**COST EMF - MED (Action BM1309):**
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STSM Report:

# Nanosecond pulsed electric fields on liposomes for potential drug delivery applications

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**STSM Reference:** ECOST-STSM-BM1309- 110515-060042

**STSM dates:** FROM 11th May 2015 TO 30th May 2015

**Abstract:**

The delivery of chemotherapeutic drugs through the use of nanosecond pulsed electric fields (nsPEF) is one of the most promising techniques which allows a controlled release of drugs, encapsulated in a nano-carriers (i.e.liposomes), by the application of an electrical stimulus. The goal of this STSM mission has been to test the real capabilities of liposomes to mediate the drug delivery into cell membrane under nsPEF exposure. In vitro experiments were performed in order to test the feasibility of the liposome membrane poration under the application of a 10-ns electric pulse with the electric field intensity predicted by numerical models.

## A. Purpose of the STSM

The aims of this STSM were: (i) to understand the possibility to use the liposome as a drug delivery system; (ii) to understand the role of the liposome dimension in that process; (iii) to test the feasibility of the liposome membrane poration through in vitro experiments.

## B. Work Description

The main goal of this STSM mission was to test the real capabilities of liposomes to mediate the drug delivery into cell membrane under nsPEF exposure through the application of an experimental approach. The main issue is related to the different dimensions between cells and liposomes and to the possibility to electroporate both of them with the application of the same pulsed electric field intensity without causing drastic effects to the biological cells.

Thanks to a previous collaboration with the Institute Gustave Roussy regarding the microdosimetric modelling, it has been possible to identify the intensity of the nsPEF signal to be used in the experimental activity for the poration of the liposome membrane.

Based on the results obtained by microdosimetric models in-vitro experiments for the exposure of liposomes under 10-ns pulsed electric field were carried out in collaboration with a Post-Doc researcher Marie Breton from the host institution. The experimental setup consisted of a HV generator (FPG 10-1SM10, FID Technology, Burbach, Germany) that can deliver 10-ns duration pulses with amplitude between 4.5 kV and 10 kV. The signal waveform was monitored with a LeCroy Oscilloscope. For the exposure of the vesicles a 1 mm cuvette was used, filled with 55µl of solution.

The main idea was to expose liposomes, containing inside quenched calcein (concentration of 80 mM) as fluorescent dye, to one 10-ns electric pulse and, after the exposure, reading the fluorescence to see if the

electric field of a precise intensity was able to porate the liposome membrane and release the calcein outside, as predicted by numerical models. As first step three different types of vesicles (with different lipids) were prepared: DSPC (1,2-Distearoyl-sn-glycene-1-phosphocholine), POPC (1-palmitoyl-2-sn-glycero-3phosphocholine) and DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine). The size was measured using a Malvern nano-sizer. Based oh these measurements, the DSPC lipid was chosen since it was the only one containing two well distinguished liposome populations: one of 362 nm of diameter and another one of 62 nm of diameter (fig.1). The other two lipids solutions contained only aggregates or many different liposomes populations in the same solution.

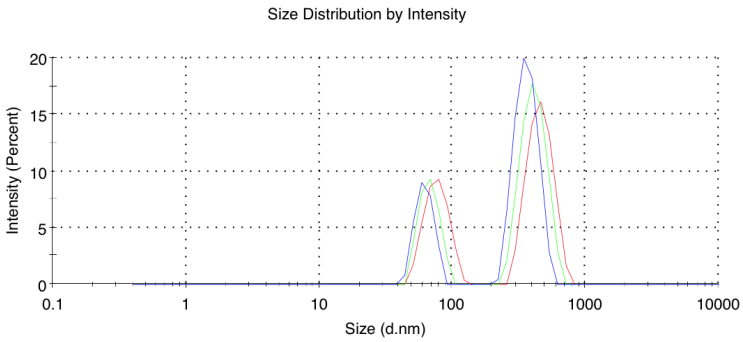


Fig1.: Size distribution measurements: the 62 nm liposomes on the left side and the 362 nm on the right side; three different measurements have been carried out.

Because we could not use an extruder to have only one population of liposomes in our sample we used two different solutions of DSPC: one containing both populations of liposomes and another one containing the smallest population (62 nm) obtained by the filtration of the initial solution using a 200 nm filter.
The cuvette for the exposure was filled with 55 µl of solution made by 50 µl of DSPC (filtered or not filtered) + 5 µl of EDTA, necessary to avoid any interaction between calcein and iron ions coming from the electrodes in the cuvette, in order to maximize the fluorescence of the vesicles. After the exposure of each solution to one electric pulse of 10ns duration, the fluorescence value was calculated using a GloMax®-Multi+ Microplate Multimode reader comparing the fluorescence before and after the electric pulse. After the fluorescence reading, we added to each solution 20µl of Triton X-100, to have a positive control releasing the 100% of fluorescence, and we checked again the fluorescence value. Moreover in order to have more concentrated liposome solutions some experiments were performed evaporating the DSPC sample for 1 or 2 hours before the exposure to the electric field. The Savant AES 1010 SpeedVac® concentrator was used for the evaporation at 43°C, in full vacuum conditions. Conductivities were measured using a Mettler Toledo -S30K- Conductivity Meter.

## C. Results

According to the electric field intensity suggested by the numerical models in vitro experiments were carried out considering a solution with liposomes of two different populations as described above. In order to have a concentrated solution of vesicles for the first experiments the DSPC solution was evaporated for 2 hours and the conductivity. Because of the high conductivity value (~ 2 S/m) the highest field we were able to deliver was around 3 MV/m and, as predicted by the numerical model, no electroporation occurred. After this first trial, the DSPC sample was evaporated for 1 hour in order to reduce the conductivity, and hence to achieve an higher electric field amplitude. Under these conditions it was possible to apply an electric field of 4 MV/m. After the exposure through the fluorescence reading it was possible to appreciate slightly differences in the fluorescence between the not-exposed and the exposed samples but still it was not enough to confirm the liposome electroporation as predicted by the numerical models. This behaviour can be justified by the

presence of the two different populations in the same samples and to the possibility to have in the exposed solution much more liposomes of smallest dimension for which 4 MV/m, as predicted by the simulations, is not sufficient to porate the liposome membrane.

Such results are an important indication for further experiments in which it will be possible to realize vesicles of only one size, as 400 nm liposome, in order to obtain the electroporation with a lower amplitude value, easily achievable with the current nanopulsers.

## D. Future collaboration with host institution

As indicated in description of obtained results, it will be necessary to optimize the protocol for the electric field exposure, in order to rich in the same time the right conductivity value and the electric field intensity necessary to porate liposome membrane as suggested by numerical models. Additionally, some experiments related to an ensemble made by cell and liposome will be interesting to verify the feasibility of the cell and liposome membrane poration obtained almost with the same electric field intensity. For these reasons it will be fundamental a future collaboration with the host institution to confirm or not the real feasibility of electroporation of the cell and the liposome membrane using the electric field amplitude predicted by the numerical models.

## E. Expected Publications

These preliminary results need further work both in terms of modelling and experimentally. In particular, clear evidence of liposome electroporation is needed in order to move toward a scientific publication; such an activity is already planned with the host institution.

**Confirmation by the host institution of the successful execution of the STSM:**

Elena has performed an excellent work during her stay. She remained in contact with her mentors in Rome, and the work could progress under a coordinated way. She had good contacts with the entire hosting group. The conditions for the experiments displayed some difficulties to match with the conditions defined by the modeling. However it was interesting to progress in the experimental protocols. I thus fully confirm the execution of the STSM by Elena della Valle.

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