The Versatility of Two-Photon Absorption Laser Scanning Lithography in Poly(ethylene glycol) Hydrogels

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Statement of Purpose: A variety of photolithographic technologies have been applied to biomaterials in order to pattern bioactive ligands into spatially controlled topographies. Two-photon absorption laser scanning lithography (TPA-LSL) allows for the creation of three dimensional microenvironments through the use of a computer controlled, tightly focused laser beam to initiate crosslinking of ligands in a minute focal volume. In this work, TPA-LSL is applied to the micropatterning of fluorescently labeled monoacrylate PEG-RGDS in poly (ethylene glycol) diacrylate (PEG-DA) hydrogels. Specifically, we characterize the effect of laser scan speed and power on fluorescence intensity, and therein, PEG-RGDS concentration. We also demonstrate the versatility of TPA-LSL by producing micropatterns of varying size and 3D shape. Finally, we report the micropatterning of multiple, overlapping moieties with differing fluorescent labels to serve as a proof of concept for patterning an array of bioactive ligands within a PEG-DA hydrogel.

Methods: *PEG-DA Hydrogel fabrication*

A glass coverslip was piranha etched and incubated with 85 mM 3-(Trimethoxysilyl)propyl methacrylate (ph 4.5) in ethanol to introduce surface acrylate groups to the glass. A prepolymer solution of 10% (w/v) PEGDA in HBS with 10 µL mL^{-1} of 300 mg mL^{-1} 2, 2-dimethoxy-2-phenylacetophenone (DMAP) in *N*-vinyl pyrrolidone (NVP) was then prepared and injected between an acrylated coverslip and a glass slide separated by a .5 mm spacer. The hydrogels were pre-crosslinked and immobilized to the acrylated coverslip through a 45 second exposure to UV light (365 nm).

Fluorescent monoacrylate PEG-RGDS synthesis

Acrylate-PEG-SCM (Laysan) was reacted with RGDS at a ratio of 1.2:1 in DMSO. The resulting monoacrylate PEG-RGDS was then further reacted with one of several amine reactive fluorophores in 0.1 M sodium bicarbonate buffer (ph 8.3) and purified via dialysis.

TPA-LSL Patterning Strategy

The pre-crosslinked hydrogel was incubated in a solution of fluorescent monoacrylate PEG-RGDS (50-100 nmol) in HBS with 10 µL mL^{-1} of 300 mg mL^{-1} DMAP in NVP. The hydrogel was then positioned on the stage of an LSM 510 META NLO confocal microscope. Patterns were designed using the region of interest function in the LSM software, and the laser power, scan speed, microscope objective and Z plane were specified to create patterns of desired dimensionality and concentration. A two-photon titanium/sapphire laser tuned to 720 nm was then scanned across regions of interest to initiate crosslinking of free acrylate groups in desired, free-form 3D patterns. The hydrogel was then washed with HBS to remove unbound fluorescent acrylate-PEG-RGDS and the resulting pattern was imaged using a confocal microscope.

Patterning of Multiple Fluorescent Ligands

Patterned hydrogels were then incubated in a similar solution of monoacrylate PEG-RGDS (50-100 nmol) labeled with a fluorophore of a different excitation and emission spectrum. As described above, the two photon laser tuned to 720 nm was then be used to create a second pattern with dimensionality and degree of crosslinking specified via patterning conditions.

Results: *TPA-LSL patterns of varying size*

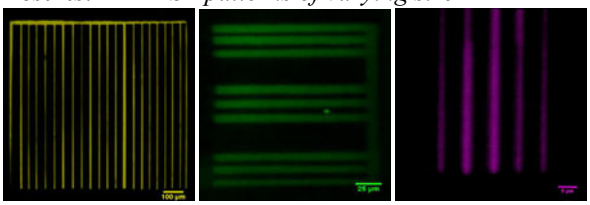

Figure 1. Patterns of fluorescent acrylate-PEG-RGDS in PEG-DA hydrogels created via TPA-LSL with 10X (L), 20X(C) and 63X(R) objectives. Scale bars 100 µm (L), 25 µm(C) and 5 µm(R).

Patterns of Varying PEG-RGDS Concentration

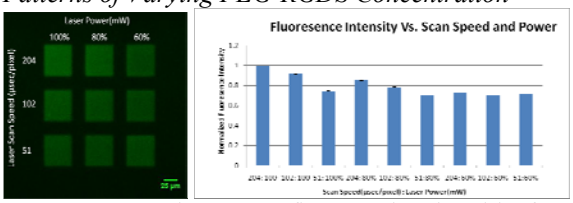

Figure 2. Patterns vary in PEG-RGDS fluorescence intensity and therefore concentration due to changing pattering conditions.

Three Dimensionality of TPA-LSL

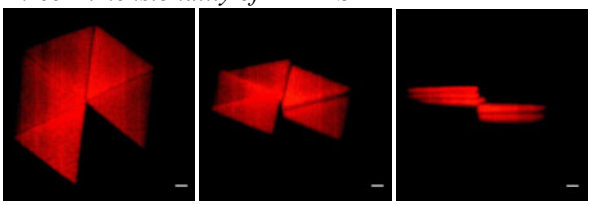

Figure 3.3D projection of fluorescent-acrylate-PEG-RGDS pattern in PEG-DA.

Patterning of Multiple Fluorescent Ligands

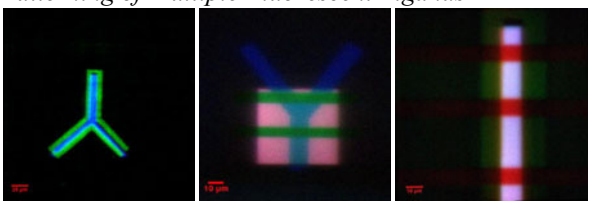

Figure 4. Patterns of two juxtaposed fluorescently labeled acrylate-PEG-RGDS ligands (left) and three different overlapping ligands (center and right) in PEG-DA hydrogels.

Conclusions: In this work, we significantly advance the versatility of TPA-LSL. Specifically, we demonstrate the ability to pattern fluorescent acrylate-PEG-RGDS ligands with distinct three dimensional shape in size ranges between 1 mm and 2 µm. Additionally, we show the ability to control concentration based on scan speed and laser power. Finally, we show the ability to pattern multiple overlapping fluorescent ligands with micrometer range precision within a single PEG-DA hydrogel. Future research will explore the application of newfound TPA-LSL patterning techniques in degradable PEG based hydrogels with a variety of different cell types. Specifically, we plan to evaluate cell phenotype and migration when exposed to multiple types of bioactive ligands in three dimensions.