Three-dimensional laser micromachining and imaging of biocompatible polymers

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Abstract: Micromachining of three-dimensional structures in biocompatible elastomers with a femtosecond laser oscillator is demonstrated. This technique may be applicable to controlling the topography of scaffolding for cell micropatterning in tissue engineering.

OCIS codes: (220.4000) Microstructure fabrication; (180.6900) 3D microscopy; (140.3440) Laser-induced breakdown

1. Introduction

Cell micropatterning for the purposes of tissue engineering is achieved by producing the appropriate topography within a scaffolding material that is biocompatible as well as pliable. Two-dimensional cell patterning has been performed on the surfaces of bulk polymers microfabricated using a soft lithography technique adapted from conventional photolithography [1]. Polymer scaffolds, typically generated by heating or chemically treating the polymer, provide a porous structure with high surface area desirable for cell attachment and subsequent growth and differentiation. Techniques for controlling the precise three-dimensional shape of these scaffolds include melt molding [2], membrane lamination [3], and 3D printing [4]. All of these methods, with the exception of melt molding, require fabrication to be performed layer by layer.

Laser ablation has also been recognized as a tool for micropatterning surfaces [5] with the use of ultraviolet lasers. At visible and near-infra-red wavelengths, where the material is transparent, femtosecond lasers can be used to produce laser-induced breakdown that is highly localized to the focal volume. This is due to the multi-photon absorption, which is nonlinearly dependent on the pulse intensity. Local heating subsequently occurs, which accumulates if the pulse repetition rate is faster than the ~1 μs relaxation time of the material. The depth at which defects are produced in the bulk material is limited by the working distance of the optics and the group-velocity dispersion of the material. Recent work in the micromachining of Corning 0211 glass [6] has shown that the threshold for breakdown can be reached with a femtosecond laser oscillator modified to produce pulses of 15 nJ energy, in combination with a high numerical aperture (NA) objective. We demonstrate that defects in polydimethylsiloxane (PDMS), a biocompatible elastomer, can be produced with as little as 1.5 nJ pulses from a standard (unmodified) femtosecond oscillator. The resulting features are tested for biocompatibility by incubation with fibroblasts.

2. Experimental

Polydimethylsiloxane (PDMS) elastomer substrates, (Slygard 184 Silicone Elastomer Kits available from Dow Corning), are prepared by casting a 10:1mixture of base and curing agent into the form of 75 mm circular slabs, 1-13 mm thick. The prepolymer solution is thoroughly mixed at room temperature and degassed at 20-50 mtorr for 60 minutes prior to casting. The slabs are subsequently cured at 75°C for 5 hours.

The PDMS samples are then micropositioned beneath a 100X oil-immersion objective (NA=1.25, working distance 0.23mm). Immersion oil is applied directly to the surface of the PDMS, which is removed with soap and water after completion of the micromachining process. The output of a Ti:Sapphire 120 femtosecond laser oscillator producing 5 nJ pulses at 800 nm with a repetition rate of 80 MHz is directed through a shuttering system into the objective. Shutter speeds of 0.1-1 s were used initially to produce localized defects in the bulk PDMS. During exposure, a transient white continuum light is emitted from the location of the focal volume, which is indicative of plasma generation in the laser-induced breakdown process.

Holes are drilled into the PDMS by positioning the focus to the maximum desired depth and translating upward during exposure. Channels are produced by translating laterally at a constant speed of 100-200 μm/s during exposure. Three-dimensional structures are produced by simultaneous movement of the positioning stage in two or

three directions, however it must be kept in mind that features which lie beneath others must be machined first. The samples exhibit laser-induced damage for pulse energies as low as 1.5 nJ, and are typically machined at energies slightly above this threshold.

Drilled holes and surface channels were tested initially for biocompatibility with rabbit dermal fibroblasts. In order to sterilize the samples for use as cell culture substrates, the machined PDMS is soaked at room temperature in a solution of ethanol and deionized water (70% ethanol by volume) for 48 hours. The samples are then collagen coated and incubated with the fibroblasts overnight. Culturing is performed in an inverted geometry because the PDMS floats in solution.

3. Results and Discussion

As illustrated in the upper right panel of Fig. 1, defects were produced in the PDMS with a maximum depth of 180 μm, as limited by the working distance of the objective used. Given longer working distances, the maximum depth is expected to be limited only by the group-velocity dispersion of the material that acts to broaden the temporal laser pulse shape. In glass this might reasonably be expected to have a detrimental effect only after several millimeters.

The defects are typically 40-60 μm in diameter and spherical in shape, with some cracking of the bulk PDMS visible near the edges. However, faster shuttering might be expected to control the amount of local heating and reduce this effect, as well as lowering the minimum possible spot size. The dark substance, which is a by-product of the laser-induced breakdown, is loosely packed and can be removed. It also appears to be an inert and biocompatible substance, as shown on the right side of Fig. 2.

The left panel of Fig. 1 illustrates a typical channel produced below the surface of the PDMS. It has the appearance of a “string of pearls” which is likely due to a change in the local heating and optical properties of the defect area, preventing breakdown from occurring too near a defect site. As a result, holes or other multiple-depth features must be machined from the bottom up. These limitations may be relaxed as improved control over exposure times and sample positioning are implemented.

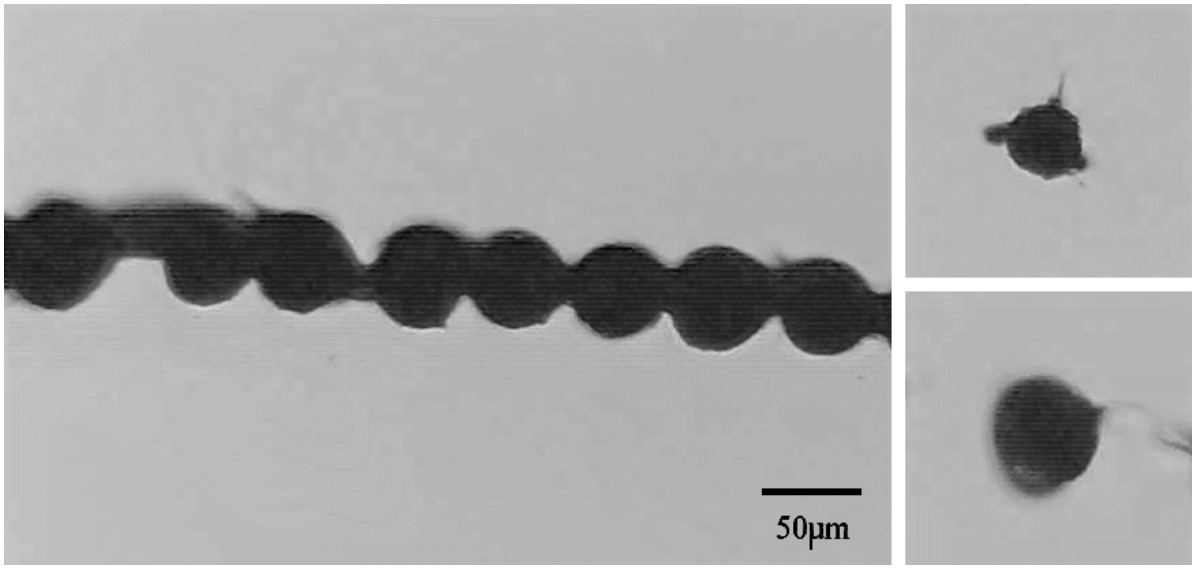

Fig. 1. 10X microscope images of micromachined features in PDMS. Left: 50 μm wide channel ~100 μm below the surface. Upper right: 40 μm defect produced 180 μm below the surface. Lower right: A hole of 52 μm width and 120 μm depth drilled up to the surface of the material.

Biocompatibility of micromachined features extending over the surface of the PDMS is illustrated in Fig. 2. After incubation over a time period less than 24 hours, fibroblasts are observed growing both in and around the laser-machined defect sites. One fibroblast (left panel of Fig. 2) is connected with a second, double-nuclei cell located immediately outside a laser-machined surface channel. Another fibroblast (right panel of Fig. 2) is shown growing into a large laser-machined hole containing remnants of the dark by-product of the laser-induced breakdown. These clearly demonstrate that the laser machining process is not detrimental to the biocompatibility of the PDMS.

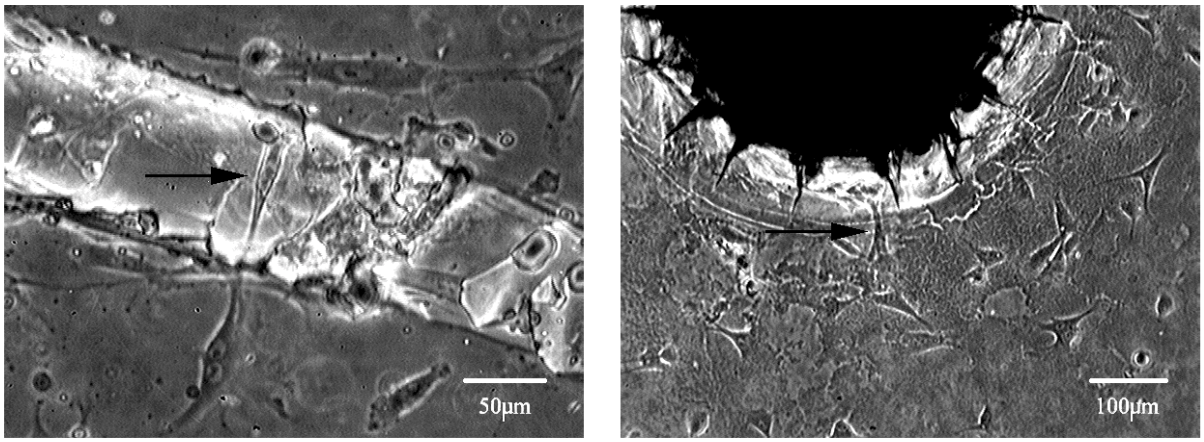

Fig. 2. Inverted microscope images of PDMS surface after micromachining and incubation with fibroblasts. Left: Fibroblast cell (indicated by arrow) attached to a 108 μm wide surface channel (20X magnification). Right: Fibroblasts in the vicinity of a 400 μm hole. The cell indicated by the arrow appears to be growing into the hole (10X magnification).

4. Conclusion

Features 30-100 μm in size have been micromachined in bulk PDMS using a conventional femtosecond laser oscillator. Pulses as low as 1.5 nJ in energy can produce laser-induced breakdown in the elastomer when focused by a high-NA (100X) microscope objective. Defects as low as 180 μm below the surface of the PDMS have been generated, and improved performance is anticipated with the use of longer working distance optics. The ability to drill holes and make channels of arbitrary depth has been demonstrated, in addition to other, more complex, three-dimensional structures.

The resulting surface features have been briefly tested for biocompatibility with fibroblast cells, and after overnight incubation indicate growth and attachment in and around these features. This technique may therefore prove to be a useful tool for generating complex three-dimensional structures for cell micropatterning in tissue engineering. It is likely to be applicable to other biocompatible, biodegradable polymers used to construct porous scaffolding, enabling additional control over scaffold topography. Complex three-dimensional devices, which mimic the natural structure of the extracellular matrix, could then be designed for a specific organ of interest. As engineered tissues become increasingly complex and three-dimensional, advanced imaging techniques such as optical coherence tomography and multi-photon microscopy will find application for analyzing the three-dimensional growth of these constructs.

5. References

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