Advances in the Fabrication of Surface Modified Micro-fluidic Devices in Non - Fluorescing UV Cured Materials.

M.P.C. Watts; Optical Associates Inc & Impattern Solutions.
mpcwatts@oainet.com
N. Cramer, R. Sebra, H. Simms, K. Masters, T. Haraldsson, K. Anseth, and C. Bowman,
Department of Chemical and Biological Engineering,
University of Colorado

ABSTRACT

The challenge in manufacturing disposable bio micro-fluidic devices centers on making complex structures with controlled wetting and adhesion characteristics that can be used with fluorescence detection at a very low cost of < $1 a part. We will report on a new low fluorescence UV curable material that can be patterned in the Contact Liquid Photolithographic Polymerization (CLiPP) process developed at U Colorado [1], or by imprint.

A generic micro fluidic device can be thought of as a liquid delivery, reaction, and detection package. The mechanics of fluid delivery require a series of delivery ports, chambers, and valves that form a complex internal structure. The CLiPP process was invented to make these complex structures by using a contact mask to image a UV curable liquid. The material is crosslinked in the exposed region, and the uncrosslinked liquid washed away. Multiple layers with embedded structures can be made by repeat exposures. Closed cells are formed by lamination, or by temporarily filling the structure with wax and them capping with UV material. The process is illustrated below in Figure 1.

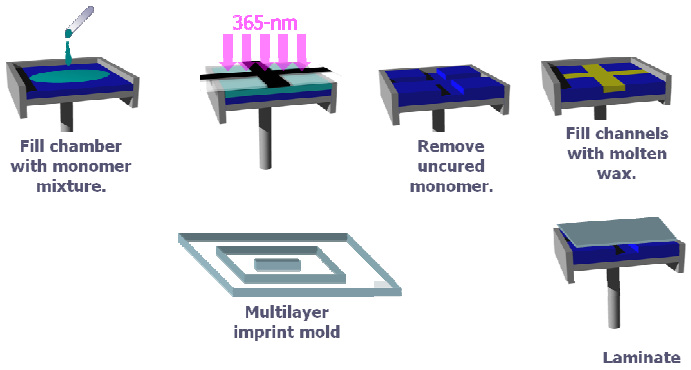


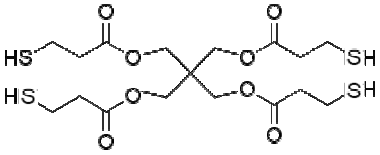
Figure 1 The CLiPP process sequence 1) Fill the chamber with monomer, 2) expose to UV, 3) Rinse away uncrosslinked monomer, 4) Fill pattern with wax, and repeat process for multiple layers. Patterns can also be created using a multilayer mold in an imprint process. The covered channel can also be formed by lamination.

The CLiPP process uses a crosslinking polymer as a “negative resist”. Similar to any crosslinking negative resist process, the feature size is limited to 3-4 x the feature thickness due to swelling of the feature. Smaller features, where the feature size is half the feature thickness, can be created using a imprint process. A typical microfluidic device with 100 um features can be created with a UV mask printed on a photo quality ink jet printer, further reducing protoype costs. For mass production, a multilayer imprint mold can be used, and a top applied by lamination.

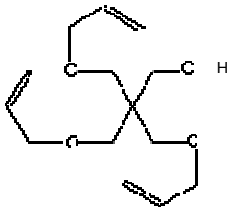
In principle any UV crosslinking polymer can be used in the CLiPP process. However, "thiol-ene" materials have a number of properties that make them particularly suitable for the creation of thick films, and surface grafted films. In particular they can be formulated to produce very low background fluorescence, which is important in bio applications where fluorescent tags are used to detect binding events.

THIOL-ENE MATERIALS

A typical formulation is a stoiciometric mixture of a multifunctional thiol (SH group) and a multifunctional vinyl group (= group) shown in Figure 2.



pentaerythritol tetra(3-mercaptopropionate) or "thiol



allyl pentaerythritol or "ene"

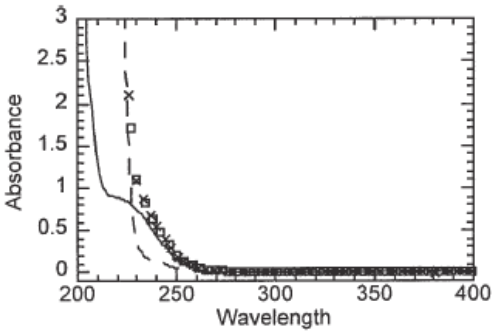
Figure 2 Components of a low fluorescence "thiol –ene"

Thiol-enes use a chain transfer polymerization process illustrated below in Figure 3. The thiol absorbs a photon to form a free radical and self initiate the polymerization.

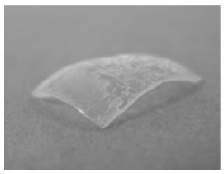
$R – (SH)_4 + h\square \rightarrow R – (SH)_3\ S.$
Thiol + photon produces a thiol free radical
$R – (SH)_3\ S. + CH_2{=}CH\ R' \rightarrow R – (SH)_3\ S\ CH_2CH.R'$
Thiol free radical reacts with vinyl group to produce alkyl radical
$R – (SH)_3\ S\ CH_2CH.R' + R – (SH)_4 \rightarrow R – (SH)_3\ S.$
Chain transfer from alkyl radical back to any thiol

Figure 3 Chain transfer thiol-ene polymerization [2] . Because the chain transfer occurs with any available thiol group, the molecular weight grows "step wise". A traditional vinyl polymerization adds to the propagating radical in "chain growth".

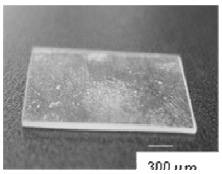
There are several important consequences of using the step growth polymerization. First, there is much less stress built into these step growth materials as they polymerize and shrink, so flat free standing films can be fabricated (Figure 4 b&c). When low stress is combined with low absorption (Figure 4a), ultra thick sections 20 mm can be UV cured as is illustrated in Figure 4d . Second, the surface will be populated with unreacted thiol groups. These can then be used to initiate grafting reactions to locally change the surface wetting properties or attach biologically active species (Figure5). This grafting will be used in examples to be discussed in the next section.



a) UV absorption spectrum of a self initiated thio-ene



b) UV cured acrylic film that bows from internal stress



c) UV cured thiol-ene film that stays flat

d) Ultra thick UV cured thiol-ene

Figure 4 Examples of thick section UV cured materials a) the absorption spectrum, b) & c) a comparison of internal stress, and d) ultra thick UV cured spheres.

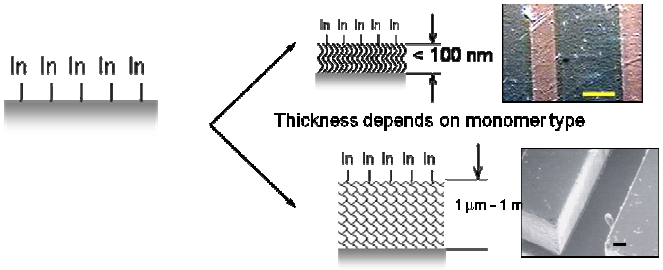


Figure 5 Grafting of surface in which a monomer is placed in contact with the surface, and UV is used to create initiation sites at the surface. Layers of varying thickness can be formed by changing conditions.

Finally, the low absorption of these materials shown in Figure 4a leads to very low levels of background fluorescence.

LOW FLOURESCENCE MATERIALS

Flourescent tags are one of the most common detection vehicles in analytical biochemistry, so any plastic microfluidic device must have very low background fluorescence. To show the low fluorescence of self initiated thiol-enes, 3 different materials were compared as thick (1.5mm) films:

1. A commercial polycarbonate based on Bisphenol A.
2. Acrylic - 50/50 mixture of Ebecryl 4827 (urethane diacrylate) and triethylene glycol diacrylate with 1.4 wt% Irgacure 184
3. Thiol-Ene with no added photoinitiator,a stoichiometric mixture of pentaerythritol tetra(3-mercaptopropionate) and allyl pentaerythritol

Samples were cured under an American Ultraviolet high intensity ultraviolet light source for 10 minutes. The polycarbonate was used as received.

Fluorescence pictures were taken with FITC, TRITC, and DAPI filters in a Nikon Eclipse TE300 fluorescence microscope. The table (Figure 6) shows the color camera image of the fluorescence from the different materials, and the relative intensity of the color signal in bits for the RBG color that best corresponds to the emission wavelength for the different filter sets. The background fluorescence of the thiol-ene was an order of magnitude lower than the other two materials.

Substrate 1.5 mm thick	DAPI 400-450 nm excitation	FITC 475-500 nm excitation	TRITC 525-575 nm excitation
Polycarbonate	B117	G90	R85
Acrylic (urethane diacrylate/triethylene glycol/ Initiator)	B255	G67	R26
Thiol-Ene	B23	G5	R2

Figure 6 Background fluorescence of thiol-enes, acrylics, and polycarbonate under different excitation filters, showing the color image from the detection camera, and the bit value of the RGB channel corresponding to the emission wavelength of interest.

APPLICATIONS

A wide range of microfluidic elements have been fabricated; , including channels, conductors, resistors, mixers, moving elements, valves, and pumps. A number of working devices have also been made using a variety of formulations, two examples will be described.

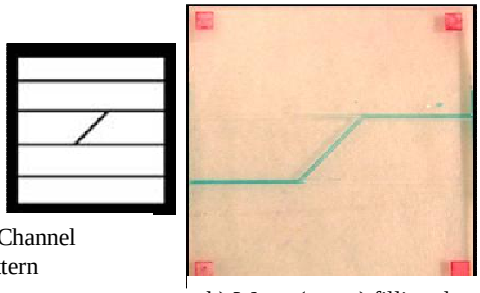
The unique feature of CLiPP is that different materials can be used at each layer, so that the inside surface of the device can be engineered. Most microfluidic devices are made in hydrophobic polymers that have the advantage that the device does not swell in water. Unfortunately the hydrophobic surface also make the cell difficult to fill without bubbles, and proteins adhere to the surface (non specific binding). Devices are often washed with a surface treatment to reduce protein adhesion.

In CLiPP, the surface is modified by using the residual initiators in the surface. This initiator is activated in the presence of a second monomer which is then grafted on the surface. This second monomer can be hydrophilic or contain bio sensor molecules such as antigens. Examples of the different water contact angles that can be achieved by grafting are shown in Figure 7.

Grafted Monomer	Contact Angle
Control: Substrate without graft	50 ± 2°
Trifluoroethyl acrylate	86 ± 4°
Tert-butyl acrylate	72 ± 1°
N-octyl acrylate	65 ± 1°
2-hydroxyethyl methacrylate	43 ± 3°
PEG (375) monoacrylate	7 ± 2°

Figure 7 Water contact angle of modified thiol-ene surfaces

A spontaneously filling device can be built using this technology as shown in Figure 8.



a) Channel pattern

b) Water (green) filling the hydrophilic channel

Figure 8 Hydrophyllic monomer graft is patterned in the central "S" channels. Aqueous solution is directed by hydrophilicity to fill the channel.

Another example of a device is a series of raised pads treated to induce cell growth in a series of distinct locations as shown in Figure 9.

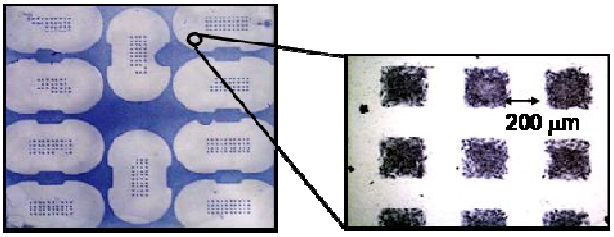


Figure 9 Discrete regions of cell cultivation on a grafted thio-ene surface.

CONCLUSIONS

Thio-ene's can be used to fabricate microfluidic devices with low background fluorescence using the Contact Liquid Photolithographic Polymerization. These materials can have an order of magnitude lower fluorescence than conventional initiated UV cured materials.

[1] T. Haraldsson et al; 3D polymeric microfluidic device fabrication via contact liquid photolithographic polymerization (CLiPP); Sensors and Actuators B 113 (2006) 454–460

[2] N. Cramer et al; Initiation and Kinetics of Thio-ene Photopoymerization without Photoinitiators, J. Poly Sci A, Vol 42, 5817 (2004)