Local Modification and Laser-welding Production of Functionalized Microfluidic Chips

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laser-welding production of
functionalized microfluidic chips**

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Local modification and laser-welding production of functionalized microfluidic chips

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To my family and my girlfriend

*[...] devant cette nuit chargée de signes et
d'étoiles, je m'ouvrais pour la première
fois à la tendre indifférence du monde.*

Albert Camus - L'étranger

*[...] gazing up at the dark sky spangled
with its signs and stars, for the first time,
the first, I laid my heart open to the
benign indifference of the universe.*

Albert Camus – The Stranger

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Chapter 1

General Introduction

INTRODUCTION

Microfluidics emerged in the early 1990s and was rapidly adopted in various fields of science and technology.[1] The concept of microfluidics is a multidisciplinary field intersecting engineering, physics, (bio)chemistry and (bio)nanotechnology. In microfluidics, from a physics and engineering perspective, one aims to understand and tune the behavior of fluids that are constrained to a very small volume (e.g. a microfluidic channel). Microfluidics is described as a technology that is able to manipulate small amounts of liquids (10^{-9} to 10^{-18} liters).[1] An everyday example of a microfluidic channel would be a blood vessel, such as a capillary vessel. Fluids behave differently in such small spaces (e.g. capillary) as they would in larger spaces (e.g. a water main) – **Figure 1.1**. Whether the water main is made of PVC or inox-steel does not significantly affect the behavior of the water flow. However, at the microscale, the material and its surface properties play a huge role in the interaction between the micro-capillary and the fluid.

Figure 1.1. Illustration of different objects for a sense of scale. (a) a typical water drain pipe; (b) a hand with some highlighted blood vessels; (c) a microfluidic chip; (d) a microfluidic channel with patches of a (e) chemical monolayer.

Typical materials used in the fabrication of microfluidic devices are paper,[2, 3] silicon,[4, 5] gold, glass,[6-11] and polymers such as cyclic olefin co-polymers (COC).[5, 11-22] These materials range from hydrophilic (e.g. glass) to hydrophobic (e.g. COC). This

means that if one would construct two microchannels with the exact same geometry, but one made of glass and another of COC, the interaction of a water-based solution with the surface of the channel would be very much different. Such surfaces can be modified, to fit the specific application. For example, if the solution contains biological components, they will stick to the surface of the channel; this is called biofouling. To prevent the surficial aggregation of biological components, one must apply an anti-biofouling coating. This is e.g. relevant for applications in which the coating also contains antibodies, which would allow the microfluidic device to be used as a point-of-care (PoC) device, and which can turn it into a cutting-edge medical device for the detection of bio-markers and diseases.[8, 11, 23] Additionally, instead of testing drugs on animals, it is also possible to mimic organs by depositing cells inside a microfluidic device, the so-called “organ-on-a-chip”. [24] Alternatively, also non-biological applications can be envisaged, and a microfluidic device can be turned into an in-flow catalytic microreactor when it has a catalytic site/coating that is reactive towards an analyte within a solution (a molecule, or a biological component).[25]

The aforementioned applications rely on (or are greatly aided by) the ability to modify and functionalize the surface of a microchannel. Choosing where to immobilize molecules on a surface and thus achieve spatial and chemical control for the modifications is extremely desirable. Soft-lithography is commonly used to locally modify open surfaces.[26] Soft-lithographic methods imply some degree of direct contact between the surface and the technology (e.g. a stamp) that is creating a pattern. Micro-contact printing is the most famous of those techniques.[27] This method is very similar to stamping a piece of paper with ink (e.g. a fluorescent dye), the deposited ink pattern being obtained due to the relief patterns of the stamp.

However, enclosed surfaces such as microchannels are not amenable to localized

functionalization. Since we are not able to physically reach the inside of a channel and place the molecules directly, other methods need to be developed, such as light-induced reactions together with masks that block out the light where one does not wish the surface to be modified. This would thus constitute a classic example of photolithography (from the Greek for writing ($\gamma\rho\acute{\alpha}\phi\epsilon\iota\nu$) in stone ($\lambda\acute{\iota}\theta\omicron\varsigma$) with light ($\phi\omega\varsigma$), in which light is either used to activate the surface or to activate the solution in the desired region. Photolithography is one of the preferred approaches towards creating patterns of any desired shape on a surface, especially if looking into scaling-up processes.[28] It is an easy way to project features, very much like a ombromanie or shadowgraphy (**Figure 1.2**).

Figure 1.2. The projection of a shadow is a simple way of patterning surfaces. On the left, an ombromanie illustration shows how folded hands work as a mask to produce a shadow resembling a duck (Ombromanie, Premièr série, Paris, 1860). On the right, a collimated light source (i) shines on a photolithographic mask (ii). The projected light (iii) is used to locally irradiate a surface (iv).

This thesis will thus focus on the light-induced localized functionalization within microfluidic channels with a specific focus on glass and plastic (e.g. cyclic olefin co-polymer COC) substrates, since they correspond to a major section of the industry.[10, 29-31]

Localized functionalization inside microfluidic devices

The miniaturization of chemical processes allows for an improved control over myriad parameters that otherwise would require a great resource expenditure to tune in a larger, industrial scale. A microfluidic system offers portability, speed and lower costs due to requiring a significant lower amount of reagents. However, as mentioned above, surficial properties play a huge role in how fluids and analytes behave. Therefore, the ability to locally modify surficial properties in very specific locations is of great interest to both academia and industry. The examples of surface functionalization on glass and COC found in literature are many.[14-18, 20-22, 29] In this thesis, the methods that are able to achieve a localized functionalization are of particular interest.

Glass surfaces

Related to glass surfaces, a very interesting work done in 2001[32] showed how an ablative method allows for the patterning of hydrophobic/hydrophilic structures (**Figure 1.3**) onto silicon oxide. This method has a major disadvantage in the many steps that are required for the end product.

Figure 1.3. An ablative process via a light-induced removal of a surface-bound layer, the authors create hydrophilic/hydrophobic contrast (Reproduced with permission from [32]).

Alternatively, light-induced chemistry on hydrogen-terminated silicon substrates has been extensively studied by groups such as Hamers,[33] Chabal,[34] Buriak,[35] Lewis,[36] and Zuilhof.[37] An interesting review by Buriak,[35] shows how the reaction of an alkene with this semiconductor surface in the presence of light goes through a plurality of mechanisms. One of such mechanisms relies on the homolytic cleavage of the Si-H bond. This radical mechanism allows for the formation of very dense, patterned nanolayers.

Chabal et al. attempted to transfer this known light-induced chemistry onto hydrogen-terminated silicon oxide (H-SiO_3), but indicated that the reaction with an alkene does not proceed.[38] The wavelength (254 nm) used in this paper is strongly absorbed by the used alkene (indene) that is to be immobilized onto the H-SiO_3 surface. This coupled with the lower reactivity of the Si-H bond (compared to H-Si(111)) led the authors to conclude that the reaction cannot proceed (in that particular set of conditions) – **Figure 1.4.**

Figure 1.4. Chabal's system in which light-induced hydrosilylation of hydrogen-terminated glass was not observed (Reproduced with permission from [38]).

Other strategies (see **Figure 1.5** for an overview) such as the ones explored by Ter Maat show how a light-induced reaction of an alkene with the OH-terminus of glass (HO-SiO_3) allows for the patterning of glass.[39, 40] This method makes use of light of wavelengths that are quite incompatible with many interesting functional moieties, and additionally increases the likelihood of forming multilayers. Multilayers are obtained when instead of just immobilizing one molecule, one stacks several of them, typically by surface-bound oligomerization or polymerization processes. It is not necessarily an undesirable situation (multilayers are typically more stable than monolayers, and contain more functional groups per surface area), but multilayer formation tends to increase the surface roughness and decrease the reproducibility.

Figure 1.5. A compilation of the different types of light-induced reactivity between alkenes and silicon or silicon-oxide (glass).

COC surfaces

Plastics, such as cyclic olefin copolymers, have been increasingly used in the microfluidics industry. These materials are cheaper than glass, moldable and typically relatively inert to standard chemicals. However, inertness is undesirable if the goal is to covalently attach molecules onto its surface. To achieve this, several methods have been explored in literature, typically using harsh conditions. One ubiquitous treatment of the COC and other plastic surfaces is the use of an oxygen or air plasma to induce significant oxidation of the C-H bonds into the oxidized products. This has the disadvantage of creating a myriad functional group ranging from ketone to aldehydes and carboxylic acids. In addition, plasma methods are not compatible with enclosed surfaces such as microfluidic devices, as the plasma simply cannot diffuse into intricate enclosed structures. Therefore, we envisaged other methods towards reaching this goal.

Shilov pioneered in the 1970s the conversion of methane into methanol, via a Pt-catalyzed oxidation.[41] Since then, the activation of the C-H bond via metal-assisted catalysis has become a world of research on its own, and it is arguably one of the hottest

topics in current synthetic chemistry (**Figure 7**).[42] Yet it is typically performed for molecules in solution or gas phase.[43] A natural follow-up of this state-of-the-art would be to study whether we can develop a metal-induced activation of a COC solid surface, in water and at room temperature (compatible with the enclosed polymeric microchannel and many industry safety standards).

Figure 1.7. A schematic of the oxidation states of carbon (adapted from [44]) The controlled conversion of a C-H bond onto a C-OH bond is a chemical holy grail, only second to the alchemical reaction of turning lead into gold, as it amounts to converting (currently) inexpensive alkanes onto added-value molecules.

Bonding of substrates

Monolithic glass microfluidic devices are commonly fabricated via direct fusion bonding, followed by thermal annealing. This means that two or more substrates, of similar coefficient of thermal expansion (CTE), are pressed together at high temperatures (e.g. for glass $> 500\text{ }^{\circ}\text{C}$) resulting in a partial melt of the glass, binding the substrates together (**Figure 1.8**). A mismatch in CTE results in damage of the substrates and ineffective bonding.[45]

Figure 1.8. A simplified schematic of the direct fusion bonding via thermal annealing between two substrates. The bottom substrate has an indentation, that upon fusion with the top substrate, creates a microfluidic channel.

In addition, such high temperatures throughout the entire wafer are often incompatible with more delicate pre-treatments. One cannot simply attach organic coatings, metals with a low melting point, or fast-oxidizing metals to a surface and fabricate the device via this route, as such functionalities will simply be destroyed in the thermal annealing process. A number of different low-temperature bonding process options has been developed, such as a e.g. “glue” between substrates. However, to envisage a fabrication process that is used to produce

devices used in a ubiquity of situations, we are faced with quite a number of restrictions in regards with the materials used for the fabrication of the device.

Laser-assisted bonding

Laser-assisted bonding technologies have been developed as a way to counter the shortcomings of the methods described above.[46-48] In some reports, glass was bonded with silicon without any intermediate layer via the local welding of glass onto silicon by a Nd:YAG laser (1064 nm, 12 ns). This system requires pressure between the substrates to aid with the welding step.[49] Such a system, however, would not work on bonding two glass substrates, as glass is transparent to the wavelength used.

Outline of this thesis

Chapter 2 - Local Light-Induced Modification of the Inside of Microfluidic Glass

Chips - addresses our goal of a method that uses a maximum of 2 steps to locally attach a molecule of interest onto a glass surface. The method of attachment was defined to be photochemical and using a wavelength higher than 300 nm. Such requirements come from the transmission of glass being significantly higher at wavelengths > 300 nm when compared with the 254 nm light used in literature. In addition, light of wavelength below ~ 285 nm has been shown to initiate photoreactivity in a variety of functional groups leading to undesirable side-reactions. The produced surfaces should have high shelf-life so that the method can generate industrial interest.

Chapter 3 - Mild Photochemical Biofunctionalization of Glass Microchannels

explores a way of further increasing reactivity of the surfaces produced in the previous chapter. The light used should have a wavelength higher than the traditional 320 nm such that it allows the usage of more useful moieties, specifically including the active ester of *N*-hydroxysuccinimide – NHS. After attachment of the NHS function onto the surface we investigate whether the direct attachment of larger biomolecules is feasible, for which we take the example of an amino-terminated DNAzyme, with the aim to construct a catalytic microreactor, that would convert 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) – ABTS – into its cationic radical $ABTS^{\bullet+}$ in the presence of hydrogen peroxide.

In Chapter 4 - Mild and selective C-H activation of COC microfluidic channels

we investigate a metal-catalyzed approach towards the extremely mild and selective activation of the C-H bond present in COC substrates, comparing it with the standard air

plasma approach. The goals here were that the developed method should be solution-based, so that it is able to functionalize enclosed substrates such as microfluidic channels. In addition, it should be extremely mild and selective, meaning that we would like to convert the C-H bond in water at room temperature, preferably only to C-OH functionalities, and avoid conversion to overoxidized species such as carboxylic acids. We then investigate whether having a COC-OH surface allows us to use those hydroxyl groups as anchors to the chemistry described in **Chapter 2** and **3**, and its resistance against standard organic solvents. This opens the possibilities for the use of this modified material to a wide variety of applications, ranging from diagnostics (by immobilizing antibodies), via organ-on-a-chip applications (attaching cells), to in-flow catalysis (by immobilizing a catalyst).

In **Chapter 5 – Laser-welding of pre-functionalized glass substrates: a fabrication and chemical stability study** – we evaluate the feasibility of an a priori modification of the substrate before laser welding-assisted direct bonding. The direct bonding is achieved via a welding step where there is localized laser irradiation of an intermediate chromium oxide layer. We attached a thermally-sensitive fluorescent dye to the glass substrate before the welding procedure. Via the profiling of the extension of thermally-decomposed dye, the heat propagation can thus be indirectly evaluated.

In **Chapter 6**, the work described in this thesis is summarized. In addition, we discuss some ideas and topics where the research could either be improved or achieved via alternative approaches, including some leads from unfinished work that was performed within this project.

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Chapter 2

Local Light-induced Modification of the Inside of Microfluidic Glass Chips

Based on:

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2.1. ABSTRACT

The ability to locally functionalize the surface of glass allows for myriad biomedical and chemical applications. This would be the case if the surface functionalization can be induced using light with wavelengths for which standard glass is almost transparent. To this aim, we present the first example of a photochemical modification of hydrogen-terminated glass (H-glass) with terminal alkenes. Both flat glass surfaces and the inside of glass microchannels were modified with a well-defined, covalently attached organic monolayer using a range of wavelengths, including sub-band-gap 302 nm ultraviolet light. A detailed characterization thereof was conducted by measurements of the static water contact angle, X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and scanning Auger microscopy (SAM). Germanium attenuated total reflection Fourier transform infrared (GATR-FTIR) indicates that the mechanism of the surface modification proceeds via an anti-Markovnikov substitution. Reacting H-glass with 10-trifluoro-acetamide-1-decene (TFAAD) followed by basic hydrolysis affords the corresponding primary amine-terminated monolayer, enabling additional functionalization of the substrate. Furthermore, we show the successful formation of a photopatterned amine layer by the specific attachment of fluorescent nanoparticles in very discrete regions. Finally, a microchannel was photochemically patterned with a functional linker allowing for surface-directed liquid flow. These results demonstrate that H-glass can be modified with a functional tailor-made organic monolayer, has highly tunable wetting properties, and displays significant potential for further applications.

2.2. INTRODUCTION

The ability to locally modify silicon oxide or glass surfaces has facilitated many biomedical and chemical applications.[1-6] Local surface modification can be achieved on planar surfaces using a wide range of non-contact methods, including inkjet printing,[7] photolithography,[8] and plasma deposition.[9, 10] However, the inside of glass microchannels is far less amenable to these patterning methods.[11, 12] Soft lithography methods, e.g., microcontact printing,[13] are feasible only on exposed channel surfaces before sealing. This requires the properties of the modified exposed surfaces to be compatible with postprocessing steps, such as high-temperature fusion bonding of glass to glass. Conversely, photolithographic methods rely on and are limited so far by the absorption of light by both the glass and the reagents. As a result, commonly used glass modification chemistries like silane,[14] phosphonic acid,[15] arsonic acid[16] or catechol-based[17] approaches do not allow the local modification of discrete areas with fine details or unusual geometries at the inside of a glass microchannel by means of single-step photolithography.[14] In contrast, the photochemical attachment of alkenes has allowed the local formation of densely packed, stable organic monolayers with reactive functional groups on exposed surfaces.[18, 19] Because the reaction is photochemical in nature, it enables local modification of the inside of a glass microchannel[20] and, for example, the subsequent local attachment of fragile biologically active materials, such as DNA-enzyme hybrids. These were posteriorly used to construct a functioning and regeneratable enzyme cascade in a microchannel made from fused silica.[21] However, this photochemical reaction was shown to be limited to wavelengths lower than 285 nm. This puts constraints on the nature of the functional groups that could be attached in one step, as many organic moieties start to undergo photochemical transformations at those wavelengths. In addition, the routine application in standard borosilicate glass microchips is hampered, as glass absorbs a

significant portion of the light intensity in the range of e.g. 254 - 285 nm. Furthermore, this alkene attachment onto hydroxylated surfaces yields multilayer formation for many functional groups.[22, 23] While this likely increases the overall stability and total number of attached functionalities, it often has poor controllability and reproducibility issues. Finally, the absorbance of such wavelengths by glass may lead to (local) heating, requiring attention to prevent secondary reactions. Given these limitations and boundary conditions, alternative approaches are required for photochemical modifications that are easy to apply and also scalable in industry.

Our current studies were prompted by photoinduced hydrosilylation chemistries demonstrated on silicon surfaces.[24-27] The direct homolytic cleavage of the Si-H bond on silicon surfaces is feasible by wavelengths ≤ 355 nm ($\geq \sim 3.5$ eV), i.e., significantly longer than feasible with alkene-on-OH photochemistry.[18] Because of the fact that the transmission of borosilicate, with a thickness typical of those of glass microchips (ca. 1 mm), using wavelengths down to 300 nm, is $> 84\%$ (see Appendix I Figure S4), it is of significant interest to combine such direct hydrosilylation chemistry with the functionalization of glass microchannels. Because previous attempts in this direction have been reported not to yield Si-H dissociation-based reactivity,[28, 29] a new approach is needed. Herein we present a novel photochemical method for the local functionalization of hydrogen-terminated flat glass surfaces and the inside of glass microchannels using wavelengths > 300 nm. This was achieved by the conversion of the typical hydroxyl functions present on the glass surface to a hydrogen-terminated layer (H-glass) by modification with triethoxysilane, (H-Si(OC₂H₅)₃) or trichlorosilane (H-SiCl₃) in the first step, followed by light-induced reactions with 1-alkenes using light of 302 nm (254, 330 or 365 nm were also studied). The resulting functionalized surfaces were analyzed in detail by static water contact angle measurements (SCA) to study the wettability and packing density. X-ray photoelectron spectroscopy (XPS) was

complemented by density function theory (DFT) calculations to study the composition and thickness of the attached layer. Additionally, attenuated total reflection IR measurements using a germanium ATR crystal (GATR-FTIR) were used to derive information regarding the short-range order, packing density, and the presence of functional groups within the monolayers.[30] Moreover, this photochemistry applied in combination with lithography to microfluidic channels was used to form local patterns within microchannels to steer fluid flow (**Scheme 2.1**). Finally, we show that the stability of the hydrogen-terminated surfaces enables stepwise local modifications with different functionalities within the same microfluidic channel (multiplexing).

Scheme 2.1. Schematic representation of the local functionalization of the interior of standard glass microchips via light-induced reactions of 1-alkenes with H-glass surfaces.

2.3. EXPERIMENTAL SECTION

1-Hexadecene was obtained from Sigma Aldrich and distilled twice before use. Acetone (Aldrich, semiconductor grade VLSI PUNARAL Honeywell 17617), dichloromethane (DCM, Sigma Aldrich) and n-hexane (Merck Millipore) were used for

cleaning before modification and Milli-Q water (resistivity $18.3 \text{ M}\Omega \times \text{cm}$) for rinsing after hydrolysis process. All other chemicals were purchased from Sigma Aldrich and used as received. 10-Trifluoro-acetamide-1-decene (TFAAD) was synthesized based on literature methods,[31] with improved yield (72%, versus 65% in literature) and an optimized purification (for synthetic procedure see Appendix I).

2.3.1. Substrate preparation

For monolayer formation on flat substrates 0.7 mm thick Borofloat 33 borosilicate glass substrates (Schott) were sonicated for 10 min in semiconductor-grade acetone, subsequently dried with argon, and cleaned further using an air plasma (Harrick Scientific Products, Inc. Pleasantville, NY) for 10 min. The covalent surface modification was conducted directly afterward. The microchannels (40 mm long, 5 mm wide, $100 \mu\text{m}$ high and $18.5 \mu\text{L}$ internal volume, Micronit Microfluidics BV.) were prepared by flushing DCM extensively, dried with argon and cleaned further using air plasma for 30 min.

2.3.1.1. Formation of hydrogen-terminated modification of glass (H-glass)

Using triethoxysilane ($\text{H-Si}(\text{OC}_2\text{H}_5)_3$): For the gas-phase modification with $\text{H-Si}(\text{OC}_2\text{H}_5)_3$ (chemical vapor deposition, **CVD**), plasma-cleaned glass substrates ($1 \times 1 \text{ cm}$) were placed for 8 h with $\text{H-Si}(\text{OC}_2\text{H}_5)_3$ in a desiccator under vacuum to saturate the vessel with the silane in its gaseous form. Curing of the resulting H-glass was done in a vacuum oven (16 h, 180°C , 10 mbar). **$\text{H-Si}(\text{OC}_2\text{H}_5)_3$ - CBD:** For the liquid-phase modification with triethoxysilane (chemical bath deposition, **CBD**), plasma-cleaned glass substrates ($1 \times 1 \text{ cm}$) were immersed in a 10 mL cyclohexane solution of 1 mM $\text{H-Si}(\text{OC}_2\text{H}_5)_3$ at r.t. for 10 min. Afterward the substrate was washed copiously with cyclohexane and dichloromethane, and dried under argon.

Using trichlorosilane: 500 μL of H-SiCl_3 inside a glove box was transferred into a flask (MBRAUN MB 200B), which was further connected to a closed T-valve. This system was taken outside of the glovebox and connected to a desiccator containing the plasma-cleaned glass substrates under argon atmosphere (see Appendix I Scheme S1). The desiccator was then placed under vacuum (10 mbar) for 1 h. Subsequent closing of the vacuum and opening the connection to the silane-containing flask fills the desiccator with trichlorosilane gas, initiating the deposition. The deposition was quenched after 20 min by applying a vacuum of 10 mbar and refilling with argon before exposing the modified substrates to air (a base-containing trap was used to protect the pump from hydrochloric acid being generated due to contact with ambient humidity). The substrates were sonicated in dichloromethane for 20 min to remove physisorbed silane residues and subsequently dried with argon. The surfaces were directly used for surface characterization/modification or stored in the glovebox until further use (with the exception of stability studies, where it was stored under air).

2.3.1.2. Photochemical surface modification

A drop of the respective 1-alkene was placed on an H-glass slide in a glovebox. Another borosilicate glass slide was placed on the drop and gently pressed against the first slide,[19] both homogenously spreading the alkene and also mimicking a closed glass microfluidic channel. The surfaces were then illuminated with a UV pen lamp (254, 302, 330 or 365 nm, Jelight Company, Irvine, CA, USA), which was placed approximately 4 mm above the surface. The entire setup (lamp and substrate) was covered in aluminum foil, and the sample irradiated for 16 h. After irradiation, the substrates were extensively rinsed with distilled dichloromethane and hexane and dried under argon. The surfaces were directly used for surface characterization or stored under air.

2.3.2. Photolithography

Photolithography was performed with a 302 nm lamp, as in the aforementioned setup, in combination with a gold electron microscope grid (SEM F1, Gilder Grids). This photolithographic mask was placed on top of the H-glass slide with a drop of 1-alkene. After spreading of the liquid, a borosilicate glass slide (SCHOTT) was placed on top of the mask as a cover, above which the pen lamp was placed (ca. 4 mm distance). Samples were irradiated for 16 h, removed from the glove box and cleaned as described above. The patterns were examined with optical microscopy, scanning electron microscopy (SEM), and scanning Auger microscopy (SAM).

Microchannels were modified by applying a mask to the bottom side of a commercially available glass microchip (Micronit Microfluidics BV.; see **Scheme 2.1**). Insertion of the chip into a holder (Fluidic Connector Pro, Micronit) was followed by flushing and filling of the system with 1-hexadecene. A 302 nm lamp was placed on top of the mask (ca. 4 mm distance) and used to irradiate for 16 h. The flows were visualized using a Canon 600D with a macro lens ($f = 50$ mm, $f/1.8$ ISO100).

2.3.3. Microfluidic mask fabrication

The mask used for the microfluidic chip modification was fabricated by applying an opaque tape on a glass slide (as support), and cutting the desired pattern (sketched on SolidWorks 2015) with a VersaLaser VLS2.30 (Universal Laser Systems) at 100% power and 70% speed (200 μ m resolution).

2.3.4. Monolayer characterization

2.3.4.1. Static water contact angle measurements (SCA)

Static water contact angles (SCA) were measured using a Krüss DSA-100 goniometer. Droplets of 3 μL were dispensed on the surface, and contact angles measured with a CCD camera using a tangential method (method 2). The reported value is the average of at least five droplets of at least three different samples, and has an error of $\pm 1^\circ$ between samples.

2.3.4.2. Germanium Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (GATR-FTIR)

GATR-FTIR spectra were recorded with a Bruker Tensor 27 FT-IR spectrometer, using a commercial variable-angle reflection unit (Auto Seagull, Harrick Scientific). A Harrick grid polarizer was installed in front of the detector and was used for measuring spectra with *p*-polarized radiation with respect to the plane of incidence at the sample surface. Single channel transmittance spectra were collected at an angle of 25° using a spectral resolution of 2 cm^{-1} and 2048 scans while flushing with dry N_2 . Obtained spectra were referenced with a clean H-glass substrate (H-glass substrates were referenced with freshly plasma-cleaned glass).

2.3.4.3. X-ray Photoelectron Spectroscopy (XPS).

XPS spectra were recorded on a JPS-9200 photoelectron spectrometer (JEOL, Japan). The analysis was performed under ultra-high vacuum conditions using a monochromatic Al $K\alpha$ source at 12 kV and 20 mA and an analyzer pass energy of 10 eV. A takeoff angle ϕ of 80° was used, with a precision of $\pm 1^\circ$. All XPS spectra were analyzed with Casa XPS software (version 2.3.15). The binding energies were calibrated on the hydrocarbon (CH_2) peak with a binding energy of 285.0 eV. Because of the electrostatic charging of the surface

during the measurements, a charge compensation was used with an accelerating voltage of 2.8 eV and a filament current of 4.80 A.

2.3.4.4. Atomic Force Microscopy (AFM).

AFM images (512×512 pixels) were obtained using a scanning probe microscope (JSPM-5400, JEOL Ltd.) in tapping mode (AC-AFM) with a standard silicon cantilever (resonant frequency 70 kHz, force constant 1.7 N/m, OMCL-AC240TS-R3, Olympus, Tokyo, Japan) at a scan speed of 1 $\mu\text{m/s}$. Images were flattened with a third-order flattening procedure using WinstpmII software.

2.3.4.5. Scanning Electron Microscopy/ Scanning Auger Microscopy (SEM/SAM).

Morphologies of TFAAD micropatterns were analyzed by SEM/SAM. Measurements were performed at room temperature with a scanning Auger electron spectroscopy system (JEOL Ltd. JAMP-9500F field emission scanning Auger microprobe). SEM and SAM images were acquired with a primary beam of 0.8 keV. The take-off angle of the instrument was 0° . For Auger elemental image analyses, an 8 nm probe diameter was used.

2.3.5. Computational Procedures.

B3LYP/6-311G(d,p), M11L/6-311G(d,p), MP2/6-311G(d,p), MP2/6-311+G(d,p), and MP2/6-311++G(d,p) calculations were used to optimize geometries and study the Si-H bond dissociation energies on silicon and glass surface, for which we used $\text{H-Si(OSiH}_3)_3$ and $\text{H-Si(SiH}_2\text{SiH}_3)_3$ models, respectively (see details in Appendix I Figure S3). Electronic core level calculations (using natural bond orbital (NBO) analysis) were used to obtain the energies of the C 1s core levels to mimic C 1s XPS spectra.[32] The effect of the bulk substrate on C-Si bound monolayers was mimicked by attaching the organic species to a C-Si(OSiH₃)₃ moiety. The geometries of the different systems were for this purpose

optimized at the B3LYP/6-311G(d,p) level of theory. All of the calculations were performed with GAUSSIAN09 version D1. GaussView 5.0 was used for the visualization of output files.

2.4. RESULTS AND DISCUSSION

2.4.1. Preparation of Si-H-terminated glass (H-glass)

In order to use light-induced alkene modification of glass substrates at wavelengths higher than 285 nm,[18] we envisaged the use of hydrogen-terminated glass substrates (H-glass) in the first step. Such hydrogen-terminated (Si-H terminated) glass substrates were made via two approaches onto plasma-cleaned glass substrates: using chemical vapor deposition (CVD) or chemical bath deposition (CBD) of triethoxysilane, and CVD of trichlorosilane (see Appendix I Figure S2.1). CVD has intrinsic advantages in terms of handling and scale up, allowing the simultaneous modification of a great number of surfaces, whereas CBD is easier in terms of handling procedures. With both triethoxysilane and trichlorosilane, $\text{O}_3\text{Si-H}$ groups are deposited onto the surface, turning the standard Si-OH termination of glass into Si-H termination, thereby bestowing an entirely new chemical reactivity onto glass via an easily scalable reaction.

The presence of the Si-H bonds was confirmed by GATR-FTIR measurements for all three types of H-glass, which reveal the characteristic Si-H stretching vibration at 2250 cm^{-1} (**Figure 2.1**), but for triethoxysilane-based H-glass, C-H stretching bands ($2800 - 3000\text{ cm}^{-1}$) were also observed.[33] Montanari *et al.* have shown that complete removal of such ethoxy groups is possible by outgassing at $500\text{ }^\circ\text{C}$. [34-36] These conditions are, however, not compatible with delicate glass microstructures, for which milder reaction conditions were evaluated and optimized. An easy-to-use optimum was found after 16 h of curing at $180\text{ }^\circ\text{C}$ under vacuum. This curing process effect is shown in the IR spectra before (**Figure 2.1b**) and

after curing (**Figure 2.1c**), and demonstrates the significant reduction of ethoxy chains upon this relatively mild and microchip-compatible curing step.

XPS analysis confirms this curing step (see **Figure 2.1 right**): wide scans of a plasma-cleaned glass substrate typically indicate $< 1\%$ carbon, while modified surfaces via triethoxysilane - CVD show values of 9 ± 2 and $2 \pm 1\%$ before and after curing, respectively. Yielding a strong reduction of residual carbon, and the slightly higher carbon content after curing compared to those of plasma-cleaned surfaces can be attributed to a combination of trace residual ethoxy groups and atmospheric contamination due to handling of the sample under air.

For comparison, we also evaluated the use of chemical bath deposition using triethoxysilane. The degree of silanization and the amount of carbon residue present on the surface (14% C1s by XPS) were difficult to control,[37] and the stability of the resulting layer was comparatively low. In addition, XPS Si2p narrow scans clearly indicate polymerized multilayers composed of Si-O-Si bridges between the molecules leading to shoulder formation of the characteristic Si2p peak from 103 eV ($\text{Si}(\text{-O})_4$) to 101.9 eV ($\text{Si}(\text{-O})_3$)[38] (see Appendix I -Table S2.1). Hence, further studies via chemical bath deposition of triethoxy silane were no longer pursued, and only CVD-prepared substrates were studied in the remainder of this investigation.

To further minimize the carbon contamination, chemical vapor deposition of trichlorosilane was performed. Due the high reactivity of H-SiCl_3 , this process required strictly anhydrous reaction conditions at room temperature for 20 min before quenching. Upon extended deposition times, multilayer formation was observed, reducing reproducibility and increasing roughness (Ra) from $0.6 \pm 0.1 \text{ nm}$ (20 min) to $20.5 \pm 12.1 \text{ nm}$ (60 min) (see Appendix I - Table S2.2). The static water contact angle (SCA) of thus prepared H-glass was determined to be $85 \pm 1^\circ$. This higher contact angle reflects a higher density of Si-H

termination than obtained from triethoxysilane ($82 \pm 1^\circ$). The XPS wide scan indicates a negligible amount of carbon (1%), similar to a freshly plasma-cleaned glass surface ($< 1\%$) (see Appendix I Figure S2.2). Thus we conclude, that chemical vapor deposition of H-SiCl₃ can be a reliable method for further surface chemistry studies. The reactivity of this silane allows for short deposition times, while enabling the study of carbon contaminant-free substrates. Interestingly, H-glass was stable under air (in a closed, opaque container, r.t.) for at least a month as measured by SCA and GATR-FTIR (**Figure 2.1e**). Minimal variation of the $\nu(\text{Si-H})$ intensity ($\sim 1\%$) was observed, although gradually increasing noise in the C-H stretching region indicates some slight atmospheric contamination over this month of storage.

Figure 2.1. (left) GATR-FTIR spectra and comparison of the Si-H intensity and amount of residual carbon as observed between the various preparation methods: (a) method H-Si(OC₂H₅)₃ – CBD, (b) H-Si(OC₂H₅)₃ - CVD before curing (c) H-Si(OC₂H₅)₃ - CVD, after curing. Stability test of H-glass samples modified by H-SiCl₃: (d) freshly prepared and (e) after 1 month under air (in a closed, opaque container). (right) Comparison of C1s atomic ratios obtained from XPS wide scans of H-glass substrates compared to plasma-cleaned glass. Atomic ratios were determined by integrating the XPS C1s, O1s, and Si2p signals.

2.4.2. H-glass modification with 1-alkenes

The obtained H-Si(OC₂H₅)₃-based H-glass substrates were subsequently used for the light-induced modification with 1-alkenes, building upon light-induced hydrosilylation chemistry.[27, 28, 39, 40] 1-Hexadecyl monolayers were prepared by placing a drop of 1-hexadecene on an H-glass substrate, onto which a second standard glass slide was gently pressed (see Appendix I, Scheme S2.2). The film-separated slides were irradiated for 16 h to complete the reaction. Reacting for longer than 16 h did not give any change in SCA or XPS results. To obtain insight in the effect of the wavelength of irradiation, lamps with $\lambda = 254$, 302, 330 or 365 nm were used, and the resulting monolayers were analyzed by SCA measurements. Obtained values are shown in **Figure 2.2a**, overlaid with the glass transmittance at the used wavelengths. For the irradiation with 254 nm light (4.9 eV), it is clear that despite the high energy per photon, the reduced transmittance of light through the glass (17.4%) greatly hinders the ability to form monolayers at this wavelength. Light of 302 nm (4.1 eV) has a considerably greater transmittance through 0.7 mm of glass (83.7%). The highest static water contact angle of H-glass-based 1-hexadecyl monolayers was obtained after irradiation at this wavelength: $102 \pm 1^\circ$. For longer wavelengths (330 and 365 nm), the transmittance is near 100%, but the reaction is occurring only poorly (330 nm) or not at all (365 nm).

Figure 2.2. Photochemical monolayer formation on H-glass using 1-hexadecene and light of different wavelengths (▨ - H-Si(OC₂H₅)₃ (A), ▩ - H-SiCl₃ (B)): (a) Comparison of the resulting static water contact angles upon irradiation with monochromatic light and corresponding borosilicate glass transmittance values at that wavelength (—●—). (b) XPS-based carbon content present on modified H-glass dependent on the applied irradiation conditions (see Appendix I Figure S2.5). Notes: SCA's of non-irradiated H-glass, based on H-Si(OC₂H₅)₃ and H-SiCl₃, prior to modification are shown as a reference. H-Si(OC₂H₅)₃-based H-glass (B) was only used for modification with 302 nm irradiation. Lines were added to guide the eye.

Analogously, H-SiCl₃-based H-glass substrates were modified with 1-hexadecyl monolayers. As mentioned above, this silanization method allows for a contaminant-free substrate, making it appropriate for further detailed studies on the ordering and mechanisms of the monolayer formation. Therefore, irradiation in the presence of 1-hexadecene was studied with the optimal wavelength at 302 nm for 16 h (**Figure 2.2**). The measured static water contact angle of $109^\circ \pm 1^\circ$ is similar to reported values for alkene-derived methyl-terminated monolayers on H-terminated Si(111).[41] As the Si-H IR signal on H-SiCl₃-based H-glass was significantly higher than obtained by those of H-Si(OC₂H₅)₃, these results

suggest that the surface density of Si-H moieties on H-glass plays an important role in determining the overall monolayer density.

The thickness of the obtained monolayers after 16 h of irradiation with 302 nm light, on HSiCl₃-based H-glass, was calculated according to **equation 2.1** with a C/Si ratio of 0.44 derived from the atomic percentages of XPS wide scans (see Appendix I Figure S2.6):[42]

$$d_{ML}(\text{\AA}) = \lambda_{Si,C} \sin(\phi) \cdot \ln(1 + C/Si) \quad (2.1)$$

With $\lambda_{Si,C}$ being attenuation length of Si2p photoelectrons in the hydrocarbon layer, and ϕ being the takeoff angle between the surface and the detector. $\lambda_{Si,C}$ was calculated to be 3.9 nm.[43] The thickness of the hydrocarbon layer was thus calculated to be 1.6 ± 0.1 nm, which is similar to the calculated length of a single 1-hexadecyl molecule (1.9 nm, determined by Chem3D) at a $32.6 \pm 0.6^\circ$ tilt angle from the surface normal, and confirms monolayer formation. As an additional control, we irradiated plasma-cleaned OH-terminated glass surfaces with 1-hexadecene (302 nm, 16 h), showing no monolayer formation, in line with the literature^[18] (See Appendix I Figure S2.7). This ascertains that the described photochemical reaction at 302 nm is highly selective toward the H-glass surface. These results on H-glass show a marked improvement compared to previously reported results on hydroxyl-terminated fused silica and that of other oxides, where irradiation in the presence of 1-alkenes yields multilayer formation.[18, 19]

The quality of the H-SiCl₃-based monolayer is further validated by XPS and GATR-FTIR (see **Figure 2.3**). C1s narrow scans of these monolayers (**Figure 2.3a**) can be deconvoluted into two main peaks: the main C-C peak at 285 eV (93%), and a peak at 286.8 eV (~ 7%) corresponding to the one carbon atom per surface-bound chain (O₃Si-CH₂-), in excellent agreement with the theoretical ratio of 15:1 and also supported by B3LYP/6-311G(d,p)-derived C1s XPS simulations of 1-hexadecyl monolayer on glass.[44]

The level of short-range ordering of the hexadecyl monolayers on H-glass can be studied via GATR-FTIR measurements. The characteristic methylene symmetric and antisymmetric C-H stretching vibrations are visible at 2850 and 2918 cm^{-1} , respectively (**Figure 2.3b**), and can be compared to those of e.g. liquid 1-hexadecene (2854 and 2926 cm^{-1} , respectively) and disordered hexadecyl monolayers on hydroxyl-terminated glass (2854 and 2924 cm^{-1} , respectively).[18] As such, the monolayer on H-glass results are similar to well-ordered monolayers of alkylsilanes on flat glass substrates, and display a high degree of short-range order.[45] Such high order was only achieved upon 302 nm irradiation. With either shorter wavelengths (254 nm) or longer ones (330 nm) disordered, incomplete monolayers were formed, as inferred from only minimal changes in the XPS. Upon irradiation with 365 nm, no monolayer formation was evident, and e.g. no characteristic CH_2 IR vibrations were observed (see **Table 2.1**) The IR-inferred short-range order is thus nicely in line with the water contact angle and XPS data (*vide supra*), and indicate the formation of a high-quality alkyl monolayer onto H-glass.

Figure 2.3. (a) XPS experimental C1s narrow scan of a photochemically (302 nm, 16 h) obtained 1-hexadecyl monolayer on H-glass overlaid with a DFT-based simulation (dotted)

of the C1s narrow scan. (b) C-H stretching vibration region of the GATR-FTIR spectrum of a 1-hexadecyl monolayer on H-SiCl₃-based H-glass.

Table 2.1. GATR-FTIR data of the antisymmetric and symmetric C-H stretching vibrations of 1-hexadecyl monolayers on H-glass (in cm⁻¹).^a

Irradiation	254		302	330	365
(nm)					
Method	H-Si(OC ₂ H ₅) ₃	H-Si(OC ₂ H ₅) ₃	H-SiCl ₃	H-Si(OC ₂ H ₅) ₃	H-Si(OC ₂ H ₅) ₃
v_a(CH₃)	2958	2955	2955	2960	- ^b
v_a(CH₂)	2922	2920	2918	2924	- ^b
v_s(CH₂)	2854	2850	2850	2854	- ^b

^a GATR-FTIR data: p-polarized light, angle of incidence 25°. ^b No peaks were detected.

These data can be compared with those obtained on other surfaces. For example, the homolytic cleavage of the Si-H bond on H-terminated Si(111) requires irradiation at ~ 350 nm (3.5 eV) or shorter wavelengths.[25] Furthermore, our group showed that alkene attachment onto OH-terminated glass has an energy threshold of ca. 285 nm.[18] Whereas this may involve homolytic SiO-H bond cleavage, the observed multilayer growth over a prolonged reaction time may also be interpreted to be due to light absorption by a tail of the π - π^* band of the alkene. Upon hydrosilylation onto H-glass no such multilayer formation is observed for irradiation at 302 or 330 nm. Because the wavelength is likely short enough to break the Si-H bond homolytically but is not absorbed significantly by the alkene, we conclude that there is a threshold energy below which grafting does not occur. Furthermore, we hypothesize that it corresponds to the homolytic dissociation energy of the Si-H bond. To evaluate this energy threshold, MP2/6-311++G(d,p) calculations were performed on the Si-H

dissociation energies using a H-Si(OSiH₃)₃ model. These calculations indicate that Si-H bond cleavage on H-glass requires 3.76 eV (330 nm), while on hydrogen-terminated silicon that analogous process has a calculated dissociation energy threshold of 3.41 eV (364 nm), i.e. close to experimental observation (see Appendix I Figure S2.3 and Table S2.3). These threshold wavelengths are clearly above the energy required for π - π^* excitation of the used alkenes, e.g. 4.76 eV (260 nm) for hexadecene[18] (see Appendix I Figure S2.1), making this process extra interesting due to the minimization of alkene-based (photo-)reactions.[46]

2.4.3. Mechanistic study of monolayer formation by 1-alkenes

To investigate the mechanism of the photochemical reaction of alkenes onto H-glass substrates, surfaces were modified via H-SiCl₃ in order to avoid the presence of residual ethoxy groups on the surface and subsequently reacted with 11-fluoroundec-1-ene.[18, 47] This allows the distinction between attachment via an anti-Markovnikov mechanism (**Figure 2.4a**) that does not introduce a methyl group onto the surface and a CH₃-introducing Markovnikov addition (**Figure 2.4b**) by GATR-FTIR spectra comparison. As significant CH₂ vibrations at 2920 cm⁻¹ and 2850 cm⁻¹ become visible upon monolayer attachment, but no trace appears of an antisymmetric methyl stretching vibration (~ 2955 cm⁻¹), we infer the occurrence of an anti-Markovnikov addition (**Figure 2.4d**). This result is also in line with the high water contact angles and high degree of short-range order discussed in the previous section, both of which would be reduced with the presence of a footprint-increasing methyl group close to the surface. In this way, upon light-induced monolayer attachment H-glass reacts thus different from the photochemical attachment of 1-alkenes on OH-terminated glass, which occurs via Markovnikov addition.[18] XPS wide scans of 11-fluoro-1-undecenyl monolayers (**Figure 2.4c**) indicate peak intensities and atomic ratios of C/F, which correlate well with the number of carbons in the chain (11:1) and a C/Si ratio indicative of a monolayer formation without concurrent multilayer formation (See Appendix I Figure S2.8).

Figure 2.4. (top) Mechanistic pathways of photochemical reaction of 11-fluoroundec-1-ene onto H-SiCl₃-based H-glass (302 nm, 16 h): (a) Anti-Markovnikov addition and (b) Markovnikov addition. (c) XPS wide scans, with the inset showing the zoomed F1s region (d) C-H stretching vibration region of the GATR-FTIR.

2.4.4. Introducing chemical functionalities on H-glass

The ability to selectively modify glass substrates with additional functionalities is a crucial goal in the work presented here. Amino-terminated surfaces are an attractive starting point for covalent attachment of biomolecules or nanoparticles to substrates. H-glass surfaces were functionalized into amino-terminated substrates in a two-step procedure, as shown in **Scheme 2.2**. First, 10-trifluoro-acetamide-1-decene (TFAAD) modified glass surfaces were obtained via irradiation at 302 nm of H-glass in the presence of a drop of TFAAD for 16 h. The choice of TFAAD is dictated by the desire to have low vapor pressure (to minimize evaporation) and minimal side reactions upon irradiation at this wavelength.[46] Subsequent

deprotection to yield the amine-terminated surface was performed by a brief basic hydrolysis (0.12 M NaOtBu in DMSO, 65 s) followed by extensive rinsing with water. The static water contact angle is 77° for the TFAAD and decreases to 55° for the resulting amine-terminated monolayers, which can be explained by the more hydrophilic character of terminal amines compared to that of the fluorine terminated surface.

Scheme 2.2. Schematic representation of the photochemical attachment of TFAAD to H-glass, followed by deprotection of the monolayer resulting in a primary amine-terminated surface enabling further modification.

GATR-FTIR spectra of TFAAD and NH₂-terminated monolayers are depicted in **Figure 2.5a**. A sharp peak at 1705 cm⁻¹ in the IR data of the TFAAD monolayer is attributed to the carbonyl stretching vibration from the amide functional group, which disappears upon deprotection. Both monolayers are disordered as concluded from the positions of the CH₂ stretching peaks (2928 and 2853 cm⁻¹ for the TFAAD monolayer, and 2930 and 2855 cm⁻¹ for the amine-terminated monolayer, respectively). These results are comparable to those previously reported for TFAAD monolayers on diamond or metal oxide surfaces.[48-50] Furthermore, the absence of a CH₃ peak is yet another indication of an anti-Markovnikov addition to the surface. XPS measurements confirm the clean formation of a monolayer. From the XPS wide scan the C:Si ratio was calculated to be 0.36 (see Appendix I Figure S2.9), which gives a monolayer thickness of 1.2 ± 0.2 nm according to Equation 2.1.

This is consistent with the calculated length of a single TFAAD molecule of 1.5 nm (determined by Chem3D) at a $36.9 \pm 1.2^\circ$ tilt angle with respect to the surface normal.

Figure 2.5. (a) GATR-FTIR spectra of an H-glass sample after photochemical modification with TFAAD and further deprotection, using H-glass as a reference spectrum. (b) XPS C1s and DFT-based theoretical values (dotted) for C1s are overlaid, (c) F1s narrow scans of TFAAD monolayer, NH₂-terminated monolayer and H-glass; (d) deprotection of TFAAD by NaOtBu over time, with areas of F1s and N1s normalized to t_0 (TFAAD) values.

In addition, the C1s XPS spectrum (**Figure 2.5b**) as well as B3LYP/6-311G(d,p)-derived XPS simulations, which yield a reasonable agreement with experimental data, confirm the attachment of intact TFAAD molecules. The observed deviations are due to electrostatically induced binding energy shifts from an embedded dipole layer[51] (details in Appendix I). A comparison of the XPS narrow scan spectra shows that deprotection for 65 s induces 71% loss of the F1s peak at 688.3 eV (**Figure 2.5c** and **2.5d**). The N1s peak area

decreases in total area by only 6%, indicating a largely intact amine group (**Figure 2.5d**). This reaction time is an optimized value: shorter reactions yield less deprotection, while at longer times the monolayer starts to be degraded. These results are comparable to previously reported TFAAD deprotections on other surfaces.[50, 52]

2.4.5. Photolithography

To demonstrate the ability to functionalize substrates locally, proof-of-concept photolithography was performed. Therefore, a contact mask (gold electron microscope grid) was used to pattern the surface with TFAAD by locally blocking irradiating light (see the experimental section for details). The reaction takes place within irradiated regions only, as indicated by Scanning Electron Microscopy (SEM) (see **Figure 2.6a** and **2.6b**), with a resolution of 10 μm (see **Figure 2.6c**) and Scanning Auger Microscopy (SAM) (see **Figure 2.6d**), which reveals the local attachment of a monolayer and of F atoms onto the surface, respectively. In addition, the pattern can be made visible without any instrumentation by blowing wet air over the surface. This instrument-free technique allows to visualize the pattern due to the different wettability of the reacted and non-reacted area, albeit at lower resolution (See Appendix I Figure S2.10). Finally, after deprotection of the TFAAD, the resulting amine-terminated patterned substrate was made to react with p-phenylene-diisothiocyanate (in acetone, 0.1 M) for 30 s immediately followed by washing with acetone and immersion in a solution of NH_2 -terminated fluorescent, 4 nm-sized Si nanoparticles (DMSO, 50 mg/mL). After extensive washing and sonication in water and DCM, the pattern was visualized using fluorescence microscopy (See **Figure 2.6e** and Appendix I Scheme S2.3).

Figure 2.6. Photo-attachment of a patterned TFAAD monolayer onto H-glass: (a) Scanning electron microscopy (SEM) image, (b) Close-up image of the fraction of the SEM image showing the frontier between TFAAD functionalized area and nonfunctionalized H-glass, the red line represents a cross section (c) measuring the SEM-based pixel intensity between these areas, which drops fully over a distance of 10 μm; (d) Scanning Auger Microscopy (SAM) image for F 1s (green) at 686 eV of the same area; (e) Fluorescence microscopy picture of the deprotected primary-amine substrate after reaction with a diisocyanate and coupling to amine-terminated fluorescent Si nanoparticles.

2.4.6. Photolithography in microfluidic glass channels

Building on the successful photolithography on flat, light-exposed H-glass surfaces, the local photochemical attachment of 1-hexadecene onto enclosed surfaces was achieved. As a proof of concept, the local modification of the inside of a microchannel of a commercial microfluidic chip with 1-hexadecene was studied using opaque tape locally attached onto the

microchip (302 nm, 16 h; see **Figure 2.7**). Because of the shown wettability properties of functionalized flat surfaces, we reasoned that the static water contact angle of the thus obtained locally bound monolayers might be sufficiently high to give indications of monolayer formation via surface-directed liquid flow.[11, 53] To increase this surface-driven effect, the remaining hydrogen-terminated (non-alkylated) areas of the microchannel were photo-oxidized[54, 55] to standard, OH-terminated glass via irradiation at 302 nm for 16 h in the presence of water and air in order to locally enhance the hydrophilicity (**Figure 2.7**). Subsequently, aqueous solutions introduced into these patterned channels are confined to the hydrophilic surface area as long as exerted pressure onto the liquid, to induce flow, is maintained below a critical value (in our set-up to a flow of 500 $\mu\text{L}/\text{min}$).[8] In other words: a 1.5 nm monolayer suffices to steer a 100,000 nm high flow. To visualize this process a solution of dyed water was introduced into the microchannel at a flow rate of 50 $\mu\text{L}/\text{min}$. As clearly seen (**Figure 2.7a**), the water avoids the hydrophobic area creating air pockets and swirls through the microchannel, yielding a mixing laminar flow (see Appendix I – Videos). This demonstrates that local functionalization by light-induced monolayer attachment is feasible, and that the attached hydrophobic layer can function as a passive directional flow actuator and thus as a fluid mixer. Although the quality of the prototypical ‘mask’ and lamps results in a not sharply defined patterning (LASER spot size resolution of 200 μm), the proof of concept is clear. In addition, the quality of the locally attached monolayers is high, as indicated by the stability of the swirling laminar flow (patches were not wetting) up to 4 h of continuous flow of water through the chip (longer times will lead to the eventual diffusion of air into the water flow). In addition, storage under ambient conditions of the chip for two months after the initial use, and reiterating experiments displayed identical results, proving the high quality and potential of these monolayers.

Figure 2.7. (top) Schematic representation of the photolithographic attachment of 1-hexadecene on the inside of a microchannel, followed by photo-oxidation of the remaining Si-H moieties for enhanced contact angle contrast. (a) Macro-photograph of a meandering microfluidic channel (width 5 mm, height 100 μ m) with local pockets of air due to patterned hydrophobicity. [Note: real-time videos 1,2 and 3 are linked in Appendix I.] (b) Schematic representation of the used photolithographic mask placed on top of the microchannel.

We have also shown that this chemistry can be used for surface-directed liquid flow. Since water does not wet hydrophobic patches, we hypothesized that the 1-hexadecene-derived monolayer may function as a capillary stop valve. To reveal this, a section of the channel (height 100 μ m) inside a microchip made from glass was patterned with an hexadecyl monolayer (height 2 nm), and water was introduced into the channel by means of capillarity. Water gently filled the channel until it reached the monolayer and stopped (see **Figure 2.8**). Then, by applying pressure to the inlet, the flow eventually resumed after reaching a certain pressure threshold. The effect is retained upon emptying the channel and drying it. Storing the chip in the dark for 2 months maintains the same passive stop-valve effect.

Figure 2.8. (a) Capillary flow of water inside a hydrophilic channel made in glass stopped by a hydrophobic patch (b) channel after a pressure pulse triggered the bursting of the valve.

2.5. Conclusions

We have shown the successful local modification of the inside of a glass microchannel with light using wavelengths longer than 300 nm via a two-step procedure including uniform silanization (H-glass) followed by local photochemical attachment of alkene derived monolayers. Modification of H-glass with 1-hexadecene yielded hydrophobic surfaces (110°). The mechanism of the hydrosilylation reaction was confirmed to proceed via an anti-Markovnikov addition to the surface. Local modification with a 1-alkene bearing a protected amine (TFAAD), and subsequent deprotection resulted in a locally amine-functionalized surface, which could be used to covalently pattern glass with nanoparticles. The procedure yields high-quality monolayers (well-ordered, stable for months) and provides the first approach to locally modify standard commercial glass microfluidic chips with a widely-increased range of chemical functionalities compared to those in previous methods. The locally attached monolayer can be used to direct the flow of water within microchannels. As such, this easy-to-perform methodology for the local functionalization of microchips has significant potential for a wide range of applications.

2.6. Appendix I.

Tables with silanization method comparison (SCA, XPS, AFM). Theoretical calculations for Si-H bond energy. Schematics for CVD setup, photochemistry setup, SiNP reaction. XPS of the monolayers. Micrograph of hydrophobic pattern.

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Chapter 3

Mild Photochemical (Bio-)Functionalization of Glass Microchannels

Based on:

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3.1. ABSTRACT

The ability to locally modify the inside of microfluidic channels with bioactive molecules is of ever-rising relevance. In this paper, we show the direct photochemical coupling of a *N*-hydroxysuccinimide-terminated ω -alkene onto hydrogen-terminated silicon oxide, and its subsequent functionalization with a catalytically active DNAzyme. To achieve this local attachment of a DNAzyme, we prepared hydrogen-phenyl-terminated glass (H- Φ -glass) by the reaction of glass with H-SiPhCl₂. The presence of a radical-stabilizing substituent on the Si atom (i.e., phenyl) enabled the covalent modification of bare glass substrates and of the inside of glass microchannels with a functional organic monolayer that allowed direct reaction with an amine-functionalized bio-molecule. In this study, we directly attached an NHS-functionalized alkene to the modified glass surface using light with a wavelength of 328 nm, as evidenced by SCA, G-ATR, XPS, SEM, AFM and fluorescence microscopy. Using these NHS-based active esters on the surface, we performed a direct localized attachment of a horseradish peroxidase (HRP)-mimicking hemin/G-quadruplex (hGQ) DNAzyme complex inside a microfluidic channel. This wall-coated hGQ DNAzyme effectively catalyzed the in-flow oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate) [ABTS] in the presence of hydrogen peroxide. This proof-of-concept of mild bio-functionalization will allow the facile preparation of modified microchannels for myriad bio-relevant applications.

3.2. INTRODUCTION

The localized functionalization of silicon oxide and glass surfaces with organic functional molecules is crucial for a wide range of advanced applications of glass-based (bio-)hybrid materials.[1-4] Most surface modifications on glass make use of silane chemistry,[5-7] although other molecule-to-surface linkages[8] like phosphonic acid,[9] arsonic acid,[10] or catechol-based[11] have also been used. While these oxygen-based anchor-groups have proven their value for thermal anchoring reactions, they are incompatible with single-step photolithographic patterning. Photolithographic patterning is the preferred way of functionalizing enclosed surfaces, such as microfluidic channels.[12] In previous work, we showed that precisely positioned patterns could be achieved in glass capillaries using the photochemical attachment of alkenes.[13-15] However, the light-induced reaction on those surfaces required UV light with $\lambda < 285$ nm, precluding the use of standard commercial glass microchannels. To circumvent these limitations, we recently reported a method for light-induced hydrosilylation of hydrogen-terminated glass (H-glass), which was obtained by surface-activation of glass with HSiCl_3 . This chemistry allowed the use of lower energy light ($\lambda = 302$ nm) to locally modify the inside of microfluidic channels with functional groups that facilitated further modifications;[16] this was previously only feasible using hydrogen-terminated silicon substrates.[17-19] Specifically, we installed nanometer-sized hydrophobic-alkyl or fluoro-alkyl patches, *de facto* molecular phaseguides[20] or “nanodikes”, at chosen positions inside a microchannel in order to regulate its flow.[16] However, our previous approach proved incompatible with photosensitive *N*-hydroxysuccinimide (NHS) ester moieties due to the short wavelength required (302 nm) for the functionalization of the surface. This was particularly unfortunate as the direct photochemical attachment of such a moiety would be highly advantageous for a variety of microfluidics-based biological applications, *e.g.* in diagnostics (immobilization of antibodies) and in-flow biocatalysis. To

solve this, we aimed to reduce the Si-H bond energy to such a level that light of significantly lower energy could be used. This lower energy light would be compatible with the use of (NHS)-containing ω -alkenes – which are photoreactive with wavelengths < 310 nm – and as such would allow, for the first time, the direct photochemical attachment of bio-functionalizable groups onto hydrogen-terminated glass.[21-23] We hypothesized that the presence of a phenyl ring on the Si atom would stabilize the intermediate Si $\cdot$ radical,¹⁶ [24] [25], [26] thereby facilitating the photochemical homolytic Si-H bond cleavage at a longer wavelength, and thus the direct attachment of NHS-functionalized ω -alkenes on H-glass surfaces.

Indeed, we now demonstrate that the addition of a phenylsilane (phenyldichlorosilane – H-SiPhCl₂) with radical-stabilizing properties to a glass surface allows for the lowering of the reaction energy threshold to longer wavelengths, *i.e.* 328 nm (**Scheme 3.1**). Importantly, this seemingly minor elongation of the wavelength from 302 to 328 nm had significant effects on the applicability of the technique, as this now allows the direct photolithographic coupling of NHS-terminated ω -alkenes. The resulting functionalized surfaces were analyzed by X-ray photoelectron spectroscopy (XPS) to study the composition and thickness of the attached layer. In addition, attenuated total reflection IR measurements using a germanium crystal (GATR-FTIR) were used to derive information regarding the short-range order, packing density, and the presence of functional groups within the monolayers.[27] Atomic force microscopy (AFM) was used to study the topography and surface roughness. Ambient desorption ionization high-resolution mass spectrometry, namely DART-HRMS, allowed (quick) detection and quantification of surface-bound low molecular weight species such as NHS.[28, 29] Scanning electron microscopy (SEM) and fluorescence microscopy were used to visualize patterned surfaces. To show that the NHS-functionalized surface is only one step removed from biofunctionalization, we locally attached a horseradish peroxidase (HRP)

mimicking hemin/G-quadruplex (hGQ) DNAzyme[30] with a pending amine moiety to the inside of a glass microchannel, turning it into a biomimetic reactor.

Scheme 3.1. Schematic of the fabrication of various H-glasses and the subsequent photochemical hydrosilylation. (a) H-glass, reactive at a wavelength of 302 nm, with R being a compatible end group; (b) Φ -glass, reactive at 328 nm, with R_1 being a photo-sensitive group such as NHS; (c) mixed $H_5\text{-}\Phi_1$ -glass, with a small fraction of Ph moieties at the surface, reactive at wavelengths of 328 nm, but more homogeneous than pure Φ -glass.

3.3. EXPERIMENTAL SECTION

Acetone (semiconductor grade VLSI PUNARAL, Honeywell), dichloromethane (DCM, Sigma Aldrich), and *n*-hexane (Merck Millipore) were used sequentially for cleaning glass surfaces before modification. Milli-Q water (resistivity $18.3 \text{ M}\Omega \times \text{cm}$) was used for all aqueous solutions. Amine-functionalized DNA was purchased from Integrated DNA Technologies, sequence: $5'\text{-H}_2\text{N-C}_{12}\text{H}_{24}\text{-CTGGGAGGGAGGGAGGG-3'}$. Fe(III)-protoporphyrin IX (hemin chloride) was purchased from VWR international.

Dichlorophenylsilane ($\text{H SiPhCl}_2 \geq 98\%$), trichlorosilane (H SiCl_3 99%) and all other chemicals were purchased from Sigma Aldrich and used as received. *N*-succinimidylundecyl-1-enate (**C11NHS**) was synthesized based on literature methods.[23, 31] Silicon nanoparticles were synthesized based on literature methods.[32]

3.3.1. Substrate preparation

For monolayer formation on flat substrates, 0.7 mm thick (1×1 cm) borosilicate glass substrates (Borofloat 33, Schott) were sonicated for 10 min in acetone, dried with argon, and cleaned further using an argon plasma for 15 min, followed by an oxygen plasma (Harrick Scientific Products, Inc. Pleasantville, NY) for 10 min. The covalent surface modification procedure was performed directly afterwards. Microreactors made from Borofloat glass (R150.332.2, Micronit – internal volume 18.7 μL , channel width 300 μm , depth 150 μm) were exposed to argon plasma for 1 h, followed by oxygen plasma for 30 min. The argon plasma is instrumental in removing any carbon and fluorine-containing contaminants that can become visible in XPS. Alternatively, the use of an etching solution such as KOH or piranha solution is also effective and recommended for more intricate microfluidic channels.

3.3.2. Formation of hydrogen-phenyl-terminated glass (H- Φ -Glass)

Solutions of varying concentrations and ratios of H SiPhCl_2 and H SiCl_3 [pure H SiPhCl_2 (55 mM); 5:1 H SiCl_3 (63 mM)/ H SiPhCl_2 (12 mM); 12:1 H SiCl_3 (63 mM)/ SiPhCl_2 (5 mM)] were prepared in anhydrous cyclohexane inside a glove box and stored in there until used. These solutions, in closed glassware and under argon, were removed from the glove box and the plasma-cleaned glass substrates (1×1 cm²) were immersed in the silane solution for 20 min. Afterwards the substrates were washed copiously with cyclohexane and dichloromethane, and dried under argon. For the microfluidic chip, the 5:1 silane solution was flushed into the channel; the flow was then stopped to allow a

homogeneous silane deposition for 20 min. Subsequently, the channels were flushed with DCM, at 100 $\mu\text{L}/\text{min}$ for 30 min, to ensure no clogging occurred due to any unreacted silane.

3.3.3. Photochemical surface modification

The H- Φ -glass substrates were inserted in a closed vessel, to prevent solvent evaporation, and immersed in a 35 mM solution of **C11NHS** in trichlorobenzene. The substrates were then irradiated with a pen lamp (328 nm, Jelight Company, USA – company indicator: 330 nm) that was placed at a distance of approximately 1 cm from the substrate. A long-pass filter (Edinburgh Instruments) was placed between the samples and the lamp, and required to remove wavelengths $\ll 320$ nm in the emitted spectrum (**Figures S3.1 and S3.2**). The setup (lamp and half of the substrate-containing vessel) was covered in aluminum foil, and the sample irradiated for 16 h (see **Figure S3.3** for the experimental setup). After irradiation, the substrates were extensively rinsed with distilled DCM, sonicated for 1 min in DCM, and dried under argon. The surfaces were directly used for surface characterization, or were stored under argon. For the microfluidic chip, the NHS solution was flushed into the channel, and then the flow was stopped to allow a homogeneous reaction for 16 h (*vide infra*, see also **Figure S3.4**).

3.3.4. Photolithography

Photolithography was performed with a 328 nm light lamp, as in the aforementioned section, in combination with a gold electron microscope grid (SEM F1, Gilder Grids) as a mask. This photolithographic mask was placed on top of an H₅- Φ ₁-glass slide with a drop of a 35 mM solution of **C11NHS** in trichlorobenzene. After spreading of the liquid, the sample was inserted in the closed vessel along with unmasked substrates as a control. The setup (lamp and half of the substrate-containing vessel) was covered in aluminum foil, and the

sample irradiated for 16 h. After irradiation, the substrates were extensively rinsed with distilled DCM, sonicated for 1 min and dried under argon. The surfaces were directly used for surface characterization or stored under argon. The NHS-terminated surfaces were then further reacted with fluorescent amino-terminated SiNPs (50 mg/mL in DMSO), and cleaned by sonication in water. The patterns were then examined with fluorescence microscopy and scanning electron microscopy (SEM).

3.3.5. DNAzyme Attachment and hGQ formation

For the attachment of the G-quadruplex, the NHS-modified flat glass was reacted with a 100 μ M buffered solution of a NH₂-terminated synthetic DNA fragment for 24 h (18 mM PBS (pH 7.4) with 100 mM KCl). The surfaces were then rinsed with MilliQ water and sonicated for 2 min. These DNA-modified substrates were then subjected to a 100 μ M solution of Fe(III)-protoporphyrin IX in DMSO for 30 min. The surfaces were then rinsed with MilliQ water and sonicated for 2 min. These DNAzyme-modified samples were then characterized by GATR-FTIR, XPS and AFM.

Similar to a flat glass substrate, the NHS-containing microchannels were filled with a 100 μ M solution of the DNAzyme (18 mM PBS, pH 7.4, 100 mM KCl) and left to incubate overnight. Following extensive rinsing with Milli-Q water for 2 h (at a flow rate of 100 μ L/min), the channels were dried by flushing with argon. The protocol proceeded with the hGQ attachment by filling the channel with a 100 μ M solution of Fe(III)-protoporphyrin IX in DMSO. The system was left to incubate overnight, rinsed for 2 h with Milli-Q water (at a flow rate of 100 μ L/min), and finally flushed with argon. The hGQ-containing substrates were directly used for the catalytic experiments.

3.3.6. Peroxidation experiments

Peroxidation reaction was carried out at 25 ± 1 °C in 100 μ M PBS (pH 6.0) with 5 μ M ABTS diammonium salt and 5 μ M H₂O₂. The microfluidic chip (R150.332.2) was placed in a holder (Fluidic Connect Pro, Micronit) and solutions of ABTS and the H₂O₂ were injected into the inlets at variable flow rates, using a Chemyx syringe infusion pump. Formation of the oxidized product (ABTS⁺•) was monitored by collecting the solution that exited the chip, and UV-Vis analysis thereof with a Cary UV-Vis Spectrophotometer (350–500 nm). Control experiments were performed by eliminating each of the components at a time: (i) ABTS (75 μ L/min) and H₂O₂ (75 μ L/min) flushed through a hGQ-modified chip; (ii) ABTS (75 μ L/min) and H₂O (75 μ L/min) flushed through an NHS-modified chip that was exposed to hemin; (iii) ABTS (75 μ L/min) and H₂O₂ (75 μ L/min) flushed through an unmodified chip.

3.3.7. Monolayer characterization

3.3.7.1. Static water contact angle measurements (SCA)

Static water contact angles (SCA) were measured using a Krüss DSA-100 goniometer. Droplets of 3 μ L were dispensed on the surface, and contact angles measured using a tangential method. The reported value is the average of at least five droplets on at least three different samples.

3.3.7.2. Germanium Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (GATR-FTIR)

GATR-FTIR spectra were recorded with a Bruker Tensor 27 FT-IR spectrometer, using a commercial variable-angle reflection unit (Auto Seagull, Harrick Scientific). A Harrick grid polarizer was installed in front of the detector and was used for measuring

spectra with *p*-polarized radiation with respect to the plane of incidence at the sample surface. Single-channel reflectance spectra were collected at an incident angle of 25° using a spectral resolution of 4 cm⁻¹ and 1024 scans while flushing with dry N₂. The resulting spectra were divided by a background reference, the reference used was the spectrum of the very last step before that modification step: DNA-modified glass was referenced with **NHS**-terminated glass, which was in turn referenced with H_x-Φ_y-glass; lastly, the H_x-Φ_y-glass substrates were referenced with a freshly plasma-cleaned glass substrate.

3.3.7.3. X-ray Photoelectron Spectroscopy (XPS)

XPS spectra were recorded on a JPS-9200 photoelectron spectrometer (JEOL, Japan). The analysis was performed under ultra-high vacuum conditions using a monochromatic Al K α source at 12 kV and 20 mA and an analyzer pass energy of 10 eV. A takeoff angle ϕ of 80 ± 1°. All XPS spectra were analyzed with Casa XPS software (version 2.3.15). The binding energies were calibrated on the hydrocarbon (CH₂) peak with a binding energy of 285.0 eV. Because of the electrostatic charging of the surface during the measurements, a charge compensation was used with an accelerating voltage of 2.8 eV and a filament current of 4.80 A.

3.3.7.4. Computational Procedures

M11/6-311+G(d,p), M11L/6-311+G(d,p), B3LYP6-311+G(d,p), and B97D/6-311+G(d,p) calculations were used to optimize geometries and study the Si–H bond dissociation energies (BDE) on H-glass and Φ-glass for which we used H–SiO₃Si₃H₃O₃ and H–SiPhO₂Si₃H₄O₃ models, respectively (**Table S3.1**, **Figure 3.1**). All of the calculations were performed with Gaussian 09 version D1.[33] GaussView 5.0 was used for the

visualization of output files. The Si–H BDE were calculated using these methods as the enthalpy change of the following reaction in the gas phase at 298.15 K, 1.013×10^5 Pa:

3.4. RESULTS AND DISCUSSION

3.4.1. Evaluation of the substituent effect on the Si–H dissociation energy

To study the feasibility of longer-wavelength initiation of the radical-mediated hydrosilylation reaction, we performed theoretical calculations of the effect of a range of small substituents on the homolytic Si–H bond dissociation energy of small glass models. We limited ourselves to those substituents (R) on the top of a surface (that would look like $(-\text{O})_2\text{SiR-H}$) that can be easily obtained as RHSiCl_2 , preferably from a commercial source. We found that radical stabilization is not easily achieved on Si; in fact, only when $\text{R} = \text{Ph}$ did we calculate a small but significant lowering of the homolytic Si–H bond cleavage. Specifically, both H-glass and the so-called Φ -glass were simulated by two clusters as representatives ($\text{H-SiO}_3\text{Si}_3\text{H}_3\text{O}_3$, $\text{H-SiPhO}_2\text{Si}_3\text{H}_4\text{O}_3$) using various density functional theory (DFT) methods (see **Figure 3.1**). This revealed that the Si–H bond strength (typically 95 kcal on H-glass) is reduced by $\sim 4\text{--}5$ kcal/mol upon replacement of a H by a Ph, which yields a corresponding red-shift in the optical absorption spectrum ($\sim 15\text{--}20$ nm for glass, **Table S3.1**), this absorption shift was inferred from the bond energy shift.[26]

Figure 3.1. Schematic representation of the Si-H bond dissociation and resulting HOMO of (a) Φ -glass and (b) H-glass. Blue – silicon, red – oxygen, grey – carbon, white – hydrogen.

3.4.2. Synthesis of hydrogen-phenyl-terminated glass (H- Φ -glass)

Hydrogen-terminated (Si-H) glass substrates were made on plasma-cleaned glass substrates using chemical bath deposition (CBD) of trichlorosilane (H-SiCl_3), dichlorophenylsilane (H-SiPhCl_2) and mixtures thereof. This reaction adds $(-\text{O})_3\text{Si-H}$, $(-\text{O})_2(\text{Ph})\text{Si-H}$, or a mixture of these onto the surface of the glass substrates, and thereby turns the standard termination from Si-OH into Si-H. Trichlorosilane-prepared surfaces yield nicely defined surfaces,[16] pure dichlorophenylsilane-prepared glass possessed rougher surfaces, and mixed surfaces obtained from glass treated with 5:1 or 12:1 $\text{H-SiCl}_3/\text{H-SiPhCl}_2$ ($\text{H}_5\text{-}\Phi_1$ -glass and $\text{H}_{12}\text{-}\Phi_1$ -glass, respectively) yielded intermediate roughnesses (**Figure S3.5**). The presence of Si-H bonds with two different vibrational frequencies were predicted computationally (**Table S3.2**) and confirmed by GATR-FTIR measurements for varying ratios of the silanes, which reveal characteristic $\text{O}_3\text{Si-H}$ stretching vibration at 2250 cm^{-1} (**Figure 3.2c**) in addition to the substituted $\text{O}_2\Phi\text{Si-H}$ at 2180 cm^{-1} (**Figure 3.2a and S3.6**), in the mixed monolayer the $\Phi\text{-Si-H}$ peak is visible as a shoulder-peak at *ca.* 2180 cm^{-1} (**Figure**

3.2b). At higher ratios of Si–H to Φ -Si–H (12:1), it becomes hard to quantify the amount of Φ Si–H due to the low intensity of the peak. The red shift in the Si–H stretch energy is consistent with a reduction in the electronegativity of the substituents (i.e., replacement of O with Ph).[34]

Figure 3.2. GATR-FTIR of glass functionalized with varying ratios of HSiCl_3 and HSiPhCl_2 .

(a) pure Φ -glass, (b) H_5 - Φ_1 -glass, (c) H_{12} - Φ_1 -glass.

Modification of glass to Ph-H-glass increases the static water contact angle (SCA) from $< 10^\circ$ to $102 \pm 5^\circ$ (pure Φ -Si–H), whereas mixed H_{12} - Φ_1 -glass surfaces display an SCA of $89 \pm 1^\circ$, *i.e.* close to the value of 85° for H-glass itself.[16] This higher contact angle reflects an increasing fraction of phenyl groups on the surface. The XPS wide scans across multiple samples indicate an increasing amount of carbon with the increasing amount of Φ -Si–H groups (see **Figure S3.7**; XPS Si 2p narrow scans can be seen in **Figure S3.8**). We conclude that chemical bath deposition of a mixture of $\text{Cl}_3\text{Si-H}$ and $\text{Cl}_2\text{PhSi-H}$ is a reliable method to obtain H- Φ -glass surfaces.

3.4.3. Surface modification of H- Φ -glass with a NHS-terminated ω -alkene

NHS-modified glass surfaces were obtained via irradiation of H₅- Φ ₁-glass in a solution containing an ω -NHS-functionalized 1-dodecene (35 mM in trichlorobenzene; λ = 328 nm; 16 h). Formation of NHS-terminated surfaces was apparent from GATR-FTIR, which displayed three characteristic peaks at 1748, 1790 and 1815 cm⁻¹ (**Figure 3.4**).[23] Furthermore, the presence of NHS was confirmed via DART-HRMS, which yields strong signals for the succinimide anion (m/z 114.0165; NHS⁻) and the corresponding [2 NHS⁻ + H⁺]⁻ species, in line with previous findings.[28, 29] Interestingly, we observed that H₅- Φ ₁-glass that was functionalized using 328 nm and **C11NHS** produced a signal intensity of surface-bound NHS that was a full order of magnitude higher than that of similarly NHS-functionalized H-glass (**Figure S3.9-3.10**).

Figure 3.3. Overlay of: the absorption spectrum of the **C11NHS** (solid red line); transmittance spectrum of the 320 nm long pass filter (solid black line); emission spectrum of the 328 nm lamp filtered through the 320 nm long pass filter (dotted blue line).

We would like to highlight the following three crucial experimental features. First, the choice of trichlorobenzene as a solvent was dictated by the lack of labile protons, thus preventing side reactions where the solvent (such as isopropylalcohol, toluene or mesytelene)

might attach to the hydrogen-terminated silicon oxide surface, as observed by Chabal and co-workers.[17] Second, the vapor pressure of trichlorobenzene is lower than lesser-substituted analogues, such as chlorobenzene, and a low vapor pressure is required to keep the solvent on the glass surface for a sufficient amount of time to allow prolonged irradiation (16h).[35] Third, the 328 nm pen lamp with a 320 nm long-pass filter is crucial, as the reaction of H- Φ -glass and NHS-terminated alkene using a 302 nm pen lamp with a 280 nm long-pass filter (see **Figure S3.1-S3.2** for the lamps emission spectra with and without filters), yielded extensive NHS degradation and polymerization (**Figure S3.11**), also evidenced from heavy coloring of the solution.

Figure 3.4. (a) Schematic representation of the photochemical attachment of **C11NHS** to H₅- Φ ₁-glass. (b) GATR-FTIR spectra of an H₅- Φ ₁-glass sample after photochemical modification with C11NHS using H₅- Φ ₁-glass as a background reference. (c) Vibration modes of the NHS moiety.[36]

The thickness of the **C11NHS** monolayers on H₅-Φ₁-glass, after 16 h of irradiation with light of 328 nm wavelength, was calculated from the atomic percentages of XPS wide scan spectra, after blank subtraction, according to **equation 3.1**. This resulted in a normalized Si^{NHS}:Si^H ratio of 0.908 ± 0.0183 (see **Figure 3.5**):[37]

$$\ln(Si_{2p}^{NHS}/Si_{2p}^H) = d/\lambda_{Si,C}(1/\cos\phi) \quad (3.1)$$

With $\lambda_{Si,C}$ = attenuation length of Si_{2p} photoelectrons in the hydrocarbon layer (3.94 nm),[38, 39] and ϕ = takeoff angle between the surface and the detector. This yielded a thickness of the hydrocarbon layer of 2.18 ± 0.45 nm, *i.e.* in line with that expected for a dense monolayer of NHS-functionalized chains, as this is just slightly larger than the calculated length of a single **C11NHS** molecule (1.6 nm, determined by Chem3D). A schematic depiction of the types of carbon atoms that are distinguishable by XPS is shown in **Figure 3.5c**. The signal of the C1s narrow scan of the NHS-ester-terminated monolayer on H-Φ-glass could be deconvoluted into three peaks (**Figure 3.5d**), at 285.1 eV for aliphatic and aromatic carbons, 286.4 eV for $-\underline{C}H_2-C(=O)$ and Si- $\underline{C}H_2$, and at 289.7 eV for $\underline{C}=O$. The ratio between these three peaks for a NHS-ester terminated monolayer was theoretically predicted to be 9:3:3 for pure silicon oxide, free of other carbon sources. However, in our case the presence of the phenyl group is expected to add carbon to the lowest energy peak, and the narrow scan peak-ratio of 13:2:2 is thus in line with efficient NHS attachment (1.5 C_{Ph} per **C11NHS** attached; corresponding approximately to the attachment site ratio of 5:1 of SiH:SiPhH). Thus, we can confirm that under these conditions, we have covalently attached NHS on the surface and that we do not (or only to a very minor degree) observe the formation of multilayers with the method described above. Analogously, modification of H₅-Φ₁-glass with 10-trifluoro-acetamide-1-decene (TFAAD)[16] under irradiation of 328 nm light was also successful.

Figure 3.5. XPS wide scans of (a) H₅-Φ₁-glass and (b) NHS-terminated glass; (c) XPS C 1s narrow scan in which the various C atoms of the NHS-terminated glass are indicated; (d) shows the different C atoms of the NHS-terminated Φ-glass.

3.4.4. Modification with nanoparticles

To demonstrate the ability to functionalize substrates locally, photolithography was performed on the H₅-Φ₁-glass surfaces using 328 nm light. A contact mask (gold electron microscope grid) was used to pattern the surface with **C11NHS** by locally blocking irradiating light (see the experimental section for details). The reaction with the alkene takes place within irradiated regions only (i.e. the dark patches in **Figure 3.6a**). The surface patterned with **C11NHS** can be directly modified by immersion of the glass slide in a

solution of NH_2 -terminated fluorescent Si nanoparticles (4 nm in diameter, 50 mg/mL in DMSO; **Figure S3.12**). After extensive washing and sonication in water and DCM, the patterned SiNPs were visualized by SEM and fluorescence microscopy (see **Figure 3.6b-d**); a photolithographic resolution of ca. 10 μm was obtained (**Figure 3.6c**).

Figure 3.6. Photo-attachment of C11NHS onto H₅-Φ₁-glass followed by direct attachment of NH_2 -terminated Si nanoparticles. (a) Scanning electron microscopy (SEM) image of the SiNP patterned substrate. (b) Zoomed area of the substrate showing the boundary between the SiNP functionalized areas and non-functionalized H₅-Φ₁-glass; the red line represents a cross section. (c) Plot of the pixel intensity between modified and non-modified regions. (d) Fluorescence microscopy picture of an H₅-Φ₁-glass patterned with amine-terminated Si nanoparticles.

3.4.5. Modification with DNA

To demonstrate the immediate applicability of such NHS-modified surfaces, we attached a guanine-rich DNA sequence that can form, in the presence of hemin, a

catalytically active DNAzyme.[40] Specifically, the so-called “EAD2 G-quadruplex” sequence that was used possessed an amino moiety on a C₁₂ spacer at the 5'-end of the oligonucleotide (5'-H₂N-C₁₂H₂₄-CTGGGAGGGAGGGAGGG-3'). In the presence of suitable cations like K⁺ or NH₄⁺ and hemin,[41-43] this DNA fragment forms a highly effective horseradish peroxidase (HRP)-mimicking hGQ DNAzyme, which oxidizes ABTS in the presence of hydrogen peroxide.[44]

For the attachment of the DNAzyme-forming sequence on the glass-substrates, the modified glass reactor was incubated overnight with a 100 μM solution of EAD2 in 18 mM PBS buffer (pH 7.4). Analysis of the EAD2-functionalized glass by GATR-FTIR revealed complete disappearance of the C=O stretching vibrations of the NHS ester in the 1748 to 1815 cm⁻¹ range and the appearance of characteristic N-H modes at 1608 cm⁻¹ and specifically characteristic guanine-related N-H bending vibrations at 1582 and 1630 cm⁻¹. [45] Additionally, carbonyl stretching vibrations at 1730 cm⁻¹ matched the expectations of a guanine rich surface. We note that the intensity of the peaks discussed above are an order of magnitude higher than those of NHS, providing additional evidence for DNA attachment which contains a high number of carbonyl groups (**Figure S3.13**). Furthermore, substitution of the NHS-ester moiety by DNA was confirmed by XPS. Figure 7 shows the XPS narrows scans of the C1s and N1s energy ranges of the DNA modified surface. The C1s narrow spectra (**Figure 3.7**) further corroborated DNA immobilization due to presence of C-N/O and enhanced C=O regions. Complexation of hemin [Fe(III)-protoporphyrin IX chloride] to the G-quadruplex structure (in presence of K⁺) (**Figure S3.14**) was apparent from two Fe 2p peaks in XPS at 707 and 720 eV (**Figure S3.15**). Having proof of the attachment of the hGQ DNAzyme structure onto the H₅-Φ₁-glass surface, we proceeded to study the oxidative activity of the immobilized hGQ DNAzyme inside a microfluidic channel.

Figure 3.7. XPS (a) C1s and (b) N1s narrow scan of (i) DNA-functionalized glass that was obtained after reaction of amino-functionalized DNA with (ii) NHS-terminated glass.

3.4.6. In-flow DNA catalysis

Having modified a commercial microfluidic glass chip (R150.332.2, Micronit) into H₅-Φ₁-glass, we then proceeded to apply a photomask to *ca.* 50% of the channel total area. The microchannel was subsequently filled with **C11NHS** and the setup was irradiated with light of $\lambda = 328$ nm for 16 h (See **Figure S3.4**). After this, the channel was modified with a G-rich DNA oligonucleotide (see Methods, **Figure S3.14**), which was subsequently converted in the horseradish peroxidase-mimicking hGQ DNAzyme complex. Next, we proceeded to study the oxidative capability of our hGQ-modified microreactor. We used ABTS, as in the presence of the hGQ DNAzyme catalyst and H₂O₂, this is converted into a colored radical that can be easily detected by UV-Vis spectroscopy.[46] The radical ABTS^{•+} has an absorption maximum at 414 nm and is easily distinguished from the ABTS absorption maximum (320 nm) (**Figure 3.8**).

Figure 3.8. Local attachment of the hGQ DNAzyme allows the localized catalytic oxidation of ABTS into its cationic radical $\text{ABTS}^{+\bullet}$. The insert, (a), shows that increasing residence time (from 2.2 to 10.8 s) yielded more of the oxidation product.

The colored radical was produced by injecting H_2O_2 and ABTS into the inlets of an hGQ-modified microreactor, which was apparent from the UV-Vis analysis of the collected solutions. As expected, the amount of produced $\text{ABTS}^{+\bullet}$ increased with increasing residence times (see **Figure 3.9**). To monitor the amount of time the substrate is exposed to the wall-coating, a simplified residence time was calculated with equation 2. Where t is the residence time, Q_V is the volumetric flow ($\mu\text{L}/\text{min}$ the sum of Q_V from both inlets), V_T is the volume ($9 \mu\text{L}$) of the section of the channel that has been modified.

$$t = \frac{V_T}{Q_V} \times 60 \quad (2)$$

Importantly, neither the injection of H_2O_2 and ABTS into an unmodified microreactor, nor the injection of ABTS without H_2O_2 in the hGQ-modified microreactor yielded oxidation products (blanks have residence time of $t = 0$ s).

Figure 3.9. Oxidation of ABTS inside a microreactor as function of the residence time. UV-Vis absorbance at 415 nm corresponding to the radical $\text{ABTS}^{+\bullet}$ (dashed line).

3.5. CONCLUSION

We have prepared covalently attached monolayers onto glass surfaces using light with a wavelength of 328 nm, i.e. significantly extending upon the previously reported minimally required wavelength of 302 nm. This was achieved by introducing a radical-stabilizing hydrogen-phenyl-silane functionality ($(\text{-O})_2(\text{Ph})\text{Si-H}$ moiety) on the surface, creating H- Φ -glass. Under these conditions, the degradation of NHS was minimized, allowing immediate attachment of amino-functionalized molecular entities. We showed this for amino-terminated silicon nanoparticles, and amino-terminated guanidine rich DNA (EDA2). Regarding this last biomimetic catalyst, we were able to perform in-flow oxidation of ABTS by H_2O_2 inside a glass microreactor. Such a one-step light-induced attachment of NHS functionalities allows for smooth photopatterning of glass for a wide variety of amino-terminated substances, including nanoparticles and DNA, but also the attachment of antibodies can be envisioned.

Thus this work further widens the scope of future applications of biofunctionalized glass microfluidic chips.

3.6. ASSOCIATED CONTENT

Appendix II. Emission profiles of Jelight lamps with and without a long pass filter. Reaction setup schematics. AFM images of the silanized glass. GATR-FTIR and XPS spectra of modified surfaces. DART-HRMS measurements of NHS-modified glass. NH₂-SiNP characterization details.

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Chapter 4

Mild and selective C-H activation of COC microfluidic channels

Based on:

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4.1. ABSTRACT

Plastics, such as cyclic olefin copolymer (COC), are becoming an increasingly popular material for microfluidics. COC is used, in part, because of its (bio)-chemical resistance. However, its inertness and hydrophobicity can be a major downside for many bioapplications. In this paper, we show the first example of a surface-bound selective C-H activation of COC into alcohol C-OH moieties under mild aqueous conditions at room temperature. The nucleophilic **COC-OH** surface allows for subsequent covalent attachments, such as of a H-terminated silane. The resulting hybrid material (**COC-Si-H**) was then modified via a photolithographic hydrosilylation in the presence of ω -functionalized 1-alkenes to form a new highly-stable, solvent-resistant hybrid surface.

4.2. INTRODUCTION

Cyclic olefin copolymers (COCs) are emerging as an increasingly popular substrate material for microfluidics. This is mostly due to their optical transparency, chemical resistance, low water absorption properties and good biocompatibility.[1] However, COC being quite inert toward chemical modification, of course, hinders the ability to covalently functionalize the surface.[2] A series of methods have been investigated that endow functionalities to COC, as well as reducing its hydrophobicity, (static water contact angles of COC are typically $>95^\circ$).[1] For instance, covalent coupling of a silane in plasma conditions can be used to modify COC surfaces.[3] Other reported modification strategies also rely on harsh conditions such as sputtering deposition of a SiO₂ thin layer,[3] oxygen-plasma pretreatment,[2, 4] UV/ozone oxidation,[5] UV-photografting,[6] or UV-induced catalytic oxidation.[7, 8] Plasma/gas based approaches may work on exposed surfaces, but face restrictions caused by the limited diffusion distance of the reactive species into the narrow

microfluidic channels. In addition, despite the transparency of COC at short wavelengths, prolonged UV exposure yellows COC and can turn it opaque, reducing transmittance.[9-11]

Additionally, common polymeric substrates for microfluidic applications have a disadvantage in their low solvent resistance. Thermoplastics, such as COC, show increased solvent resistance, but despite the modification with polymeric functional coatings the applications are mostly using polar solvents.[12-15] Usage of fluorinated-polymers although shown to be resistant to organic solvents, yields hydrophobic surfaces that are not functionalizable.[16]

Copper is known to be an instrumental component in many oxidases present in nature.[17, 18] Copper(II) complexes have been used to oxidize alkanes under mild conditions.[19] Norbornane, one of the constituents of COC, has structural similarities to cyclohexane, which has been reported to be a suitable substrate for mild peroxidative oxidation.[20] It has been reported for cyclohexane (Cy) that a radical chain mechanism leads to the formation of alkyl hydroperoxide intermediates (CyOO[•]), which by addition of suitable additives might be converted into alcohols (CyOH).[21] We thus reasoned that oxidation of a COC surface with these solution-based methods should be feasible, to create alcohol (**COC-OH**) and carbonyl (**COC=O**) functional groups. Specifically, we were interested in appropriate methodologies that are able to preferentially form **COC-OH**, as a monofunctional surface coating would increase the definition of and control over surface properties. **COC-OH** alcohol groups would provide surface-bound nucleophilic anchors that, similarly to Si-OH on glass, allow for, for example, the covalent attachment of a very wide array of molecules.[22] As a point in case, we demonstrate here the attachment of a silane,[8] as this allows photochemical patterning at a later stage.

We have recently reported on the deposition of a hydrogen-terminated silane on glass (H-glass) and subsequent UV irradiation (302 nm) in the presence of terminal alkenes.[23] We then improved this approach by incorporating a radical-stabilizing substituent (H- Φ -glass), thus reducing the activation energy threshold to light with a wavelength of 328 nm.[24] This allowed the direct attachment of an NHS-containing alkene and subsequent localized DNA attachment. Now typically commercial microfluidic devices are manufactured (and functionalized) in parts and then later bonded together. However, many (bio)chemical functions that can be introduced locally with our method are not compatible with the use of the high temperatures needed for bonding. Additionally, such thermal treatments result in a loss of imbued hydrophilicity (by plasma or otherwise) due to surficial polymer reorganization. There are two ways to approach this problem, either to find a method that allows for low-temperature bonding of high- T_g COC (while keeping the COC-surface chemistry unchanged), or to develop a method to modify COC posterior to the bonding procedure. Our paper addresses the later approach.

In this paper, we therefore show that this approach can be adapted to modify COC substrates into a new hybrid material, hydrogen-terminated-(phenyl)-silanized COC (**COC-Si- Φ_1 -H₅**), by modifying **COC-OH** with a mixture of trichlorosilane and phenyldichlorosilane (5:1 molar ratio). This modification of COC allows for a mild photolithographic covalent attachment ($\lambda = 328$ nm) of terminal alkenes onto COC surfaces, including enclosed surfaces such as the inside of microfluidic channels (**Scheme 4.1**).

Scheme 4.1. Scheme for (i) the modification of COC into a nucleophilic surface, **COC-OH**, via (ii) copper-catalyzed peroxidative oxidation followed by a mild reductive washing step; (iii) Subsequent silanization with $\text{HSiCl}_3/\text{HSiPhCl}_2$ (5:1 molar ratio) yields the **COC-Si- Φ_1 -H₅** hybrid material; (iv) This hybrid material is then used for mild light-induced hydrosilylation with a terminal alkene.

4.3 EXPERIMENTAL SECTION

Acetone (Aldrich, semiconductor grade VLSI PUNARAL Honeywell 17617), dichloromethane (DCM, Sigma Aldrich) and *n*-hexane (Merck Millipore) were used for cleaning before modification. All other chemicals were purchased from Sigma Aldrich and used as received. 10-Trifluoro-acetamide-1-decene (TFAAD) was synthesized based on literature methods.[23, 25, 26] COC 6013 was purchased from TOPAS.

4.3.1 Oxidation of the COC surface

For monolayer formation on flat substrates cyclic olefin copolymer, COC 6013 (TOPAS), was milled into 1×1 cm pieces and sonicated for 30 min in ethanol/acetone (1:1),

subsequently dried with argon, and placed under vacuum for 1 h at room temperature. Surface modifications were conducted directly afterwards.

COC is quite inert and lacks nucleophilic groups on the surface. These groups act as covalent anchoring points for the intended subsequent silanization steps. Two oxidation methodologies were thus studied. A simple way of oxidizing the COC substrate is by using an air plasma for 30 s. This method is not compatible with bonded microfluidic channels. Solution-based oxidation of COC was performed via peroxidative oxidation of the COC surface for 20 min. The surface was immersed and sonicated (37 kHz, RT) immediately after mixing a 5 mL solution of $\text{Cu}(\text{OAc})_2$ (73 mg, *cr* 20 mM) in 15 mL of 30% H_2O_2 (*cr* 22.5% (v/v)), rinsed with ethanol, and blow-dried with N_2 flow to remove surficial water, followed by sonication in a methanolic solution of NaBH_4 (112 mM) for 5 min. Sonication prevents nucleation of gas bubbles on the COC surface, which hinder the surface modification. Freshness of the hydrogen peroxide, and thus its high concentration, is necessary for a proper oxidation of the surface.

Oxidation inside a microfluidic channel was performed by flushing the abovementioned solution for 20 mins at a 50 $\mu\text{L}/\text{min}$ flow rate (channel dimensions: width 300 μm , height 150 μm).

4.3.2. Formation of hydrogen-terminated-Si-COC (H-SiCOC)

Gas phase modification of COC with HSiCl_3 was performed as previously described in Chapter 2.

4.3.3. Formation of hydrogen-terminated-(phenyl)-Si-COC (H- Φ -SiCOC)

A 1:5 molar mixture of $\text{Cl}_2\text{PhSi-H}$ and $\text{Cl}_3\text{Si-H}$ was added to semiconductor grade acetone under bubbling N_2 . The oxidized COC surfaces ($1 \times 1 \text{ cm}$) were immersed for 20

min. Afterwards, the substrates were washed copiously with ethanol, dried under argon and stored in an opaque container. For the microfluidic chip, the solution was flushed and allowed to react for 20 min. After which, acetone was copiously flushed through.

4.3.4 Photochemical surface modification

A drop of TFAAD was placed on a H-SiCOC or H- Φ -SiCOC slide in a glovebox. An appropriate cutoff filter (295 or 320 nm) was placed on the drop and gently pressed against the first slide,[27] homogenously spreading the alkene, mimicking a closed glass microfluidic channel while blocking the reaction from undesirable high energy emissions. The surfaces were then illuminated with a pen lamp (pen lamp 302 or 328 nm, Jelight Company, Irvine, CA, USA), which was placed approximately 1 cm above the surface. A cutoff filter is necessary due to a strong presence of higher energy wavelengths (**Figure S4.1-S4.2**). The entire setup (lamp and substrate) was covered in aluminum foil, and the sample irradiated for 16 h. After irradiation, the substrates were rinsed with acetone, sonicated in ethanol/acetone for 5 min and quickly dried under argon. The surfaces were directly used for surface characterization or stored under air.

4.3.4.1. Photolithography

Photolithography was performed with a pen lamp, as in the aforementioned setup, in combination with a gold electron microscope grid (SEM F1, Gilder Grids, **Figure S4.3**). This photolithographic mask was placed on top of the H-SiCOC or H- Φ -SiCOC slide with a drop of TFAAD. After spreading of the liquid, a cutoff filter was placed on top of the mask as a cover, above which the pen lamp was placed (ca. 1 cm distance). Samples were irradiated for

16 h, removed from the glove box and cleaned as described above. The patterns were examined with optical microscopy, scanning electron microscopy (SEM).

Photolithography on the microfluidic devices were performed by first flushing TFAAD, followed by irradiation under the same conditions as described above (the light-induced reaction was carried out on a stopped-flow). The photomask used was a thick aluminum foil taped to a section of the device, blocking light on half of the chip.

4.3.4.2. Monolayer characterization

4.3.4.2.1. Static water contact angle measurements (SCA)

Static water contact angles (SCA) were measured using a Krüss DSA-100 goniometer. Droplets of 3 μL were dispensed on the surface, and contact angles measured with a CCD camera using a tangential method (method 2). The reported value is the average of at least five droplets of at least three different samples.

4.3.4.2.2. Germanium Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (GATR-FTIR)

GATR-FTIR spectra were recorded with a Bruker Tensor 27 FT-IR spectrometer, using a commercial variable-angle reflection unit (Auto Seagull, Harrick Scientific). A Harrick grid polarizer was installed in front of the detector and was used for measuring spectra with *p*-polarized radiation with respect to the plane of incidence at the sample surface. Single channel transmittance spectra were collected at an angle of 25° using a spectral resolution of 4 cm^{-1} and 1024 scans while flushing with dry N_2 . Obtained spectra were referenced with a clean silanized COC substrate (silanized COC substrates were referenced with oxidized COC).

4.3.4.2.3. X-ray Photoelectron Spectroscopy (XPS)

XPS spectra were recorded on a JPS-9200 photoelectron spectrometer (JEOL, Japan). The analysis was performed under ultra-high vacuum conditions using a monochromatic Al K α source at 12 kV and 20 mA and an analyzer pass energy of 10 eV. A takeoff angle ϕ of 80° was used, with a precision of $\pm 1^\circ$. All XPS spectra were analyzed with Casa XPS software (version 2.3.15). The binding energies were calibrated on the hydrocarbon (CH₂) peak with a binding energy of 285.0 eV. Because of the electrostatic charging of the surface during the measurements, a charge compensation was used with an accelerating voltage of 2.8 eV and a filament current of 4.80 A.

4.3.4.2.4. Atomic Force Microscopy (AFM)

AFM imaging was performed under air using both a Digital Instruments NanoScope V Multimode Scanning Probe Microscopy (Bruker, MA) and a JSPM-5400 (Jeol, Japan) Scanning Probe Microscope in AC-AFM (“tapping”) mode with a noncontact silicon nitride cantilever with a stiffness of 0.58 N/m (Veeco Metrology, Santa Barbara, CA) at a scan speed of 1 $\mu\text{m/s}$, in the Scan-assist imaging mode. Images were flattened with a second-order flattening procedure using Nanoscope Analysis software (v1.5) and WinSPM software (v 5.0).

4.3.4.2.5. Scanning Electron Microscopy/ Scanning Auger Microscopy (SEM/SAM)

Morphologies of TFAAD micropatterns were analyzed by SEM. Measurements were performed at room temperature with a scanning Auger electron spectroscopy system (JEOL Ltd. JAMP-9500F field emission scanning Auger microprobe). SEM images were acquired with a primary beam of 0.8 keV. The take-off angle of the instrument was 0°.

4.4. RESULTS AND DISCUSSION

In order to be able to covalently silanize a COC substrate, the surface first needs to be activated. Activation of a COC surface into a nucleophilic anchor-containing surface can be achieved via oxidation of the alkane surface into C-OH groups (e.g. alcohol or acid). A ubiquitous surface oxidation method makes use of oxygen or air plasma, modifying the C-H terminus into a C-Ox (e.g., alcohol, acid, ketone, aldehyde). This plasma method was evaluated and compared with a mild oxidation based on exposure to an aqueous solution of hydrogen peroxide (22.5% (v/v)) and copper acetate (20 mM) (**Figure 4.1**).

An air plasma-based treatment of COC substrates for 10 s gives immediate results. The static water contact angle drops from 100° to <30°, indicating the rapid formation of hydrophilic surface groups. From GATR-FTIR measurements it becomes clear that a variety of surficial carbonyl species are present (**Figure 4.1a**), as expected under the harsh oxidation conditions provided by plasma. Wide range XPS data reveal an increase in total oxygen content, while XPS C1s narrow scans show many peaks to indicate that multiple functional groups are now present on the surface (due the presence of various oxidized forms of carbon, e.g. (C-OH) 286.9 and 289.3 (C=O)). Unfortunately, this surface-modification technique is not able to homogeneously functionalize the inside of intricate COC channels because of plasma-diffusion limitations in microfluidic devices.[28]

In order to bypass the limitations stated above, we looked into other C-H activation chemistries.[20, 21, 29, 30] Due to the similarities between COC and (cyclo)hexane, we hypothesized that this copper-catalyzed water-based chemistry could also work on a COC surface. To test this, we first exposed COC to 30% (v/v) H₂O₂ and no change in the water contact angle was observed, even after simultaneous sonication for 30 mins. However, upon the addition of copper(II) acetate, a contact angle of 83 ± 2° was obtained in just 30 min.

Shul'pin reviewed how the addition of additives, such as PPh_3 , in the washing/quenching solution can be used to favor the decomposition of the $\text{CyOO}^\bullet$ species into the alcohol (CyOH) counterparts instead of into ketones or other minor overoxidation products such as esters.[21] However, attempts to use PPh_3 to favor the conversion of $\text{COC-OO}^\bullet$ to **COC-OH** did not yield satisfactory results. The PPh_3 oxidation adduct (O=PPh_3) forms a sticky precipitate that whitens the substrates and clogs microfluidic channels. Adding a small amount of nitric acid (20 mM) in the oxidation solution, increased the yield slightly and shows some tuning ability towards the alcohol product is possible; similar results were found in solution.[31] However, we found an easy alternative method by using methanolic NaBH_4 in the washing step (by sonication for 5 mins) of the copper-catalyzed oxidized surfaces. This yielded further a reduction in the water static contact angle to $72 \pm 2^\circ$ (**Table S4.1**). These values are close to those of other **COC-OH** substrates found in literature.[7, 8] Our method represents a clear improvement in that it is much milder (no UV required, room temperature, aqueous solution), and leaves far less contaminants on the surface.

To further corroborate this, the surfaces were analyzed by GATR-FTIR (**Figure 4.1b-d**). The presence of alcohol (3174 cm^{-1}) and carbonyl groups (1735 cm^{-1}) indicates that the surface has undergone oxidation. Evidently, the intensity of carbonyl-related stretching peaks is reduced compared to what is observed upon air plasma-treatment, suggesting that the aqueous $\text{Cu}/\text{H}_2\text{O}_2$ oxidation is mild compared to plasma treatment. Of specific interest is the basically flat IR spectrum in the C=O region (**Figure 4.1d**) upon NaBH_4 washing. Finally, the formation of $\text{C}\equiv\text{O}$ and C-OH bonds implies a lower amount of surficial CH and CH_2 bonds (compared to the reference background), leading to the inversion of those peaks (i.e., positive peaks around $2850\text{-}2950 \text{ cm}^{-1}$).

XPS wide scans show that our peroxidative treatment yields a clear increase of oxygen content when compared to bare COC, and less than for plasma-treated COC (**Figure**

4.2). In **Figure 4.2(ii)** the C1s narrow scans indicate a predominance of C-C bonds (285.0 eV) for all substrates, while in the oxidized substrates C-OH and C=O are distinctively present (286.8 and 289.3 eV). In **Figure 4.2(iii)**, the O 1s narrow scans show the presence of C-O-H (532.7 eV – 57.6%) and O-C=O (533.8 eV – 42.4%) for the COC that was subject to plasma treatment; COC that has been subjected to the $\text{Cu/H}_2\text{O}_2$ or $\text{Cu/H}_2\text{O}_2/\text{HNO}_3$ oxidation show a predominant presence of C-O-H (532.4 eV – 56% or 68%) along with the less prevalent C=O (531.4 eV – 17.2% or 11.6%), O-C=O (534.0 eV – 26.5% or 20.1%), and copper oxide (529.9 eV) contaminants due to just washing with water for analysis purposes.[32] COC that was oxidized with $\text{Cu(II)/H}_2\text{O}_2$ and washed with methanolic NaBH_4 shows a quantitative formation of surficial C-OH (Figure 2 (iii-d) 532.4 eV).[33]

Figure 4.1. GATR-FTIR of a COC surface after oxidation by (a) air plasma for 10 s; (b) copper-catalyzed peroxidative oxidation for 30 min and (c) copper-catalyzed peroxidative oxidation with HNO_3 as additive for 30 min; (d) COC-OH surface (COC oxidized with $\text{Cu(II)/H}_2\text{O}_2$ mixture followed by washing with methanolic NaBH_4 (with unmodified COC used as a reference background)).

In line with the IR and C 1s XPS results, the inclusion of the reductive NaBH_4 washing step yields a near-quantitative conversion to alcohols, free of Cu oxide contaminants.[34] While traditional plasma activation is a viable and operationally simple option in many instances, the resulting presence of basically only one type of oxidized carbon is a major improvement compared to air/oxygen plasma methods.[35]

Figure 4.2. XPS wide scans (i), C1s (ii) and O1s (iii) narrow scans of (a) COC subjected to air plasma; (b) COC oxidized with $\text{Cu(II)/H}_2\text{O}_2$ mixture; (c) COC oxidized with $\text{Cu(II)/H}_2\text{O}_2/\text{HNO}_3$ mixture, and (d) COC oxidized with $\text{Cu(II)/H}_2\text{O}_2$ mixture and washed with methanolic NaBH_4 ; (e) bare COC.

As previously mentioned, the ability to generate OH-terminated COC surfaces allows for a wide variety of functionalization chemistries. In the current paper, we present as an example the first of those under current development. Given the increasing use of COC in the fabrication of microfluidic devices, chemistries that allow for plastics photolithography are extremely desirable.[36] In line with our previous work done on borosilicate glass,[23, 24] we aimed to modify **COC-OH** with a 5:1 mixture of trichlorosilane and dichlorophenylsilane. With this, we envisioned to fabricate a hybrid material, hydrogen-(phenyl)-terminated silanized COC (**COC-Si- Φ ₁-H₅**), that would have a similar reactivity as the previously reported H- Φ -glass.

Figure 4.3. (a) XPS wide scan of (b) a **COC-Si- Φ ₁-H₅** substrate. (c) GATR-FTIR of the **COC-Si- Φ ₁-H₅** substrates, showing the two Si-H stretching vibrations (**COC-OH** was used as reference background).

Of specific interest were the findings that H- Φ -glass was shown to be highly stable in air for months, while smoothly reactive towards alkenes using light of ~ 328 nm.[23, 24]

The hybrid material **COC-Si- Φ_1 -H₅** was prepared along these lines and analyzed. Static water contact angles (85° for **COC-Si-H** and 90° for **COC-Si- Φ_1 -H₅**) were similar to the ones we reported for analogous silicon-based substrates.[23, 24] XPS wide scans (**Figure 4.3a**) show the presence of Si at 102 eV, indicating the formation of an ultrathin layer on top of COC. Analysis by GATR-FTIR, while using **COC-OH** as a reference background, confirms the presence of two different Si-H stretching bands, which we attributed to O₃Si-H at 2249 cm^{-1} and to O₂ Φ Si-H at 2185 cm^{-1} (**Figure 4.3c**).

Having characterized the **COC-Si- Φ_1 -H₅** substrates, we proceeded with modifying it further via light-induced hydrosilylation. For this, we chose 10-trifluoro-acetamide-1-decene (TFAAD) as developed by Hamers[37-40] as a reactivity probe. This alkene is useful to evaluate attachment, due to the IR-active carbonyl group and three C-F bonds with characteristic XPS C1s and F1s signals, and can be easily converted into an amine, upon deprotection.

COC-Si- Φ_1 -H₅ substrates were modified with TFAAD using 328 nm light. The TFAAD-substituted **COC-Si- Φ_1 -H₅** substrates were characterized by GATR-FTIR using **COC-Si- Φ_1 -H₅** as reference background. The carbonyl stretching peak from TFAAD was clearly present at 1705 cm^{-1} (**Figure 4.4**).[23] The reversal of the Si-H peaks at 2249 nm and 2185 nm indicates that there are less Si-H bonds than before the hydrosilylation, i.e., that the alkene reacted with the hydrogen-terminated surfaces forming Si-C bonds. Wide range XPS scans show the presence of fluorine, while C1s narrow scans indicate the presence of CF₃ groups at 293.5 eV (**Figure S4.4**). This further confirms covalent attachment of TFAAD onto the **COC-Si- Φ_1 -H₅** surfaces.

Figure 4.4. (a) XPS wide scan of (b) a **COC-Si- Φ_1 -H₅** substrate modified with TFAAD in the presence of 330 nm light for 16 h. (c) GATR-FTIR of the TFAAD-modified substrates, showing inversion of the two types of Si-H stretching vibrations and presence of the carbonyl from TFAAD.

Similarly to the results obtained on glass, AFM measurements of these surfaces show that the roughness does not change considerably upon modification (RMS roughness from 1.9 ± 0.2 nm to 2.0 ± 0.5 nm) (**Table S4.2**), which is another significant improvement over plasma-based oxidations.^[21]

Photolithography on open substrates was performed by positioning a contact mask (circular TEM grid with circles and spokes, **Figure S4.3**) on top of the **COC-Si- Φ_1 -H₅** substrates during the light-induced hydrosilylation reaction. We then planned to visualize the resulting pattern by scanning electron microscopy (SEM) (**Figure 4.5**, **Table S4.3**).

Figure 4.5. (a) Photograph and SEM image (b) of a TFAAD-patterned $\text{COC-Si-}\Phi_1\text{-H}_5$ after exposure to DCM (30 min); (c, d) close-ups of the regions denoted by the dashed squares in b.

Interestingly, when cleaning the modified substrate with dichloromethane (DCM) for 5 mins to allow for SEM measurements, we observed that the pattern had slowly become visible by naked eye (**Figure 4.5a**). Further exposure to DCM showed clear degradation by the solvent of the non-exposed areas, while the TFAAD-modified areas resisted solvent damage effectively (**Figure 4.5b-d**). Overall, the ability to covalently bind an ultrathin, yet highly protective coating that can be further modified,[38, 41] opens the use of these substrates for many applications.

We then finally proceeded to modify the channel of an already bonded COC microreactor (Micronit) into **COC-OH** by flowing the oxidation solution for 20 mins at 50 $\mu\text{L}/\text{min}$ and washing for 5 mins (50 $\mu\text{L}/\text{min}$) with methanolic NaBH_4 . Silanization was carried out and a fully modified **COC-Si- Φ_1 -H₅** microfluidic channel was photopatterned with TFAAD (see Appendix III for details). We then tested the ability of the TFAAD patches to protect the device from a prolonged flow of DCM (30 min at 50 $\mu\text{L}/\text{min}$). As can be seen from **Figure 4.6b**, the coating provides significant protection even to harsh solvents such as DCM. In the absence of a protective coating (**Figure 4.6b-c**), the polymer swells and cracks, starting as stripe-like cracks at the side of the channel (**Figure 4.6b**). In addition, the bonding strength between the COC top and bottom substrates is severely weakened and leakage occurs. Since the TFAAD moiety is by no means optimized for this purpose, functional group optimization will likely provide significant possibilities for further improvement of the protection.

Figure 4.6. Microscope image of plastic microchip with (a) a TFAAD-modified COC channel; (b) a partially TFAAD-modified COC channel (the faint orange part on the left was coated; the right part is uncoated) after exposure to a flow of DCM (white arrow indicates flow direction; 50 $\mu\text{L}/\text{min}$; 30 min), showing significantly more damage in the uncoated part; (c) untreated COC submitted to the same treatment.

4.5. CONCLUSIONS

In conclusion, we have demonstrated a mild aqueous C-H activation method to modify the surface of cyclic olefin copolymers (COC). This method can be applied to open plastic substrates as well as to bonded microchannels, and yields highly defined alcohol-terminated COC surfaces without carbonyl-containing moieties. Due to their nucleophilic character, such surface-bound alcohol moieties can be used for a very wide array of surface

modifications. When, for example, reacted with hydrosilanes (such as $\text{Cl}_3\text{Si-H}$ or $\text{Cl}_2\text{PhSi-H}$), this surface yields a hydrogen-terminated Si-COC surface. This new hybrid surface is highly stable in air and photo-patternable via a mild light-induced hydrosilylation with terminal alkenes. The TFAAD functional monolayer attached in this fashion enhanced the resistance of the COC to organic solvents (e.g., DCM). In addition, TFAAD is well known for its ability to undergo further surface modifications for, for example, biological applications.[38, 41, 42]

This research opens a door towards the mild activation of what was considered a highly inert substrate that required harsh modification techniques. This technique is currently transposed to other C-H containing polymer analogues, *e.g.* polyphenyl ethers/oxides (PPEs/PPOs), and speeding up the process via the photochemical attachment of 1-silanols rather than 1-alkenes – which is ca. two orders of magnitude faster on H-terminated Si(111)[43] - is also under investigation in our laboratories.

4.6. ACKNOWLEDGMENTS

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4.7. Appendix III. SCA and AFM images of the COC before and after silanization and hydrosilylation. XPS narrow scans and SEM of TFAAD modified COC.

4.8. REFERENCES

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Chapter 5

Laser welding of pre-functionalized glass

substrates:

A fabrication and chemical stability study

Based on:

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5.1. ABSTRACT

Low-temperature bonding of glass substrates is of great interest in the field of microfluidic-based biosensing, and we study how laser welding could be used for this. This technology allows for the modification of glass channels with temperature-sensitive materials prior to bonding. We study the effects of the welding process by investigation of the thermal degradation of a fluorescent monolayer throughout the bonding procedure.

5.2. INTRODUCTION

The integration of microfluidics with bio-chemical systems has paved the way to applications such as “organ-on-a-chip” and biomimetic micro-reactors. Commonly chosen substrates for the fabrication of such micro-reactors are borosilicate glass and silicon.[1] These substrates are structured and then bonded together to form a monolithic microfluidic device. The direct fusion bonding of two substrates is a reliable method to have a permanent bond between borosilicate glass and silica substrates.[1-3] This process typically makes use of the Van der Waals interaction between substrates to obtain a pre-bond, which are then fused together via annealing (e.g. using temperatures of up to 600 °C for glass or 1200 °C for fused silica).[4, 5] This process is time-consuming due to the gradual temperature ramp required, and the scope of technique is limited, as only combinations of materials that have similar thermal expansion coefficients are suitable.

Furthermore, if bio-chemical substances or coatings need to be present prior to bonding, substantial heating of the substrates is undesirable.[6, 7] Several approaches towards a lower glass bonding temperature have been developed over the years.[2] Of particular interest for glass-only systems is a two-step plasma activation treatment that allows to fabricate a nanofluidic device at a relatively low temperature (200 °C).[8, 9] The method was

recently improved to allow for room-temperature bonding using a O_2/CF_4 plasma in the surface-activation step. However, the surface chemistry is not well-understood and requires further studies; e.g. the rise in contact angle in comparison to regular O_2 plasma might indicate the formation of Si-C-F and Si-F surficial groups which would greatly hinder any surface modification.[10] Recent advances in silicon direct bonding use relatively low-temperature methods,[11] but still rely on elevated temperatures (250 °C) and surface activation procedures that are incompatible with biological components. Another approach would be the welding of transparent materials (such as glass or polymers) by means of ultrashort laser pulses while clamping the layers together with high force (40 MPa, such that the distance between substrates is $\lambda/4$). This is attractive since glass components can be joined at room temperature without the use of adhesives.[12-15] This opens other challenges, such as focusing the light at the interface between the substrates. Also, the bonding strength is dependent on the weld seam alone instead of on the entire surface contact area.

In this paper, we study a new laser welding method and its thermal effects on a model chemical monolayer. To achieve the bonding of two glass substrates, we deposit a thin chromium film on the bottom substrate (**Figure 5.1a**), while on the top substrate a covalently bound functional monolayer has been attached via standard surface modification chemistry.[16] Next, bringing the glass surface of the top substrate into close contact with the thin film metal layer of the bottom substrate allows for the subsequent local bonding between substrates via laser welding (**Figure 5.1b,c**). The glass substrate is transparent to the IR wavelength used (1064 nm), while the chromium layers effectively absorb the energy, melt and react with the glass, so as to weld the substrates together.[17] Heat propagation through the glass is a possible issue that partially determines the scope of this method. Therefore, we used fluorescein (which is thermally degraded above 315 °C) covalently bound on the surface

as a simple candidate to investigate the chemical compatibility of this approach via local fluorescence microscopy studies (**Figure 5.1**).

Figure 5.1. (a) Modified substrates with a functional monolayer and with a chromium thin film. (b) Pressing the substrates together allows for a good contact, which is followed by laser welding (c). The heat generated by the laser reaches the outer layers of the functional surface, causes thermal degradation (bleaching) of part of the monolayer.

5.3. EXPERIMENTAL SECTION

Glass substrates were obtained from SCHOTT (Borofloat 33). The top substrate had a thickness of 1.1 mm and the bottom substrate a thickness of 0.7 mm. The holes were manufactured via powderblasting. The substrates were cleaned by sonication in semiconductor-grade acetone. The top substrates were cleaned and activated via an oxygen-plasma treatment, after which (3-aminopropyl)triethoxysilane (APTES) was deposited via chemical vapor deposition. These amino-terminated substrates were subsequently immersed in a solution of fluorescein isothiocyanate (FITC) (50 mM in ethanol) and allowed to react for 30 min. The fluorescently labelled substrates were then washed with ethanol and acetone, dried and stored in an opaque container until used (**Figure 5.2**).

The bottom substrates were coated with a 100 nm chromium layer, on top of which a positive photoresist was spin-coated. The microfluidic channel was etched in the chromium and subsequently the glass to a depth of 100 μm . Afterwards the resist was stripped but the chromium film was left on the glass to facilitate subsequent welding.

To study the effect of the laser-welding and channel width (1, 3 and 5 mm) on the stability of the surface functionalization, we manufactured 18 chips in total. Half of the chips had the surface functionalized only with APTES, the other 9 with APTES followed by FITC (Table S5.1).

Figure 5.2. Surface modification procedure. The activated top glass substrate is modified with APTES and FITC. The bottom substrate has a chromium layer which is then protected with a photoresist, followed by local exposure to light and localized etching; thus forming a microfluidic channel.

Laser welding protocol

Welding was performed using a DPLNexus Marker (1064 nm, 12 W, diameter of the beam at the focal point approximately 8 μm). The bonding strength was then evaluated using the crack-opening method (where a blade is inserted between substrates until substrate delamination occurs).[18] To further test the bonding strength, water was pumped through the

chip while the outlet was blocked, and the fluid pressure was increased until failure of the weld was observed.[19]

5.4. RESULTS AND DISCUSSION

The welding step was performed by means of a laser locally heating the thin chromium film layer, generating a local glass-metal-glass joint (**Figure 5.3**). As can be seen in **Figure 5.3d-e**, the amount of chromium remaining in its metallic form in the welding area appears to have been reduced down to the point of either being optically transparent or reacted altogether. Upon obtaining efficient welding settings, the crack-opening method (via insertion of a blade between substrates) showed fracture of the bottom substrates up to the weld seam and not further (**Figure S5.1**). Pressure tests indicated a stable bonding up to ca. 2 bar, upon which there was separation of the substrates. These leakage-free devices were deemed to be suitable for most common applications. Optimal laser settings were found to be 45% power (5.4 W), a pulse repetition frequency of 45 kHz, a pulse duration of 3 ns and a welding speed of 300 $\mu\text{m/s}$.

Figure 5.3. (a-c) Photographs of welded devices of differing channel widths – 1, 3 and 5 mm; (d-e) laser welding seams that bind top and bottom glass substrates together

The details of the welding procedure determine the thermal budget. The optimized procedure keeps the temperature at which the fluorescent dye degrades confined to a distance of *ca.* 550-650 μm from the focus point of the laser, i.e. confined to *ca.* 100-200 μm from the wall inside the microfluidic channel (**Figure 5.4, Table S5.1**).

Figure 5.4 Fluorescence microscopy pictures of the edge of a microfluidic channel: (a) FTIC-modified substrates before welding, (b) FTIC-modified substrate after welding; (c) Fluorescence intensity profiles after the laser welding procedure. Three channel widths were studied and the normalized fluorescence intensity was plotted to evaluate thermal bleaching.

Thus, one can conclude that the described method is an efficient way of creating leakage-free glass microfluidic chips via the usage of a commercial laser scribe at considerably lower temperatures than needed for fusion bonding. The moderate bleaching of

the heat-sensitive dye indicates a potential for the pre-functionalization of substrates with temperature-sensitive substances prior to bonding.

For the welding-heating studies a low-cost nanosecond laser was used, which delivers the energy in relatively long pulses when compared to pico- and femtosecond lasers. Preliminary work demonstrates that the width of the weld seam can be reduced when a picosecond laser is used (**Figure 5.5**). Further studies need to be performed to determine whether a shorter pulse length will also confine the heat-affected zone to a smaller region.

Figure 5.5 Picosecond laser welding seams that bond top and bottom glass substrates together. An extremely reduced weld width is obtained.

5.5. CONCLUSIONS

We have described a new laser-welding method and studied its thermal impact on the fabrication of a leakage-free glass microfluidic chip that was pre-functionalized with a thermally sensitive fluorescent model molecule (fluorescein). The thermal bleaching tests show that the temperature rise is enough to bleach fluorescein up to ca 650 μm from the laser impact point. This welding method allows for a quick and easy fabrication of pre-functionalized microfluidic devices. Using the described method, the substrates used can be

pre-functionalized with a chemical coating from a certain distance from the weld, whereas that is not possible at all with direct fusion bonding.

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Chapter 6

General Discussion

Abstract

This chapter gives an overview of the main achievements of this thesis. Herein, we also discuss what questions remain unanswered and how to possibly improve the described systems for future work.

Throughout **this thesis**, one main objective was clear: to develop methodologies that enable the attachment of a chosen molecule on a specific location onside an enclosed surface, such as a microfluidic channel (**Figure 6.1**). Striving to achieve this goal, we also discovered new chemistries and developed new protocols. All of the steps and discoveries were carefully analyzed and characterized.

Figure 6.1. Schematic of the overall goal of this thesis. The localized modification of the inside of microfluidic channels.

Chapter 2 describes a novel attachment chemistry, using a combination of silanization and hydrosilylation. The chemistry described allowed for the light-induced (302 nm) local modification of enclosed surfaces (silicon oxide), which until this work was limited to light of wavelengths lower than 285 nm.[1-3] Interestingly, the set of reactions herein described was reported not to work in previous studies,[4, 5] and proves the value of revisiting so-called established data, detailed observations and analysis. After extensive characterization and analysis, we proceeded to modify both exposed and enclosed glass surfaces with a variety of molecules. The methodology that we developed proved to be very robust and reproducible. As a proof-of-concept we showed how this chemistry can be used to fabricate localized “molecular phaseguides”[6] by introducing a hydrophobic barrier in order to passively steer the water flow, a “nano-dyke” (**Figure 6.2**). Other applications for this hydrophobic/hydrophilic local functionalization can be the immobilization of cells within

specific locations of a channel, bypassing the need for the fabrication of e.g. pillars, micro-phaseguides and other cell-trapping microstructures. This method can thus contribute to the creation of organ-on-a-chip type of technologies.

Figure 6.2. (left) Schematic of the chemical approach for Chapter 2. (a-c) Simple use of a molecular phaseguide to act as a passive stop-valve.

The light-induced hydrosilylation reaction with hydrogen-terminated silicon oxide was novel and the mechanism not yet understood. In order to study the mechanistic pathway, we reacted an omega-fluoro terminal-alkene. We concluded that the addition to the surface proceeds via an anti-Markovnikov addition, in line with previous findings on silicon.⁵ For future work, the deuteration of the surface, and thus the conversion of $\text{O}_3\text{Si-H}$ to $\text{O}_3\text{Si-D}$ could provide more direct evidence of the mechanistic pathways. The position in an infrared spectrum of the $\text{O}_3\text{Si-D}$ stretching vibration is different from that of $\text{O}_3\text{Si-H}$ vibration.[7] Concurrently, there are also clear differences on the C-D stretching vibrations if the addition to the surface proceeds via an anti-Markovnikov mechanism, yielding HC-D groups (**Figure 6.3a**) or Markovnikov yielding a terminal $\text{H}_2\text{C-D}$ group (**Figure 6.3b**).

Figure 6.3. Hydrosilylation reaction with deuterium-terminated silicon oxide surface. (a) Anti-Markovnikov addition and (b) Markovnikov addition.

The deuteration of the surface can be achieved via two different approaches. The first approach would proceed via the synthesis of a deuterium-terminated silane precursor, such as $\text{Cl}_3\text{Si-D}$, that upon surficial polymerization yields a $\text{O}_3\text{Si-D}$ surface. Such methods have been described in literature and make use of complex, hazardous chemistry.[8-12]

Another approach would start with the deuteration of the surface. Formation of a deuterium-terminated glass could be achieved by first silanizing the OH-terminated glass ($\text{O}_3\text{Si-OH}$) with trichlorosilane, forming hydrogen-terminated glass ($\text{O}_3\text{Si-H}$). This intermediate surface would subsequently be subjected to a deuterium (D_2) plasma that would convert the surface into a $\text{O}_3\text{Si-D}$. Some preliminary results indicate that this approach is feasible (**Figure 6.4**).

Figure 6.4. (a) Schematic of the deuteration reaction, superimposed on a photo of H-glass substrates under a D₂ plasma; (b) simulated infrared spectra for Si-H and Si-D (MP2-6-311G++(d,p)); (c) GATR-FTIR of H-glass after D₂ plasma.

At the end of **Chapter 2**, in order to achieve surface-directed control of the water flow, we enhanced the hydrophilicity/hydrophobicity contrast of the functionalized microchannel. This was done by first immobilizing a hydrophobic patch, followed by the irradiation of the remaining Si-H with light in the presence of water, modifying the group into the hydrophilic Si-OH. One can make the logical leap that if water reacted, it is likely that molecules of the formula R-OH (e.g. alcohols or silanols) are also reactive with the H-glass surface under UV irradiation (**Figure 6.5**).

Figure 6.5. Reaction of H-glass with an R-OH molecule (e.g. alcohol) in the presence of light.

While the chemistry in **Chapter 2** allowed local modification in standard/commercial glass microchips using light of 302 nm, this wavelength is, unfortunately, incompatible with a range of moieties that are highly interesting for bio-functionalization. This is due to the fact that these functional groups are still photo-active at that wavelength, with concomitant degradation (especially the N-hydroxysuccinimide moiety). Therefore, novel chemistry needed to be developed to allow the one-step photochemical attachment of such materials, e.g. containing the NHS-moiety, onto glass.

Chapter 3 describes one of the possible approaches to this problem. By using a novel silanization method, where one of the silane precursors has a radical-stabilizing substituent, we stretched the effective energy window, to allow the subsequent hydrosilylation reaction to take place at longer wavelengths (328 nm). This seemingly minor change is enough to avoid photo-degradation of photo-sensitive moieties such as NHS, and allows its direct attachment onto an enclosed glass channel. Subsequently, for proof-of-concept, we proceeded with the one-step attachment of a DNA-zyme.

One of the drawbacks of the method described in **Chapters 2** and **3** is the amount of time required for the reaction to be complete (16 h). A possible way of improving upon this, would be by switching to a more optimized light source. Commercial Jelight pen lamps have undesirable mercury emission peaks (e.g. at 254 nm) that constitute a majority of the

emission intensity. By blocking these undesirable emissions, we avoid secondary reactions via photodegradation of sensitive moieties. However, blocking roughly 90% of the emission spectrum of the lamp means a significant lower number of photons will interact with our surface, thus slowing down the process.

Another approach would be via the usage of another substituted silane. The synthesis of a substituted-phenyl dichlorosilane might not be trivial at all, but such a compound (e.g. dichloropentafluorophenylsilane) could be thought of as an alternative. To preliminary investigate this, we looked more carefully at this C_6F_5 group. The fluorine atoms would be small enough that in terms of steric hindrance the substituent would not be very different from the original dichlorophenylsilane. A fluorinated-phenyl substituent could provide further stabilization to the Si-centered radical, allowing us to use light of wavelengths longer than the ones used in **Chapter 3 (Figure 6.6)**. However, a quick calculation (B3LYP-6311++G(d,p)) on a very simple model $(C_6F_5)(CH_3)Si\bullet$ shows that additional fluorine atoms do not induce significant stabilization of the Si-centered radical compared to $(Ph)(CH_3)-Si\bullet$ (ca. 5 nm). It is therefore clear that the silicon-centered radical stability is not trivially modulated.

Figure 6.6. Mixture of trichlorosilane and a fluorinated phenyl silane yields a $H_5-F_5\Phi$ -glass that might react at longer wavelengths.

A completely different approach would be to immobilize a photoinitiator (such as benzophenone) to the surface in catalytic amounts. In **Figure 6.7**, as an example we show an

alkyne-substituted benzophenone being attached to the surface. Alkynes are known to undergo thermal (and light-induced) addition to Si–OH and Si–H surfaces.[13–16] This benzophenone moiety is excitable by light of wavelengths above 354 nm.[17] This ketyl radical would then have enough reactivity to either abstract the nearby hydrogen[18] or react with an alkene/alkyne/alcohol in solution and thus initiate the radical chain-mechanism. To favor the reaction with the surface by any formed radicals, a diluted solution of the e.g. alkyne would be used.

Figure 6.7. The addition of a photoinitiator to the surface might allow the usage of very long wavelengths for the hydrosilylation reaction.

Chapter 4 describes a novel method for the modification of plastic surfaces. In particular, it focuses on the activation of surficial C–H bonds, ubiquitous in plastics. Plastics are becoming increasingly used in the field of microfabrication, such as microfluidics. They are cheap, and relatively inert, with exception of solvents that are structurally alike (*similia similibus solvuntur*). However, this inertness hampers the ability to chemically modify them. Cyclic olefin copolymers are considered pretty much chemically unreactive unless in harsh conditions. A few methods are described, but overall the most ubiquitous method for the activation of COC is the usage of an oxygen/air plasma. This method easily creates an oxidized surface. Plasma is easily applied on an open surface, not so much on an enclosed substrate due to diffusion limitations. Furthermore, plasma creates a variety of oxidized

species (COC–OH; COC–COOH; COC=O; etc), while to control the properties of a surface, one should minimize variations thereof. Prolonged plasma also etches away the polymer and roughens the surfaces.[19]

We strived for finding a simple, water-based C-H functionalization method, so that it can be used by flushing the reacting liquids along an enclosed surface such as a microfluidic channel. Such an approach should thus also be significantly milder and selective than plasma treatments. While this was basically the Holy Grail, we in fact discovered that the simple immersion of a COC surface into a solution of copper acetate with hydrogen peroxide is enough to oxidize C-H bonds. COC was thus converted into an alcohol-terminated surface (COC–OH) without any side-products (**Figure 6.8**).

Figure 6.8. Schematic for the activation of COC's C–H bond into a C–OH bond.

This catalyst, although extremely convenient as it is a simple commercial copper salt, might not be the most active. A wide variety of copper and other transition metal catalysts has been reported in literature for the activation of C–H bonds. Different metals and ligands should thus be studied to further optimize this method.[20-22]

Figure 6.9. Examples of catalysts found in literature for the activation of C–H bonds in liquid and gaseous alkanes.

Furthermore, we demonstrate that the addition of nitric acid to the oxidation mixture increases the yield of -OH moieties, in analogy with literature reports.[20] In the future, for the system described herein, the ratios of catalyst/acid should be further studied and optimized.

We thus show a very simple method, that is compatible with enclosed surfaces, with prompt results. Such surfaces can be used to a wide variety of chemistries. As a point-in-case, we show that the chemistry described in **Chapter 2** and **3** is applicable to this activated plastic. A new hybrid material was thus created and shown to be more resistant to harsh organic solvents than uncoated COC.

Figure 6.10. SEM figure of a COC surface that has been modified by our procedures and then immersed in a harsh organic solvent (dichloromethane) for 30 mins. Our treatment grants some protection.

For future studies, the attachment of more interesting moieties such as multiplexed antibodies will give further relevance to these plastic micro-technologies, further contributing to the field of bio-medical devices and e.g. flexible/wearable plastic-based bio-sensors.

In **Chapter 5**, we address the goal of allowing surface modifications in specific locations inside enclosed substrates with an *a priori* functionalization approach that involves

a novel way to make such devices. Fabrication of microfluidic devices usually requires a top and bottom substrate, which after micro-structuring are subsequently bonded. Typical bonding procedures employ temperatures that are not compatible with the organic or biological moieties that would be of use to immobilize in the channel. Hence, in the previous chapters we strived to develop methodologies that enabled modification *a posteriori* of the bonding procedure, i.e. enclosed substrates. In this chapter, we proceed with the surface modification before the bonding procedure, and used a low-temperature laser welding methodology. The assumption here was that the laser will only heat the substrate locally, leaving the organic moieties inside the channel intact.

Figure 6.11. Schematic for the laser-welding – surficial molecular stability studies.

We tested this by modifying a glass surface with a thermally-sensitive fluorescent dye and evaluating the fluorescence left after the welding procedure in dependence to the distance of the laser light spot that was used for welding. Thus, we could evaluate how thermally destructive the procedure is. As various factors come into play, we tested three channel designs. Under the conditions described in more detail in the chapter, it was concluded that there is some damage of the fluorescein monolayer up to 600 μm of the laser weld seam, or 100 μm from the channel wall. For future work, further testing should be performed and

perhaps improvements are possible. This technology has a large potential for easy and quick fabrication of bio-sensing microdevices with relatively wide channels. The next paragraph will address the possible ways of evaluating the technique and possible ways to improve it.

Using thinner glass slides will help dissipate the heat faster and thus decrease the distance from the welding laser spot from which there is monolayer bleaching. Additionally, since metal is an excellent heat conductor, the chromium layer should be at a larger distance from the channel wall (**Figure 6.12**); this will create a small 100 nm tall gap in between the welded glass slides that for all intents and purposes is negligible in terms of fluidic dynamics. Lastly, using a smaller laser incidence spot will also effect that less energy is required for the welding.

Figure 6.12. Schematic of a possible way to improve the laser welding system. Note that the image is not to scale.

In conclusion, the choices for the chemistries to be investigated and developed were always based on a mix of pragmatism, time constraints and a wonder for the exotic. Through our endeavor to create new technologies, i.e. applied chemistry, we found new reactions, i.e. fundamental chemistry, and characterized them as best as possible. Overall, a lot was learned, and many aspects can be improved, but the ground is set and we can now start climbing – **Figure 6.13.**

Figure 6.13. The stage of development of this research (from the perspective of the author).

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Appendix I

Supporting Information to Chapter 2

Local Light-induced Modification of the Inside of Microfluidic Glass Chips

Silanization

Figure S2.1. Schematic of the silanization methods: **A** using triethoxysilane by chemical vapor deposition (CVD) or chemical bath deposition (CBD), **B** using trichlorosilane by chemical vapor deposition (CVD).

Scheme S2.1. Schematic representation of the CVD setup. Desiccator volume of 5L with the surfaces to be modified. Flask containing 500 μL of silane (flask volume of 2 mL) under Argon overpressure (filled in a glove box). A, B and C are closed

1. Open A to vacuum
2. Open B
3. Let the system be in vacuum (10 mbar) for 1h
4. Close A
5. Open C and let the silane evaporate
6. Close C and B
7. Allow the silane to deposit and react with the surface for the desired amount of time
8. To quench the reaction. Open A and B to vacuum.
 - a. Use a base-containing trap to prevent damage to the pump
9. Refill with Argon
10. Close all valves
11. Disconnect the desiccator from the system and sonicate the silanized substrates in DCM for 20 min.

Table S2.1 Comparison between used silanization methods.

Method	Scheme	XPS Si2p	SCA	$\nu(\text{Si-H}) \text{ cm}^{-1}$
H-Si(OEt)₃ (CVD)			80±1°	2245
H-Si(OEt)₃ (CVD _{cured})			82±1°	2250
H-Si(OEt)₃ (CBD)			74±2°	2240
H-SiCl₃ (CVD)			85±1°	2250

Figure S2.2 XPS Wide scans for (a) freshly plasma-cleaned glass, (b) H-Si(OEt)₃ (CVD) pre-curing, (c) H-Si(OEt)₃ (CVDcured), (d) H-Si(OEt)₃ (CBD) and (e) H-SiCl₃ (CVD)

Table S2.2. SCA and AFM measurements of roughness, topography and amplitude of glass at different silanization time of H-SiCl₃ (CVD) – image size is 2 × 2 μm².

Time (min)	SCA (°)	R _a (nm)	Topography	Amplitude
0	<10	0.4 ± 0.1
20	85±1	0.6 ± 0.1
30	89±2	6.4 ± 2.2
40	92±2	15.1 ± 1.1
50	94±3	14.9 ± 3.5
60	96±3	20.5 ± 12.1

Theoretical calculations on Si-H bond energy

The Si-H bond dissociation enthalpies D_o^{298K} were estimated from the direct expression

$$D_o^{298K} = {}_fH^\circ(\text{Si}) + {}_fH^\circ(\text{H}) - {}_fH^\circ(\text{SiH}) \quad (1)$$

Where ${}_fH^\circ(X)$ are the enthalpies of formation for the species X.

Figure S2.3. Models used to calculate the Si-H bond dissociation enthalpies at 298K in eV.

Value shown on arrow by MP2/6-311++G(d,p) method.

Table S2.3. Comparison of Calculation Methods for the for Si-H Bond Dissociation Enthalpies at 298K.

Method	H-glass D_o^{298K}		H-silicon D_o^{298K}	
	eV	nm	eV	nm
M11L/6-311G(d,p)	3.99	311.1	3.63	341.9
MP2/6-311G(d,p)	3.83	323.9	3.48	356.5
MP2/6-311+G(d,p)	3.75	330.3	3.41	363.8
MP2/6-311++G(d,p)	3.76	329.7	3.41	363.2
B3LYP/6-311G(d,p)	3.87	320.5	3.61	343.8

Figure S2.4. (a) UV-Vis Transmittance spectra of borosilicate glass (H-glass is the same), and quartz glass. (b) UV-Vis Absorbance spectra of 1-hexadecene TFAAD and glass.

H-glass hydrosilylation modification setup

Scheme S2.2. Schematic representation of the setup for the photochemical modification of 1-hexadecene monolayers on H-glass flat surfaces.

1-hexadecene

Figure S2.5. XPS Wide scan of hexadecyl monolayer on H-Si(EtO)₃-based H-glass after modification for 16h under irradiation at different wavelengths: (a) 254 nm, (b) 302 nm, (c) 330 nm, (d) 365 nm.

Figure S2.6. XPS Wide scan (a) and GATR-FTIR (b) of hexadecyl monolayer on H-SiCl₃-based H-glass after modification for 16h under 302 nm irradiation.

Figure S2.7. XPS Wide scan of OH-glass reacted with 1-hexadecene for 16 h under 302 nm irradiation.

11-fluoro-1-undecene

The C:Si ratio was calculated to be 0.285. This ratio is then converted into monolayer thickness according to equation 1. The thickness of the hydrocarbon layer after 16 h of irradiation was thus calculated to be 1.0 ± 0.1 nm. This is similar to the calculated length of a single 11-fluoro-1-undecenyl molecule (1.3 nm, determined by Chem3D) at a $39.7 \pm 0.7^\circ$ tilt angle with respect to the surface normal. This is indicative of a monolayer formation without contribution of multilayer formation and/or other carbon contaminants. This was further confirmed by C1s narrow scans shown in Figure S7(a) and deconvoluted into 4 main peaks with a theoretical and experimental ratio of 1:1:1:8. The first peak at 287.7 eV (8.4%) corresponds to the carbon bound to the fluorine ($-\text{CH}_2\text{F}$). The second peak at 286.2 eV (8.3%) corresponds to the carbon, which is next to the carbon bound to fluorine ($\text{CH}_2\text{-CH}_2\text{F}$), which is similar to previously reported by our group i.e. 287.9 eV and 285.9 eV respectively.¹ The third peak at 286.8 eV (8.2%) corresponds to the carbon bound to the silicon in the glass substrate ($\text{O}_3\text{Si-CH}_2\text{-}$). The final main peak at 285.0 eV (75.0%) – which was used as a reference peak – corresponds to the rest of the aliphatic chain (C-H_2). In addition, the C1s XPS spectrum was simulated using B3LYP/6-311G(d,p)-derived method, yielding a reasonable agreement with experimental data.

Figure S2.8 (a) XPS narrow scan C1s (b) DFT calculated C1s and (c) XPS narrow scan F1s of 11-fluoroundecenyl monolayers on H-glass.

TFAAD (10-trifluoro-acetamide-1-decene)

Synthesis: 66 mg of tetrabutylammonium bromide (0.25 mmol, catalytic) and 12.1 g 10-undecenoyl chloride (60.0 mmol, 1.00 eq.) were combined and cooled to -3 °C. Aqueous sodium azide solution (3.90 g in 15 mL H₂O) was added dropwise, keeping the temperature below 5 °C. The reaction mixture was stirred for 24 h at 0 °C. After addition of 15 mL of cold DCM, the organic phase was washed two times with 15 mL of cold H₂O and dried over NaSO₄. (During the whole workup process solvents and solutions were kept as cool as possible.) The resulting acyl azide was directly reacted with 6.0 mL trifluoroacetic acid (78 mmol, 1.30 eq.). The reaction mixture was allowed to warm up, stirred at RT until gas formation stopped and refluxed at 40 °C overnight. Washing three times with 15 mL of saturated sodium bicarbonate solution, drying over sodium sulfate and removal of the solvent under reduced pressure gave the crude product. Purification was achieved by column chromatography (100% DCM; R_f 0.8) and Kugelrohrdestillation (0.4 mbar, 176 °C), which yielded the desired product with a purity of 98% (GC-MS).

[C₁₂H₂₀F₃NO, M = 251.29 g/mol] Yield: 10.8 g (43.1 mmol, 72 % over two steps)

¹H NMR (400 MHz, CDCl₃) δ 6.54 (s, 1H, 11-H), 5.79 (ddt, ³J_{HH} = 16.9, 10.2, 6.7 Hz, 1H, 2-H), 5.02 - 4.89 (m, 2H, 1-H), 3.34 (q, ³J_{HH} = 6.8 Hz, 2H, 10-H), 2.03 (q, ³J_{HH} = 7.0 Hz, 2H, 3-H), 1.57 (p, ³J_{HH} = 7.1 Hz, 2H, 9-H), 1.42 - 1.20 (m, 10H, 4,5,6,7,8-H).

¹³C NMR (101 MHz, CDCl₃) δ 157.4 (q, ²J_{CF} = 36.6 Hz, C-12), 139.2 (C-2), 115.9 (q, ¹J_{CF} = 187.9 Hz, C-13), 114.6 (C-1), 40.1 (C-10), 33.9 (C-3), 29.4 - 29.0 (C-4,5,6,7,9), 26.8 (C-8).

ESI⁺ (MeOH): calc.: $m/z = 252.1570$ [M+H]⁺, 274.1389 [M+Na]⁺; det.: $m/z = 252.1562$ [M+H]⁺, 274.1379 [M+Na]⁺.

Surface-bound TFAAD

Both XPS wide and C1s narrow scans have peak intensities and atomic ratios correlating well with the number of carbons in the chain (theoretical C:F 4.0, C:N 12.0; experimental C:F 4.1, C:N 12.7 – see **Figure S**). The recorded C1s narrow scans are deconvoluted into 4 main peaks, namely the CF₃ group at 293.4 eV, the C=O group at 289.2 eV, a peak at 286.8 eV belonging to the (O)₃Si-C, and (H)₂C-N bond and the main hydrocarbon C-C group at 285.0 eV.

Figure S2.9 XPS Wide scans of TFAAD monolayer on H-glass before (a) and after (b). deprotection by 0.12 M NaOtBu.

Photolithography

H-glass was patterned with 1-hexadecene patterned and subsequently photo-oxidized in the presence of water and air by irradiation of 302 nm for 16h. Posteriorly cleaned by dichloromethane/hexane and exposed to water vapor while being visualized on a microscope.

Figure S2.10. Micrograph of water vapor condensing on glass modified with a pattern of hexadecyl monolayers.

Attachment of amine-terminated fluorescent Si nanoparticles:

Scheme S2.3. Schematic representation of the deprotection of TFAAD and subsequent tagging of the amino-terminated surface by p-phenylene diisothiocyanate, followed by a fluorescent amino-terminated silica nanoparticle.

Surface-driven flow

- Video 1 shows a dye flowing at 50 $\mu\text{L}/\text{min}$ through a meandering hydrophilic path.
 - http://pubs.acs.org/doi/suppl/10.1021/acs.langmuir.5b04621/suppl_file/la5b04621_si_002.mp4
- Video 2 shows a comparison of laminar flow versus mixing regimes via demineralized water flowing through a non-modified channel versus a photochemically modified channel.
 - http://pubs.acs.org/doi/suppl/10.1021/acs.langmuir.5b04621/suppl_file/la5b04621_si_003.mp4
- Video 3 shows the abovementioned flow from 50 $\mu\text{L}/\text{min}$ until 500 $\mu\text{L}/\text{min}$.
 - http://pubs.acs.org/doi/suppl/10.1021/acs.langmuir.5b04621/suppl_file/la5b04621_si_004.mp4

- Video 4 shows a hydrophobic patch acting as a passive flow stop-valve.

- <https://www.youtube.com/watch?v=NdMTNbtj7x4>

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Appendix II

Supporting Information to Chapter 3

Mild Photochemical (Bio-)Functionalization of Glass Microchannels

Figure S3.1. Overlay of the emission profile of the 330 nm lamp with (a) and without (b) a 320 nm long pass filter.

Figure S3.2. Overlay of the emission profile of the 302 nm lamp with (a) and without (b) a 280 nm long pass filter.

Figure S3.3. Schematic representation of the reaction setup for bare glass surfaces in a perspective (a) and top view (b); gray line represents the tin foil used to cover the setup and protect the user from UV light.

Figure S3.4. Schematic representation of the reaction setup for microfluidic device. (a) simplified view of a long pass filter (i) stacked on top of a photolithographic mask (ii), which

are then placed on a H- Φ -glass modified microfluidic reactor (iii). (b) SolidWorks 3D rendition of our photolithographic - Fluidic Pro setup where a UV source (iv) illuminates only a part of a microchannel that is filled with an NHS-terminated ω -alkene.

Figure S3.5. AFM image (10×10 μ m) of glass modified with: (a) pure dichlorophenylsilane - roughness $R_a = 9.5 \pm 1.5$ nm; (b) 5:1 mixture of trichlorosilane:dichlorophenylsilane - roughness $R_a = 1.0 \pm 0.5$ nm; (c) H₅- Φ ₁-glass after reaction with NHS-terminated ω -alkene $R_a = 1.2 \pm 0.8$ nm;.

Figure S3.6. GATR-FTIR spectrum of glass slide functionalized only with dichlorophenylsilane. Aromatic stretching vibrations (Ph-H) are visible in the range 3100-3000 cm^{-1} ; the main (Ph)Si-H at 2180 cm^{-1} ; Si-Ph overtones (2000-1660 cm^{-1}).

Figure S3.7. Schematic of glass modified for 20 min in air with different silanization mixtures in anhydrous cyclohexane. (a) pure dichlorophenylsilane; (b) mixture 5:1 trichlorosilane:dichlorophenylsilane; (c) 12:1 trichlorosilane:dichlorophenylsilane.

Figure S3.8. XPS Si 2p narrow scans of (a) H₅-Φ₁-glass – fwhm 1.68 eV; (b) H-glass – fwhm 1.64 eV; (c) freshly plasma-cleaned glass – fwhm 1.53 eV.

Theoretical calculations on Si-H bond energy¹

	H-glass (HG) (O ₃ Si—H)	GlassPh (ΦG) (O ₂ PhSi—H)	HG-ΦG
M11	97.7	93.6	4.1
M11L	98.3	94.1	4.2
B97D	95.3	90.4	4.9
B3LYP	96.5	91.3	5.2

Table S3.1. Theoretical bond dissociation energies calculated via several DFT methods (kcal/mol).

	Experimental	Scaling factor			
		0.9679	0.957	0.952	0.952
		B3LYP- 6311+G(d,p)	B97D- 6311+G(d,p)	M11- 6311+G(d,p)	M11L- 6311+G(d,p)
v(Si-H) / cm⁻¹	2250	2290	2214	2186	2323
v(PhSi-H) / cm⁻¹	2180	2188	2142	2123	2231

Table S3.2. Theoretical wavenumber for Si-H stretching vibrations calculated via several DFT methods.

Figure S3.9. DART-HRMS TIC (total ion current) and EIC (Extracted Ion chromatogram) of 10 mmu mass window around theoretical mass of target analyte, NHS ($m/z = 114.0165$). In the middle figure presents the integrated intensity of 0.5 min for H- Φ -glass - Lamp 330 nm long pass filter of 320 nm (standard deviation is 7.2% across hexaplet samples).

Figure S3.10 – DART-HRMS TIC (total ion current) and EIC (Extracted Ion chromatogram) of 10 mmu mass window around theoretical mass of target analyte, NHS (m/z 114.0165). The middle figure presents the integrated intensity of 0.5 min for H-glass - Lamp 330 nm long pass filter of 320 nm (standard deviation is 18 % across hexaplet samples).

Figure S3.11. (a) GATR-FTIR and (b) XPS wide scan of a 5:1 H- Φ -glass surface modified with C11NHS via irradiation of a 302 nm lamp with a 280 nm long-pass filter for 16 h.

Figure S3.12. (a) Microwave-synthesized water-soluble amine-terminated Si NPs. (b) TEM and (c) HRTEM images of Si NPs showing lattice fringes of an individual NP (left insert) and electron diffraction pattern obtained in “transmission” geometry (right insert). (d) DLS histogram and (e) optical absorption and fluorescence spectra of these NH₂-terminated Si NPs. (f) Photographs displaying visual fluorescence upon irradiation with 254 and 365 nm.

Figure S3.13. GATR-FTIR spectrum of **C11NHS** monolayer before (a) and after (b) DNA attachment (referenced with H- Φ -glass).

Figure S3.14. A guanine rich surface-bound DNA G-quadruplex structure yields, in the presence of K^+ and hemin [Fe(III)-protoporphyrin IX chloride], a horseradish peroxidase mimicking hGQ DNAzyme.

Figure S3.15. XPS Fe2p narrow scan of H- Φ -glass modified with an iron-containing hGQ complex.

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Full reference of Gaussian 09:

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Appendix III

Supporting Information to Chapter 4

Mild and Selective C-H Activation of COC

Microfluidic Channels

Figure S4.1. Overlay of the emission profile of the 328 nm lamp without (a) and with (b) a 320 nm long pass filter.

Figure S4.2. Overlay of the emission profile of the 302 nm lamp without (a) and with (b) a 280 nm long pass filter.

Material	SCA image	SCA (°)
COC		95
COC+H ₂ O ₂		95
COCOx Plasma		<30
COCOx Cu Oxidation		82
COCOH		72°
HSiCOC		85°
TFAADSiCOC		77°

Table S4.1. Water static contact angle of the substrates. After each modification step, the substrates were rinsed with ethanol, and acetone, and subsequently dried with a nitrogen gun to be directly analyzed by water static contact angle. Note: As is typical of polymeric substrates, contact angles of oxidized COC will increase over time. WSCA will stabilize upon the formation of the hybrid HSi-COC surface and subsequent steps. The hybrid material displays some ceramic surficial properties and as such the WSCA will have negligible change over time.

Material	Topography	Roughness (nm)
COC		1.7±0.5
COC-Ox (Plasma)		1.8±0.8
COC-OH		1.6±0.2

H-SiCOC	An atomic force microscopy (AFM) image of an H-SiCOC substrate. The image shows a brownish, textured surface with some bright spots. A green square highlights a specific region of interest. A scale bar in the bottom left corner indicates 1 μm. Several small red and blue numbers are scattered across the image, likely representing height or phase values.	1.9±1
TFAAD-SiCOC	An atomic force microscopy (AFM) image of a TFAAD-SiCOC substrate. The image shows a brownish, textured surface with some bright spots. A green square highlights a specific region of interest. A scale bar in the bottom left corner indicates 1 μm. Several small red and blue numbers are scattered across the image, likely representing height or phase values.	2.0±0.5

Table S4.2. Atomic force microscopy images and roughness measurements of the substrates.

Figure S4.3. Gold contact mask used for the photolithography of exposed surfaces.

Figure S4.4. XPS C1s narrow scan of a COCSiΦTFAAD surface.

Time of exposure	SEM pictures of TFAAD modified COC after exposure to DCM
0
5 min

30 min
16 h

Table S4.3. SEM pictures of TFAAD modified COC after exposure to DCM

Figure S4.5. To maximize contact with the oxidizing solution, the COC substrate was kept immersed in the aqueous solution by a plastic holder.

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Appendix IV

Supporting Information to Chapter 5

Laser welding of pre-functionalized glass
substrates: A fabrication and chemical stability
study

Figure S5.1. (a) a schematic of the insertion of a blade between substrates to induce a fracture between bonded substrates; (b) a photograph of the broken device, after delamination the top surface with the chromium layer fractured and broke loose. The black line indicates the edge of the bottom glass layer which is still intact; (c) microscopy picture of a laser-welded microfluidic chip after applying the crack-opening method. Delamination occurs only until the weld and not further.

Channel width (mm)	Fluorescence before welding	Welded #1	Welded #2	Welded #3	Welded APTES
1
3
5

Table S5.1. Fluorescence microscopy pictures of the modified surfaces before the laser welding procedure (The chromium coated substrate was put on top to give a contrast and an idea of how the channel would look like after the welding.). And after the laser-welding, where APTES-modified surfaces that were not reacted with FITC and were used as a blank.

Summary

The ability to locally functionalize the surface of glass and polymers allows for myriad biomedical and chemical applications. When we started this research enclosed surfaces such as microfluidic channels were not easily amenable to localized functionalization. Furthermore, pre-functionalization of microfluidic devices was incompatible with the typical high-temperature substrate bonding procedures. The work described in this thesis was performed to tackle these two issues.

In **Chapter 1**, an introduction is provided to concepts that are central to microfluidics and surface functionalization. Focus is given to techniques that allow for localized surface functionalization, specifically on silicon oxide and glass surfaces. Although contact methods are often easy and near-ubiquitously used, non-contact methods, such as photolithography, are preferred for scaled-up production and are compatible with enclosed surfaces. In addition, plastics – preferably so-called inert plastics – are emerging as a consistent and cheap substrate for the fabrication of microtechnologies. Therefore, previous methods for the modification of such plastics, e.g. cyclic olefin co-polymers (COC), are described. Most of these methods rely on harsh conditions, e.g. plasma, to oxidize the plastic surface. However, in solution there are reported methods to selectively oxidize C-H bonds on alkanes, under mild conditions. Solution-based procedures are much more compatible with enclosed structures when compared to methods relying on gases or plasmas, as the latter exhibit several diffusion issues. Finally, we address the emergence of laser-based techniques and other low-temperature glass bonding methods which will allow for the pre-functionalization of microfluidic devices prior to the bonding step of their fabrication. An outline of this thesis is provided at the end of the chapter.

In **Chapter 2**, we present the first example of a photochemical modification of hydrogen-terminated glass (H-glass) with terminal alkenes. Both flat glass surfaces and the

inside of glass microchannels were modified with a well-defined, covalently attached organic monolayer using a range of wavelengths, including 302 nm ultraviolet light. Mechanistic studies of the surface modification showed that it proceeds via an anti-Markovnikov substitution. Reacting H-glass with 10-trifluoro-acetamide-1-decene (TFAAD) followed by basic hydrolysis afforded the corresponding primary amine-terminated monolayer, enabling additional functionalization of the substrate (with fluorescent nanoparticles). A microchannel was photochemically patterned with a functional linker allowing for surface-directed liquid flow, and a passive flow stop-valve. H-glass was shown to be modifiable with a functional tailor-made organic monolayer, has highly tunable wetting properties and presents potential for many applications that are explored in the following chapters.

In **Chapter 3**, we studied how to increase the wavelength used in the surface modification approach described in the previous chapter, from 302 to 328 nm. Thus, we show the direct photochemical coupling of a *N*-hydroxysuccinimide-terminated (NHS) ω -alkene onto hydrogen-terminated silicon oxide, and its subsequent functionalization with a catalytically active DNAzyme. In this chapter, we prepared hydrogen-phenyl-terminated glass (H- Φ -glass) by the reaction of glass with H-SiPhCl₂. The presence of a radical-stabilizing substituent on the Si atom enabled the covalent modification of bare glass substrates and of the inside of glass microchannels with a functional organic monolayer that allowed direct reaction with an amine-functionalized bio-molecule. Using these NHS-based active esters on the surface, we performed a direct localized attachment of a horseradish peroxidase (HRP)-mimicking hemin/G-quadruplex (hGQ) DNAzyme complex inside a microfluidic channel. This wall-coated hGQ DNAzyme effectively catalyzed the in-flow oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate) [ABTS] in the presence of hydrogen peroxide. This proof-of-concept of mild bio-functionalization will find use in myriad bio-relevant applications.

In **Chapter 4**, we explored the modification of plastics, such as cyclic olefin copolymer (COC), which are becoming an increasingly popular material for microfluidics. COC is used, in part, due to its (bio)-chemical resistance. However, its inertness and hydrophobicity can be a major downside for many bio-applications. In this chapter, we showed the first example of a surface-bound selective C-H activation of COC into alcohol C-OH moieties under mild aqueous conditions at room temperature. The nucleophilic **COC-OH** surface allowed for subsequent covalent attachments, such as of a H-terminated silane. The resulting hybrid material (**COC-Si-H**) was then modified via a photolithographic hydrosilylation in the presence of ω -functionalized 1-alkenes to form a new highly-stable, solvent-resistant hybrid surface. The work described in this chapter shows great potential for multiple types of plastics, and further shows that the hydrosilylation chemistry described in the previous chapters can be applied onto a multitude of surfaces that contain nucleophilic groups (such as -OH), and thus be able to react with hydrogen-terminated silanes.

In **Chapter 5**, we explored low-temperature bonding methods of glass substrates. These methods are of great interest in the field of microfluidic-based biosensing. We evaluated how laser welding could be used for this, by studying how the compatibility of this technology with the, prior to bonding, modification of glass channels with temperature-sensitive materials. The effects of the welding process were studied by investigating the thermal degradation of a fluorescent monolayer throughout the bonding procedure. Furthermore, these leakage-free devices, as well as preliminary work using picosecond lasers, shows that there is a great potential for improvement and wide applicability.

In **Chapter 6**, we summarize the results obtained in this thesis. In addition, we discuss some ideas and topics where the research could either be improved or achieved via alternative approaches, including some leads from unfinished work that was performed within this project.

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*As armas e os barões assinalados
Que da Ocidental praia Lusitana,
Por mares nunca dantes navegados
Passaram ainda além da Taprobana,
Em perigos e guerras esforçados
Mais do que prometia a força humana
E entre gente remota edificaram
Novo Reino, que tanto sublimaram;*

Camões, Os Lusíadas – Canto I – 1572

*Arms and the heroes of illustrious fame,
Who from the western Lusitanian shore,
Remote unnavigated seas explored, –
Far beyond Taprobana's distant isle, –
And 'midst the perils of adventurous war,
With more than human constancy endured,
In remote climes, a mighty empire raised
And aggrandized by great and glorious deeds;*

Camões, The Lusiad – Canto I – 1572

List of Publications**Articles**

- **R. Rijo Carvalho**, S. P Pujari, S. C. Lange, R. Sen, E. X. Vrouwe, H. Zuilhof: *Local light-induced modification of the inside of microfluidic glass chips*, Langmuir, 2016, 32(10), 2389–2398 - 10.1021/acs.langmuir.5b04621
- **R. Rijo Carvalho**, S. P Pujari, D. Gahtory, E. X. Vrouwe, B. Albada, H. Zuilhof: *Mild photochemical biofunctionalization of glass microchannels*, Langmuir, 2017, Article ASAP - 10.1021/acs.langmuir.6b03931
- **R. Rijo Carvalho**, S. P Pujari, E. X. Vrouwe, H. Zuilhof: *Mild and selective C-H activation of COC microfluidic channels*, ACS Applied Materials & Interfaces, Article ASAP - 10.1021/acsami.7b02022
- **R. Rijo Carvalho**, S. Reuvekamp, H. Zuilhof, M. Blom, E. X. Vrouwe: *Laser welding of pre-functionalized glass substrates: a fabrication and chemical stability study*, Manuscript submitted for publication
- **R. Rijo Carvalho**, S. P Pujari, E. X. Vrouwe, H. Zuilhof: *Local visible-light induced modification of the inside of glass and COC microfluidic channels*, Proceedings of 20th International Conference on Miniaturized Systems for Chemistry and Life Sciences, 2016, 1142-1143 - ISBN 9780979806490
- R. Sen, D. Gahtory, **R. Rijo Carvalho**, M. M. J. Smulders, B. H. Albada, F. van Delft, H. Zuilhof: *DNA constructs and metal-free click reactions on covalently-modified mica*, Angewandte Chemie Int. Ed. 2017, 56, 4130 – 4134 - 10.1002/anie.201701301

Patents

- **R. Rijo Carvalho, S. P. Pujari, E. X. Vrouwe, H. Zuilhof:** *Photochemical modification of a solid surface*, Micronit Microtechnologies / Wageningen University & Research - Priority date 19-02-2016 – EP/US/WO Patent 17156039.4 - 1371
- **R. Rijo Carvalho, E. X. Vrouwe, H. Zuilhof:** *Mild modification of polymeric substrates*, Micronit Microtechnologies / Wageningen University & Research — Priority date 22-03-2017 – NL N2018559 *Patent pending*
- **R. Rijo Carvalho, et al -** *Photochemical modification of a solid surface* - Surfix BV. *in preparation*

Astrophotographs

- **R. Rijo Carvalho,** *Milky Way at the Višnjan Observatory*, 2016, *Secrets of Superhero Science*. By Barry W. Fitzgerald, BW Science, Eindhoven, 345, Print, ISBN: 978-90-825040-0-2

Discipline specific activities		
Dynamic Covalent Chemistry and Complex Chemical Networks	IECB	2011†
Molecular Encapsulation	EPFL	2012†
Nanoparticles and Dynamic Materials	WIS	2012‡
Applications of Nano-sized Compounds and Materials	UCAM	2012†
Dutch Soft Matter Meeting	WUR	2012† [±]
Analysis in SEDPHAT	LHSK	2012
Fundamentals of Nanotechnology	WUR	2013
Dynamic Molecular Nanostructures	RUG	2013†
Molecules: Synthesis and Properties	NWO	2013
Bruker Nano AFM Course	Bruker (DE)	2013
III Electrochemistry and Innovation Days	E-INOV	2013‡
Dynamics of Self-assembly	TU Delft	2013‡
FP7 Dynamol Meeting	Solvay	2014† [±]
Minacned Flow Chemistry Hands-On Training Course	Minacned	2014
The 20th International Conference on Miniaturized Systems for Chemistry and Life Sciences	MicroTAS	2016
Internships		
Synthesis of Substituted Cyclodextrins	KTU-Germany	2012
Surface-Confined Organometallic Oligomers	WIS-Israel	2013
General Courses		
Crystallography Workshop	Global Phasing	2012
Presentation Skills	WUR	2013†
Share your results	WUR	2013†
Electrochemistry Workshop - SSCP and AGNES	WUR	2013
Solidworks Training	WUR	2014
Research and Development in an Industrial Setting	Solvay	2014 [±]
Entrepreneurship / Commercialization / Intellectual		
Mock job interview, Feedback and Career Management	Solvay	2014†
Career Perspectives (CCP)	VLAK	2015
Workshop: How to manage your supervisor	VLAK	2015† [±]
Workshop: How to survive your PhD	WASS	2016† [±]
Optionals		
Organization Dutch Soft Matter Meeting	WUR	2012 [±]
Project Proposal	Micronit/WUR	2012
PhD Excursions	PCC/ORC	2012-2016
PhD Study Trip	PCC	2013†
Soft Matter Meetings	PCC	2012-2015†
Symposia	PCC	2012-2015†
Colloquia	ORC	2015-2016†
Surface Group Meetings	ORC	2015-2016†

† (oral presentation) ‡(poster presentation) [±](organizer)

About the author

Rui Pedro was born on 18 January, 1985 in Oeiras e São Julião da Barra, Portugal. Sci-fi, astronomy, and MacGyver have been a constant presence and source of inspiration, ever since he was a child.

As an adult, he dived into a Bachelor in Chemistry at the Superior Technical Institute in Lisbon. An internship with Prof. A.M. Martins (OARC-IST) provided ample training in anhydrous and organometallic chemistry. Further studies with Prof. C.A.M. Afonso (iMed.UL, FFUL), he developed a taste for mechanistic studies and modification of natural products. In 2009, he researched under Prof. A. L. Pombeiro (CCC-IST) financially supported by a Santander-Totta research grant for the “Activation of small molecules”, concomitantly doing a Masters in Chemistry at the same institute. He continued under Prof. Pombeiro’s supervision, financed by two grants of the Science and Technology Foundation (FCT). This research was then used in his MSc Thesis in 2010.

In 2011, Rui ventured north to The Netherlands and joined Micronit Microtechnologies BV. as an Early Stage Researcher - part of a FP7 Marie Curie ITN – DYNAMOL. The goal was to create new technologies that would allow the local functionalization of the inside of microfluidic channels. Several instructive secondments were had throughout (DOC-KTU, DOC-WIS).

In June 2015, he joined the Department of Organic Chemistry at Wageningen University & Research (ORC-WUR) as an external PhD Student, under the supervision of Prof. Han Zuilhof. This very productive collaboration between Wageningen University and Micronit Microtechnologies resulted in several papers and patents within a short period. The main results obtained in the period June 2015 - December 2016 are described in this thesis.

Rui currently works as a R&D Scientist at Surfix BV, focusing on the localized functionalization of a plurality of substrates. All the while keeping close collaborations with his previous associates.

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