

3D-reconstruction of cell-cell vaccines with focus on functional membrane exchange

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Background: Many tumour vaccination strategies are currently under investigation. One promising strategy is the total cell to cell fusion. Functional intact tumour cells are fused with Antigen-presenting cells (APC), such as dendritic cells or B-cells. Electroporative cell fusion is commonly employed to fuse cells. Different electroporative fusion-protocols are published but there are still many open questions about optimal fusion efficiencies, and often functional tests are limited to cell culture experiments. Here we describe for the first time a method to reconstruct electro fused cell-cell vaccines by using high resolution microscopy. Using this innovative technique it is now possible to freely rotate cellular aggregates in 3D-space and clearly visualise membrane exchange between the fusion partners.

Experimental design / results:

The RCC-line ACHN (ATCC) was fused with B-cells using an optimized medium and Biorad Genepuls II. Conductivity measurements during and after the application of the electric field pulse could be established as a tool for the optimization of field pulse parameters necessary for field-induced formation of membrane pores. Therefore respective technical requirements were created. A theoretical analysis of measured gradients on the basis of electro-thermodynamics is possible and critical parameters could be extracted for fused cells. Ahead of fusion, RCC cells were stably transfected using eGFP and B-cell membranes were made visible using CD19-PE antibody. DAPI staining was added to visualize nuclei. Directly post electro fusion, aggregates were cast in glycerin-gelatin on microscope slides [$1 \cdot 10^5 / 100 \mu\text{l}$]. Covered with cover slips, cells were analyzed using the Olympus Confocal Microscope FV500. A stack of 20-30 2D pictures (based on overlays of three pictures for each fluorescence channel on every section level), each $\approx 100\text{nm}$ thin, were produced from selected cell-cell aggregations. Received picture stacks were imported to 3D Software Imaris[®] (Bitplane) using the internal surpass function to reconstruct free rotating images of the analyzed aggregates. Zoom function in combination with every possible section view allows exact membrane analysis on fused cells.

Conclusions: 3D reconstruction of aggregates after cell-cell fusion via electro fusion is a powerful tool to observe membrane exchange between fusion partners. This technique could therefore be used as quality and quantity control of antibody marked cell-cell electroporative fusion. Also additional information e.g. about the number, the presence or loss of nuclei after fusion are clearly visible.

A high resolution 4D analysis of cell-cell fusion, including time is currently under investigation.