**Novel 3D hyaluronic acid-based breast cancer *in vitro* model** **as tool** **for transfection tests**

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Electrochemotherapy is used in the clinical practice since it represents a promising approach for the intracellular delivery of chemotherapeutic drugs by means of electroporation (EP). Furthermore, EP is a valuable tool to deliver nucleic acids encoding molecules useful for anti-tumor treatment, regenerative medicine, or investigation of intracellular environment.

In general, the set-up of voltage pulse applied to electroporate cell membrane is evaluated by using cells in suspension that are not representative of the *in vivo* conditions because they are isolated and lack of the cell-cell and cell-extracellular matrix (ECM) interactions. Indeed, in the tissues cells are surrounded by ECM whose composition and physical characteristics are different for each tissue. Since the efficiency of EP depends not only on cell size and density but also by medium conductivity, various tridimensional (3D) *in vitro* models, such as spheroids and hydrogel-based cultures, have been proposed in order to obtain a microenvironment resembling the tissue structure.

Herein, to mimic a 3D environment supporting cell growth and matrix production, scaffolds with myxoid characteristics were designed. Human breast cancer HCC1954 and MDA-MB231 cells were seeded on cross-linked and lyophilized matrices composed of hyaluronic acid and ionic complementary self-assembling peptides functionalized with IKVAV adhesion motif. Cultures were characterized at various time points (1, 3, and 7 days from seeding): cell morphology and growth, and matrix deposition were evaluated by means of phase contrast microscopy, resazurin-based assay, and histochemical staining, respectively.

The EP of cell membrane in 3D culture was set up by applying a sequence of 8 voltage pulses with different amplitude through a plate electrode. In fact, the EP procedure, performed using a sequence of square voltage pulse at a suitable amplitude (pulse length 100 µs, 1 Hz), is a safe method for introducing exogenous molecules into cells. The efficiency of EP was verified using propidium iodide (PI) and Hoescht 33442 staining, whereas cell viability through Presto Blue assay. Moreover, cell morphology and viability were determined until 3 days from electroporation.

Starting from these data, a sequence of 8 voltage pulses with an amplitude of 1300 V/cm was applied on 3D cultures for the transfection of a plasmid vector encoding the Green Fluorescent Protein (pEGFP). A commercially available transfection reagent was taken as control, following the manufacturer’s instructions. Furthermore, to verify the reliability of our 3D model, the two transfection methods were also tested on 2D cultures grown on tissue culture-treated polystyrene plates at 7 days from seeding. At 48 h from transfection of 2D cultures, the percentage of fluorescent cells were similar for the two transfection methods: about 12 % and 15% in EP-treated cultures and control ones, respectively. Similar results were obtained in 3D cultures, where the number of GFP-positive cells increased ranging from 24 to 72 h from EP. In 3D cultures, EP led to a percentage of transfected cells higher than 50 %.

Collectively, our preliminary results suggest that the proposed scaffold is suitable to produce 3D cultures useful for *in vitro* studies on gene electrotransfection.