Electric Stimulus Response of Chitosan Microbeads Embedded with Magnetic Nanoparticles for Controlled Drug Delivery

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***Abstract*—Drug Delivery Systems (DDS) is suitable for drug delivery over direct pharmaceutical administration of drug due to enhanced bioavailability, precision targeting and localization, and defined characteristics of drug release. It is envisioned that controllable DDS could facilitate maintaining drug concentration within prescribed therapeutic limits over desired duration or provide a burst release of drug when needed. Majority of DDS suffer from an uncontrollable diffusion profile, leading to passive drug release over time, or poor control of external or internal stimuli. In this paper, we demonstrate a novel DDS platform based on chitosan microbeads embedded with magnetic nanoparticles (MNP) that is responsive to electric stimulation. The DDS was fabricated by embedding Fe_3O_4 MNP (ϕ = 12 nm) within biocompatible chitosan microbeads cross-linked with glyoxal. Various types of electric stimulations (e.g. bipolar rectangular, sinusoidal) were applied with the sample suspended on interdigitated electrodes on a Surface Acoustic Wave (SAW) resonator chip. The spectrophotometric analysis of the absorbance post-stimuli shows statistically significant drug release compared to control samples. The highest drug elution recoded is 6.6 times to control for 20 V_{pp}, 100 Hz, bipolar rectangular pulse for 30 seconds. These results indicate the possibility of controllable DDS development using electrical stimulus.**

I. INTRODUCTION

Since the inception of Drug Delivery Systems (DDS) by Bangham in 1960s [1], followed by extensive work on delayed release from liposomes by Gregoriadis [2], active drug delivery has seen a surge in different biocompatible biomaterials that exhibited behavior regulated by various stimuli. The stimuli consisted of both physical and chemical approaches; from external light to *in vivo* pH and enzymatic changes [2-8].

Chitosan has been in use as DDS since 1990s because of its several attractive properties [9-11]. We ingrained MNP within the chitosan microbead based DDS to develop a stimuli responsive substrate, and loaded them with alizarin dye to simulate drugs. MNP embedding will also enhance imaging contrast and enable tracking of DDS through MRI [12,13], as well as can aid in confining DDS by applying a fixed magnetic field. Drug release with electric stimulation has previously been tested on hydrogels [14,15].

This work is supported by FedEx Institute of Technology Innovation Grant (2012-537865), University of Memphis.

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II. MATERIALS AND METHODS

A. Materials

Chitosan with 83.46% Degree of Deacetylation was obtained from Chitinor (Norway). Paraffin, Glyoxal, Hexane, Methanol, Acetone, Nitric Acid were sourced from Fisher Scientific (USA) while Alizarin Red S and Glacial Acetic Acid from Acros (USA). The Span 80 was procured from Sigma Aldrich (USA).

The Ferric Oxide nanoparticles (MNP) used in this work were made by aqueous solution method [16] and were obtained from Department of Physics, University of Memphis. A TEM image is shown in Fig. 1. The average diameter was reported as 12nm.

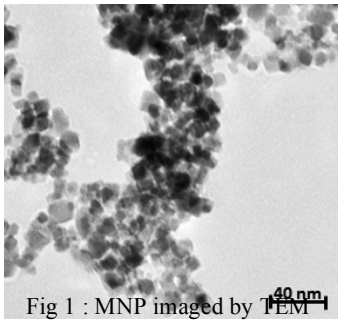


Fig 1 : MNP imaged by TEM at 150000x

The electrical stimulation was applied with a 433.92 MHz Surface Acoustic Wave (SAW) resonation chip (Model: R880, EPCOS AG, Germany). We chose an off-the shelf SAW resonator to show proof-of-concept at a low cost on a scale that is sufficient for measurement.

B. Chitosan Microbead Preparation

Chitosan is obtained by treating chitin, a constituent of crustacean exoskeletons, with an alkali. It is a cationic polysachharide constituted of glycosamine and N-acetylglucosamine copolymers and has been widely used as a biomaterial with varied applications due to its biocompatibility, biodegradability, non-toxicity and drug loadability [9-11].

Chitosan with MNP microspheres were prepared by modified emulsification technique as reported by Jain *et al* [17]. For the experiment, chitosan was used to make 4% wt solution in 5% acetic acid. 1:1 ratio of this solution was dissolved in liquid paraffin containing 0.5 gm of Span 80. Glyoxal in 4.5% v/v with 50% w/w of MNP and Alizarin red S were added to the mixture, and the dispersion was stirred overnight. Alizarin was chosen as an anionic drug substitute for visual confirmation of dye release. The microspheres were separated using a centrifuge and washed with hexane,

followed by methanol and acetone and then dried at 50ºC. Fig 2 shows a scanning electron microscope (SEM) image of the chitosan microbeads. The zeta potential was measured at 37.76 mV, indicating good stability in solution without any clumping or settling.

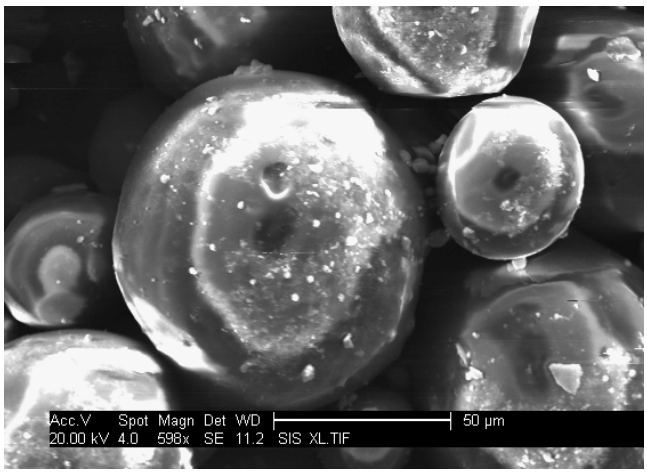


Fig. 2 : Image of a chitosan microbead observed with an SEM at 598x to focus on the apparent smooth surface devoid of any aberrations.

Around 960 beads from the SEM images were analyzed using ImageJ software (NIH, USA) for a good statistical estimate of the size distribution (Fig. 3). The data fits well with a Gaussian distribution curve with a median particle size of 74 µm and standard deviation of 31.4. The size median was at 73µm with the major concentration between 50-80 µm. The beads were noticed to be spherical and possess a smooth surface, without any surface deformities.

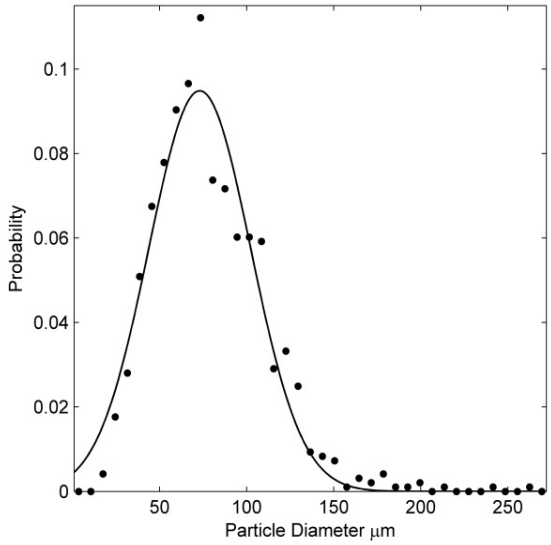


Fig. 3 : Particle size distribution of chitosan microbeads with a fitted Gaussian curve.

C. *Exposing SAW resonator*

An acoustic SAW resonator chip was exposed by swabbing with 4M nitric acid on the top metal enclosure of the package. The dimension of the resonator package was 5 mm × 3.5 mm × 1.45 mm and weighed approximately 0.1g. Its terminals are Nickel-Gold plated and has a center frequency of 433.820MHz. The resonator has a pair of interdigitated

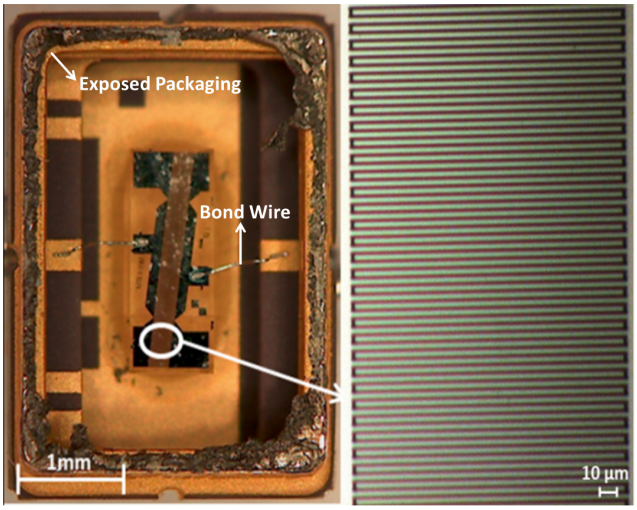


Fig. 4 : **(L)** Exposed SAW resonator with interdigitated electrodes encircled **(R)** Portion of interdigitated electrodes, viewed at 20x in a light microscope.

integrated electrodes within the cavity (Fig. 4). The interdigitated electrode separation is 3.6 µm.

The two electrodes of the chip are connected to two pins of the chip via a pair of bond wires that allows application of electrical stimulation to the electrodes. The exposed chip was affixed on a microscope slide to limit movement while experimentation.

D. *Experimental Procedure*

At the beginning of each experiment, the chip was exposed and dried thoroughly. The DDS sample was mixed with Phosphate Buffered Solution (PBS). A 10 µl of sample was applied to the cavity of the chip using a micropipette. For each case, electrical stimulation was administered for 30s. The treated samples were then carefully collected with a micropipette, and collected in a centrifuge tube. A strong fixed magnetic field of a neodymium magnet (Model: Cylinder N50, Applied Magnets, USA) was applied for 2 mins, causing any MNP residue to settle down at the bottom.

The two pins of the resonator chip that were wired to the interdigitated electrodes were connected to an arbitrary waveform generator (Model: DG4062, Rigol USA) in series of a shunt resistor. The schematic is shown in Fig. 5. An oscilloscope (Model: Tektronix TDS1001B, USA) was used to capture the waveforms across the chip (via Ch. 1) and across the shunt (via Ch. 2). The setup was subjected to two types of electric pulses: bipolar rectangular, and sinusoidal, both with an applied potential of 20 V_{pp} at 500 Hz and 1 kHz. The magnitude of the applied potential was kept 20 V_{pp} for all experiments to enable direct comparison for waveform and frequency. The rectangular pulses had a 10% duty cycle. The supernatant was collected and stored for subsequent analysis. For the control batch, the same setup was followed; the samples were allowed to settle in PBS for 30s in the chip without any electrical pulses, and then collected. The amount of drug eluted post-stimuli was compared with a control using a spectrophotometer.

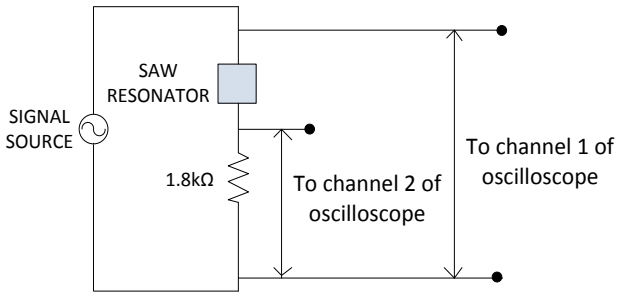


Fig. 5 : Schematic diagram for the experimental setup.

Four types of pulses, as summarized in Table I, were applied on 2 samples; each time with a new resonator chip loaded with a fresh sample. A representative oscilloscope screen capture of the voltage and current waveforms for rectangular and sinusoidal waveform applied to the SAW chip are given in Fig. 6. The distinct spikes at the corner of the pulses is caused by the capacitive nature of interdigitated electrodes which is typical. The current waveforms can be found from channel 2 by dividing the captured voltage data by 1.8 kΩ.

Table I : Pulse type applied for Set I

Test	Pulse Type	No. of repetition
1	Control, no pulse	2
2	Bipolar rectangular, 20 V_{pp}, 1kHz	2
3	Bipolar rectangular, 20V_{pp}, 500Hz	2
4	Sinusoidal, 20V_{pp}, 1kHz	2

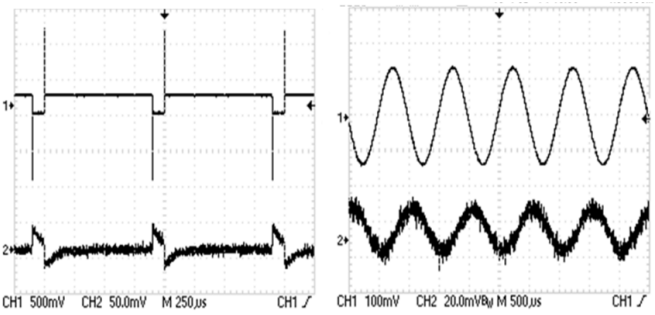


Fig. 6 : **(top)** Applied waveforms of 20V_{pp} bipolar rectangular pulse measured by a 10x probe oscilloscope; **(bottom)** Similar waveforms of 20V_{pp} sinusoidal pulse measured by the oscilloscope.

E. Measurement and Analysis

The treated supernatant after the experiments were collected on a microplate, and the associated absorbance were measured by a spectrophotometer (Model: Synergy H1 microplate reader, BioTek US, Winooski, VT) at 350 nm monochromatic wavelength. A one-tailed t-test was done to assess the significance of difference recorded in average absorbance compared to control. The samples were also analyzed with a Scanning Electron Microscope (SEM) to investigate surface deformities.

III. RESULTS

The results of the absorbance test were analyzed and presented in Fig. 7. It shows statistically significant release of alizarin when bipolar rectangular pulse was used, with the lower frequency showing a higher amount of dye compared to the higher frequency of the same pulse. For the test hypothesis that the stimulated samples release more dye, the power of one-tailed t-test was calculated as 82.3% for 1kHz and 100% for 500Hz, which are both higher than the accepted adequacy norm of 80%. No commendable dye release was observed for sinusoidal pulses.

We also repeated the same control and stimulation protocol on plain chitosan microbeads without magnetic nanoparticles or dye in them, to eliminate any possibilities that the PBS or chitosan in itself may be causing electrochemical effects that could be confused with dye release. A lower frequency of 100Hz was used to exacerbate the changes, if any. The results in Fig. 8 indicate that no statistically significant difference between stimulation and control was observed. Thereby it is confirmed that the absorbance detected was that of dye in the experiments.

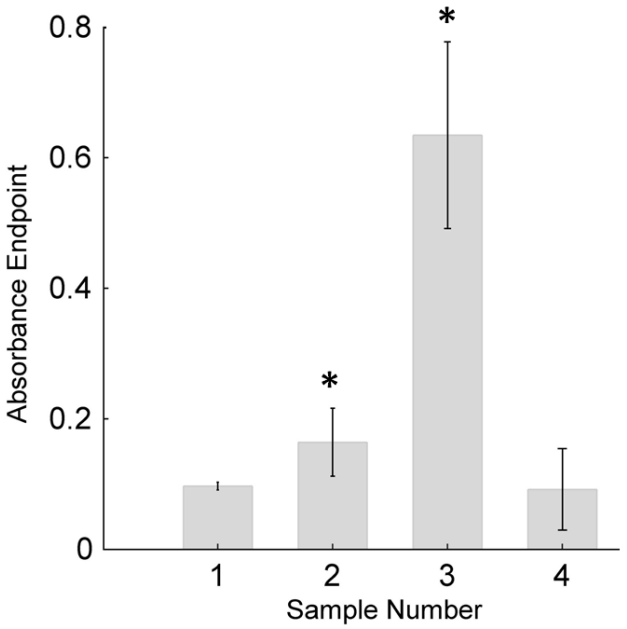
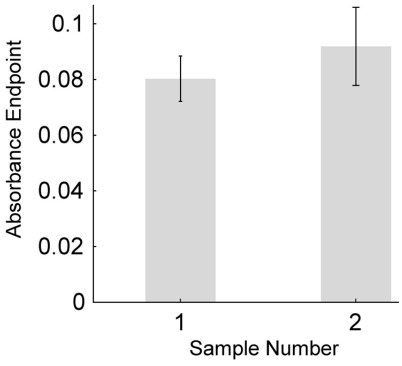


Fig. 7 : Average absorbance endpoint measured at 350nm plotted between control (○), 20V_{pp} 1kHz bipolar rectangular (□), 20V_{pp} 500Hz bipolar rectangular (◊) and 20V_{pp} 1kHz sinusoidal (x). The errorbars represent standard error, star (*) represents statistically significant difference

Fig. 8 : Average absorbance endpoint measured at 350nm plotted between control, 20V_{pp} 100Hz bipolar rectangular for plain chitosan microbead



The SEM images of the samples prior (Fig. 9a) and after (Fig. 9b and 9c) application of stimulation (20V_{pp}, 500Hz, bipolar rectangular electric pulses) shows significant rupturing and shrinking of microbeads subjected to electrical

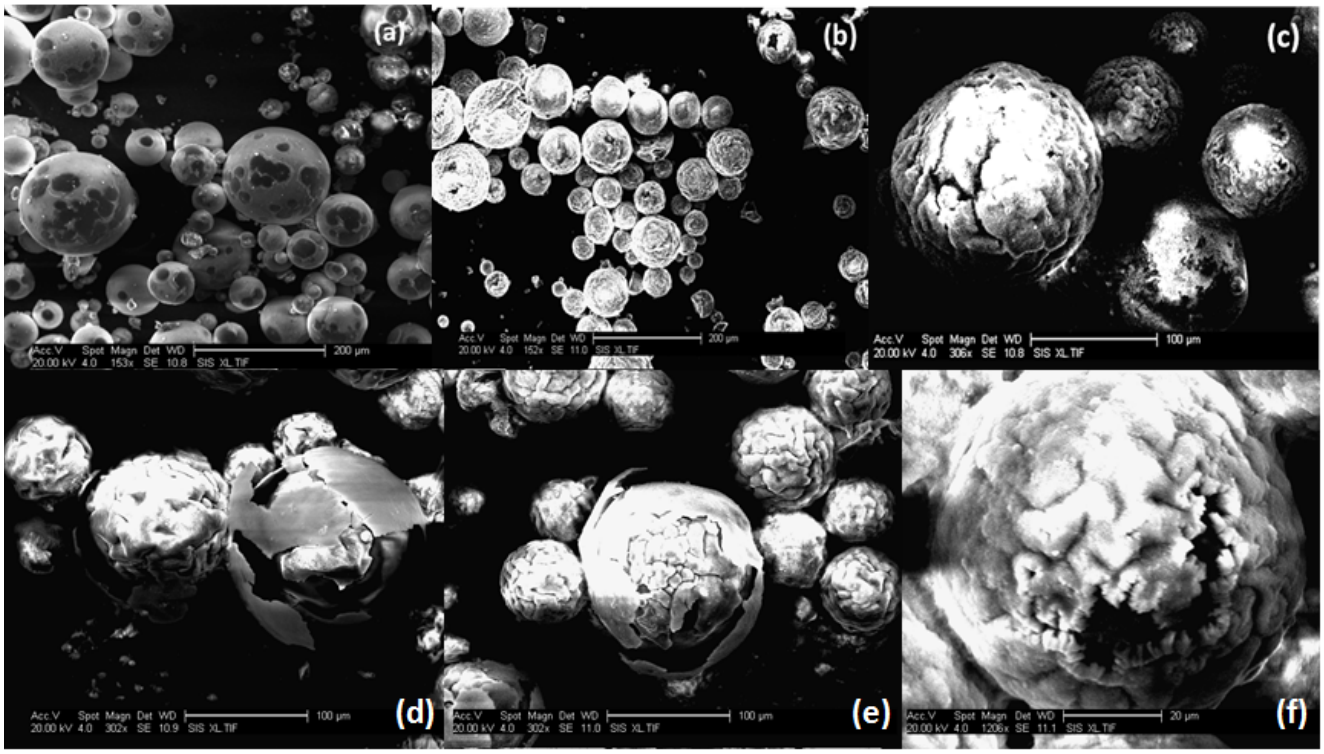


Fig 9 : SEM images of (a) Control Beads at 153x and Stimulated Beads at (b) $20V_{pp}$ 500Hz bipolar rectangular viewed at 152x (c) $20V_{pp}$ 500Hz bipolar rectangular viewed at 306xx (d) 10V DC viewed at 302x (e) 10V DC viewed at 302x (f) 10V DC viewed at 1206x

stimulus to which the observed release of dye can be attributable. Since the severity of the phenomenon seemed to be inversely proportional to frequency, a fresh batch of beads were stimulated at 10V DC for 10 seconds to observe the effect of maximum stimulus on the bead structure (9d, 9e, 9f). The effects are much more distinct and noticeable. The stimulus was limited to 10s to prevent heating and melting of the chip terminals.

V. CONCLUSIONS

A new DDS platform was fabricated that contains embedded MNP within chitosan microbeads. Various types of electrical stimulation pulses were applied to observe and quantify alizarin dye release. From the observed quantification experiments and statistical analysis, it is found that the application of bipolar rectangular electric pulses caused significant release of alizarin. The dye release for sinusoidal stimulus was insignificant. For practical DDS, alizarin will be substituted with therapeutic drugs. The work provides a proof-of-concept that electric stimulation can be used as an effective and controllable means to develop a stimulus responsive active DDS based on chitosan microbead embedded with MNP.

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