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GEBZE TECHNICAL UNIVERSITY
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AMINE REACTIVE POLYMERIC MATERIALS FOR PATTERNED BIOIMMOBILIZATION AND ORTHOGONAL FUNCTIONALIZATION

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A THESIS SUBMITTED FOR THE DEGREE OF
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ASST. PROF. DR. TUĞÇE NİHAL GEVREK CİVAN

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2023

T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
BİYOTEKNOLOJİ ENSTİTÜSÜ

DESENLİ BİYOİMMOBİLİZASYON VE ORTOGONAL FONKSİYONLANDIRMA İÇİN AMİN REAKTİF POLİMERİK MALZEMELER

NİSA DEMİRBİLEK

YÜKSEK LİSANS TEZİ
BİYOTEKNOLOJİ ENSTİTÜSÜ

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GEBZE
2023

SUMMARY

Polymers possessing both reactive and antifouling groups find many applications in biomedical fields. These polymers facilitate strong immobilization of molecules of interest while minimizing non-specific immobilization of other biological materials, such as proteins and cells. Since amine groups are abundant in biomolecules, polymers that contain amine-reactive groups are highly utilized for the immobilization of biomolecules. The abundance of molecules containing amine groups, coupled with the fact that amine groups readily react with many reactive groups such as epoxy, isocyanate, or aldehyde, highlights the importance of developing new methods for preparing amine-reactive polymeric platforms. Herein, we studied two approaches for amine-mediated (bio)immobilization. In the first part of the thesis, zwitterionic hydrogel films containing ortho-nitrobenzaldehyde were synthesized on glass surfaces through free radical polymerization. These reactive surfaces were first reduced to alcohols and then regio-selectively activated using UV light. We demonstrated the patterned immobilization of small molecules as well as biomolecules on these surfaces. In the second part of the thesis, we synthesized polymers with pendant epoxide and isocyanate groups. The different reaction kinetics between epoxide-amine and isocyanate-amine reactions allowed for the orthogonal conjugation of different molecules containing amine groups to the polymeric platforms. Consequently, we were able to attach two different amine-containing molecules to these polymers. Furthermore, by conjugating through the isocyanate-amine reaction, we were able to anchor the polymers onto the glass surface using the epoxide groups. We believe that both studies have potential applications in various bio-immobilization-based bioapplications.

Key Words: Ligth-Responsive Hydrogels, Zwitterionic Hydrogels, Orthonitrobenzyl (o-NB) Groups, Amine Reactive Hydrogels&Polymers, Bioimmobilization.

ÖZET

Reaktif ve antifouling grupları birlikte barındıran polimerler, biyomedikal alanlarda çok uygulama bulmaktadır. Bu polimerler, proteinler ve hücreler gibi diğer biyolojik materyallerin spesifik olmayan immobilizasyonunu en aza indirirken ilgili moleküllerin güçlü bir şekilde sabitlenmesini kolaylaştırmaktdır. Biyomoleküllerde amin grupları bol olduğundan, amin-reaktif gruplar içeren polimerler, biyomoleküllerin immobilizasyonu için yüksek oranda kullanılmaktadır. Amin grubu içeren moleküllerin bolluğu, ayrıca amin gruplarının epoksi, izosiyanat veya aldehit gibi birçok reaktif gruba kolaylıkla tepki vermesi, aminle tepkimeye girebilen polimerik platformların hazırlanması için yeni yöntemlerin geliştirilmesinin önemini vurgulamaktadır. Bu çalışmada, amin aracılı (biyo)immobilizasyon için iki yaklaşım üzerinde çalışılmıştır. Tezin ilk bölümünde, serbest radikal polimerizasyonu yoluyla cam yüzeyler üzerinde orto-nitrobenzaldehit içeren zwitteriyonik hidrojel filmler sentezlenmiştir. Bu reaktif yüzeyler öncelikle alkol haline indirgenmiş ve ardından UV ışığı kullanılarak bölgesel olarak aktive edilmiştir. Bu yüzeyler üzerinde küçük moleküllerin yanısıra biyomoleküllerin desenli immobilizasyonu gösterilmiştir. Tezin ikinci bölümünde ise, yan dallarında epoksit ve izosiyanat grupları taşıyan polimerlerin sentezi gösterilmiştir. Epoksit-amin ve izosiyanat-amin reaksiyonları arasındaki farklı tepkime kinetiği, amin grupları içeren farklı moleküllerin polimerik platformlara ortogonal konjugasyonuna izin vermiştir. Sonuç olarak, bu polimerlere iki farklı amin içeren molekülün bağlanabildiği gösterilmiştir. Ayrıca, izosiyanat-amin reaksiyonuyla konjugasyon sonrası, boşta kalan epoksit grupları sayesnide polimerlerin cam yüzeye kaplandığı gösterilmiştir. Her iki çalışmanın da çeşitli biyo-immobilizasyon temelli biyo-uygulamalarda potansiyel kullanım alanları olduğuna inanıyoruz.

Anahtar Kelimeler: Işığa Duyarlı Hidrojeller, Zwitteriyonik Hidrojeller, Ortonitrobenzil (o-NB) Grupları, Amin Reaktif Hidrojeller&Polimerler, Biyoimmobilizasyon.

ACKNOWLEDGEMENTS

First of all, I would like to express sincere appreciation to my invaluable advisor, Assist. Prof. Dr. Tuğçe Nihal GEVREK CİVAN, who has been with me every step of the way from the beginning of my undergraduate education to my master education, never withholding her support for even a single day. I am grateful for her endless patience and trust in me during this long and difficult journey.

I would like to express my great appreciation to Assoc. Prof. Dr. Erman KARAKUŞ who has been a guiding light to me with his knowledge and experiences, for his support in every opportunity.

I would like to express my gratitude to Aleyna TURANLI for her endeavor in the synthesis of the NBAA monomer at the beginning of the study and friendship during our laboratory works.

My deepest thanks go to Onur Alp AKSAN for his constant support, endless encouragement, patience, and valuable love through the times I was confused and stressed. Moreover, I would like to thank him for his endless help with XPS and UV-Vis spectroscopy measurements.

I would like to express my endless thanks to all my friends who have been with me and their support during this journey.

Finally, I would like to express my endless gratitude to my dear mother Nesrin DEMİRBİLEK, and my dear father Murat DEMİRBİLEK who always being there for me throughout my educational journey, for their unwavering support in every decision I made and their endless love.

This research has been supported by the Scientific and Technological Research Council of Turkey (TUBITAK) 2210-C Graduate Scholarship Program and GTU BAP (Project No. 2022-A-101-08). I would like to thank TUBITAK for their financial support.

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LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviations and Acronyms		**Explanations**
°C	:	Celsius
3D	:	Three Dimension
µL	:	Microliter
cm^2	:	Centimeter square
g	:	Gram
h	:	Hour
mg	:	Miligram
mmol	:	Milimol
pH	:	Power Of Hydrogen
ACBR	:	5-Hydroxy-2-nitrobenzaldehyde
ACVA	:	4-4'Azobis (4-cyanovaleric acid)
AlBN	:	Azobisisobutyronitrile
AHMA		6-Azidohecyl methacrylate
ATRP	:	Atom transfer radical polymerization
BA	:	4-butylaniline
BSA		Bovine serum albumin
BPO	:	dibenzoyl peroxide
DCM	:	Dichloromethane
DEGMA	:	Di(ethylene glycol) methyl ether methacrylate
DMAPs	:	2-(Methacryloyloxy)ethyl]dimethyl-(3 sulfopropyl)ammonium hydroxide
DMF	:	Dimethylformamide
DMSO	:	Dimethyl sulfoxide
DNA	:	Deoxyribonucleic acid
FTIR	:	Fourier-transform infrared spectroscopy
FuMaMA	:	Furan-protected maleimide-containing methacrylate
GMA	:	Glycidyl methacrylate
H_2O	:	Water

HDMS	:	Hexadecyltrimethoxysilane
ICM	:	2-isocyanatoethyl methacrylate
IPN	:	Interpenetrating networks
$NaBH_3CN$	:	Sodium cyanoborohydride
NaOH	:	Sodium hydroxide
NBAA		Novel Ortho Nitrobenzyl Aldehyde Monomer
NHS	:	N-Hydroxysuccinimide
NMR	:	Nuclear magnetic resonance
NO_2	:	Nitrogen dioxide
OHMA		6-oxohexyl methacrylate
o-NB	:	ortonitrobenzyl
PAA	:	Polyacrylic acid
PAOI	:	Poly(2 (Acryloyloxy)Ethylisocyanate)
PC	:	Polycarbonate
PEG	:	Poly(Ethylene) Glycol
PEGMEMA	:	Poly(Ethylene Glycol) Methyl Ether Methacrylate
PETA	:	Pentaerythritol Tetraacrylate
PETMP	:	Pentaerythritol Tetrakis(3-Mercaptopropionate)
PVA	:	Polyvinyl Alcohol
PVC	:	Polyvinyl Chloride
RAFT	:	Reversible Addition Fragmentation Chain-Transfer
RAFT-CTA	:	4-Cyano-4-[[(dodecylthio)carbonothioyl]thio]pentanoic acid
RNA	:	Ribonucleic Acid
SEM	:	Scanning electron Microscope
TEA	:	Triethylamine
TFBA	:	4-(Trifluoromethyl)benzylamine
THF	:	tetrahydrofuran
TMSMA	:	3-(Trimethoxysilyl)propyl methacrylate
TMST	:	3-(Trimethoxysilyl)propyl methacrylate
UV	:	Ultraviolet
XPS	:	X-ray photoelectron spectroscopy

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1. INTRODUCTION

1.1. Polymers

Polymers are large molecules which are made of several monomers connected by covalent bonds. Basically, the structural formation of polymers is composed of identical and many repeating units. These numerous monomer structures are transformed into polymer molecules by forming chemical bonds with each other as a result of the polymerization reaction under appropriate conditions. They can have a variety of molecular structures, physical, and chemical characteristics, and molecular weights. Polymers can be classified in a number of ways based on their characteristics such as obtained source, structure of chain, polymerization type, repeating units, and types of molecular forces [1][2] (Figure 1.1).

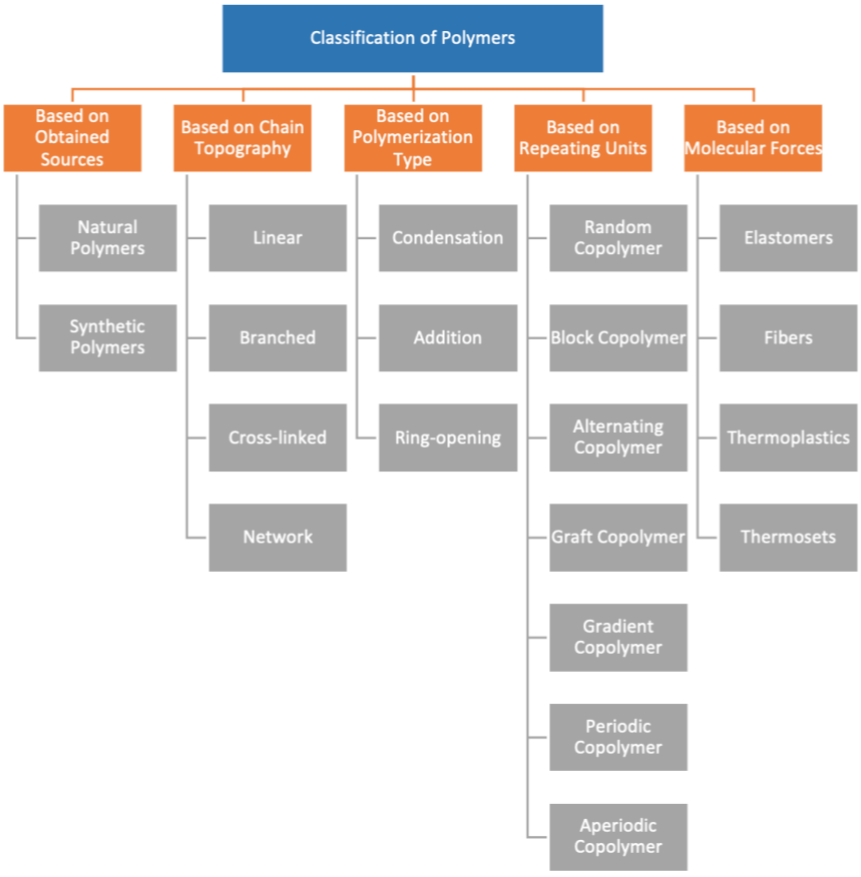

Figure 1.1: Polymer classification based on different characteristics.

1.1.1. Classification of Polymers Based on Obtained Sources

Generally, polymers can be divided into two categories which are natural and synthetic based on the occurrence or source. Polymers derived from animals and plants are called natural polymers. Some of the examples of natural polymers are cellulose and starch which are examples of polysaccharides, proteins, nucleic acids such as DNA or RNA, silk or etc. Natural polymers can be found everywhere in our daily life. Synthetic polymers are as diverse as natural polymers. These types of polymers are produced in laboratory and called as man-made polymers. PVC, polystyrene, elastics, synthetic rubbers are some of the examples of synthetic polymers.

1.1.2. Classification of Polymers Based on Chain Topography

In terms of molecular structure, the physical characteristics of polymers not only depend on their molecular weight and shape, but also on the structure of their molecular chains. Polymers are classified as linear, branched, crosslinked, or network based on their structure of chain. Linear chains do not have any branching and they have very basic structure. In this type of polymer, end-to-end addition of units is repeated to build a linear chain. There exist strong hydrogen and van der Waals bonds between these chains. In branched types of polymers, they consist of a primary chain with at least one branch in an irregular manner. As compared to linear polymers, branched polymers are weaker. Low-density polyethene is the best example for branched polymers. Linear chains are covalently bonded to each other in different directions to create a three-dimensional network structure in cross-linked type of polymers. These polymers tend to swell in water and are insoluble in solvents. The most common example of cross-linked polymers is resins. Complex polymers that are tightly connected to create a complex three-dimensional network are known as networked polymers.

1.1.3. Classification of Polymers Based on Polymerization Type

Polymers are formed by polymerization reactions. There are two common polymerization reactions to obtain polymers, condensation reaction and addition reaction [2]. Initiation, propagation, and termination are the three processes that often constitute polymerization. In condensation polymerization, molecules containing two or more functional groups react to form larger molecules. They are generated by a series of reactions resulting in long chain. Polymerization begins with two molecules reacting to produce a dimer. The resulting dimers can react with another dimer to form a tetramer or react with a monomer to generate a trimer. In this polymerization type, small molecules such as water or ammonia can be eliminated during the reaction. The incorporation of monomers into continuously growing polymer chains is known as addition polymerization. In addition, polymerization, the monomers combine directly to create the macromolecule chain without elimination of small molecules such as water. Alkenes, aldehydes, acetylenes, or other similar compounds with unsaturated bonds are polymerized by addition polymerization. Polymerization of monomer structures such as propylene and styrene, which mostly contain unsaturated bonds, can be given as an example. In addition, cyclic ethers, acetals, esters, amides, and siloxanes are polymerized by ring-opening polymerization.

1.1.3.1. Free Radical Polymerization

Free radical polymerization is the process of creating a polymer unit by sequentially adding electrically neutral free radicals. Free radical polymerization is a kind of chain-growth polymerization and contains three essential steps which are initiation, propagation, and termination. Polymerization starts on radicals, and chain growth continues with radicals. One or two radicals are generated from the initial molecule during the first stage of polymerization, called initiation [3]. A generated radical initiator assaults a monomer during the propagation step. The chain might develop as hundreds or even thousands of monomers join. Polymerization stops with the termination step. Extremely active radicals are undergoing a number of reactions that reduce their activity and thus stop the polymerization process. There are different ways to stop polymerization process. One of them occurs when the two active chains

interact with each other. Another one occurs, when an actively growing chain reacts with the initiator radical [4]. Moreover, termination may occur as a result of reaction with impurities.

1.1.3.2. Reversible Addition − Fragmentation Chain-Transfer (RAFT) Polymerization

Reversible addition-fragmentation chain-transfer polymerization is also known as a type of living or controlled chain growth polymerization because it closely resembles the characteristics of living polymerization while benefitting from the adaptability of a radical process [5]. It is unique and widely used approach for regulating the molecular weight and molecular weight distribution of a free radical polymerization. This approach has various applications that extend from materials science to biology. The mechanism starts with activation of radical source. In order to achieve a balance between active and dormant species, the radical species add to the RAFT agent. Basically, the whole process consist of introducing monomers between an R- and Z-C(=S)S-groups which are belonging from RAFT agent that are constitute the α and ω end-groups of most of the resultant polymeric chains [6] (Figure1.2). This method enables the creation of polymers with foreseeable functionality as well as polymers with an established polymer architecture. In addition, free radical initiators such as azobisisobutyronitrile (AlBN) or dibenzoyl peroxide (BPO) are often employed for initiating free-radical chain growth with continuous thermal breakdown [7].

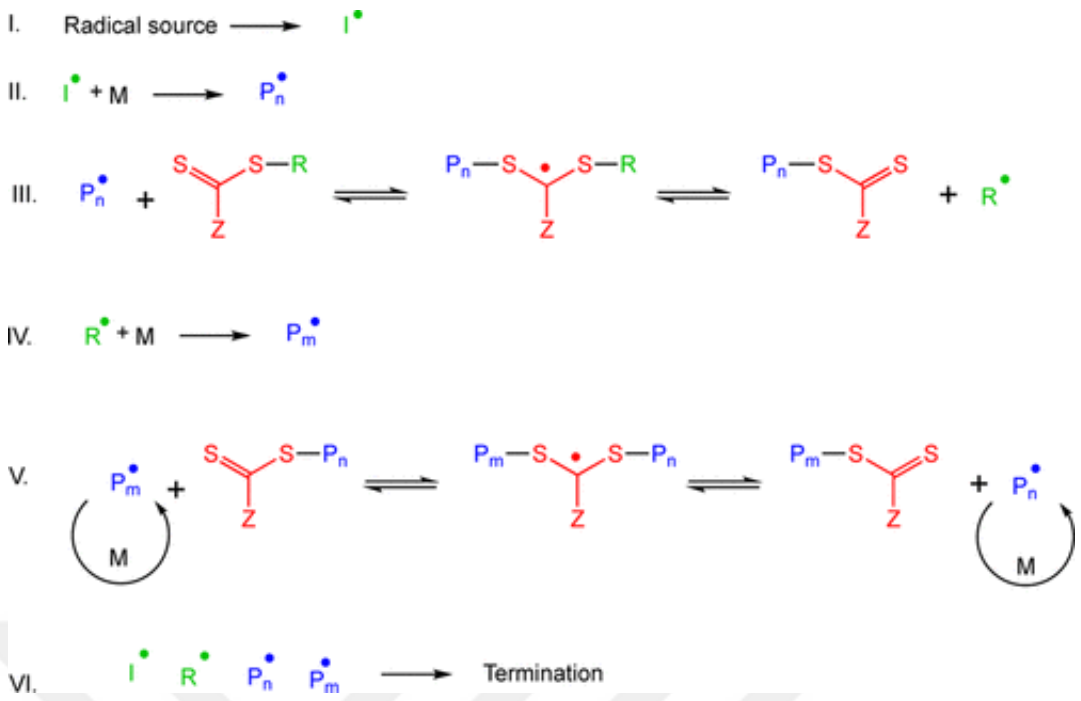

Figure 1.2: Reaction mechanism of RAFT polymerization.

1.1.4. Classification of Polymers Based on Repeating Units

Some polymers only contain one kind of repeating unit, whereas others have several distinct kinds. There are two main types of polymers based on their repeating units, that are homopolymers and copolymers. The polymer made up repeating of a single kind of monomer unit is called as homopolymer. Copolymers are defined as polymers with two distinct kinds of monomers in the chain structure. Copolymers may generally be classified as random copolymers, block copolymers, graft copolymers, alternating copolymers, and periodic copolymers due to various configurations of comonomers (Figure 1.3) [8]. Moreover, gradient copolymers and aperiodic copolymers are two new forms of copolymers that have been discovered in recent years.

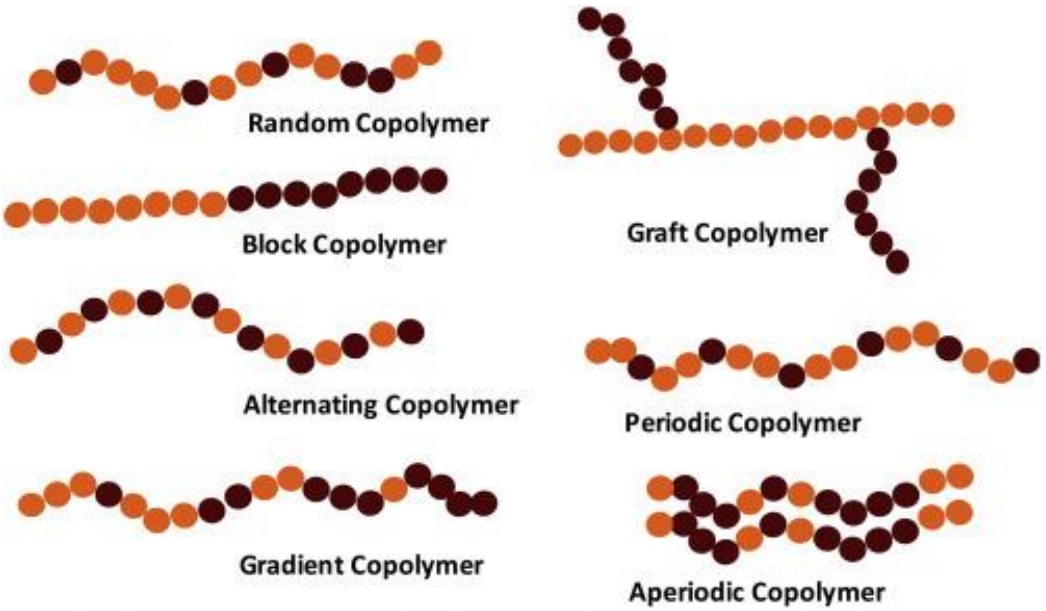

Figure 1.3. Various types of copolymers.

Random copolymers also called as statistical copolymers which are composed of a random assembly of monomer units. Block copolymers consist of long segments, referred as blocks, made up of various homopolymers. Monomer units are arranged in a certain order to form alternating copolymers. Graft copolymers are formed by connecting two polymer chains with different chemical structures from one place to another repeating unit as branches. Periodic copolymers are made up monomer units arranged in a repeating pattern. Gradient and aperiodic copolymers have been defined in the literature recently. Monomer contents gradually varying along the chain in type of gradient copolymers. In aperiodic copolymers, the distribution of the monomer sequence is irregular, however maintains the same arrangement throughout all chains [9]. Aperiodicity is not the same as randomness and this creates a difference between random copolymers and aperiodic copolymers. The same monomer sequence should be maintained throughout all chains of an aperiodic copolymer.

1.1.5. Classification of Polymers Based on Types of Molecular Forces

In accordance with the characteristics of the polymerization process or the type of mechanism, all polymers can be divided into four main groups which are elastomers, fibers, thermoplastic, and thermosets polymers [10]. Thermoplastics are most used types of polymers in daily life. Their structure is mostly made up of linear

chains. They have a low strength and can be repeatedly heated or cooled to change their form. Their chemical composition and bond formation are not change during heating or cooling processes and these polymeric materials regain their characteristics. The most popular examples of thermoplastic polymers are teflon, polyethylene, and polyvinyl chloride [10]. In contrast to thermoplastic polymers, thermoset polymers can not melted and reshaped due to their crosslinked and irreversible characteristics. These types of polymers become rigid during the heating process. In addition, chain and bond structures may fracture and degrade at high temperatures. As their name indicates, elastomers are polymers which have high elasticity that can regain their initial shape after stretching. This flexibility is caused by weak intermolecular interactions, primarily Van der Waals forces, between polymer chains. Fibers have dipole-dipole attraction which is strong intermolecular attraction forces between their highly ordered chains. They have restricted flexibility and have the greatest tensile strength between other polymers.

1.2. Functional and Reactive Polymers

Functional polymers have wide range applications in various field such as biomedical engineering, biotechnology, medicine, or material science due to their controllable and functionalizable characteristics. Numerous applications are made possible through the addition of functionality to drug molecules, imaging agents, or biomolecules. The characteristics of this class of materials are primarily determined by the existence of chemical functional groups which are different from the backbone chains. Polymers containing aromatic groups (such as polystyrenes), unsaturation, and heteroatoms, whether integrated into the backbone or attached as pendant groups (such as poly(acrylate)s, poly(methacrylate)s), have been shown in the literature to be valuable components for the synthesis of functional polymers [11].

1.2.1. Post-Polymerization Modification

The post-polymerization is mostly utilized synthesis method for obtaining functional and reactive polymers. Post-polymerization is relied on direct polymerization or copolymerization of monomers and enables inclusion of functionality which is incompatible with the process of polymerization. However, they can be quantitatively transformed into a wide range of different functional groups in a following phase. In represented schema explains general process of generating functional polymers through post-polymerization modification (Figure 1.4) [12]. Since monomer has chemoselective feature, polymerization occurs in a controlled manner. In this way, polymer can be functionalized with a wide range of different types of functional groups in the following step. Polymers can be modified various ways such as atom transfer radical addition, Michael-type addition or reaction with aldehydes and ketones. Post-polymerization gives opportunity to design different kinds of functional polymers which would not be possible with direct polymerization. A diversified library of functional polymers with the same average chain lengths and chain length distributions can be potentially produced from a single reactive polymer precursor with post-polymerization.

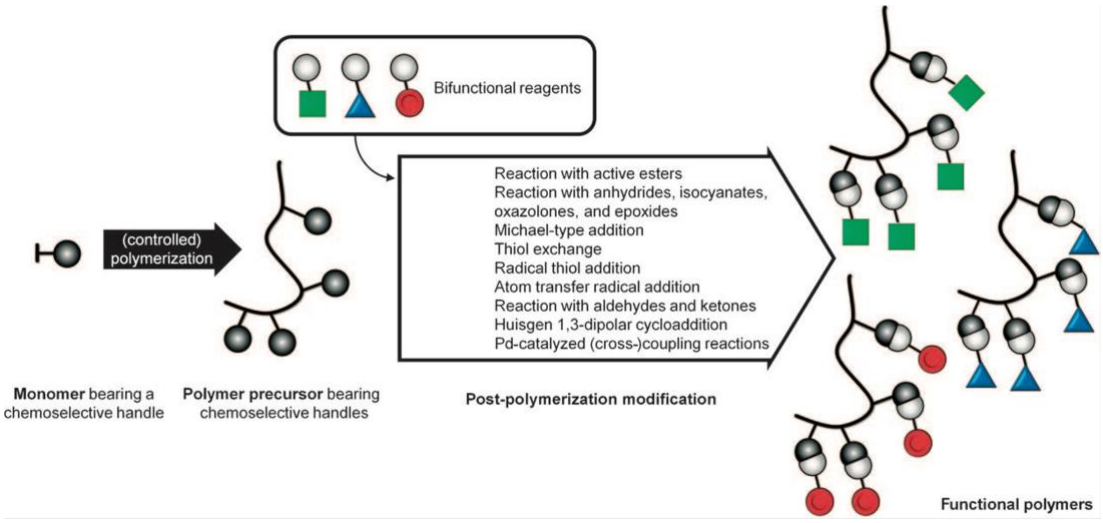

Figure 1.4: Obtaining functional polymers via post-polymerization modification.

Some of the important advantages for post-polymerization reactions are obtaining high yield products, easy purification processes, short reaction times and

basic reaction conditions [13]. Furthermore, the post-polymerization modification avoids away from monomers that are not stable during polymerization or difficult to manufacture in large enough amounts. There are several reactions to obtain this type of modificated polymers including Michael additions, radical thiol addition, Pd-catalyzed (cross-)coupling reactions, and epoxide ring opening reactions and these reactions can be classified as click-reactions. The epoxide ring opening reactions employ with commonly obtainable monomer (glycidyl methacrylate, GMA) that is easily adaptable to reversible deactivation radical polymerization methods such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer. In addition, investigations on GMA polymers and their post-modifications with various amines such as propylamine, methylpropylamine, and butylmethylamine have been reported [14].

1.3. Reactive Groups for Post-Modification Polymerization with Amines

The polymer chain must include reactive groups which can be easily and effectively functionalized with molecules containing complementary reactive groups, in order to carry out the post-polymerization modification [15]. The great relevance of amine and thiol groups for bio-applications has led to substantial research into polymers reactive towards these functional groups. Polymers that have functional groups like carbonates or activated esters like N-hydroxysuccinimide (NHS) or pentafluorophenyl can give reaction with amine-containing compounds. Amines can typically react with active esters such as NHS, enabling selectivity without the requirement for protective groups due to their superior nucleophilicity compared to other functional groups (for example, alcohols) [12]. On the other hand, thiol-containing compounds react with polymers which include maleimide, pentafluoro styrene, and thiosulfonate groups. Additionally, maleimide is one of the most often employed functional groups for biomolecular immobilization studies due to its strong reactivity towards thiol-containing compounds under mild conditions [16]. In addition, isocyanates and epoxide groups are both reactive to amines and thiols. Isocyanate reactions with active hydrogen including groups like amines or alcohols are effective and precise under specific reaction conditions and they react quickly and often

selectively with both amines and thiols [17]. The polymerization of monomers with activated ester, disulfide, acetal, and epoxy groups also employed with the RAFT method. For instance, Hawker et al. established a synthetic method to create linear copolymers with pendant cross-linkable isocyanate functionalities utilizing commercially available monomers for efficient crosslinking [18]. They obtained intramolecularly cross-linked nanoparticles through reaction of linear copolymers with diamines in dilute solution.

Especially, since isocyanates react with various functional groups including hydroxyl, amine, and thiol, the production of polymeric materials incorporating isocyanate groups has been well explored. The conjugation of a wide range of functional compounds into complex macromolecular structures has been demonstrated to be very efficient through isocyanate-based click reactions [17]. For instance, Flores et al. developed RAFT homopolymerization of unprotected isocyanate-containing monomer which is 2-(acryloyloxy)ethyl isocyanate [17]. They also established the post-polymerization modification of poly(2 (acryloyloxy)ethyl isocyanate) (PAOI) homopolymers with model amines, alcohols, and thiols.

In other example, Tarakci et al. developed functionalizable reactive hydrogels which were containing isocyanate functional groups [19]. These hydrogels were synthesized by using 2-isocyanatoethyl methacrylate (ICEMA) and poly(ethylene glycol) methyl ether methacrylate (PEGMEMA) with a crosslinker that was pentaerythritol tetraacrylate (PETA). The reactive isocyanate groups were functionalized with several amine or thiol containing molecules such as 3-amino-1-propanol, 4-(trifluoromethyl) benzylamine and 4-fluoro-benzyl mercaptan. Moreover, they were investigated that the hydrogel coatings could potentially be used to biofunctionalization studies by using bioactive ligands.

Hensarling et al. developed functional and micropatterned polymer brush surfaces by using thiol-isocyanate click reactions [20]. According to the Figure 1.5, which is schematic representation of their work, silicon surfaces were functionalized using a chlorosilane derivative, followed by photopolymerization of 2-isocyanatoethyl methacrylate and a photoinitiator at wavelength of 365 nm and created NCO reactive groups. After that, obtained isocyanate-functionalized polymer brushes were functionalized with thiols in the presence of a catalyst under laboratory conditions. Moreover, these brushes were functionalized with amines without the need of catalyst

in a very short time, 12 minutes. In this way, they developed a multifunctional and patterned polymer brush surfaces via thiol-isocyanate click reactions.

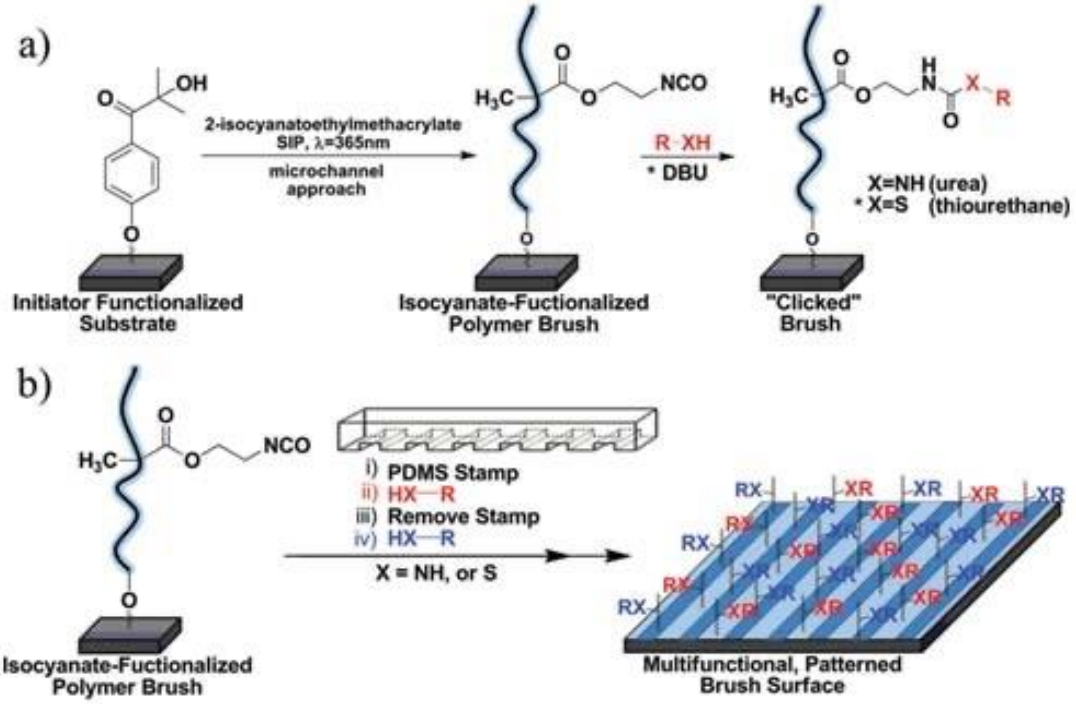

Figure 1.5: a) Surface-initiated photopolymerization of 2-isocyanatoethyl methacrylate followed by X-isocyanate functionalization (X can be thiol or amine). b) Successive X-isocyanate reactions are illustrated schematically as a way of patterning NCO-containing polymer brush surfaces.

Furthermore, epoxide groups are other groups used for functionalization of amines, alcohols, or thiols, as mentioned before. For instance, Saha et al. employed an amine-epoxy click reaction between an alkyl amine and diglycidyl ether to demonstrate polymerization in just one step (Figure 1.6) [21]. In this way, a novel class of main-chain cationic polymers has been discovered using one-step polymerization with an amine-epoxy click reaction in acceptable conditions from commercially available and inexpensive small molecular building blocks.

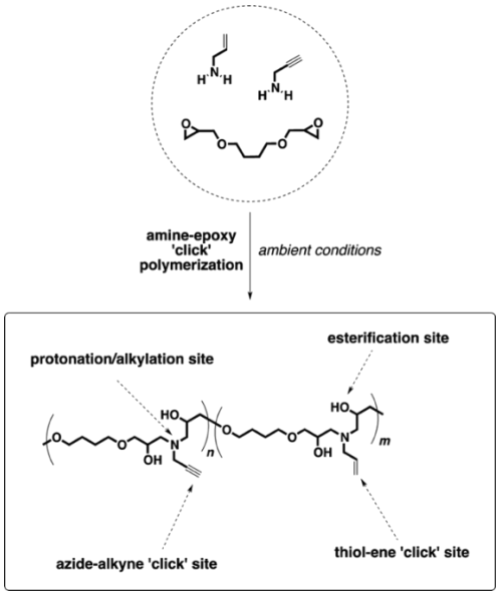

Figure 1.6: One step polymerization with amine-epoxy click reaction in suitable conditions to obtain multiple functionalized presented novel class of main-chain cationic polymers.

In another example from literature, Cengiz et al. demonstrated that a new route for developing reactive and functionalized hydrogels through thiol-epoxy coupling chemistry [22]. In this study, they used thiol-epoxy chemistry to create a hydrophilic network for hydrogels using commercially available precursors which were pentaerythritol tetrakis(3-mercaptopropionate) (PETMP), and diglycidyl ether terminated poly(ethylene glycol) (PEG). The expected functionalized hydrogel has been produced by converting hydroxyl units into ester functionalities.

Furthermore, Fantoni et al. showed another approach for epoxide-alcohol chemistry [23]. In their study, they demonstrated that the polymerization of bio-based and sustainable epoxy monomers with alcohols using a catalyst which was imidazole. The successful polymerization was followed by ^{1}H-NMR analysis. In this way, they were able to produce controlled changing copolymerization between alcohol and difunctional epoxy monomers.

1.4. Orthogonal Functionalization

The term of orthogonality was firstly introduced in the chemistry by Merrifield in 1977 [24]. His method is based on eliminating specific protecting groups in the solid-phase synthesis of peptide oligomers attached to polymer resins by altering

reaction conditions. Systems having independent chemical reactivity and their selective production within a single pot referred to orthogonality [25]. Orthogonality provides full selectivity in chemical reactions that prevents cross-reactivity and allows for reaction sequences to be carried out in any order while still producing the same outcome. These kinds of chemical processes made it simple to produce complicated compounds like functionalized copolymers and polymers. A great deal of study regarding modifying polymeric materials using reactive functional groups for different purposes have been published in the literature. Many orthogonally functionalizable polymers uses reactive groups which undergo conjugation with molecules having various reactive group [15]. In the literature, there are not many studies regarding sequential modifications of polymers utilizing different molecules carrying the same group. In an example from literature, Cengiz et al. produced an orthogonally functionalizable amine reactive copolymer employing an azide-containing monomer and an activated ester monomer including N-hydroxy succinimide [26]. In their study, firstly, methacrylate monomer including N-hydroxy succinimide carbonate was synthesized. After that, copolymerization with other monomers, such as PEG methacrylates, allowed for the integration of this reactive monomer as side chains. Orthogonal functionalization was achieved by copolymerization of carbonate monomer with an azid monomer and NHS methacrylate monomer. The first functionalization was achieved from pendant reactive groups by using amines to obtain carbamates. The second functionalization was done by 1,3-dipolar cycloaddition and amidation. Moreover, this study has potential to use in drug delivery studies.

In other example from literature, Sanyal et al. fabricated and functionalized micro-patterned hydrogels through orthogonal thiol-ene click reactions (Figure 1.7) [27]. First, they synthesized orthogonally functionalizable thiol reactive copolymer by using furan-protected maleimide-containing methacrylate (FuMaMA) and PEG-based monomer with atom transfer radical polymerization (ATRP) in the presence of PEG-based macroinitiator. After that, obtained copolymer was heated in toluene at 100°C and it caused to partial activation of a part of the maleimide groups and produces an orthogonally functionalizable copolymer through the retro Diels-Alder reaction. They could potentially be sequentially functionalized with two distinct thiol-containing compounds using the Michael type thiol-ene and radical thiol-ene conjugation. This novel method essentially employs a single parent polymer which may be thermally

altered to produce two orthogonally reactive alkene functionalities. They also showed that the orthogonal functionality of polymers through the production of patterned hydrogels.

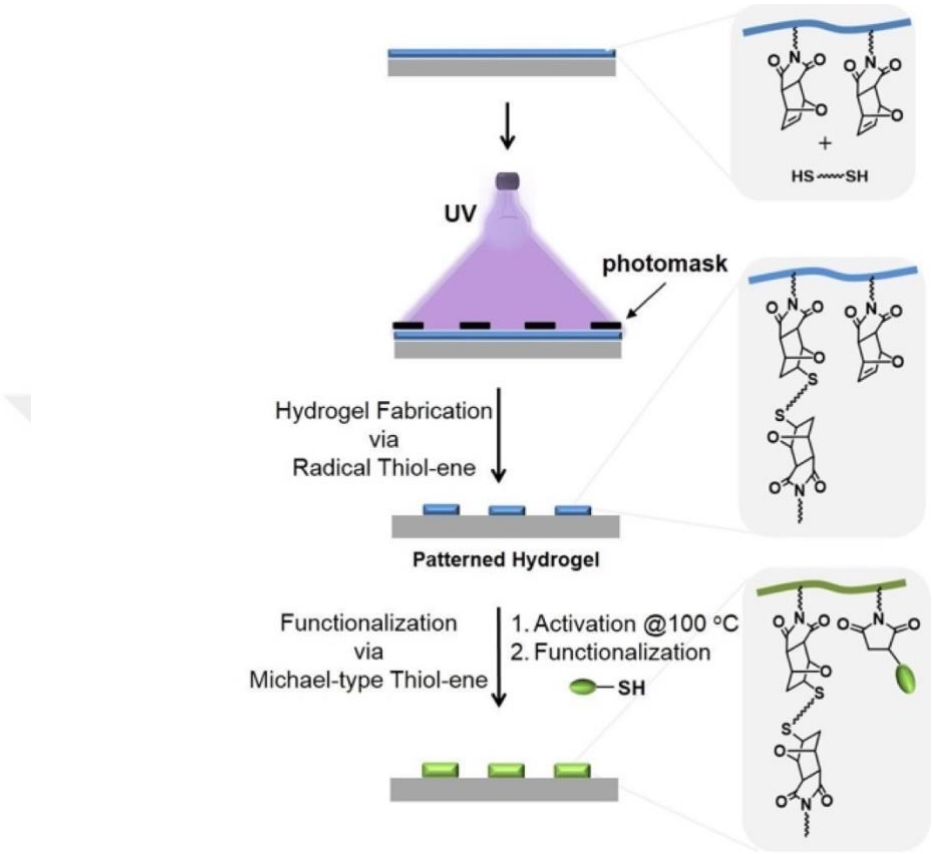

Figure 1.7: General representation of developed micro-patterned hydrogels via orthogonal Thiol-ene click reactions.

Uysal et al. established that the orthogonal multifunctionality of aliphatic polycarbonate (PC) through two distinct reactions which are thiol-ene click reactions and Michael addition [28] (Figure 1.8). In their approach, firstly, they synthesized PC polymer using ring opening reactions. After that, pendant acrylate and ally groups of synthesized PC scaffold were orthogonally functionalized via Michael addition and photo-induced radical thiol-ene click reactions with using several thiol compounds such as thiophenol, cysteine and 1-octanethiol. In this way, they obtained biocompatible and non-toxic orthogonally functional PC copolymers with using two distinct reactions. Furthermore, their developed copolymer has potential to use in biomedical field.

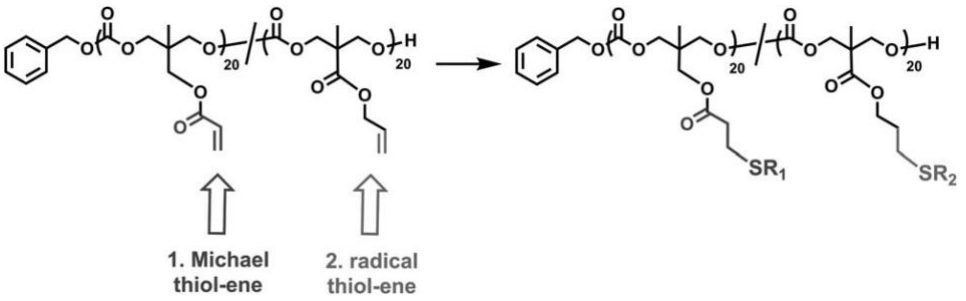

Figure 1.8: PC copolymer were orthogonally multifunctionalized via Michael addition and photo-induced radical thiol-ene reactions.

Different types of functional polymers can be obtained via orthogonal chemistry. For instance, Hiltebrandt et al. developed a light induced star shaped orthogonal polymers [29]. Their approach includes a bifunctional oligomer carrying two different photoactive moieties which are tetrazole and benzaldehyde. When this oligomer was exposed to UV radiation with wavelengths ranging from 260 to 320 nm, the tetrazole moiety was transformed into a nitrile imine reactive intermediate. The benzaldehyde group was deactivated through imine formation with hexylamine under UV radiation to demonstrate orthogonality. As a result, the developed system promotes the possibility of an orthogonal reaction sequence depending on the wavelength. Based on this research, it has been proven that it is possible to synthesize complicated macromolecular systems.

Orthogonal click reactions provide a variable route to produce a wide range of polymer structures. Complex macromolecular systems, multifunctional materials and novel polymer structures can be produced through orthogonal chemistry [25]. The development of novel generation materials is made possible by orthogonal systems that result from the integration of many polymerization processes into a single system.

1.5. Hydrogels

Hydrogels are cross-linked, three-dimensional (3D), hydrophilic networked polymers which are insoluble in water. Their hydrophilic nature allows them to hold significant amount of water [30]. Hydrophilic functional groups such as OH, NH_2 or COOH in the structure of hydrogels enable them to absorb water, whereas cross-links between the network chains prevent the hydrogel from breaking [31]. Their network structure keeps stable in the swollen phase because of the crosslinking property.

Hydrogels have potential to use in various biomedical applications such as tissue engineering, drug delivery, contact lenses or wound dressing due to their similarities to living tissues, soft, flexible, and porous structures, biodegradability, biocompatibility, and high oxygen permeability potential [32]. Additionally, hydrogels which respond to external triggers which include pH, temperature, electrical, magnetic, or light are referred as smart hydrogels. [33]. Smart hydrogels enable precise manipulation of fundamental material characteristics including porosity, swelling ability, physical structure, and elasticity. As a result, several applications ranging from medical to industrial have become available through the usage of these types of hydrogels. Moreover, there are several types of hydrogels, which can be classified in different ways based on the source of the polymer, preparation method, crosslinking technique, physical structure, or electric charge. [34] (Figure 1.9). In addition to this classification, numerous other hydrogel types have been reported in the literature. Some examples include stimuli responsive hydrogels such as temperature sensitive, light sensitive, pH sensitive, and biodegradable hydrogels or smart hydrogels.

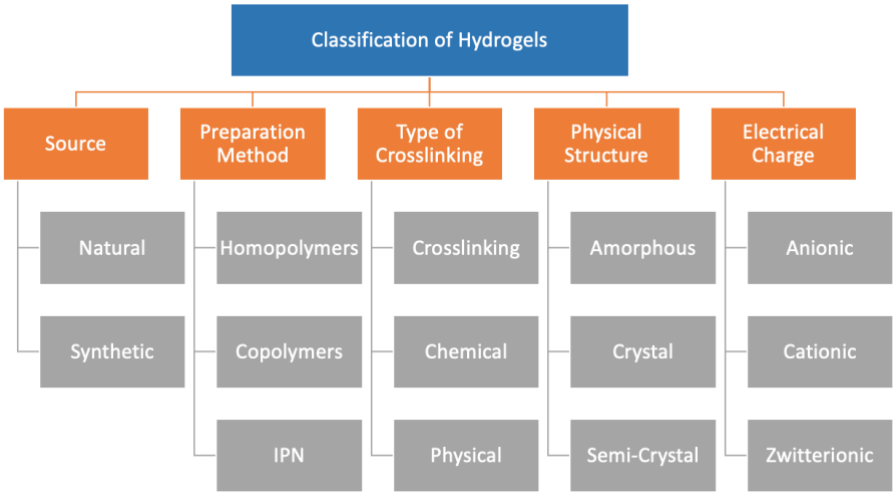

Figure 1.9: Hydrogel classification schema.

1.5.1. Classification of Hydrogels Based on Source

Basically, there are two types of hydrogels which are natural and synthetic based on their obtained source. Natural hydrogels are obtained from natural sources such as alginate, collagen, gelatin or fibrins and they have excellent biocompatible and

biodegradable properties [35]. This non-toxic characteristic makes them suitable materials for bio-applications. However, natural hydrogels have weak mechanical properties due to their composition. Synthetic hydrogels have better mechanical properties when compared to natural hydrogels. They can be constructed to have proper structure to impart biological activities to the hydrogel, such as biodegradability. Most frequently used synthetic polymers to manufacture synthetic hydrogels are polyethylene glycol (PEG), polyacrylic acid (PAA) or polyvinyl alcohol (PVA).

1.5.2. Classification of Hydrogels Based on Preparation Method

Hydrogels can be classified as homopolymers, copolymers and interpenetrating networks (IPN) according to their preparation method. Homopolymers can be defined as basic hydrogels that can be obtained through the crosslinking of a single kind of hydrophilic monomer [36]. Copolymeric hydrogels are composed of at least two distinct monomers which are arranged in block or alternating configuration. Copolymers are divided as random, alternate, block, and graft copolymers depending on the manner in which two distinct monomers are ordered. Two different cross-linked synthetic or natural polymer constituents create interpenetrating networks (IPN). One of the advantages of IPN hydrogels is that they allow more efficient drug loading in drug delivery studies.

1.5.3. Classification of Hydrogels Based on Type of Crosslinking

Generally, two main types of crosslinking which are physical and chemical for obtaining 3D structure of hydrogels. Physical networks occur through physical interactions such as Van der Waals interactions, ionic interactions, hydrogen bonds, or hydrophilic interactions. Polymer chains in chemically crosslinked networks are attached to each other by covalent bonds, which form permanent crosslinks, in contrast to temporary crosslinks in physically crosslinked networks [37]. Furthermore, physical interaction and chemical cross-linking are most common methods of synthesis of hydrogels. Physical hydrogels are generated by ionic, H-bonding, or supramolecular association. Due to their weak bonding, these hydrogels are also known as reversible

gels [38]. In contrast, to create a durable, non-reversible and permanent hydrogel, the chemical cross-linking approach employs covalent bond interaction between polymer chains.

1.5.4. Classification of Hydrogels Based on Physical Structure

According to the classification of hydrogels based on their physical structure, amorphous, crystals, and semi-crystals are main three types of hydrogels. Amorphous hydrogels have macromolecule chains that are arranged in a random order and mostly in groups [39]. In addition, amorphous hydrogels have a glassy and transparent structure. In crystalline configuration, the chains are assembled in an ordered manner, in contrast to the amorphous structure. These types of hydrogels are form like rigid. The semi-crystalline structure, which is a complex mixture of amorphous and crystalline structures, is made up of irregular amorphous blocks as well as regular and dense crystal blocks.

1.5.5. Classification of Hydrogels Based on Electrical Charge

Hydrogels are classified into three categories which are anionic, cationic, and zwitterionic depend on the ionic charge in the polymer chain. Anionic hydrogels formed by the combination of ionic charged monomers. The structure of anionic hydrogels is mostly composed of negative fixed ions. Cationic hydrogels contain mostly positive fixed ions in their structure when compared to anionic hydrogels (Figure 1.10) [40].

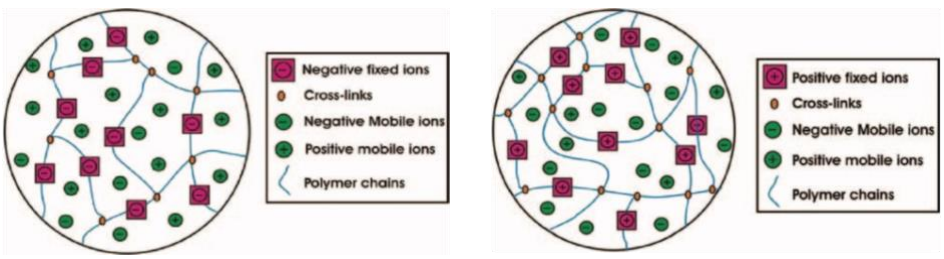

Figure 1.10**:** Schematic representation of structure of anionic and cationic hydrogel, respectively.

Zwitterionic hydrogels have both positive and negative charges and these types of hydrogels have excellent antifouling properties as well as hydrophilic groups in their structure. This makes them suitable materials for biomedical applications in different purposes.

1.5.6. Zwitterionic Hydrogels

Zwitterionic materials have both positively and negatively charged functional groups in their structure. The structural stability and tunable regulation capabilities of zwitterionic hydrogels are very remarkable. These types of materials have gained attraction due to their excellent antifouling characteristics [41]. Antifouling ability provides potential application for zwitterionic materials in biomedical field. Even though polyethylene glycol (PEG) has been known as one of the most popular antifouling materials due to its limited interaction with proteins, it has some limitations. For instance, PEG based materials are susceptible to oxidation when it interacts with biological medium and that leads to significant protein adsorption [42]. Also, short-term stability is another problem which causes efficiency degradation in avoiding non-specific protein adsorption. Consequently, zwitterionic group-containing monomers have been employed as an alternative to PEG in the synthesis of hydrogels.

1.5.7. Stimuli Responsive Hydrogels

Stimuli responsive hydrogels show structural or mechanical changes in response to environmental stimuli or triggers such as temperature, pH, magnetic or electric field or light [43]. Because of their smart and controlled properties, stimuli responsive hydrogels have become attractive in biomedical applications such as drug delivery, tissue engineering, and wound dressing. They can exhibit a switchable sol-gel transition when external signals are provided [44]. For instance, signals like light, temperature, magnetic or electric field can be considered as physical triggers. On the other hand, chemical or biological triggers or pH changes can be regarded as bio-chemical triggers and these kind of stimuli responsive hydrogels are often employed

for the development of bio-functional materials. Besides that, the most studied types of hydrogels are pH sensitive, temperature sensitive, electro-sensitive and light sensitive or photoresponsive hydrogels among the stimuli responsive hydrogels.

1.5.8. Light Sensitive (Photoresponsive) Hydrogels

Since light is non-invasive, effective, easy to operate, inexpensive, and widely available, light-responsive hydrogels are more appealing [45]. As a result of the application of the appropriate wavelength of light, this type of hydrogels produce a response and cause structural changes. Generally, the conversion of photoreactive functional groups which are incorporated to the polymer structure causes structural and conformational changes [43]. Basically, these types of hydrogels consist of a polymeric network and a photoreactive group. When a photoreactive group interacts with a light source, it catches the optical signal and turns into a chemical signal using a photo-reaction process such as cleavage, isomerization, or dimerization [46]. Photoreactive molecules can undergo reversible or irreversible reactions depending on ability of molecules to return their original structures [47]. As a result of this light exposure, it can cause changed hydrogel behaviors such as shrinking, partial disintegration, degradation, or crosslinking (Figure 1.11) [48]. Moreover, light sensitive hydrogels can also become functional under this light exposure.

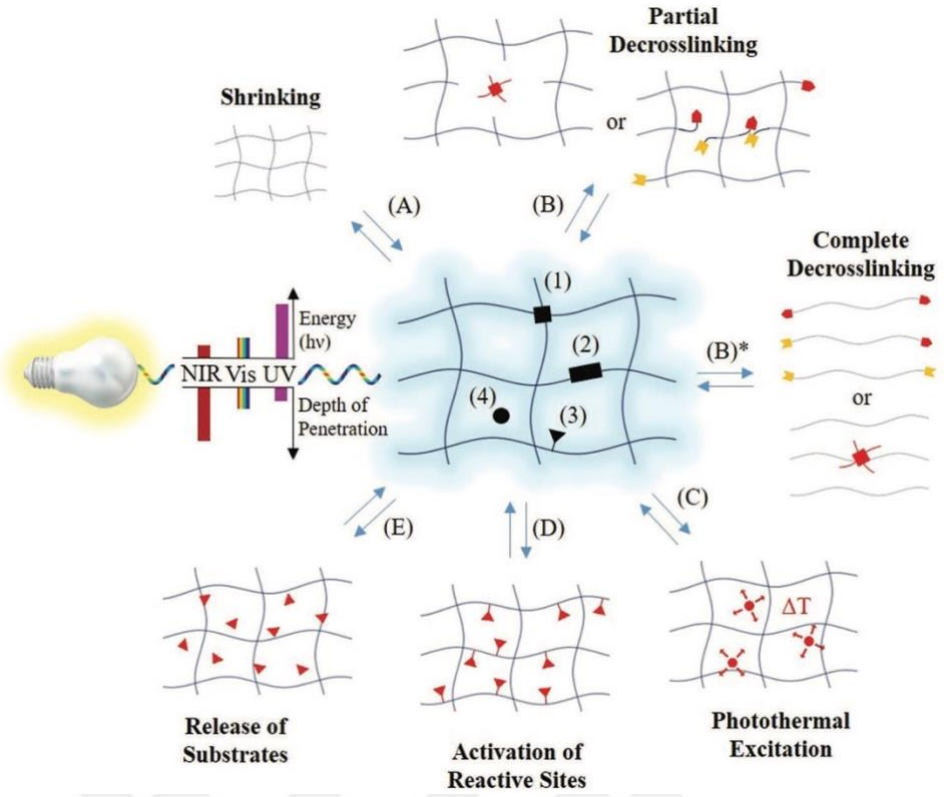

Figure 1.11: Various hydrogel behaviors as a result of light exposure (UV,Vis or NIR).

1.5.8.1. Preparation of Light Sensitive Hydrogels

Light sensitive hydrogels can be designed to expand, shrink, or aggregate when exposed to UV light or visible light. There are various approaches to obtain light responsive hydrogels. One of them is the incorporation of chromophores into the hydrogels (Figure 1.12A) [46]. In this mechanism, chromophores convert light energy into heat energy, which weakens hydrogen bonds and causes polymers to assemble into gels. In the other gelation mechanism, hydrogels become uncrosslinked (Figure 1.12B). During light irradiation, chromophore units embedded in the hydrogel matrix undergo isomerization, photocleavage, or photooxidation. As a consequence, it alters the chemical or physical characteristics of hydrogels. In third mechanism, hydrogel gained swelling feature due to external and internal osmotic pressure [Figure 1.12C]. The reason of that formed significant number of ions after light exposure.

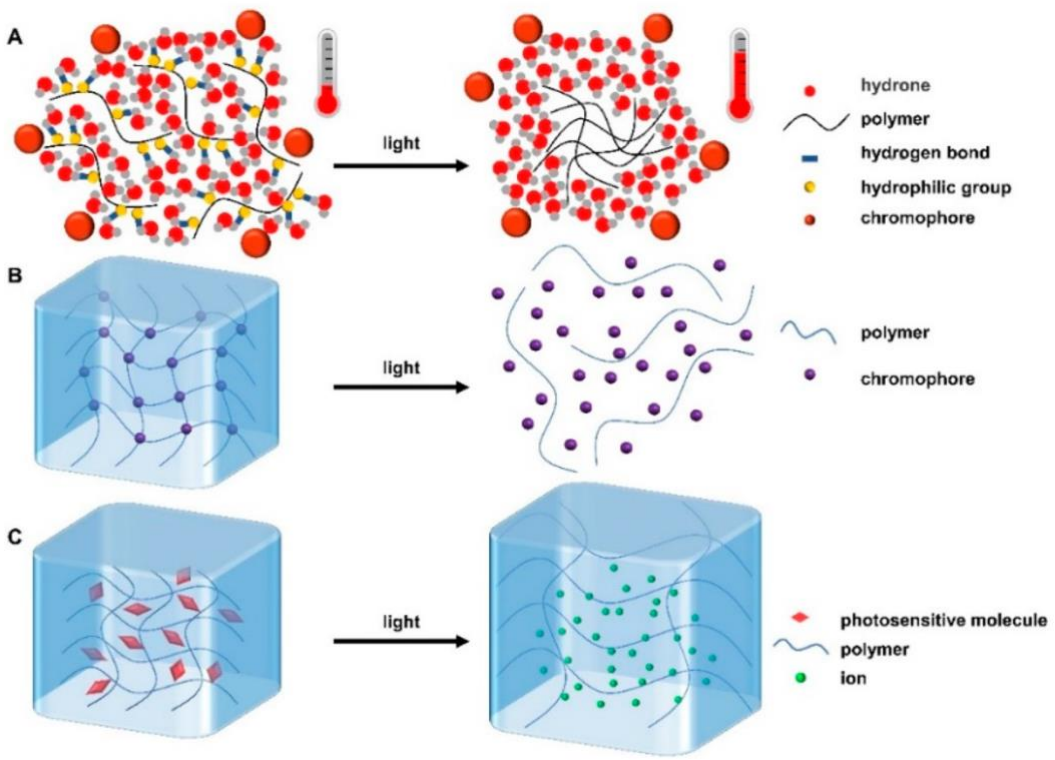

Figure 1.12: Representation of three different gelation of photoresponsive hydrogels.

Furthermore, the choice of the photoreactive group and the fundamental composition of the gels are crucial for obtaining photosensitive hydrogel system. Azobenzene, spiropyran, coumarin, diarylethene, triphenylmethane, and o-nitrobenzylester derivatives are some of the groups which are frequently employed in research on photosensitive materials (Figure 1.13) [49]. Among those photosensitive hydrogels, the ortonitrobenzyl (o-NB) ester is the most preferred photosensitive group due to its high sensitivity and appropriate UV wavelength which is 365 nm for biological applications. [47].

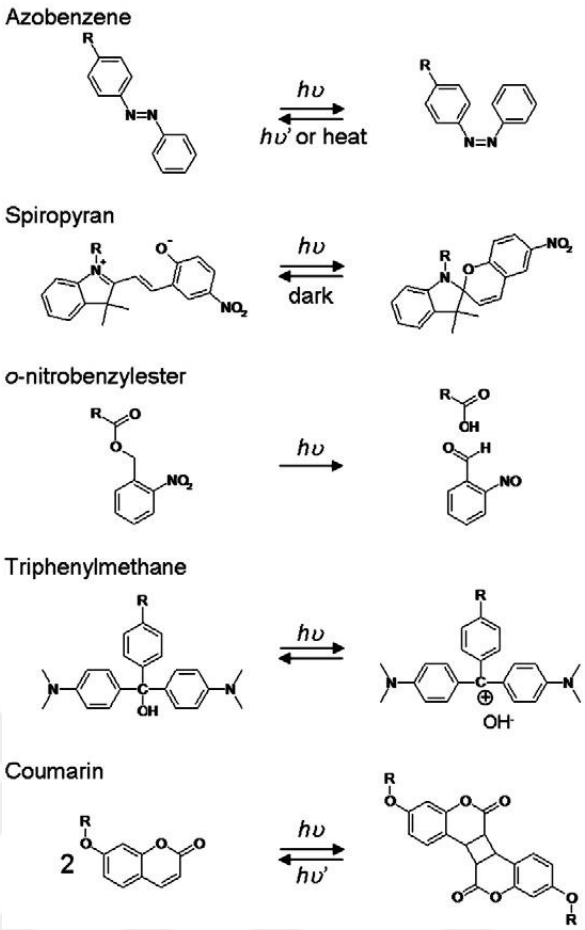

Figure 1.13: Most frequently utilized photosensitive groups for photoresponsive hydrogels and their reaction mechanisms.

1.6. Hydrogel Coatings and Applications

Hydrogel coatings have been attracting more attention in both biomedical field and non-medical applications due to their numerous possible application such as drug delivery, tissue engineering, antibacterial surfaces, biosensor, etc. [50]. In terms of chemical composition and network topologies, various hydrogels can be designed, which makes them desirable candidates for use as coating materials. They offer a three-dimensional (3D) soft material-based interface which may be changed by choosing an appropriate coating material and further improved by suitable functionalization [51].

The physical method is a simple way to modify the surface of hydrogel coatings with biological compounds. However, this approach may not be very suitable for long term applications due to its unstable nature [52]. In physical approaches, hydrogels are attached to the surface via non-covalent interactions. Thus, hydrogel coatings

containing these weak bonds are easily separated from the surface. Due to this concern, covalent bonding is utilized to attach hydrogels to surfaces, aiming to enhance their adhesion and longevity. This approach results in hydrogel coatings that exhibit notably improved durability and long-term stability. Within the existing literature, various techniques for hydrogel coating are documented, including surface bridge method, surface initiation method or hydrogel paint method [50]. Especially, surface initiation method is most known and widely used method for preparing hydrogel coatings. Polymeric materials are fabricated via free radical polymerization in this method. Basically, active radicals on the surface of the substrate start the polymerization process. Coating of hydrogels on surfaces could be achieved by generating active radicals on the surface of the substrate and adding grafting sites onto the surface [53].

For instance, Sanyal et al. developed multifunctional, transformable, and biocompatible clickable hydrogel coatings on a titanium surfaces [54]. In this study, titanium is modified by using dopamine methacrylate and the PEG based hydrogels containing furan protected maleimide groups synthesized by photopolymerization. Furan protected maleimide group was transformed into thiol-reactive maleimide groups via retro Diels–Alder reaction for functionalization by nucleophilic thiol-ene reactions and Diels-Alder reactions. The developed hydrogel surfaces were also functionalized with biotin-benzyl-tetrazine followed by TRITC-labelled ExtrAvidin, to show multifunctionality of hydrogels. This approach enables more diversified uses of hydrogel coatings by mixing numerous antibacterial agents, drugs, and biomolecules to create surface functionalization via various click reactions.

In another example from literature, Leng et al. produced enzymatically degradable hydrogel coatings on the nanoflower-like structure of ZnO structure of titanium [55]. They prepared nanoflower structure of ZnO on the titanium surfaces through hydrothermal method to achieve hydrogel coatings. Thereafter, they synthesized hybrid hydrogel which containing gelatin methacrylate and hyaluronic acid methacrylate. Hyaluronic acid methacrylate was utilized to impart the enzymatically degradable characteristic, whereas gelatin methacrylate offered the biocompatible property of hydrogels. When fabricating hydrogel coatings on ZnO-titanium surfaces, they followed photo-crosslinking of gelatin methacrylate and hyaluronic acid methacrylate. Moreover, they checked antibacterial activity of ZnO through enzymatically degradation of hydrogel coatings. Additionally, the

developed hydrogel coatings served as an effective buffer zone for soft tissue integration and cell ingrowth. It has a unique ability to regulate biological behavior and displays excellent soft tissue compatibility.

Wu et al. fabricated 3D printed antibacterial hydrogel coatings as a biological immobilization interface for the artificial joint prosthesis [56] (Figure 1.14). In this study, they utilized 3D printing technology to printed antibacterial chitosan-gelatin hydrogel on laser-treated titanium alloy surface. Moreover, the developed chitosan-gelatin hydrogels were immersed into the nano-silver solution to produce antibacterial hydrogel coatings and increase to adhesion and proliferation of bone cells. Developed strategy offers an opportunity for growth new bone tissue and the fixation of the prosthesis and bone interface through degradation of 3D printed chitosan-gelatin and nanosilver containing hydrogel coating. Future developments in the field of prosthetic joint replacement will make this method ideal for biological fixation interfaces.

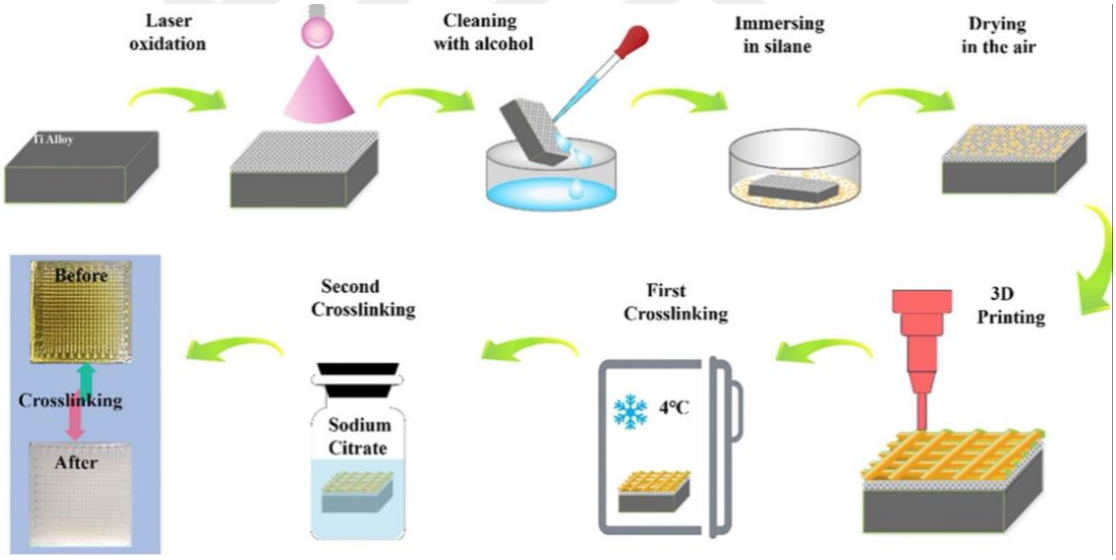

Figure 1.14: The developed strategy for creating 3D printed chitosan-gelatin hydrogel coatings on titanium alloy surface.

1.6.1. Patterned Hydrogel Coatings

In particular, drug delivery studies and the creation of microarray platforms for biomolecular immobilization have significant potential for using patterned hydrogels. It expands to possibilities for bioapplications such as cell seeding, drug loading, DNA

immobilization or biomolecule immobilization into the created patterns on hydrogel surfaces. There are different methods to obtain patterned hydrogels such as photolithography [57], microfluid patterning [58], or electrochemical deposition [59]. In the literature, various studies about patterned hydrogels were reported. For example, Cengiz et al. produced multifunctional patterned hydrogel interface which including maleimide groups as a side chain of hydrogel [60]. Maleimide groups were utilized in their work as a handle for effective functionalization employing thiol-maleimide and Dies-Alder addition reactions, as well as enhanced to generate clickable patterned hydrogel system (Figure 1.15). In the beginning, they fabricated functionalizable hydrogel with using PEGMEMA and FuMaMA copolymers under UV exposure. They utilized various patterning methods such as molding in capillaries and photolithography with a photomask to obtain micro-patterned hydrogels. Thereafter, they selected a thiol including dye which is BODIPY-SH and furan containing dye which is BODIPY-furan, respectively to demonstrate functionalization of patterned hydrogel interface through thiol-maleimide and Dies-Alder addition reaction.

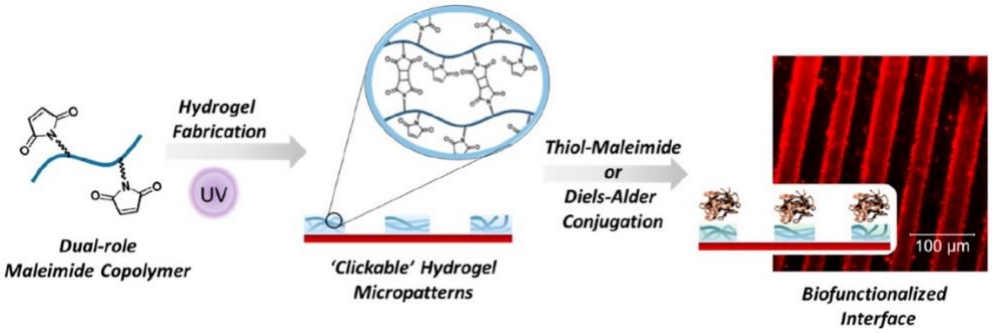

Figure 1.15: Develop clickable and biofunctionalized hydrogel interface system including maleimide groups.

In another example, protein functionalized hydrogel surfaces were created to use as a template for cell growing [61]. Shain et al. synthesized biomimetic hydrogels including acrylamide and patterned by using soft protein lithography or microcontact printing method. Basically, the aim of their research was to successfully immobilize a variety of proteins and peptides such as laminin and fibronectin onto a biocompatible hydrogel surface. In addition, their developed patterned hydrogel surface can be used for the regulated growth of a variety of neural cell types such as LRM55 rat astroglioma cells and primary hippocampal neuron cells. This research has shown that

patterned hydrogels may be used for a variety of purposes, including the examination of creating functional neuron cells.

In another example, Hahn et al. illustrated the use of photoactive PEG-diacrylate hydrogel surfaces for a straightforward, affordable photolithographic approach for patterning the surfaces of versatile, solvated substrates [62]. They created their transparent photolithographic masks in preferred patterns by using laser jet printer and placed on the hydrogel surface directly. After the UV activation of hydrogels through photomask, monoacrylated materials were immobilized to hydrogel surface by binding covalently on the light allowed region of photomask. However, the unpatterned regions remain bioinert characteristics. In this way, they developed a basic, useful, and cheap technique for surface patterning multiple bioactive peptides and cells onto hydrogel surfaces with using UV light. Furthermore, the 3D structures can be obtained by using transparency-based photolithography and it enhances to covalent immobilization of biomolecules both in 2D and 3D. Since developed method is easy, cheap, and versatile, it can be used in different applications of biotechnology and tissue engineering.

1.6.2. Photopatterning

Photopatterning allows for the modification of the characteristics of the hydrogel which enables for both spatial and temporal control while the desired reaction happens only when light is utilized [63]. In the post-gelation patterning, a synthetic hydrogel including a reactive light-sensitive group is a necessary component [64]. As mentioned before, o-nitrobenzyl ester groups are most frequently used photolabile moieties for creating patterned hydrogel systems. In this way, generating patterns occur via photo-modification reaction. Among the photopatterning techniques, mask photolithography is most used technique to create patterns on hydrogels. In this technique, the surface of hydrogel can be covered with a mask before being exposed to light, allowing the selectively irradiated unmasked portions to trigger a photo-reaction and generate structural and biochemical patterns. This technique commonly preferred in immobilization or removing of biomolecules. Furthermore, lots of patterning methodology can be found for various applications and they have also some advantages and disadvantages depending on material type, required equipment or

modifications [64]. For instance, mask photolithography has some significant advantages such as the absence of the need for specialized and expensive equipment. Furthermore, it includes a basic application procedure that allows them to easily modify clinical environment. However, there are certain drawbacks, such as the requirement of photolabile groups. In addition, this method gives poor resolution patterns when compared to other techniques.

For instance, William et al. demonstrated orthogonal photopatterning of hyaluronic acid hydrogels with using various thiol containing fluorescent molecules [63]. In their study, they synthesized hyaluronic acid functionalized NorHA hydrogels that contain norbornene groups. They checked photopatterned ability of hydrogels with using several fluorescent molecules through a 100 μm stripe photomask in the presence of UV light (Figure 1.16). Thereafter, they also showed sequential photopatterning ability of NorHA hydrogels by using different types of photomasks such as stripe, line, and triangle. Consequently, it was proven that different molecules can be sequentially patterned in just one region, successfully producing complicated shapes of molecules with this study. In other words, orthogonal secondary reactions can be carried out by determining the conditions in which pendent norbornenes persist in the gel.

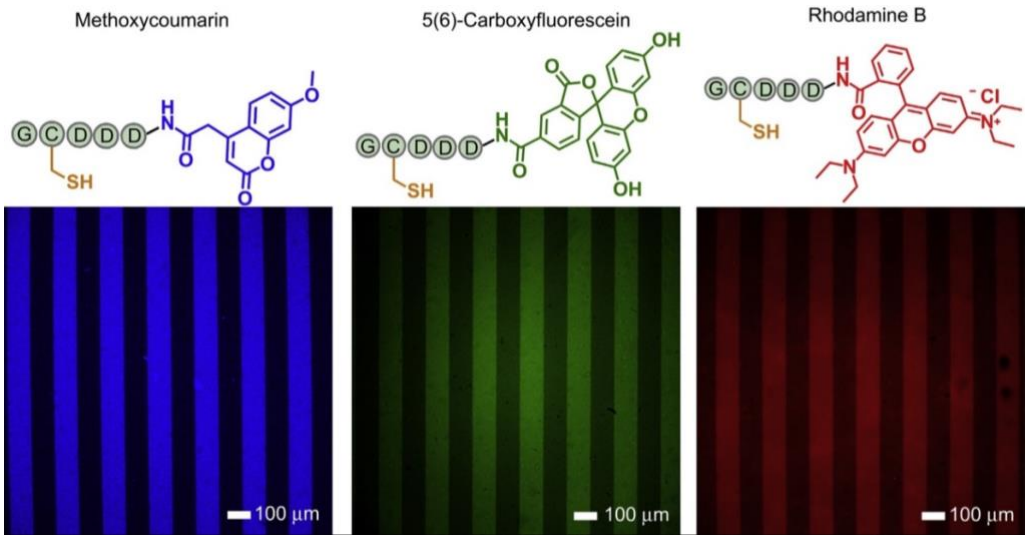

Figure 1.16: Different thiol containing fluorescent dye molecules were immobilized into synthesized and photopatterned hydrogels.

1.7. Bioimmobilization and Reactive Hydrogels

Bioimmobilization is a highly effective method for immobilization of free biomolecules in solution, allowing for the short, long, or permanent localization of a biomolecule on or inside the support [65]. Surfaces including immobilized biomolecules have several applications for different purposes such as therapeutic, wound healing, biosensors, or drug delivery studies. Numerous types of biologically functional molecules such as enzymes, antibodies, antibiotics, drugs, DNA or RNA can be immobilized by chemically or physically on the hydrogel or polymeric surfaces [66]. Furthermore, immobilized biomolecules are more resistant and stabile to environmental changes including temperature, pH, or light.

There are various methods for immobilization of biomolecules, but most commonly used methods are classified as covalent bonding, physical entrapment, and physical adsorption [67]. The most common interactions in the physical adsorption and entrapment approaches are often weak bindings such van der Waals interactions, electrostatic interactions, and affinity recognition. These approaches do not require the use of any modified biomolecules or reducing agents. Since they involve weak attachment ability and interactions, biomolecules may be escaped from substrate. Furthermore, changes in the environment could have an impact on immobilized biomolecules. In contrast to covalent bonding and chemical attachment, these approaches rely on physical attachment of biomolecules.

Covalent bonding is the most stable method for biomolecule immobilization among them. With the assistance of a chemically active functional groups, biomolecules are bound to a specific soluble or insoluble base using the covalent bonding technique [65]. Various modifications are become possible with using covalent binding method for biomolecule immobilization with easy and versatile designing possibilities and strong binding capability. Numerous modifications are become possible with using covalent binding method for biomolecule immobilization with easy and versatile designing possibilities and robust binding ability. Amine coupling and thiol coupling are popular interactions for this method and thiol and amine groups are commonly found in hydrogels designed for direct binding of biomolecules. Reactive groups in hydrogels make it possible to functionalize with specific molecules or biomolecules.

1.7.1. Reactive Hydrogels

Some reactive groups, such as -OH, -COOH, or NH_2, play a crucial role in the covalent bonding of biomolecules to this kind of supports in bioimmobilization studies. Generally, click chemistry is frequently used reactions for functionalize reactive hydrogels with biomolecules [68]. The functionalization of amine groups is very popular for bioimmobilization studies in the literature. When the surfaces contain amine reactive groups, biomolecules including amine bearing molecules can accomplish surface immobilization by chemically interacting with the appropriate molecules.

1.7.1.1. Amine Reactive Hydrogels

Active esters [69], epoxies [70], succinimide carbonates [22], and aldehyde molecules can rapidly react with amine groups. For instance, Cengiz developed dual reactive hydrogels by incorporating aldehyde and azide groups including methacrylate monomers which are AHMA and OHMA [71]. This developed system includes aldehyde and azide groups which can react with alkyne and amine molecules, respectively. Moreover, pyrene and rhodamine-NH_2 dye molecules were employed through Huisgen-type click and Schiff base reactions, respectively, to demonstrate the dual reactivity of hydrogels. Consequently, it was demonstrated that hydrogels can be functionalized by different amine and alkyne containing molecules as well as different types of reactions. The developed hydrogel approach has a potential use in multiresponsive detection applications.

In another study, epoxy-containing amine-reactive amphiphilic polymer-coated plastic surfaces were produced with antifouling properties and amine-containing biomolecules were immobilized via epoxy chemistry [72]. In the beginning, they synthesized two amphiphilic polymers which including three main components that are hydrophobic moiety acts as anchoring group, PEGMA and a functional group, carboxylic acid, for biomolecule immobilization. Thereafter, obtained amphiphilic polymers were coated onto copolystyrene substrate as a plastic surface. Furthermore, a soft lithographic technique which is microcontact printing was used to create micropattern of biomolecules. In addition, created system was used to detect anti-BSA

by using protein A, as a model protein. Moreover, FTIC-labeled BSA patterns were investigated in fluorescence microscope result. Streptavidin and antibodies were also employed as model biomolecules and could be specifically immobilized on created surfaces imprinted with biotin and protein A, respectively, via microcontact printing. Consequently, it was shown that the potential applications of biomolecule immobilization or detection on polymer coated plastic surfaces could be possible in designing biosensors or biochips with this study.

Spring et al. fabricated polyethylene glycol-based NHS-ester containing hydrogel coated glass surfaces for immobilization of amine containing molecule, biotin-amine, and Cy3-avidin complex used as protein immobilization [73] (Figure 1.17). They developed a 3D microarray platform for detecting small molecule proteins by reducing non-specific interactions in their investigation. Following the small molecule microarray experiment was carried out with biotin-amine, immobilization of the Cy3-avidin complex was done. As a result, they demonstrated that protein immobilization could be selectively fixed to reactive microarrays.

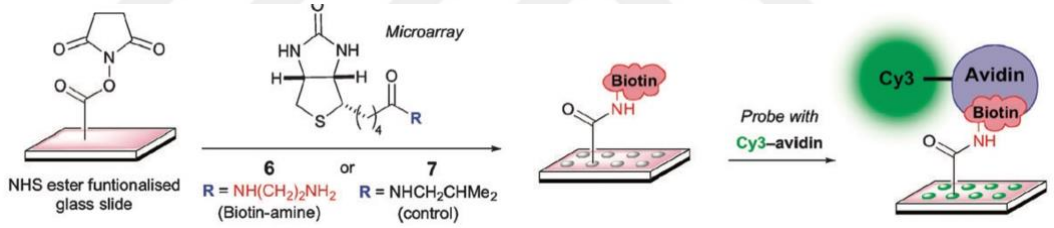

Figure 1.17: NHS-ester functionalized hydrogel coated glass surface and functionalization of the surface with protein ligand complex.

1.8. Orthonitrobenzyl (*o*-NB) Groups

Ortho nitrobenzyl groups are highly preferred and commonly employed in the construction of photosensitive hydrogel systems. Orthonitrobenzyl groups are classified as irreversible light responsive molecules. Typically, o-NB groups exhibit their maximum absorption when exposed to light with wavelength between 300 and 365 nm [74]. Thereafter, decomposition happens within minutes. The fundamental principle is based on the decomposition of an o-NB group after interaction with UV light, resulting in the simultaneous formation of nitrosobenzaldehyde (or nitrosoketone) and a carboxylic acid (or alcohol). This decomposition mechanism

includes three crucial steps. In the beginning, nitrobenzyl is stimulated by irradiation, and photoinduced H atom transfer occurs, leading to the formation of the primary acinitro intermediate. Following that, a benzoisoxaline derivative formation occurs through a molecular rearrangement. Finally, a carboxylic acid releases from the molecule and the nitrosobenzaldehyde molecule is formed [75]. The detailed decomposition mechanism is shown in Figure 1.18 [47]. Furthermore, the generated nitrosobenzaldehyde molecule contains aldehyde groups and these groups are reactive for amine-containing molecules.

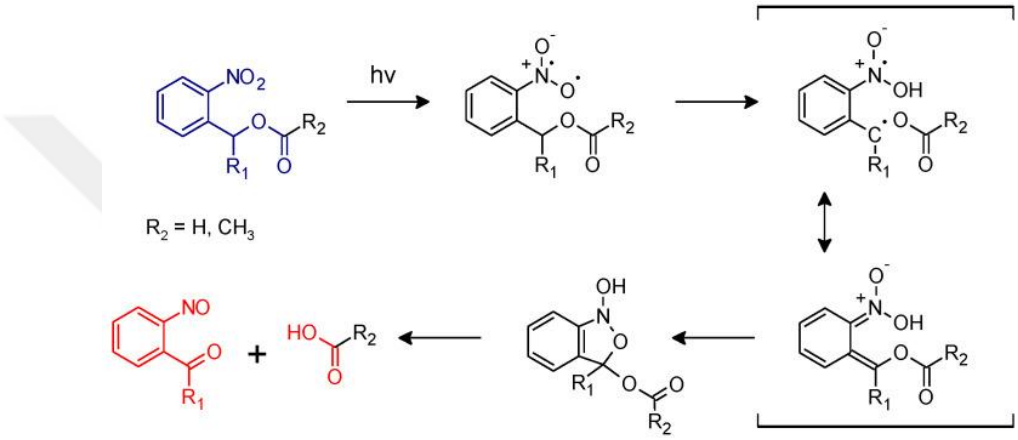

Figure 1.18: The decomposition mechanism of orthonitrobenzyl molecule by the light.

1.8.1. Orthonitrobenzyl (o-NB) Group Containing Hydrogels and Their Applications

o-NB chemistry is employed in the construction of various photosensitive polymer networks in the literature [74,76]. For instance, Spatz et al. demonstrated versatile and practical method for decorating materials with biologically active compounds utilizing an on-demand cleavable orthonitrobenzyl containing N-Hydroxysuccinimide (NHS) linker [77]. In the study orthonitrobenzyl containing N-Hydroxysuccinimide (NHS) linker was conjugated to the surfaces through a click-chemistry. They also demonstrated that protein can be immobilized on surfaces and cells can be grown on these surfaces after the binding of peptides that allow cells to attach. Thus, they showed that the release of cells and biomolecules from the surface can be achieved by cleavage of the nitrobenzyl group when exposed to UV light.

Moreover, o-NB group is commonly used in drug delivery and tissue engineering fields. For instance, Yang et al. used o-NB based crosslinkers for crosslinking hydrophilic poly(ethylene glycol) chains [78]. In their study, the o-nitrobenzene groups converted to o-nitrosobenzaldehyde via 365 nm light exposure. As a result, they developed a hydrogel system that can be employed as a scaffold for tissue engineering studies as well as a drug delivery system for drug release studies.

Furthermore, photosensitive hydrogels with o-NB containing crosslinkers can be employed to produce unique hydrogels that can be further developed on target by selective photodegradation. In recent investigations, these hydrogels have been employed as three-dimensional matrices for cell orientation studies. For example, Kasko et al. used o-NB groups to benefit from their photodegradable property [79]. In their study, they demonstrated that hydrogels can be degraded by light after cell seeding and that cells are not negatively affected by light. The side branches of the gelatin were modified with acrylate molecules bearing o-NB group, and then gelation was performed under 405 nm light. After the cell seeding, the degradation of the hydrogels was occurred by the nitrobenzyl groups, when exposed to 365 nm light and cells were released.

In another example in the literature, Cai et al. provided a synthetic technique for creating biomimetic hydrogels for three-dimensional cell culture with using o-nitrobenzyl alcohol photochemistry [80]. In their study, the side branches of the hyaluronic acid were modified with nitrobenzyl alcohol and vinyl sulfone and then gelation was occurred with the enzymatically degradable crosslinker. Thereafter, photolysis occurred via o-NB groups on the hydrogel over the photomask with 365 nm light exposure. Consequently, they functionalized the resulting nitroso aldehyde groups with BSA-c(RGDfC). Subsequently, it was shown that cells grown in a different medium (collagen) migrated following the pattern onto the new hydrogel with the created RGD pattern.

2. MATERIALS and METHODS

2.1. Materials

2.1.1. Materials and Devices

5-Hydroxy-2-nitrobenzaldehyde (ACBR), Triethylamine (TEA) (Merck), and acryloyl chloride (Thermo Scientific (Santa Cruz Biotechnology) were used for monomer synthesis. 2-(Methacryloyloxy)ethyl]dimethyl-(3 sulfopropyl)ammonium hydroxide (DMAPs) (ChemCruz), 4-4'Azobis (4-cyanovaleric acid) (ACVA) (Santa Cruz Biotechnology) and pentaerythritol tetraacrylate (PETA) (Sigma Aldrich) were utilized without further purification.

Sodium cyanoborohydride (NaB_3CN) was obtained from TCI and used for reduction of hydrogels. Hexadecyltrimethoxysilane (HDMS) was obtained from ChemCruz and used for preparation of hydrophobic cover glasses. Acrylated surfaces were prepared by using 3-(Trimethoxysilyl)propyl methacrylate (TMST) and obtained from Sigma Aldrich. Sodium hydroxide (NaOH) was used to clean the glass surfaces and purchased from Merck. Silica gel 60 was used for column chromatography and purchased from ISOLAB. Silica gel plates were used for thin layer chromatography and purchased from Merck (Kieselgel 60 F_{254}, 20 cm x 20 cm). 4-(Trifluoromethyl)benzylamine (TFBA) (TCI), streptavidin (Invitrogen), 4-butylaniline (BA) (97%, Sigma Aldrich), and fluoresceinamine isomer-I (Sigma Aldrich) were used for bioimmobilization and functionalization studies. Rhodamine-amine was synthesized according to literature procedure [76].

During the synthesis and washing processes, solvents such as methanol (ISOLAB Chemicals), toluene (Merck), tetrahydrofuran (THF) (Sigma Aldrich), dichloromethane (DCM) (99%, ISOLAB), dimethylformamide (DMF) (Sigma Aldrich), and dimethyl sulfoxide (DMSO) (Merck) were used. Technical solvents such as ethyl acetate and hexane were purchased from EMBOY. Chloroform-D1 was purchased from Sigma Aldrich and used as NMR solvent.

4-Cyano-4-[[(dodecylthio)carbonothioyl]thio]pentanoic acid (RAFT-CTA) (97%, TCI), 2-isocyanatoethyl methacrylate (ICM) (98%, Chem Cruz.), di(ethylene

glycol) methyl ether methacrylate (DEGMA) (95%, Sigma Aldrich), glycidyl methacrylate (GMA) (97%, Fluka), and were used without further purification and utilized for polymerization studies.

Table 2.1: Devices used during the thesis study.

Name	Model	Location
Rotary Evaporator	Heidolph	Gebze Technical University
FTIR Spectroscopy	Perkin Elmer 100	Gebze Technical University
NMR Spectroscopy	Varian INOVA 500 MHz	Gebze Technical University
Fluorescence Microscope	Zeiss Observer	Bogazici University
Scanning Electron Microscope	Thermo Fisher Scientific	Bogazici University
UV Light Source	Black-Ray UV Lamp (365 nm, 100 watt)	Gebze Technical University
Size Exlusion Chromatography	PSS-SDV	Bogazici University
X-ray Photoelectron Spectroscopy	Phoibos 100, SPECS GmbH	Gebze Technical University
UV-Vis Spectroscopy	Shimadzu 3600i Plus	Gebze Technical University

2.2. Methods

2.2.1. Amine-Reactive Zwitterionic Hydrogel Coatings

2.2.1.1. Synthesis of Novel Ortho Nitrobenzyl Aldehyde Monomer (NBAA)

5-Hydroxy-2-nitrobenzaldehyde (0.00448 mmol, 0.75 g) was dissolved in 25 mL of dry THF in a round bottom flask with a magnetic bar. After that, 1.5 mL of TEA was added into the solution and placed into ice bath to cool to 0℃. Then, 0.5 mL of acryloyl chloride was slowly added into the solution. The solution was returned to room temperature and stirred at room temperature in the dark for overnight. At the end of the reaction, the solvent was evaporated by *vacuo* and the product was purified by

column chromatography on silica gel eluting with ethyl acetate/hexane (1/9) to obtain pure product (0.578 g, 98.68 %).

2.2.1.2. Preparation of Glass Surface with TMSMA

Microscope slides were cut into four pieces and obtained surfaces with 2.5 cm x 2.5 cm dimension. Glass surfaces were placed into NaOH (5 g, 200 mL dH_2O) solution for 1 day to clean surfaces. After that, glass surfaces were washed with dH_2O and acetone, respectively then dried in an oven at 70℃. 0.5 mL of 3-(Trimethoxysilyl)propyl methacrylate (TMSMA) was dissolved in 50 mL toluene in a flat bottom flask and glass surfaces were placed into the solution and a septum was used to close the flask. Glass surfaces containing flask was left for 16 h. Thereafter, glass surfaces were washed with toluene and methanol, respectively and dried with argon gas. They were kept in refrigerator at 4℃ for later uses.

2.2.1.3. Preparation of Hydrophobic Cover Glass

Cover glasses (1.8 cm x 1.8 cm) were placed into NaOH (5 g, 200 mL dH_2O) solution for 1 day to clean surfaces. Thereafter, surfaces were washed with dH_2O and acetone and placed in an oven at 70℃ to obtain dry surfaces. 0.4 mL of hexadecyltrimethoxysilane (HDMS) was dissolved in 20 mL toluene in a flat bottom flask. Then, cover glasses were placed into the solution and left for 16 h. Finally, surfaces were washed with toluene and methanol, respectively and stored in refrigerator at 4℃ for later uses.

2.2.1.4. Synthesis of Ortho Nitro Benzyl Aldehyde Containing Hydrogels on Glass Surfaces

Various hydrogels were synthesized with changing ortho nitro benzyl alcohol containing monomer to zwitterionic monomer ratio and obtained three different (90:10, 80:20 and 50:50) hydrogels. These hydrogels were named as S1 (90:10), S2 (80:20) and S3 (50:50).

2.2.1.4.A. Preparation of S1

DMAPs (0.090 g, 0.322 mmol) was dissolved in 150 μL DMSO at 70℃. Stock solutions of ACVA (0.010 g in 100 μL DMSO) and PETA (0.0126 g in 100 μL DMSO) were prepared. Thereafter, synthesized monomer (0.0079 g, 0.0358 mmol), 150 μL of DMAPS (0.090 g, 0.322 mmol), 10 μL of ACVA and 10 μL of PETA were mixed in a glass vial. 25 μL of solution was dropped onto methacrylated glass surface and covered with prepared hydrophobic cover glass. Then, prepared surfaces were placed in 70℃ oven for 3 h for initiation of radical polymerization. After that, hydrophobic glasses were removed and surfaces were washed with DMSO, dH_2O and THF, respectively and dried with argon gas.

2.2.1.4.B. Preparation of S2

DMAPs (0.080 g, 0.286 mmol) was dissolved in 150 μL DMSO at 70℃. Same stock solutions of ACVA (0.010 g in 100 μL DMSO) and PETA (0.0126 g in 100 μL DMSO) which were prepared in S1 was used. Thereafter, synthesized monomer (0.01583 g, 0.0716 mmol), 150 μL of DMAPS (0.080 g, 0.286 mmol), 10 μL of ACVA and 10 μL of PETA were mixed in a glass vial. 25 μL of prepared solution was dropped onto methacrylated glass surface and covered with hydrophobic cover glass. After that, prepared surfaces were placed in 70℃ oven for 3 h. Finally, hydrophobic glasses were removed, and surfaces were washed with DMSO, dH_2O and THF, respectively and dried with argon gas.

2.2.1.4.C. Preparation of S3

DMAPs (0.050 g, 0.179 mmol) was dissolved in 150 μL DMSO at 70℃. Same stock solutions of ACVA (0.010 g in 100 μL DMSO) and PETA (0.0126 g in 100 μL DMSO) which were prepared in S1 was used. Then, synthesized monomer (0.0396 g, 0.179 mmol), 150 μL of DMAPS (0.050 g, 0.179 mmol), 10 μL of ACVA and 10 μL of PETA were mixed in a glass vial. 25 μL of prepared solution was dropped onto methacrylated glass surface and covered with hydrophobic cover glass. Thereafter,

prepared surfaces were placed in 70℃ oven for 4 h. Finally, hydrophobic glasses were removed, and surfaces were washed with DMSO, dH_2O and THF, respectively and dried with argon gas.

2.2.1.5. Reduction of Hydrogel Surfaces with $NaBH_3CN$

S1, S2 and S3 surfaces were reduced with $NaBH_3CN$ to convert the aldehyde group to alcohol. $NaBH_3CN$ (0.025 g, 0.00066 mmol) was dissolved in a glass vial which contain 750 μL of THF and 750 μL of EtOH. 50 μL of prepared solution was dropped onto each surface. Prepared samples were placed in an ice containing bath and reaction was occurred at 0℃ for 3 h in dark condition. Thereafter, each surface was washed with THF, EtOH and dH_2O respectively. Surfaces were dried with argon gas.

2.2.1.6. UV Activation of Hydrogel Surfaces

S1, S2 and S3 surfaces were exposed to 365 nm UV irradiation after treated with $NaBH_4$ to produce amine reactive nitrosobenzaldehyde units. Each surface was placed under UV light for 30 minutes. Thereafter, surfaces stored in refrigerator at 4℃ for later uses.

2.2.1.7. Functionalization of Hydrogel Surfaces with TFBA

UV activated surfaces (S1, S2, and S3) were used for immobilization of amine containing molecule, TFBA. 10 μL of TFBA was dissolved in 400 μL of THF. Thereafter, 20 μL of TFBA solution was dropped on UV activated surfaces and covered with hydrophobic cover glass. After incubation for 3 hours in the dark, cover glass was removed, and surfaces were washed with THF and dried with argon gas.

2.2.1.8. Preparation of Reactive Micropatterns on Hydrogel Surfaces

Reactive micropatterns were created with using reduced S1, S2 and S3 surfaces. Each surface was exposed UV light for 30 minutes through photomask placed on the surfaces. Surfaces stored in refrigerator at 4℃ for bio-immobilization studies.

2.2.1.9. Functionalization with Amine-containing Dye Molecule, Fluoresceinamine

To show functionality of micropatterns, amine-containing dye molecule, fluoresceinamine was immobilized on these created regions. According to this purpose, 2.3 mg of fluoresceinamine was dissolved in 460 μL of DMF. Thereafter, 20 μL of fluoresceinamine solution was dropped on micropatterned surfaces (S1, S2, and S3) and covered with cover glasses. After 3 hours incubation in the dark, cover glasses were removed. Surfaces were thoroughly washed with DMF and dried with argon gas. Functionalized micropatterns on surfaces were observed via fluorescent microscope.

2.2.1.10. Biomolecule Immobilization

2.2.1.10.A. Functionalization with Fluorescently Labelled Biomolecules, Biotin-PEG2-Amine/Streptavidin

Micropatterned S1, S2 and S3 surfaces were functionalized with Biotin-PEG_2-amine and streptavidin, respectively. In first step, 2.3 mg of Biotin-PEG_2-amine was dissolved in 460 μL of DMF. After that, 20 μL of dissolved solution was spread over the micropatterned surfaces and covered with cover glasses. Surfaces were incubated for 3 hours in the dark. At the end of the 3 hours, cover glasses were removed, and surfaces were washed with DMF, then dried with argon gas. In the second step, 100 μL of streptavidin was dissolved in 900 μL of PBS. 20 μL of prepared solution was dropped on biotin treated surfaces and covered with cove glasses. After 30 minutes incubation time, surfaces were washed with prepared PBS solution (1 tablet dissolved in 200 mL dH_2O) and dried with a stream of argon. Biomolecule functionalized micropatterns on surfaces were observed via fluorescent microscope.

2.2.2. Amine-Reactive Dual-Functionalized Polymers and Their Surface Modifications

2.2.2.1. General Polymerization of Poly(ICM-r-GMA-r-DEGMA) (P1 and P2)

2.2.2.1.A. Preparation of P1

To obtain isocyanate and epoxy functional dual-reactive copolymers, P1 and P2 were synthesized by using reversible addition-fragmentation chain-transfer (RAFT) polymerization. Firstly, 2.5 mL of dry THF was used to dissolve RAFT-CTA (0.0153 g, 0.0038 mmol), ACVA (0.00213 g, 0.0076 mmol), GMA (0.30 mL, 2.284 mmol, 40 eq.), and DEGMA (0.53 mL, 2.855 mmol). Prepared solution was purged with nitrogen gas for 30 min. Following that, 80 μL of ICM (0.571 mmol, 10 eq.) was added to the mixture. Thereafter, the mixture was heated to 65 °C in an oil bath and stirred for 6 h. When the reaction was complete, volatiles were removed under vacuo and the resulting polymer was washed with cold ether to eliminate unreacted monomers and reagents to obtain **P1** (58.4% yield).

2.2.2.1.B. Preparation of P2

RAFT-CTA (0.0153 g, 0.0038 mmol), ACVA (0.00213 g, 0.0076 mmol), GMA (0.56 mL, 1.4275 mmol, 25 eq.) and DEGMA (0.53 mL, 2.855 mmol) were dissolved in 2.5 mL of dry THF. The prepared solution was purged with nitrogen gas for 30 min. After that, 200 μL of ICM (1.4275 mmol, 25 eq.) was added to the mixture. The prepared mixture was heated to 65 °C in an oil bath and stirred for 6 h. Finally, volatiles were removed under vacuo and the resulting polymer was washed with cold ether to eliminate unreacted monomers and reagents to obtain **P2** (37% yield).

2.2.2.2. Functionalization of Copolymer P1 with Amine-containing Molecule, TFBA (P1-TFBA)

First of all, obtained copolymer P1 (100 mg) was dissolved in 100 μL of dry THF. Thereafter, 16 μL of TFBA (0.09015 mmol) was added into polymer solution. The reaction was conducted for 1 h at 25 °C in dark with gentle stirring. After the reaction was complete, the residue was washed with cold ether. Following that, the volatiles were removed under vacuum to produce functionalized polymer, P1-TFBA (91.61% yield).

2.2.2.3. Functionalization of Copolymer P2 with Amine-containing Molecule, TFBA (P2-TFBA)

The obtained copolymer P2 (90 mg) was dissolved in 100 μL of dry THF. After that, 22 μL of TFBA (0.1352 mmol) was added into the dissolved polymer solution. The reaction was conducted for 1 h at 25 °C in dark with gentle stirring. After the reaction was complete, the residue was washed with cold ether. Following that, the volatiles were removed under vacuum to produce functionalized polymer, P2-TFBA (74.8% yield).

2.2.2.4. Functionalization of P1-TFBA with 4-Butylaniline (P1-TFBA-BA)

Firstly, the obtained functionalized polymer P1-TFBA (30 mg) was dissolved in 30 μL of dry THF. Then, 34 μL of 4-butylaniline (0.01892 mmol) was dissolved in 126 μL of dry THF and added into the polymer solution. The reaction was carried out for 18 h at 65 °C in the oil bath with stirring. At the end of the reaction, volatiles were removed under vacuo and the resulting dual functionalized polymer was washed with cold ether to eliminate unreacted monomers and reagents (78.85% yield).

2.2.2.5. Functionalization of Copolymer P1 with Rhodamine-amine (P1-Rhodamine)

The obtained copolymer P1 (26.5 mg) was dissolved in 46 μL of dry THF. Then, 8 mg of rhodamine-amine (0.01892 mmol) was dissolved in 126 μL of dry THF and dissolved rhodamine-amine solution was added into the polymer solution. The reaction was conducted for 1 h at 25 °C in dark with gentle stirring. At the end of the reaction, volatiles were removed under vacuo and obtained polymer with cold ether to remove unreacted monomers and reagents (79.6% yield).

2.2.2.6. Polymeric Coatings on Glass Surfaces

2.2.2.6.A. Polymeric Coating on Glass Surfaces for P1-TFBA

First, glass surfaces (1.35x1.55 cm^2) were treated with NaOH solution (5 g in 200 mL dH_2O) for overnight. After that, surfaces were washed with dH_2O and acetone, respectively and placed in an oven at 70℃ to obtain dry surfaces. The obtained functionalized polymer, P1-TFBA (0.005 g) was dissolved in 100 μL DMF. Thereafter, 20 μL of prepared solution was dropped on treated glass surfaces and spread over. Prepared surfaces were placed into the oven at 70℃ for 18 h. Then, surfaces were washed using dry DMF and THF, respectively and dried with argon gas.

2.2.2.6.B. Polymeric Coating on Glass Surfaces for P2-TFBA

NaOH (5 g in 200 mL dH_2O) treated glass surfaces (1.35x1.55 cm^2) were used for coatings. The obtained functionalized polymer, P2-TFBA (0.005 g) was dissolved in 100 μL DMF. After that, 20 μL of prepared solution was dropped on treated glass surfaces and spread over. They were placed into the 70℃ oven for 18 h. Finally, surfaces were washed using dry DMF and THF, respectively and they were dried using an argon stream.

2.2.2.6.C. Polymeric Coating on Glass Surfaces for P1-Rhodamine

NaOH (5 g in 200 mL dH_2O) treated glass surfaces (1.35x1.55 cm^2) were used for coatings. 5 mg of P1 was dissolved in 100 μL DMF. 20 μL of prepared rhodamine-amine solution (8 mg, 0.01892 mmol in 126 μL of dry THF) was spread over on treated glass surfaces and placed into the oven at 70℃ for 18 h. Finally, surfaces were washed using dry DMF and THF, respectively and they were dried using an argon stream.

3. RESULTS and DISCUSSION

3.1. Amine-Reactive Zwitterionic Hydrogel Coatings

In the first part of the study, UV-activable, o-NB group containing amine reactive zwitterionic hydrogel surface coatings were synthesized and functionalized with amine containing molecules and biomolecules. At first, the o-NB group containing aldehyde monomer was synthesized and subsequently used for fabrication of zwitterionic surfaces onto the glass surface. After that, aldehyde group was reduced to alcohol to obtain deactivated hydrogel surfaces by using $NaBH_3CN$. Thereafter, spatially controlled activation of hydrogel surfaces was obtained through UV irradiation. Moreover, different patterned photomasks were used to create reactive micropatterned regions of the surfaces. Finally, biomolecular immobilization was achieved by using various amine bearing biomolecules and visualized via fluorescent microscopy (Figure 3.1).

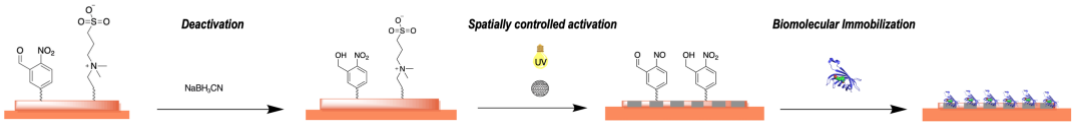

Figure 3.1: General schema of obtaining UV-activable, o-NB group containing amine reactive zwitterionic hydrogel surface coatings and their biomolecular immobilization.

3.1.1. Synthesis of Novel Ortho Nitrobenzyl Aldehyde Monomer (NBAA)

5-hydroxy-2-nitrobenzaldehyde was reacted with acryloyl chloride in the presence of triethyl amine in DCM at 0℃. As a result of synthesis, ortho nitrobenzyl containing aldehyde monomer was obtained (Figure 3.2). The purification of synthesized monomer was done by column chromatography with 54.4% yield.

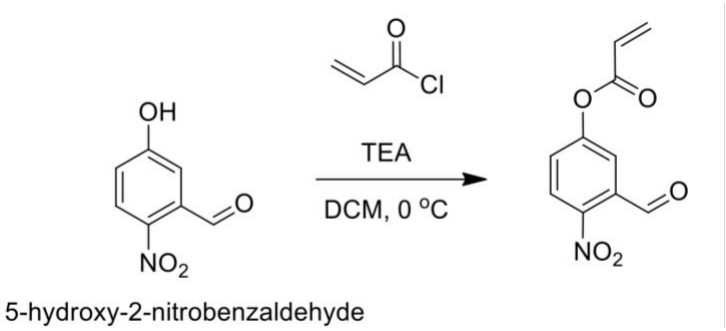

Figure 3.2: Synthesis of novel ortho nitrobenzyl aldehyde monomer.

The synthesized molecule was chemically characterized by FTIR and ^{1}H-NMR measurements (Figure 3.3 and Figure 3.4). According to FTIR spectrum result, the presence of a peak at 1741 cm^{-1}, which is absent in the initial material, provides confirmation of the existence of an ester group in the resulting structure. Additionally, the presence of a peak at 1507 cm^{-1} in the resulting molecule, which is also present in the starting material, indicates that the N-O stretching associated with the NO_2 groups remained intact during the synthesis process. In the NMR spectrum result, the indicated peaks were appeared in expected regions (Figure 3.4). The occurrence of the peak at 10.5 ppm was indicated the protons in the aldehyde group of the obtained monomer. Three aromatic protons of benzene ring appeared at 8.2, 7.7, and 7.5 ppm. Moreover, disappearance of the peaks of hydroxide groups showed that the achievement of the targeted structure.

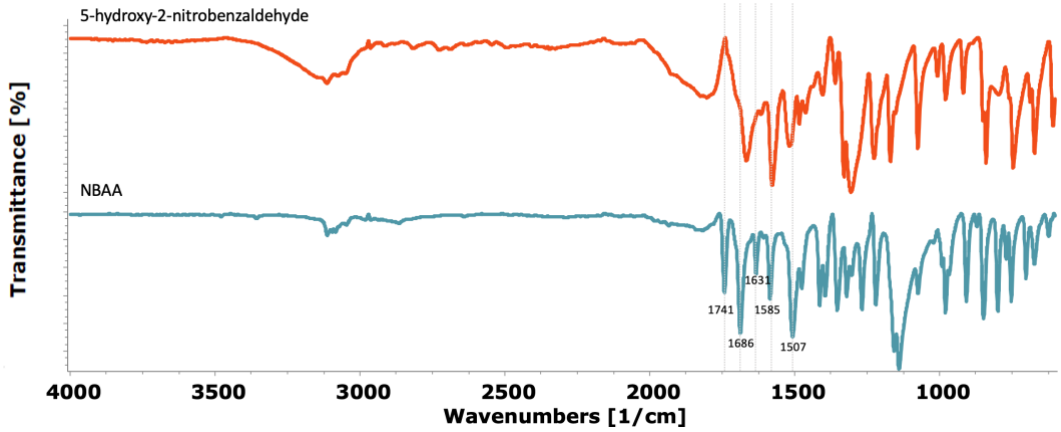

Figure 3.3: FTIR spectrum of synthesized ortho nitrobenzyl aldehyde monomer and 5-hydroxy-2-nitrobenzaldehyde.

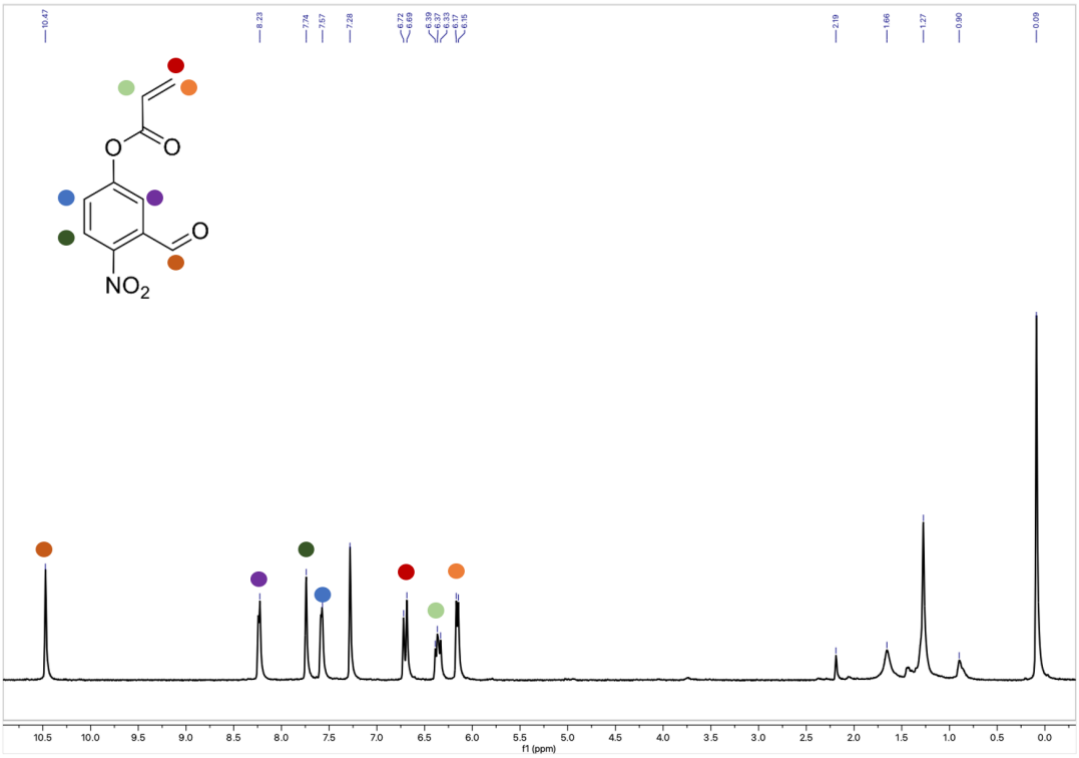

Figure 3.4: ^{1}H-NMR result of synthesized ortho nitrobenzyl aldehyde monomer.

3.1.2. Synthesis of Ortho Nitrobenzyl Aldehyde Containing Hydrogels on Glass Surfaces (S1, S2 and S3)

The synthesized monomer, along with the zwitterionic monomer and PETA crosslinker, was used to prepare hydrogel films on glass surfaces. First of all, the glass surfaces were modified with TMSMA to prepare the hydrogels as covalently bonded films on the glass surface. Following that, the surfaces were covered with the prepared hydrophobic cover glasses and placed at 70℃ to initiate the radical polymerization. Finally hydrophobic cover glass was removed, and hydrogel film was washed with organic solvents and dried in vacuum (Figure 3.5).

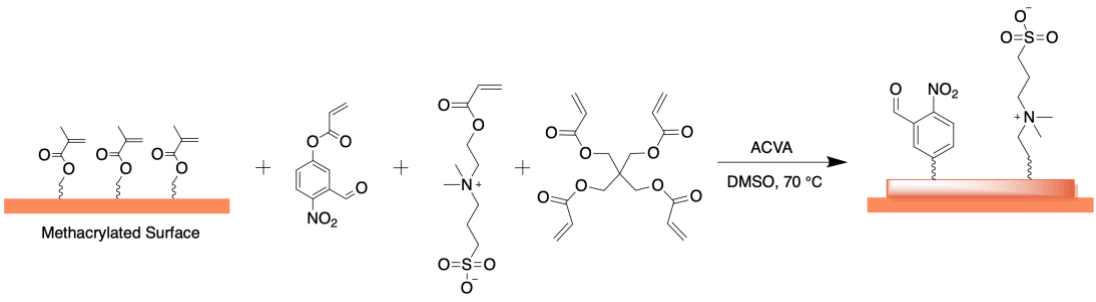

Figure 3.5: Synthesis of hydrogel on modified glass surface.

Changing NBAA to zwitterionic monomer ratio, three different hydrogels were synthesized on glass surfaces (S1, S2 and S3) to monitor the altered ratio of NPAA in those hydrogels and analyze the chemical composition as well as functional groups present, FTIR measurements were conducted (Figure 3.6). The ester related carbonyl stretching vibration was observed in the FTIR result at 1720 cm^{-1}. It was suggested that aldehyde peak which expected to appearance around 1730 cm^{-1} in the FTIR spectrum was not observed due to the suppression of the ester peak. Moreover, peak at 1530 cm^{-1} was indicated as nitro peak and coming from ortho nitrobenzyl aldehyde monomer. As expected, it was observed that the nitro peak at 1530 cm^{-1} increased as the ratio of synthesized monomer increased (S1 to S3).

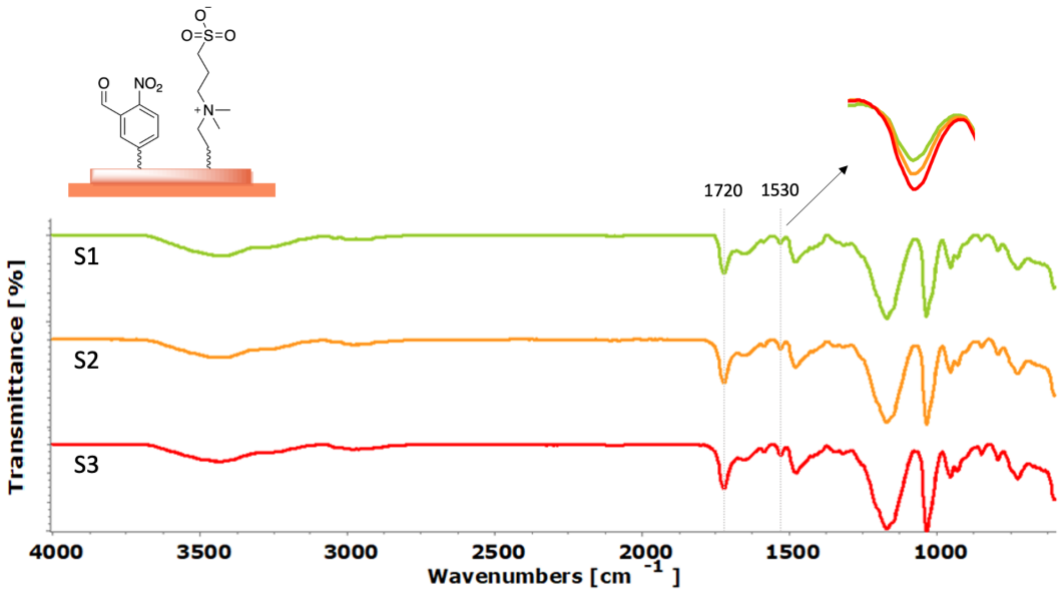

Figure 3.6: FTIR spectrum of S1, S2 and S3.

In addition, the thickness of the hydrogel surface was determined through SEM analysis. To accomplish this, hydrogels on glass were sectioned in the middle, and cross-sections were visualized. Based on the SEM results, the thickness of S1 was measured to be 51 micrometers (Figure 3.7).

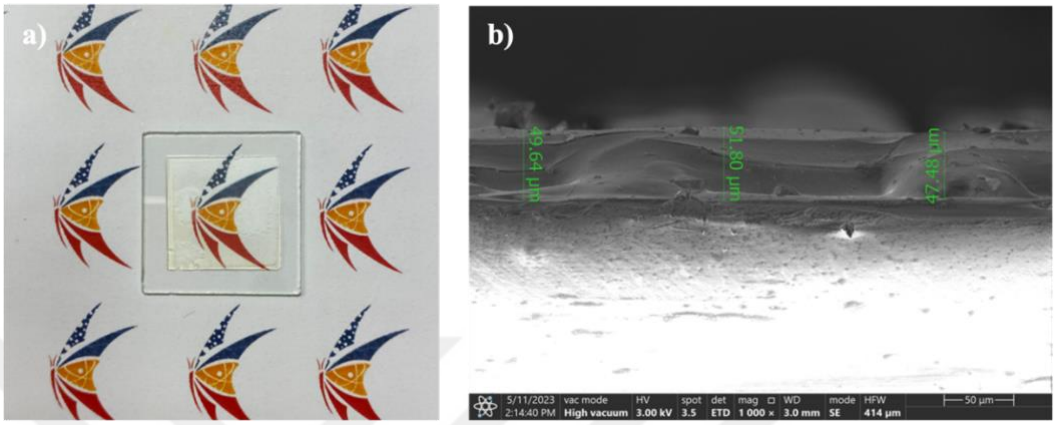

Figure 3.7: a) Image of prepared S1 surface. b) SEM image of the edge of the S1 surface.

3.1.3. Reduction of Hydrogels (S1, S2 and S3) with $NaBH_3CN$

The aldehyde groups of each hydrogel surface (S1, S2, and S3) were reduced to alcohol by using sodium cyanoborohydride at 0℃ (Figure 3.8). The reduction process was essential since it allowed to construct reactive micropatterns on the hydrogel surfaces. In the beginning, the obtained hydrogel surfaces were completely active to aldehyde units. As a result of the reduction step, the hydrogel surfaces were deactivated allowing for further spatially controlled activation through UV light with photomasks. The resulting hydrogels were analyzed by FTIR spectroscopy (Figure 3.9).

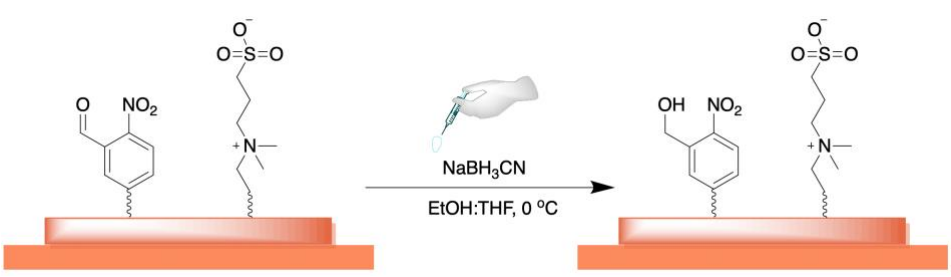

Figure 3.8: Reduction of S1, S2 and S3 with $NaBH_3CN$.

The ester peak at 1720 cm^{-1} stay remained in the FTIR spectra as expected (Figure 3.9). However, since the expected aldehyde peak was not seen in the previous step, reduction step also could not be followed by FTIR spectrum. This might be due to the ester peak appearing excessively large, and suppressing the predicted aldehyde peak. Despite the lack of observable changes in the FTIR spectroscopy, we proceeded with the assumption of successful reduction and moved on to the next step.

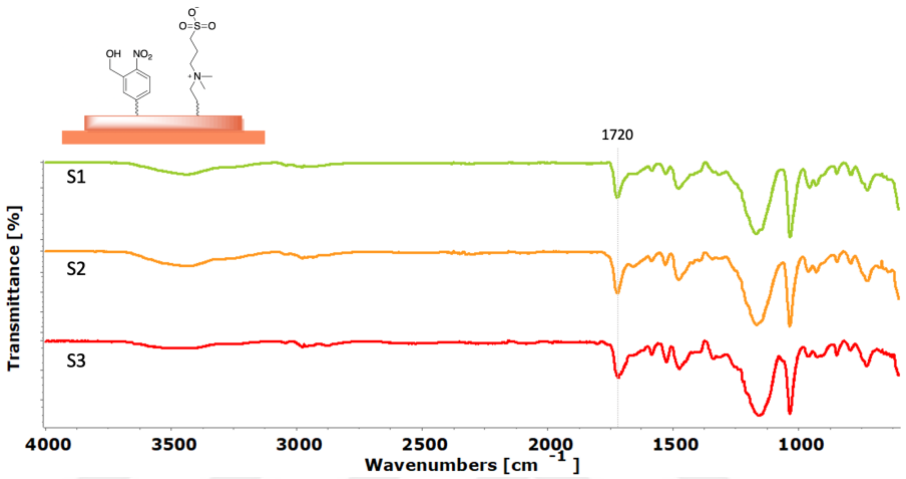

Figure 3.9: FTIR spectrua of reduced S1, S2 and S3 surfaces.

3.1.4. UV Activation of Hydrogels

Hydrogels were exposed to 365 nm UV irradiation for 30 min to produce amine reactive nitrosobenzaldehyde units (Figure 3.10). After UV exposure, formation of the nitrosobenzaldehyde units was proven by FTIR spectroscopy (Figure 3.11). Obtained UV-activated surfaces were used for immobilization of amine containing molecules and biomolecules in further steps.

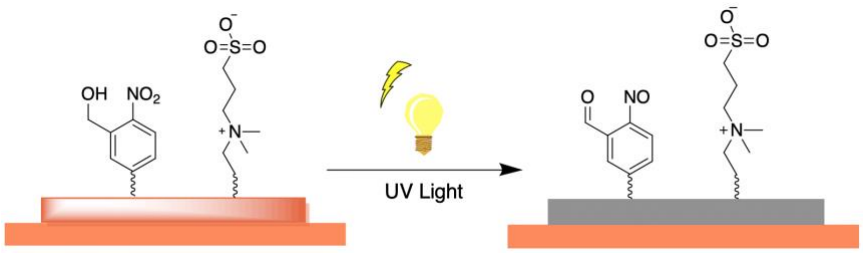

Figure 3.10: General illustration of UV activation for reduced S1, S2, and S3 surfaces.

Furthermore, it has been proven that UV activation occurs with the proportional decrease in the intensity of the nitro band at 1530 cm^{-1} of the S1, S2 and S3 surfaces with FTIR result. Moreover, the ester peak at 1720 cm^{-1} stay remained in the FTIR spectrum result, as expected.

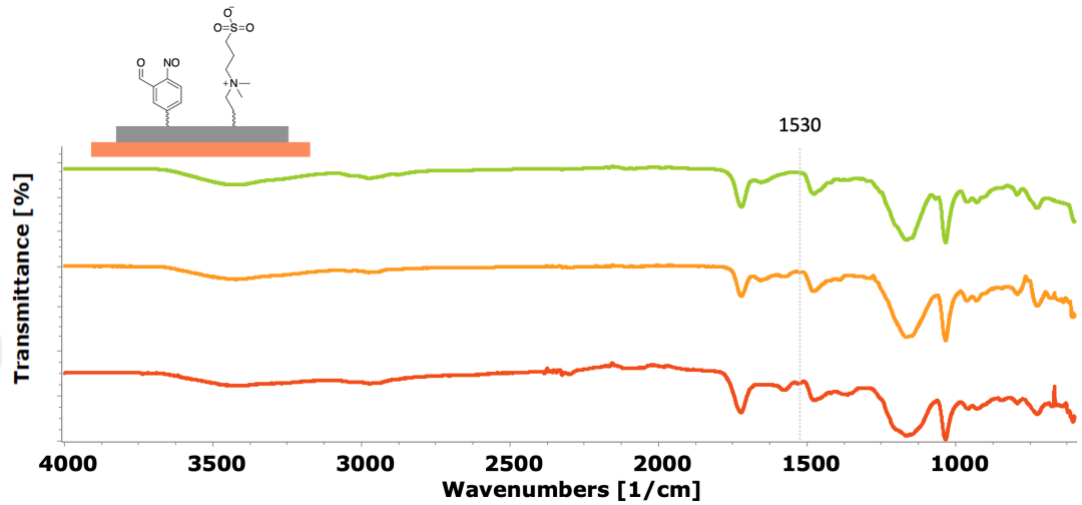

Figure 3.11. FTIR spectrum of UV activated S1, S2, and S3 surfaces (after $NaBH_3CN$ treatment).

The FTIR spectroscopy result of the hydrogel surface with the highest amount of monomer, S3, was given step by step to help understand the effect of UV treatment on hydrogel surfaces (Figure 3.12). It was clearly seen that NO_2 peak at 1530 cm^{-1} was significantly decrease due to the UV exposure.

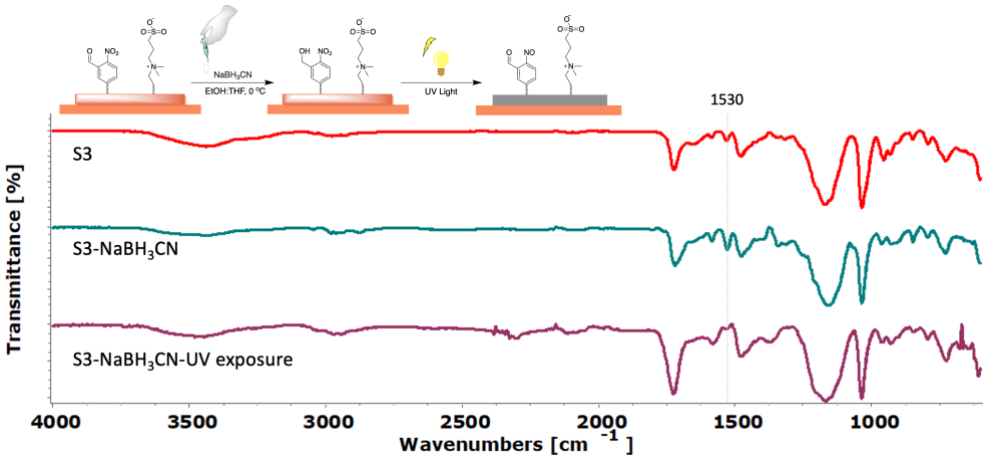

Figure 3.12: FTIR spectroscopy results of the stages up to UV application of S3.

3.1.5. UV-Vis Spectroscopy Measurements of S1, S2 and S3

UV-Vis spectroscopy measurements were conducted for hydrogel films which produced in three different ratios (S1, S2, and S3). The visible light which is range between 400 and 700 nm was selected for the measurements, and the light transmittance was recorded for each surface. S3 had highest light transmittance of 99.44% at 600 nm, when compared to S2 (98.01%) and S1 (97.25%) (Figure 3.13b). This difference can be attributed to the lower content of zwitterionic monomer present in S3 compared to the other samples. In other words, transparency is directly influenced by the amount of zwitterionic monomer. Hydrogel with the lowest concentration of zwitterionic monomer (S3) tend to be more transparent, while the one with the highest concentrations (S1) appear less transparent (Figure 3.13a). Consequently, light transmittance and transparency of the hydrogel films have been proven both qualitatively and quantitatively.

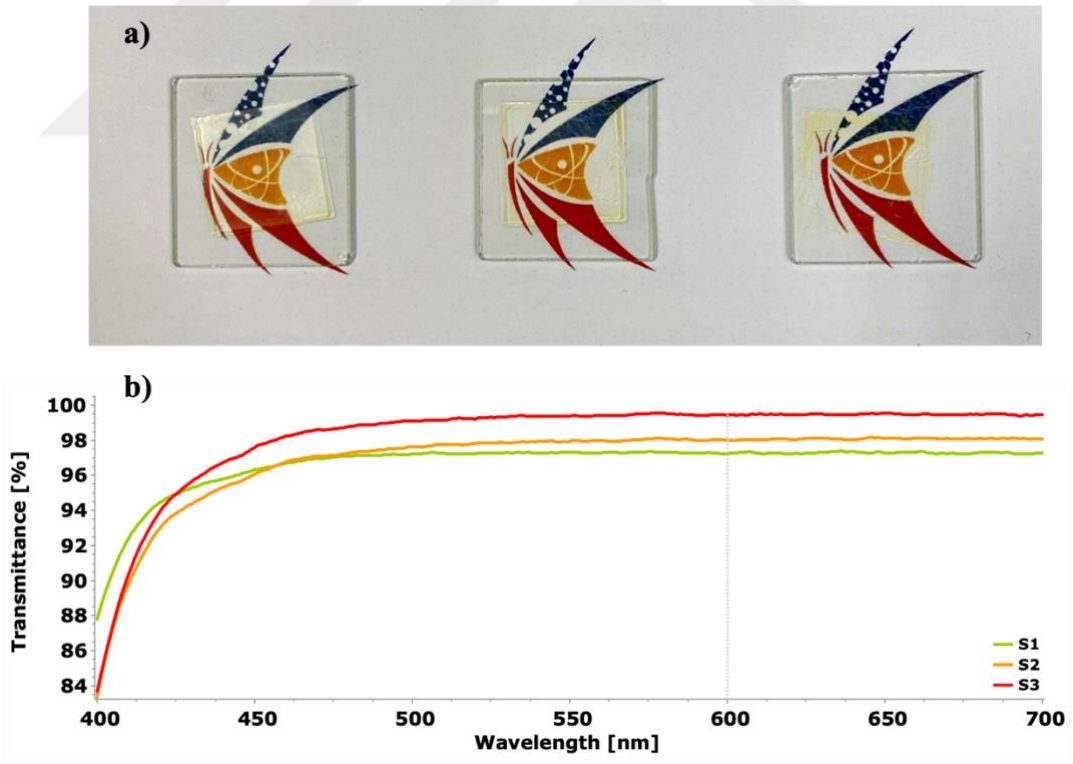

Figure 3.13: a) Prepared hydrogel films which are S1, S2 and S3, respectively.b) UV-Vis spectroscopy of S1, S2 and S3 in range between 400 to 700 nm.

3.1.6. Conjugation of TFBA to UV-Activated Hydrogels

When the UV light was exposed onto the hydrogel surfaces, the o-nitrobenzyl group converted into the nitrosobenzaldehyde units. In this way, obtained hydrogel surfaces include amine-reactive units. These amine-reactive regions were functionalized by binding amine-containing molecules. According to this purpose, the amine-containing model molecule, TFBA, was immobilized on the UV light-activated surfaces (Figure 3.14). Successfully immobilization of TFBA on surfaces was controlled by FTIR spectroscopy (Figure 3.15) and XPS measurement (Figure 3.19). It was expected that TFBA molecule can only conjugated to UV activated surfaces. In order to determine whether the TFBA molecule only attaches to UV-activated surfaces, a control sample which was not exposure from UV light, was prepared. Prepared TFBA solution was directly dropped on surfaces and same reaction condition was applied. The TFBA molecule did not bind to non-UV-activated surfaces, as expected, and this hypothesis was supported by FTIR spectroscopy (Figure 3.15). In FTIR spectrum result, the characteristic peak of the TFBA molecule was not appear at 1326 cm^{-1} in the control sample. Moreover, it was also shown that the nitro peak at 1530 cm^{-1} was not disappear in control sample.

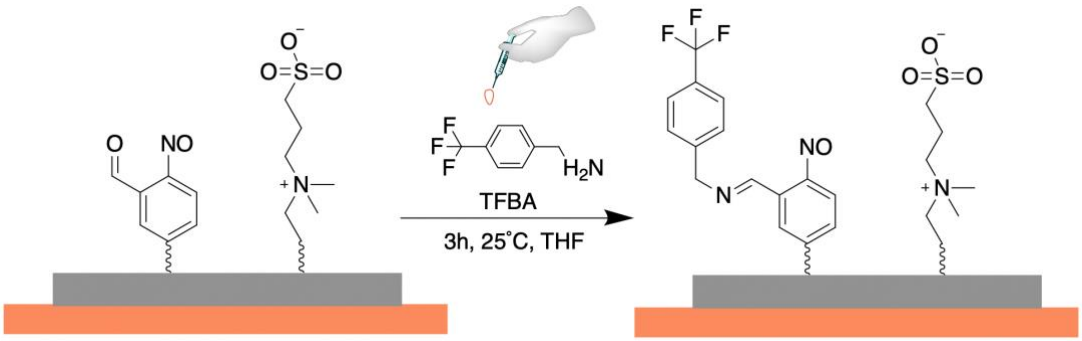

Figure 3.14: General illustration of TFBA immobilization on UV activated surfaces.

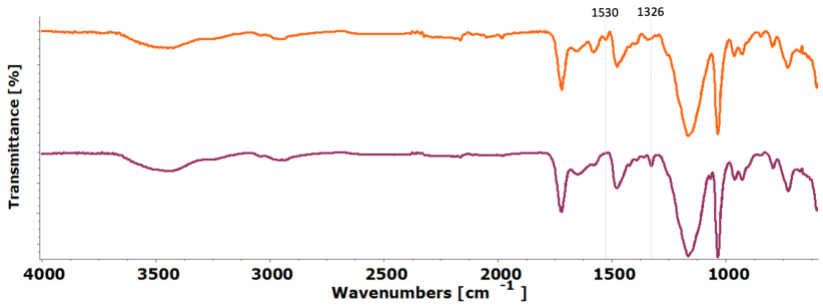

Figure 3.15: Comparison between control (S2-reduced) and S2-reduced-UV sample by following FTIR spectrum peaks.

Furthermore, the peak at 1326 cm^{-1} was coming from TFBA molecule for each surface in FTIR spectrum (Figure 3.16). Simultaneously, a regular increase and a trend in the TFBA peak at 1326 cm^{-1} was observed in the FTIR spectrum as the monomer ratio increased (S1 to S3). Also, to better understand all over the changes in hydrogel surfaces, FTIR spectrum of S3, S3-$NaBH_3CN$, S3- $NaBH_3CN$-UV and S3 S3-$NaBH_3CN$-UV-TFBA was given (Figure 3.17). It was clearly seen that an amine containing molecule was successfully attached on obtained S3 surface.

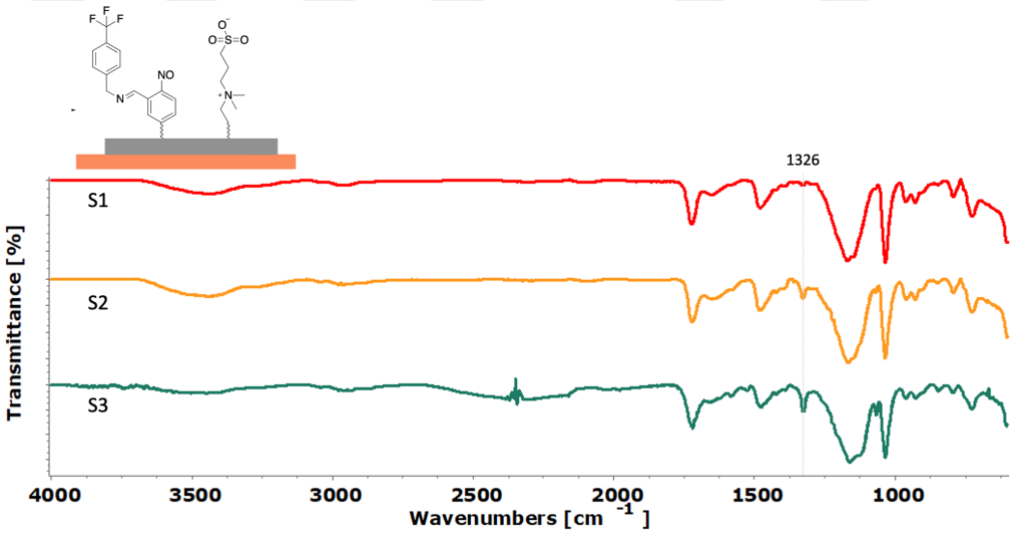

Figure 3.16: FTIR spectrum of TFBA immobilized S1, S2 and S3 surfaces.

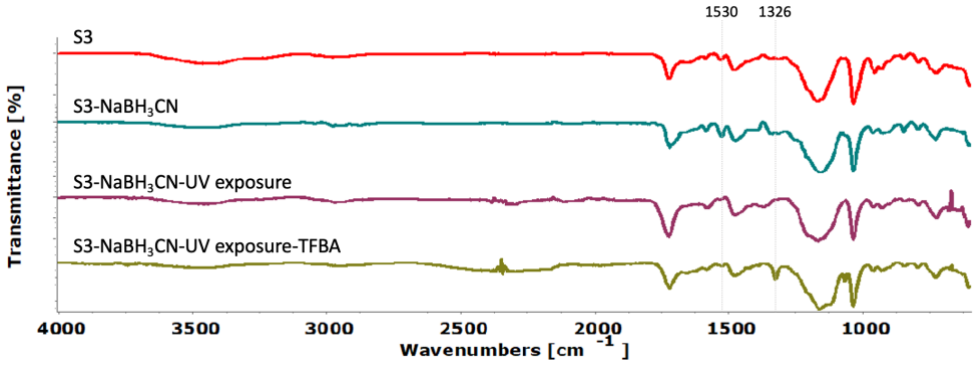

Figure 3.17: FTIR spectrum of all of the treatments on S3 hydrogel surface.

Moreover, XPS analysis was done for each surface, S1, S2, and S3 to demonstrate TFBA attachment on surfaces by following their F atoms (Figure 3.18). According to the XPS spectrum result, each surface had F atom with expected contained ratio (Table 3.1). Since S3 contained higher ratio of nitrosobenzaldeyde unit the peak intensity of F 1s and ratio of F/C atoms were higher than S1 and S2 like as in FTIR spectrum result (Figure 3.16). Moreover, according to the Table 3.1, S atom was present in each surface due to the zwitterionic comonomer.

Table 3.1. Atom percentages of each surface after functionalization with TFBA.

Sample	Atom percentage				
	F 1s	O 1s	N 1s	C 1s	S 2p
S1-TFBA	1.98	25.57	3.41	63.22	5.82
S2-TFBA	2.67	22.46	4.05	66.50	4.33
S3-TFBA	6.15	17.56	5.50	66.78	4.01

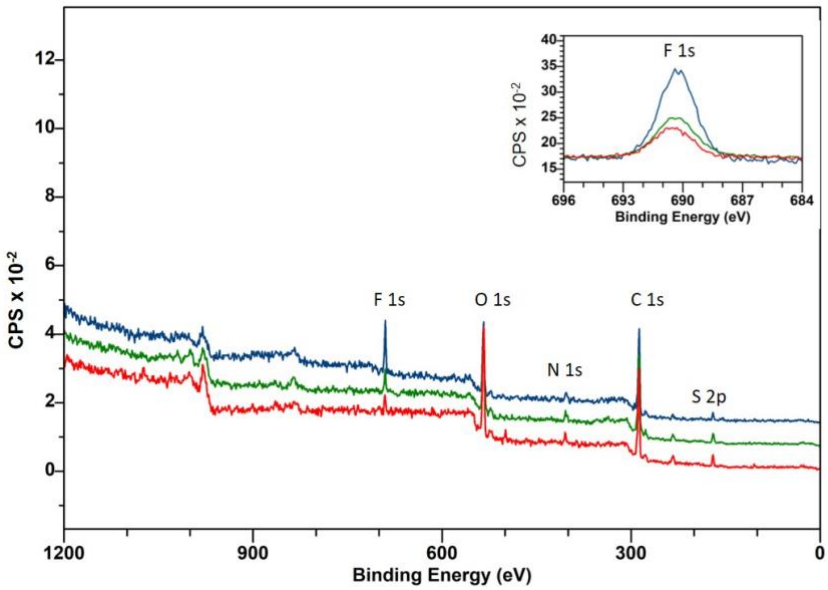

Figure 3.18: XPS spectrum result of S1, S2 and S3 after TFBA treatment and F 1s atom comparison for each surfaces (inset).

3.1.7. Functionalization of UV-Activated Hydrogels with Fluorescein-amine

Hydrogels were exposed to 365 nm UV irradiation through photomask for 30 min to produce amine reactive nitrosobenzaldehyde units containing patterned surfaces (Figure 3.19). Nitrosobenzaldehyde groups were produced only in the targeted micropatterns by sending UV light through the photomasks which were placed on the hydrogel surfaces. Molecules which are in reduced state were exist in non-transparent regions of photomasks. In this way, it was possible to obtain site-specific reactive micropatterns on hydrogel surfaces. To vary the patterns on the surfaces, two different types of photomasks were employed and each patterns were observed with fluorescence microscope. On the other hand, the presence of zwitterionic groups on the surfaces helped to minimize non-specific biomolecule adsorption for biomolecule immobilization studies.

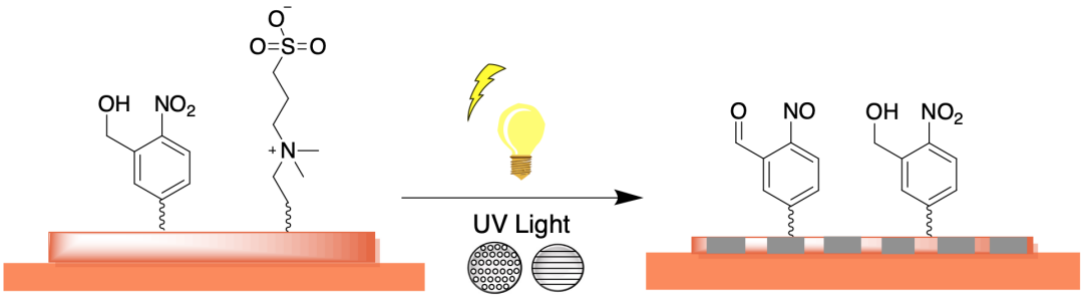

Figure 3.19: General illustration of UV activation of reduced S1, S2 and S3 surfaces through different patterned photomasks.

Following to UV activation through a photomask, functionalizability of micropatterns was shown by immobilization of an amine containing dye molecule, fluorescein-amine (Figure 3.20). In addition, fluorescein-amine was immobilized on non-UV activated surfaces without photomasks to control succesful binding of this molecule with a control sample (Figure 3.21a and Figure 3.21b). The formation of amine-reactive units as a result of the conversion of the o-nitrobenzyl group to nitrosobenzaldehyde under the effect of UV light has been demonstrated by several kinds of amine containing molecules. Therefore, since orthonitro benzyl groups will not convert to aldehydes on gel surfaces that have not been exposed to UV light, molecules containing amines are not expected to bind to the hydrogel surfaces. As a result, no significant fluorescence signal was obtained in the fluorescence microcope with the control samples.

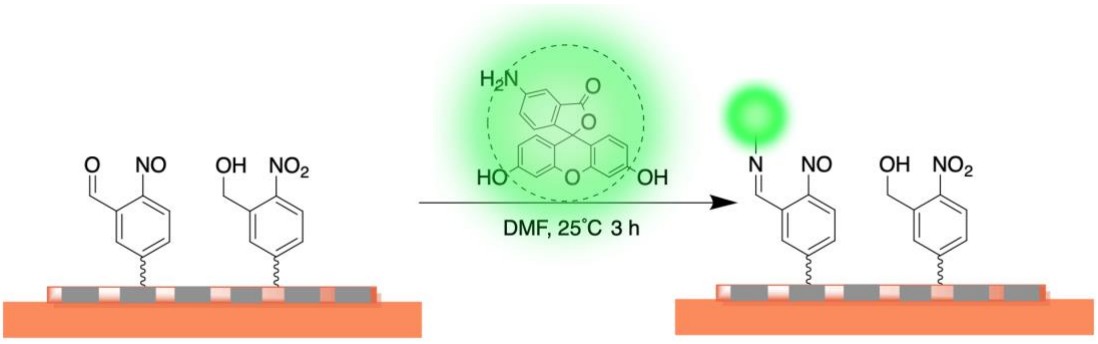

Figure 3.20: Illustration of fluorescein-amine immobilization of UV activated surfaces.

Fluorescence microscopy analysis was done to follow succesfull attachment of fluorescein-amine. According to the fluorescence microscope result, the green color

was examined on micropatterned regions (Figure 3.21c and Figure 3.21d). In Figure 3.21b, fluorescein-amine was directly immobilized on non-UV activated surfaces, as a control sample. Fluorescein-amine was not bind to the surface and give black colour in the fluorescence microscope, as expected.

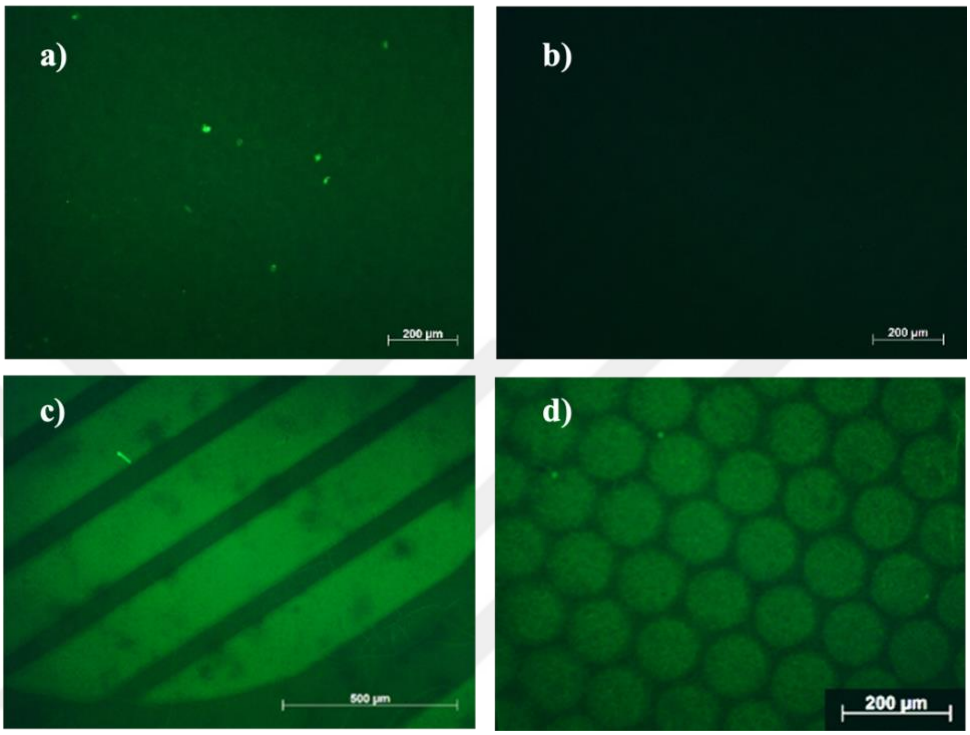

Figure 3.21: Fluorescence micsrocope images of fluorescein-amine containing S1. a) S1 with fluorescein-amine. b) Control sample. c) S1 with fluorescein-amine under stripe photomask. d) S1 with fluorescein-amine under circular photomask.

Furthermore, striped and circular photomasks were used to create different micropatterns on hydrogels surfaces. In Figure 3.21c, striped fotomask was used to create micropatterned regions on S1. Circular photomask was used in S1 surface and the amine reactivity of the hydrogel surface was reconfirmed by fluorescein-amine molecule using a different pattern photomask (Figure 3.21d).

3.1.8. Biomolecule Immobilization

After confirming the amine-reactivity of the hydrogel surfaces by fluorescein-amine functionalization, the study was diversified by immobilization of biomolecules to these UV-activated nitrosobenzaldehyde regions. According to this purpose, firstly,

biotin-amine was immobilized on UV-activated hydrogel surfaces at room temperature for 3 hours. The biotin molecule, which is utilized to immobilize biomolecules, is a molecule that mediates binding of streptavidin to the surfaces. Since streptavidin possesses a strong affinity for biotin, it will only adhere to the surface in the presence of biotin. The biotin-amine molecule bonded to the reactive hydrogel with aldehyde groups due to its amine functionality. Following that the fluorescently labeled streptavidin molecule was immobilized to the biotinylated surfaces at room temperature for 30 minutes (Figure 3.22).

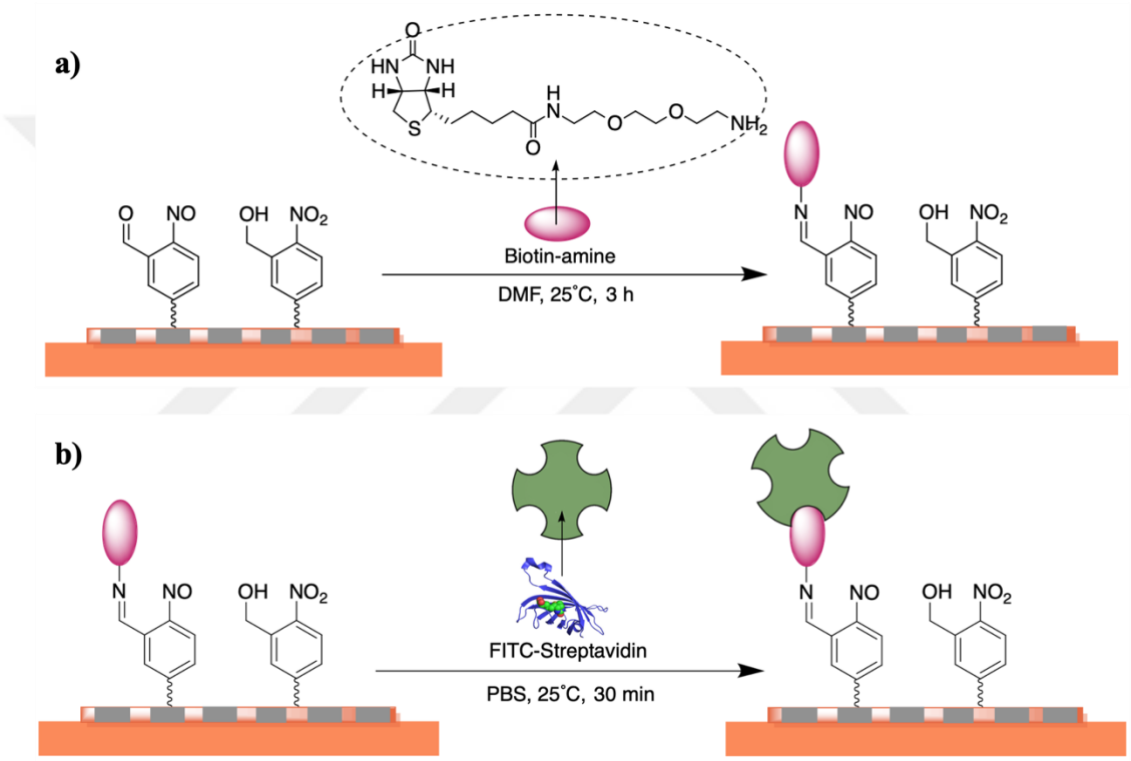

Figure 3.22: a) Schematic representation of biotin-amine immobilization of UV-activated hydrogel surfaces. b) Schematic representation of sreptavidin immobilization of UV-activated hydrogel surfaces.

To investigate the usability of the prepared surfaces for biomolecular immobilization, streptavidin immobilization was tested on biotinylated surfaces. Firstly, the UV-activated surface (S1) and the control, S1-reduced, were incubated in a biotin solution for 3 hours and then washed with an organic solvent. Subsequently, both surfaces were incubated in FITC-labeled streptavidin and washed with an aqueous solution and visualized under fluorescence microscope. According to the fluorescence microscope results, the UV-treated hydrogel sample exhibited a

fluorescent color indicating streptavidin binding (Figure 3.23a), while no fluorescent color was observed in the control group (Figure 3.23b). After confirming the feasibility of biomolecular immobilization on the surfaces, patterned bioimmobilization was attempted using photomasks. Photomasks with linear or circular features were placed on the hydrogel film and exposed to UV irradiation. Subsequently, the photomasks were removed, and the surfaces were incubated with biotin-amine, followed by streptavidin. The figure illustrates the successful achievement of patterned bioimmobilization (Figure 3.23c and Figure 3.23d). The protected areas, which lacked nitrosobenzaldehyde units, prevented the conjugation of biotin-amine, thereby ensuring the specific immobilization of streptavidin without any non-specific binding. As a result, hydrogel surfaces were fabricated to be specifically activated, while the non-activated parts exhibited antibiofouling properties, which are highly demanding for bioimmobilization studies. Consequently, micropatterned hydrogel surfaces that can be activated regioselectively by light, were biocompatible, and minimize non-specific adsorption have been developed for bioimmobilization studies.

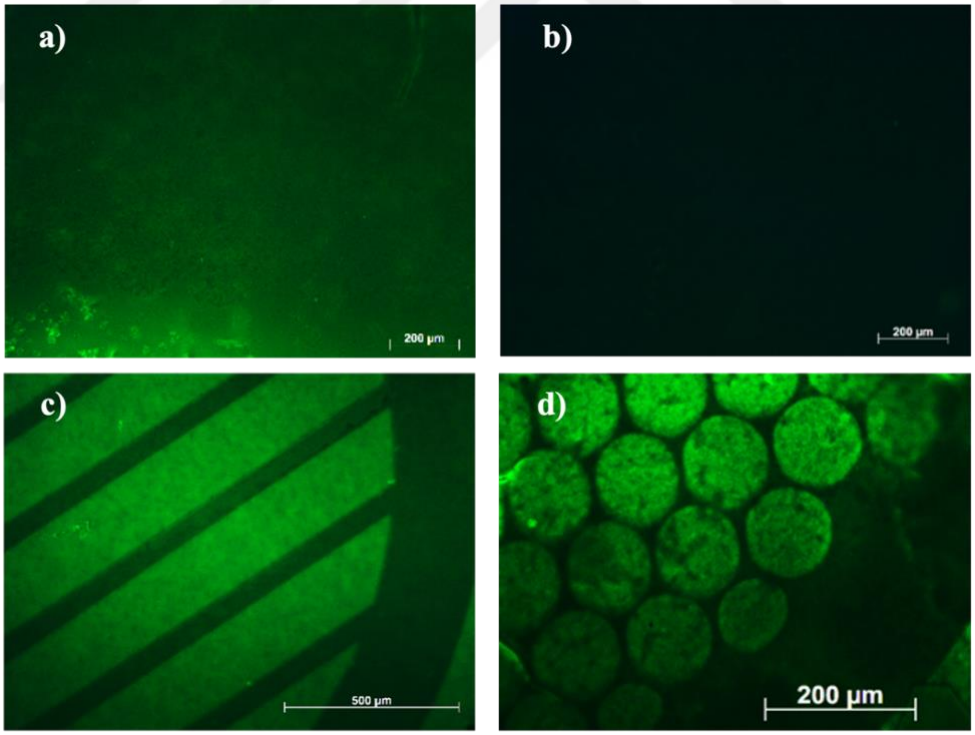

Figure 3.23: a) UV-activated S1 surface with biotin-streptavidin bioimmobilization. b) Control sample without UV activation after biotin-streptavidin bioimmobilization. c) S1 after biotin-streptavidin bioimmobilization under linear photomask. d) S1 after biotin-streptavidin bioimmobilization under circular photomask.

3.2. Amine-Reactive Dual-Functionalized Polymers and Their Surface Modifications

In this part of the study, hydrophilic polymers containing epoxy and isocyanate groups in their side branches were synthesized for the first time. In this way, isocyanate and epoxy functional dual-reactive copolymers were synthesized by RAFT polymerization. Afterwards, the orthogonal functionalization of polymers with various amine-containing molecules has been shown. Furthermore, it has also been demonstrated that the possible coating applications of polymers after isocyanate-amine functionalization, the side chain epoxide groups were used for attaching the polymers to a glass surface (Figure 3.24).

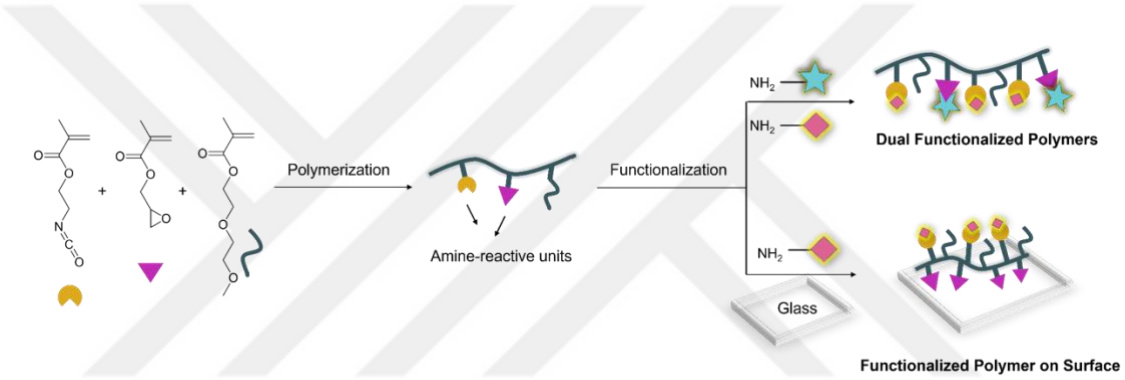

Figure 3.24: General illustration of dual functionalization of reactive polymers and their surface modification.

3.2.1. Synthesis of Copolymers Poly(ICM-r-GMA-r-DEGMA) (P1 and P2) with Using Reactive Monomers

To obtain copolymers which was poly(ICM-r-GMA-r-DEGMA), isocyanate, glycidyl and diethylene(glycol) group containing methacrylate monomers were used by using RAFT polymerization. 4-cyano-4-[((dodecylthio)carbonothioyl]thio]pentanoic acid was used as RAFT agent and ACVA used as azo-based initiator in this polymerization. The polymerization was carried out 65 °C in an oil bath for 6 h (Figure 3.25). As a result, the isocyanate and epoxide groups were both reactive to amine. On the other hand, the isocyanate group reacts more rapidly with an amine at room temperature, but the epoxide-amine reaction without a catalyst takes longer reaction times and higher temperatures. Furthermore,

the epoxide group of synthesized polymers was also used as a surface anchoring group. Also, diethylene(glycol) group promotes hydrophilicity while inhibiting non-specific protein adsorption.

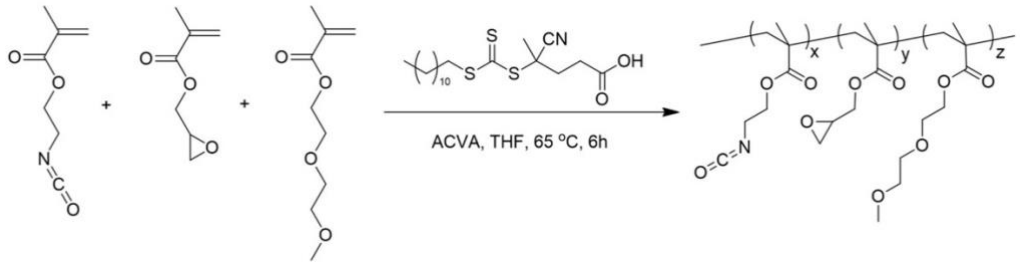

Figure 3.25: Synthesis of pendant isocyanate and epoxide-containing copolymers using reactive monomers.

Two different copolymers were synthesized (P1 and P2) by changing the ICM, GMA and DEGMA monomers ratio. For the P1 and P2 copolymers, ICM, GMA, and DEGMA have feed mole ratios of 10:40:50 and 25:25:50, respectively. The synthesized P1 and P2 were chemically characterized by FTIR and ^{1}H-NMR measurements (Figure 3.26 and Figure 3.27). According to the ^{1}H-NMR result, observed peaks at 2.85 ppm (CH, GMA), 3.41 ppm (CH_3, DEGMA) and 4.13 ppm ($COCH_2$, DEGMA and $COCH_2CH_2$ ICM) were consistent with the molecular structure.

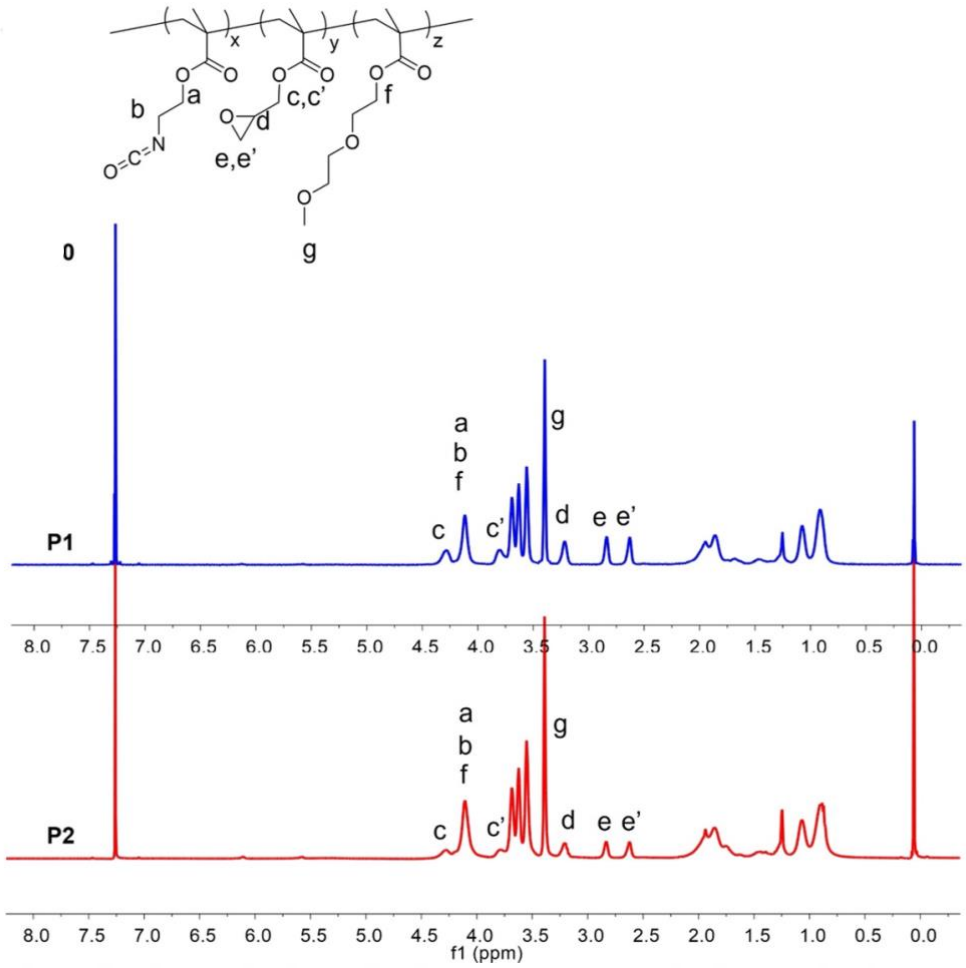

Figure 3.26: ^{1}H-NMR results of P1 and P2.

According to the FTIR result, presence of both reactive groups which are isocyanate and epoxide groups were confirmed. For P1 and P2, the peak at 2272 cm^{-1} was coming from isocyanate group with asymmetric stretching vibration of N=C=O. Simultaneously, a regular increase in the NCO peak at 2272 cm^{-1} was examined because P2 has higher amount of the isocyanate group when compared to P1. Furthermore, peak at 908 cm^{-1} was attributed to the epoxy ring in both P1 and P2. The epoxide group indicated peak intensity for P1 was higher than P2 due to the higher ratio of GMA monomer in P1.

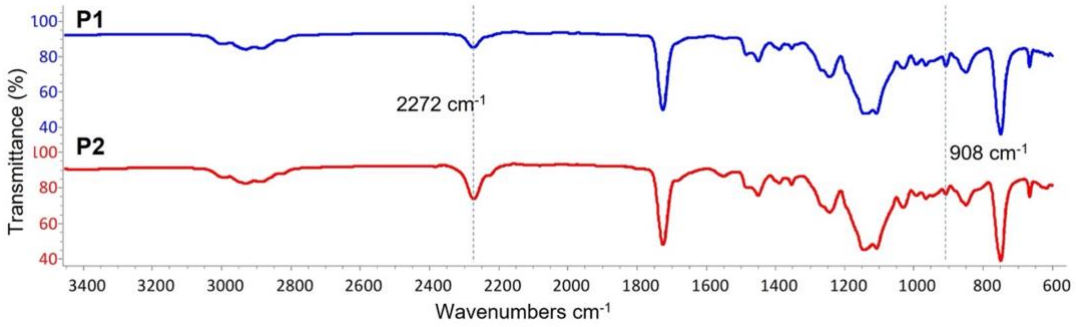

Figure 3.27: FTIR spectrum of P1 and P2.

Lastly, size exclusion chromatography analysis (GPC) was done to determine moleculer weight and dispersity of copoloylmers P1 and P2 (Table 3.2 and Figure 3.28). Before GPC analysis, the actual monomer ratio of copolymers was calculated with using their ^{1}H NMR peak integration values. For this purpose, peaks at at 2.85 ppm (CH, GMA), 3.41 ppm (CH_3, DEGMA) and 4.13 ppm ($COCH_2$, DEGMA and $COCH_2CH_2$ ICM) in the ^{1}H NMR spectrum were used. As a result of this, calculated ICM:GMA:DEGME ratio was found as 5:43:52 and 14:30:56, respectively for P1 and P2. According to the GPC result, M_n values were found as 14.6 kDa and 14.5 kDA, respectively for P1 and P2. Moreover, polydispersity index of P1 and P2 was found as 1.42 and 1.55, which is suitable values for polymer samples.

Table 3.2: Monomer Compositions and Molecular Weight Analysis of P1 and P2.

Copolymer[a]	**ICM:GMA:DEGMA (feed ratio)**[b]	**ICM:GMA:DEGMA (calculated)**[c]	**M_n**[d]	**PDI**[d]
P1	10:40:50	05:43:52	14.6 kDa	1.42
P2	25:25:50	14:30:56	14.5 kDa	1.55

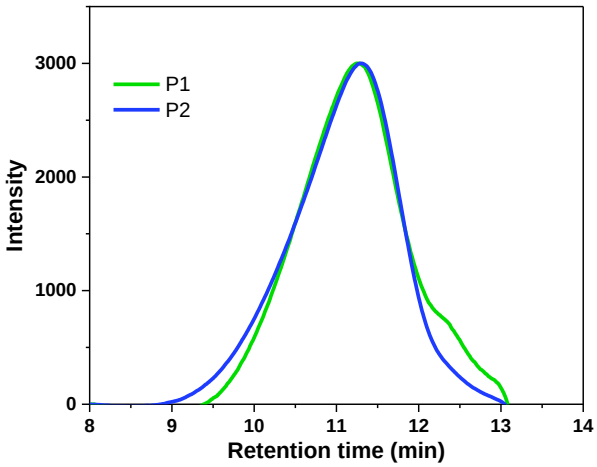

Figure 3.28: GPC analysis result for P1 and P2.

3.2.2. Dual-Functionalization of Copolymer P1 and P2 with Amines

Dual functionalization of copolymers was started with amine-containing model molecule, TFBA. Primarily, pendant isocyanate units on the copolymers P1 and P2 were functionalized with TFBA molecule for 1 h at room temperature. Following that, the functionalized polymer's epoxide group was functionalized with another amine-containing molecule, butylaniline (BA) for 18 h at 65 °C (Figure 3.29). In contrast to isocyanate-amine reaction, this epoxide-amine reaction requires longer reaction times and higher temperature.

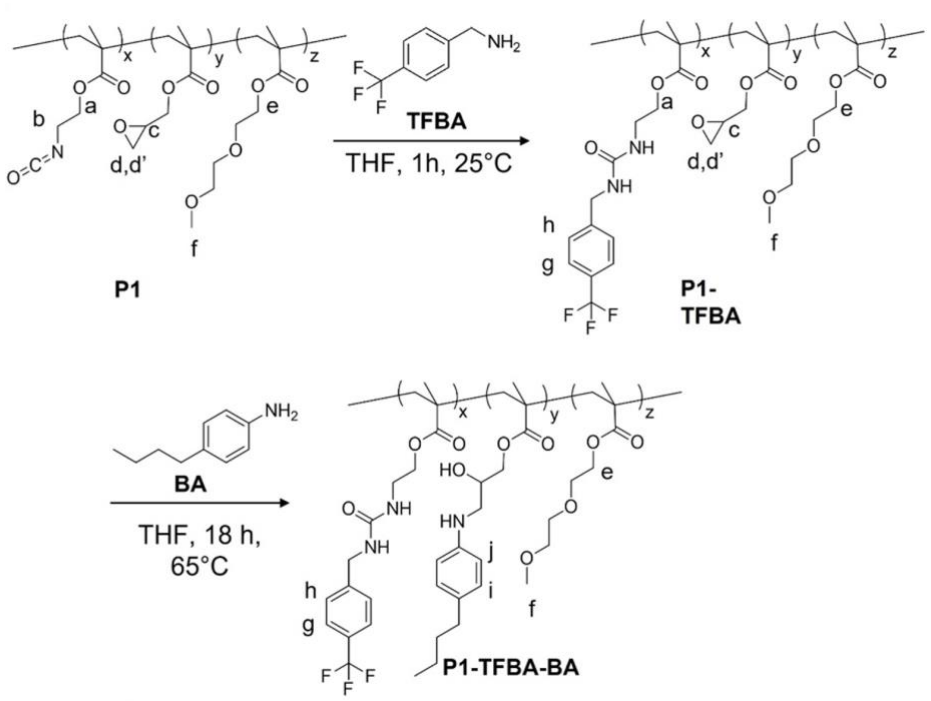

Figure 3.29: Dual functionalization of copolymer P1 with TFBA and Butylaniline.

Dual functionalized polymers were chemically characterized by FTIR and ^{1}H-NMR analyses (Figure 3.30 and Figure 3.31). According to the FTIR result of P1 after TFBA functionalization (P1-TFBA), peak at 1326 cm^{-1} was attributed to presence of TFBA molecule with C-CF_3 stretching. It was also confirmed with disappearance of peak at 2272 cm^{-1} which was coming from isocyanate group. Moreover, peak at 908 cm^{-1} which was coming from epoxide group was still protected after TFBA functionalization. Afterwards, another amine molecule, butylaniline, was used to functionalize of epoxide group of the P1-TFBA polymer. According to the FTIR spectrum, peak at 1522 cm^{-1} was contributed with butylaniline with aromatic C=C stretching vibration. Another proof of successful functionalization was the occurrence of peak at 817 cm^{-1}, which was coming from benzene ring of the butylaniline molecule. In addition, peak at 908 cm^{-1} which was attributed to epoxide group disappeared due to complete ring opening (Figure 3.30).

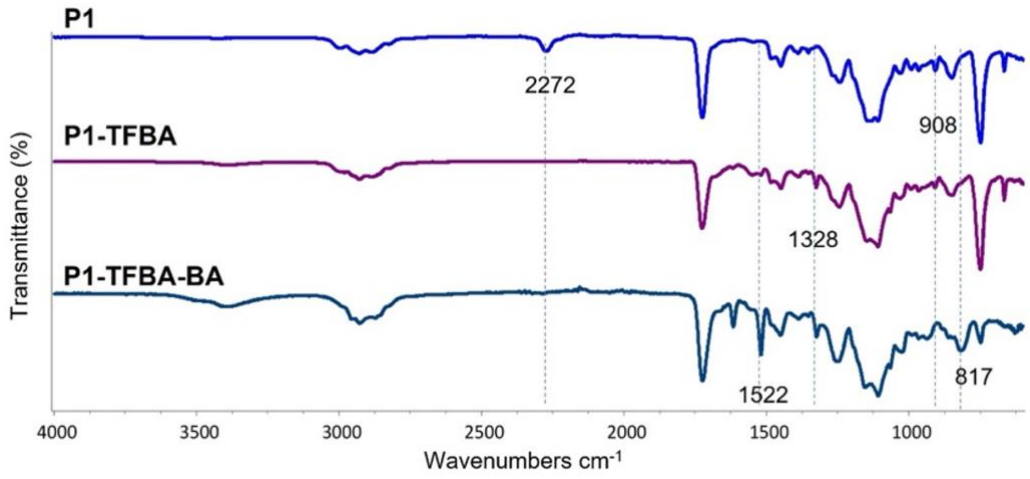

Figure 3.30: FTIR specturum analyses for P1, P1-TFBA and P1-TFBA-BA.

According to the NMR results of dual functionalized polymer, peaks at 7.56 and 7.40 ppm were attributed to the TFBA aromatic protons. After functionalization of butylanile, new peaks formation occurred at at 6.99 and 6.58 ppm and they were indicated as aromatic proton signals. Moreover, peaks at 2.84 and 2.65 were indicate characteristic epoxide protons and they were disappear after functionalization of butylaniline molecule (Figure 3.31).

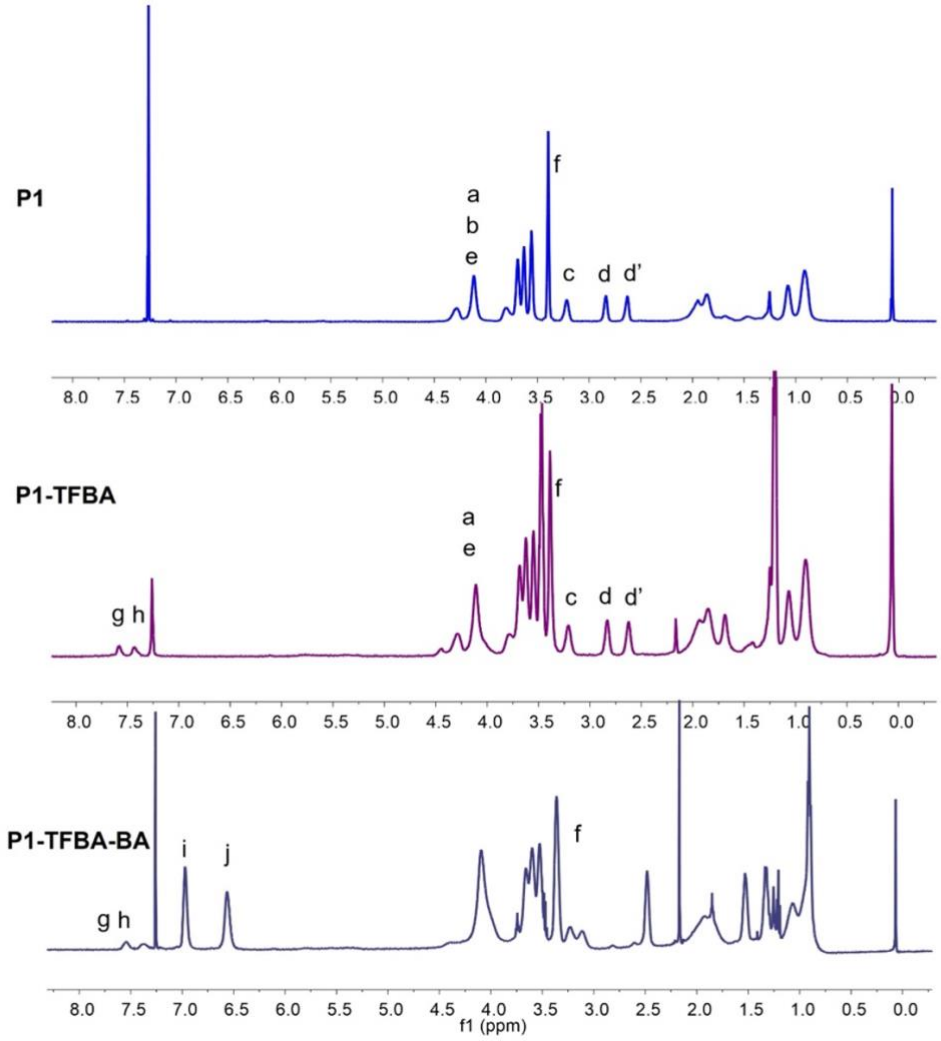

Figure 3.31: NMR results of P1, P1-TFBA and P1-TFBA-BA.

Furthermore, functionalized polymers P1-TFBA and P2-TFBA comparised with in FTIR and NMR analyses (Figure 3.32 and Figure 3.33). According to the FTIR spectrum, peak at 1326 cm^{-1} which related to C-F streching, was higher for P2-TFBA when compared to P1-TFBA. The reason of that the P2 copolymer included higher amount of isocyanate. In NMR result of P2-TFBA, specific TFBA aromatic protons were observed like as P1-TFBA (Figure 3.33). As expected, spesific TFBA aromatic protons in P2-TFBA were higher than P1-TFBA in NMR result due to isocyanate content.

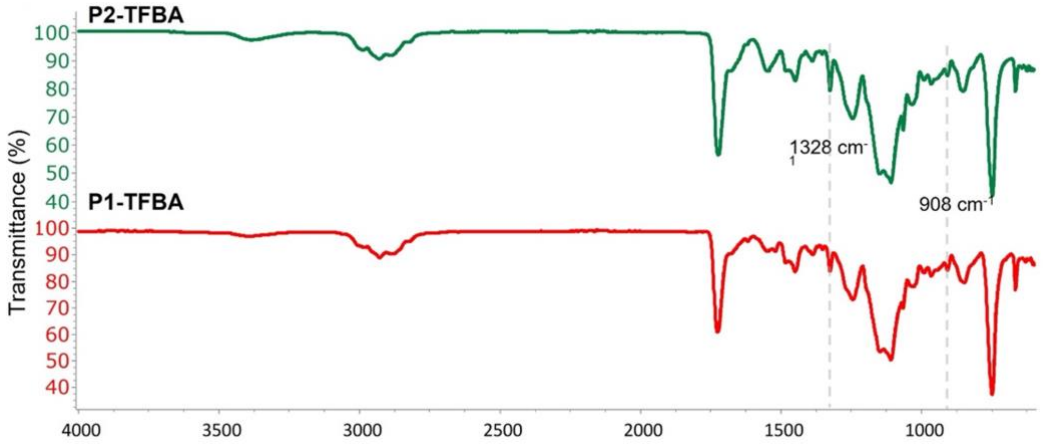

Figure 3.32: Comparision of P2-TFBA and P1-TFBA in FTIR spectrum.

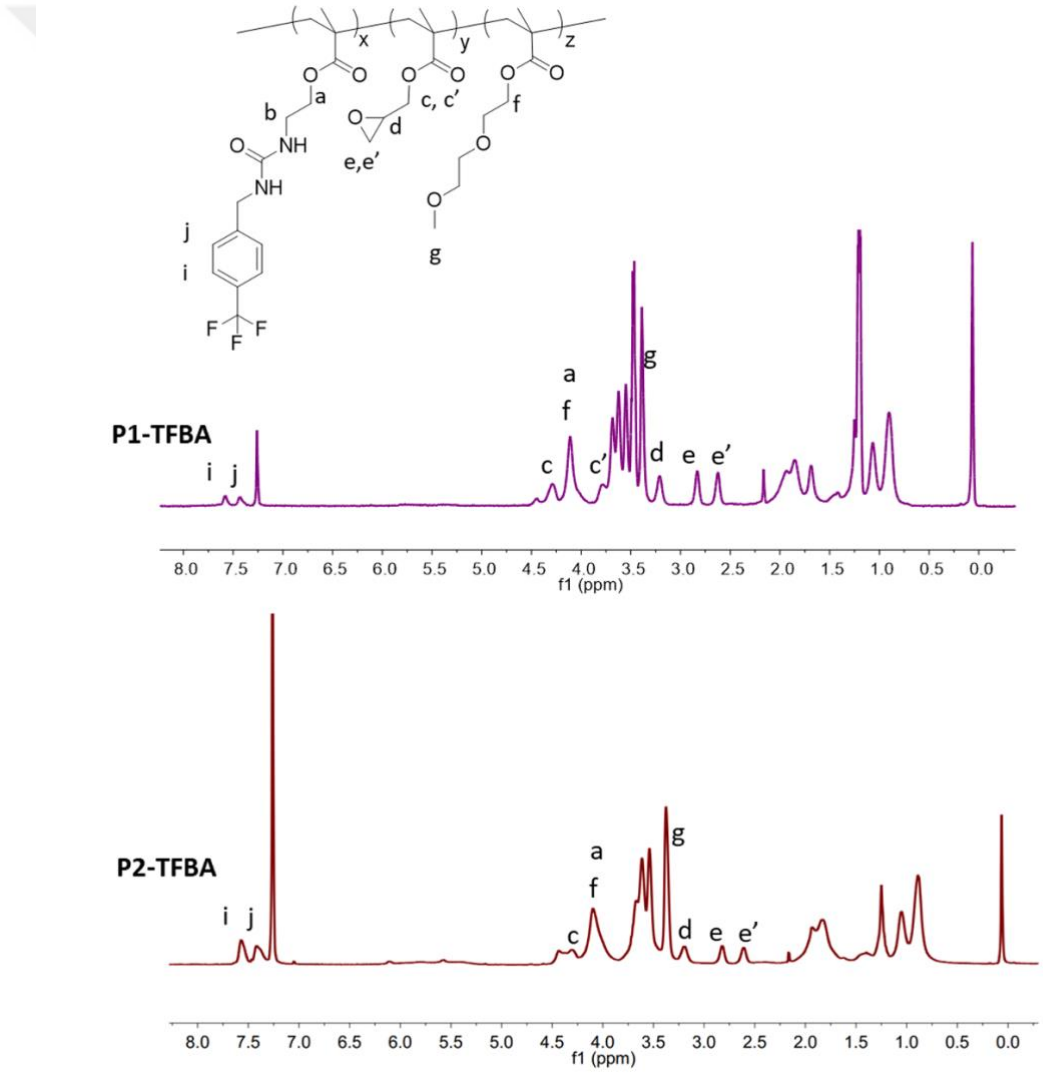

Figure 3.33: NMR results of P1-TFBA and P2-TFBA.

3.2.3. Polymeric Coatings on Glass Surfaces

3.2.3.1. Polymeric Coatings on Glass Surfaces for P1-TFBA and P2-TFBA

The epoxide chains of the functionalized polymers were used as surface anchoring group for surface coating through epoxy-amine reaction. Functionalized polymers P1-TFBA and P2-TFBA were used to coat on glass surfaces. They were dissolved in DMF and spread over NaOH treated glass surfaces. To obtain TFBA functionalized polymeric coated surfaces, they were cured at 70 °C for 18 h (Figure 3.34).

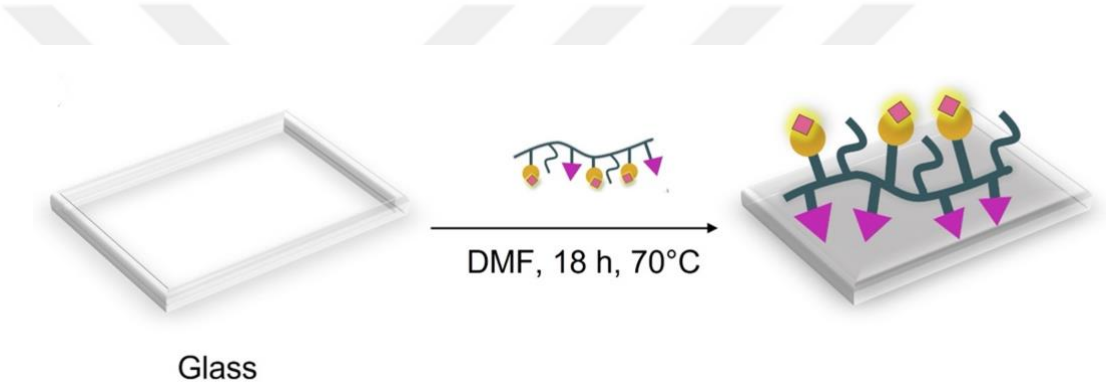

Figure 3.34: Schematic representation of polymeric coatings on glass surface after functionalization of TFBA via epoxy-amine reaction.

The chemical compositions of the coated surfaces were determined via XPS measurements (Figure 3.35). XPS measurements also verified the existence of polymeric coatings. According to XPS result, F1s signal at 690 eV in the survey verified F atoms of TFBA molecule on polymeric coating. Since P2-TFBA contained higher ratio of isocyanate groups, the peak intensity of F1s and ratio of F/C atoms were higher in P2-TFBA like as in FTIR spectrum result of P2-TFBA and P1-TFBA. Moreover, atom percentages of each atom for P1-TFBA and P2-TFBA were calculated based on XPS analysis result (Table 3.3). According to result, percentage of F atom for P2-TFBA sample was higher than P1-TFBA, as expected due to the higher percentage of isocyanate groups in P2-TFBA sample.

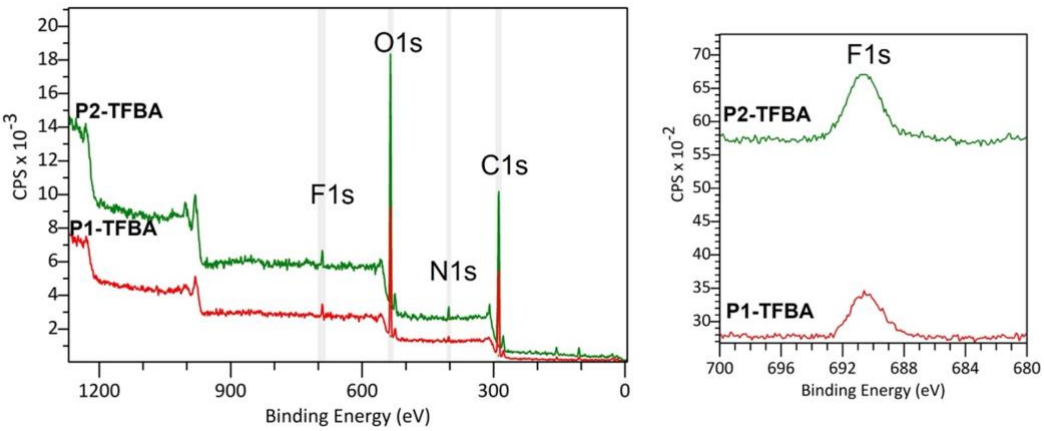

Figure 3.35: XPS measurement of coated glass surfaces for P1-TFBA and P2-TFBA.

Table 3.3: Atom percentages of each P1-TFBA and P2-TFBA surfaces.

Sample	Atom percentage			
	F 1s	C 1s	N 1s	O 1s
P1-TFBA	0.96	72.05	1.47	25.52
P2-TFBA	1.26	69.23	1.64	27.88

3.2.3.2. Polymeric Coatings on Glass Surfaces for P1-Rhodamine

As before, the epoxide groups on the functionalized copolymer were employed as surface anchoring group for glass surfaces. First, copolymer P1 was functionalized with a fluorescent dye containing amine molecule which was rhodamine-amine via isocyanate-amine reaction at 70 °C for 18 h. After that, functionalized polymer P1 was coated on glass surface through epoxide groups of the polymer (Figure 3.36). Chemical characterizations of coated P1-Rhodamine polymer were done by using FTIR and ^{1}H-NMR analyses (Figure 3.37 and Figure 3.38).

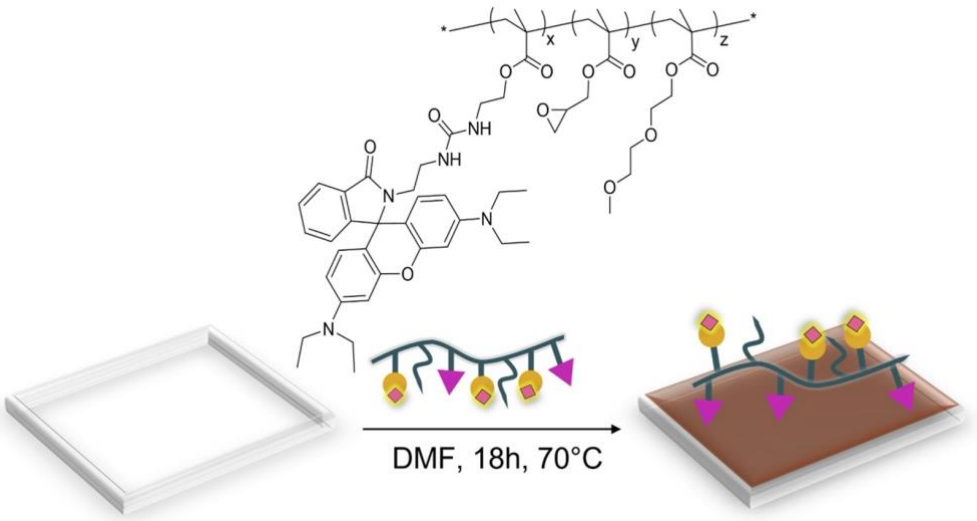

Figure 3.36: Schematic representation of P1-Rhodamine polymer on glass surface.

According to the FTIR result of P1-Rhodamine polymeric surface, peak at 2272 cm^{-1} disappeared after functionalization with rhodamine-amine via isocyanate-amine reaction (Figure 3.37). Peaks at 1551 cm^{-1} indicating urea, 1613 cm^{-1} and 1514 cm^{-1} indicating aromatic C=C were newly formed in FTIR spectrum as a result of conjugation of rhodamine-amine. Furthermore, peak at 908 cm^{-1} indicating epoxide group still protected after functionalization.

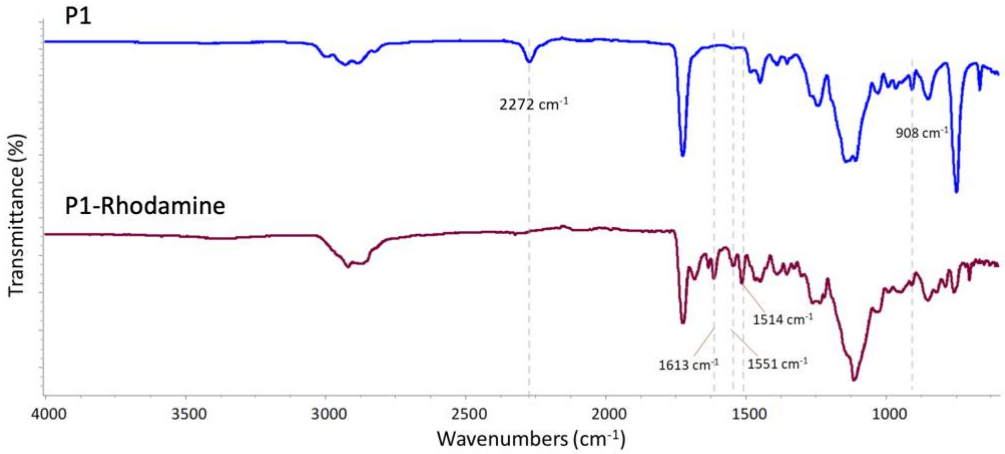

Figure 3.37: Comparison of P1 and P1-Rhodamine in FTIR spectrum.

^{1}H-NMR result was also confirmed successful conjugation of rhodamine-amine for P1 copolymer (Figure 3.38). The ^{1}H-NMR results clearly showed that the glycidyl groups (c, d, and d') of the copolymer were still present in the P1-Rhodamine polymer.

In addition, new peaks formation occurred due to the conjugation of rhodamine-amine molecule. It was suggested that indicated letters (h, i, j, k, l, m, and n) were coming from rhodamine-amine.

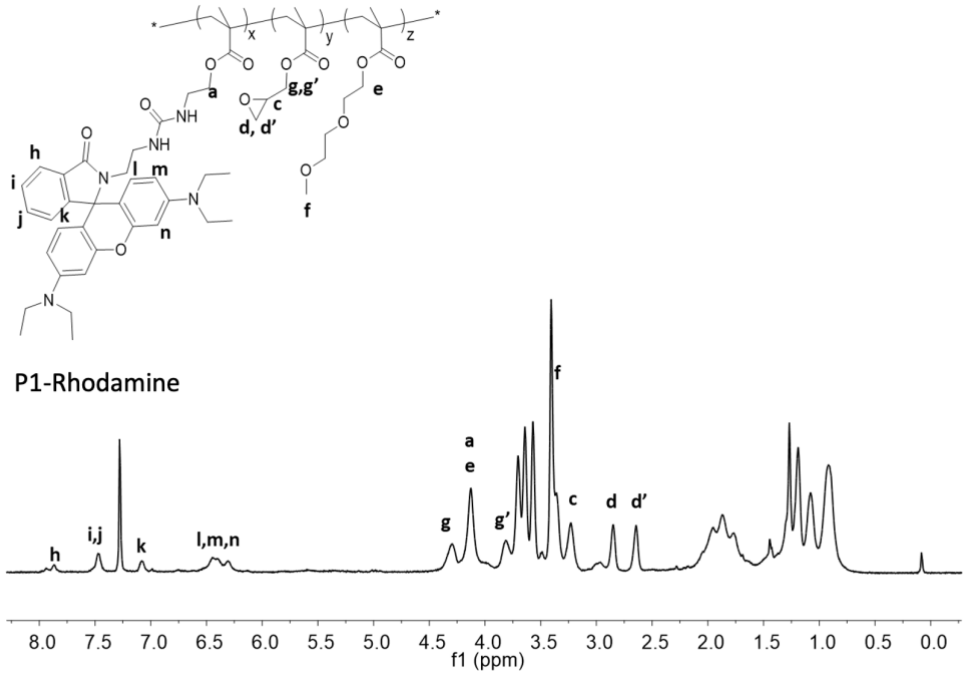

Figure 3.38: ^{1}H-NMR result of P1-Rhodamine polymer.

Lastly, the coated surface of P1-Rhodamine was examined with fluorescence microscope to observe fluorescence property of functionalized polymer (Figure 3.39). The coated surface had a slight-yellow color (Figure 3.39a-I), when compared to non-treated surface (Figure 3.39a-II). Although the functionalization was proceed on glass surface, it did not lost transparent property. According to fluorescence microscope result, the red color was obtained due to fluorescence property of rhodamine-amine molecule (Figure 3.39b).

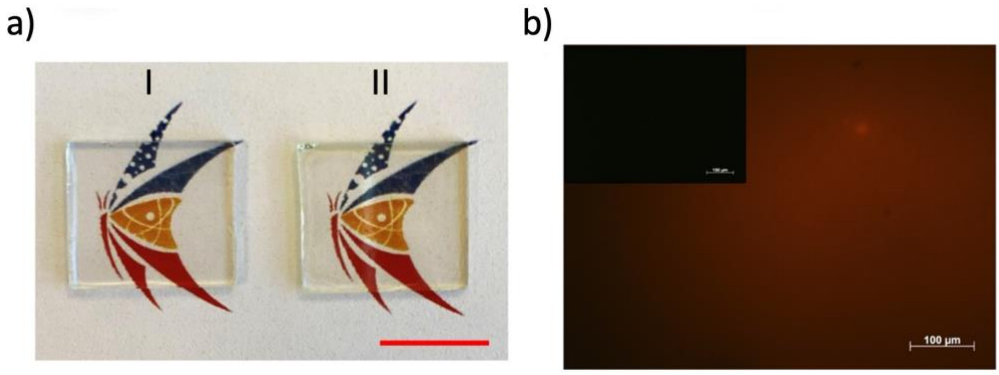

Figure 3.39: a) Non-coated (I) and coated (II) surfaces (scale bar is 1 cm). b) Fluorescence microscope images of P1-Rhodamine and non-coated glass surface (inset).

4. CONCLUSION

In the first part of the study, UV-activable, orthonitrobenzyl group containing amine reactive zwitterionic hydrogel surfaces in various ratios (10:90, 20:80, 50:50) were developed for biomolecular immobilization studies. Furthermore, photomasks with various shapes were utilized to produce different patterns on the hydrogel surfaces. In this way, amine reactive nitrosobenzaldehyde groups were produced in the light-transmitting regions of the photomask. Functionalizability of reactive micropatterns was shown by amine containing dye molecules and fluorescently labeled bio-molecules. Non-specific binding in non-UV-activated regions of the photomask was prevented with the help of zwitterionic groups. It is thought that this approach will make a great contribution in biomedical field, especially in bioimmobilization and biosensor studies.

The second part of the study reports the synthesis of copolymers containing isocyanate and epoxide groups as well as the examination of their orthogonal functionalization using various amine-containing molecules. In order to demonstrate the potential of coating applications, functionalized polymer generated by isocyanate-amine reaction was coated onto glass surfaces through epoxide groups. Furthermore, it has been shown that the number of isocyanate and epoxy groups in the polymer chain can be regulated by adjusting the feed rates of the monomers. It is possible to anticipate that this method might be improved to create varied functional surfaces and polymers for utilizes in materials and biomedical fields owing to the discovered system's ease of use and efficacy in functionalization.

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BIOGRAPHY

Nisa DEMİRBİLEK achieved her bachelor's degree from the Department of Molecular Biology and Genetics at Gebze Technical University in 2021. Following that, she started master's degree program in the Institute of Biotechnology at Gebze Technical University in the same year. Her master thesis was supported by TÜBİTAK 2210/C program. She has two published article which titled ""Colorimetric and Electrochemical Detection of SARS-CoV-2 Spike Antigen with a Gold Nanoparticle-Based Biosensor" and "Pendant Isocyanate and Epoxide- Containing Copolymers: Synthesis, Sequential Dual-Functionalization with Amines, and Surface Modifications". Moreover, she made two poster presentations during her master education.

APPENDICES

Appendix A: Copyrights of Figures/Tables/Schemas Taken from Literature

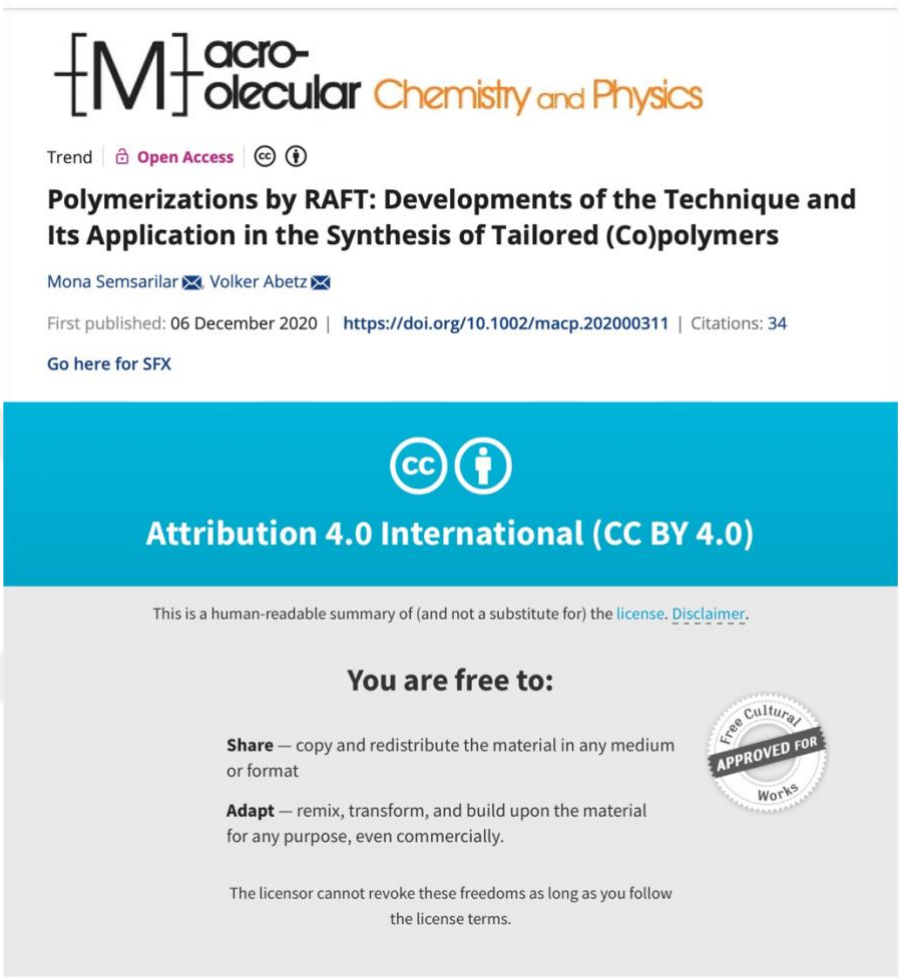


Figure A.1: Copyright of article with named “Polymerizations by RAFT: Developments of the Technique and Its Application in the Synthesis of Tailored (Co)polymers”.

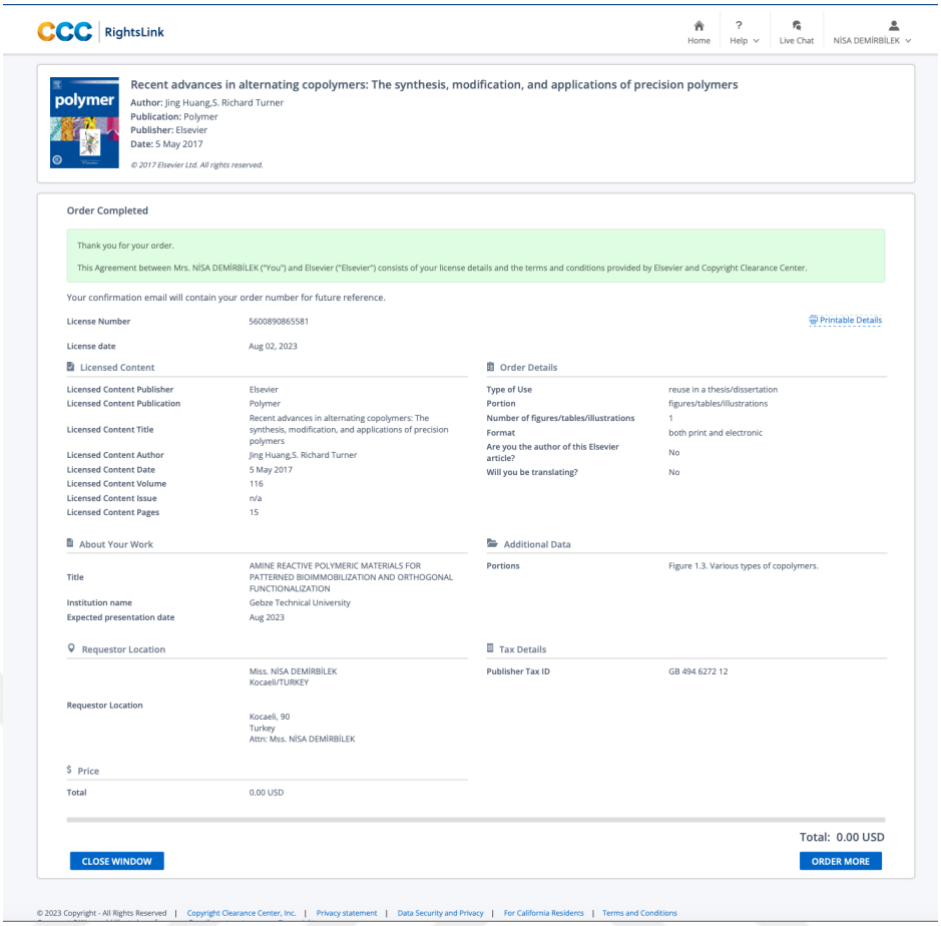


Figure A.2: Copyright of article with named “Recent advances in alternating copolymers: The synthesis, modification, and applications of precision polymers”.

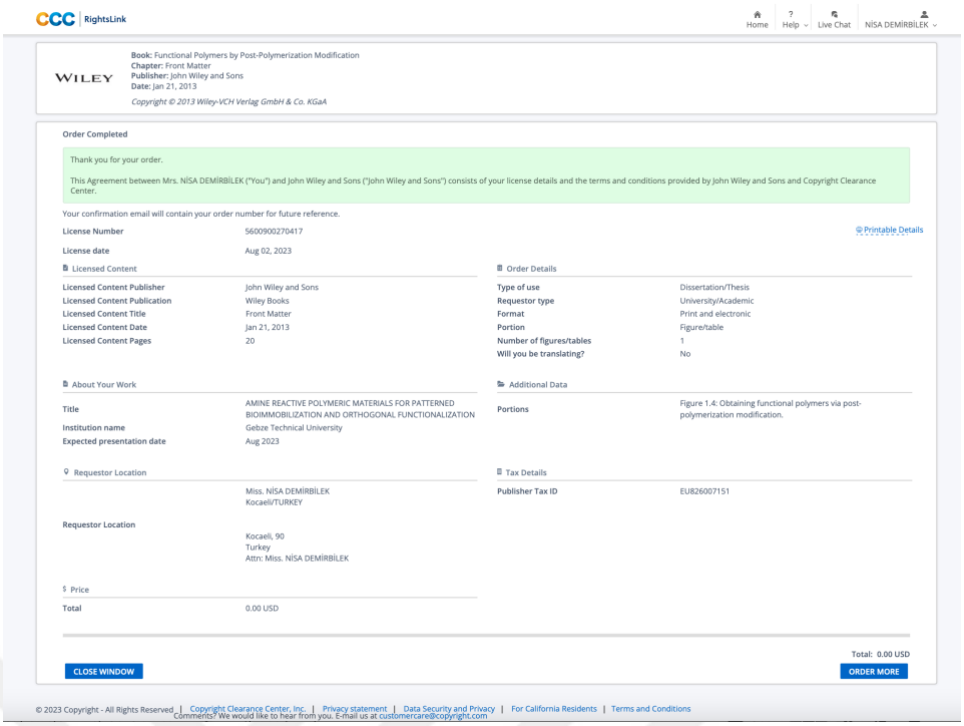
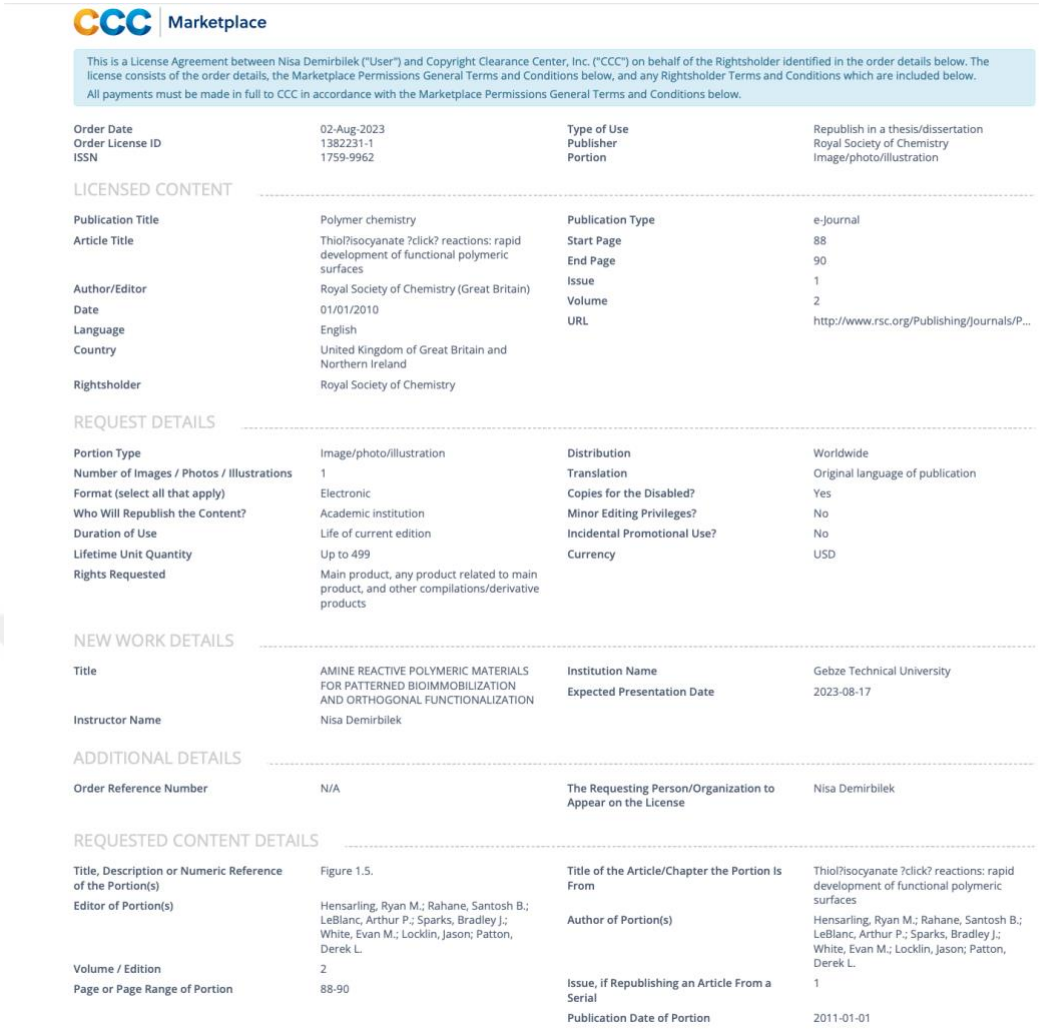


Figure A.3: Copyright of book chapter with named “Functional Polymers by Post-Polymerization Modification”.



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Publication Title	Polymer chemistry	Publication Type	e-Journal
Article Title	Thiol?isocyanate ?click? reactions: rapid development of functional polymeric surfaces	Start Page	88
		End Page	90
Author/Editor	Royal Society of Chemistry (Great Britain)	Issue	1
Date	01/01/2010	Volume	2
Language	English	URL	http://www.rsc.org/Publishing/Journals/P...
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		Expected Presentation Date	2023-08-17
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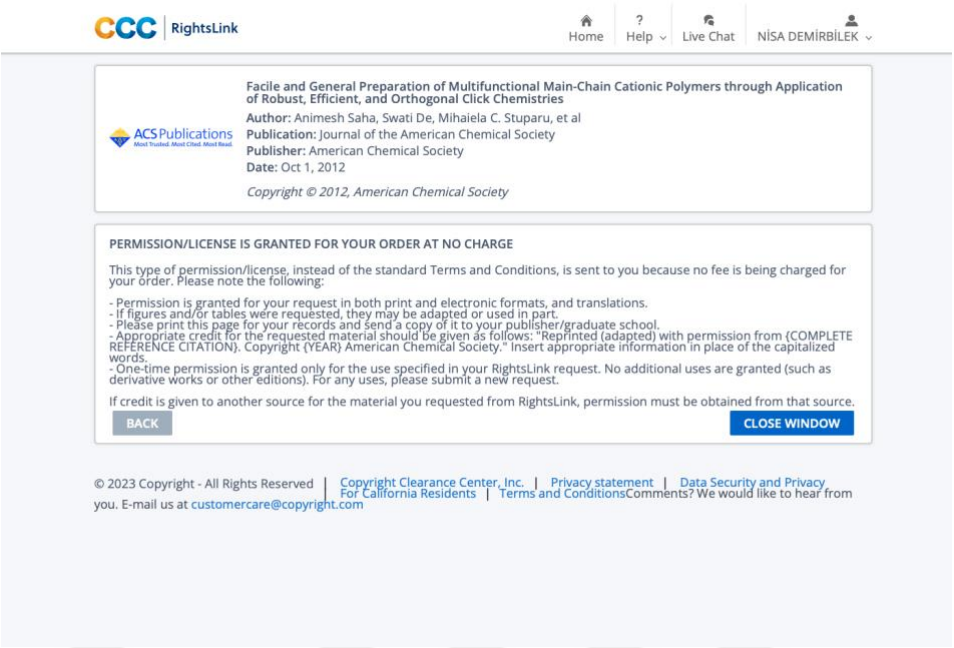

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ACS Publications

Facile and General Preparation of Multifunctional Main-Chain Cationic Polymers through Application of Robust, Efficient, and Orthogonal Click Chemistries

Author: Animesh Saha, Swati De, Mihaiela C. Stuparu, et al
Publication: Journal of the American Chemical Society
Publisher: American Chemical Society
Date: Oct 1, 2012

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Article Title	Orthogonal thiol-ene 'click' reactions: a powerful combination for fabrication and functionalization of patterned hydrogels.	Publication Type	e-Journal
		Start Page	8894
Author/Editor	Royal Society of Chemistry (Great Britain)	End Page	8897
Date	01/01/1996	Issue	63
Language	English	Volume	53
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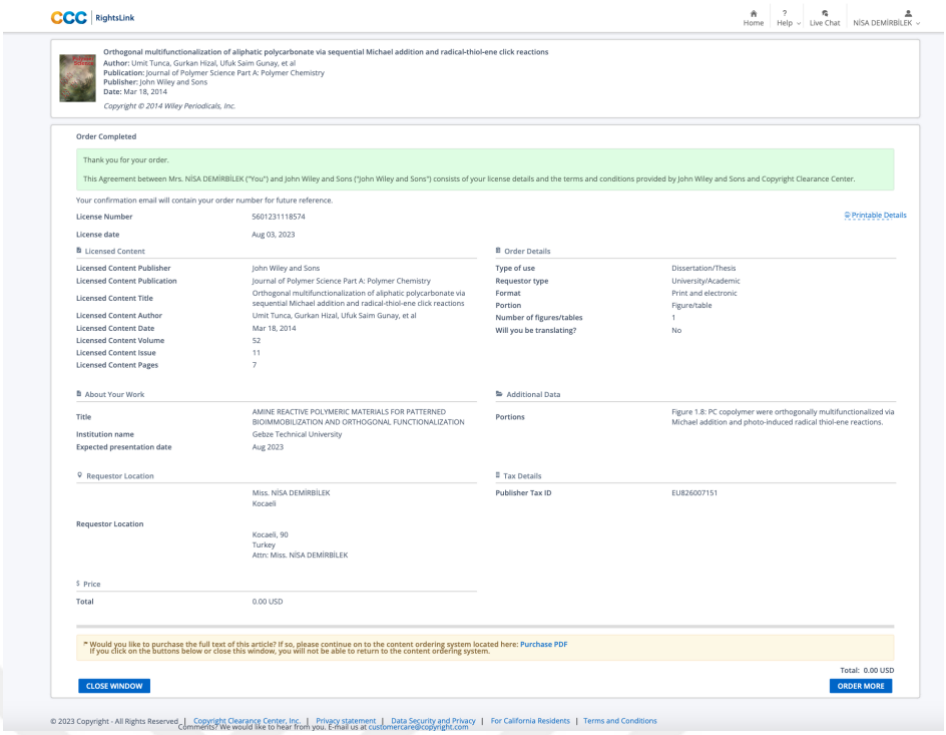

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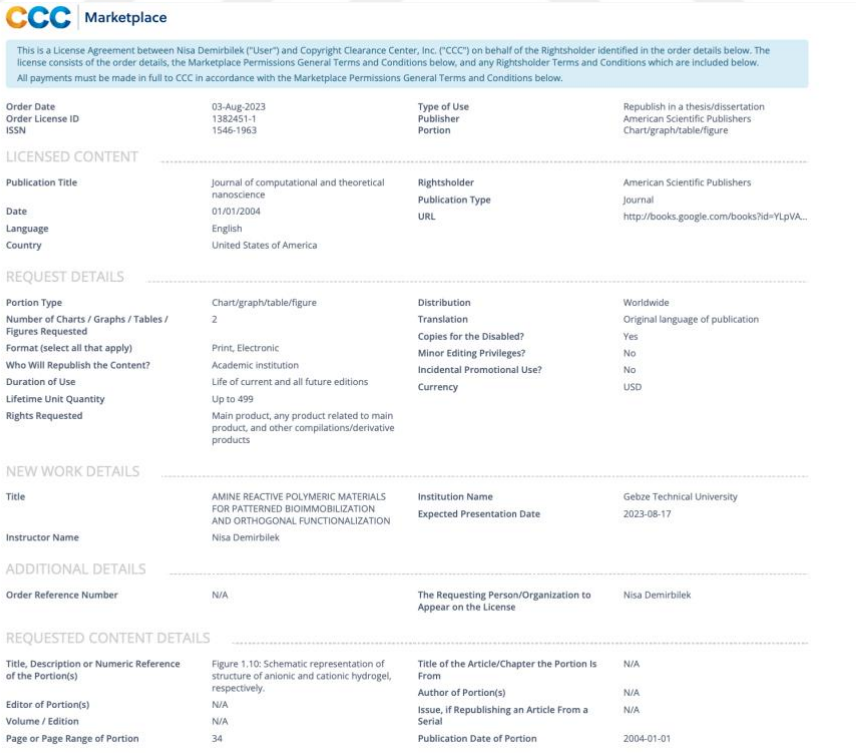

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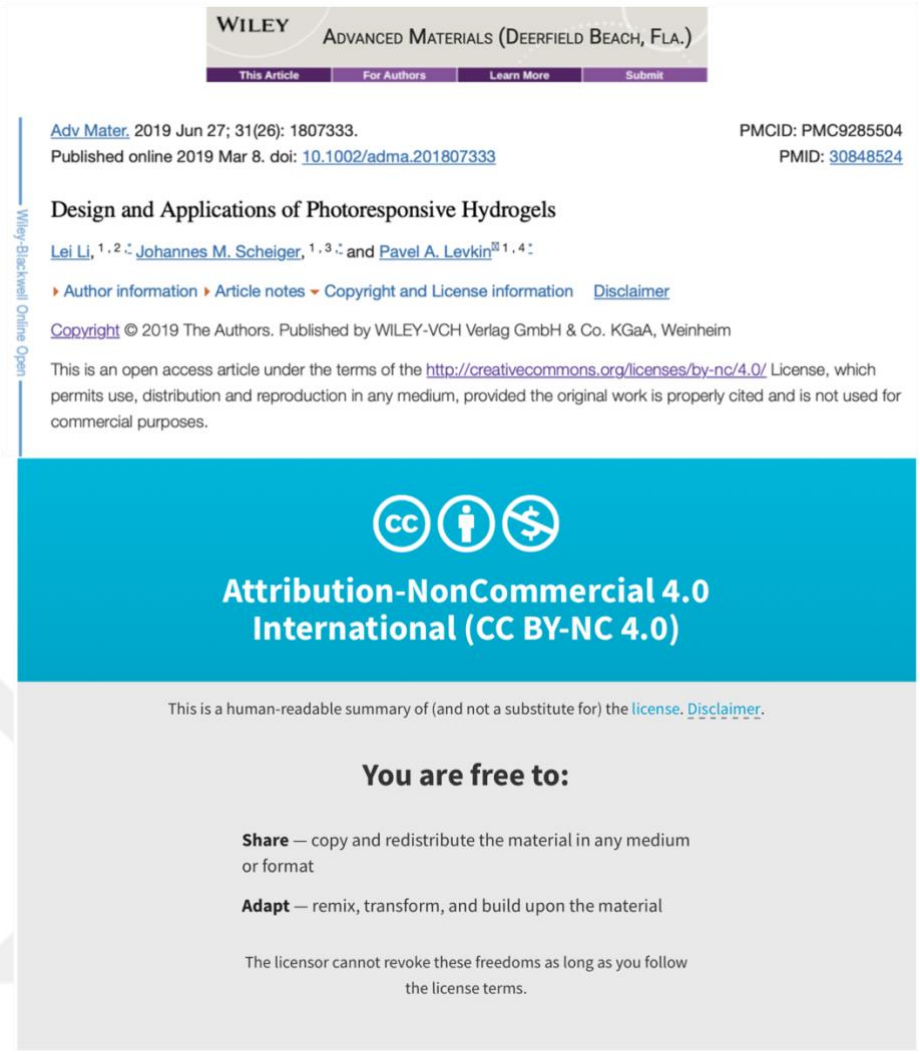

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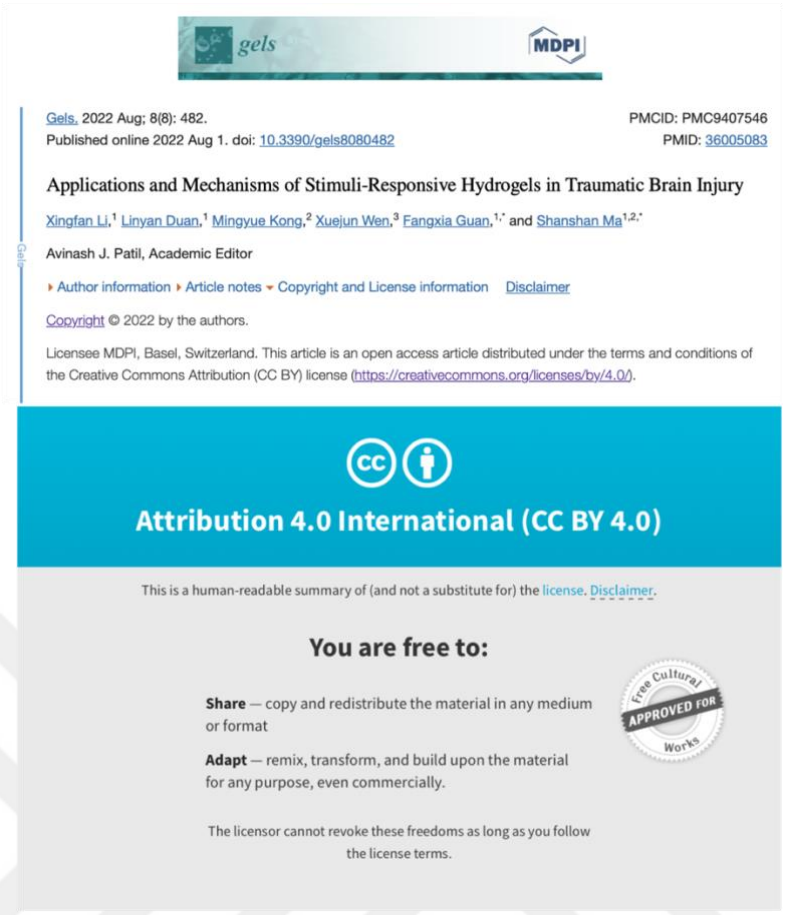

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Published online 2022 Aug 1. doi: 10.3390/gels8080482

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Applications and Mechanisms of Stimuli-Responsive Hydrogels in Traumatic Brain Injury

Xingfan Li,[1] Linyan Duan,[1] Mingyue Kong,[2] Xuejun Wen,[3] Fangxia Guan,[1,*] and Shanshan Ma[1,2,*]

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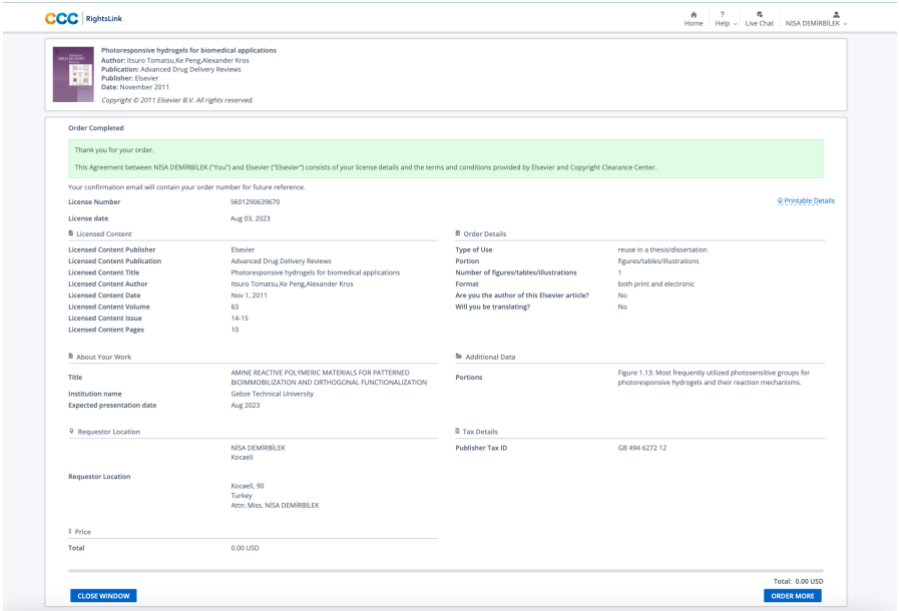

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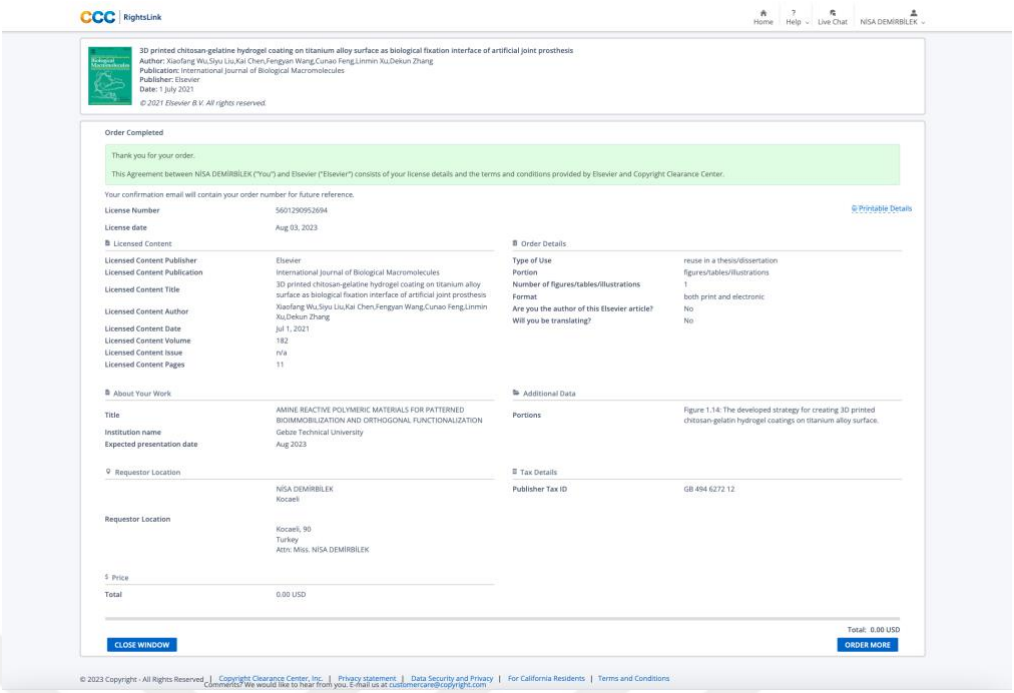

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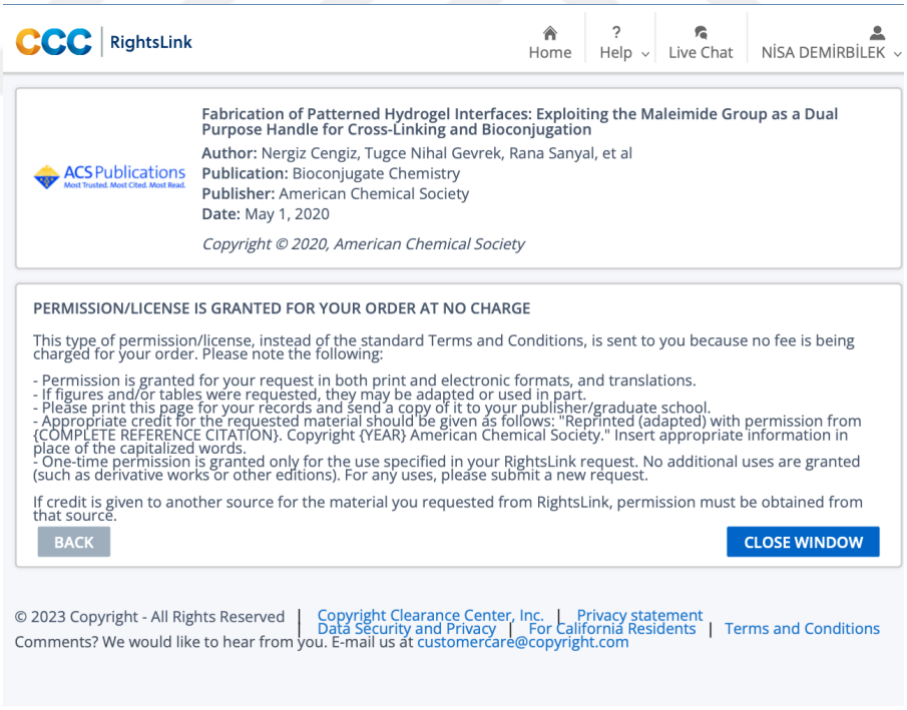

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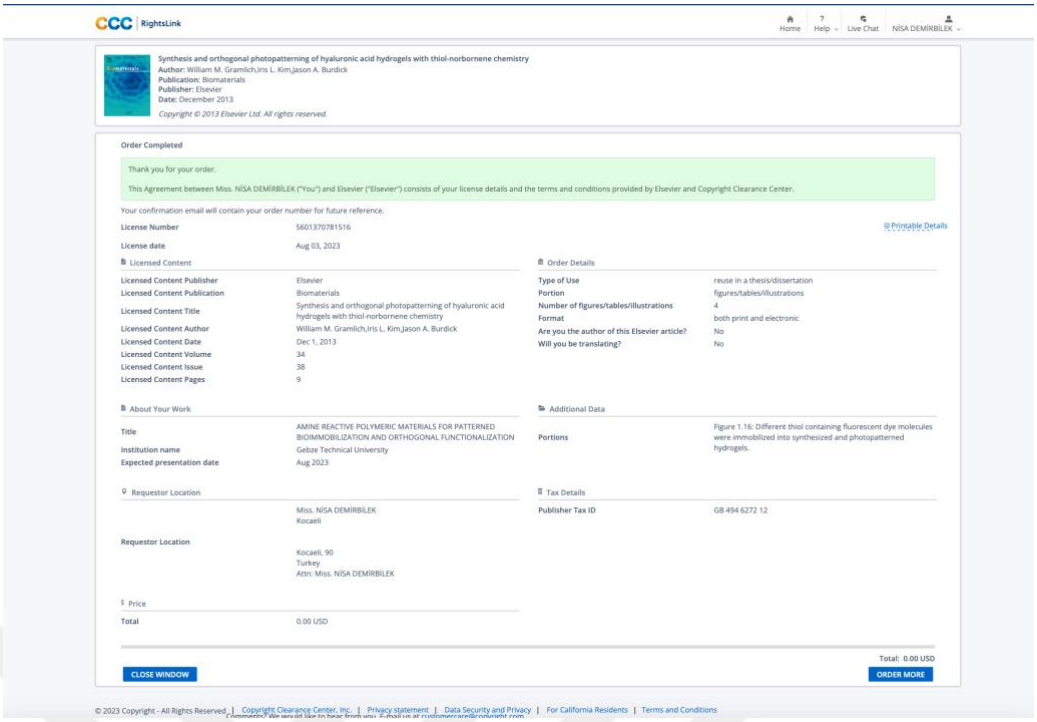

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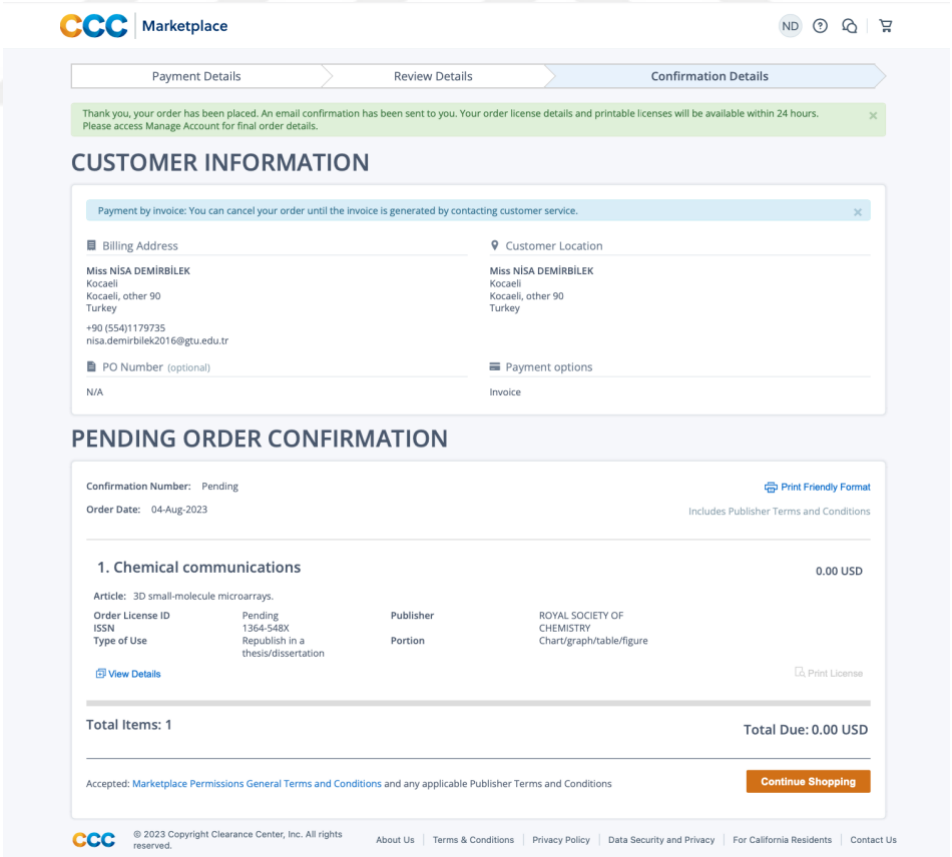

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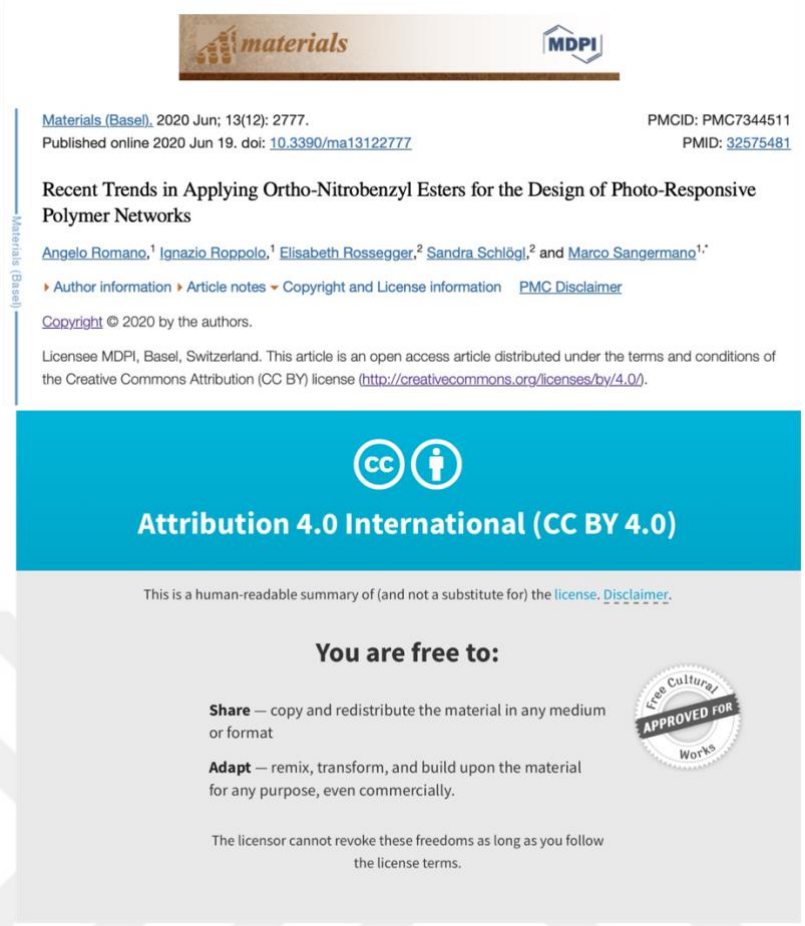

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Recent Trends in Applying Ortho-Nitrobenzyl Esters for the Design of Photo-Responsive Polymer Networks

Angelo Romano,[1] Ignazio Roppolo,[1] Elisabeth Rossegger,[2] Sandra Schlögl,[2] and Marco Sangermano[1,*]

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