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Journal
Lab on a Chip, 17(2)

ISSN
1473-0197

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Publication Date
2017-01-17

DOI
10.1039/c6lc01338d

Peer reviewed

HHS Public Access

Author manuscript

Lab Chip. Author manuscript; available in PMC 2022 July 21.

Published in final edited form as:

Lab Chip. 2017 January 17; 17(2): 267–273. doi:10.1039/c6lc01338d.

Plasma free reversible and irreversible microfluidic bonding†

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Abstract

We demonstrate a facile, plasma free process to fabricate both reversibly and irreversibly sealed microfluidic chips using a PDMS-based adhesive polymer mixture. This is a versatile method that is compatible with current PDMS microfluidics processes. It allows for easier fabrication of multilayer microfluidic devices and is compatible with micropatterning of proteins for cell culturing. When combined with our Shrinky-Dink microfluidic prototyping, complete microfluidic device fabrication can be performed without the need for any capital equipment, making microfluidics accessible to the classroom.

Introduction

Fabrication of a functional microfluidic device necessitates a substantial seal between the device and substrate for leakproof encapsulation of the channels and chambers. This crucial step has been the focus for developing novel and versatile bonding techniques. While there are many different materials used for fabricating microfluidic chips, replica molding with polydimethylsiloxane (PDMS) is currently one of the most common prototyping procedure;[1] however, as PDMS does not readily adhere to most substrates, an adhesion step is required to strongly bond the PDMS device and substrate together. The ubiquitous method for sealing PDMS-based devices is *via* oxygen plasma treatment of both the PDMS and the substrate's surfaces before placing them in contact with each other immediately after activation. Oxygen plasma treatment activates the surfaces of both the PDMS device and glass substrate by replacing Si–CH_3 bonds with Si–OH groups. The surfaces bond irreversibly when the reactive −OH groups are put in contact with each other, forming a covalent Si–O–Si bond between the glass and the PDMS.[2] Although this process produces a strong and irreversible seal, it is a time sensitive step and necessitates access to an oxygen plasma machine. Moreover, this bonding method limits throughput because of the time dependency of the surface activation and the limited size of a typical oxygen plasma chamber. Additionally, once contact between the activated surfaces is made, removing the surfaces is no longer possible, making microfluidic chips that require tight alignment tolerances, such as 3D devices, difficult. Due to these limitations, alternate methods have been developed for irreversibly sealing microfluidic chips on glass and alternative substrates. For example,

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c6lc01338d

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popular alternatives include utilizing: corona treatment, partially cured PDMS, or chemical cross-linkers.[3–6]

While irreversible bonding is often sufficient for many microfluidic operations, there are certain circumstances where a reversible seal is advantageous.[7] For instance, in cell culture systems, where subsequent harvesting of the cell or tissue sample is required, easy access to the channels is desirable. However, research focused on reversible microfluidic bonding is limited,[7] with many of these methods requiring extra components or processing to create a reversible seal.[8–10] Alternatively, simpler sealing methods have also been proposed. Thompson *et al.* used double-sided tape to seal their PDMS devices.[11] They reported a bonding method that can withstand high-pressure operation. More recently, Shiroma *et al.* have reported a simple sandwich bonding method that produces a strong seal by sandwiching a glass coverslip against the channels with PDMS.[12]

Overall, methods for creating irreversibly or reversibly sealed microfluidic devices typically require capital equipment or specialized components, adding complication to the fabrication process. While fabrication of single layer devices is achievable with the aforementioned methods, the process for creating more specialized chips, such as multilayers devices or channels and chambers with functionalized surfaces, becomes more difficult. For example, any surface modification made on the microfluidic channels, chambers, or substrate must be able to withstand the subsequent bonding procedure used afterwards.

This need for compatibility between the adhesion layer and surface modification is exemplified with cell patterning within a sealed fluidic chamber. Micropatterning is one of the most widely used methods to spatially grow cells in a deterministic pattern; when combined with a microfluidic environment, it allows for greater control and manipulation of the cells.[13] Micropatterning is normally achieved by functionalizing the surface of the substrate in a specified pattern for cell adhesion; however, it is difficult to pattern cells within a fluidic device because the compatibility between the patterned area and the bonding step must be considered. While many have reported methods on the micropatterning of open substrates,[14–17] there have been relatively few reported methods for micropatterning within a fluidic device.[18–20] Moreover, the reported methods are often laborious, multistep processes meant for laboratories specialized in microfluidics, which greatly limits accessibility of this technology to general laboratories. Having a simple fabrication method without an additional adhesion layer would not only provide greater versatility of the device for cell research, but also increase accessibility of the platform to nonspecialized laboratories.

In this Technical Innovation, we demonstrate a simple and versatile plasma free bonding method that can achieve both a reversible and irreversible seal with microfluidic devices. Following convention, we choose to define irreversible bonding as a seal that can withstand greater than 207 kPa (ref. 21) in which the polymer surface is compromised upon removal; a reversible seal, on the other hand, allows for the device to be removed and then reapplied without any damage. Our process allows for facile fabrication of multilayer PDMS devices while also being compatible with micropatterning technique for patterned cell growth within a fluidic chamber.

Instead of applying an adhesive layer to bond the PDMS device and substrate together, we use a PDMS-based adhesive polymer as the substrate for direct adhesion of PDMS devices. The adhesive polymer can also be used to mold microfluidic devices. When cured, the polymer mixture exhibits high adhesion, which is leveraged as a sealing mechanism for a reversible seal against glass. Conversely, an irreversible bond can be achieved between the cured adhesive polymer and PDMS after a simple heat treatment of the two polymers in contact with each other. We applied the adhesive polymer with PDMS to demonstrate a facile process for fabricating an irreversibly bonded multilayer 3D microfluidic device (Fig. 1a and b); we also show the fabrication of a reversibly sealed device against glass. Lastly, we demonstrate the compatibility of this system with micropatterning by creating a large array of square islands for cell culturing within a fluidic chamber. Importantly, with this approach, laboratories and classrooms without any capital equipment can easily fabricate a larger variety of microfluidic devices.

Experimental

Device fabrication and bonding

The adhesive polymer is a mixture of a silicone-based soft skin adhesive (MG 7-9850, Dow Corning®) and traditional PDMS (Sylgard 184, Dow Corning®). Both polymers were first mixed separately and then combined to form the final adhesive mixture. The PDMS was prepared by mixing the cross linker and base at a 1:10 ratio by weight, and the soft skin adhesive was prepared by mixing part A and part B components at a 1:1 ratio by weight. The adhesive polymer mixture was then formed by combining the uncured PDMS and soft skin adhesive at a 1:40 ratio, respectively, by weight. Next, the final adhesive mixture was used to mold the microfluidic devices following the traditional replica molding process,[22] cured, and bonded to a glass substrate for a reversible seal (Fig. 2a). Alternatively, for an irreversible bond, cured adhesive polymer was spun coat onto a glass slide and cured; the cured adhesive polymer was then used as a substrate to bond traditionally molded PDMS devices (Fig. 2b). The PDMS device was placed directly on the cured adhesive polymer substrate, and heat treated to create an irreversible seal.

Burst pressure test

The bond strength of the interface was measured *via* a burst pressure test[11] for three different conditions: PDMS device to glass substrate (control), adhesive polymer device to glass substrate, and PDMS device to adhesive polymer substrate. For each condition, the pressure within a 3 mm diameter chamber was increased incrementally until failure occurred. The master mold for the chambers were fabricated by adhering 3 mm diameter circles, cut from Frisket Film (Grafix®), onto a flat PMMA surface. Afterwards, either PDMS or the adhesive polymer was poured into the molds, degassed for 15 minutes, and cured for at least 3 hours. The glass substrates were prepared by drilling inlet holes through cleaned glass slides; afterwards, commercially made press fit tubing connectors (Grace Bio-Labs, Inc.) were then adhered over the holes to serve as inlets for the tubing. The adhesive polymer substrate was fabricated by spin coating an additional layer of the 1:40 ratio adhesive polymer on the glass substrate at 800 rpm for 60 seconds and allowed to fully cure. Afterwards, the inlet holes were cleaned.

Device assembly occurred by placing the cured device chamber side down onto the substrate so that the center aligned with the inlet and press fit tubing (ESI† Fig. s1). Slight pressure was applied to ensure full contact between both surfaces. The devices were then heat treated in an oven at 120 degrees Celsius for 90 minutes.

The burst pressure test set up consisted of a closed tubing system that connected the 3 mm chamber to a 20 ml syringe and digital manometer (Dwyer Series 490). The pressure of the system was controlled using a syringe pump, which decreased the volume of the syringe by 0.5 ml intervals at a rate of 2 ml min^{-1}. Measurements were taken once the pressure equilibrated; the last stable pressure before bond failure for each device was reported. To determine reusability, three separate burst pressure measurements were taken for the same set of adhesive polymer devices bonded to the glass. After each test, the adhesive polymer was removed from the glass slide, washed with isopropyl alcohol, and dried in an oven at 60 degrees Celsius for 30 minutes. The glass substrate was also cleaned in the same manner. Both the glass slide and adhesive polymer device were additionally cleaned with Scotch® tape 3 times between testing.

Swell test

To compare the degree of swelling between traditional PDMS and the adhesive polymer, a swell study was done with five different solvents. Solid squares of the adhesive polymer and traditional PDMS, respectively, were made using the same replica molding process as described above. A set of 5 squares was used for each solvent. The pieces were submerged in separate containers and imaged with a DSLR camera (Canon EOS Rebel T3i) while immersed in solvent. After full immersion for 24 hours at room temperature, the pieces were then imaged again. The length of each edge was measured before and after from the digital image using ImageJ software. The solvents examined were acetone, isopropyl alcohol (IPA), ethanol, water, and dimethyl sulfoxide (DMSO).

Microfluidic chip fabrication

To demonstrate the reversible sealing capability of the adhesive polymer, gradient generating devices were fabricated using the Shrinky-Dink procedure, first developed by Grimes *et al.*,[23] and reused multiple times. AutoCAD® drawings of both designs were printed onto pre-stressed polystyrene (PS) using a laser printer. The PS was then shrunk in an oven at 160 degrees Celsius, allowing the ink to reflow to create rounded protrusions. The adhesive polymer was then poured into the mold, degassed for 15 minutes in vacuum, and cured at 60 degrees Celsius for 2 hours. A thin layer of PDMS was subsequently cured on top to serve as mechanical support for the inlet and outlet tubing insertion. Inlets and outlets were punched through the adhesive polymer and PDMS bilayer using a biopsy punch (Miltex®), and the surface of both the glass slide and the adhesive polymer were cleaned prior to bonding. The devices were placed chamber side down onto the cleaned glass slides and baked at 120 degrees Celsius for 90 minutes. For the gradient generator, the channels were primed with 70% ethanol before flowing blue and yellow food dye at a flow rate of 0.001 μl min^{-1}. This

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c6lc01338d

process also serves as a general proof-of-concept that the entire microfluidic device can be made without any capital equipment of clean room access.

A multilayer micromixer was fabricated by stacking alternating layers of PDMS and adhesive polymer (Fig. 1). The positive mold for each layer was fabricated by laser cutting the outline of the channel shape in Frisket Film adhered onto a flat PMMA sheet. Afterwards, the Frisket Film surrounding the channel was removed leaving only the positive channel structure. PDMS was used to mold the first and third layer of the device while the adhesive polymer was used to mold the middle layer. Once fully cured, the negative mold was then released, and inlet and outlet holes were punched using a biopsy punch. The device was then assembled onto a glass slide laminated with a layer of pre-cured adhesive polymer; each layer of PDMS and adhesive polymer were stacked sequentially, with the first layer adhered onto the pre-cured adhesive polymer glass slide. Slight pressure was applied and the construct was heated for 90 minutes at 120 degrees Celsius. Afterwards, the channels were primed with 70% ethanol, and food dye were flowed through the inlets. As can be seen from Fig. 1a, blue and yellow food dye were individually flowed through the first and second layer of the multilayer chip; the two food dye mixed in the vertical column connecting all three layers before flowing through the third layer. Fig. 1b shows the exploded view of the multilayer chip with the inlet and outlet holes aligned.

Cell patterning and culture

To show the facile integration of micropatterning within a fluidic device, cell patterning was performed by plating human stem cell-derived cardiomyocytes (hES2-7E) on the adhesive polymer within a PDMS fluidic chamber. A large oblong shaped fluidic chamber with a height of 1.52 mm was molded using PDMS; inlet and outlet holes were punched into opposite corners. A thin layer of the adhesive polymer was then spun coat onto a microscope slide, and allowed to cure at 60 degrees Celsius for 3 hours. Traditionally, silicone polymers display poor cell adhesion due to the materials' high surface hydrophobicity.[24] Pruitt *et al.* demonstrated that proteins necessary for cell adhesion can be covalently bonded to PDMS *via* an organosilane process using 3-glycidoxypropyltrimethoxysilane (GPTMS).[25] The adhesive polymer on the microscope slides were plasma treated with oxygen for 3 minutes and then incubated in a methanol solution of 20% GPTMS. To pattern in a deterministic manner, a shadow mask was applied to the adhesive polymer prior to the plasma treatment. Following the organosilane treatment, the surface was sealed by placing the PDMS chamber on top. The construct was then sterilized *via* autoclave, in which the high temperature helps to strengthen the bond between the PDMS and the adhesive polymer. After sterilization, Matrigel (Corning®) was flowed into the construct. Cardiomyocytes were then loaded at a density of 6.3×10^5 cells per ml. The contractility was confirmed and quantified with an optical flow-based method.[26]

Results and discussion

Characterization of bond strength and swelling

The soft skin adhesive is a FDA-approved, PDMS-based platinum catalyzed elastomer. By introducing varying amounts of standard PDMS to the soft skin adhesive, the stiffness and

tackiness of the polymer can be tuned. The adhesive nature of the polymer was leveraged as the bonding mechanism for sealing the device to the substrate through direct contact. A 1:40 ratio of the PDMS to the soft skin adhesive was found to have an optimal stiffness for molding while maintaining enough adhesion to bond to glass. However, the ratio can also be adjusted for other applications.

The adhesive polymer formed a reversible, bond when placed directly onto an untreated glass substrate; this bond is stronger than that of the PDMS control. Fig. 3a shows a cross-sectional schematic of the control, reversible, and irreversible conditions. Despite the increased bond strength, the polymer can still be reversibly removed without harming the channel footprint. The bond between the adhesive polymer and glass failed after 79 ± 5 kPa. As seen in Fig. 3b, this failure pressure is fourfold higher than that of the PDMS control, which failed after 21 ± 1 kPa. Failure of the bonds occurred *via* concentric delamination from the edge of the chamber outward towards the edge of the chip. Post removal, the adhesive polymer chambers were then washed and re-bonded to a glass substrate for reuse. We found no significant loss in the burst pressure with subsequent reuse of the devices (ESI† Table t1). This bond strength is sufficient for many microfluidic applications including: gradient generation, droplet generation, and cell culturing.[27–29]

Alternatively, an irreversible seal can also be achieved by bonding cured PDMS to a cured adhesive polymer substrate. As previously stated, Sia *et al.* defines irreversibly sealed devices as capable of withstanding 207–345 kPa;[21] the bond strength between the PDMS device and adhesive polymer substrate was able to withstand a pressure of 229 ± 2 kPa (Fig. 3b). In fact, bond failure did not occur at this point, but, rather, the upper limit of the manometer was reached. Moreover, there was no visual indication of delamination at this pressure, and subsequent removal of the PDMS chambers tore the adhesive polymer substrate. The boundary between the bonded region and the chamber of the substrate post-device removal is indicated in the inset image of ESI† Fig. s2, which shows a top down view taken using a 3D laser scanning microscope (Keyence VK-X 100 series); the bonded region was torn during the removal, while the chamber region remained undisturbed. Consequently, the adhesive polymer is softer than PDMS, and when a tensile stress is applied to remove the PDMS device, the adhesive substrate mechanically fails before the PDMS does. Although the PDMS device cannot be reused afterwards, this method provides a simple way for device removal by leveraging the adhesive polymer as a sacrificial layer. Moreover, this method is compatible with current microfluidic fabrication using PDMS replica molding and eliminates the need for oxygen plasma treatment.

There was no significant difference in swelling between the PDMS and adhesive polymer for all the solvents tested (ESI† Fig. s3), suggesting that the swelling behavior of the adhesive polymer is similar to PDMS. The solvents chosen were the most commonly found in a standard laboratory, and moreover, often used in cell culture protocols. The pieces were found to have swelled the most in IPA, followed closely by acetone; the swelling in the other solvents tested was found to be negligible. However, even the most significant swelling remained at 7% or below, making the adhesive polymer suitable for standard use within a common lab.

Gradient generator

A concentration gradient was created by reversibly bonding an adhesive polymer gradient generator device to glass (Fig. 4a). The channel height and width were approximately 32 μm and 180 μm, respectively, and blue and yellow food dye was flowed through the inlet to generate the gradient. After the initial operation, the gradient generator was removed, cleaned, and re-bonded. Fig. 4b–d shows the step-wise removal of the gradient generator from the glass slide after the second use. While the adhesive polymer can still mold conventional micron-sized channels, the polymer itself is still softer than PDMS. Thus, channels with lower aspect ratios will be more likely to deform and collapse onto the substrate with applied pressure. However, the adhesive polymer stiffness can be optimized to mold lower aspect ratio geometries.

3D microfluidics

Mixing in a 2D microfluidic environment is difficult to achieve due to the natural laminar flow regime of the small channel; however, this problem can be alleviated by introducing a 3D geometry that disrupts the laminar flow.[30,31] The 3D microfluidic chip is a three-layer micromixer interconnected with holes punched through each layer. The device consists of two inputs that allow fluid flow to travel through two separate layers before mixing and exiting through the last layer; in other words, blue and yellow food dye flowed through the first and second layer, individually, before mixing and flowing through the third layer. The layers are bonded irreversibly together by having alternate layers of PDMS and adhesive polymer.

Moreover, because the PDMS and adhesive polymer will not irreversibly bond until heat treated, this fabrication process allows for multiple attempts to position each layer. If the initial placement is not fully aligned, then the device can be removed and realigned. With the traditional plasma bonding method, the surface activation of the PDMS is time sensitive, and therefore the alignment and bonding of each layer must be done immediately upon activation, typically in a single attempt. As the adhesive polymer and PDMS do not irreversibly bond until after prolonged exposure to heat, multiple alignment attempts can be made for each layer without a significant effect on the bond.

Cell patterning

A large patterned square array was created on the adhesive polymer prior to sealing the microfluidic chip. As seen in Fig. 5a, functionalization of the surface for adhesion occurs right before the fluidic component is sealed over the substrate. Afterwards, human stem cell-derived cardiomyocytes were loaded and patterned on the substrate within the fluidic chamber. Fig. 5b shows two square islands of cardiomyocytes patterned on the substrate. Contractility was assessed using an optical flow based method, which generates motion vectors following the cardiomyocyte's contraction and relaxation, as seen in Fig. 5c principal component analysis (PCA) was then used to summarize the motion vectors generated from the optical flow into one variable that automatically discerns the contraction and relaxation phase of a contractile event (Fig. 5d). Contractility was evident within two days of cell seeding, and the cells were viable up to 150 days. Additionally, as discussed above, the PDMS chamber can still be easily removed from the adhesive polymer layer for easy access

to the cells. ESI† Fig. s2 shows the fluidic chamber and the subsequent removal of the device from the substrate.

We demonstrated that selective micropatterning can be performed on the adhesive polymer substrate and then sealed by a microfluidic device in a facile manner. The micropatterned areas is not affected by an additional adhesion layer (see process flow in Fig. 5a). Accordingly, other micropatterning techniques such as microcontact printing can be used to create functionalized patterns prior to sealing. Moreover, because the adhesive polymer is used as the substrate to bond PDMS devices, existing designs can easily be integrated with micropatterned surfaces. Due to the characteristic adhesiveness of the substrate, the PDMS chip can seal over any excess patterned area, allowing for a larger tolerance for device alignment. Thus, it is possible to create different patterns over a larger area without concern for alignment or bonding, making it simpler to integrate micropatterned cell culturing with microfluidics.

Conclusions

We demonstrated a simple and versatile system for fabricating both reversibly and irreversibly sealed microfluidic chips. The adhesive polymer used in this Technical Innovation demonstrates similar properties to PDMS, and we have successfully cultured fragile hESC-CM with this material for >150 days, but further characterization is ongoing. While the adhesion strength is lower than other values reported in the literature and the polymer stiffness is softer than that of PDMS, this system still meets common microfluidic operations while providing fabrication advantages. More importantly, the polymer shows promise in simplifying the fabrication procedure for PDMS-based devices.

Use of the adhesive polymer can be easily integrated into the standard PDMS soft lithographic process flow, simplifying the fabrication procedure while also allowing for higher throughput. When combined with the Shrinky-Dink microfluidic rapid prototyping method, fabrication of a completed microfluidic device can be accomplished from start to finish without the need for specialized equipment, such as an oxygen plasma machine, or a cleanroom. This would allow for microfluidics in a classroom or low resource setting area. This bonding method also enables simple fabrication of 3D microfluidic devices. Moreover, certain micropatterning techniques can be directly integrated into the fabrication procedure. Importantly, this process allows researchers and teachers who are not in specialized microfluidic laboratories, such as those in the biological field, to be able to fabricate and implement a microfluidic platform in a low cost and simple manner.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to thank Erik Werner and Da Zhao for graciously lending us equipment for this study. This work was supported by the National Science Foundation I/UCRC FRP (MicroSURF for Enhanced Diagnostics 1535592). Cell line and support for E. K. Lee was provided by subcontract from Novoheart, LLC. This study was partially supported by a contract from Novoheart. Principal Investigator Michelle Khine has an equity interest in

Novoheart. The terms of this arrangement have been reviewed and approved by the University of California, Irvine in accordance with its conflict of interest policies.

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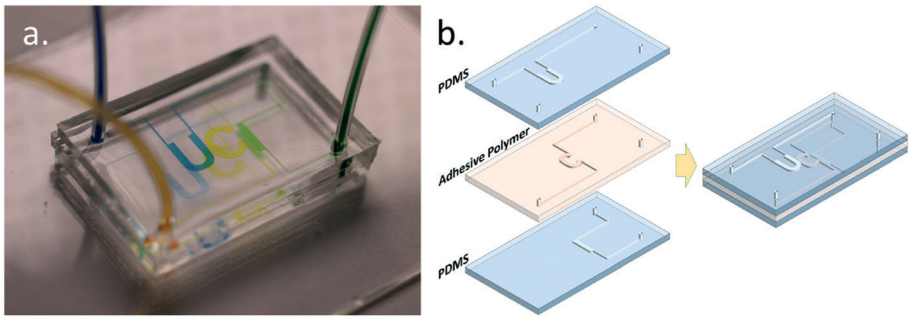

Fig. 1.

(a) Image of the 3D micromixer. (b) Schematic of the different layers. The top and bottom layers were molded using PDMS while the middle layer was molded with the adhesive polymer. The device was assembled on a glass slide coated with cured adhesive polymer.

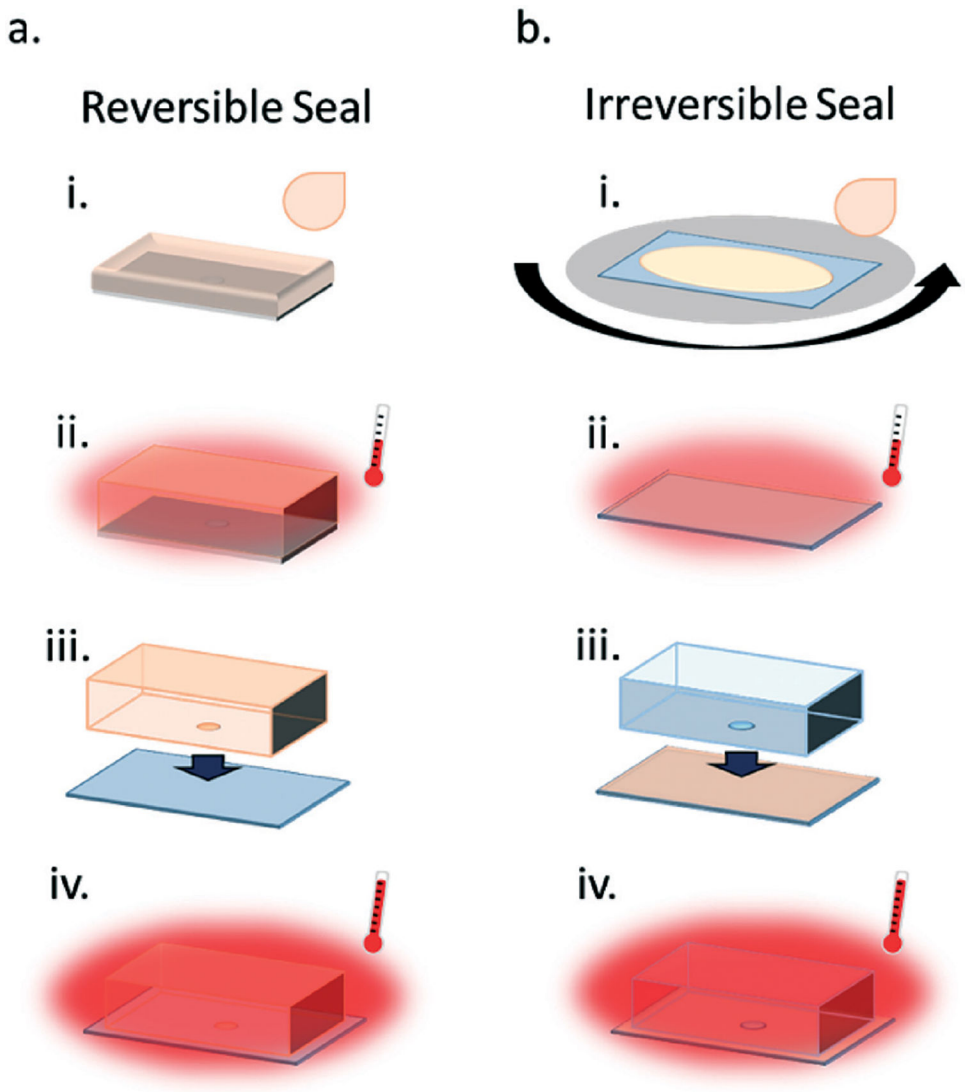

Fig. 2.

(a) Process flow for fabricating the reversibly sealed device. (i) A master mold was first casted with adhesive polymer. (ii) The polymer was then cured at 60 degrees Celsius. (iii) Afterwards, the polymer was removed and placed onto a clean glass substrate. (iv) The construct was then heated at 120 degrees Celsius for 90 minutes. (b) Process flow for fabricating the permanently sealed device. (i) A thin layer of the uncured adhesive polymer was first spun coat onto a glass slide. (ii) The polymer was then cured at 60 degrees Celsius. (iii) A traditionally casted PDMS microfluidic mold was placed onto the cured adhesive polymer substrate. (iv) The entire device was heat treated at 120 degrees Celsius for 90 minutes.

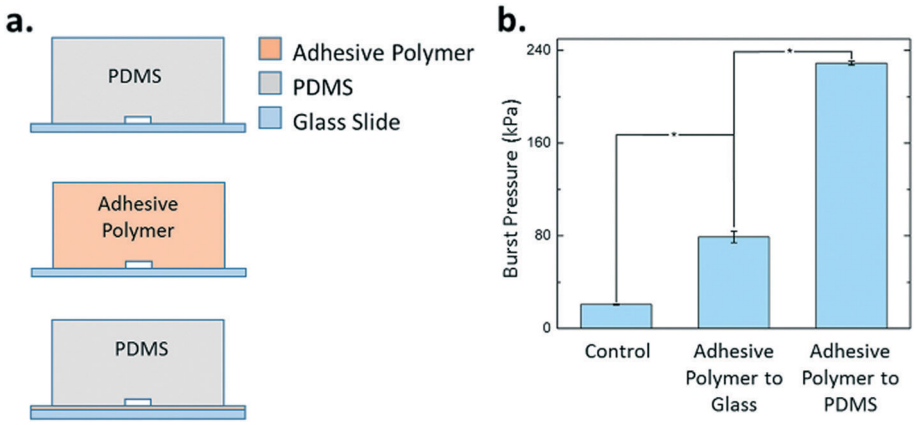

Fig. 3.

(a) Cross sectional diagram of the three test conditions for the pressure burst test. PDMS adhered directly onto the glass slide served as the control. (b) Graph of the last stable pressure before bond failure occurred for each of the conditions.

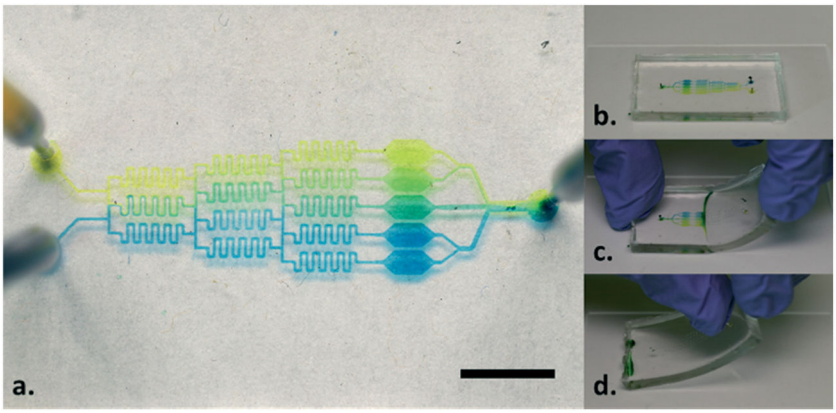

Fig. 4.

(a) Reversibly sealed microfluidic gradient generator with blue and yellow food dye. Scale bar is 10 mm. (b–d) Sequence for removal of the adhesive polymer device from the glass substrate after second use.

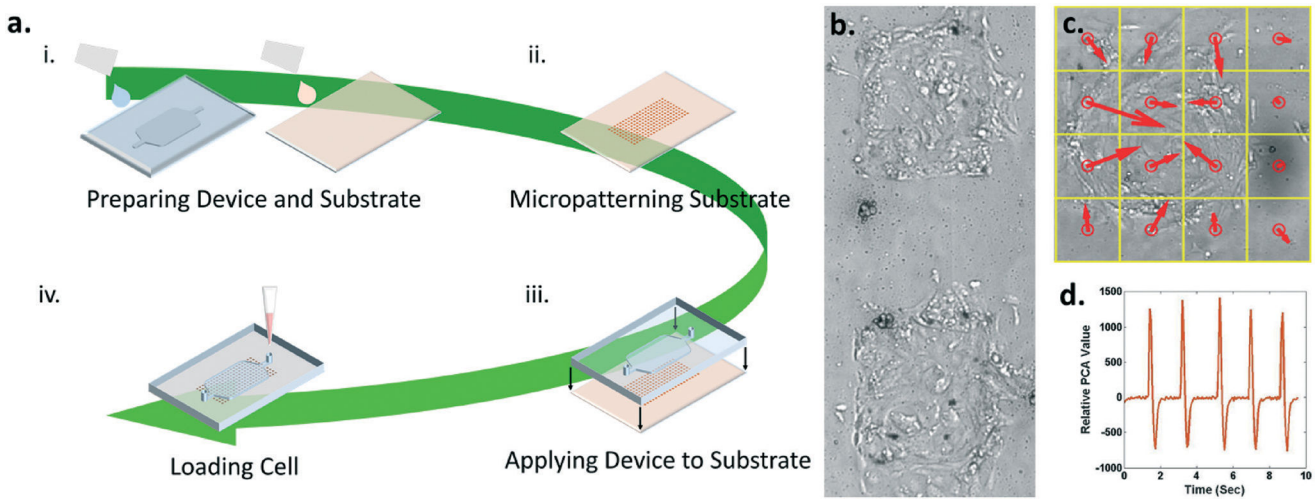

Fig. 5.

(a) Process flow for sealing the micropatterned substrate within a PDMS device. (i) The PDMS device is molded *via* replica molding, and the substrate is made by depositing a layer of adhesive polymer over a glass slide *via* spin coating. (ii) The micropattern is formed on the cured adhesive polymer substrate. (iii) The PDMS device is sealed against the substrate through direct contact. (iv) Cells are loaded into the construct. (b) Two patterned square islands with live cardiomyocytes. (c) Motion vectors (red arrows) of the cardiomyocyte contractions generated using optical flow. (d) Graph of the first PCA from the optical flow.