Lamination of a Biodegradable Polymeric Microchip

by

Jina Kim

Submitted to the Department of Materials Science and Engineering in Partial Fulfillment of the Requirements for the Degree of

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Signature of Author.....
Department of Materials Science and Engineering
May 26, 2006

Certified by.....
Michael J. Cima
Sumitomo Electric Industries Professor of Engineering
Thesis Supervisor

Accepted by.....
Caroline A. Ross
Professor of Materials Science and Engineering
Chair, Departmental Undergraduate Committee

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ABSTRACT

This work builds on the initial design of a polymer microchip for controlled-release drug delivery. Currently, the microchip employs a nonbiodegradable sealant layer, and the new design aims to fabricate it only of biodegradable parts. Experiments were conducted to evaluate two potential designs that are fabricated via lamination, and a final design was proposed based on the results.

Design 1 sought to replace the sealant directly with a PLA backing layer, but the laminated backing layer was found to leak in ^{14}C-dextran release experiments. Design 2 used a laminated film instead of the original injected membrane. The laminated film was optimized to a 200-μ m thick poly(D,L-lactic-*co*-glycolic acid) 2A membrane, and the film-laminated microchip was shown to release ^{14}C-dextran within a 40-day period. The final proposed design was based on Design 2, which demonstrated more potential as a future means of drug delivery.

Thesis Supervisor: Michael J. Cima
Title: Professor of Materials Science & Engineering

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1 Introduction

The method of drug delivery is a key component of effective therapy for patients. Currently, implantable, controlled-release devices are a major area of study because they would insure drug adherence and efficacy. Controlled release is a more favorable mechanism than traditional drug delivery because it supplies a constant, effective concentration of drug to the body. With traditional drug delivery, the body only experiences an effective drug dosage for a short span of time.

Controlled release comes in two variations: sustained and pulsatile. Sustained release means the drug is supplied at a constant rate and concentration; it is mostly achieved via diffusion through or degradation of a polymer. Pulsatile release, in which drug is released periodically, is more favorable because it mimics the body's natural pattern of distributing chemicals. Devices sometimes use an external stimulus to trigger periodic release. Biodegradable polymer microchips have been developed as one method of achieving pulsatile release without the need for a stimulus.

The current polymer microchip design, from previous research, is mostly biodegradable. However, it uses a nonbiodegradable sealant layer that would complicate its use in the future, raising questions of biocompatibility and convenience. This work evaluates two designs and proposes a final design to fabricate the microchip from only biodegradable parts.

Proposed Design 1 seeks to replace the sealant directly with a PLA backing layer. Design 2 replaces the original membrane with a laminated film, which could help eliminate the need for a sealant layer altogether in the fabrication process. After evaluation of both designs, a final design based on the Design 2 is proposed. A fully

biodegradable polymer microchip would be a promising technology advance in drug delivery and patient treatment.

2 Background

Controlled release involves regulating the release time or rate of a chemical. The method of delivery for a drug affects its efficacy, and exceeding the optimal range of drug concentration can be toxic to the body. With conventional drug system like tablets or injections, the drug concentration profile in the body initially peaks then decreases rapidly. Hence, the time experienced in the therapeutic concentration range is short. Controlled release, which is either sustained or pulsatile, offers a more effective mode of drug delivery.

2.1 Sustained Release

Sustained release delivers drug at a constant, continuous rate over a period of time. Thus far, researchers have achieved sustained release mostly via polymers that release drug at a constant rate via diffusion through the polymer or degradation of the polymer. These systems come in several micro- and macroscopic forms: polymer implants, microspheres, and oral tablets (Santini et al. 2000).

One example of a marketed sustained release product is Gliadel, used in treatment of malignant brain tumors. Basically, it is a polyanhydride wafer, implanted at the time of surgery, which delivers carmustine (BCNU) as the polymer degrades over time. BCNU is incorporated in the polymer matrix composed of 1,3-bis(p-carboxyphenoxy)propane (CPP) and sebacic acid (SA) in a 20:80 molar ratio (Dang et al.).

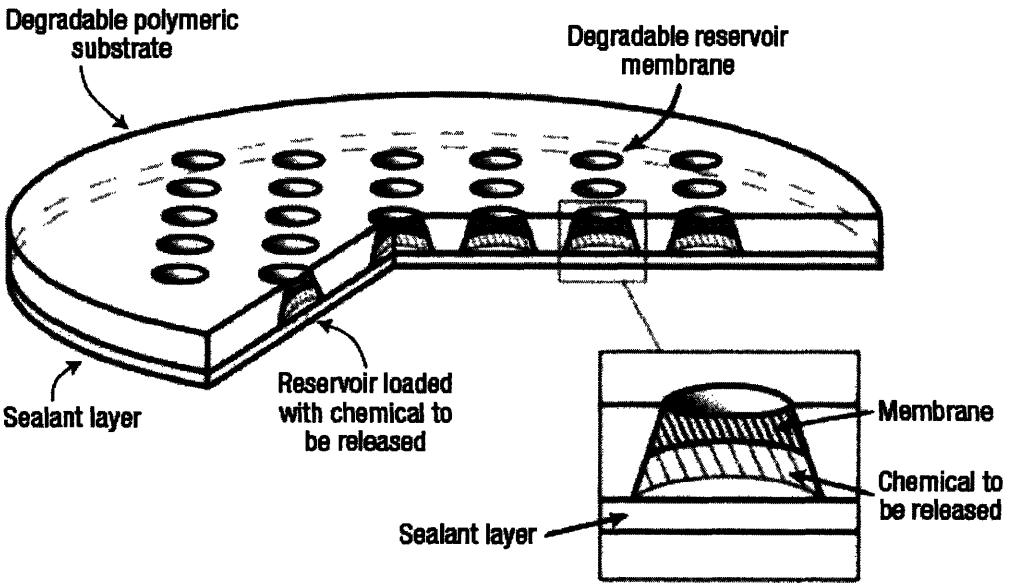
2.2 Pulsatile Release

Pulsatile release, the second type of controlled drug delivery, provides a pulsed pattern of drug delivery at specific time intervals. This system is more preferred as it mimics how the human body naturally produces certain substances like insulin and gonadotropin. Pulsatile release can be designed as an externally regulated or self-regulated system. An externally regulated system responds to application of an outside stimulus, such as light (Mathiowitz et al. 1989), ultrasound (Kost et al. 1989), and enzymes (Fischel-Ghodsian et al. 1988). For example, transdermal delivery systems can be induced to produce pulsatile release in the presence of ultrasound or voltage pulses. Self-regulated delivery does not require an outside stimulus to activate pulsatile drug delivery.

Biodegradable polymeric microchips are a promising approach for a self-regulated, controlled-release biodegradable drug delivery system (Figure 1, 2). The devices consist of a poly(L-lactic acid) (PLA) body and poly(D,L-lactic-*co*-glycolic acid) (PLGA) membranes. The type of membrane, which degrades at different rates depending on molecular mass and thickness, in each reservoir determines when the drug will be released.

Grayson et al. have demonstrated that pulsatile chemical release is possible with the microchip device (2003). In a single microchip device, four different reservoirs were injected with a different PLGA copolymer membrane. Drug was released in a pulsatile pattern as the reservoir membranes opened. Water uptake and swelling of the membrane has been proposed as a mechanism to cause rupture and subsequent drug release. Polymers with greater molecular mass have higher mechanical strength retention, which

leads to later membrane rupture (Grayson et al. 2003). This polymeric device allows for self-regulated drug delivery and biocompatibility.



(Grayson et al. 2003)

Figure 1. Schematic for a biodegradable polymeric microchip. An injected membrane covers the smaller opening of each reservoir. Once the appropriate chemical is placed in the membrane-filled reservoirs, the entire device is sealed with a non-biodegradable sealant.

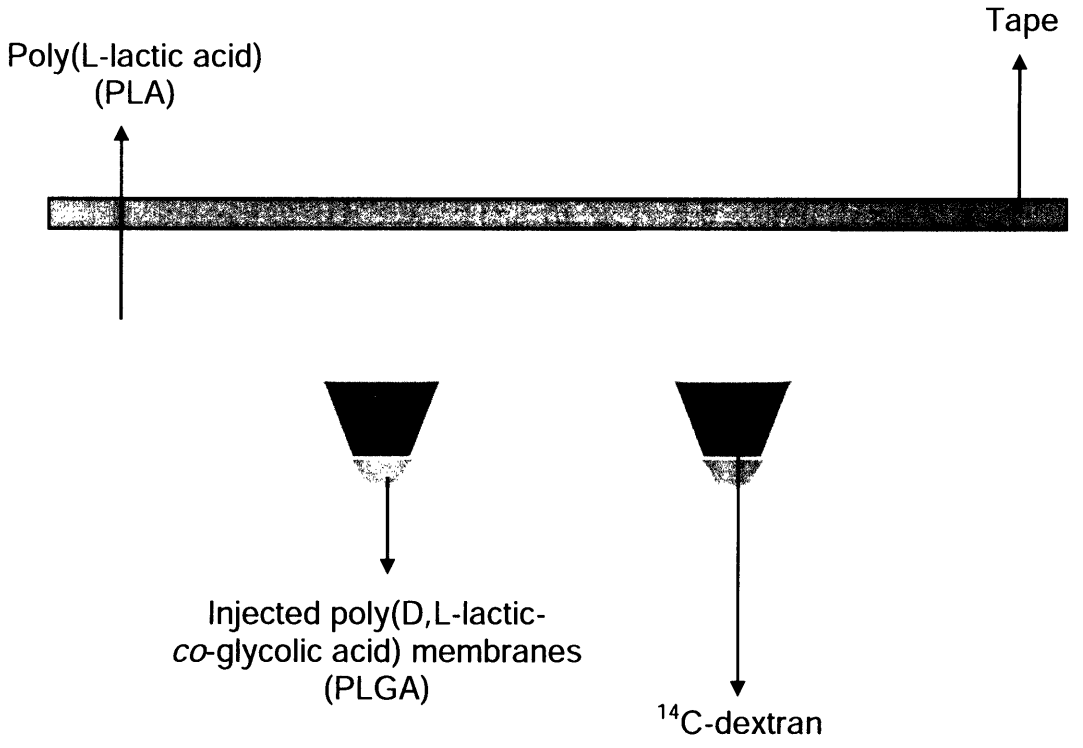


Figure 2. Simplified schematic for the original biodegradable polymeric microchip, shown in Figure 1. The chemical used in these microchip experiments is ^{14}C-dextran, whose release will be measured with a scintillation analyzer.

However, in these studies, the microchip devices were sealed with a tape that is not resorbable by the body, which brings about various concerns. If this device were to be implanted during surgery, a second surgery would be required to remove the nonbiodegradable sealant. A fully biodegradable microchip would eliminate the need for a second surgery after implanting the drug delivery device. Questions of the sealant's biocompatibility also arise. A fully biodegradable device would be completely biocompatible. This work evaluates two potential ways of fabricating a microchip device composed only of biodegradable, laminated parts. A final design is proposed based on the results described here.

3 Procedures

3.1 Film casting

Poly(D,L-lactic-*co*-glycolic acid) 2A (PLGA-2A, relative molecular mass 12,000, density = 1.303 g/mL) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Aldrich), dichloromethane (DCM), or chloroform (CF, Sigma) to achieve 5, 10, and 15% v/v solutions with each solvent. The solutions were injected in 177-µl volumes into silicone gaskets, backed by a glass slide, that each contained two 15 mm-wide, 2 mm-thick cylindrical holes. Each hole was covered with Teflon-coated aluminum foil to prevent the membrane from adhering to the slide. Total amount of injected solution depended on the desired thickness of the dried film (Table 1). Each 177-µl injection was followed by a ten-minute incubation.

Table 1. PLGA-2A Injection volumes (µl) depending on film thickness.

	5% PLGA-2A	**10% PLGA-2A**	**15% PLGA-2A**
Film thickness (µm)			
50	176.71	88.36	58.90
100	353.43	176.71	117.81
150	530.14	265.07	176.71

3.2 Microchip fabrication

Poly(L-lactic acid) (PLA, relative molecular mass 194,000 (M_t 194K), T_m=176°C, Medisorb 100 L; Alkermes) microchips were produced via a two-step system on a Carver Lab Press, model C. First, finely chopped PLA powder was compressed with 10,000 pounds at room temperature in a die to form a cylindrical chip. The preform was melted and remolded at 180°C, with and without the conical protrusions that create reservoirs in

the final microchip. The PLA microchip was polished to expose both ends of the conical reservoirs for a final thickness between 480 to 560 μm.

Polished PLA microchips with reservoirs were suspended on glass slides, and each reservoir was injected with 200 nl of PLGA/HFIP for a predicted membrane thickness of 150 μm. The devices were dried in a vacuum oven at 80°C for 48 hours. Three reservoirs with well-formed membranes in each device were selected and loaded with chemicals. After drying solutions for 10-15 minutes at room temperature and pressure, the large-end side of the device was sealed with Ideal 9144 Masking Tape (American Biltrite; Lowell, Massachusetts).

Two sizes of microchip devices were fabricated in the lab. Both were approximately 12 mm in diameter. The large-capacity microchip is about 1 mm thick, while the small-capacity microchip is 500 μm thick.

3.3 Microchip lamination

Lamination was used in two cases: in "mock laminating" a thinner, solid PLA chip to a PLA microchip or in attaching a PLGA-2A film to microchip. In the first scenario, PLA microchips were overlayed with a PLGA-2A film, and the combination was pressed with a force of approximately 2000 pounds for 5 to 20 minutes with the membrane facing upwards, in contact with a Teflon block. In "mock lamination", a secondary PLA chip without reservoirs was pressed against a microchip with injected membranes, but the two pieces were not actually attached. In lamination, the microchip is pressed between two plates, in which the upper one is heated to approximately 40°C

and the lower is maintained at room temperature. All laminations were performed on a Model C Lab Press (Carver).

^{14}C-dextran release. The ^{14}C-dextran was dissolved in deionized water, and injected into three reservoirs in each microchip. The microchips were placed in phosphate buffer saline (PBS) and maintained at 37°C to mimic the body's internal environment. At each timepoint, the PBS and microchip were swirled in each vial, and 500 µl PBS was removed to be analyzed in the TriCarb Liquid Scintillation Analyzer (Perkin-Elmer). The amount removed was replaced with fresh PBS to maintain a constant volume in the vial.

4 Design Proposal

4.1 Design 1 Proposal

The first proposed design replaces the nonbiodegradable sealant layer with a biodegradable PLA backing layer (Figure 3). This new layer would be thinner than the microchip and adhere to the microchip via lamination with a PLA film.

This design has its advantages and concerns. Since the drug is still released through a membrane individually injected into each reservoir, it would be easier to vary the type of membrane loaded in each of the microchip's 36 reservoirs. This would enable the device to behave with a pulsatile mechanism as the drug diffuses at different rates, depending on the membrane polymer (Grayson 2003). However, a major concern is whether the backing layer would serve as an adequate seal and prevent unwanted leakage from the device. And during fabrication, the injected membranes may also rupture

inadvertently due to the high force they experience during the lamination of the backing layer.

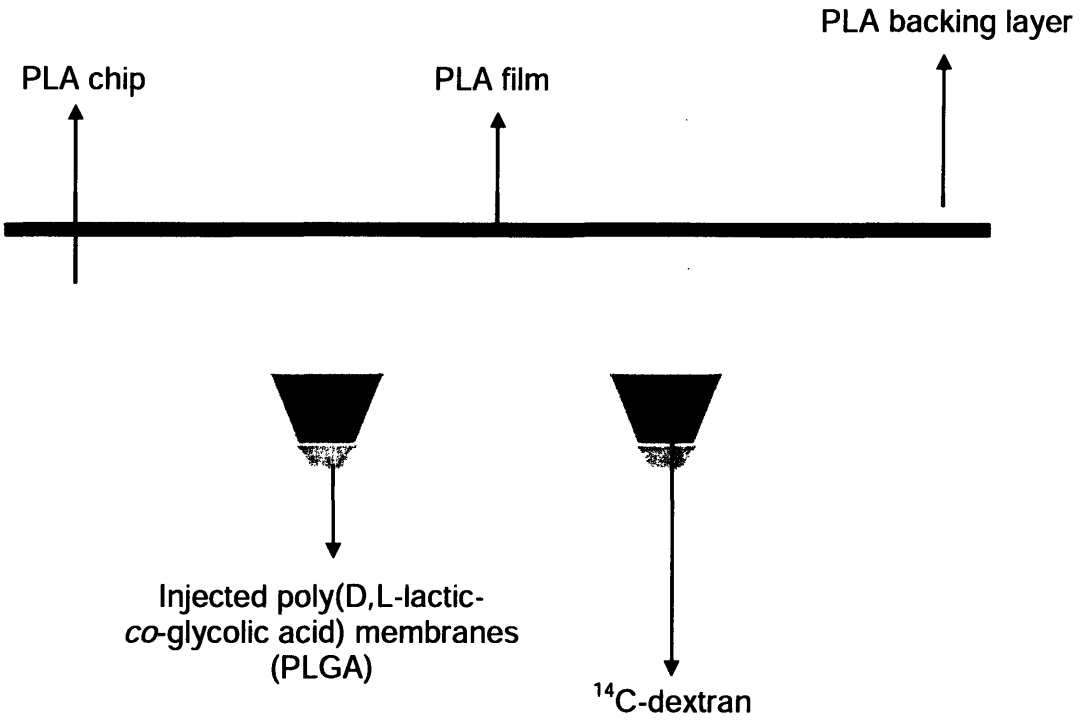


Figure 3. Simplified schematic for Design 1.

4.2 Design 2 Proposal

The second design replaces the injected membrane of the original microchip (Figure 4). Drug will diffuse from the device via a PLGA-2A film laminated over the reservoirs. If this design were successful, the microchip fabrication process would be altered so the device only has one open side, and the other side is closed.

Since individual membrane injection is eliminated in this design, the fabrication process would be faster. However, since even the smallest polymer film will cover multiple reservoirs, each one could not hold a different membrane, which is possible with

injected membranes. This limits the customizability and pulsatile behavior of the polymer microchip.

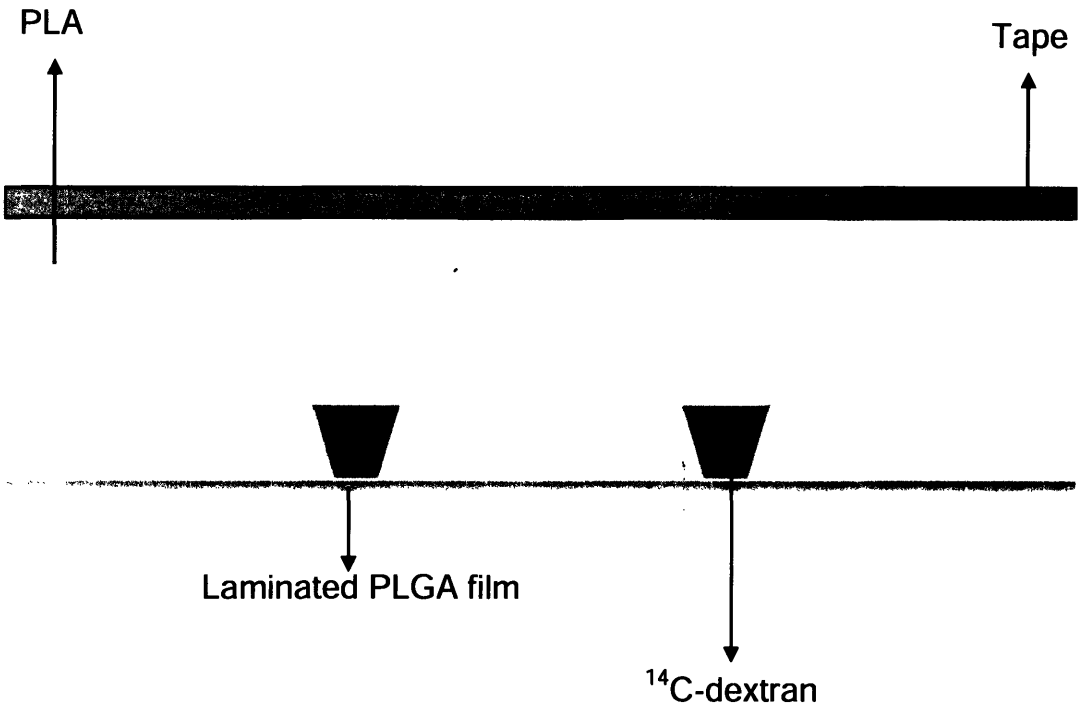


Figure 4. Simplified schematic of Design 2.

5 Results

5.1 Design 1 Results

PLA film cast in HFIP provides greater adhesion between PLA chips than one cast in CF. 50-μm thick films were cast with 5% PLA/HFIP or 5% PLA/CF and dried for 48 hours at room temperature. One of each type of membrane was used to join two solid PLA chips.

After approximately 24 hours of incubation, the chips glued with PLA/CF film could be pried apart easily with tweezers. The PLA/HFIP film provided a better adhesive

between the two chips as they could not be tweezed apart. After an additional 24 hours, the PLA/HFIP-glued chips were easily forced open with a razor, most likely due to greater evaporation of the solvent as the film dried.

Lamination does not affect injected PLGA-2A membrane behavior. Two microchips with injected PLGA-2A membranes were covered with a solid, thin PLA piece of the same radius and mock-laminated with 0.75 metric tons for twenty minutes. This experiment mimicked the process of applying a PLA backing layer onto the microchip without actually laminating them together. Three reservoirs on each microchip were loaded with ^{14}C-methylated dextran via injection, and the device was sealed on the open side. Once the device was placed in PBS solution, ^{14}C would leak from the device if the injected membranes had been ruptured in the lamination process.

The amount of ^{14}C that leaked from the microchip into PBS solution is measured in disintegrations per minute and recorded as a fraction of the total amount of ^{14}C injected in each microchip. Hence, a ratio of 1 means that all ^{14}C has exited the device. Both the unlaminated (control) and mock-laminated microchip membranes ruptured after 4.8 days in solution, indicating that the lamination process did not affect the membrane behavior in solution (Figure 5).

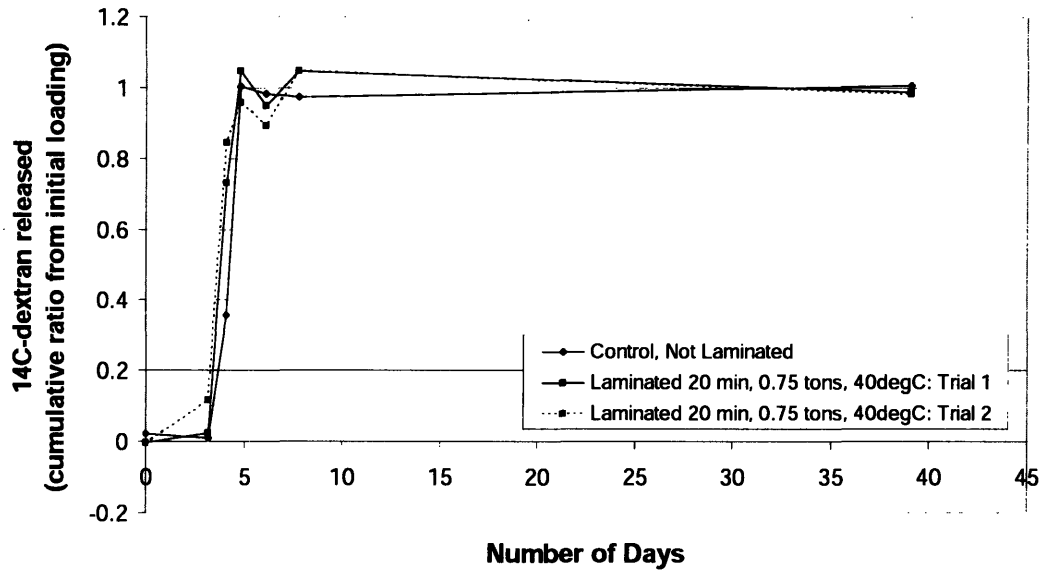


Figure 5. The PLGA-2A injected membranes, laminated or not, ruptured and released virtually 100% of the ^{14}C-dextran after 4.8 days. The DPM ratio signifies the fraction of ^{14}C that has exited the device, relative to the amount that was loaded.

Laminated PLA backing layer does not provide a satisfactory seal. Two large-capacity, nine-well microchips were sealed with a thinner secondary PLA chip of a smaller radius but still covered all the reservoirs. ^{14}C-dextran was injected into the reservoirs. The devices were laminated at 0.75 metric tons for 5 or 20 minutes with the upper plate heated to 105°F. The open side of the microchip was then sealed with tape. By isolating the backing layer, this experiment determined the rate of radioactive leakage via the backing layer alone.

Whether the PLA backing layer was laminated in 5 or 20 minutes, both leaked 40 to 80% of the total ^{14}C loaded into the microchip over forty days (Figure 6). An ideal seal would have shown negligible ^{14}C release. The PLA backing layer was not a successful method of sealing the device, and its performance did not significantly depend on how long the backing layer was laminated.

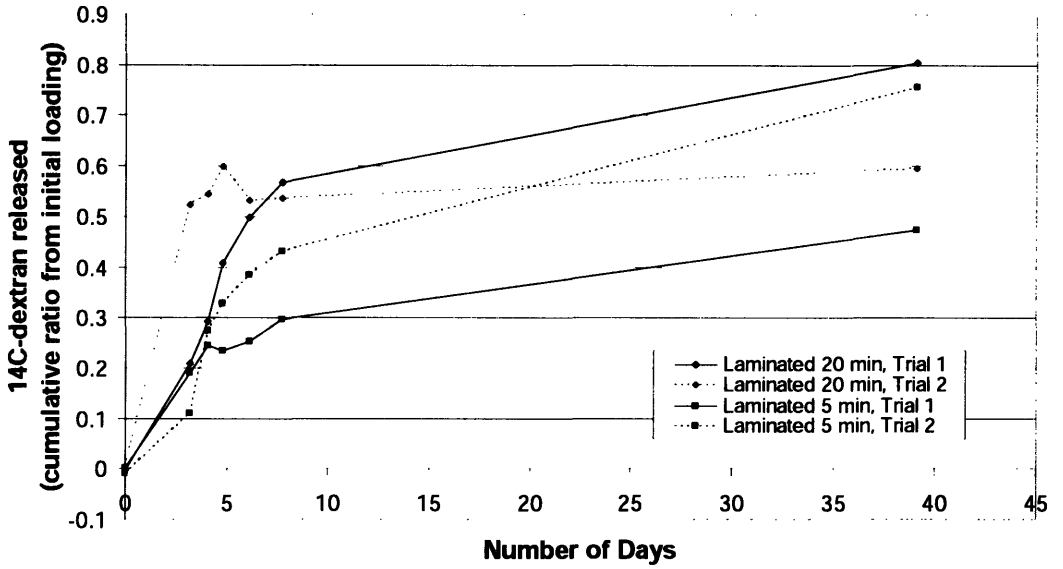


Figure 6. An ideally sealed microchip device would have leaked virtually none of the loaded ^{14}C. However, whether the PLA backing layer was laminated in 5 or 20 minutes, both leaked 40-80% of the ^{14}C over forty days. The ^{14}C-dextran release ratio indicates the fraction of ^{14}C that has leaked from the device, relative to the amount that was initially injected. This result suggests that the PLA backing layer is not a sufficient seal for the microchip device.

5.2 Design 2 Results

5.2.1 Design 2: Film Optimization

A 200 μm-thick PLGA-2A film cast in dichloromethane and dried at room temperature for 48 hours was the most favorable candidate for lamination. Before any film production, a scoring system to fairly evaluate each type of membrane was developed (Table 2). Scores ranged from 1 to 5 in 0.5 increments. The score indicated the level of film deformation after drying and detaching it from the glass. It did not consider the film performance in the lamination process. A film with a score of 3 would be considered a highly favorable specimen for future testing.

Table 2. Film Scoring System

Score	Description
1	Film flows if not held flat.
2	Film stretched when tweezed and remains attached to glass.
3	Film detaches from slide with minimal deformation.
4	Film breaks with tweezed, remains attached to glass.
5	Film is cracked, already detached from slide.

100 and 200-μm thick films were cast from 5% and 10% PLGA-2A in HFIP, DCM, and CF. After 24 hours, all films were too viscous to use as a film. A 48-hour incubation produced better dried films that ranked closer to the ideal score of 3 (Table 3).

300-μm and 450-μm thick films cast in 15% PLGA-2A in DCM and CF were also dried in vacuum at room temperature after an initial 24-hour room temperature incubation. They became brittle and possessed large bubbles. Bubble formation was observed within 5 minutes of starting vacuum, and they grew to excessive sizes within 30 minutes.

Table 3. Film Scores for Incubation Time Optimization

Solvent	% v/v	Film Thickness (μm)	Score after 24 hours	Score after 48 hours
HFIP	5	100	1.5	2
		200	1.5	2
	10	100	1.5	2
		200	1.5	2
DCM	5	100	2	4
		200	2	2.5
	10	100	2	4
		200	2	2.5
CF	5	100	2	2.5
		200	2	2.5
	10	100	2	2.5
		200	2	2.5

Film production was optimized by casting films on Teflon-coated aluminum foil.

Initially, all films were cast on plain glass slides, but they were found to strongly adhere

to the surface. To minimize interaction between the slide and film, two different approaches were tested. First, the glass was silanized (Sigmacote, Aldrich) and dried for several minutes. 10% PLGA-2A/DCM membranes were cast on the silanized glass and incubated at room temperature for 48 hours. They detached more easily and with less deformation than from non-silanized glass.

Second, the 10% PLGA-2A/DCM films were cast on glass covered with Teflon-coated aluminum foil and also dried for 48 hours at room temperature. This method proved the most effective, as it allowed for the films to be tweezed away from the gasket with less deformation than either normal glass or silanized glass.

5.2.2 Design 2: Film Lamination

A PLGA-2A film is a potential substitute for injected membrane. A 200-μm PLGA-2A film cast in dichloromethane was laminated over a 36-well small microchip. Carbon 14-dextran was injected into the reservoirs, and the microchip was sealed on the open side. This experiment determined the behavior of a PLGA-2A film, as opposed to an injected membrane version.

The PLGA-2A film demonstrated different release behavior than the PLGA-2A injected membrane. Within a 40-day span, the PLGA-2A film released nearly 100% of ^{14}C, but it did not completely rupture as the membranes did after 5 days (Figure 7). However, the exact behavior of the injected membrane is unknown because the release experiment lacked data points between 10 and 40 days. This initial result shows that in the future, the PLGA-2A film may be a useful component for controlled release from the microchip.

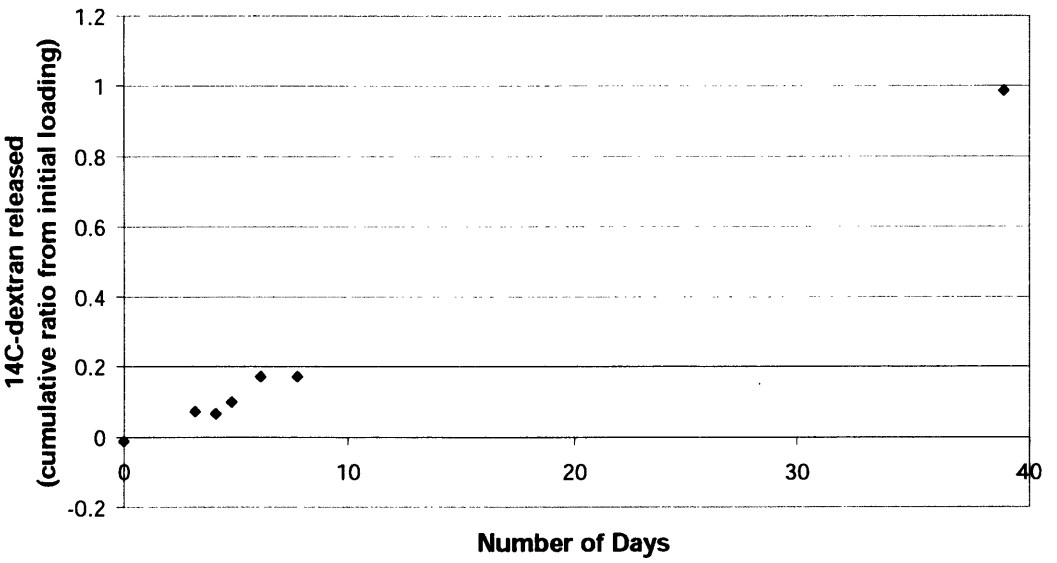


Figure 7. Within a 40-day span, the PLGA-2A film released nearly 100% of ^{14}C. The PLGA-2A film may be a useful component for controlled release from the microchip. This behavior is different from that of the injected PLGA-2A membrane, but its exact nature is still uncertain as time points between 10 and 40 day are lacking.

6 Discussion

Results show that a PLA backing layer is an insufficient seal for the device, but a PLGA-2A film is a potentially useful substitute for the injected membrane. A new design and fabrication process for a fully biodegradable polymer microchip design is proposed, based on Design 2 (Figure 8).

The polymeric microchip would only be open on one side, which contains larger reservoir openings, instead of both as it is in the original device. The openings would be sealed with a polymer film that allows for drug diffusion.

To fabricate a new design prototype, the microchip would no longer be polished so far as to truncate both ends of the conical reservoirs, which is the current standard.

Instead, the pointed end would remain sealed with the PLA chip. The target drug is loaded into the reservoirs, and the PLGA-2A film is laminated over the well openings.

This new design will also have concerns that must be addressed. Unlike the original device, drug now would diffuse through the larger end of each reservoir. The rate of drug diffusion for the new design may be significantly different from that of the original.

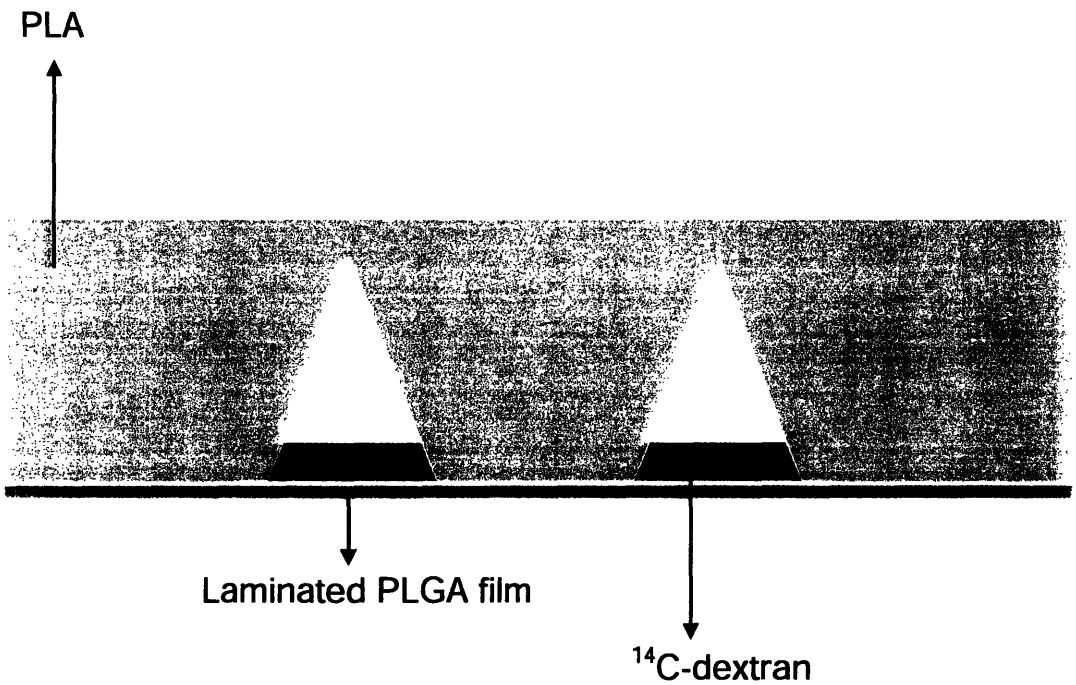


Figure 8. Simplified schematic for final, proposed biodegradable microchip design. To fabricate this new design, the microchip would no longer be polished to expose both ends of the reservoirs, and their conical shape would be preserved. A laminated PLGA film would cover the open ends of the reservoirs, allowing chemical to diffuse.

7 Future Work

Future studies should involve additional trials to further evaluate the two initial design proposals and later, the final design prototype. These will lend confidence to the conclusions drawn in this work.

In experiments for Design 1, solid PLA chips of a smaller radius were laminated as backing layers for large-capacity microchips. This difference in size between the backing layer and actual microchip could have attributed to increased leaking. In future trials, microchips should be laminated with backing layers of equivalent size.

For Design 2, further trials should be conducted to affirm the results obtained for a microchip with laminated film. Additional trials will also provide better information about the behavior of the film and how it allows chemical to diffuse, in comparison to the injected membranes. Especially, another ^{14}C-dextran release should be conducted and monitored more closely with frequent analyses for the laminated microchip.

If the new design demonstrates potential after supplementary trials for Design 2, the film lamination procedure should be optimized. This work used 0.25 metric tons to laminate the film, but a range of forces should be tested to determine the amount that will effectively impede leakage from the film while not tearing it.

Finally, for the newly proposed design, its release behavior may be different from the original device because the drug is now loaded and diffused from the larger end of each reservoir. The film itself also may cause release in a manner very different from the injected membrane. The chemical release behavior of the newly proposed design should be studied since it is the major element of any microchip device.

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