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Radiosensitization of oral tongue squamous cell carcinoma by nanosecond pulsed electric fields (nsPEFs)

Jinsong Guo [a], Yu Wang [b], Jing Wang [c,*], Jue Zhang [a,b,**], Jing Fang [a,b]

[a] College of Engineering, Peking University, Beijing, 100871, China
[b] Academy of Advanced Interdisciplinary Studies, Peking University, Beijing, 100871, China
[c] Department of Oral Medicine, School of Stomatology, Lanzhou University, Lanzhou, Gansu, China

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ABSTRACT

Nanosecond pulsed electric fields (nsPEFs) are a non-thermal and non-toxic technology that induce a myriad of biological effects. They have been proven to be effective in tumor shrinkage, but few studies focus on its radiosensitization in oral tongue squamous cell carcinoma. The purpose of this research was to study the radiosensitization effect of nsPEFs on a human oral tongue cancer cell line Tca8113 and to investigate the potential antitumor mechanism. A Tca8113 cell line was tested respectively by MTT assay, clonogenic assay, flow cytometry assay, annexin V-FITC/PI assay, mitochondrial potential assay and total nitric oxide assay. Our results showed that nsPEFs had a time and field strength dependent inhibition effect on Tca8113 cells. The sensitization enhancement ratio (SER) of nsPEFs was 1.453 ± 0.038. Furthermore, radiation induced G2/M arrest was augmented by treatment with nsPEFs. We observed many more Tca8113 cells showing early apoptosis after nsPEFs combined with radiotherapy. Additionally, the NO concentration was significantly increased after nsPEFs treatment. These findings indicate that combination of nsPEFs with radiotherapy can enhance the radiosensitivity of Tca8113 cells and nsPEFs could be a potential radiosensitizer for oral tongue squamous cell carcinoma.

1. Introduction

Oral tongue squamous cell carcinoma (TSCC) is one of the most common malignancies of the head and neck region. Recent survey data suggests that the incidence of TSCC is actually increasing in young and middle age populations [1,2]. Despite advances in cancer treatment, the 5-year survival rate has remained at about 50% over the past several decades [3]. Usually, operation and irradiation are the mainstay treatments for TSCC patients. However, surgical treatment for patients often produces a significant adverse effect on speech function, chewing and swallowing ability. Further, there is a high recurrence rate after surgery [4]. Thus, how to further improve the sensitivity of oral tongue cancer cells to radiation has become a research hotspot. Recently, looking for new, safe and effective radiosensitizers has become a novel strategy in oral tongue cancer treatment.

Since the 1960s chemical sensitization in radiotherapy has already been studied [5]. Up until now, we have obtained some stable and high radiosensitizing effect radiosensitizers, such as misonidazole (MISO), taxanes and sirolimus. With advances in new radiosensitizers, the survival of TSCC patients have improved significantly [6–8]. However, it is difficult to avoid the toxicity effects induced by the chemical radiosensitizers, especially for the advanced cancer patients and those with TSCC recurrence after radiation therapy. Overall, we need to find an effective sensitization technology for radiotherapy in TSCC treatment without toxic side effects.

Typically, the effects of pulsed electric fields on biological cells have been investigated since the late 1950s. More recently, the duration of the electric fields has been shortened to nanoseconds [9]. Many previous studies have found that nanosecond pulsed electric fields (nsPEFs) are able to induce a series of medical and biological effects. The pertinent areas include tumor shrinkage, wound healing, sterilization, and plant growth promoting [10–13]. Meanwhile, the present work describes in detail the anticancer activity of nsPEFs, including cell apoptosis, cytochrome C release, calcium release and DNA damage [14]. Additionally, some studies have also showed that nsPEFs have synergistic effects on low concentration chemotherapy drugs in oral cancer cell lines [15,16]. In particular, Nuccitelli R completed the first-in-human safety trials of nsPEFs in basal cell carcinoma (BCC). The results indicated that nsPEFs are safe and may offer a fast and scarless alternative to cancer treatment [17]. Also, Shan Wu, et al. found that nsPEFs treatment did not create permanent damage to the skin or normal tissues and only left shallow marks on the skin that faded completely in two weeks [18]. Moreover, we concluded that nsPEFs can effectively induce cell cycle arrest at G2/M phase [19,20]. Moreover, we inferred that NO was positively involved in the early growth effects of *Haloxylon ammodendron* seed

* Corresponding author.
** Correspondence to: J. Zhang, College of Engineering, Peking University, Beijing 100871, China.
E-mail addresses: lzukqwj@126.com (J. Wang), zhangjue@pku.edu.cn (J. Zhang).

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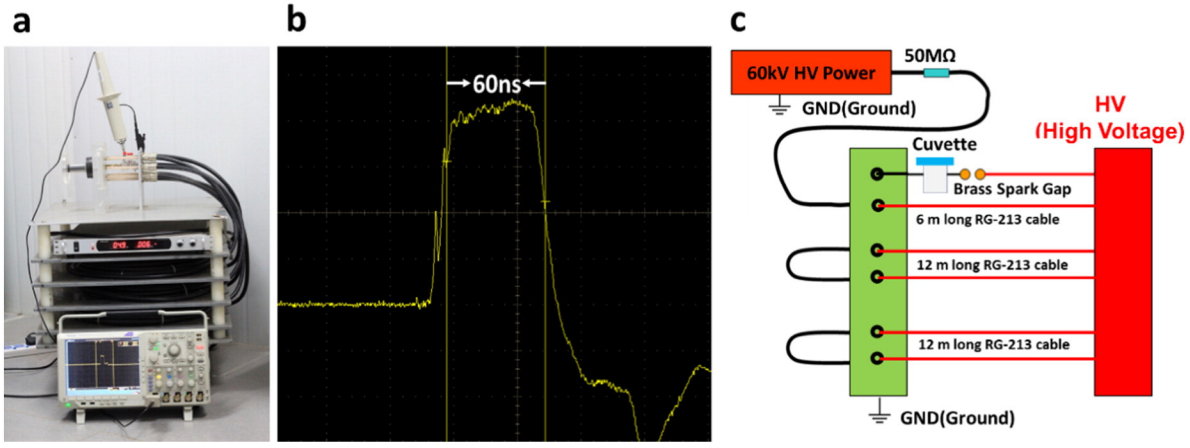


Fig. 1. a.) The photo of 60 ns nsPEFs generator used in this experiment. b.) Typical oscillogram of 60 ns pulse generator. c.) Circuit diagram of the basic Blumlein pulse forming system. Two cable length of 12 m and one cable length of 6 m were chosen in order to generate 60 ns pulses.

after nsPEFs exposure [13]. However, so far, there is no study focusing on the effect of nsPEFs for radiosensitization in oral tongue squamous cell carcinoma.

The present study aimed to investigate the proliferation, apoptosis and cell cycle effects of nsPEFs combined with radiation in a human tongue squamous cell carcinoma Tac8113 cell line, providing a new theoretical basis of nsPEFs for radiosensitization in oral tongue squamous cell carcinoma.

2. Materials and methods

2.1. Cell line and cell culture

Human squamous cell carcinoma Tca8113 cells were cultured in RPMI-1640 medium (GIBCO BRL, Rockville, MD) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT). No antibiotics were used in the preparation of the medium. Cells were grown under 5% CO_2 in a humidified atmosphere at 37 °C. Cells in the logarithmic growth phase were used to perform the experiments described follows.

2.2. nsPEFs treatment

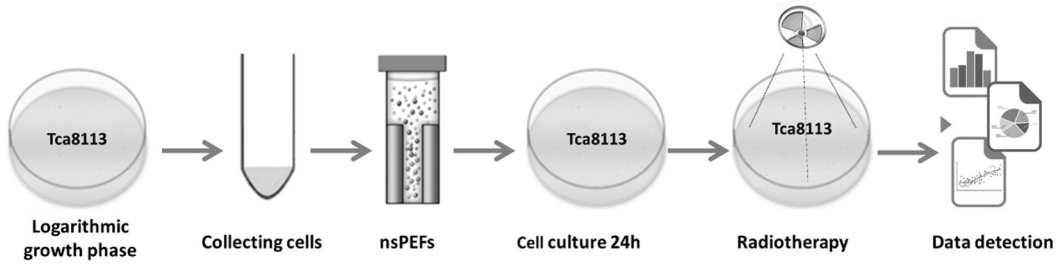
In this study, the nsPEF generator was applied as previously described [21,22]. A Blumlein line pulse generator (Fig. 1A.) produced nearly rectangular 60 ns pulses that were delivered to the Tca8113 cells by a 2 mm cuvette. The electric field strength varied from 10 kV/cm to 50 kV/cm. A digital phosphor oscilloscope (DPO4054, Tektronix) with a probe (P6015A, Tektronix) was utilized to monitor the voltage waveform (Fig. 1B.). Tca8113 cells were counted with a hemocytometer, and 2.0×10^6 cells suspended in 500 μL culture medium were added to 2 mm gap cuvettes (Biosmith, San Diego, CA). Then the experimental cuvettes were exposed to 20 pulses of nsPEFs of 60 ns duration at an electric field strength of 10, 20, 30 and 50 kV/cm, respectively. Cuvettes that did not undergo nsPEF treatment served as the control group. The time between each pulse was about 1 s.

2.3. Radiation platform

To simulate clinical conditions, Tca8113 cells were irradiated with X-rays at 6 MV at room temperature using a linear accelerator (Elekta Precise, Stockholm, Sweden) under the source-to-skin distance (the distance from the radiation source to the central surface of the plate – 100 cm), and the dose rate was 2.0 Gy/min.

2.4. Cell proliferation assay

The effects of nsPEFs on Tca8113 cells were determined with a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The MTT assay uses a MTT dye which acts as a substrate for viable cell reductase enzyme. This enzyme reduces MTT yellow dye to purple colour formazan which is in proportional to viable cell eventually [23]. When cells began to grow exponentially, they were harvested and resuspended at a concentration of 2.0×10^6 cells/ml. A 500 μl cell suspension (1.0×10^6 cells) was placed in a 2 mm gap cuvette (Biosmith) and then treated with nsPEFs at electric fields of 0, 10, 20, 30 and 50 kV/cm. After incubation for 24, 48, and 72 h, 20 μL MTT (5 mg/ml) was added to each well, and cells were further incubated at 37 °C for 4 h. The medium was then removed and 200 μL of DMSO was added to dissolve the reduced formazan product. MTT dye intensity was then read on a micro plate reader (BioRad) at 492 nm. The survival rates under different field strengths were calculated according to the following formula [24]: Survival rate = A value of tested well/A value of control well × 100%. Using this same method, we investigated the *anti-*



Scheme 1. A schematic diagram of the experimental arrangement, including logarithmic growth phase cells preparation, cells collecting, nsPEFs treatment, cell culture, radiotherapy of Tca8113 cells and data detection.