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## Pulsatile release of biomolecules from polydimethylsiloxane (PDMS) chips with hydrolytically degradable seals

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### Abstract

We demonstrate, for the first time, a robust novel polydimethylsiloxane (PDMS) chip that can provide controlled pulsatile release of DNA based molecules, proteins and oligonucleotides without external stimuli or triggers. The PDMS chip with arrays of wells was constructed by replica molding. Poly(lactic acid-co-glycolic acid) (PLGA) polymer films of varying composition and thickness were used as seals to the wells. The composition, molecular weight and thickness of the PLGA films were all parameters used to control the degradation rate of the seals and therefore the release profiles. Degradation of the films followed the PLGA composition order of 50:50 PLGA > 75:25 PLGA > 85:15 PLGA at all time-points beyond week 1. Scanning electron microscopy images showed that films were initially smooth, became porous and ruptured as the osmotic pressure pushed the degrading PLGA film outwards. Pulsatile release of DNA was controlled by the composition and thickness of the PLGA used to seal the well. Transfection experiments in a model Human Embryonic Kidney 293 (HEK293) cell line showed that plasmid DNA loaded in the wells was functional after pulsatile release in comparison to control plasmid DNA at all time-points. Thicker films degraded faster than thinner films and could be used to fine-tune the release of DNA over day length periods. Finally the PDMS chip was shown to provide repeated sequential release of CpG oligonucleotides and a model antigen, Ovalbumin (OVA), indicating significant potential for this device for vaccinations or applications that require defined complex release patterns of a variety of chemicals, drugs and biomolecules.

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**Keywords:** Plasmid DNA; Non-viral gene delivery; Transfection; Chips; Pulsatile release; Oligonucleotides; Poly(lactic acid-co-glycolic acid) (PLGA); polydimethylsiloxane (PDMS)

### 1. Introduction

There are a number of examples in which pulsatile release of drugs and proteins are desirable [1–5]. For example, we and others have shown that multi-dose vaccines delivered weeks apart stimulate significantly more potent antigen-specific immune responses than single doses [6–8]. We have also shown that the orders in which immunostimulants and antigens are released are critical for enhancing the antigen-specific immune response [6,8]. Other examples in which pulsatile release is advantageous include the delivery of insulin and gonadotropin-releasing hormones [9–11]. Pulsatile release of these proteins would mimic the human body's natural release profiles [11]. To achieve pulsatile release of

proteins or hormones or to optimize delivery of multi-dose drugs and vaccines, a number of approaches have been investigated. These include delivery systems that respond to changes in pH [12], temperature [13], electric [14] and magnetic fields [15] or exposure to triggers such as ultrasound [16,17], enzymes [18] or light [19]. A promising approach for pulsatile release of drugs has been the development of chips with wells that release drugs on demand [10,20,21]. For example, silicon based micro-chips can provide controlled release of single or multiple chemicals on receipt of an electrical trigger signal [21]. The release mechanism is based on the electrochemical dissolution of thin anode membranes covering reservoirs that contain the agent to be released. Chips have also been prepared that are composed entirely of biodegradable polymers [10,20]. A drawback to a chip in which the core base is prepared entirely out of a biodegradable polymer, is the high build-up of acidic oligomers can stimulate

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undesirable inflammatory responses and necrosis of tissues [22]. In addition, the biodegradable bases or silicon bases are delicate and susceptible to fracture if not handled carefully [23,24].

In this study, we prepare poly(dimethylsiloxane) (PDMS) chips with multiple reservoirs by replica molding. PDMS is a robust material and devices made from it can be bent and dropped without altering performance. PDMS is chemically inert, has low polarity, low electrical conductivity and does not swell in aqueous environments [25,26]. The cost of PDMS is also substantially lower than many materials including silicon and most biodegradable polymers. The reservoirs are covered with biodegradable seals to achieve pulsatile release of DNA and other molecules. The prototype we have developed takes advantage of the differential degradation rates of poly(lactic acid-co-glycolic acid) (PLGA) polymer films of varying composition and thickness whilst simultaneously removing the possibility of high concentrations of localized acidic oligomers by using inert and flexible PDMS as the main chamber. We show that this PDMS chip can provide controlled pulsatile and sequential release of molecules such as plasmid DNA, oligonucleotides and antigenic proteins.

## 2. Materials and methods

### 2.1. Materials

Poly (D, L-lactic acid-co-glycolic acid) polymers (PLGA) molar ratio: 50/50, inherent viscosity: 0.41 dL/g in HFIP, molar ratio: 75/25, inherent viscosity: 0.47 dL/g in CHCl<sub>3</sub> and molar ratio 85/15, inherent viscosity 0.99 dL/g in CHCl<sub>3</sub> were purchased from Absorbable Polymers International (Pelham, AL USA). All PLGAs were amorphous and had Tgs that ranged from 40 to 48 °C. Silicone base and silicone elastomer curing agent were obtained from Sylgard (Midland, MI USA). Deoxyribonucleic acid (DNA) free acid, ovalbumin and crude oligonucleotides from herring sperm obtained from Sigma–Aldrich (St. Louis, MO). CpG ODN 1826: 5'TCCATGACGTTCCCTGACGTT 3 was obtained from Coley Pharmaceutical Group (Wellesley, MA). Solvents were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma–Aldrich (St. Louis, MO).

### 2.2. Fabrication of PDMS

A template was prepared with cube features (0.3 cm width × 0.3 cm length × 0.3 cm depth) aligned and adhered on a plastic substrate with interval spacings of 0.9 cm across and 1.3 cm down. The PDMS chip was formed by replica molding. A liquid PDMS pre-polymer in a mixture of 1:10 base polymer: curing agent was vigorously stirred and degasified by sonication. The PDMS mixture was then poured onto the mold. The PDMS chip was cured at 55 °C for 24 h and peeled off the master.

### 2.3. Film fabrication

PLGA films were manufactured using a melt pressing method. Briefly, approximately 1–3 g of PLGA pellets was melted on a glass plate. The temperature was raised by 5 °C

every 3 min until the pellets melted. The melted polymer was covered with another glass plate and the two plates were placed between compression vice clamps. Thickness of the films was varied by applying different degrees of pressure from the compression clamps (settings 1 to 8) on the glass plates. The melting polymer was solidified at room temperature for 10 min. Next, the films (on glass) were soaked in distilled deionized water (ddH<sub>2</sub>O) for a few hours. Films were cut to 0.8 cm width × 0.8 cm length square pieces after peeling off the glass plate. The films were then air and vacuum dried. Thicknesses of the film were determined using vernier caliper measurements.

### 2.4. Swelling of PDMS with solvents

The degree of swelling is expressed by the swelling ratio.  $S = D/D_0$  where  $D$  is the length of the solid PDMS in the solvent and  $D_0$  is the length of the dry solid PDMS. Swelling was measured at room temperature by comparing the length of solid pieces of PDMS before and after being immersed in a solvent. Solvents were obtained from Sigma–Aldrich Co (St. Louis, MO). The PDMS chips were 4.8 cm width × 4.9 cm length × 0.5 cm depth in dimensions. The length of one edge of the square to the opposite edge was measured. The PDMS chip was then immersed in acetone, dichloromethane or toluene for 24 h at room temperature. The pieces were imaged using a light microscope (Olympus BX40) with a CCD camera attached. Six PDMS chips per solvent were measured and the results recorded as mean ± standard deviation.

### 2.5. In vitro release studies

PDMS chips were loaded with 50 μL of 20 mg/ml of DNA, CpG ODN, plasmid DNA or Ovalbumin (OVA) in each well. Toluene was dabbed around the edges of each well and left for 30 min. Loading of biomolecules and sealing was carried out in a laminar flow hood to prevent any contamination. PLGA films were then placed over the well and left for a further 2–3 h. PDMS chips sealed with PLGA films were placed in a sonicator bath to confirm the integrity of the seal. The *in vitro* release experiments were performed by immersing the PDMS chips with PLGA seals in aqueous isotonic phosphate buffer medium maintained at pH 7.4 and 37 °C. The glass containers containing the chips were agitated at 100 rpm. The samples were collected every 12 h up to 60 days and the isotonic *in vitro* medium was replaced at each collection time. All experiments were performed in triplicate. The amount of DNA, plasmid DNA and CpG ODN released from chips was analyzed spectrophotometrically at 260 nm using a SpectraMax Plus<sup>384</sup> Microplate Spectrophotometer (Molecular device). The amount of OVA released was measured using a BCA protein assay kit (Pierce).

### 2.6. pH change analysis

PLGA films of varying composition but fixed thickness and size were placed in glass scintillation vials, each containing 10 mL of phosphate-buffered saline (PBS, Fluka) for various time periods up to 60 days in a 37 °C environment with shaking

at 100 rpm. The pH of the PBS was monitored during the course of degradation. All measurements were expressed as means  $\pm$  standard deviation (SD) relative to the initial values.

### 2.7. Weight loss analysis

PLGA films were weighed and placed in glass scintillation vials containing 10 mL of PBS pH 7.4. Samples were placed at 37 °C environment with shaking at 100 rpm. At each time point, the films were air-dried overnight, vacuum-dried for 72 h and placed at 37 °C incubator for 1 week. The weight of these samples was then recorded using three samples for each time point. Percent weight remaining was calculated using the following formula: % weight remaining =  $m_2/m_1 * 100$ , where  $m_1$  and  $m_2$  are the film weights determined initially and after degradation for time  $t$ , respectively. All measurements were expressed as means  $\pm$  standard deviation (SD) relative to the initial values.

### 2.8. Nuclear Magnetic Resonance Spectroscopy (NMR)

Proton  $^1\text{H}$  NMR spectra were recorded on a Bruker Avance-300 NMR spectrometer (Bruker, Billerica, MA).  $^1\text{H}$  NMR chemical shifts were measured in parts per million (ppm) relative to  $\text{CHCl}_3$  in  $\text{CDCl}_3$ .

### 2.9. Scanning electron microscopy (SEM)

The surface morphology of films was characterized by scanning electron microscopy (SEM, Hitachi S-4000, Hitachi High Technologies America Inc, Pleasanton, CA). Air-dried films were placed on adhesive carbon tabs mounted on SEM specimen stubs. The specimen stubs were gold-coated using a sputter coater (E550 Emitech sputter coater) set at 10 mA for 10 s. The gold coating thickness was approximately 5 nm. The films were examined with the SEM operated at 1 kV accelerating voltage.

### 2.10. Cell culture

Human embryonic kidney cells (HEK293) cell lines were obtained from the American Type Culture Collection (ATCC,

Rockville, MD). The cells were maintained in DMEM supplemented with 10% FBS, streptomycin at 100  $\mu\text{g}/\text{ml}$ , penicillin at 100 U/ml, and 4 mM L-glutamine at 37 °C in a humidified 5%  $\text{CO}_2$ -containing atmosphere.

### 2.11. Amplification and purification of plasmid DNA (pDNA)

VR1255 plasmid is a 6.4-kb cDNA encoding firefly luciferase driven by the cytomegalovirus (CMV) promoter/enhancer. The pDNA was transformed in *Escherichia coli* DH5 $\alpha$  and amplified in Terrific Broth media at 37 °C overnight with a shaking speed of 300 rpm. The pDNA was purified by an endotoxin-free QIAGEN Giga plasmid purification kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol. Purified pDNA was dissolved in saline, and its purity and concentration were determined by UV absorbance at 260 and 280 nm.

### 2.12. Transfection experiments

HEK293 cells were seeded into 24-well plates at a density of  $8 \times 10^4/\text{well}$  24 h before transfection. One  $\mu\text{g}$  of pDNA was complexed with polyethylene imine (PEI, 25K and branched, Sigma–Aldrich) at an N:P ratio of 5:1 and added to the cells in Opti-MEM medium (serum-free) and incubated for 4 h at 37 °C, followed by further incubation in serum containing medium for 44 h. After the incubation, cells were treated with 200  $\mu\text{l}$  of lysis buffer (Promega). The lysate was subjected to two cycles of freezing and thawing, then transferred into tubes and centrifuged at 13,200 rpm for 5 min. Twenty microliters of supernatant was added to 100  $\mu\text{l}$  of luciferase assay reagent (Promega) and samples were measured on a luminometer for 10 s (Lumat LB 9507, EG&G Berthold, Bad Wildbad, Germany). The relative light units (RLU) were normalized against protein concentration in the cell extracts, measured by a BCA protein assay kit (Pierce). Luciferase activity was expressed as relative light units (RLU/mg protein in the cell lysate). The data were reported as mean  $\pm$  standard deviation for triplicate samples. Every transfection experiment was repeated at least twice.

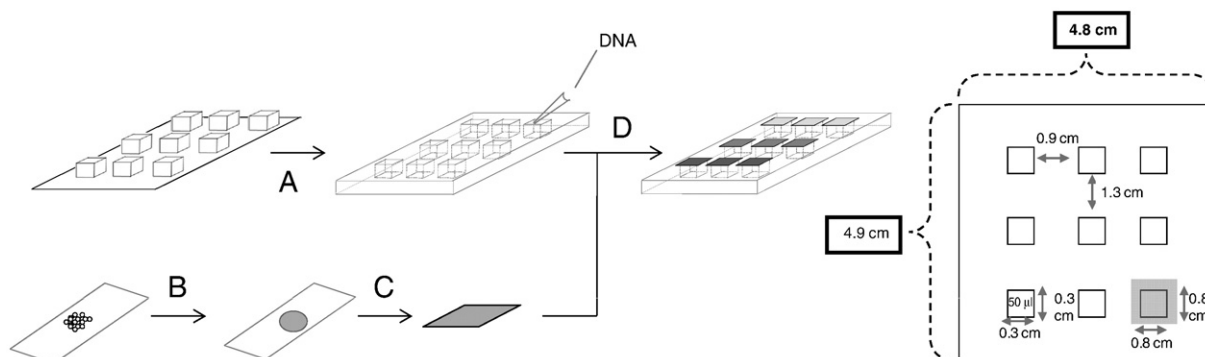


Fig. 1. Schematic of preparation of prototype PDMS chip with degradable seals. A) Liquid PDMS pre-polymer is poured over a template with positive cube features in a 3  $\times$  3 array. B) PLGA pellets are placed on a glass plate and melt-pressed into films using controlled compression clamps. C) The PLGA films are cut to 0.8 cm  $\times$  0.8 cm square pieces. D) Each well in the PDMS chip is filled with 50  $\mu\text{l}$  of 20 mg/ml of DNA and sealed with the PLGA film using toluene.

### 3. Results and discussion

Fig. 1 shows our general approach to preparing chips that provide pulsatile release. First, a template containing 3×3 positive features was prepared. The features were spaced 0.9 cm apart across and 1.3 cm apart down. Each feature was a cube 0.3 cm×0.3 cm×0.3 cm. The PDMS chip with 9 wells was prepared by replica molding. This involved the casting of prepolymer over the template. The PDMS was cured over the template at 55 °C overnight and the replica was peeled from the template. Replica molding allows for any number of features and wells to be prepared [27,28]. The 9 reservoirs on each chip were loaded with 50 μL of 20 mg/ml of Herring sperm DNA, plasmid DNA, CpG ODN or OVA protein. PLGA films were prepared by melt pressing PLGA pellets between two glass plates, cooling and then releasing the film in deionized water over a few hours followed by removal using a sharp blade. Control over the pressure applied to the glass plates using mechanical vice clamps allowed PLGA films to be prepared with thicknesses varying from 90 to 550 μm. The PLGA films were cut to 0.8 cm width×0.8 cm length square pieces. Alternative approaches that we have explored to producing the PLGA films include solvent casting. Solvent casting produced films in which thickness was dependent on the concentration of the PLGA/dichloromethane solution and was highly reproducible from one batch to another (less than 7% standard deviation). The solvent casting approach also allows us to prepare PLGA films that are much thinner (up to 5 μm thickness) than the melt-pressed PLGA films. A drawback to the solvent casting approach, however, is that PLGA films prepared using this methodology readily fractured when removed from the glass plates. In addition, previous studies on solvent casted PLGA films have reported accelerated degradation as a result of the processing conditions [29]. Each sheet of PLGA film prepared using the melt-pressing method can produce enough seals for up to 18 PDMS chips. This ensured that we had enough seals of identical thickness for each composition of PLGA for repeat experiments. The standard deviation of the thickness of the PLGA sheet between one location of the same sheet to another was less than 4%.

Using a solvent swelling approach to form degradable polymer seals over the wells is attractive because it avoids the need for time-consuming and harsh chemical reactions and allows for easy sealing of the wells. To seal the PLGA films over the wells in the PDMS chips, we evaluated a series of organic solvents that swell PDMS and PLGA. An appropriate choice of solvent forms an interpenetrating network at the interface between the PLGA film and the PDMS chip sealing the film over the well [30]. Solvents tested include acetone, dichloromethane (DCM), and toluene. PDMS was most efficiently swollen by toluene generating a mean swelling ratio (*S*) value of 1.29±0.03. This compared to mean *S* values of 1.20±0.02 and 1.05±0.01 for DCM and acetone respectively. These values are consistent with those reported by Whitesides and colleagues [31]. When toluene was used as the solvent to seal the wells in the PDMS chip, the PLGA films remained intact when placed in an ultrasound bath. In contrast,

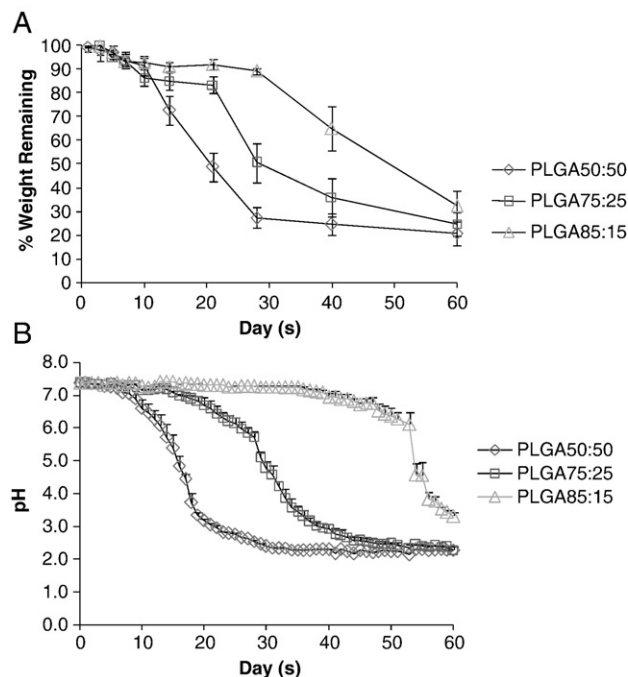


Fig. 2. Results of *in vitro* degradation study of 50:50 (534 μm), 75:25 (559 μm) and 85:15 (469 μm) PLGA films. A) Percentage mass of PLGA films remaining and B) pH of media in which the PLGA films were incubated. Results are presented as the mean ± standard deviation. The sample size was 3.

PLGA films sealed using acetone were rapidly displaced. <sup>1</sup>H NMR analysis of the PLGA seals removed from the PDMS chips after they had dried showed that all residual solvents used to form the seal had been removed/evaporated. Films were prepared from PLGAs with three distinct compositions. Careful control over inherent viscosity (*M<sub>w</sub>*), copolymer ratio, width and size of the PLGA seals are all parameters that allow for tailored degradation rates [29]. The PLGAs we used had compositions of 85:15, 75:25 and 50:50 lactide to glycolide ratios with corresponding inherent viscosities of 0.99 dL/g, 0.47 dL/g and 0.41 dL/g respectively. Three seals were prepared for each film with thicknesses varying from 469 to 559 μm. Degradation of PLGA occurs by hydrolysis of the ester bonds into lactic and glycolic acid [29,32,33]. Weight loss and pH changes are commonly used to characterize PLGA degradation and previous studies have shown that these two measures are directly correlated to decreases in molecular weight, swelling and polydispersity [22,29,34]. Initially the weight remained relatively constant; then a small percentage drop was observed followed by a plateau for a few weeks. This was followed by a more significant decrease in mass. The time-frames for each of these phases were dependent on the composition of the PLGA film. The 85:15 films maintained 93.1% of the day 0 value after 1 week and were still 89% of the day 0 value at 4 weeks. The 75:25 films maintained 93.7% of the day 0 value after 1 week of degradation in PBS, but only 50.5% of the day 0 value remained at 4 weeks. The 50:50 films were 93.6% of their day 0 mass by week 1 and were 27.3% of the day 0 mass by week 4. At relatively constant thicknesses in the range of 450 to 560 μm, the PLGA films showed mass losses in the order of 50:50

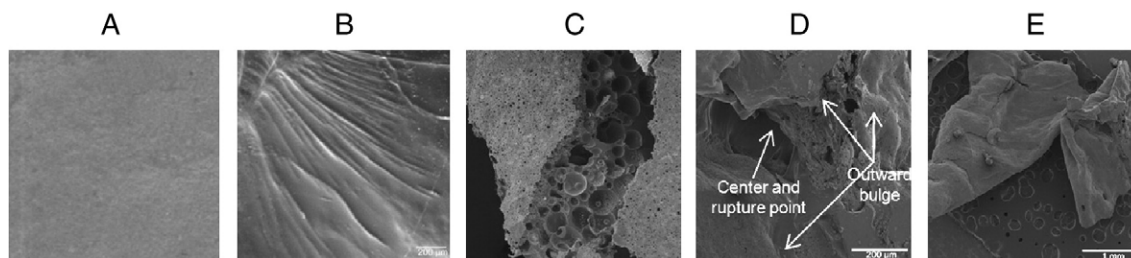


Fig. 3. SEM photomicrographs of 75:25 PLGA films from PDMS chips at various time-points during incubation in aqueous isotonic medium at 37 °C at 100 rpm. A) The initial film is smooth in morphology, B) once the films begin to take water up, they swell generating wave-like features. C) As the film degrades, small pore features appear on the surface with a cross-section revealing large pore structures through the film. D) The osmotic pressure pushes outwards as the PLGA degrades until the film reaches its rupture point at the center. E) The film ruptures releasing the contents of the well.

PLGA > 75:25 PLGA > 85:15 PLGA at all time-points beyond week 1. Previous studies have shown that thicker films show faster loss than thinner films, that higher Mw PLGA films degrade slower than low Mw PLGAs and that PLGA films degrade faster at higher temperatures [22,29,34]. Mass loss measurements strongly correlated to pH measurements with no significant change in the pH of PBS solution measured up to 1, 2, and 6 weeks for PLGA films of 50:50, 75:25 and 85:15 compositions respectively. This was followed by a rapid drop in pH due to the release of acidic polymer degradation products in the solution. The time course for the pH drops seen in Fig. 2B strongly correlated with the weight loss profiles observed in Fig. 2A. The gross appearance of all the PLGA films changed over time during degradation. The initially transparent films became whitish due to water absorption. All 9 PLGA films were initially non-porous, with smooth surfaces as examined by scanning electron microscopy (Fig. 3A). Initially the films began to swell (due to water uptake) with wave-like features appearing on the surface (Fig. 3B). As the PLGA films sealing the wells in the PDMS chips began to degrade, the films swelled outwards with extensive micropores developing (Fig. 3C). Eventually the internal osmotic pressure caused the swollen porous degrading PLGA film to stretch at the center (Fig. 3D) until it ruptured thereby releasing the contents of the well into solution (Fig. 3E). The PLGA seals that were thicker burst a few days quicker than the thinner seals at all PLGA compositions presumably because the thicker films have a greater extent of autocatalytic activity that accelerate degradation. Figs. 4 and 5 shows that the dominant parameter in controlling the time-point at which the seal burst was the composition of the PLGA film. Release of DNA strongly corresponded to mass loss and pH changes. DNA was released from wells with PLGA seals in the order of 50:50 > 75:25 > 85:15 compositions (Figs. 4 and 5). Pulsatile release of DNA could be separated on the week scale by using varying compositions of PLGA as seals to the wells. Finer control over the pulsatile release of DNA could be achieved on the day scale by varying the thickness of the PLGA films. For example, the first wave of DNA was released from wells with 50:50 PLGA seals from day 8 to day 15 (Fig. 5A). Within this period, the 534 μm 50:50 PLGA seal burst at day 8 and the 117 μm 50:50 PLGA seal ruptured at day 13. Wells with 75:25 PLGA seals released DNA between days 20 and 27 and wells with 85:15 PLGA seals released DNA between days 28

and 37. As the composition of the PLGA film changed from 50:50 to 85:15, the impact of thickness of the PLGA film on time-point of DNA release also increased. For example, the

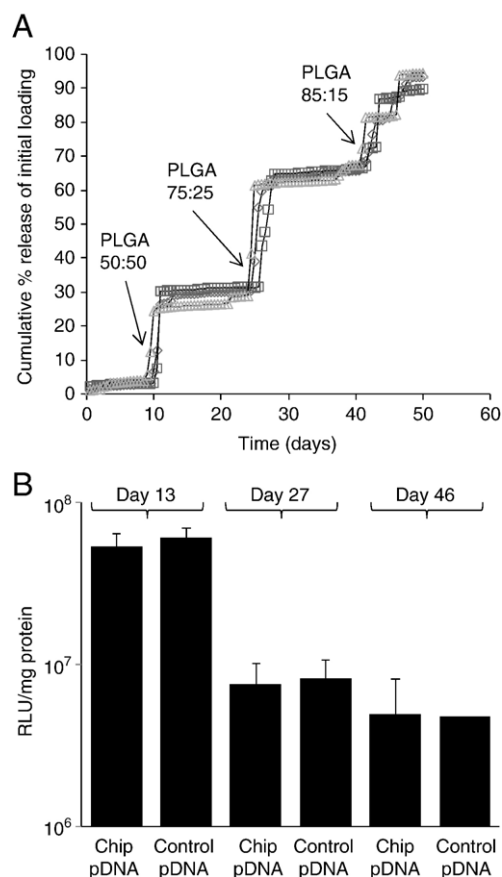


Fig. 4. A) Cumulative percentage of initial DNA loading released from PDMS chips. Each symbol (triangle, square or diamond) represents data collected for a separate PDMS chip. Each PDMS chip had a total of 9 wells with 3 of each well sealed with a 50:50 PLGA film (534 μm), a 75:25 PLGA film (559 μm) or a 85:15 PLGA film (469 μm). The release times of the DNA increased as the ratio of lactide: glycolide increased. Experiments were carried out in aqueous isotonic medium at 37 °C *in vitro*. B) Luciferase activity from plasmid DNA (pDNA) released at time-points after films had burst. One μg of the pDNA released from the wells were complexed with polyethyleneimine (PEI, 25K and branched) at an N:P ratio of 5:1 and incubated with Human Embryonic Kidney 293 (HEK293) cells for 4 h. Cell harvesting and luciferase assays were performed 44 h after transfection as described in the Materials and methods section. Data are represented as mean ± standard deviation (n = 3).

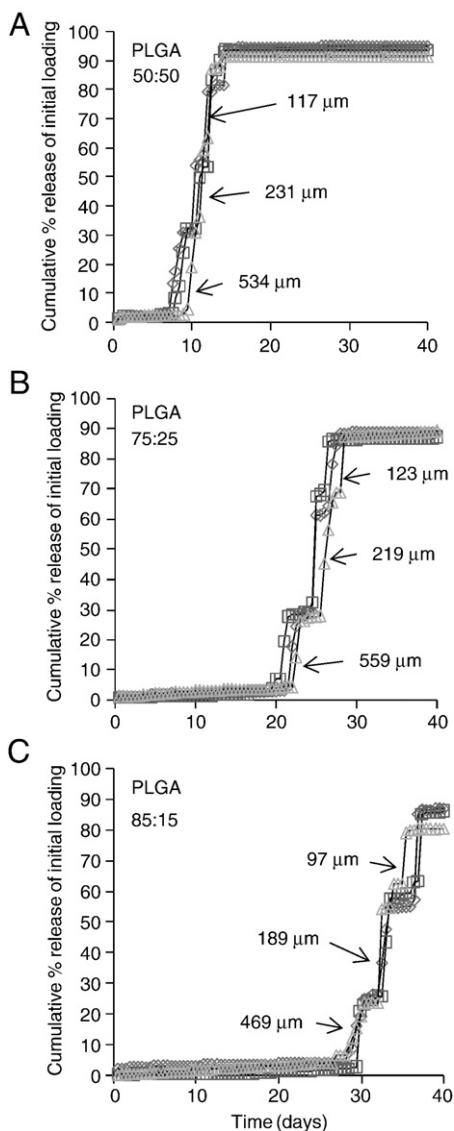


Fig. 5. Effect of PLGA film thickness on DNA release from wells in PDMS chip. Fig. 5 shows the cumulative percentage of initial DNA loading released from PDMS chips. Each symbol (triangle, square or diamond) represents data collected for a separate PDMS chip. A) Each PDMS chip had a total of 9 wells with 3 of each well sealed with a 50:50 PLGA film (534  $\mu\text{m}$ ), a 50:50 PLGA film (231  $\mu\text{m}$ ) or a 50:50 PLGA film (117  $\mu\text{m}$ ). B) Each PDMS chip had a total of 9 wells with 3 of each well sealed with a 75:25 PLGA film (559  $\mu\text{m}$ ), a 75:25 PLGA film (219  $\mu\text{m}$ ) or a 75:25 PLGA film (123  $\mu\text{m}$ ). C) Each PDMS chip had a total of 9 wells with 3 of each well sealed with an 85:15 PLGA film (469  $\mu\text{m}$ ), an 85:15 PLGA film (189  $\mu\text{m}$ ) or a 85:15 PLGA film (97  $\mu\text{m}$ ). The release times of the DNA increased as the thickness of the PLGA films decreased. Experiments were carried out in aqueous isotonic medium at 37 °C *in vitro*.

difference in release of DNA from wells with 534  $\mu\text{m}$  thick 50:50 PLGA seals to 117  $\mu\text{m}$  thick 50:50 PLGA seals is 5 days. However, the difference in release of DNA from wells with 469  $\mu\text{m}$  thick 85:15 PLGA seals to wells with 97  $\mu\text{m}$  thick 85:15 PLGA seals is 9 days. DNA release from wells in the PDMS at later time-points often followed a biphasic release profile. Furthermore, the longer the time taken to release the DNA, the lower the overall percentage recovery. PDMS chips prepared with 50:50 PLGA films as seals to the wells resulted in

between 91 and 95% recovery of the initial DNA loading. PDMS chips prepared with 75:25 PLGA and 85:15 PLGA films as seals to the wells resulted in 87–89% and 80–87% recovery of the initial DNA loading respectively (Fig. 5). It is presumed that this loss is due to time-dependent absorption of the DNA to the walls in the PDMS wells. To confirm that the PDMS chip is able to deliver functional biomolecules, plasmid DNA loaded into the wells and released following similar profiles to those shown in Fig. 4A were tested for transfection efficiency in a model HEK293 cell line. Control pDNA incubated in identical temperature and solvent conditions in Eppendorf tubes were also tested simultaneously to determine the impact of the PDMS device on pDNA functionality. At day 13, 1  $\mu\text{g}$  of pDNA released from the PDMS chips and delivered using a common 25K branched PEI gene delivery vector at a nitrogen:phosphate (N:P) ratio of 5:1 generated  $5.33 \times 10^7$  RLU/mg protein transgene expression in HEK293 cells. By day 46, the transfection efficiency of pDNA in the PDMS chips had fallen by 11-fold to  $4.9 \times 10^6$  RLU/mg protein. However, a comparison to the control pDNA showed no significant difference suggesting that the fall in transfection efficiency was primarily due to the storage conditions and temperature of the pDNA and not the PDMS chip or pulsatile release itself. This is consistent with our previous laboratory observations that plasmid DNA not stored at 4 °C reduces its transfection potency in a time-dependent manner. These results therefore confirm that the PDMS chip can provide pulsatile delivery of functional biomolecules without altering potency. Finally, we have previously reported that two doses of a vaccine containing CpG oligonucleotides and a model antigen, Ovalbumin (OVA) stimulates much more potent immune responses than a single dose [6,8]. In addition, careful control over the sequence of delivery of toll-like receptors such as CpG ODN has been shown to substantially improve the overall antigen-specific immune responses [35]. To demonstrate that this PDMS chip can provide sequential release of CpG ODN and OVA, we

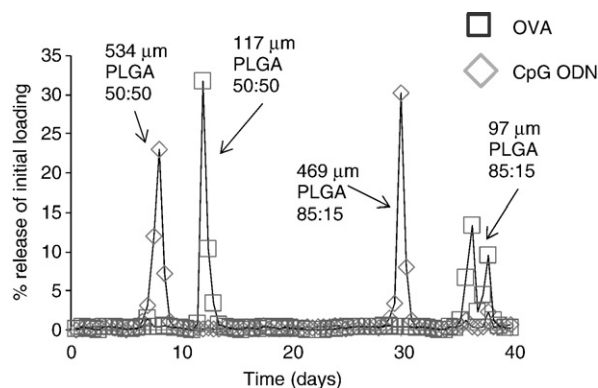


Fig. 6. Percentage release of initial loading of a model antigen, Ovalbumin (OVA) and immunostimulatory molecule CpG ODN from a PDMS chip. 8 wells on the PDMS chip were utilized with 2 wells loaded with CpG ODN sealed with a 50:50 PLGA film of 534  $\mu\text{m}$  thickness, 2 wells loaded with OVA sealed with a 50:50 PLGA film of 117  $\mu\text{m}$  thickness, 2 wells loaded with CpG ODN sealed with a 85:15 PLGA film of 469  $\mu\text{m}$  thickness, 2 wells loaded with OVA sealed with a 85:15 PLGA film of 97  $\mu\text{m}$  thickness. Experiments were carried out in aqueous isotonic medium at 37 °C *in vitro*.

loaded 4 wells with CpG ODN and 4 wells with OVA in a 9 well PDMS chip. Four PLGA films of differing thickness and composition (PLGA 50:50 534  $\mu\text{m}$ , PLGA 50:50 117  $\mu\text{m}$ , PLGA 85:15 469  $\mu\text{m}$ , PLGA 85:15 97  $\mu\text{m}$ ) were selected to ensure sufficient intervals between release of CpG ODN and OVA. Fig. 6 shows that CpG ODN and OVA could be delivered in sequential pulses 4–6 days apart and the sequence could be repeated from the same PDMS chip 18 days later suggesting significant potential for this device in vaccination applications and therapeutic strategies that require multiple drug and dosing profiles.

#### 4. Conclusion

In summary, we demonstrate a new prototype PDMS based chip that can provide controlled pulsatile release of proteins, antigens, oligonucleotides, DNA and a wide variety of other biomolecules without any external trigger signal. The PDMS chamber is cheap to build, robust, inert and provides flexibility of size and number of reservoirs. Defined pulsatile release profiles can be achieved by careful control over the Mw, thickness and composition of PLGA degradable films that form a seal over the wells in the PDMS chip. Using this approach, multiple compounds can be released independently from the same device, which allows for the possibility of a myriad of complex release patterns.

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