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Abstract: We are evaluating the effects of laser-induced cell lysis in microfluidic devices fabricated from PDMS and PMMA. To measure the spatial extent of damage following laser-induced cell lysis, adherent cells and viability assays are used.
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1. Introduction

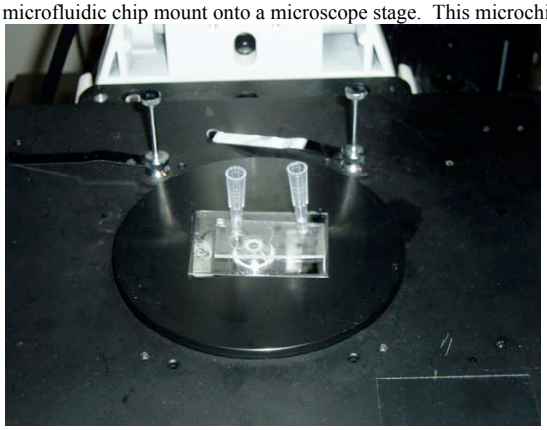
Micro-fluidic devices are believed to have great promise in the analysis of chemical and biochemical systems[1]. One area of significant interest is in conducting rapid chemical analysis of single cells for biological, medical and pharmaceutical applications[2]. The use of pulse laser micro-beam as a method for achieving rapid cell lysis has been investigated by several groups[3-4]. This method not only has the advantage over other cell lyses techniques because it is very rapid but it also allows you to access cells anywhere in a transparent microchip. A series of viability assays are used to assess the viability of cells cultured in the microchips and to measure the spatial extent of damage following laser-induced cell lysis. These assays include the fluorescent dye calcein AM, which stains viable cells and propidium iodide, which stains non-viable, cells. These assays were used to asses the effects of laser pulse energy, geometric confinement and material stiffness on the extent of damage in the microchip.

2. Method

To understand the effects of laser pulse energy, geometric confinement and material stiffness on the extent of damage from laser-induced cell lysis in a microfluidic chip, a laser-microscope platform was designed and constructed to perform a series of experiments. The system included an inverted microscope (Nikon TE 300), which functioned as the platform for imaging the experiments, a frequency doubled Nd:YAG laser (λ=532nm, t_p = 6ns, Continuum Inc.) and a 12-bit CCD camera (Photometrics CoolSNAP fx, Roper Scientific, Inc.), which was installed on the side port of the microscope.

PDMS microchips were fabricated using the soft lithography technique called replica molding. In this process, a silica mold containing the desired pattern for the device is made using lithographic techniques and a two part liquid silicon solution (monomer base and curing agent) is mixed in a 10:1 base to curing agent ratio and poured over the pattern. The polymer solidifies as it cures due to the cross-linking of the monomer chains. The resulting solid with the imprinted pattern is now bonded to a No.1 glass slide. PMMA microchips are created by taking a large sheet off PMMA and cutting it into 1” x 3” rectangles. The channels are micro-machined into the polymer in a machine shop and a drill press to create the reservoirs. This piece of pattern PMMA is then bonded to a 35 x 50 cm PDMS coated No.1 glass slide.

Figure 1: A PDMS microfluidic chip mount onto a microscope stage. This microchip has to reservoir added to



increase the volume of the reservoirs.

The cells used in the experiments are RBL cells which are cultured in modified eagle Medium in tissue culture (Invitrogen, Carlsbad) flasks at 37^{o}C with 5% CO2. The experiments to evaluate the effects of Extent of Damage resulting from varying the laser pulse energy, modifying the geometric confinement and hanging material stiffness were all conducted by inoculation the microchips with cells, culturing the cell until they are confluent in the microchips and then performing laser-induced cell lysis. The laser-induced cell lysis process involves the delivery of a single laser pulse to a region of the microchip. The cells are then loaded with a mixture of two fluorescent dyes, calcein AM and Propidium iodide, and then imaged using fluorescent microscopy techniques.

3. Results

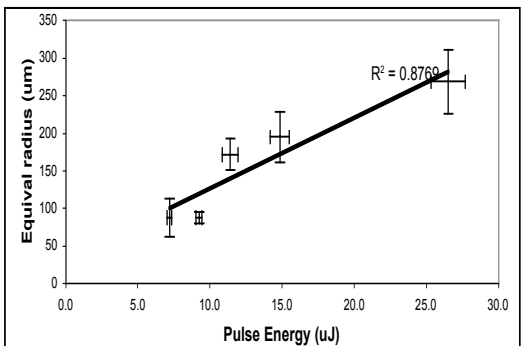
The resulting damage zone from laser induced cell lysis can be determined by measuring the area of damage from the post cell lysis fluorescent images. The area of damage is outlined by the fluorescent dye and the area void of all cells. This is illustrated in figure 2

a. b. c. d.

Figure: 2 Shows micrographs of RBLs in a (a. & b.) 100μm and (c. & d.) 200μm microchip post lysis. Figures 2b. & 2d. are fluorescent micrographs with cells loaded with 10μM calcein AM

The plots of the damage are versus laser pulse energy from an open Petri dish, the PDMS microchips and the PMMA microchips were compared. These plot are shown below in figure 3 a-b

a. b.



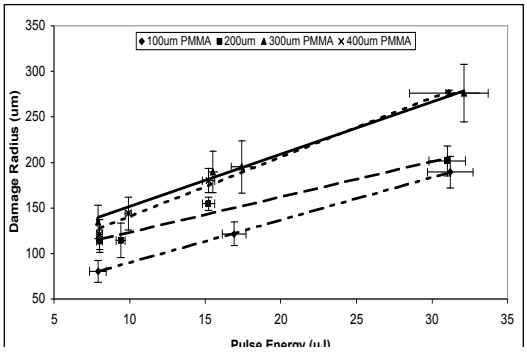


Figure 3a. shows a plot of the equivalent radius of damage Petri Dish as a function of laser pulse energy. Figure 3b. shows a plot of the equivalent radius of dame in a PMMA microchip as a function of laser pulse energy.

4. Conclusions

We have develop a system for measuring and comparing the extent of damage in a microfluidic device made from a soft polymer such as PDMS and a harder, more mechanically rigid polymer, such as PMMA. It was shown that laser-induced cell lysis in the PDMS microfluidic chips produce smaller areas of damage than both PMMA microfluidic chips and an open Petri dishes when using the same laser pulse energy. It is believed that the mechanical compliance of PDMS absorbs mechanical energy of the cavitation bubble that may lead to the observed smaller damage zone. The area of damage in the PMMA chips is between that of the PDMS microchips and Petri dish. The rigidity of PMMA relative to PDMS is likely responsible for the larger damage zones.

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