With adequate local anesthesia, the main side-effect of electrochemotherapy is an unpleasant contraction of muscles located in the vicinity of the electrodes. Since the pulse repetition frequency of 1 Hz is most often used, each pulse in the train of 8 pulses causes the contraction of muscles innervated by the excited nerves. Electrochemotherapy with pulse repetition frequency above the frequency of tetanic contraction would reduce the number of individual muscle contractions and would improve patient comfort. Recent experimental studies where electrochemotherapy with high frequency pulses (e.g. 1 kHz [52] and 5 kHz [12, 53]) was performed have shown that using higher repetition frequency reduces the unpleasant contractions to a single one and shortens the treatment time, while the efficacy of electrochemotherapy measured by the tumor response to treatment was not different comparing to the standard 1 Hz protocol. Results of our simulations for high repetition frequency electrochemotherapy (HV pulses: 1500 V/cm, 8×100 μs, 1 kHz) for two electrode geometries (plate electrodes - Figure 5; needle electrodes – Figure 8) show that the increase of pulse repetition frequency from 1 Hz to 1 kHz causes an increase of bulk tissue temperature that is still low and unlikely to induce thermal damage (note that time scale is in ms). This result was validated in the most recent electrochemotherapy study on patients where, for the first time, pulse repetition frequency of 5 kHz was used and has proven to be safe and even more effective in tumor treatment than standard repetition frequency of 1 Hz [12]. However, from our simulation results we observe that near the electrodes temperature rise may be significant (Figure 5 trace T_2, Figure 8 trace T_3) if increased conductivity is assumed.

Simulation results for electrotransfection protocol with train of LV pulses (250 V/cm, 8×50 ms, 1 Hz) for both plate (Figure 6) and needle electrode (Figure 9) show that temperature increase in the tissue is far from being unimportant. The simulated train of electrophoretic pulses is likely to cause localized thermal damage, especially if we assume highly conductive tissue. This numerical result can explain tissue damage observed in some *in vivo* electrotransfection studies. For the success of electrotransfection, and minimization of thermal damage, careful optimization of pulsing protocol is necessary. This is particularly important if needle electrodes will be used. Therefore, to be within the 'safe range' either the pulse amplitude, duration or number should be reduced.

When comparing a pair of needles to parallel plate electrodes, with needles it is harder to avoid local tissue heating and to achieve, in a larger tissue volume, local electric field between reversible and irreversible permeabilization threshold. This is an important guideline for clinicians when considering appropriate electrode type for cancer electrochemotherapy or gene electro transfer.

5 CONCLUSIONS

Model-based analysis of Joule heating in electroporation-based treatments enables to predict temperature rise in tissue and electrodes during and after pulse delivery. This analysis provides useful insight into the extent of tissue thermal damage (if any) and provides important guidelines for development of electrodes and safe protocols for electrochemotherapy and gene electro therapy. Our results show that at specific pulse parameters at least locally tissue heating might be significant (i.e. tissue temperatures to grow in excess of 43ºC). For electrochemotherapy, this is not critical since regions of increased temperature are most likely irreversibly electroporated. However, DNA electrotransfer may be unsuccessful due to heating-related DNA damage.

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