Electronic Supplementary Information

Fabrication of nickel molds.

A Graphtec Craft Robo Pro CE5000-40 Cutting Plotter (Graphtec America, Inc.) was used to plot CAD designs directly from a .dwg or .dxf file with a programmed step size of 10 μm. An 80 μm thick vinyl adhesive film (3M Scotchcal 220, Gerber Scientific Products, Inc.) was used as the positive electroplating mask. Following plotting, features to be plated up were removed manually using fine-tipped forceps. The plotted design was overlaid with laboratory tape prior to removal from the backing substrate. Feature heights greater than 80 μm were formed by layering one or more vinyl adhesive films over the first prior to cutting. Optimal electroplating uniformity was achieved by the addition of 500 μm wide perimeter boundary features within 500 μm of the designs.

Stainless steel sheets (0.030" thick 304 grade steel with a "mirror-like" finish; #9785K51, McMaster-Carr, Inc.) were machined into circular 90 mm diameter wafers and washed with isopropanol and distilled water. Following a nickel strike of the wafer at 235 A-m^{-2} for 2 min at 50-55 ^{o}C in a continually-stirred Wood's Nickel Strike Solution (240 g-L^{-1} $NiCl_2$•6 H_2O, 25% v/v HCl) with an electrolytic nickel anode, the sticker mask was transferred to the steel wafer and the transfer tape or film was removed. The exposed features were electroplated to the desired height in a continually-stirred commercial bright nickel plating solution (#42027, Alfa Aesar, Inc.) at 55 ^{o}C. Multi-level features were fabricated by uncovering regions with the tallest features, and later removing the sticker mask from lower height features. For a repeatable and uniform plating rate, the sticker mask-coated wafer and the nickel anode were held in place in a custom acrylic manifold. A laboratory power supply (GPS-3030DD, Instek, Inc.) was used in current control mode to regulate the plating current density. Any remaining adhesive following sticker removal was dissolved with a 1:1 mixture of acetone and toluene. Care should be taken with handling and disposal of nickel salt solutions as nickel contact can cause allergic reactions in some people and ingestion or inhalation has been associated with increased cancer risk.

Hot embossing plastic microfluidic devices.

Zeonor 1060R and 1420R cyclo-olefin copolymer (COC) sheets (1 mm thick) were obtained directly from Zeon Chemical, Inc. Extruded 250 μm thick THV500 samples were kindly provided by Dyneon, Inc. Hot embossing was performed in a hot embossing insert built in-house (see Fig. S1) attached to an H6231Z 10 Ton Hydraulic Press (Grizzly Industrial, Inc.). A sheet of polymer was sandwiched between the nickel mold and a sheet of glass and placed between the heated platens. Best results were obtained at pressures of 5-15 MPa for 0.5-2 min and >10 ^{o}C above the polymer's glass transition temperature (T_g). Devices were cooled under pressure and de-embossed 10 ^{o}C below T_g.

Heated platen insert design details.

The insert consists of two 4.5" diameter 3/8" thick aluminum platens, each with a 300W concentric ring heating element (A203/120, Omega, Inc.). Teflon sheets (1/4" thick, 4.5" diameter) and a Teflon washer (1" diameter) were used for thermal insulation. The insert components were attached together using headless screws. The temperature of each platen was independently maintained using two T-type thermocouples (5TC-GG-T-24-36, Omega, Inc.) and two PID controllers (CN7823, Omega, Inc.) connected to the heating elements *via* solid state relays (SSRL240DC25, Omega, Inc.).

Device assembly.

Via holes in COC devices were drilled using a 1/32” milling bit operated at low speed (approximately 100-200 rpm) to minimize surface roughness. Via holes in THV500 devices were made using a 1 mm biopsy punch. Following drilling of via holes, devices were cleaned with distilled water and isopropyl alcohol and dried with nitrogen prior to bonding. Rapid bonding of the embossed COC sheets was accomplished using a solvent-assisted lamination approach modified from the work of Miserere *et al.*[14] We identified o-xylene as an optimal solvent for bonding COC devices due to the absence of hazing or cracking, compared to cyclohexane[21] and hexadecane,[14] and low toxicity and carcinogenicity. Specifically, pipetting 1:1 o-xylene:isopropyl alcohol to cover the featureless polymer sheet and incubating for 30 s (Zeonor 1420R) or 10 s (Zeonor 1060R) resulted in a thin low-Tg layer that could be laminated to the embossed sheet at temperatures that would not distort the channels. The sheets were quickly dried with nitrogen prior to bonding at or slightly below the polymers’ T_g in a Peach 3500 Photo Pouch Laminator (PEACH 3500, Oregon Laminations, Inc.) at the slowest speed setting. THV500 devices were washed briefly with acetone and dried prior to laminator bonding. Devices with integrated PDMS membrane valves were bonded using a UV/O_3 cleaner (Jelight Company, Inc.) by activating each COC surface as well as both sides of the 250 μm PDMS membrane (B&J Rubber Products, Inc.) for 2.5 min. If necessary, a permanent bond was achieved by heating the assembled device for >10 min at 70 ^{o}C or more.

Droplet generator operation.

Droplets were generated using the droplet generator fabricated from THV500, with Droplet Generator Oil (#186-3005, Bio-Rad, Inc.) as the continuous phase. The aqueous phase consisted of standard PCR mix with 1.5 mg/mL BSA (A7906, Sigma-Aldrich) and 5% w/v Pluronic F68 for stabilizing droplets during PCR. Droplets were generated at flow rates of 83 μL/min (oil phase) and 30 μL/min (PCR mix).

Imaging and characterization.

Bright field and fluorescence microscopy images were taken with a Retiga EXi CCD camera (Q Imaging, Inc.) mounted on a Nikon SMZ1500 dissecting microscope fitted with a 515 nm long pass filter (OG515LP, Nikon, Inc.) and custom built illumination module containing a 470 nm Luxeon Star LED (LXML-PB01-0030, Quadica Developments, Inc.) and FITC excitation filter (FM475-35, Thorlabs, Inc.). Scanning Electron Microscopy (SEM) was performed on a Hitachi S-2460N. Nickel molds were imaged directly while polymer devices were sputtered with approximately 15 Å of gold using a Polaron SEM Coating System. Height and lipping characterization of nickel molds was performed using a Dektak 3030 Surface Profiler. Values are expressed as means with 95% confidence intervals unless stated otherwise.

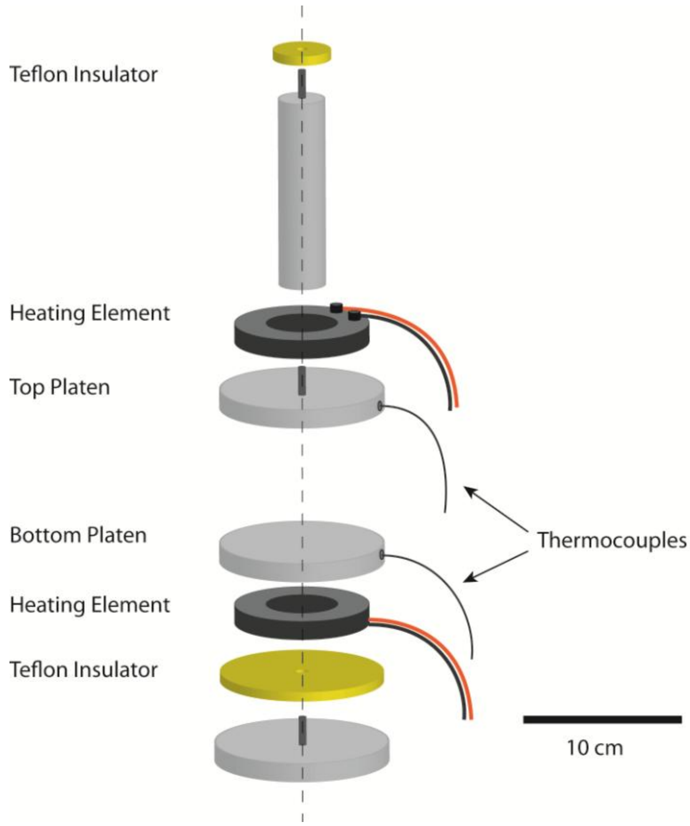

Figure S1. Exploded rendering of the insert designed to convert a hydraulic press into an inexpensive hot embossing machine for easy access to hot embossing of thermoplastic microfluidic devices. Platens machined from 3/8" aluminum stock, thermally insulating 1/4" Teflon sheets, and a 1" wide aluminum force transduction rod were fastened together and to the press with headless screws. Two concentric heating elements are independently regulated by two PID controllers (not shown) that receive feedback from the thermocouples inserted into the platens.

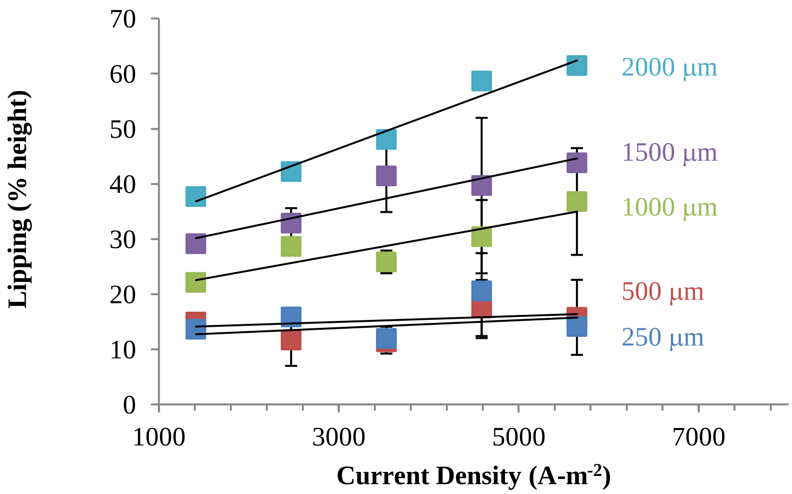

Figure S2. Lipping of feature edges expressed as a percentage of feature height increases both as a function of average current density and distance of the boundary feature from the studied feature. To generate these data, 1.5 mm wide boundaries were placed at the specified distances from 1.5 mm wide features and electroplated at various current densities. Error bars show standard deviation of the mean.

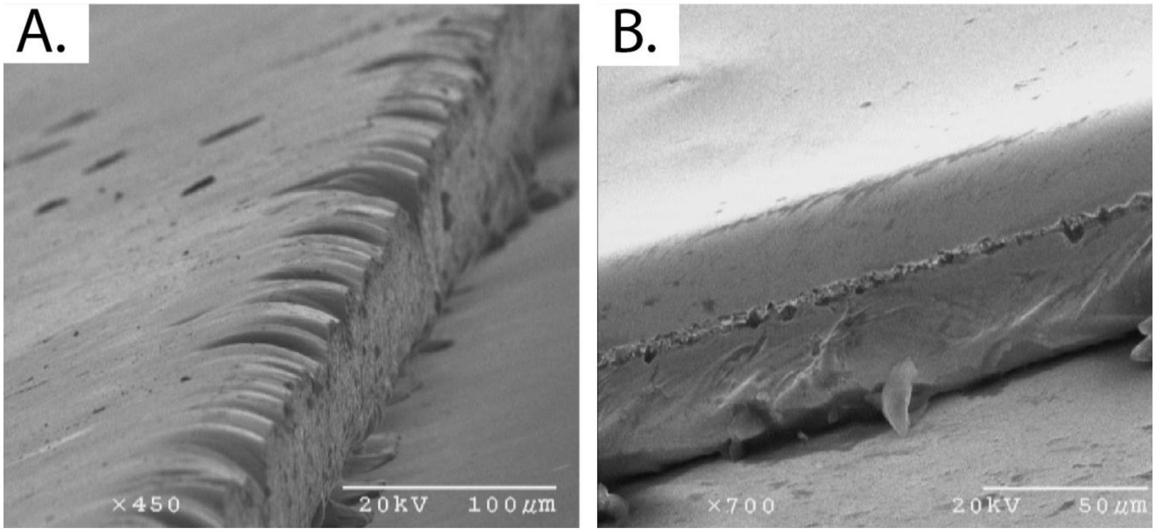

Figure S3. Details of nickel molds illustrating the incorporation of electroplating boundaries. SEM images of electroplated features show pronounced lipping and uneven edges in an unbounded feature (A) and smooth and uniform edges with decreased lipping in a feature adjacent to a boundary (B).

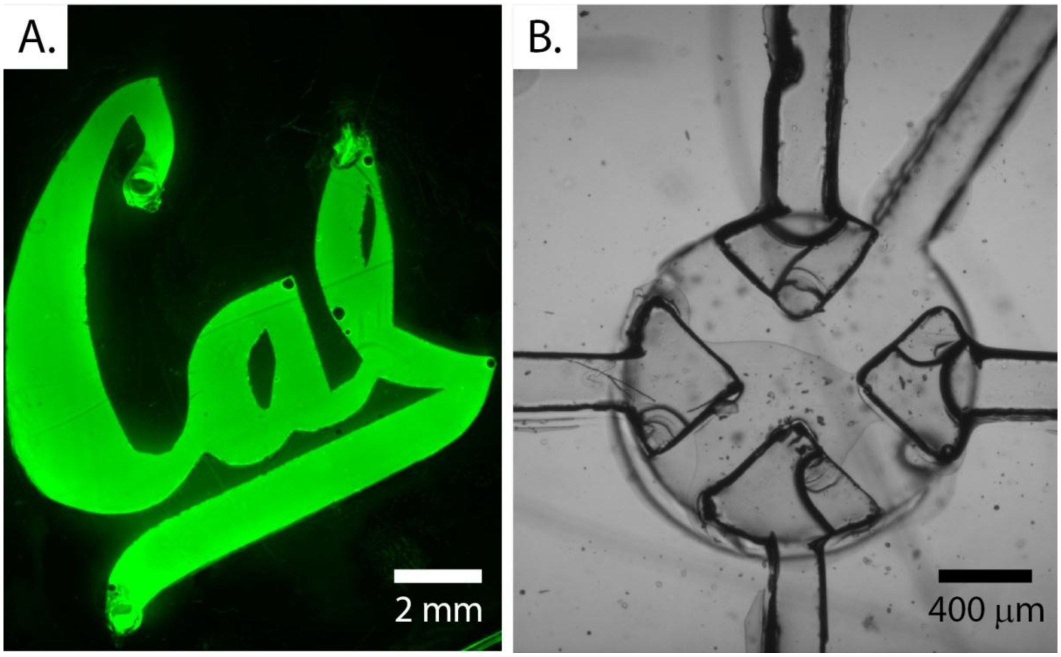

Figure S4. Images of sticker mold-fabricated and assembled functional devices. (A) Extremely wide low aspect ratio channels filled with calcein to demonstrate the uniform 50 μm channel height after bonding. (B) The rapid prototyping method can be used to produce functional components, such as a four-way pneumatic valve made with an integrated PDMS membrane.