

RAPID PHOTOCHEMICAL SURFACE PATTERNING OF PROTEINS IN THIOL-ENE BASED MICROFLUIDIC DEVICES

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ABSTRACT

The ability to immobilize biomolecules at specific locations on the surface of solid supports is central to many biochip applications. This paper reports the rapid one-step photochemical surface patterning of biomolecules in thiol-ene microfluidic chips. Adjusting the stoichiometric ratio of “thiol” and “ene” monomers present in the microfluidic chip bulk material provides a simple and efficient way of tuning the chip’s surface chemistry. Here, thiol-ene chips displaying an excess of functional thiol groups at their surfaces are functionalized with biotin and streptavidin in a controlled fashion using photolithography. We also present quantitative data on the number of functional groups available for surface modification on thiol-ene substrates and their stability.

KEYWORDS

Thiol-ene polymers, biomolecule immobilization, surface functionalization, biochips.

INTRODUCTION

Thiol-ene formulations have recently generated a lot of interest in microfluidic fabrication [1, 2]. In thiol-ene polymerization, shown in Figure 1(a-b), a monomer containing multiple sulfhydryl functional groups (“Thiol”) adds to a second monomer featuring multiple alkene functional groups (“Ene”) to form a crosslinked polymer. A unique advantage of the thiol-ene polymerization reaction is the ability to select the nature and density of the functional groups present on the surface of the substrates by altering the reactant ratios. An excess of thiol monomers in the reactants will therefore result in the presence of unreacted thiols on the surface of the polymeric substrate. These unreacted thiols have previously been used as anchors for attaching polymer films [3] and allyl-functionalized red dye [2] to thiol-ene substrates. The thiol-ene photoreaction is considered bioorthogonal, *i.e.* it does not interfere with native biochemical processes due to its specificity for alkenes and robustness in aqueous buffer [4]. Therefore, it is an attractive reaction scheme for the attachment of biomolecules inside biochips made of thiol-ene polymer. Jonkheijm *et al.* [5] demonstrated the photoimmobilization of alkene-functionalized biotins onto thiol-modified silicon surfaces using the thiol-ene reaction. However, the procedure is labor intensive, requiring several steps to yield the desired thiol-terminated surfaces (plasma enhanced chemical vapor deposition and silanization to activate the silicon wafer substrate, attachment of polyamidoamine dendrimers to the silicon oxide surfaces followed by an aminocaproic acid spacer, coupling of the spacer to cystamine followed by reduction of the disulfide groups to yield the desired thiol functional groups). We demonstrate here that microfluidic chips made of thiol-ene can be rapidly and selectively functionalized with biomolecules.

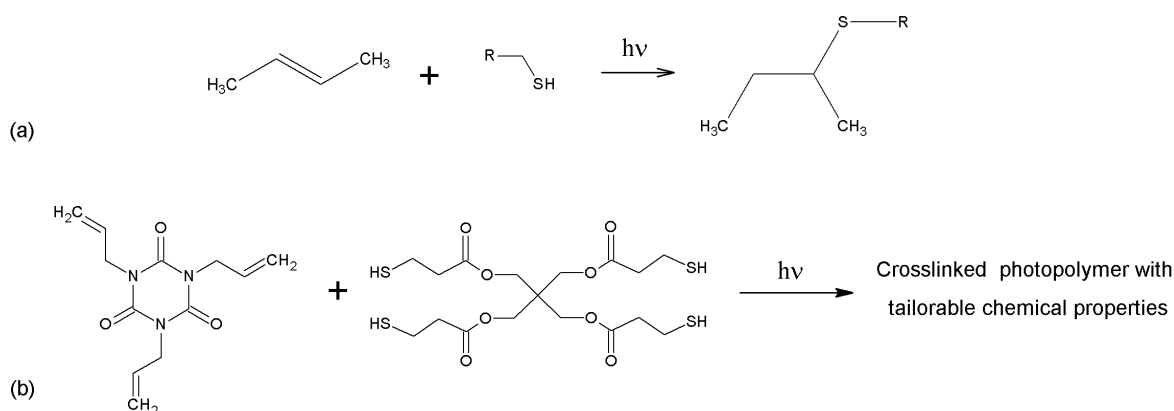


Figure 1. (a) The general thiol-ene reaction and (b) the thiol-ene reaction between a tetra-functional thiol and a tri-functional allyl yields a crosslinked photopolymer with tailorable chemical properties.

EXPERIMENT

Thiol-ene substrates were prepared by casting various monomer mixtures in a PDMS mold followed by UV curing (2 minutes, ~ 40 mW/cm² at 365 nm). The ratios of tetrathiol (pentaerythritol tetrakis-(3-mercaptopropionate)) and triallyl (1,3,5-triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione) monomers were adjusted to ensure that an excess of thiol groups would be present on the surface of the polymers. Ellman’s reagent (Figure 2) was used to evaluate the sulfhydryl group density at the polymer surfaces as a function of monomer mixture composition. Square substrates were incubated for 10 minutes with Ellman’s reagent. After removal of the substrates, the absorbance of the reaction fluid was measured at 412

nm. The number of thiols on the surface of the thiol-ene substrates was evaluated from the molar extinction coefficient of the 2-nitro-5-thiobenzoate reaction product.

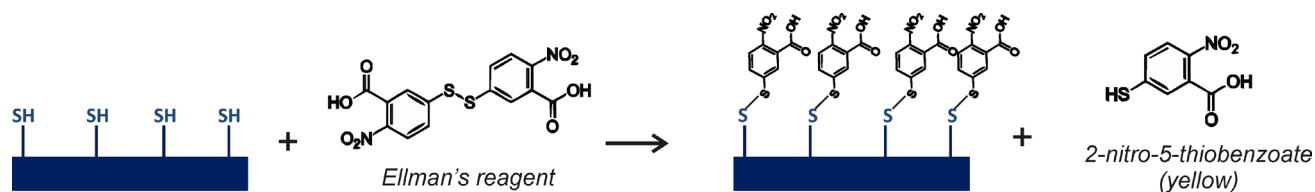


Figure 2. Determination of the thiol surface coverage using Ellman's reagent

Surface functionalization was achieved by exposing a drop of biotin alkyne to UV light (Figure 3(a)), either through a photomask or directly through the microfluidic channel walls of the chip. After exposition, the unreacted biotin was thoroughly washed with phosphate buffer saline solution (PBS, pH 7.4) and the functionalized surface was incubated with fluorescently labeled streptavidin (Figure 3(b)) for 10 minutes and rinsed again thoroughly with PBS.

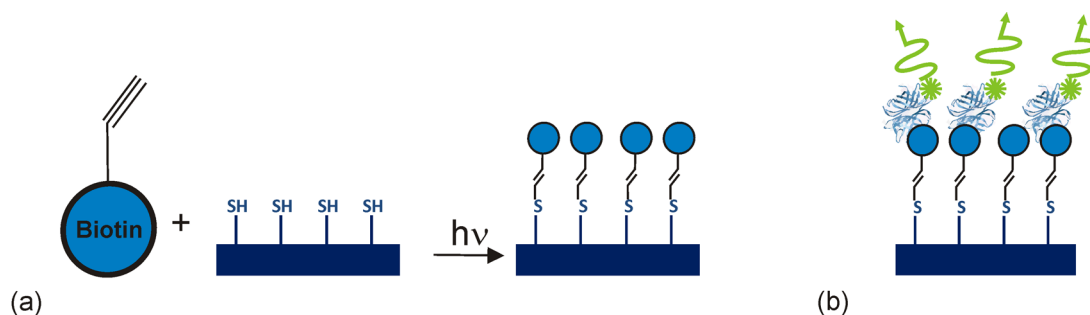


Figure 3. (a) Photochemical patterning of biotin-alkyne to thiol-ene substrates using the thiol-yne reaction. (b) Immobilization of fluorescently labeled streptavidin on the biotin functionalized surface.

RESULTS AND DISCUSSION

Figure 4 shows that the thiol surface coverage ranges between 0 and 280 SH/nm² for mixture compositions ranging from 0-120% excess thiol. However, 90% excess thiol was found to be the highest possible ratio practically usable for the fabrication of microfluidic chips and was used in further studies. Figure 3 also shows that thiol groups remain present after storage and plasma treatment.

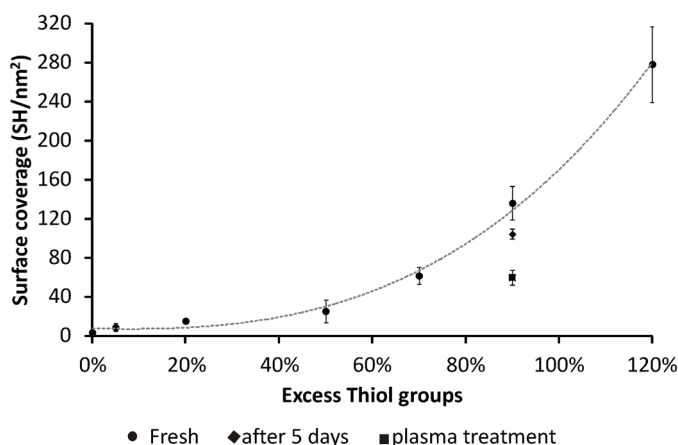


Figure 4. Thiol group density on the microfluidic chip surface as a function of initial monomeric mixture composition

The thiol-ene reaction was used to immobilize an olefin functionalized biotin directly to the thiol-ene chip surface, without any additional modification. After removal of the unreacted biotin, the surface was incubated with fluorescently labeled streptavidin for visualization. Figure 5 shows the variation in the amount of biotin/streptavidin immobilized as a function of irradiation time (without photoinitiator). Figure 6(a-b) shows that patterns as small as 20 microns could be replicated with high fidelity using a photomask. Figure 6(c-d) shows that immobilization of biotin in enclosed thiol-ene microchannels was also possible.

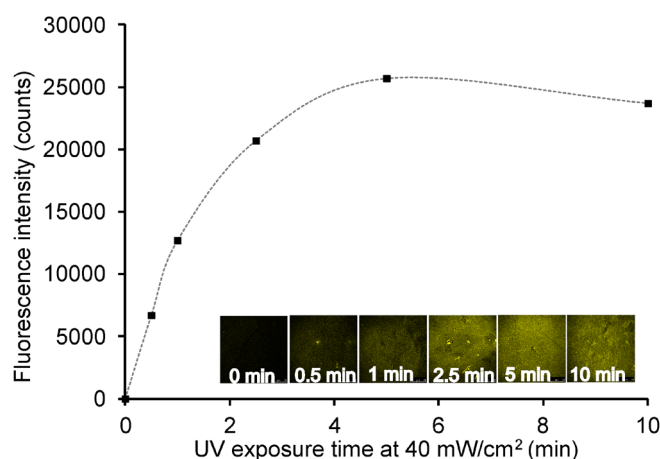


Figure 5. Fluorescence detection of the immobilized labeled streptavidin as a function of UV irradiation time.

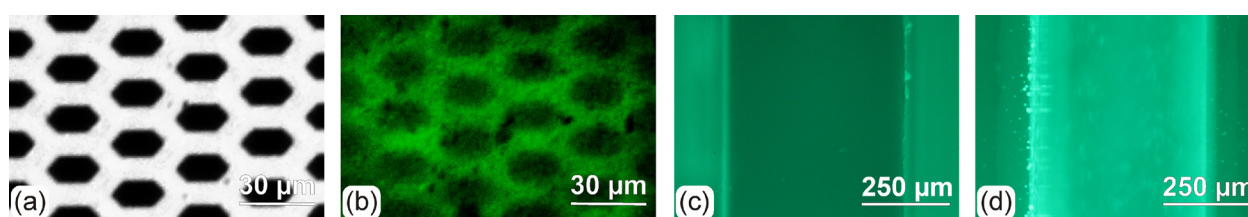


Figure 6. (a-b): Photolithographic patterning of biomolecules on thiol-ene surfaces. (a) Chromium mask used for the photolithographic patterning of (b). (b) Micrograph of the patterned biotin / labeled streptavidin. (c-d) Biotin functionalization in closed microchannels. The pictures show the thiol-ene microchannel before (c) and after (d) the photolithographic grafting of biotin and immobilization of fluorescently labeled streptavidin.

CONCLUSIONS

These results demonstrate the vast potential of thiol-ene as a substrate material in the fabrication of biochips where rapid and selective surface patterning of biomolecules is required.

ACKNOWLEDGMENTS

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