The Effectiveness of Electroporation-based Nanocurcumin and Curcumin Treatments on Human Breast Cancer Cells

Wan-Ying Lin[1], Christy Cooper[2,3], Ignacio Camarillo[1], Lisa M. Reece[2,3], Leon Clah[1], Arutselvan Natarajan[4], Luca G. Campana[5], and Raji Sundararajan[6]

[1]Dept. of Biological Sciences, College of Science, Purdue University
[2]Dept. of Basic Medical Sciences, College of Vet. Medicine, Purdue University
[3]Birck Nanotechnology Center, Discovery Park, Purdue University
[4]Dept. of Radiology, School of Medicine, Stanford University, CA
[5]Sarcoma and Melanoma Unit, Veneto Region Oncology Research Institute, University of Padova, Padua, Italy
[6]Electrical and Computer Engineering Technology Dept., College of Technology, Purdue University, West Lafayette, IN

Abstract—**The objective of this research is to propose a safe, effective and affordable cancer treatment using electrical pulses and the natural turmeric herb extract, curcumin. Curcumin has anti-inflammatory, anti-oxidant, and anti-tumor properties, but its use is limited due to its short bioavailability. To enhance its efficacy, we have encapsulated curcumin within nanoparticles to extend its bioavailability and tested it on MCF-7, human breast carcinoma cells. We administered eight, 1200V/cm, 100µs electrical pulses to deliver curcumin and nanocurcumin into cells and study their anti-tumor response over a period of 72 hours. The results suggest that the synergy of nanoencapsulated curcumin combined with electrical pulses may provide an important novel alternative for increasing cancer treatment efficacy. Further, electroporation-based, nanocurcumin delivery method for breast cancer may be especially effective in the treatment of inoperable and chemo-resistant patients.**

I. Introduction

Worldwide, breast cancer (BC) is the most common cancer diagnosed in women, with approximately 1.3 million new cases per year [1].With an estimated 232,240 new cases of invasive breast cancer (Stages I-IV), and an estimated 64,640 new cases of ductal carcinoma *in situ*, Stage 0, occurring, one woman in every 13 minutes will die from breast cancer in the US [1, 2]. Conventional breast cancer treatments like chemotherapy, radiation therapy, and surgery have drawbacks and may not be applicable to all patients [2, 3]. There have been many drugs developed to treat cancer overall but they have exhibited

limited success, due to inefficient delivery methods that allow molecules to cross the hydrophilic/hydrophobic lipid bilayers of cellular plasma membranes [4]. To overcome this limitation, electrochemotherapy (ECT) has been a process utilized over the years.

ECT is the local application of electrical pulses directly to tumor tissue to render the cells permeable to impermeant drugs such as bleomycin [2, 4–6]. Once the bleomycin has been introduced into the cell, there occurs a most potent cytotoxic effect. However, clinical experience to date, while encouraging, is still not optimal for drug treatment using ECT with bleomycin [5]. Aside from effective and targeted delivery of cancer treatment, patients face other problematic issues. Typical side effects are systemic in the case of drug therapies, while the side effects reported for radiation therapy are local or locoregional [7]. It is therefore necessary to find a gentler drug with higher efficacy.

One bioactive constituent, turmeric, has a history of over 6,000 years of medicinal utilization [8]. Curcumin, the yellow pigment of the turmeric, is derived from the rhizomes of *Curcuma longa*, a herbaceous perennial plant of the ginger family [9-11]. Turmeric is a naturally occurring polyphenolic phytochemical that exhibits antiseptic, anti-inflammatory, antioxidant, chemopreventive, and chemotherapeutic properties [10]. There has been evidence to show its effective cytotoxic, antiproliferative, and apoptotic activity *in vitro*, as well as tumorigenesis suppression within rat models [8], making curcumin attractive as a chemotherapeutic agent [12]. The drawback is that its bioavailability in animals and humans remains low which relates to its inadequate absorption into tissues and its rapid metabolism. To illustrate, curcumin bioavailability from standardized curcumin extract was poor on colorectal cancer patients in some clinical studies [11, 12]. To increase curcumin's bioavailability, it is possible to use electrical pulses to locoregionally uptake this compound [10] as recently shown in our hands using the human breast cancer cell line, MCF-7. We have utilized curcumin-loaded nanoparticles (nanocurcumin) to assess the apoptotic activity within the MCF-7 cells and show that the nanocurcumin increases programmed cell death with this particular cancer cell type.

II. MATERIALS & METHODS

A. MCF-7 Cancer Cell Line

Estrogen receptor positive MCF-7 (human, Caucasian, breast adenocarcinoma) cells were used. Cells were cultured in 90% MEM media + 10% fetal bovine serum (FBS, ATTC, Manassas, VA) and 1% Penicillin/Streptomycin (Invitrogen, Carlsbad, CA). Cells were grown in an incubator at 37°C at 95% humidity and 5% CO_2.

B. Electroporation (EP) of MCF-7 cells

Cells were dissociated from flask by treatment with 0.25% trypsin/EDTA solution (Invitrogen, Carlsbad, CA). Cells were counted using a Cellometer (Nexcelom Bioscience, Lawrence, MA) and resuspended in MEM media to a concentration of 250,000 cells per 0.4cm cuvette tube. A BTX 830 square wave electroporator (Genetronics, San Diego, CA) along with 0.4cm cuvettes were used for electroporation. The parameters for electroporation were 480V, 100µs, 8 pulses, at one-second interval. Cells were pulsed in media without 10% FBS or in RPMI media. Cells were removed after pulsing from the cuvettes

and seeded in 6 well plates. Cells were then incubated at 37°C in a 5% CO_2 atmosphere at 95% humidity over a period of 72 hours.

C. Experimental Design

Fig. 1 shows the experimental design where-in the cells were initially treated with or without electroporation, with and without serum, and the curcumin and the nanocurcumin. Apoptosis was checked after 72 hours to check the cell viability and growth and the type of cell death.

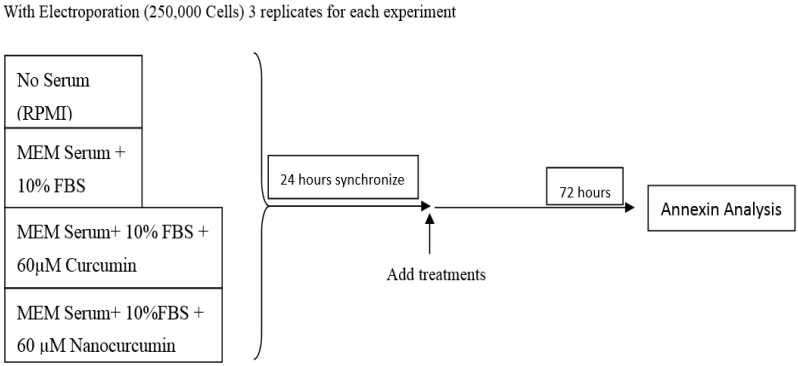

Fig. 1. Experimental Design: Procedure from treating cells to collecting data.

D. Nanocurcumin Synthesis

Nanocurcumin generation began by utilizing 250 g water-soluble glycol chitosan (cat. #G7753-500MG, Sigma-Aldrich, St. Louis, MO) conjugated to 75 mg 5-Cholanic acid (cat. #C7628-1G, Sigma-Aldrich) to form self-assembled hydrophobically-modified glycol chitosan (HGC) nanoparticles in an aqueous solution according to previously published data [13-15]. Next, mono-dispersed super paramagnetic iron oxide nanoparticles (SPION) were made by mixing 0.178 g of iron (III) oxide (FeO(OH), cat. #371254-50G, Sigma-Aldrich), 2.26 g oleic acid (cat. #364525-25ML, Sigma-Aldrich), and 5 g of 1-octadecene (cat. #O806-25ML, Sigma-Aldrich) followed by heating the mixture to 320°C with constant stirring under a nitrogen atmosphere. The resultant SPION were further purified, processed, and ultimately physically loaded into 12 mg HGC nanoparticles by using a probe-type sonicator at 4°C [14]. The HGC nanoparticles were further processed with curcumin (cat. C7727-500MG, Sigma-Aldrich) such that the curcumin was solubilized in ethanol followed by attachment to the HGC nanoparticles via adsorption onto the iron oxide surfaces of the nanoparticles [15].

E. Effect of Nanocurcumin on MCF-7 Cells

The effect of Nanocurcumin at a dose on 60µM, without electroporation, on MCF-7 cell growth and viability was determined. Control cells were treated with MEM media and 10% FBS. Cells were seeded in 6-well plates in 3 replicates at 37^0 C for 72 hours.

F. Effect of Electroporation coupled with Nanocurcumin on MCF-7 Cells

The effect of nanocurcumin, with electroporation on MCF-7 cell growth and viability was determined.

G. Electroporation (EP) of MCF-7 cells

Following electroporation and incubation for 72 hours, cell count for live and dead cells (viability), and total numbers of cells (growth) were done for each electroporation treatment. Three replicates for each treatment were counted using the MUSE flow cytometer. Annexin V and Dead Cell Assay Kit (EMD Millipore, Billerica, MA) was utilized. 100µL of Annexin V and Dead Cell Reagent was added to a 1.5mL conical tube, along with 100µL of cells in suspension was added to each tube, and incubated for a period of 20 minutes at room temperature before counting. The average was taken from three replicates in each experiment for each treatment.

H. Statistical Analysis

Results are expressed as mean $\pm$ SEM. Analyses were performed using one way and repeated measures of ANOVA.

III. Results and Discussion

A. Effect of Electroporation (EP) alone on MCF-7 Cell Growth and Viability

Fig. 2 shows the growth and viability measured at various conditions, after 72 hours, treated with and without EP and serum. It is seen that, without EP, there is enhanced uptake of the nutrients that is from the environment into the cell but not killing it (the first two conditions). The third and fourth conditions indicate that EP helps cells to take in the nutrients from the serum and shows enhanced growth. It indicates that the EP conditions used did not irreversibly damage the cells. Interestingly enough, EP seemed to enhance cell numbers in the presence of serum- probably by increasing the uptake of nutrients in the serum.

B. Effect of Nanocurcumin on MCF-7 Cell Growth and viability (without electroporation)

Fig. 3 shows the growth and viability at 24, 48 and 72 hours for the conditions with and without serum and with curcumin alone and nanocurcumin alone. There was no electroporation performed on any of these samples. The results indicate that it takes 24 hours after addition and electroporation of 60µM nanocurcumin for it to have an effect on the cells. At 24 hours, there were less cell count in the curcumin treatment which was expected as curcumin induces immediate apoptotic event after addition of 60µM curcumin and electroporation. At 48 and 72 hours, there was an increase in cell count for MCF-7 cells with curcumin, whereas the MCF-7 cells with nanocurcumin stayed relatively the same number of cell count throughout the 72 hour period. Overall, it indicates that nanocurcumin is much more effective in suppressing tumor cell growth compared to standard curcumin. The effect of cell growth after 72 hours were lower when compared to that of curcumin and without 10% FBS serum treatment.

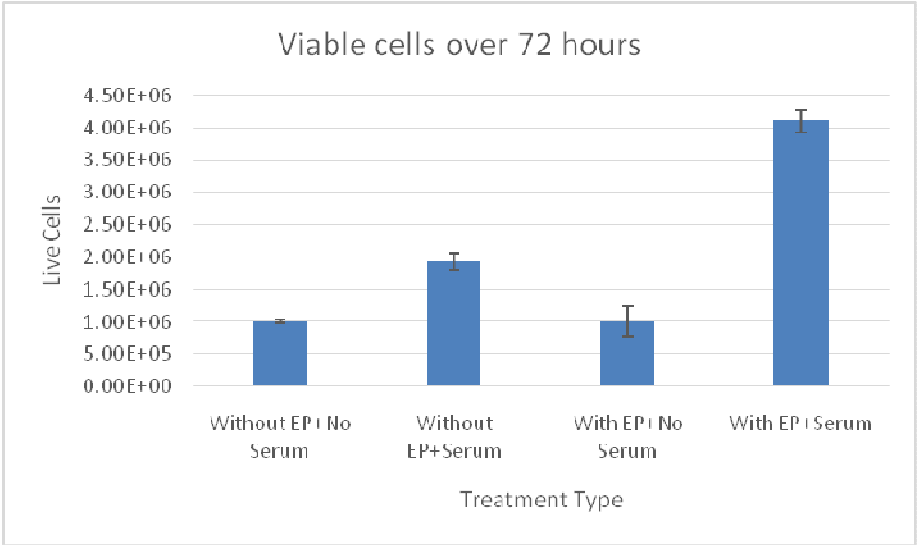

Fig. 2. MCF-7 cells growth and viability were measured at various conditions after 72h treated with and without EP and serum. Data represents mean cells count ± SEM(n=3).

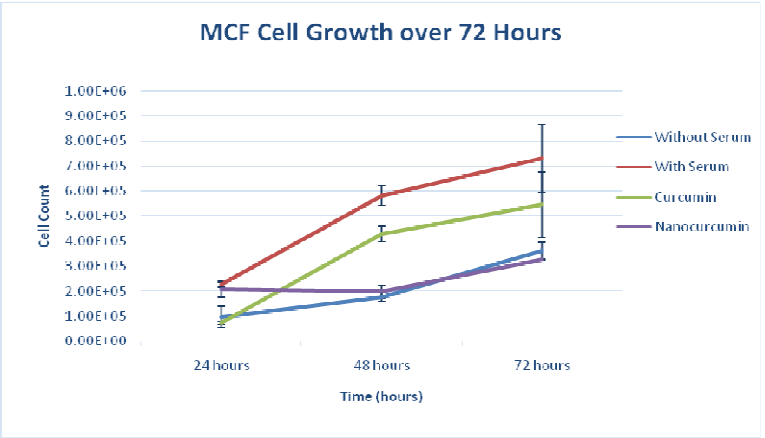

Fig. 3. MCF-7 cell growth and viability were measured at 24, 48, and 72 hours for the different treatments. Data represents mean cells count ± SEM(n=3).

C. *Effect of Electroporation on MCF-7 Cell Growth and Viability*

Fig. 4 shows the effect of curcumin and nanocurcumin with electroporation using 8 pulses of 1200V/cm, 100µs pulses at one second interval. In this case, Annexin V analysis for each treatment group was compared with the positive control, 10% FBS serum. There is cell apoptosis in response to nanocurcumin and electroporation, and there were higher late apoptosis than dead cells or live cells, indicating that nanocurcumin is more effective at inducing apoptosis of tumor cell. The effect of cell growth after 72 hours were more effective when coupled with electroporation at 1200V/cm. Similar results (Fig. 5) were also obtained by Gupta *et al* [16], using biologically-derived (skin fibroin-derived (SF))

nanoparticles on MCF-7 cells, but without electroporation. They found the entrapment and release of curcumin over eight days was highest for SF-derived nanoparticles.

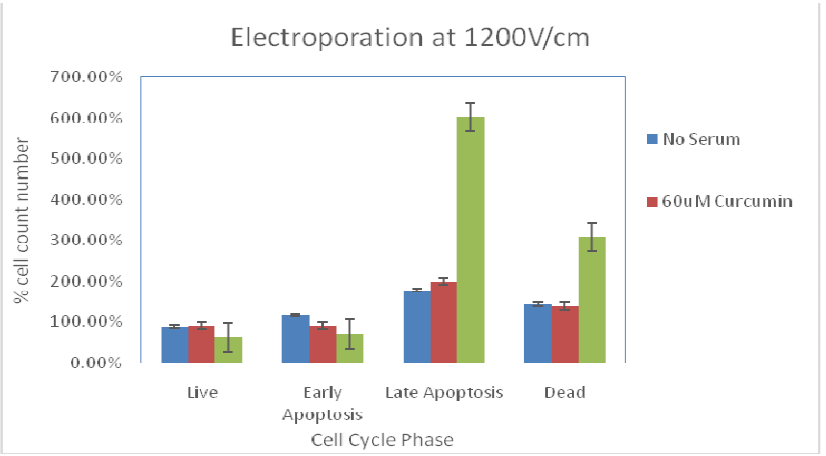

Fig. 4. Annexin V to access MCF 7 cell growth and viability. Electroporation on MCF analysis. MCF 7. Data represents mean cells count ± SEM(n=3).

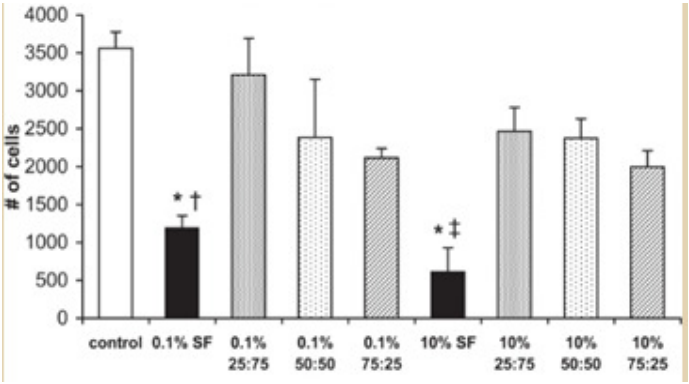

Fig.5. Cell viability by MTT assay after exposure to curcumin nanoparticles for four days (n=3).

IV. Conclusion

Treatment of MCF-7, the human breast carcinoma cells with serum, electroporation (at 1200V/cm, 100µs, 8 pulses at one second interval), curcumin and nanocurcumin indicate that:

a) EP condition used did not irreversibly damage the cells. Interestingly enough, EP seemed to enhance cell numbers in the presence of serum- probably by increasing the uptake of nutrients in the serum.
b) Nanocurcumin is much more effective in suppressing tumor cell growth compared to standard curcumin.
c) Nanocurcumin is more effective in inducing apoptosis, compared to standard curcumin.

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