Membrane hydration of cells exposed to microsecond and nanosecond intense electric pulses followed by CARS optical microspectroscopy

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Electropulsation, the application of short, intense electric pulses, is widely used in biomedical applications such as drug delivery, gene therapy, and tumor treatments. While previous studies primarily relied on molecular simulations or indirect experimental methods to study membrane electro-permeabilization, this work expands on using a wide-field Coherent anti-Stokes Raman Scattering (CARS) microspectroscopy approach to investigate hydration changes in cell membranes.

In this work, attention has been paid to the phospholipids C-H and water O-H vibrational modes in the 2900-3480 cm^{-1} range. Thanks to the special geometrical arrangement of the illumination laser beams in our Electro-CARS microspectroscope, the probed water vibrations correspond to the molecules of water located at the cell membrane surface (indicated as interfacial water molecules) and confined in the proximity of the phospholipid heads (indicated as interstitial water molecules).

By integrating Electro-CARS with a grounded closed coplanar waveguide, the approach enabled real-time tracking of vibrational modes of lipids and water molecules in human mesenchymal stem cells (HuMSC), murine fibroblasts (DC-3F), and erythrocytes. Two experimental protocols were applied: one for a post-electropulsation spectral analysis and another for real-time tracking during electric pulse application. Results show that microsecond and nanosecond pulsed electric fields (PEFs) differentially impact membrane hydration, with interstitial and interfacial water dynamics being significantly altered in HuMSC and DC-3F cells. The study also confirms membrane electro-permeabilization through fluorescence microscopy, with real-time monitoring indicating long-term hydration changes in electroporated membranes.

These findings provide key insights into the electropulsation-induced modifications of lipid membranes and water incorporation. The differential responses among cell types highlight the importance of tailored pulse parameters for biomedical applications. Electro-CARS emerges as a powerful, label-free technique for studying electroporation dynamics at high spatial and temporal resolution. Future studies will expand the dataset by analyzing additional cell types and optimizing electric pulse parameters for therapeutic applications.