

# Enhanced Nanocurcumin uptake using Electrical Pulses in Regulating MCF-7 Tumor Cell Growth and Apoptosis



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## Abstract

The objective of this research is to propose a safe, effective and affordable cancer treatment using low level electrical pulses and the natural turmeric herb extract, curcumin. Curcumin has anti-inflammatory, anti-oxidant, and antitumor properties, in addition to many other. However, it has a short bioavailability. To enhance efficacy, we have encapsulated curcumin within nanoparticles to extend its bioavailability. For this purpose, estrogen receptor positive, MCF-7 cells were studied, using eight 1200V/cm, 100 $\mu$ s electrical pulses to deliver curcumin and nanocurcumin into cells and study their anti-tumor response over a period of 72hours. The results suggest that the synergy of nano encapsulated 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.

## 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 in the USA, one woman dies every 13minutes from breast cancer [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 gaining momentum. 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 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]. Although, ECT with bleomycin is a promising therapy, it is of practical interest to find a gentler drug with high efficacy. One bioactive constituent is curcumin. Curcumin is the yellow pigment of the spice, turmeric, which has a history of over 6,000 years of medicinal utilization [7] and is derived from the rhizomes of *Curcuma longa*, a herbaceous perennial plant of the ginger family [8-10]. Turmeric is a naturally occurring polyphenolic phytochemical that exhibits antiseptic, anti-inflammatory, antioxidant, chemopreventive, and chemotherapeutic properties [9].

There has been evidence to show its effective cytotoxic, antiproliferative, and apoptotic activity *in-vitro*, as well as tumorigenesis suppression within rat models, making curcumin attractive as a chemotherapeutic agent [11]. 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. To increase curcumin's bioavailability, it is possible to encapsulate using nanoparticles and using electrical pulses to locally uptake this compound as recently shown in our lab, using the human breast cancer cell line, MCF-7. In this study, we have utilized curcumin-loaded nanoparticles (nanocurcumin) to assess the apoptotic activity within the MCF-7 cells and show that the curcumin increases programmed cell death with this cancer cell type.

## Materials & Methods

### MCF-7 cancer cell line

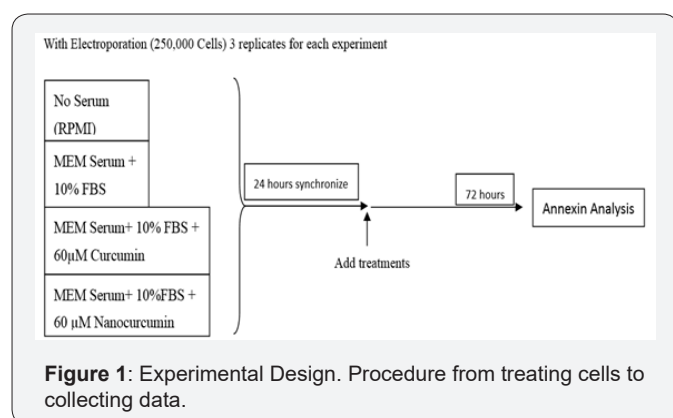
Estrogen receptor positive MCF-7 (human, Caucasian, breast adenocarcinoma) cells were used. Cells were cultured in 90% MEM media +10% FBS (ATTC, Manassas, VA) and 1% Penicillin/Streptomycin (Invitrogen, Carlsbad, CA). Cells were grown in an incubator at 37 °C at 95% humidity and 5% CO<sub>2</sub>.

### Electroporation 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 and resuspended in MEM media to a concentration of 250,000 cells per 0.4cm cuvette tubes. A BTX 830 square wave electroporator (Genetronics, San Diego, CA) along with 0.4cm cuvettes were used for electroporation. The parameters for electroporation were 1200V/cm, 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<sub>2</sub> atmosphere at 95% humidity over a period of 72hours.

### Experimental design

Figure 1



### Nanocurcumin synthesis

Nanocurcumin generation began by utilizing 250g water-soluble glycol chitosan (cat. #G7753-500MG, Sigma-Aldrich, St.

Louis, MO) conjugated to 75mg 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 [12-14]. Next, monodispersed super paramagnetic iron oxide nanoparticles (SPION) were made by mixing 0.178 g of iron (III) oxide (FeO(OH), cat. #371254-50G, Sigma-Aldrich), 2.26g oleic acid (cat. #364525-25ML, Sigma-Aldrich), and 5g of 1-octadecene (cat. #0806-25ML, 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 12mg HGC nanoparticles by using a probe-type sonicator at 4 °C. 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.

### Effect of nanocurcumin on MCF-7 cells

The effect of nanocurcumin, without electroporation, on MCF-7 cell growth and viability was determined. The effect of cell growth after 72hours were lower when compared to that of curcumin and without 10% FBS serum treatment. Control cells were treated with MEM media and 10% FBS. Cells were seeded in 6 well plates in 3 replicates at 37 °C for 72hours.

### 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. The effect of cell growth after 72hours were more effective when coupled with electroporation at 1200V/cm. There were higher late apoptosis than dead cells or live cells.

### MCF-7 cell viability and growth assay and MUSE flow cytometer

Following electroporation and incubation for 72hours, 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, 100µL of cells in suspension was added to each tube, and incubation of 20minutes at room temperature before counting. The average was taken from three replicates in each experiment for each treatment.

### Statistical analysis

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

Results and Analysis

Effect of electroporation on MCF-7 cell growth and viability

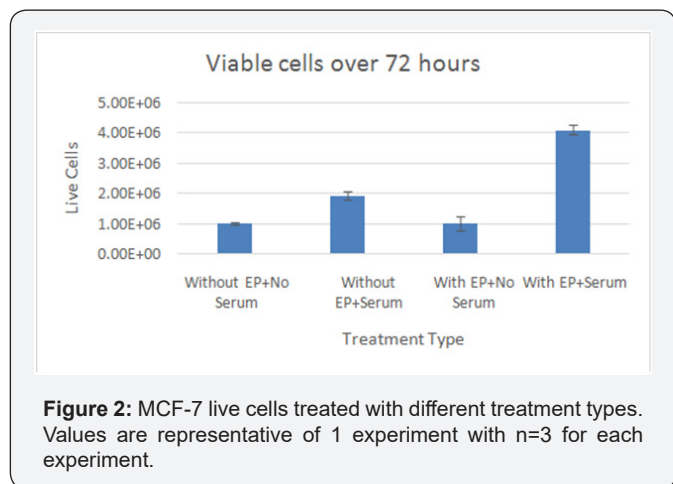


Figure 2: MCF-7 live cells treated with different treatment types. Values are representative of 1 experiment with n=3 for each experiment.

Figure 2 shows the viable cell counts over 72hours for various samples. In control samples, there is some growth observed of MCF-7 cells in the presence of serum, while there is no exposure to EP. Interestingly, the EP treatment did not result in a reduction in cell numbers when there was no serum present. This indicates the EP did not cause irreversible damage and cell death. When cells were treated with EP and serum, there was a significant 2 fold increase in cell numbers. This increase suggests that EP lead to transient openings in cell membranes that allowed serum components access to the inner cell and cytoplasm, thus promoting cell growth.

Effect of nanocurcumin on MCF-7 cell growth and viability (Without Electroporation)

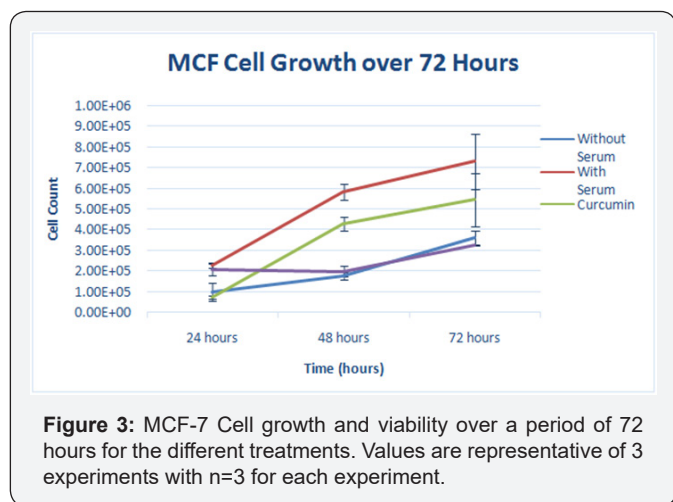


Figure 3: MCF-7 Cell growth and viability over a period of 72 hours for the different treatments. Values are representative of 3 experiments with n=3 for each experiment.

Figure 3 shows MCF-7 Cell growth and viability over a period of 72hours for the different treatments, using nanocurcumin and no electroporation. A sustained growth and doubling of cell numbers occurred in MCF-7 control cells treated with serum. It takes 48 hours after addition of the 60µm nanocurcumin to effect the cells. At 24hours, there were less cell count in the curcumin

treatment which was expected as curcumin induces immediate apoptotic event after addition of 60µm curcumin. At 48 and 72hours, 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 72hour period.

Effect of electroporation on MCF-7 cell growth and viability

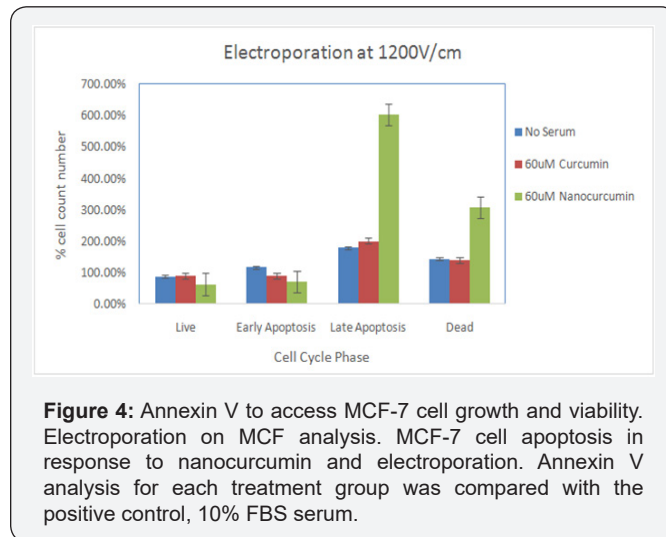


Figure 4: Annexin V to access MCF-7 cell growth and viability. Electroporation on MCF analysis. MCF-7 cell apoptosis in response to nanocurcumin and electroporation. Annexin V analysis for each treatment group was compared with the positive control, 10% FBS serum.

Figure 4 shows the Annexin assay results on cell growth. The cells were electroporated using eight, 1200V/cm, 1000µs pulses at one second interval. At the 72 hour time point, curcumin did not have a induce apoptosis in MCF-7 cells. However, in the present of EP the treatment of cells with nanocurcumin resulted in a significantly higher percentage of dead MCF-7 cells, and cells that were in late apoptosis. This could be the result of enhanced entry of nanocurcumin into the cells and due to the increased half life of curcumin that has been encapsulated.

Discussion & Conclusion

Use of nanoparticles for cancer therapy has been gaining momentum [15-18]. Rodzinski et al. [16] reported about use to magnetoelectric nanopartilces for targeted and controlled anticancer drug delivery. They studied using both *in-vitro* and *in-vivo* models and found that the nanoparticles were localized in the tumor sites. Sun et al. [17] discuss in their work on engineered nanoparticles for drug delivery in cancer therapy. In this review, they discuss the critical need for nanoparticle carriers for the delivery of various types of cancer therapeutic agents, such as hydrophilic, hydrophobic, and highly charged, and their design, synthesis and fabrication. They also indicate some general challenges in targeted delivery of nanoparticle carriers, such as endocytosis, intracellular transport, biodistribution, biocompatibility, and bioavailability, under both *in-vitro* and *in-vivo* conditions. Huang et al. [18] used gold nanaopartciles using electroporation for enhanced polyplex delivery of DNA and siRNA, into mammalian cells. Both anchor cells (NIH 3T3) and suspension cells (K562) were studied

and found 1.5 to 2x improved transfection efficiency with no significant increase of toxicity compared to delivery of plasmid only using electroporation alone. They also used BTX ECM 830 electroporator that was used in our study, with 2mm electrode gap cuvettes. The pulse parameters applied were: 625V/cm, 10ms, single unipolar pulse.

Encapsulating curcumin using polymeric nanoparticles, known as nanocurcumin and its efficacy on cancer cells was also studied by Bisht, et al. [19], on pancreatic cells. In this study on eight pancreatic cell lines, they studied the effect of polymeric nanoparticle-encapsulated curcumin. The cell lines were exposed to a 20x range of void polymeric nanoparticles (93-1852µg/mL). The size of the nanoparticles was 50nm. Viability study after 72hours indicated no cytotoxicity to the cell lines. Unlike normal curcumin, nanocurcumin readily dispersed in aqueous media and it demonstrated comparable *in-vitro* therapeutic efficacy compared to normal curcumin. Further, nanocurcumin's mechanisms of action on pancreatic cells are comparable to that of normal curcumin, including induction of cellular apoptosis, blockade of nuclear factor kappa B (NFκB) activation, and down regulation of steady state levels of multiple pro-inflammatory cytokines (IL-6, IL-8, and TNFα).

Our results indicated that use of nanocurcumin, uploaded using electrical pulses has the potential to treat various cancers that are refractory to current standard or cure. Considering that there were 14.1million new cancer cases and 8.2million deaths in 2012 and it is estimated that both the number of new cases and deaths will increase to over 20million and 10million respectively, by 2030, there is a critical need for alternate therapies. Electrical-pulse-mediated chemotherapy using nanocurcumin is a promising candidate for further studies to transfer this technique from lab to the clinic.

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