Chitosan Microbeads with MNP on Printed Electrodes for Electric Stimulus Responsive Drug Delivery

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***Abstract*— Over the last few decades, various drug delivery systems (DDS) have been fabricated to overcome limitations of direct drug administration like solubility, short circulation lifetime, low tissue specificity, etc. Several of these DDS are also capable of controlling the passive drug release profile in response to a stimulus. This work describes a novel stimuli responsive DDS that alters its normal elution profile to release a higher drug amount when electric pulses are provided as excitation. The DDS is in form of microbeads made from chitosan, containing magnetite nanoparticles and vancomycin as the drug of interest. Silver (Ag) Inter-Digitated Electrodes (IDE) on a Polyimide film were printed using a material printer and placed on the DDS. Two separate stimuli, each of 30 s duration were applied to the DDS via the IDE. The stimulated groups showed vancomycin release increased by 75% and 51% after the first and second stimuli respectively, in comparison to samples that were not stimulated. This preliminary work shows promising mechanism of a multiple- stimuli responsive DDS.**

I. INTRODUCTION

Direct systemic administration of drug has several disadvantages like poor target specificity, short elimination half-time, renal flushing, low efficacy, etc. This requires repetitive dosage, leading to patient discomfort and incompliance. Using a drug delivery system (DDS) as a carrier can provide a steric shield against macrophagal elimination thereby increasing drug bioavailability and improve tissue localization to targeted tissues [1]. However, conventional methods of DDS based drug delivery display a drug release profile with an initial peak, followed by a gradual deterioration in therapeutic levels [2]. Several researchers have reported successful attempts at using various kinds of stimuli to modify the drug release. The modification can either be to withhold the drug until stimulated (Fig. 1), or cause a substantial increment in the rate of release when excitation is given.

Drug release by electric stimulus has not been as widely researched on as other stimulation techniques. In 1984, Miller applied a low voltage of 1 V to polypyrrole films, which induced a higher glutamate release in contrast to films that were not stimulated [3]. Recently, in 2016, Szenerits *et al* implanted polypyrrole nanoparticles loaded with fluorescent molecules in mice and detected fluorescence in tissue upon applying a 1.5 V/cm electric field for 40 s [4]. There are other literature based on Poly(methyl methacrylate) [5] , guar gum hydrogel [6], Polyaniline [7], etc. Most of these findings have been shown to work on the principle of polymer substrate deconstruction due to migration of ions, thereby discharging the drug.

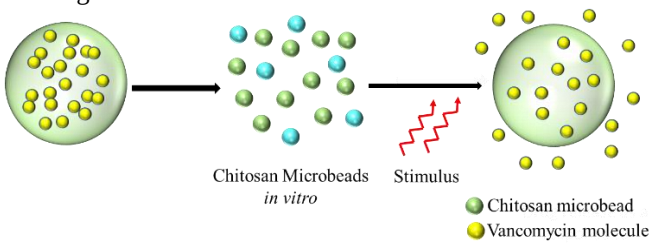

Fig. 1: Conceptualized framework for an ideal DDS, responsive to external stimuli.

The DDS under our examination is based on chitosan cross-linked with Polyethylene Glycol Dimethacrylate (PEGDMA). The microbeads are embedded with magnetic nanoparticles (MNP) and are loaded with the antibiotic. Chitosan is a biocompatible and biodegradable polymer that has been widely used for formulating implantable DDS [8,9]. Inclusion of MNP in the DDS allows DDS tracking [10] and its confinement to the target area by an external magnetic field. It can also help in intensifying MRI contrast [11].

In our preliminary work, we used off-the-shelf Inter-Digitated Electrodes (IDE) in Surface Acoustic Wave (SAW) resonators to administer electric pulses to chitosan microbeads. We observed a higher release of alizarin post-stimulus, in comparison to non-stimulated microbeads [12,13]. Lower frequencies of bipolar rectangular pulses were seen to be more effective than other waveforms. It was also noted that providing excitation to microbeads not containing MNP did not have any significant difference in alizarin elution between control and stimulated groups.

This paper describes printing our own IDE using a material printer so that the stimulation could be applied to samples on a larger, more practical scale. We also applied two sets of electric pulses, separated by an interval to investigate if the same substrate could be repetitively responsive to the same stimulus and release a higher amount of drug in both instances. Vancomycin concentration before and after each stimuli have

been compared to study the effect of stimulus on the passive diffusion profile of vancomycin.

The second section describes the procedure for making the magnetic nanoparticles and microbeads. It also explains printing the IDE and setting them up to a signal generator to provide electric stimulation. At the end of this section, the experimental timeline is illustrated, along with the statistical tools used to analyze the data. Section 3 describes the key observations obtained after the experiments. Section 4 discusses the probable mechanisms which may be causing a higher amount of vancomycin to be released from samples that were stimulated. Section 5 recapitulates the findings and importance of this work.

II. MATERIALS AND METHODS

A. MNP Preparation and Characterisation

The magnetite (Fe_3O_4) magnetic nanoparticles (MNP) were made by the process described by Kang *et al* [14]. They were imaged by a Transmission Electron Microscope (TEM) (Jeol JEM 1200) and measured (Fig. 2(a)), which revealed their average size as 12 nm. The X-Ray diffraction (XRD) and Vibrating Sample Magnetometer plots are shown in Fig. 2(b) and 2(c). The saturation magnetization, retentivity and coercivity were found to be 60.55 emu/g, 3.52 emu/g and 65.24 Oe respectively.

B. Preparation and Characterization

The chitosan microbeads were produced by a slight variant of the water-oil emulsification technique by Jain *et al* [15]. 1 g of MNP and 46 ml of distilled water were mixed together and sonicated for an hour. The solution was then added to 2 g of chitosan (Chitopharm S) and 0.4 g of vancomycin (MP Biomedicals), followed by 0.5 ml glacial acetic acid. The solution was left on an impeller overnight. On the next day, 2 g of Span 80 (Sigma Aldrich) was mixed with 75 ml each of both heavy and light mineral oils and 15 ml of PEGDMA (Mn 550, Sigma Aldrich) and positioned on an impeller heated to 60°C. The chitosan solution from the previous day was slowly added to this solution and left overnight. Next day, the solution was drained and the microbeads obtained. The microbeads were triple washed with hexanes, methanol and acetone and then dried.

The average bead size was 288.4 ± 62.2 µm. A scanning electron image (SEM) of a single microbead is shown in Fig. 2(d). The FTIR spectra (Nicolet iS 10 FTIR Spectrometer), Fig. 2(e), of the microbeads in the mid IR range (400-4000 cm^{-1}) show peaks at 1070 (C-O-C stretching), 1400 (C-H rocking), 1530 (Amide II), 1550 (N-H scissor), 1640 (C=O), 1720 (C=O), 2850 (C-H), 2920 (C-H) and 3350-3280 (N-H stretch). The broad peak in the range 2700-3600 is due to O-H stretching. The peaks correspond to those expected from chitosan, reported in other literature [16,17].

An XRD (D8/Advance, Bruker Advance X-Ray solutions) of the microbead samples with Cu-Kα radiation (λ = 0.15418 nm) is shown in Fig. 2(f). It has the major peaks known for chitosan, vancomycin and MNP [18,19], supporting the existence of these compounds in the microbead DDS.

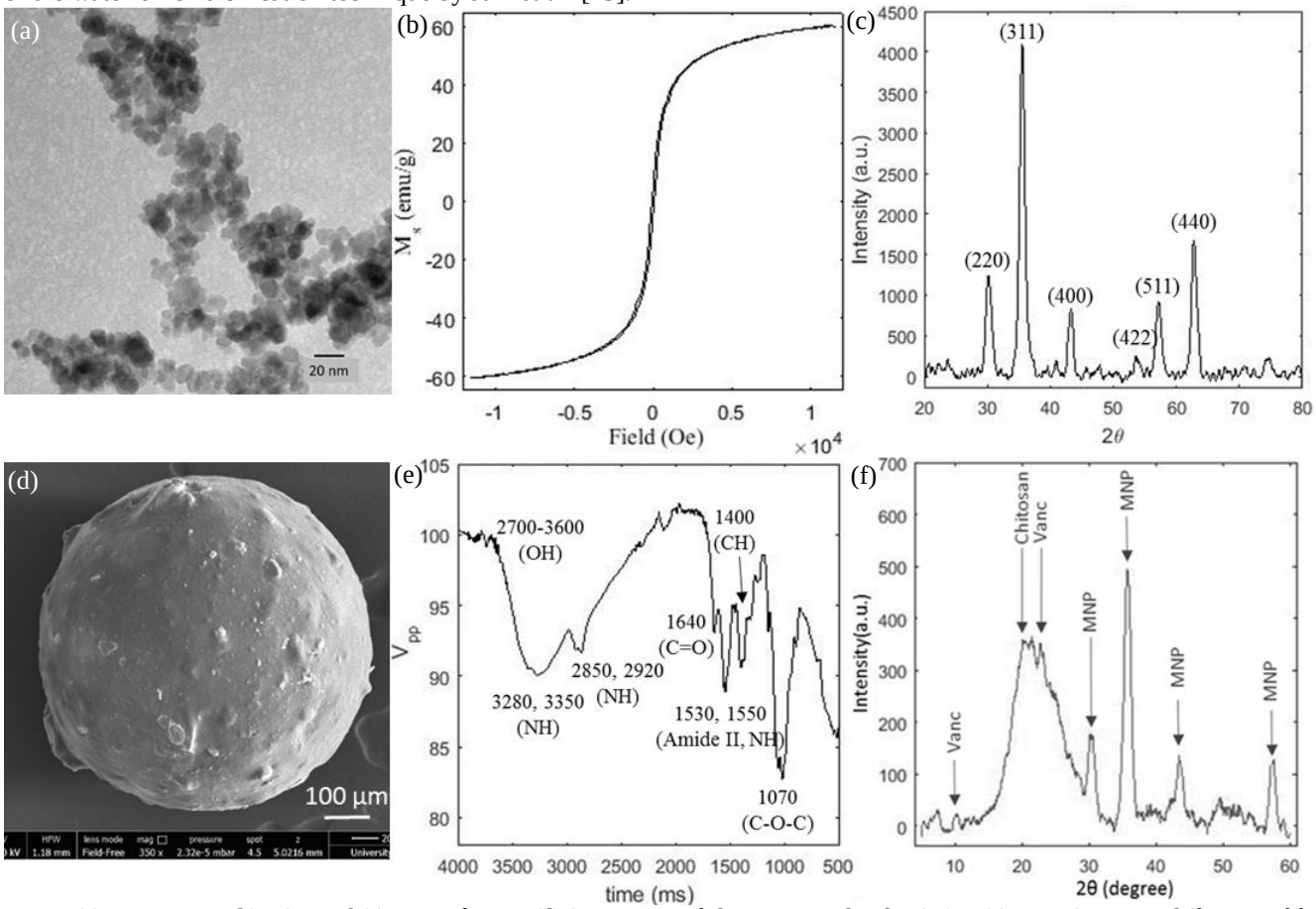

Fig. 2: (a) TEM image, (b) VSM and (c) XRD of MNP (d) SEM image of chitosan microbead at 350X, (e) FTIR Spectra and (f) XRD of final chitosan microbead containing MNP, vancomycin and PEGDMA

C. Printing IDE

A material deposition printer (DMP-2831, Fujifilm Dimatix Inc., NH) was used to print the IDE, with Silver ink (Metalon JS-B40HV, Novacentrix, USA). The ink has a viscosity of 8cP. Several different combinations of finger dimensions were examined and the design showed in Fig. 3 was found to be optimal and reliably reproducible.

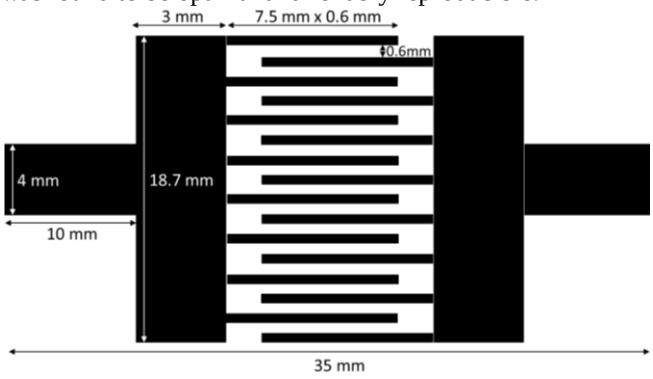

Fig. 3: IDE dimension and layout

A polyimide film was taped on the printing platen pre-heated to 60°C and the pattern in Fig. 3 was printed at 1693 dpi and 15 µm drop width. The whole substrate was then transferred into a glass plate and sintered at 250°C for 15 mins. This heating process evaporates the ink solvent, adheres the silver on to the film and helps make the traces conductive. Fig. 4 shows an image of a single IDE finger. The ink was found to have a slight propensity to spread over the substrate. The average finger width was 783.68 ± 52.6 µm, although the design was 600 µm.

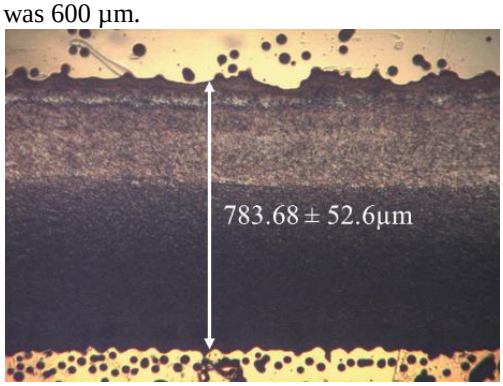

Fig. 4: Image of an IDE finger taken with a light microscope, depicting the actual width of the finger after printing process.

The next step was to make arrangements such that the samples and PBS (Phosphate Buffered Solution, 1x) are constrained on top of the IDE. A plastic tube of diameter 2.9 cm was sawed into 1" sections. Each section was glued with silicone resin to the substrate, such that the IDE is positioned at the center (Fig. 5). Wires were attached to the end terminals using silver epoxy. The whole setup was left to dry for 24 hours.

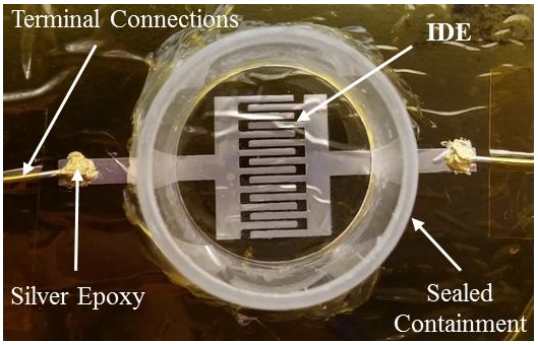

Fig. 5: IDE printed on polyamide substrate after fixing of containment

The IDE was connected in series with a 1Ω resistor and the whole setup was connected to a signal generator (DG4062, Rigol USA). Two channels of an oscilloscope (Tektronix TDS1001B, USA) were connected across the whole setup and the resistor to measure the voltage and current waveforms respectively. The schematic is shown in Fig. 6 and a photograph of the complete setup is shown in Fig. 7.

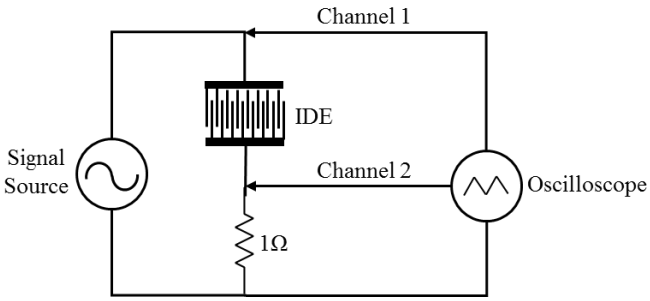

Fig. 6: Schematic diagram for IDE connection to signal generator and oscilloscope.

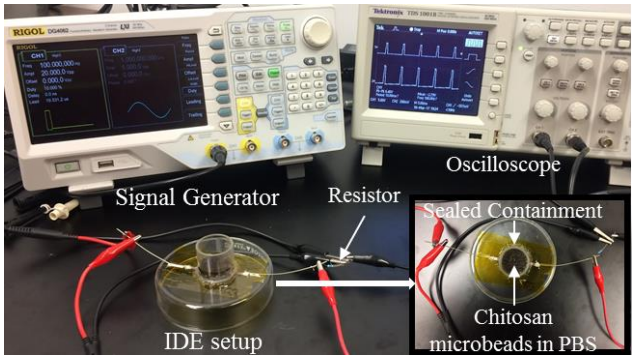

Fig. 7: The complete setup according to the schematic in Fig. 6. (inset) The IDE setup shown in Fig. 5 with DDS sample and PBS

C. Experimental Procedure

The microbeads were divided into 10 samples of 100 mg weight. They were grouped into control and test groups, each group containing 5 samples. The experimental timeline is shown in Fig. 8.

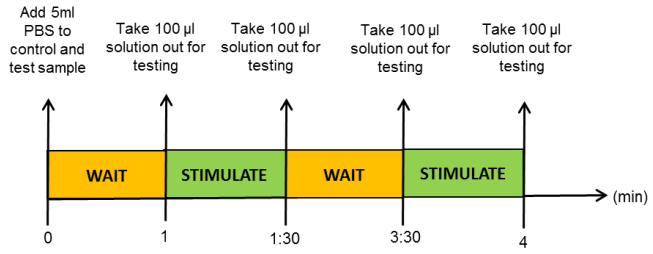

Fig. 8: Multiple stimuli timeline for electric stimulus experiments

The total duration of the experiment was 4 mins, including two different stimuli of 30 seconds each. 5 ml of PBS was added at t = 0. Excitations were provided at 1 min and 3:30 min. Each stimulus was a bipolar rectangular pulse of ~6V (peak to peak), 100 Hz and 10% duty cycle, applied for 30 seconds across the setup. 100 µl of PBS was pipetted out immediately before and after stimulation and stored to be analyzed with an HPLC.

D. Measurement and Analysis

The vancomycin concentration drawn at each time-point (Fig. 8) was assessed by High Performance Liquid Chromatography (HPLC) (Dionex Ultimate 3000, Thermo Scientific USA) at 209 nm. Assuming normal distribution of the concentration values, they were statistically analyzed by a t-test at 5% significance level.

III. RESULTS

A. Stimulus Waveforms

The voltage and current observed in both stimuli are plotted in Fig. 9 and 10. The peak current during each excitation period is in the range of 260-280 mA for an applied voltage of ~7V . The current (I) through the setup slowly decays for longer stimulation periods due to electrode degradation which is apparent in the current waveforms between first and second excitation (Fig. 9, 10). During the first stimulation, the peak current across the setup was measured as 290mA. In the second stimulation the peak current decreased to 260 mA. The impedance (Z_{IDE}) of the IDE increases due to stimulation related degradation, thus the voltage (V_{IDE}) across the IDE (equation 1) also increases in second stimulation (Fig. 10).

$$V_{IDE}(V) = Z_{IDE}\ (\Omega)\ \text{x}\ I(A) \quad (1)$$

Energy dissipated by a pulse is mathematically defined as:

Energy (mJ)= Voltage(V) x Current(mA) x Pulse width (s) (2)

Using this formula, the energy in each pulse of 10 ms is found out to be 1.84mJ in first stimulation and 2mJ for the second stimulation. Extending these values over each stimulation duration of 30 s, it was calculated that in the first and second excitation spans a total energy of 5.5J and 6J was dissipated by the circuit respectively.

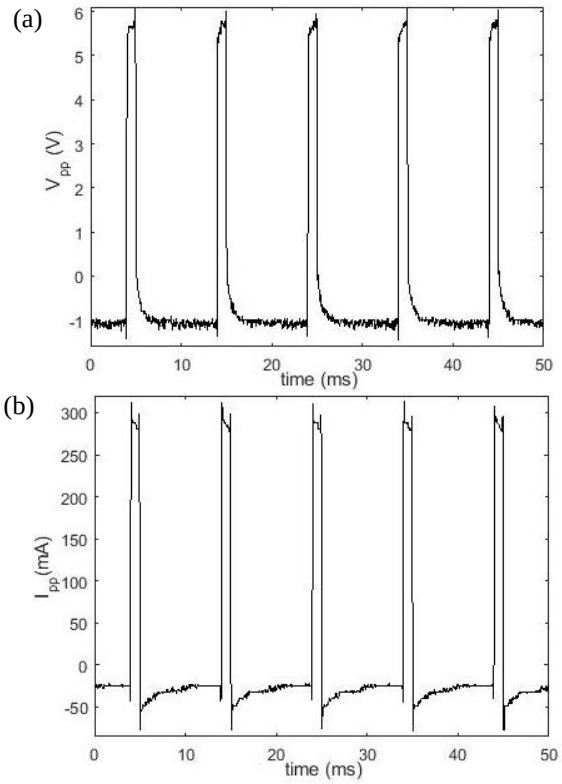

Fig. 9: (a) Voltage and (b) Current through setup for first stimulation.

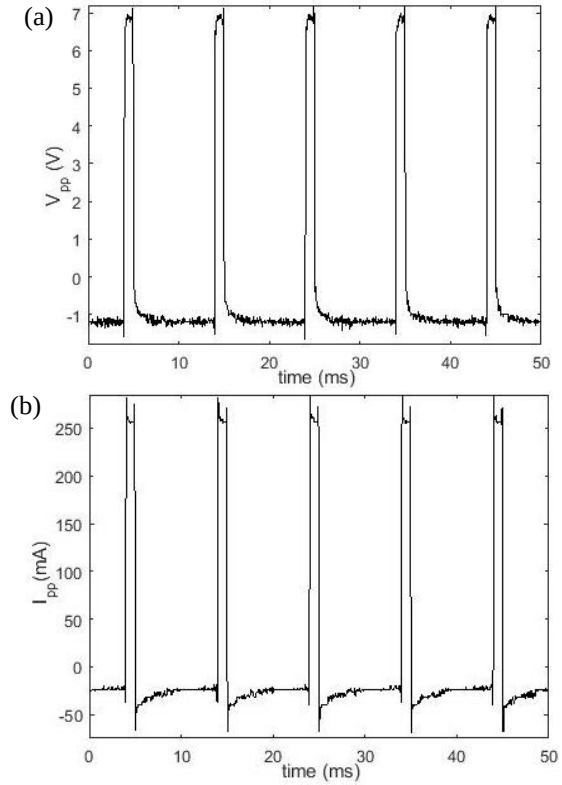

Fig. 10: (a) Voltage and (b) Current through setup for second stimulation.

B. Statistical Analysis

The t-tests test revealed that the vancomycin released by stimulated (stim) groups were statistically more significant than that released by non stimulated (control) groups for both stimuli periods ($p<<0.01$, $p<0.05$). The elution bar plots represented as average ± standard deviation, are shown in Fig. 11. Asterisks denote significant difference in elution.

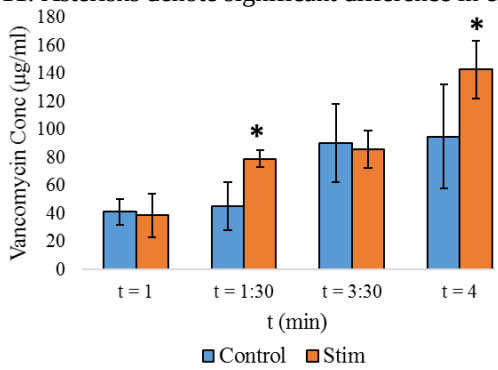

Fig. 11: Concentration of vancomycin over time with (Stim) and without stimulation (Control), eluted by chitosan microbeads.

C. SEM Imaging

The images of microbeads that were stimulated did not show any surface aberration or morphological anomalies that could have been attributed to the stimulus (Fig. 12).

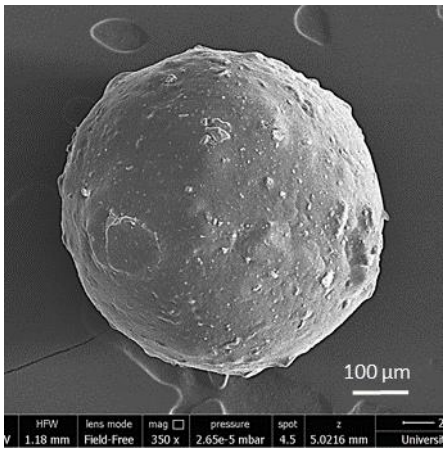

Fig. 12: SEM image at 350x of chitosan microbead after electric stimulus.

IV. DISCUSSION

The electrochemical reactions of silver electrodes with the PBS are:

$$NaCl \rightarrow Na^+ + Cl^- \quad (3)$$

$$Ag(s) + Cl^- \rightarrow AgCl(s) + e^- \quad (4)$$

$$H_2O \rightarrow H^+ + OH^- \quad (5)$$

$$\text{Anode: } Ag \rightarrow Ag^+ + e^- \quad (6)$$

$$\text{Cathode: } 2H^+ + 2e^- \rightarrow H_2 \quad (7)$$

Equations 2, 4 might be the primary cause for electrode degradation electrode degradation (as seen in Fig. 13) This electrode degradation causes gradual reduction of current through the IDE when stimulated. Future challenges will be to design electrodes that are reusable and do not degrade after repetitive stimulations.

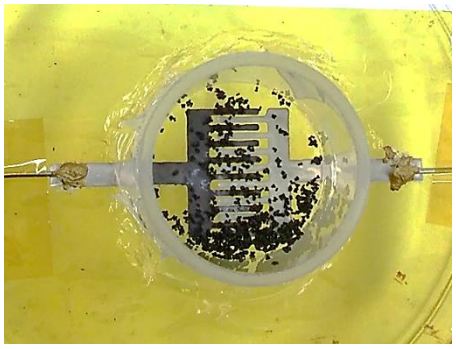

Fig. 13: IDE after two stimuli have visibly degraded.

The exact mechanism causing drug release is not clearly known at this point and is possibly a combination of more than one phenomena. Chitosan, being a cationic molecule, is known to neutralize into an insoluble deposit at cathode (equation 8) [20].

$$\text{Chit-}NH_3^+ + OH^- \rightarrow \text{Chit-}NH_2 + H_2O \quad (8)$$

It is likely that a similar reaction in the microbeads on a microscopic level might be causing degradation of the polymer matrix, thereby incrementing vancomycin release.

A temperature rise of 3°C was also observed during stimulation, which can be attributed to Joule heating [21]. Joule heating is caused by an electric current flowing through the electrolytic solution. This temperature increase may also be contributing to a slight increase in vancomycin elution, compared to non-stimulated groups.

V. CONCLUSION

A new DDS based on chitosan, MNP and carrying vancomycin was successfully shown to be responsive to multiple stimuli of *in vitro* electric pulses. The stimulated groups showed a significantly higher amount of vancomycin released by the groups that were stimulated, in comparison to non-stimulated groups. This demonstrates possible applications in pharmaceutical industries, where healthcare providers can better tune the dosage timeline according to a patient's requirements and comfort.

VI. REFERENCES

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