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Wide-field Coherent anti-Stokes Raman microspectroscopy to detect changes in membrane hydration of liposome exposed to nanosecond electric pulses

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To deep more insight into basic phenomena occurring during and after electropulsation of biological membranes, a new experimental modality has been used combining a wide field Coherent Anti Stokes Raman Spectroscopy (CARS) system [1] with a coplanar wave guide able to deliver nanosecond pulsed electric fields to different in vitro samples [2]. This setup allows to acquire CARS hyper-spectra at specific Raman bands from 2900 to 3500 cm-1 (into the so called water vibration region) as well as to acquire in real time the CARS signature at specific wavelengths with a spectral resolution of few ns. This time scale is comparable to the duration of the electrical stimulation, that we synchronised to the laser emission. As the biophysical and chemical bases of cells electropermeabilization are still debated, our setup allows the experimental assessment of the role of water molecules and phospholipid bilayers during the occurrence of this phenomenon which is used in various biotechnological, biological and medical applications.

The experiments have been conducted on liposome suspensions placed between the central and lateral (ground) electrodes of a grounded closed coplanar waveguide (GCCPW) [2]. Liposomes, that is lipid spherical unilamelar vesicles, where chosen as a suitable synthetic system to mimic phospholipid double layers as they are similar to the structure of real cell membranes. The illumination scheme of the CARS microscope followed a non-phase-matched geometry as suggested in [1]. Image of the observation plane was formed on an intensified CCD camera (PIMAX 3 Camera, Roper Technologies, Sarasota, Florida). The camera was triggered by the laser pulse using a delay generator (DG 545, Princeton instruments) with a temporal gate of 40 ns. Bandpass filters were placed in order to reflect the pump/probe and Stokes beams right after interacting with the sample of interest.

Spectra of liposomes suspensions were acquired immediately after or during electropulsation (in real-time experiments) evidencing an increase of the vibrational modes around 3345 cm^{-1} in the pulsed samples with respect to the non-pulsed ones. Pulsed samples received 2000 pulses consecutively at 10 Hz and at an amplitude of 9 MV/m. This vibrational signature at 3345 cm^{-1} is related to the so called lipid associated water molecules, representing a water structure in which the intermolecular OH bonds become weak (asymmetric OH stretch modes) corresponding to the water molecules which are not in or close to the bulk water, but the water intermingled with the lipid heads. The effective permeabilization of liposome suspensions after the electric pulses delivery was also verified looking at the release of a fluorescent dye (5-6-carboxfluorescein) included into the liposomes' core. Finally, a simple electrochemical model was also developed to explain part of our results.

In summary, CARS, employing nanosecond lasers pulses and the properties of our wide field microscope and its intrinsic ability to sense complex interferences, has provided us with an appropriate diagnostic tool. In a future, the underlined mechanism will be investigated on cells, hence taking into account recovery processes as well as the different interactions elicited by the application of longer electric pulses.

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2. C. Merla, et al., A wide-band bio-chip for real-time optical detection of bioelectromagnetic interactions with cells. Scientific Reports, 8, 5044, 2018.