PET Biochip Fabrication For DNA Sample Preparation

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Abstract

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There are many potential applications for the use of microfluidic biochips in molecular diagnostics. Two areas of research that have received considerable attention for integration into microfluidic devices are the stages of DNA amplification and detection, less investigated is that of DNA sample preparation. The focus of the bulk of this research has been on the traditional methods of micromachining in glass and silicon materials. More recently polymers have been investigated as a lower cost alternative. The reports to date of polymeric microfluidic devices have focused on bulk surface machining, or replication techniques. The alternative approach of machining and layering polymer films has shown promise for greater 3-dimensional structuring and continuous production.

It is known that some polymers fluoresce at the wavelengths commonly used for DNA fluorescence detection, reducing the signal to noise ratio. It is also known that the surface charge detrimentally impacts many DNA amplification and detection techniques by the non-specific binding of proteins. There have been few reports of microfluidic biochips fabricated from Poly(ethylene Terephthalate) (PET) for molecular diagnostics. This thesis describes an investigation into a novel method for fabricating a DNA sample preparation biochip by laser machining and the subsequent layering of PET films.

A new method of laser micromachining microfluidic structures in PET film using a direct-write 355nm frequency tripled Nd:YAG (Neodymium-doped Yttrium Aluminium Garnet; Nd:$Y_3Al_5O_{12}$) laser was investigated, with the results indicating that ablation was dominated by photothermal processes. A reproducible ablation threshold of 2.0 ± 0.3 J/cm^2 and etch rates of up to 25µm per beam pass were achieved. Compression of melt at the cut edge produced channel dimension close to the beam diameter, having a channel width down to 30 ± 5 µm for film thicknesses between 12-350 µm.

During process development it was shown that surface oxidation of the film was important for improving capillary flow, electroosmotic flow, bonding time and quality, and that it detrimentally impacted biocompatibility and fluorescence. The laser machined surfaces had a reduction in the ester component compared to the non machined surfaces. Surface oxidation techniques were developed for improved microfluidic performance and bonding using chemical saponification and UV photo-oxidation. These produced a change in the contact angle from 75° for the native PET film down to 16° and 35° respectively. However, only UV patterning by non-contact masking enabled localised surface oxidation with features down below 100 µm for limiting the impact of surface modification on biocompatibility and fluorescence.

To demonstrate the applicability of these techniques polymer microfluidic filtration chips were investigated for leukocyte filtration and particle retention for solid phase extraction. Filter membranes were achieved with pore exit dimensions down to 1µm and porosities up to 50% with non-supported spans of 1x2mm. Cake-layer formation proved to be an issue during in-line leukocyte filtration causing non-reversible filter fouling and increased backpressures, with back-flushing having limited success only when using surface modified membranes. Stable solid-phase matrices were achieved using irregularly shaped particles (25-75µm) resulting in extraction efficiencies of approximately 7-14% with the first elution containing approximately 82% of the DNA recovered.

This thesis concludes that frequency tripled Nd:YAG laser ablation of PET films can be used to produce components of a biochip for molecular diagnostics, namely microfluidics and solid phase extraction of DNA. This new laser machining method of PET film enables the fabrication of microfluidic channels more quickly than excimer based lasers, and with improved feature resolution in comparison to CO_2 lasers. Importantly, the thin film fabrication process can be used for fabricating microfluidic filtration devices entirely from polymers. It was shown that the detrimental impact of the fabrication process on device biocompatibility and fluorescence can be removed by using masking techniques to pattern the oxidised areas.

PET Biochip Fabrication For DNA Sample Preparation

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university and to the best of my knowledge and belief contains no material previously published or written by another person or persons except where due reference is made.

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Micah Atkin

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