Impedance in real-time monitoring of trophoblast spheroid invasion dynamics and future applications

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Electric Cell-substrate Impedance Sensing (ECIS) technology was developed for a primary goal on label-free and non-invasive *in vitro* cell migration detection. However, most existing technologies are limited to single biological component system analyses with constrained resolution, posing challenges for studying more complex biological processes - from adhesion and migration to invasion and differentiation.

In our recent study, invasion of trophoblast derived spheroids through the monolayer of endometrial epithelial Ishikawa cells was investigated. In addition to classic cell proliferation and monolayer formation, a three-component *in vitro* measuring model that composed of electrode-electrolyte, monolayer and spheroid was developed to study spheroid invasion dynamics.

The interface between the spheroid and the Ishikawa monolayer was successfully detected through impedance characteristics. The measuring sensitivity to invasion dynamics was evaluated and visualized by comparing the resistance, capacitance and phase at defined low and high frequency with various spheroid-electrode coverage areas, which also aligns the assumption of biological change. Frequency-dependent semi-quantitative analysis of resistance, capacitance and phase were established to understand the cell-chip behavior over time.

Moreover, we extend *in vitro* equivalent electrical fitting (EEC) model from the conventional electrode-electrolyte double-layer interface theory to a three-component model using impedance-phase spectra. According to EEC fitting, a three-step invasion process was proposed: 1) preinvasion – non-contact condition with an intact cell monolayer, 2) early-stage invasion – spheroid attachment within 6 hours after transfer, and 3) late-stage invasion – 6 to 48 hours after spheroid transfer.

Further development of this technique could expand its scope to more complex biological mechanics sensing involving multiple factors, paving the way for more accurate and intelligent sensing systems for biomedical diagnostic such as cancer invasion and endometrial receptivity.

References:

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