abstract from 15th International Conference on Biomedical Engineering, Singapore, Singapore.

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### ID: 678

**Oral Presentation** *Topics:* B4) Gene Vector Delivery, D6) Lab-on-Chip *Keywords:* electrochemical impedance spectroscopy, cytotoxiciy, gene transfection, polyethylenimine

# Real-time impedimetric monitoring of Poly(ethylenimine)s-mediated cytotoxicity during gene transfection

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Poly(ethylenimine)s (PEIs) are able to condense DNA and RNA into stable toroidal and globular nanostructures (polyplexes) and are among the most efficient and promising synthetic transfectants, but they induce severe cytotoxicity. The mechanisms of PEImediated cytotoxicity have not been fully delineated but PEI toxicity appears to predominantly depend on membrane perturbing effects in cellular compartments in which they accumulate. Electrochemical Impedance Spectroscopy (EIS) is used as a non-invasive biophysical approach for the investigation of the electrical properties of biological materials according to their physiological and morphological changes. In this work, EIS has been used to evaluate impedance changes due to the polycation perturbations on a cell population. HeLa cells have been cultured on laminin-coated oold interdigitated electrode arrays integrated into a tailor-made microfluidic cell culture platform. Multiplexed EIS data from each sensor element were acquired using a 24channel miniaturized potentiostat (30 points between 100 Hz and 100k-Hz). Two alternative sensing configuration approaches (the standard "vertical" configuration (a single working electrode (WE) versus a large, distant counter electrode (CE)), and the interdigitated configuration (WEa comb versus WEb comb)) have been used and compared on the same cell population, providing optimal detection conditions. The experiments have been performed by initially seeding about 10<sup>3</sup> cells into each chamber, continuously perfused with fresh culture medium. The platform was incubated in a humidified atmosphere. After 24 hours, different concentrations of PEIs have been introduced in the culture medium and the incubation continued for other 24 hours. Cell adhesion, growth and PEI-cytotoxicity have been detected in real-time by following impedance changes. Microscopic imaging and MTS assays have been combined to the electrochemical detection. Complementary ongoing experiments aim to monitor in real-time gene transfection in order to detect the cytotoxic effects (apoptosis and necrosis) induced by different cationic polyplexes. This approach can contributes to a clearer and detailed mechanistic understanding of polycation-modulated cellular functions and cell death and could initiate rational approaches for design and engineering of safer vectors for nucleic acid transfection.