

# Nanosecond Pulsed Electric Field Therapy that Causes Skin Tumors to Self-Destruct with a Single Treatment

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## INTRODUCTION

We have previously reported that nanosecond pulsed electric fields (nsPEF) can eliminate murine skin tumors<sup>1-3</sup>. These studies used parallel plate electrodes that were incompatible with human skin and required multiple treatments. Here we introduce new suction electrodes compatible with human skin and have identified nsPEF therapy parameters that can eliminate murine melanomas and basal cell carcinomas with a single treatment. These pulses initiate apoptosis in the tumor cells without hyperthermia. We have used both a xenograft nude mouse melanoma model system and endogenous tumors in transgenic mice. We identified the minimal field strength, pulse frequency and pulse number for this therapy. When we used these optimal pulse parameters of 2000 pulses, 100 ns in duration, and 30 kV/cm in amplitude at a pulse frequency of 5-7 pulses/s, we eliminated all 17 melanomas treated in 4 mice. This was the highest pulse frequency that we could use without raising the skin tumor temperature above 40 °C. We also demonstrate that the effects of nsPEF therapy are highly localized to only those cells located within the suction electrode and results in very little scarring of the nsPEF-treated skin.

## MATERIALS AND METHODS

**Suction Electrodes.** We have tested four different electrode configurations. All of the suction electrodes were plastic cylinders with a cup-shaped opening at one end with an inner diameter of 4 mm and a depth of 2 mm. The base of each cup had small holes drilled in it to allow a suction to be applied from the opposite end of the cylinder to pull the tumor into the cavity. The metal electrodes were positioned either as flat or cylindrical pieces in the walls of the cavity or as needles protruding from the base. This enclosure restricts nsPEF application to only those tissues located within the suction cup. We demonstrated that this design was compatible with human skin in several regions of the body.

**100 Nanosecond Pulse Generator.** We used a Blumlein line pulse generator in which the pulse duration is proportional to the length of a coaxial cable. We used a triggered spark gap at atmospheric pressure for a switch and the 100 ns pulse rise time was 15 ns. We controlled the pulse frequency and amplitude with a microcontroller-based control panel that initiated firing of a spark plug to trigger the breakdown of the spark gap.

## RESULTS

**Optimizing Pulse Number.** We investigated a wide range of pulse numbers from 500 to 2700 while using the same amplitude, duration and frequency (30 kV/cm, 100 ns, 5 Hz). The smallest pulse number that resulted in the complete remission of tumors 100% of the time is 2000 (Fig. 1). Using fewer pulses sometimes eliminated the tumor with one treatment but often required more treatments.

**Optimizing Pulse Amplitude.** We investigated a range of pulse amplitudes using the same pulse duration, number and frequency (100 ns, N=2000, 7 Hz). Field strengths lower than 30 kV/cm did not trigger complete tumor remission with a single treatment, but could sometimes do it with multiple treatments (fig. 4B). The current delivered to the tumor was dependent on field strength, as expected. The 6-pole dual electrode typically delivered 5 amps at 10 kV/cm and 20 amps at 30 kV/cm. This non-linear relationship between voltage and current is most likely due to uneven electropermeabilization of the stratum corneum by our suction electrodes. 10 and 15 kV/cm treatments failed to cause complete regression, resulting in tumors that regrew a few days post treatment. Additional treatments at these low amplitudes never completely eliminated the tumor. We conclude that the minimum amplitude for triggering complete remission with one treatment is 30 kV/cm.

**Optimizing Frequency:** -In order to minimize the treatment time, it is desirable to use the highest pulse application frequency possible. The main limitation to increasing this frequency is the consequent temperature increase in the tissue. Therefore we introduced a miniature thermocouple into the tumor during pulsing for simultaneous measurement of tumor temperature. We repeated this experiment on 10 tumors testing frequencies up to 10 Hz. Applying nsPEF at 1 Hz increased tumor temperature by 2°C, whereas 5 pulses per second increased the tumor temperature by 6-7°C. Initial skin temperature ranged from 25 to 30 °C. We decided to use a maximum frequency of 7 Hz so that the tumor temperature would never exceed 37 °C. This is well below the hyperthermia threshold of 41 °C.

## CONCLUSIONS

From the above results we conclude that the minimum nsPEF parameters required to obtain complete tumor remission with a single treatment using 100 ns pulses are 2000 pulses at 30 kV/cm and 5-7 Hz. Using these parameters, all 8 tumors treated at 5 Hz and all 9 tumors treated at 7 Hz showed complete tumor regression with a single treatment. In order to confirm complete regression, we conducted histological analysis of the treated regions by preparing a 5 µm section at 0.5 mm intervals throughout the region. Each section was carefully studied and photographed. No melanoma cells were detected in any of these sections. We have also found that these pulse parameters can eliminate endogenous skin cancers that spontaneously arise *in situ* several months after transgenic mouse pups are treated with either ultraviolet or ionizing radiation. Both endogenous melanomas and basal cell carcinomas respond to nsPEF therapy by self-destructing via apoptosis.

## REFERENCES

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