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Green Microfluidics Made of Corn Proteins

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Abstract—**Petroleum-based polymer such as Poly(dimethylsiloxane) has been widely used to make mesoscale and microscale fluidic devices. The main drawback of such devices in disposable applications is the potential environmental pollution since they are not biodegradable. Biodegradable microfluidic devices have been fabricated out of zein, a prolamin protein found in corn, that can be utilized as disposable health and environmental-friendly micro-chips. Using stereo lithography and soft lithography, micro-chambers and micro-channels features have been replicated on zein films and enclosed zein microfluidic devices are created by bonding to glass substrate using a simple vapor-deposition method. The bonding strength of the zein microfluidic devices has been found to exceed the tensile strength of the zein film and hydraulic pressure, and fluid flow through large-area complex microfluidic designs shows no leakage or distortion. High optical clarity and fluorescent imaging in the zein microfluidic devices are demonstrated by visualizing micro-particles and Rhodamine B. Zein microfluidic devices enable truly disposable microfluidics with intrinsic biocompatibility and biodegradability that can be fabricated using existing techniques.**

I. Introduction

Commercial zein is produced from corn gluten meal, which is a by-product of bio-ethanol industry [1]. The properties of zein film can be engineered for specific applications by varying the preparation techniques. Formulations of zein film with cross-linking reagents (e.g. glutaraldehyde) and incorporating plasticizers (e.g. oleic acid) improve the mechanical properties of zein film [2]. The level of water absorption of zein film is controlled by plasticizers used, and the microfluidic device made of zein can have controllable water permeability. The type of plasticizers used also affects the mechanical properties of zein films. The use of hydrophobic plasticizers instead of hydrophilic plasticizers in improving the tensile properties resulted in more stable films over a wide range of relative humidity [3]. The controlling of the zein film topography has been studied. It was found that the topography of zein varied from featureless to many wells and pores on the film surface, which can be used for delivery vehicles. Another study also demonstrated that zein film surfaces can be engineered to yield different hydrophobic/ hydrophilic properties based on solvent type and UV/ozone treatment [4].

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Zein has also been studied extensively for applications in fields of coatings, adhesives, food packaging, drug and functional food deliveries [1]. Moreover, there has been increasing interest in using zein for medical applications such as scaffold materials because of its biocompatibility. Zein matrices were found to provide human liver cell and mice fibroblast growth and attachment [5]. The cell attachment on zein matrices could be promoted by chemical modification, and thus cell culture in zein microfluidic devices is also viable.

Microfluidic components are widely used in the design of both bioanalytical and diagnostic micro-devices. It has opened many potential application areas in engineering and biomedical research fields, for instance, microscale chemical analysis [6], micro-mixing [7], and cell culture [8]. Moreover, biopolymers like zein can potentially simulate *in vivo* physiological environment, which is desirable for microfluidic devices in biological applications [9]. In our preliminary study, zein films with several microstructures such as channels, grids and wells were fabricated using the well-accepted technique of soft lithography [10, 11]. Various micro-scale features, an array of square wells with 35 µm depth and 30 µm length and 500-µm wide and 35-µm deep channels, were replicated on the surface of zein films.

In this work, we present microfluidic devices with complex fluidic pathways made from zein, fabricating using an energy-efficient vapor deposition method. We assessed the fidelity of replicated microfluidic features on zein films before and after bonding. We characterized the optical properties of the zein microfluidic devices using fluorescent and colorant solutions, and microparticles. Zein microfluidics demonstrate accurate formation of complex microfluidic channels that can withstand fluidic pressure. This work demonstrates the potential of zein microfluidics as a biodegradable device that can be used in disposable applications in medical, environmental, and agriculture settings. The advantages of zein-based devices are its excellent biodegradability, biocompatibility, gas permeability and mechanical properties.

II. Fabrication of Zein Microfluidic Devices

A. Preparation of Stereolithography and Soft Lithography masters

Stereolithography (SLA) masters with microfluidic channels, which are designed using SolidWorks (Waltham, MA, USA), a computer-aided drawing program, are fabricated using a three-dimensional printing system (3D Systems, South Carolina, USA). Soft lithography masters are prepared by replicating the SLA master using polydimethyl(siloxane) (PDMS). The PDMS solution (10:1 v base/v curing agent) is casted over the SLA master at ambient condition for 1 hour to degas and cured at 70 ^{o}C for 1 hour.

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B. Fabrication of Zein Film

Zein (90% protein) with 100 wt. % oleic acid (Sigma Aldrich, Milwaukee, WI) and 5 wt.% monoglyceride (Caravan Ingredients, Lenexa, KS) is dissolved in warm aqueous ethanol (75% v/v). The mixture is poured on top of the PDMS master and dried in a desiccator for 72 hours. The thickness of the zein film is set by controlling the volume of the zein solution during casting step.

C. Fabrication of Zein Microfluidic Devices

A 95% v/v ethanol solution is heated to 65 ^{o}C in a 500 mL beaker and a zein film with microfluidic features, held 6.8 cm above the surface of ethanol solution, is exposed to the ethanol vapor for 5 minutes. The ethanol-exposed zein film is pressed to a degreased glass slide to ensure complete contact between the two surfaces. A 500-gram weight is placed on top of the bonded zein film for five minutes at room temperature. The same bonding procedure was followed for the fabrication of zein-zein devices.

III. CHARACTERIZATION OF ZEIN MICROFLUIDIC DEVICES

A. Scanning Electron Microscope (SEM) Examination of Microfluidic Features

An electron microscope (FEI Company, Hillsboro, OR) used to visualize the cross-sectional area of the zein microfluidic devices. Based on the SEM images (Fig. 1) of the cross sections, the zein film can replicate microfluidic features, which include and 500-µm wide channels with a depth of 200 µm (Fig. 1a). The cross-sectional geometry of the channel on zein film (Fig. 1b) is slightly deviated from the original rectangular geometry of the SLA master. Due to the elastic nature of PDMS master, the replica channel on zein film appears trapezoidal.

The bulk zein film contains smooth layers of zein protein and it contains no observable defects such as cracks or discontinuities, the melted zein layer cannot be distinguished from the bulk zein film optically. Between the glass and the zein film, there is no observable gap, which shows the strong bonding between the zein film and the glass layer (Fig. 1c). There is no observable seam between the two bonded zein films using the solvent bonding technique (Fig. 1d). The width of the channel in the zein-zein microfluidic device is approximately 435 µm and the height of the channel is approximately 200 µm. Also, the micro-channel geometry after bonding in the zein-zein microfluidic device is more oval-shaped compared to the geometry on the zein-glass microfluidic device.

The vapor-deposition bonding method retains the original shape of the zein microfluidic channel well, as seen in the SEM images of zein-glass and zein-zein samples. The vapor deposition bonding method allows zein to bond to a glass slide or a zein film with less debris at the interface compared to the samples bonded by solvent bonding method. Moreover, this bonding technique is sufficient to bond zein microfluidic film with minimal channel distortion especially when bond to soft material like another zein film. Another advantage of vapor deposition method is that the inlet and outlet of zein microfluidic film can be punctured before bonding without melted zein flowing inside the channel.

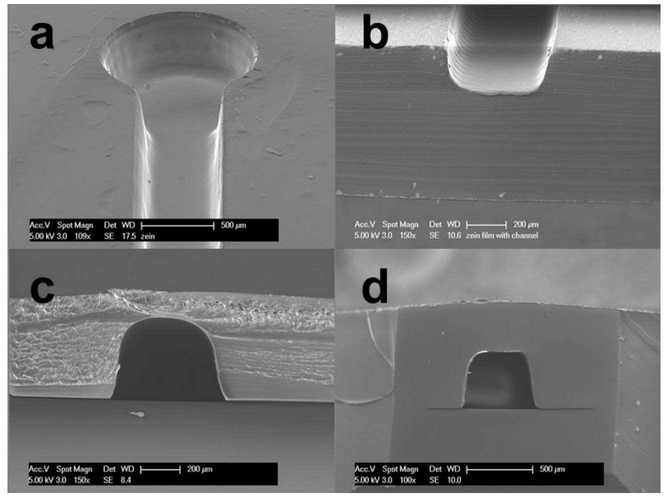

Fig. 1. Scanning electron microscope images of zein microfluidic channels. Zein film can accurately replicate the microfluidic features, which include 500-µm channel and 1-mm chamber (a, b). Cross sections of zein films bonded to glass substrate (c) and to another zein film (d) show minimal distortion of the microfluidic channel.

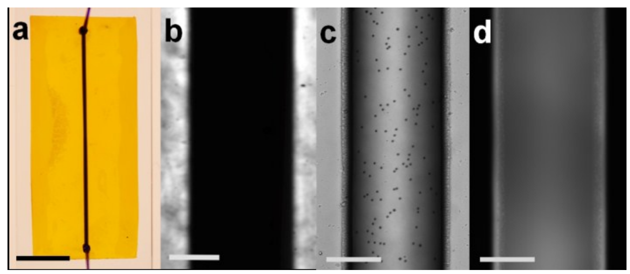

Fig. 2. Visualization of colorant, microparticles, and fluorescent molecules in zein-glass microfluidic device. (a) Macro photograph image of crystal violet stain fluid flows in a zein-glass microfluidic device with tubing at the inlet and outlet ports (scale bar: 10 mm). (b) Micro image of blue food coloring inside the zein-glass device to show the strength of channel bonding (and lack of leakage) (scale bar: 250 µm). (c) 10-µm microspheres inside the zein-glass device to show good visibility of the device (scale bar: 250 µm). (d) Rhodamine B stain inside the zein-glass device to show the low auto-fluorescent level of zein film in contrast with Rhodamine B (scale bar: 250 µm).

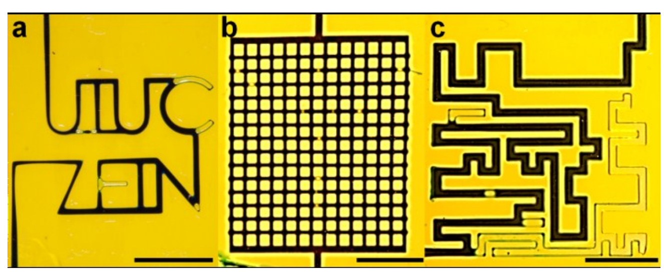

Fig. 3. Visualization of zein microfluidic devices with complex fluidic pathways. (a) Interconnected letters composed of continuous microfluidic channels, (b) a microfluidic network with channels and chambers, (c) a solved microfluidic maize maze with multiple false paths. Blue food dye was used for visual aid. All scale bars are 5 mm.

B. Optical and Fluorescence Characterization of Zein Microfluidic Devices

Several types of solutions were flowed through zein-glass devices bonded by ethanol vapor deposition (Fig. 2). Crystal violet stain solution was observed to flow only inside the channel and did not leak out at any point along the length of the microfluidic channel of zein-glass microfluidic chip with tubing at the inlet and outlet ports (Fig. 2a). Microscope images of the microfluidic channel revealed the presence of the blue food dye inside the microfluidic channel, and due to the complete encapsulation of the dye solution, the boundary between the microfluidic channel and the walls is clearly defined (Fig. 2b). Fig. 2c shows the solution containing 10-μm particles flowing through the zein-glass microfluidic devices bonded by vapor deposition method. The flow of microparticles can be observed through the glass side of zein-glass microfluidic device. Zein like other biomaterials has auto-fluorescent property. However, the auto-fluorescent level of zein is much lower than the fluorescent signal of fluorescent dyes such as Rhodamine B (Fig. 2d).

Zein microfluidic devices with complex fluidic pathways were fabricated. The food colorant was successfully flowed through an interconnected letter channel (Fig. 3a), a microfluidic network with channels and chambers (Fig. 3b), and a solved microfluidic maize maze with multiple false paths microfluidic network (Fig. 3c). A complete confinement of the food colorant through the entire length of the microfluidic channels demonstrates the quality of zein-glass bonding over a large surface area (>100 mm^2) and the resistance to hydraulic pressure.

C. Characterization of Bond Strength of Zein Microfluidic Devices

The strength of the bond between the zein film and the glass slide was measured using a pull or stretch test (Fig. 4a and b). The strength of the bond is greater than the tensile strength of the zein film as the un-bonded portion of zein film was torn apart while the bonded portion of the zein film to glass remained undistorted during the test. The tensile strength of un-bonded zein films is measured to have a mean value of 4.95 MPa and a standard deviation of 0.04 MPa (n=4). During the stretching process, film necking was observed near the portion of the zein film that was bonded to glass. Post-stretching observations revealed the zein film breakage occurred near the film-glass slide interface and the break line of the film was straight which probably due to uniform bonding across the attached area. The bonded portion of the zein film did not show any translation with respect to the glass slide.

The bonding technique used in this study was based on the thermoplastic nature of zein. Zein has been known for its adhesion properties. It was used as an adhesive for bonding glass-to-glass pieces and as a binder for cork and an adhesive for wood blocks. Zein could provide stronger adhesion than when polyvinyl acetate adhesive was utilized [12]. In our experiment, the zein surface was thermally induced and thinly coated with aqueous ethanol. This process allows zein polymer at the thin solvated layer to become more mobile and be able to diffuse across the solvated layer to another mating surface. For instance, the entire channel wall of the zein-zein microfluidic device is made of the same material and thus has the same surface properties. With ethanol assisting, the entanglement of zein polymers takes place across the interface of zein-zein microfluidic device resulting in seamless bonding.

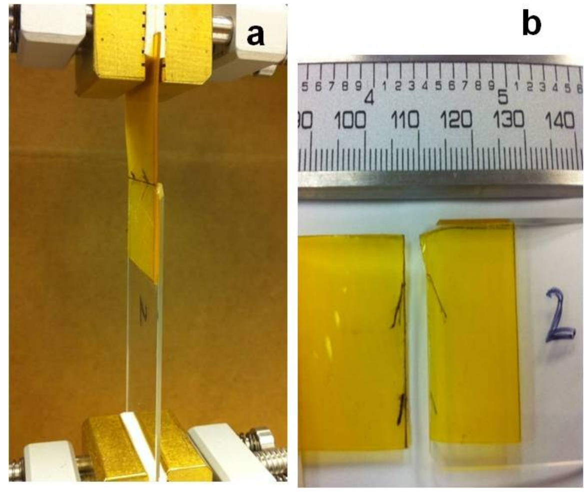

Fig. 4. Experimental set up of bonding strength test for measuring zein-glass devices bonded by ethanol vapor deposition (a) and the broken zein film after the test (b).

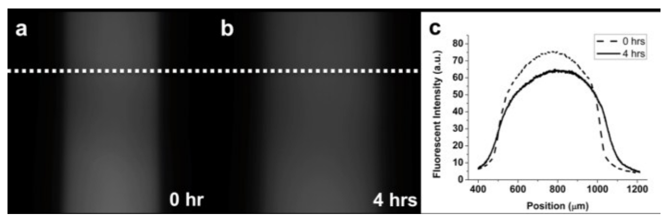

Fig. 5. Characterization of Rhodamine B absorption into zein film. Zein-zein device filled with Rhodamine B solution at the concentration of 0.1mM, the fluorescent profile was taken overtime along the white dotted line at time = 0 hour in (a) and at time= 4 hours in (b). (c) The fluorescent profile of Rhodamine B at time=0 hour (dash line) and time= 4 hours (solid line) across the channel.

A similar scenario occurred for zein-glass microfluidic device except that glass is a dissimilar material. Only the thin solvated zein layer adheres to glass slide. The excess amount of ethanol caused the geometry to be distorted, which can be avoided by using the vapor deposition bonding technique. The ethanol vapor molecules diffuse to the surface of zein microfluidic film resulting in mobile zein polymer thin layer only at the surface that bond to another mating surface.

D. Characterization of Fluid Permeability of Zein Microfluidic Device

In comparison with PDMS microfluidics, a distinctive property of the zein microfluidics device is the controllable material permeability to aqueous solutions, which potentially enables new applications. Absorption of Rhodamine B in both zein-zein and zein-glass devices was characterized by static imaging of the absorption of Rhodamine B solution. After the channel was filled with Rhodamine B solution, it was imaged up to 4 hours at 1-hour interval and the fluorescent intensity profile is obtained over the cross-section of the channel (Fig. 5a-c). The diffusion coefficient of Rhodamine B in zein matrix was calculated fitting the fluorescent intensity profile to Fick's second law of diffusion. The mean value of diffusion coefficient for zein-

zein microfluidic device and zein-glass microfluidic is $0.79 x 10^{-12} m^2/s$ and $0.17 x 10^{-12} m^2/s$ respectively ($10^{-12} m^2/s$ for polydextrose, $10^{-10} m^2/s$ for starch, and $10^{-10} m^2/s$ to $10^{-8} m^2/s$ for wheat) [13].

IV. Conclusion

For the purpose of micro-fabrication applications, zein films can be used to reliably replicate micro-scale features (e.g. channels, posts, chambers), and strongly bond to glass slides and zein films while retaining the micro-scale features. Zein film can be bonded easily and quickly to different kinds of material without requiring expensive equipment such as oxygen plasma generator. The application of zein microfluidic device can be far reaching due to its biocompatibility, bio-degradability and renewability. As a consideration for the more environmental and agricultural comparative alternative to PDMS, zein microfluidic devices have been shown to have comparable bond strength and similar processes of fabrication that do not require any new equipment beyond standard ones. The main advantages of zein over PDMS is that zein, the replenishable corn protein, has been demonstrated for biodegradability, which is important for a "greener" approach in the field of portable and disposable micro-devices. We intend to use the zein microfluidic devices in biological applications such as cell toxicity screening, cell culturing, and material absorptive applications.

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