Stimuli-Controlled Drug Delivery System Development with Implantable Biocompatible Chitosan Microbeads

Ankita Mohapatra
University of Memphis
mhapatra@memphis.edu

Nazmus Sahadat
University of Memphis
mnshadat@memphis.edu

Bashir I. Morshed
University of Memphis
bmorshed@memphis.edu

Gregory McGraw
University of Memphis
gmcgraw@memphis.edu

Alex P. Hoban
University of Memphis
aphoban@memphis.edu

Jessica A. Jenning
University of Memphis
jennings.amber@gmail.com

Warren O. Haggard
University of Memphis
whaggrd1@memphis.edu

Joel D. Bumgardner
University of Memphis
jbmgrdnr@memphis.edu

Sanjay R. Mishra
University of Memphis
srmishra@memphis.edu

Abstract

Implantable drug delivery systems (DDS) are beneficial in many medical applications including healing and generation of bone loss due to fracture, atrophy/disease excision, and complex and infected trauma sites. A major challenge is controlling the timing and dosage of specific drugs from DDS in patients to maximize therapeutic effects, rather than conventional passive diffusion or a single burst release of drugs. We are developing a new

type of stimuli-controlled DDS using electrically excited iron-oxide magnetic nanoparticles (MNP) to alter the passive elution profile of drugs from chitosan, a biodegradable and biocompatible biopolymer. We developed glyoxal cross-linked chitosan microbeads (50 to 150 micron) containing embedded biocompatible Fe_3O_4 MNPs with average diameters of 12 nm that were made via an aqueous solution technique. For experimentation, the DDS is loaded with alizarin red dye and suspended in physiological phosphate buffer saline (PBS) solution, then subjected to stimuli pulsed electric field (PEF). For PEF, a commercial surface mount SAW resonator chip was used that has interdigitated electrodes at 3.6 µm gap. Bipolar PEF of 10% duty cycle, $20V_{pp}$ and frequencies of 100 and 500 Hz were applied with a signal generator. Increased dye release was observed after PEF excitation and was quantified using a spectrophotometer. Visual comparison of surface structures with SEM imaging of treated and untreated microbeads is also presented to demonstrate the effect of PEF. It was observed that a lower frequency caused a higher dye release than higher frequencies. Stimuli responsive DDS with MNP-embedded chitosan microbeads is a promising technique for *in vivo* implantation.

Introduction

Since the discovery of "Bangosomes" by Bangham in the 1960s [1], followed by liposomes by Gregoriadis [2], several research groups have investigated these lipid vesicles as carriers for drug delivery. However, these DDSs were observed to have poor pharmacokinetic longevity and were rapidly flushed from circulation because of opsonization and uptake by mononuclear phagocytic system [2]. Numerous improvised biomaterials were discovered or developed in an attempt to deliver drug payloads at target sites before an immunological response is triggered. Simultaneously, disparate stimuli were explored to allow external control of therapeutic dose releases from DDS, rather than a single burst or uncontrollable passive diffusion release. The stimuli ranged from physical stimuli like ultrasound or light to biochemical stimuli like pH and enzymatic changes [3-9].

Chitosan and its derivatives have also been examined as a potential DDS because of biocompatibility, biodegradability, and good drug load-ability; besides its other properties, chitosan is suitable for *in vivo* delivery. The biopolymer has a low production cost and is present in abundant amounts as well. It has been fabricated in various forms, sizes, and shapes, and some of them have been shown to be inherently stimulus responsive [10-15].

In our DDS model, MNP were ingrained in chitosan microbeads and loaded with alizarin. MNP is intended to respond to a magnetic field and enhances MRI contrast, thus aiding in DDS localization and tracking [16, 17]. Alizarin was used to model a drug that provides a visual indication of increased release. PEF was applied to observe the response of stimuli on the alizarin release profile from MNP-embedded chitosan microbeads.

Materials

Chitosan

Chitosan is a positively charged linear polysachharide with random glycosamine and N-acetylglucosamine units and is obtained from chitin, after treatment by an alkali, usually sodium hydroxide.

Chitosan microbeads with MNP and alizarin were formulated by a modified emulsification technique described by Jain et al. [18]. An 83.46% deacetylated chitosan was used with 5% acetic acid to make a 4% wt solution. This solution was mixed with an equal amount of paraffin and 0.5gm of Span 80 was added. Glyoxal was chosen as a cross-linker for this batch to aid in forming microbeads. A 4.5% v/v of glyoxal with 50% w/w of MNP and alizarin were combined; then the mixture was stirred overnight. A centrifuge was used to separate the microbeads, which were washed first with hexane and then with methanol and acetone. The beads were finally dried at 50º C. The majority of the beads lay in a size range of 65-50µm. Figure 1 shows the chitosan microbeads. Their average zeta potential was measured to be 37.76mV.

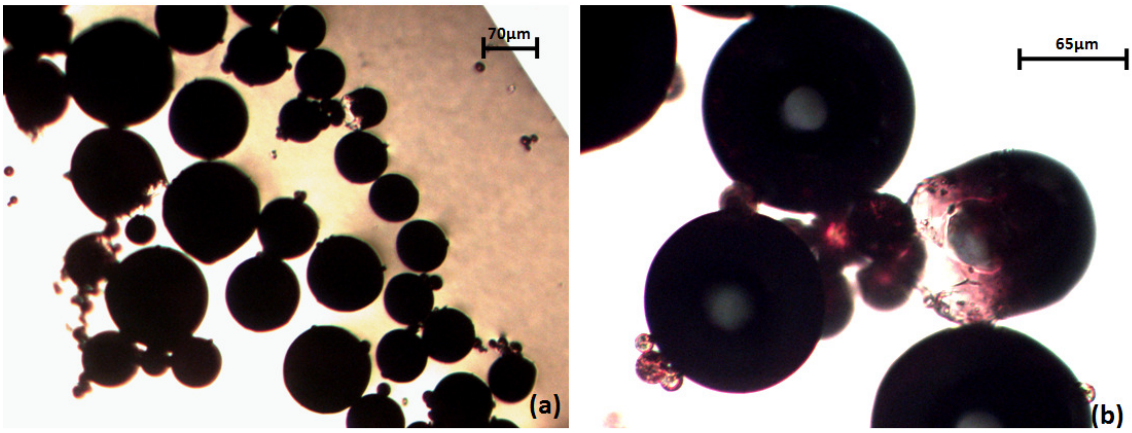

Figure 1. Chitosan microbeads with MNP and alizarin, viewed at 5x (a) and 20x (b) objective magnification under a light microscope in a bright field mode before applying stimulus

SAW Resonator

SAW (surface acoustic wave) resonator chips, sized 5 mm. x 3.5 mm x 1.45 mm (Model: R880, EPCOS AG, Germany), were obtained. The metal casing on one face was repeatedly swabbed with 4.0M nitric acid to expose the interdigitated electrodes encased inside a cavity (Figure 2). The exposed chips were washed thoroughly with deionized water and dried overnight.

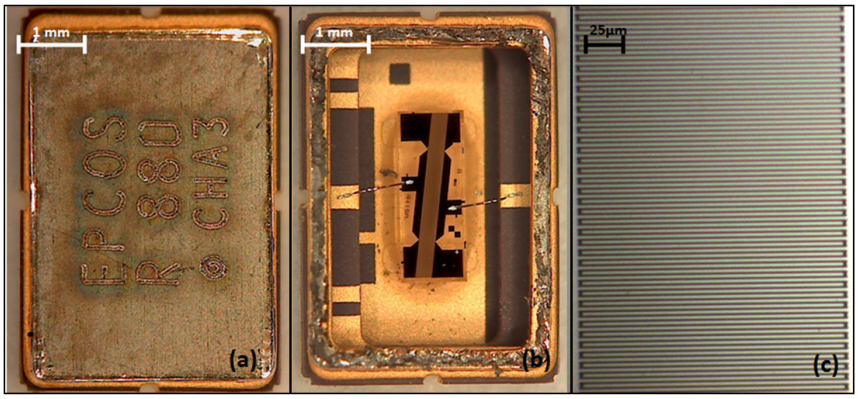

Figure 2. Unexposed SAW resonator chip (a); exposed interdigitated electrodes after acid swabbing (b); interdigitated electrodes viewed at 20x in a light microscope (c)

The chip contained a pair of interdigitated electrodes. The inter-electrode gap of fingers was 3.6µm. Two wires were soldered to the pins of the chip to provide electrical connectivity for PEF.

Magnetic Nanoparticles

The aqueous solution method reported by Kang et al. was followed for making the Fe_3O_4 nanoparticles using $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ in a 1:2 molar ratio [19]. The average diameter of MNP was 12 nm.

Method

A small amount of microbeads was transferred into the chip cavity using a spatula, followed by 20µl of PBS via a micropipette. The soldered wires from the chip were connected to a signal generator (Model: DG4062, Rigol USA, OH) and oscilloscopes for measuring waveforms, as depicted in Figure 3.

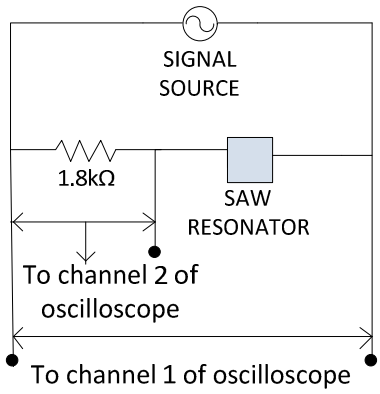

Figure 3. Schematic diagram for experimental setup

Two types of pulses were administered to the samples (Table 1, Figure 4) and experiments were repeated, each using a fresh chip. The duration of each stimulus was 30s.

Table 1. Electric pulse applied to microbeads

Test No.	Pulse type	Repetitions
1	Control	4
2	Bipolar Rectangular, 20 V_{pp}, 100Hz	4
3	Bipolar Rectangular, 20 V_{pp}, 500Hz	4

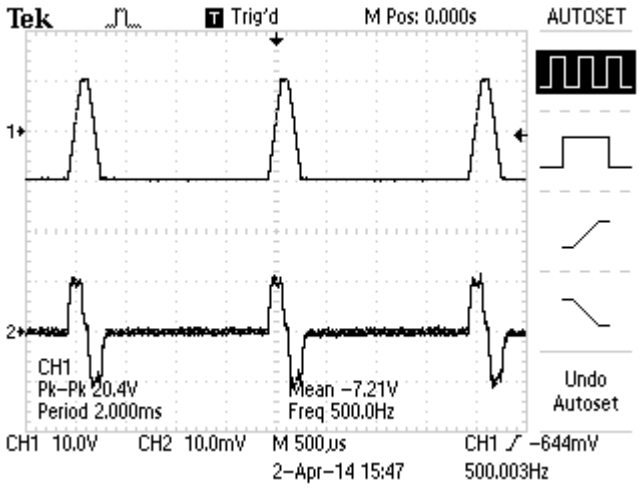

Figure 4. Applied waveforms of bipolar rectangular, 20 V_{pp}, 500 Hz

The post-stimulus PBS was collected with a micropipette and stored in a centrifuge tube. For the control samples, the same setup was followed, but no pulses were given. All tubes were brought in close proximity to a strong permanent neodymium magnet (Model: Cylinder N50, Applied Magnets, TX), ensuring that any suspended debris settled at the bottom of the centrifuge tube.

Results

The supernatant, after the debris had been pelleted, was carefully collected with a micropipette and put on a spectrophotometer (Model: Synergy H1 microplate reader, BioTek US, Winooski, VT) for reading of absorbance. A higher absorbance is expected to be directly proportional to content of alizarin released in the PBS by the microbeads. Each test case yielded two readable points. The sample absorbance was read at two wavelengths of 230 nm and 260 nm to provide affirmative data about dye release. Lower wavelengths were chosen for reading because of spectral properties of the alizarin in the PBS solution (Figure 5). However, the data observed at 230 nm can also be artifacts of the spectrophotometer and not reliable. Hence, the values were compared with a second wavelength.

The absorbance data were analyzed using one-sided unpaired t-test to provide a quantitative comparison of release profile between control and stimulated samples, as summarized in Table 2 and Figure 6.

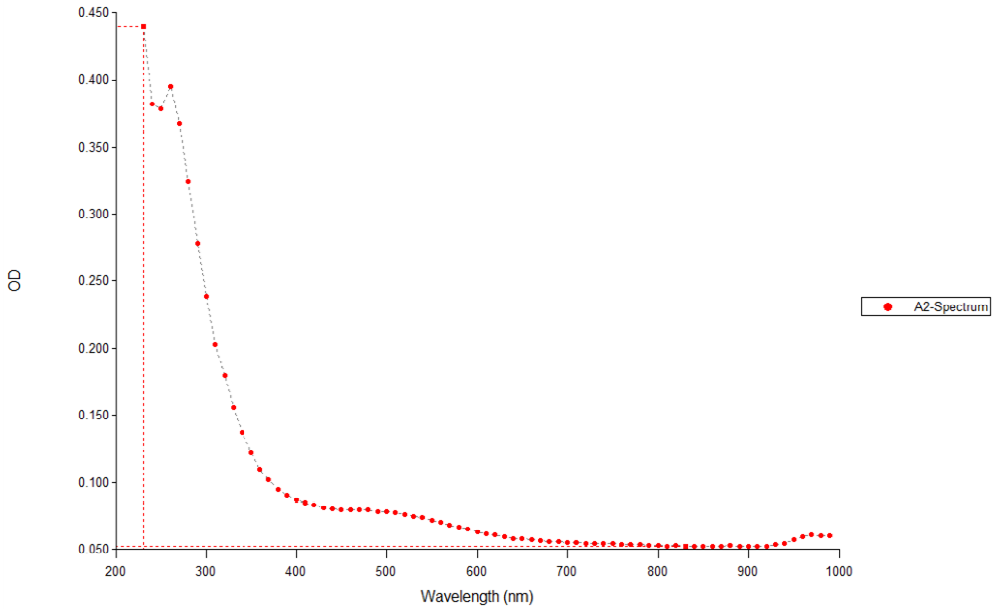

Figure 5. Absorbance of eluted alizarin from one sample, measured absorbance vs wavelength

Table 2. Electric pulse applied to microbeads, absorbance at 260 nm

Test#	P-value (one-tail)	Decision	Total Sample size	Probability Level
2	2.70E-5	Significant	8	0.05
3	1.83E-6	Significant	8	0.05

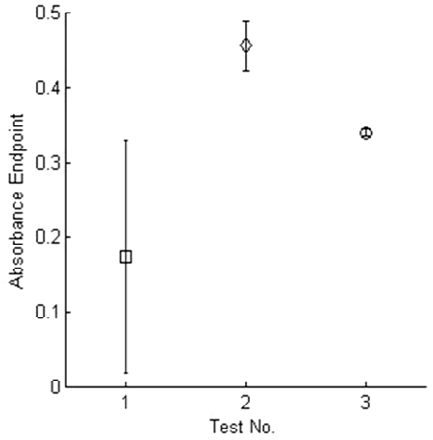

Figure 6. Average absorbance endpoint plotted between control (□), 20 V_{pp} 100Hz bipolar rectangular 10% duty cycle (◊) and 20 V_{pp} 500Hz bipolar rectangular 10% duty cycle (○) measured at 260 nm

From the statistical analysis, it can be concluded that application of stimulus caused significant dye release. Also, the lower frequency of pulses caused a higher dye release than a higher frequency.

The samples were also analyzed with a scanning electron microscope (SEM) before and after stimulus to investigate its effect (Figure 7). It was observed that samples after stimulus showed distinct evidence of rupture and surface wrinkling compared to control samples. The dye release confirmed previously can be attributed to the significant bead contortion that was not noticeable in control samples. For practical curative purposes *in vivo*, the dye can be easily substituted with any pharmaceutical payload, and the dosage administration can be controlled by PEF administered through wireless or magnetic energy transfer.

Figure 7. SEM images of samples (a) before stimulus viewed at 78x (a); and after stimulus viewed at 76x (b) and 151x (c)

This preliminary work provided affirmative proof that controlled drug delivery could be achieved by the new DDS formulated by embedding MNP within chitosan microbeads. To extend of this concept to create a functional unit that can be implanted *in vivo*, we fabricated interdigitated electrodes on a chitosan film and a glass petri dish using EMS 7620 (Electron

Microscopy Sciences, Hatfield, PA) sputter coater machine. The petri dish was attached using a stud to place the sample in the machine chamber. A mask was attached on top of the petri dish sample surface. The chamber was vacuumed by maintaining a constant pressure. An inert gas (argon) was supplied to the chamber. After ambient preparation, electrons bombarded the cathode target (Au/Pd) to coat 10 nm thickness of electrode on the petri dish surface. This fabrication was completed in two steps. First, finger electrodes were coated. The connectors were coated after precise mask placement on the coated fingers, and a 20 nm thick Au/Pd was coated on the chitosan film surface. Figure 8 (a) and (b) shows the interdigitated electrode on the petri dish and chitosan film, and Figure 8(c) shows 101x magnification of the electrodes viewed under a SEM, thus verifying continuity of the fingers. Here the electrode width and the gap between the electrodes are 0.1016 mm (4 mils) and 0.2032 mm (8 mils).

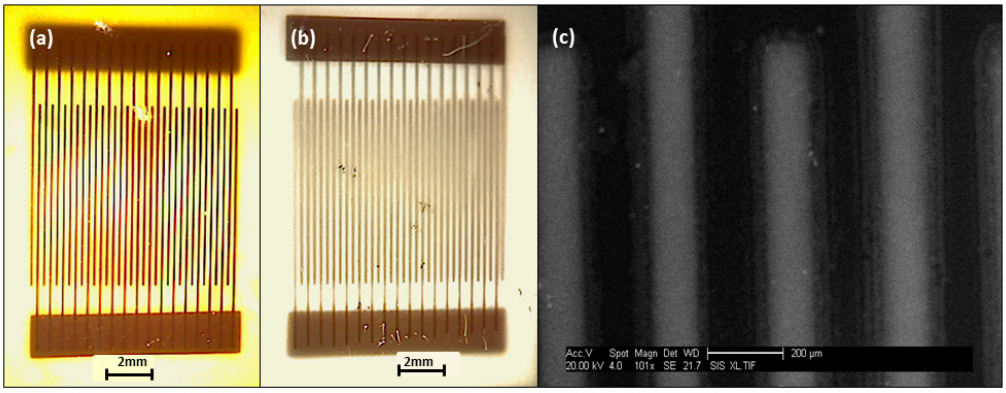

Figure 8. Light microscope images of the sputter coated interdigitated electrodes on (a) chitosan film (b) glass petri dish. (c) SEM images of electrodes at 101x

The flexible electrode-printed film thus fabricated tested with the same setup as Figure 3 *in vitro* with different electric pulses. Significant dye release was detected. We are currently targeting to develop a wireless transmitter-receiver unit that can be appended to this film for implantation and facilitate a safe generation of electric pulses *in vivo*.

Conclusions

A new DDS was formulated by embedding MNP within chitosan microbeads and investigating with different pulse types for controlled dye release. From the statistical analysis, it can be stated that the application of stimulus caused significant dye release. Also, a lower frequency of pulses caused a higher dye release than higher frequency. The SEM images support this observation, suggesting a possible mechanism for dye release as microbeads break or morphologically change. For therapeutic purposes, alizarin can be substituted by practical field drugs and injected *in vivo* in patients, and the dosage administration can be controlled by PEF administered through wireless or magnetic energy transfer.

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Biographies

ANKITA MOHAPATRA has been enrolled in the PhD program, Electrical and Computer Engineering Department, University of Memphis, since January 2013. She completed her undergraduate degree in India in 2011, majoring in Electrical Engineering, and was employed as a research assistant at Siksha 'O' Anusandhan University followed by Indian Institute of Technology, Delhi. Her research interests are mainly in smart drug delivery, novel biomaterials, signal processing, evolutionary computing, optimisation. She can be reached at mhapatra@memphis.edu

NAZMUS SAHADAT completed his undergraduate studies at Bangladesh University of Engineering and Technology in 2009 and master's in Electrical and Computer Engineering from University of Memphis in 2014. His research interests are in smart drug delivery and embedded systems. He can be reached at mnshadat@memphis.edu

BASHIR I. MORSHED is currently employed as an assistant professor in Electrical and Computer Engineering, University of Memphis. He received his PhD in Electronics Engineering from Carleton University in 2010 and was a post-doctoral fellow at Medical Devices Innovation Institute in University of Ottawa from 2010-2011. He is director of Embedded Systems Advanced Research and Prototyping Lab, University of Memphis, and his primary research area is embedded systems with applications focused on biomedical devices. He can be reached at bmorshed@memphis.edu

GREGORY MCGRAW graduated *cum laude* from the University of Memphis (UM) in 2008 with a B.S. in Biology. Following that, he attained a research position in the Immunology Department at the University of Tennessee Health Science Center (UTHSC). He will graduate with a master's in Biomedical Engineering from UTHSC/UM joint biomedical program in summer 2014 and go on to pursue his Ph.D in the same program. He can be emailed at gmcgraw@memphis.edu

ALEX PRENTICE HOBAN completed his BS in Physics from the University of Missouri and is pursuing his master's in Biomedical Engineering. He will be graduating in summer 2014. His research interest is infection. He can be reached at aphoban@memphis.edu

JESSICA A. JENNINGS is currently a research assistant professor in Biomedical Engineering at University of Memphis. She is a member of the Society for Biomaterials, Orthopaedic Research Society, and the Council on Undergraduate Research. She completed

her PhD in Biomedical Engineering from University of Alabama in 2007 and was a post-doctoral research fellow in Biomedical Engineering, University of Memphis from 2007-10. Her research interests include biomaterials, drug delivery, infection prevention, biofilm inhibition strategies, and tissue engineering. She can be reached at jennings.amber@gmail.com

WARREN O. HAGGARD has been employed as professor in University of Memphis since 2004 and currently holds the Herff Chair of Excellence in Biomedical Engineering. Before University of Memphis, he held several positions at Wright Medical Technology. He completed his PhD in Biomedical Engineering from University of Alabama in 1994, and his research interests are bone graft substitutes, functional tissue repair, modified tissue scaffolds, and local delivery of biological agents. He can be reached at whaggrd1@memphis.edu

JOEL D. BUMGARDNER is a professor in the Biomedical Engineering Department at the University of Memphis. He obtained his PhD in Biomedical Engineering from the University of Alabama at Birmingham. He is an elected Fellow (2011) of the American Institute of Medical & Biological Engineering and a JW Fulbright scholar (1994), Umeå University. His research is in biomaterials, including the biopolymer, chitosan, for dental and orthopaedic, tissue engineering, and drug delivery applications. He can be reached at jbmgrdnr@memphis.edu

SANJAY MISHRA is currently a professor at the Department of Physics at University of Memphis, which he joined in 1999. He held a post-doctoral position in Lawrence Berkeley National Laboratory, the University of California-Berkeley at the Advanced Light Source Synchrotron Facility. He completed his PhD in Physics at the University of Missouri-Rolla in 1996. His research interest is in the broad area of magnetic materials and nanocomposites. He can be reached at srmishra@memphis.edu