## **Methods and Materials**

#### Characterization of Bond Strength

Bond strength was assessed. The adherence between PO layers of the substrates was determined by a pressure regulator (SMC AR20-N01H-1Z) connected to house air. Three-layer devices were fabricated that had only one inlet for the pressure test. The house air and regulator were connected to the device via microfluidic tubing (Tygon), a blunt needle (Small Parts), and epoxy (Hardman Double/Bubble Epoxy).

### Characterization of PO optical properties

Analysis of the transmissible wavelengths of the light through the shrunk substrates for various shrinkage conditions was determined via a spectrophotometer (Perkin-Elmer Lambda 40 spectrophotometer). Three different conditions were tested: normal heating, fast heating, and heating with a heat gun (Steinel HL 1910S). Normally heated pieces were ramped as described above. Fast heated pieces were rapidly shrunk in the oven at 155 °C with no ramping. Heat gun pieces were made by placing a piece of preshrunk PO on a silicon wafer and heating with the gun on its medium setting. The single layer pieces were sized to fit into a cuvette and then analyzed with the spectrophotometer.

The autofluorescence of the PO pieces made with the aforementioned conditions were imaged with the inverted microscope with the common fluorescent filters, DAPI, FITC, and TRITC. ImageJ was used to measure the intensity of the autofluorescence. The autofluorescence of a common PS petri dish (Nunc) was also imaged to provide the basis of normalization for the autofluorescence measurements.

# **Results and Discussion**

### Characterization of Device

Uniform shrinkage of multi-layer substrate was facilitated by a step-wise heating of process as it was observed prior that quick heating of the substrate often induced undesirable shape changes in the substrate. The use of pins to adhere the layers of the substrate throughout the heating process also facilitated uniform shrinkage and alignment. The addition of pins to the middle of the substrate allowed for the design of bends and twists in the channels that did not collapse or deform in any appreciable manner during the shrinking process. The only drawback to the use of pins is the holes that they leave behind. Future work could explore the use of smaller diameter pins or wires to minimize the aesthetics of the holes. However, the holes do not compromise the bond strength between the layers nor the ability of fluid to flow without leaking.

The layers of the device were thermally bonded during the shrinking process. Thermal bonding is a technique whereby the substrates are heated to a temperature near or above the  $T_g$  of the materials.<sup>1-2</sup> Thermal bonding has several advantages over the use of pressure sensitive adhesives or solvents including channel sidewalls with chemical, optical, and mechanical properties that are the same as the bulk polymer.<sup>2-3</sup> It has also been described that the resulting bond strength can reach the cohesive strength of the bulk material.<sup>2-3</sup> The microfluidic devices described in this article showed strong adherence between the layers due to thermal bonding. The devices withstood pressures in excess of 50 psi without delaminating.

#### Characterization of the optical properties of the device

The optical properties of the PO used in our study are displayed in Figure SI1. While PO is known for its optical properties with transmission over a wide wavelength range and low autofluorescence, these desirable properties apparently diminish post-shrinkage. It seemed as though the transmission varied as a function of duration and rate of cooling after shrinkage, presumably due to whether a more crystalline versus amorphous structure ensues. It was therefore hypothesized that different heating conditions (slow, fast, and with a heat gun) could provide variation in the optical properties. Though the PO begins quite transparent, after heating, the polymer becomes far more opaque (Fig.SIa). It is seen, however, that though slower heating suggests slightly higher transmission, the differences between the three methods were not statistically significant. Despite the increase in opacity, fluorescent images may still be easily obtained with the PO chips.

Similarly, different heating techniques did not seem to significantly affect the autofluorescence of the samples (Fig. SIb). The DAPI and the FITC filter results are close for the three different conditions, but the TRITC filter results give outcomes that are distinct. The rapid heating gives the lowest florescence, followed by the ramped heating and the heat gun. All but the FITC 'rapid' condition produces pieces with autofluorescence greater than that of the PS dish. As evident in the TRITC images in this study, fluorophores of that range may be clearly distinguished from the background fluorescence of the chip. One can conclude from the evidence of these experiments that varying the heating condition does not greatly affect the optical properties of the shrinkable PO polymer used in this study. The ramp method is still preferred, however, due to its ability to limit deformation.



Figure SI1: Optical Properties of PO were tested for three shrinking conditions, which included a ramp and rapid heating, and the use of a heat gun. a) % Transmission vs. wavelength values. b) Fluorescence values normalized for a PS petri dish under typical fluorescence filters.

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