Lab on a Chip

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TECHNICAL INNOVATION

Biocompatible "click" wafer bonding for microfluidic devices

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We introduce a novel dry wafer bonding concept designed for permanent attachment of micromolded polymer structures to surface functionalized silicon substrates. The method, designed for simultaneous fabrication of many lab-on-chip devices, utilizes a chemically reactive polymer microfluidic structure, which rapidly bonds to a functionalized substrate *via* "click" chemistry reactions. The microfluidic structure consists of an off-stoichiometry thiol-ene (OSTE) polymer with a very high density of surface bound thiol groups and the substrate is a silicon wafer that has been functionalized with common bio-linker molecules. We demonstrate here void free, and low temperature (< 37 °C) bonding of a batch of OSTE microfluidic layers to a silane functionalized silicon wafer.

Introduction

Polymer labs-on-chip (LOCs) offer numerous advantages over their silicon and glass counterparts since they encompass a wider range of material properties, involve less complicated and less expensive manufacturing processes,[1] and allow for mechanically flexible components and low temperature bonding.[2] However, biocompatible bonding to functionalized substrates has proved challenging, and no entirely satisfactory method has been shown yet. The most common strategy for bonding microfluidic device parts is gluing.[3–5] While effective and biocompatible, great care must be exerted to ensure that liquid glue does not block microfluidic features,[6,7] which tends to add complexity and cost to the back-end processing. Heating thermoplastic materials above T_g (~100 °C for typical microfluidic device plastic materials) to enable thermal fusion bonding requires stringent spatial control to avoid damaging biofunctionalized surfaces, which adds unwanted complexity to the bonding step. In an innovative study conducted by Bart *et al.*,[8] activation of a fluorinated sheet (FEP) involving the room temperature reaction of EDC-NHS was shown. This sheet was subsequently used to bond at low temperature an amine activated glass top and an amine activated silicon substrate. The drawback of this method is the 15 h long bonding process, and the formation of a high molecular weight by-product that will remain in the bond. In fact, the authors point to "click" chemistry as an ideal candidate to negate the problems they encountered during their work.

The term "click chemistry", coined by Sharpless,[9] is a class of efficient and selective chemical reactions that are used to join molecules together in a rapid manner with high yield, high purity and little or no by-product.

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We have recently introduced a family of off-stoichiometry thiol-ene (OSTE) polymers developed specifically to bridge the gap between research prototyping and commercialization for lab-on-chip applications.[10] OSTE polymers are compatible with soft lithography processes for easy fabrication without clean-room access. In contrast to PDMS, these novel OSTE polymers feature tunable mechanical properties, have a large number of surface anchored thiol groups that are capable of participating in "click" reactions with many functional groups allowing for easy one-step surface chemistry modification, are surface patternable using UV-light, and are designed to soften when heated above their glass transition temperature (T_g) of 37 °C to conform with microirregularities on the surface when a light pressure is applied. The latter allows OSTE polymers to form a perfect seal to the substrate, which maximizes the adhesion forces between the device and the substrate.[10] The efficacy of the OSTE concept was previously shown on device level for sensor packaging, where an OSTE microfluidic structure spontaneously formed a leakage free bond to the gold surface of a quartz crystal microbalance, QCM,[11] and as "biostickers" where OSTE microfluidic structures spontaneously bonded under biocompatible conditions to spotted protein and DNA microarray surfaces.[12] In this contribution, we extend the versatility of the OSTE concept by utilizing "click" chemistry reactions for single step biocompatible bonding of a batch of microfluidic structures to surface functionalized wafers. This enables low complexity fabrication of large numbers of identical microfluidic devices, useful for both device development and manufacturing.

Wafer level bonding *via* "click" chemical reactions

In this paper, we extend the functionality of the OSTE polymers by utilizing the thiol excess in the material to covalently "click" bond sheets of micropatterned polymers to silicon wafers (Fig. 1A) that were chemically surface modified with either (i)

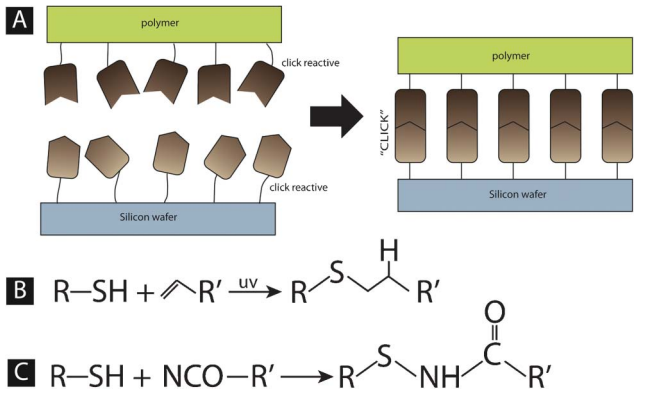

Fig. 1 The "click" chemistry reactions utilized for bonding: (A) a cartoon depicting the exact match between the "click" reactive groups, the high reaction efficiency and the suppression of side reactions; (B) the UV-initiated vinyl-thiol "click" reaction; and (C) the spontaneously occurring thiol-isocyanate "click" reaction.

vinyl silane or (ii) 3- isocyanatopropyl triethoxysilane (IPTES), which is a commonly used linker for attaching proteins. In (i) covalent bonds are formed *via* a UV-light activated radical addition between a thiol and a vinyl (Fig. 1B) to form a thioether and in (ii), a thiocarbamate bond is formed by a nucleophilic attack by the thiol on the isocyanate carbon (Fig. 1C).[13] Even though the mechanisms are very different, they both provide typical "click" chemistry characteristics.

The fact that the fabrication is carried out on wafer level leads to significant advantages, such as a reduced back-end process time and facile integration of microfluidics with CMOS electronics[14] or MEMS structures. Importantly, no leachable compounds that hamper bond integrity remain in the bond area, in contrast to solvent bonding. Furthermore, as this fabrication technique involves only low temperatures, biofunctionalization is performed on the OSTE polymer or the substrate prior to bonding, hence avoiding the need to functionalize inside closed channels.

Fabrication

To characterize the capabilities of the wafer level OSTE bonding technology, we fabricated four different devices each designed to test and characterize important characteristics such as bond strength, bond integrity and 70% stoichiometric thiol excess OSTE (OSTE-70) materials properties such as solvent resistance and permeability to small molecules:

Device 1 was designed to evaluate the wafer level bonding of a micropatterned OSTE-70 sheet to an IPTES coated Si surface with channel dimensions' of 10 mm × 0.5 mm.

Device 2 utilized the same geometries as Device 1, but vinyl silane was used to chemically surface modify the wafer.

Device 3 was designed to evaluate deformation of an OSTE-70 channel roof that was bonded to a silicon piece containing pre-etched channels and pre-treated with vinyl silane.

Device 4 was designed to measure the burst pressure of an OSTE-70 layer bonded to a vinyl silane surface modified silicon substrate.

The following three subsections give a detailed description of the fabrication of Devices 1 and 2. Fabrication of Device 3 and 4 are described in the last two subsections.

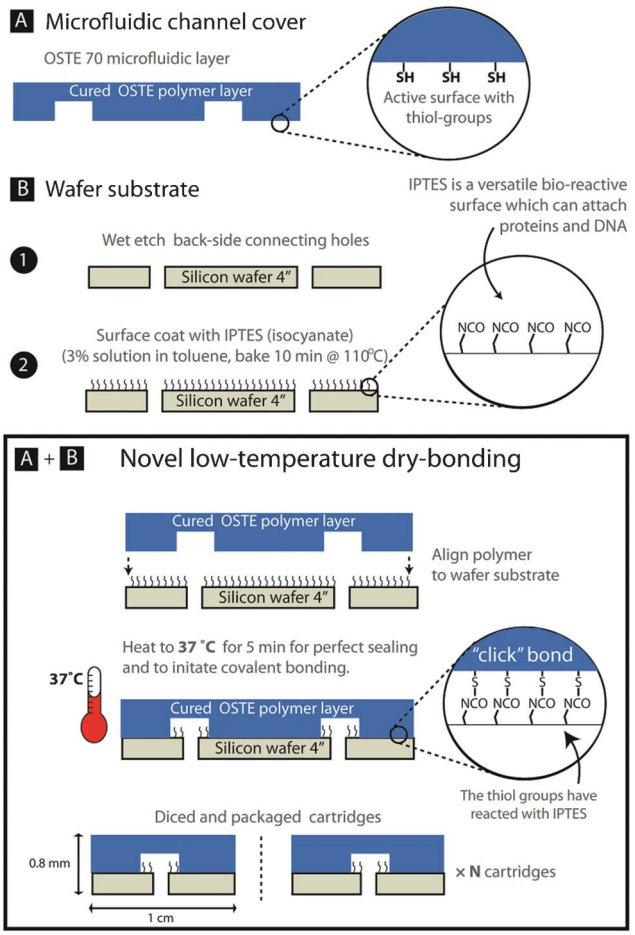

Fig. 2 The OSTE-70 fabrication process and wafer level "click" bonding illustrated for Device 1: (A) The OSTE 70 microfluidic layer; (B) The Si substrate is etched and coated with IPTES, a silane containing isocyanate or vinyl (not shown); and (A + B). The OSTE-70 polymer is transferred, aligned and bonded to the Si substrate prior to the dicing.

OSTE polymer layer fabrication

The OSTE pre-polymer was prepared with 1.7 : 1 ratio of pentaerythritol tetrakis (2-mercaptoacetate) and triallyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5H)-trione. To the mixture, 0.1% of a UV initiator, ethyl-2,4,6-trimethylbenzoylphenylphosphinate (Lucirin TPO-L, BASF AG, Ludwigshafen, Germany) was added. The components were then mixed and fabricated as the previously reported OSTE devices[10] for the microfluidic channel layer as shown in Fig. 2A.

Silicon substrate chemical surface modification

4″ silicon wafers with 2.5 μm oxide layer were etched in 30% KOH solution for 6.5 h at 80 °C and the remaining oxide layer was removed using 50% HF. The wafers with microfluidic ports (Fig. 2B1) were then immersed for 20 min in a solution consisting of either 3% w/w IPTES (3-isocynatopropyl triethoxysilane) dissolved in toluene or 3% w/w vinyl silane dissolved in methanol. The silane reactions with the native silicon oxide layer generate surface bound IPTES or vinyl, respectively. After coating, the wafers were washed thoroughly with toluene or methanol, respectively, prior to baking in the oven at 110 °C for 10 min.

Bonding and dicing

The OSTE-70 sheets and chemically surface modified wafers fabricated were then aligned manually and brought into contact. To remove voids, a light pressure was applied by hand in regions with poor contact to push the voids to the periphery of the wafer. Once satisfactory contact across the wafer was achieved, the assembly was heated on a hotplate at 37 °C for 5 min. At this temperature, the T_g of OSTE-70 was reached, and the material was softened sufficiently to conform to the silicon substrate surface and the "click" reaction between the IPTES isocyanate and the thiol occurred spontaneously to afford Device 1 (Fig. 2A + B). In the case of vinyl functionalized substrates, exposure to low wavelength UV light (EFOS Lite, unfiltered UV-light 6 mW cm^{-2}, 100 s) was used after the contacting step (in addition to heating at 37 °C) to afford Device 2.

After cooling to room temperature, the OSTE polymer regained its rigidity and the bonded stack assembly was diced using a Disco DAD 320 dice saw resulting in a large number of individual microfluidic chips.

Device 3 fabrication

A planar unpatterned OSTE-70 polymer top layer was mated to a vinyl functionalized silicon substrate that featured DRIE fabricated silicon trenches (25 μm deep × 20 μm wide). For geometrical integrity tests, 50 μm deep × 500 μm wide trenches were fabricated. After good contact between the silicon substrate and OSTE-70 was obtained, bonding reactions were initiated with UV-light (EFOS Lite, unfiltered, 6 mW cm^{-2}, 100 s).

Device 4 fabrication

For the burst pressure measurement, 2 × 2 cm^2 silicon chips with a 2 × 2 mm^2 opening located at the centre of the substrate were fabricated by etching in KOH prior to dicing. The silicon chips were subsequently surface treated with vinyl silane using the previously described protocol. An OSTE-70 polymer disc with 1.4 cm diameter and 0.5 mm thickness was centred over the opening and bonded onto the silicon at 37 °C *via* UV-illumination (EFOS Lite, unfiltered, 6 mW cm^{-2}, 100 s).

Device evaluations

Firstly, we evaluate the "click" bonding process with respect to sealing properties and burst pressures. Secondly, we evaluate the geometrical integrity of OSTE-70 sealed microchannels. Thirdly, the OSTE-70 polymer is evaluated with respect to solvent resistance and barrier properties to small molecules.

Sealing tests

100% of channels on the wafer were void-free sealed (Fig. 3A), and diced microfluidic chips are shown in Fig. 3B.

The dice cut was used to evaluate the quality of the interface between the OSTE-70 layer and the surface modified silicon in Device 1 and 2. In Fig. 3D, a very sharp and void free interface is evident, which indicates good channel sealing. To further evaluate sealing, leakage tests were performed on Device 3 where the channels etched in silicon were capillarily filled with DI water containing red and blue food dyes and observed under a microscope for 5 min. Fig. 4A shows that the liquid remained in the channel, which verifies good sealing.

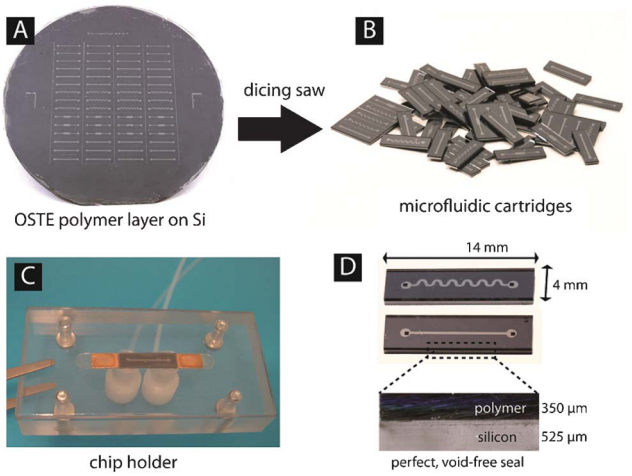

Fig. 3 Pictures from OSTE-70 processing: (A) bonded layer of OSTE-70 on an IPTES modified silicon wafer; (B) diced chips; (C) the Device 1 chip in the holder for barrier tests; (D) Cross sectional view of Device 1, demonstrating void-free seals at the bonding interface.

Pressure tests

The adhesion forces between the polymer and substrate must be sufficient to sustain normal pressures, *i.e.* 1–2 bars, typically encountered in microfluidic LOC applications. To ensure adequate bond performance, pressure tests were carried out using Device 1 and Device 4.

To examine the maximum pressure the assembled chip could withstand, the Device 1 chip was immersed in water and the channel inlets were attached to a pressurized nitrogen gas tank equipped with a pressure regulator. The pressure was slowly increased until gas leaked out or the pressure limit (4.4 bars) of the set-up was reached. The experiments showed that the channels were able to withstand 4.4 bars pressure without chip interface failure.

In the burst pressure set-up, *i.e.* Device 4, nitrogen was applied through the opening in the silicon until the polymer burst or the pressure limit was reached. As in the pressure test, the bond strength exceeded the 4.4 bars limit of the set-up.

Geometrical integrity of OSTE-70 microfluidic channels

To ensure that the softened OSTE-70 polymer allows for unblocked and undeformed channels even in demanding bonding situations, Device 3 was evaluated with respect to deformation of the flat channel roof. Fig. 4B shows that the OSTE-70 polymer does not sag over the wide trenches and the desired geometry is attained.

Solvent resistance tests

Compatibility with commonly used solvents is important since many LOC applications demand other liquids than water. To elucidate solvent compatibility, the diced Device 1 chips were immersed in the solvents listed in Table 1 for 24 h, after which device integrity was observed. As seen in the table, good

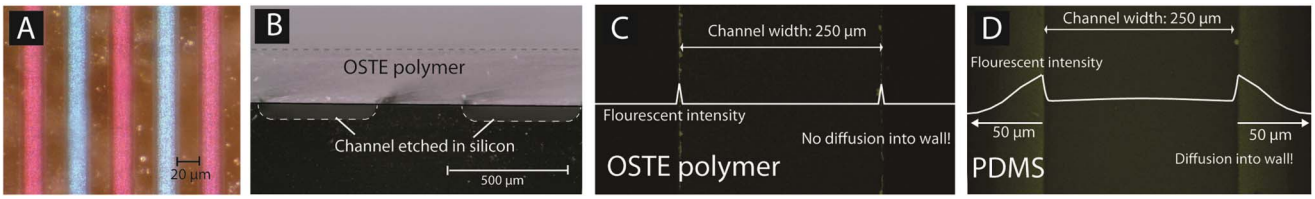

Fig. 4 Photos of leakage, channel geometries, and barrier tests: (A) No leakage was detected at the OSTE-70 polymer interface and the silicon substrate in Device 3; (B) The OSTE-70 polymer shows no sagging over the 500μm trenches (image enhanced with lines for clarity); (C) No diffusion of Rhodamine B was detected in the OSTE-70 polymer; and (D) diffusion was clearly observed in PDMS.

compatibility with alcohols and toluene was observed, but solvents with large dipole moments, *i.e.* DMSO and acetone, were not well tolerated. To increase the compatibility with these solvents, monomer substitution would be required.

In an additional experiment with the Device 1 chips, Rhodamine B was mixed with ethanol, introduced *via* capillary flow into the OSTE channel, sealed and left for 24 h. A similar test was performed using PDMS bonded to a Si substrate with oxygen plasma, for comparison. After 24 h, the ethanol with Rhodamine B still resided in the OSTE-70 channel (Fig. 4B) whereas the ethanol in the PDMS channel had completely evaporated. This shows that OSTE-70 exhibits both good sealing to substrates and good barrier properties to a low molecular weight organic molecule dissolved in ethanol, a result in agreement with the findings of Sandström *et al.*[11]

Small molecule barrier properties of OSTE

To test the long term barrier properties of the OSTE-70 compared to PDMS, an aqueous solution of Rhodamine B (50 μM aqueous) was introduced into identical OSTE-70 and PDMS microchannels, which were subsequently sealed for 24 h.

After emptying the channels, the concentration of diffused dye was analysed at 488 nm excitation and 505 nm longpass detection in a confocal laser scanning microscope (LSM 510 META; Carl Zeiss, Jena, Germany). In Fig. 4C, no diffusion of Rhodamine B was detected in the OSTE-70 channel walls after 24 h, unlike in PDMS where the Rhodamine diffused more than 40 μm into the channel wall as illustrated in Fig. 4D.

Table 1 Compatibility test of Device 1 chips in common solvents

Solvent	After 24 h
Isopropanol	No visible effect
Methanol	No visible effect
Acetone	Bulk material failure
Toluene	No visible effect
Glycerol	No visible effect
DMSO	Bulk material failure
DI water	No visible effect

Conclusions

We demonstrate for the first time a one-step, biocompatible covalent wafer-scale packaging process of microfluidic labs on chip using "click" chemistry. By not exceeding 37 °C, the process allows for batch surface bio-functionalization of the substrate before packaging, thus simplifying back end-processing and streamlining rapid prototyping of LOC's.

Acknowledgements

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