REPUBLIC OF TURKEY

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INSTITUTE OF APPLIED SCIENCES

DESIGN OF DNA- HYDROGEL BASED QUARTZ CRYSTAL MICROBALANCE

MERİÇ CANSU ÇINAR

MASTER THESIS

DEPARTMENT OF MEDICAL ENGINEERING

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ISTANBUL-2020

DECLARATION

I, the undersigned, hereby declare that this research is my own original work and that all sources have been accurately reported and acknowledged, and that this document has not been previously, in its entirety or in part, submitted at any university in order to obtain academic qualifications.

14.09.2020
Meriç Cansu Çınar

ACKNOWLEDGEMENTS

I would like to express my special thanks of gratitude to my advisors Assist. Prof. Dr. Ceyhun Ekrem Kırımlı and Assist. Prof. Dr. Özgül Gök for their guidance and support in completing my thesis dissertation.

This research has been supported by Acıbadem Mehmet Ali Aydınlar University Scientific Research Commission (ABAPKO Project No: 2019/04-05)

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Explanation
3D	Three dimensional
AC	Alternating current
APS	Ammonium persulfate saline
Au	Gold
Bis	Bis-acrylamide
°C	Celsius
C	Mass sensitivity constant
ΔD	Dissipation change
Δf_n	Resonant frequency change
Δm	Mass coupled to the surface
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
$E_{dissipated}$	Energy dissipated in one cycle of oscillation
E_{stored}	Stored energy
ECM	Extra cellular matrix
H	Hydrogen
HEMA	Hydroxyethyl methacrylate
HFIM	Poly(hexa-fluoroisopropylmethacrylate)
LCST	Low critical solution temperature
µL	Microliters
mg	Miligram
MHz	Megahertz
mL	Mililiters
n	Harmonic number
N_2	Nitrogen
Oligo A	Oligonucleotide A
Oligo T	Oligonucleotide T
Π	Number of pi

PBS	Phosphate buffered saline
PEG	Polyethylene glycol
PEO	Poly(ethyleneoxide)
pH	Potential of hydrogen
pHEMA	Poly (2- hydroxyethylmethacrylate)
PMMA	Polymethylmethacrylate
pNIPAM	Poly(N-isopropylacrylamide)
Poly A	Polyadenylic acid
Poly T	Polythymidylic acid
PPO	Poly(propylene oxide)
QCM	Quartz crystal microbalance
QCM-D	Quartz crystal microbalance-dissipation monitoring
RNA	Ribonucleic acid
SAP	Superabsorbent polymers
SH	Sulfhydryl
SPH	Superporous hydrogel
SPR	Surface plasmon resonance
ssDNA	Single stranded deoxyribonucleic acid
τ	Decay time constant
TEMED	Tetramethylethylenediamine
Thi	1,6-hexanedithiol
Tris-HCl	Tris hydrochloride
UV	Ultraviolet
UV-vis	Ultraviolet-visible
W_d	Weight of hydrogel in dry state
W_s	Weight of hydrogel in swollen state
X-Linker	Crosslinker

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SUMMARY

There is an urgent need of early diagnosis of diseases which can be life threatening such as cancer. Most of the methods are not able to detect such diseases at early stages due to the lack of sensitivity. Additionally, these methods are time consuming where the time is the most valuable concept in the life of people. The goal of this study was to represent a biosensor design which combines DNA hdyrogels and quartz crystal microbalance (QCM) together, and as a result to obtain a very sensitive biosensor which gives nearly real-time responses to the changes in hydrogel. In the design of this study, DNA hydrogel was functionalized with thiol groups to immobilize it on to the quartz crystals gold surface. By measuring the changes in dissipation rate for DNA hydrogel, the specific biorecognition elements at the sample were detected.

Key Words: Biosensors, DNA Hydrogel, Early Diagnosis, Hydrogels, Quartz Crystal Microbalance.

ÖZET

DNA- Hidrojel Tabanlı Kuvars Kristal Mikroterazi Tasarımı

Bu tezin amacı, hastalık tanısında kullanılabilecek, DNA hidrojel tabanlı bir kuvars kristal mikroterazi biyosensör sistemi tasarlamaktır. Tasarımı, tanısı yapılacak hastalığa göre kolayca değiştirilebilecek olan DNA hidrojelinin çapraz bağlı ağ iskelesi akrilamid monomeri ve bisakrilamid çapraz bağlayıcı molekülü yardımıyla oluşturulacak ve jelleşme amonyum persülfat (APS) ve N,N,N',N'-Tetrametilendiamin (TEMED) ile sağlanacaktır. Bu hidrojel, yapısındaki akrilat reaktif grupları ile tepkimeye girebilen serbest tiyol grupları içeren diğer bir çapraz bağlayıcı (heksan ditiyol) yardımıyla kuvars kristalin altın yüzeyine tutturulacaktır. Çünkü serbest tiyol gruplarının altın yüzeylerle çok güçlü koordine olarak bağlanabildikleri bilinmektedir. Fiziksel, kimyasal, morfolojik ve mekanik analizleri yapılacak olan bu hidrojellerin yapısına hangi DNA dizisinin sabitleneceği (immobilize edileceği) hidrojel bağlı biyosensörün kullanılması hedeflenen hastalığa özgü olarak seçilebilecek şekilde tasarlanacaktır.

Anahtar Kelimeler: Biyosensörler, DNA Hidrojel, Erken Teşhis, Hidrojeller, Kuvars Kristal Mikroterazi

1. BACKGROUND AND THE AIM OF THE STUDY

Since their introduction to the science world, biosensors have gained attraction due to their precise and accurate results to detect specific biological molecules in the medium. Among biosensors, quartz crystal microbalance (QCM) has attracted many scientists due to its advantages such as cost effectiveness, easy-usage, high specificity and high sensitivity. QCM detects the resonance frequency changes via its piezoelectric characteristic. These changes in resonance frequency is linked to the deposited mass on the crystal. Additionally, surface of the QCM can be modified with different components. One of these components are deoxyribonucleic acid (DNA) hydrogels. QCM with dissipation (QCM-D) is relatively more convenient to be used in DNA related studies compared to the conventional QCM. QCM-D is a nanogram sensitive tool that has to ability of simultaneously measuring the resonance frequency shift and energy dissipation changes of the hydrogels that is immobilized onto the sensor surface. In addition, QCM-D provides real-time information about viscoelastic nature of the hydrogels (1, 2, 3, 4).

Due to their 3D networks hyrdrogels have the capability of absorbing high amounts of biological fluids and water. They show similarities to the body tissues due to their hydrophilic, porous, soft and flexible nature. Hydrogels are made of synthetic or natural polymers by physical or chemical crosslinking and their porosity, softness and flexibility can be easily modified. Besides that, functional groups in their structure can be tuned accordingly to make them respond changes in pH, temperature and light. Additionally, biodegradable and biocompatible hydrogels have gained an important place in different biotechnological fields like tissue design and regeneration and targeted delivery of therapeutic agents like drugs and proteins (5,6).

DNA crosslinked hydrogels have a promising future in biomedical field. The DNA sequences in their structure can be modified according to the illness of interest.

As a result, their usage has increased in diagnostic field. If there is an interaction between biological fluid of patient and the DNA hydrogel, it gives responses by changing its volume, colour etc. Even though, QCM has been used in diagnostic field, there are a few studies which combine QCM and DNA hydrogels (1, 7, 8).

In this thesis, a DNA-based hydrogel structure was designed and synthesized while immobilizing this 3D-network onto the surface of QCM. Then the changes in its structure were monitored by measuring the dissipation rate. By this method, it is aimed to provide a more sensitive and nearly real-time diagnosis of life threatening diseases such as cancer.

2. INTRODUCTION

2.1. Biosensors

First biosensor in history was developed by Clark and Lyons who prepared enzyme electrodes in 1962. By these electrodes they managed to measure the glucose concentration (9). Since their introduction, biosensors have attracted many scientists from different disciplines because of their accurate and precise detection results (10). In general, biosensors can be described as receptor-transducer tools which can be utilized for interpretation the biochemical or biophysical properties of the medium. Main difference between the biosensors and the other sensors is that they have biological/organic recognition element which allows to detect specific biological molecules in the medium (11). Biosensors can translate biological responses into a quantifiable and processable signal. Figure 2.1 represents the fundamental elements of a conventional biosensor: a) bioreceptors are biomolecular elements that can recognize and bind to specific particle of interest. They can enzymes; nucleic acids and DNA; antigen/antibodies; biomimetic; cells and cellular structures (12, 13), b) an interface system in which biological event of interest occurs. This event generates a signal obtained by c) the transducer element convert bio recognition event into an electronic signal and amplifies it by a circuit of detector with an appropriate reference and transfers it to d) computer algorithm converts this signal into a valid physical parameter that interprets the event of interest. Results can be displayed via e) an interface to the scientists. Biosensors can be used to investigate different kinds of samples such as body fluids, cell cultures, food and environmental samples (4).

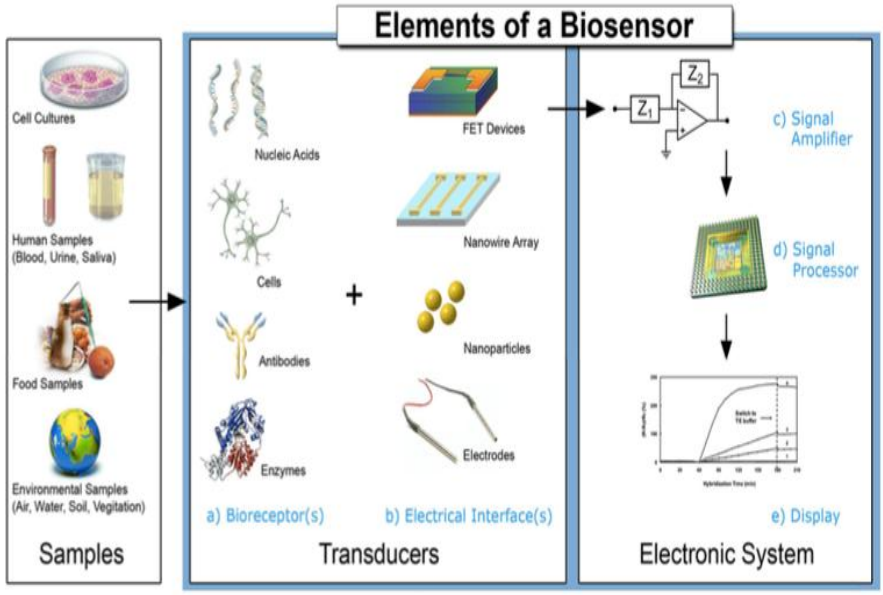

Figure 2.1: Components of a Biosensor (4)

DNA-based diagnostic tests have recently gained lots of attention in biosensor applications. In Nucleic acid biosensors, oligonucleotides with a predesigned base sequences, or a fragment of DNA or RNA, serve as sensing elements (14). Working principle of many DNA detection techniques is the hybridization between complementary parts of DNA/RNA molecules (15). Nucleic acid based biosensors are more advantageous compared to conventional ones because they are cheaper, faster, simpler and more sensitive in providing sequence-specific information. Besides that, dissimilar to enzymes and antibodies, nucleic acids can be used for multiple times (16).

2.1.1.SPR-DNA biosensors

Surface plasmon resonance (SPR) biosensors utilize an optical method to examine the events that happen in the surface of a thin metal-coated prism in touch with the solution of interest. This detection method is relied on the evaluation of

refractive index changes at the surface. After the immobilization of DNAs on the surface, sample solution flows across the surface and variation in the the angle of minimum reflectivity (SPR angle) provides a signal proportional to to the mass of the DNAs deposited on the surface. SPR principle can be seen in Figure 2.1.1.1. Although considered as being a sensitive and label-free tool if short chain DNA molecules or film with small packing density were used, changes in resonance angle is very small that sensor can not detect the biorecognition events (17,18).

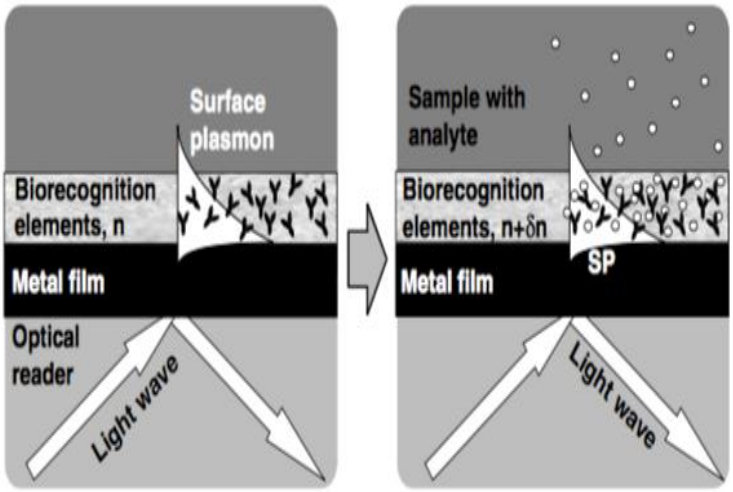

Figure 2.1.1.1: Principle of Operation of SPR affinity biosensors(19)

2.1.2. Electrochemical DNA biosensors

Lately, electrochemical DNA biosensors have gained a considerable attention owing to their high sensitivity and rapid response. In addition, they can be manufactured in small dimensions making them a good choice for DNA detection (20,21,18). An electrochemical biosensor is a biosensor that has an electrode system as transducer part. A single-chain of DNA is stabilized onto the surface of electrodes and it transforms the biological recognition occurence into an electrical signal.

Hybridization is usually monitored by looking at the increased current signals of a indicator of redox that attaches to the DNA duplex or other hybridization based electrochemical changes (conductivity or capacitance) (22, 23). Figure 2.1.2.1 shows working principle of electrochemical DNA biosensors.

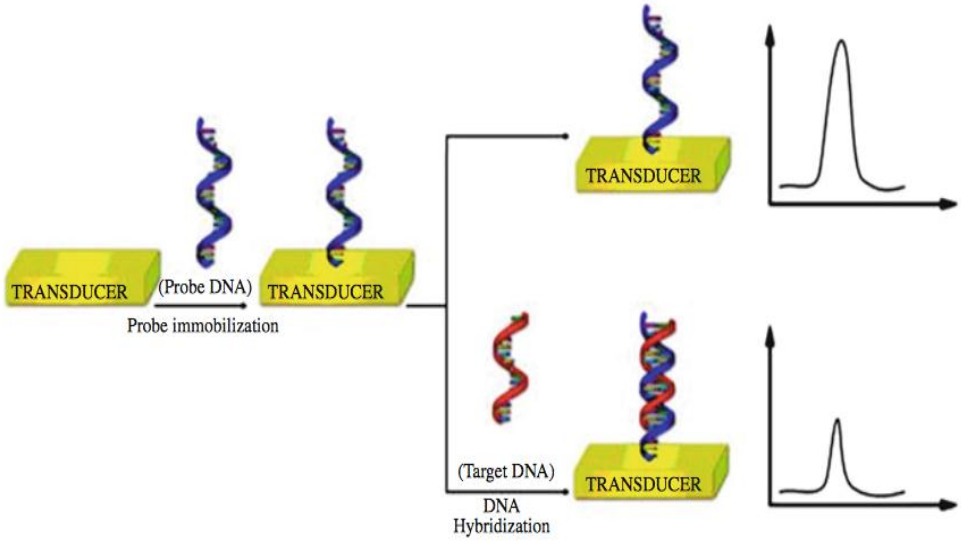

Figure 2.1.2.1: Working Principle of Electrochemical DNA Biosensors (24)

2.1.3. Label free biosensors

As mentioned earlier, biosensors utilize the highly selective binding relation between biological receptor interface and targets. To improve this binding and to transform this recognition event into a meaningful signal, some labeling components such as proteins and quantum dots are used (25, 26). Although labeling molecules enhance the binding, they can cause complexity in recognition dynamics. For instance, random bindings of labels can result in false positive and negative results. In addition, label molecules increase analysis time and costs (27).

Label free method also known as direct detection is based on the direct target receptor binding, thus the complexity of procedure decreases and reliability of the

test increases because of the absence of non-specific interactions of labeling molecules. Therefore, popularity of label free biosensors increased in various application fields such as bioanalyte recognition, environmental analysis and clinical diagnostics (28, 29, 27)

2.2. Quartz Crystal Microbalance

Quartz crystal microbalance (QCM) is a mass sensitive biosensor that can be used to identify DNA hybridization. A DNA sequence with a hundred pairs of base has a molecular weight high enough so that a mass increase can be observed as a result of hybridization with its complimentary sequence stabilized on the piezoelectric crystal surface. This increase in mass is correlated with the fundamental resonance frequency of the crystal which is the working principle of QCM (23).

Structure of a QCM sensor is composed of a thin quartz disc placed between a couple of metal electrodes (Figure 2.2.1). The working principle of quartz crystal is relied on piezoelectric effect which can be defined as an capability of a material to generate electric voltage when there is a mechanical stress applied on it. Piezoelectric effect works in the reverse direction, so quartz crystal deforms when there is an electric field applied on it. QCM is a non-invasive, label free and real time biosensor which has become popular in biological and chemical research (30, 31, 18).

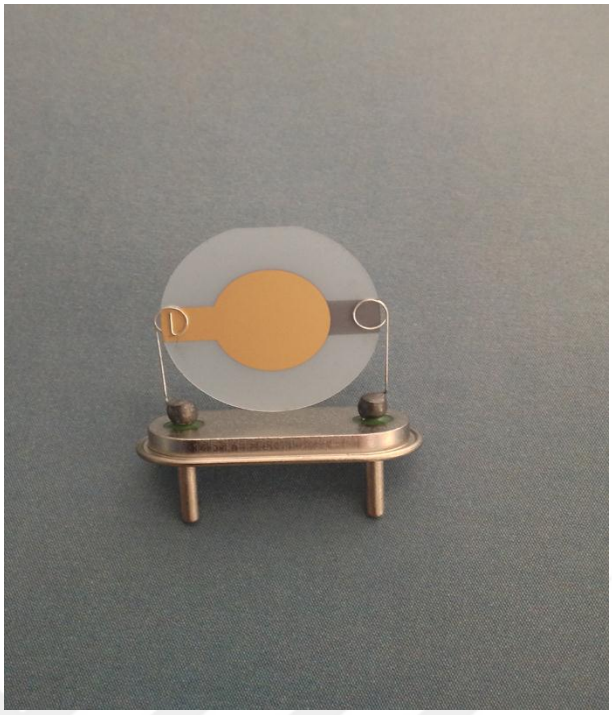

Figure 2.2.1: A Quartz Crystal with Gold Electrodes (32)

2.2.1. Quartz crystal microbalance with dissipation monitoring

Being able to simultaneously measure changes in viscoelastic and mass properties, the quartz crystal microbalance with dissipation monitoring is an advantageous technology to examine biological interactions compared to the conventional QCM (33). When there is an AC voltage application across the electrodes, crystal is excited into an oscillatory movement. Turning off the AC voltage results in a dampened oscillation of the crystal as the quartz crystal comes to rest. Dissipation can be described as the energy loss per stored energy during one oscillation cycle that is proportional to $1/\tau$, where τ represents the decay time constant. While the frequency of the crystal is associated with the mass adsorbed on the surface, dissipation is associated with the viscoelastic characteristic of the oscillating mass. Figure 2.2.2 shows the main elements of QCM-D (34).

Lately, QCM-D has gained a place in the biotechnological studies and become a useful tool answering the DNA related questions such as DNA hybridization and DNA-drug interactions.

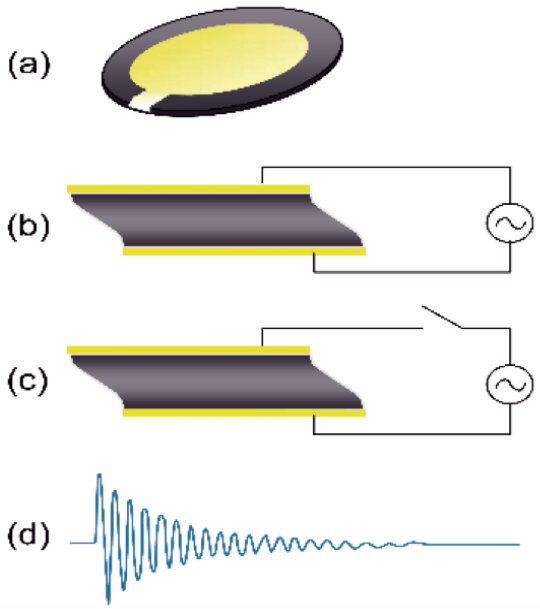

Figure 2.2.1.1: The main elements of QCM-D (a) Typical QCM-D sensor (b) Quartz crystal with AC voltage application across electrodes (c) Cutting off the AC voltage (d) The decay of oscillation (35)

2.2.2. Basic principle of QCM technologies

As mentioned before, QCM is piezoelectric in nature that means its frequency changes to the mass deposited on it. That`s why, the frequency of oscillating quartz crystal is exceptionally responsive to the alterations in the medium on it (36). The relation between resonant frequency change of the crystal and the mass deposited on its surface was established by Saurbrey (37).

$$\Delta m = -\frac{C}{n}\Delta f_n \qquad (2.2.2.1)$$

The equation above is called Saurbrey Equation and Δf_n represents the change in

resonance frequency at the nth harmonic (n = 1, 3, ...) where as Δm represents the mass coupled to the crystal surface area, and C is the mass sensitivity constant of the crystal and it is different for crystals with different resonant frequencies.

The Sauerbrey equation is applicable only for the situations when the deposited material is rigid, uniform, in the shape of thin film and the mass of it is much smaller than the mass of the quartz crystal. But Saurbrey equation becomes invalid in the case of soft deposited material and when the crystal is immersed in a liquid medium. This situation is commonly seen in biomedical applications. In this case, the frequency change becomes associated with the both of the mass of the film and to the liquid medium. The contact between the liquid medium and the soft film causes damping, which also results in the dissipation changes (ΔD).

$$D = \frac{E_{\ dissipated}}{2\pi E_{stored}} \qquad (2.2.2.2)$$

where D represents energy dissipation and dimensionless, $E_{dissipated}$ shows energy dissipated in one cycle of oscillation and E_{stored} represents the energy stored in the oscillating crystal (36).

2.3. Hydrogels

A great interest has been aroused on hydrogels because of their notable characteristics such as their modifiable physical, biological and chemical properties. Their fabrication versatility and high biocompatibility have attracted science world (38, 39). Since the publishing of first report by Wichterle and Lim in 1960 (40), scientific community worked on these materials to improve and broaden their applications for various areas like tissue engineering, wound healing, cancer research, cell therapy and sensors and drug delivery (17).

Hydrogels can be defined as three dimensional network structure (3D) that are made of synthetic or natural polymeric materials. They are capable to swell high amounts of biological fluid or water. They can be in different shapes and sizes due to their hydrophilic backbone structure (41, 6).

To prepare hydrogels, crosslink formation between polymeric materials is required. These crosslinks produce a three dimensional network structure (3D) as shown in Figure 2.3.1. Hydrogels can be constructed through physical, chemical crosslinking or with the help of radiation.

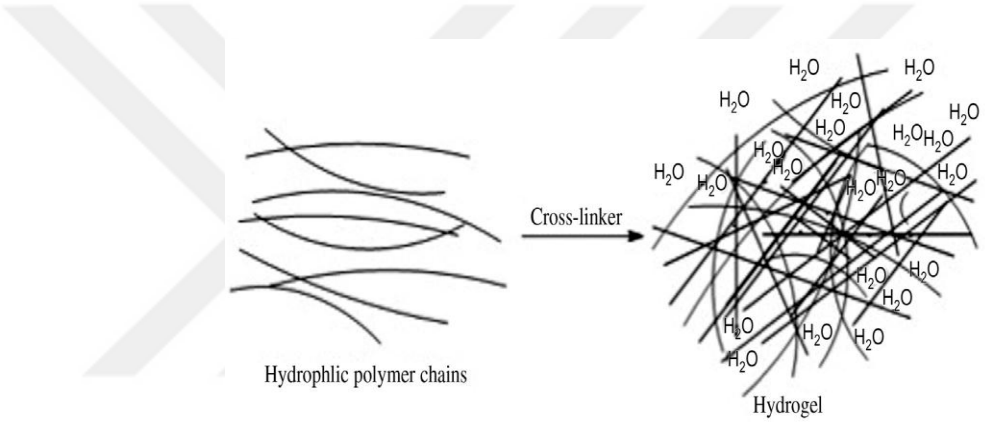

Figure 2.3.1 : 3D Hydrogel Network Structure (42)

Physical crosslinked or reversible hydrogels are formed relied on noncovalent forces such as H- bonding, ionic, electrostatic and hydrophobic interactions. These hydrogels are heterogeneous in nature since they are composed of ionically or hydrophobically linked domains and groups of molecular entanglements. Physical gels are often reversible and they have temporary junctions; they can dissolve according to the enviromental conditions such as ionic strength change and temperature (42).

2.3.1. Properties of hydrogels

Hydrophilic hydrogels have an important role in many biomedical and

pharmaceutical applications. Therefore, they must be produced by looking at their evaluated properties such as swelling, toxicity, and mechanical strength and etc. (43).

2.3.1.1. Swelling properties

To understand the swelling behaviour of hydrogels, cross-linking concept must be studied carefully. Because there are crosslinks between all polymers chains regardless of the size of the hydrogel. For this reason, crosslinking amount and crosslinking agent and capacity of water absorption are associated with each other (43). Hydrogels with higher crosslinking ratio have a tightly bound structure and is likely to swell less, whereas hydrogels with lower crosslinking ratio is tend to swell more. Because crosslinking prevents the mobility of polymer chains in the hydrogel network (44).

Another factor that alters the swelling ratio is the functional groups that hydrogels have. For example, it was noticed that hydrogels have hydrophilic functional groups are leaned to swell more in comparison with those hydrogels with hydrophobic functional groups (44).

Physical textures of hydrogels are easily affected by environmental conditions such as pH, electric signal, enyzme presence or other ionic species and temperature. These changes can show themselves as precipitation, changes in water content and size of hydrogel and swelling of hydrogels are directly influenced by these stimuli. For example, temperature fluctuations of the swelling media can change the swelling ratio of temperature sensitive hydrogels (43, 44)

Swelling ratio of a hydrogel can be calculated by the equation below:

$$Swelling\ Ratio = \frac{W_s - W_d}{W_d} \qquad (2.3.1.1)$$

where W_s is the weight of hydrogel in swollen state and W_d is the weight of hydrogel in dry state (45).

2.3.1.2. Mechanical properties

Mechanical features of hydrogels are extremely significant, especially when they are used in different biomedical technologies such as tendon and ligament repair, wound dressings, drug delivery, cartilage replacement and tissue design. Mechanical features of hydrogels should be such that it can preserve the integrity of therapeutic agent until the time of release (44).

Mechanical features of hydrogels can be modified by changing the crosslinking degree of them. As degree of crosslinking increases, hydrogels become stronger however the % elongation of the hydrogels decreases and it makes hydrogels more fragile. Therefore, to form a strong and relatively elastic hydrogel, optimum degree of crosslinking must be determined. (44).

In another useful method, scientists apply copolymerization to improve the the mechanical properties of hydrogels. The strength of hydrogels can be increased via combining them with a co-monomer in the polymeric backbone of the hydrogel (44)

2.3.1.3. Biocompatible properties

Hydrogels should pass in-vivo toxicity and cytotoxicity tests to be used in biomedical applications. Biocompability can be defined as the capability of a compound to behave with an suitable host response. This term is not only the absence of toxicity, it is also about the biofunctionality of the interested material. Material must accomplish the specific task which it is designed for and the host

response to this material must be appropriate not only systemic but also local (surrounding tissue) (46, 43).

Bicompability is especially important for the hydrogels used in tissue engineering. Because there is a continuous interaction between hydrogel and the body during cellular regeneration process, healing and scaffold degradation emerge as two main effects of hydrogel structures. In the lack of biocompability, hydrogel can give rise to damages to the connected tissues. Toxic chemicals that are utilized in the preparation of hydrogels can be dangerous for in vivo applications, if the conversion rate is not 100%. In addition, emulsifiers, stabilizers, organic solvents, crosslinkers and unreacted monomers employed in polymerization can present a challenge because they can leak to the encapsulated cells and tissues. To eliminate these kind of toxic chemicals, various purification methods can be applied such as solvent washing or dialysis (43).

There are numerous ways to test biocompability of a material. For in vitro evaluation, three primary cell culture assays are applied; a) elution (extract dilution) b) direct contact c) agar diffusion. All of these three tests are morphological tests and the results are evaluated by the observation of morphological changes in cells (43).

In vivo evaluation is based on the chemical content of the material and the tissue exposure circumstances such as degree, nature, duration and frequency of exposure. Materials of manufacture such as process contaminants, residues, predetermined additives, substances that can leach out, degradation products, other substances and their interactions in the resulted product and the properties of this final product are analyzed during the biological assessment (47).

To decrease the toxicity of the hydrogel, changing the polymerization reaction

kinetics and extensive washing methods can be applied. Besides those, to get rid of the residual initiators, alternative methods like radiation and hydrogel formation methods without any usage of initiators can be utilized (47).

2.3.2. Chemically crosslinked hydrogels

Chemically crosslinked hydrogels are formed based on covalent interactions. These hydrogels have permanent junctions and they do not dissolve in water. Chemical crosslinking methods can be listed as four subgroups; by radical polymerization, by chemically reacting complimentary groups, by high energy radiation and by using enzymes (44).In principal, three essential components of hydrogels formation are crosslinker, monomer and initiator. Besides these components, diluents such as water or other aqueous solutions can be utilized to manage the heat of polymerization and resulted hydrogels features. After the synthesis of hydrogels, they must be washed and purified to remove unreacted monomers, crosslinkers, initiators and unwanted products that was formed through side reactions. Figure 2.3.2.1 shows the basic elements and steps of hydrogels synthesis (48).

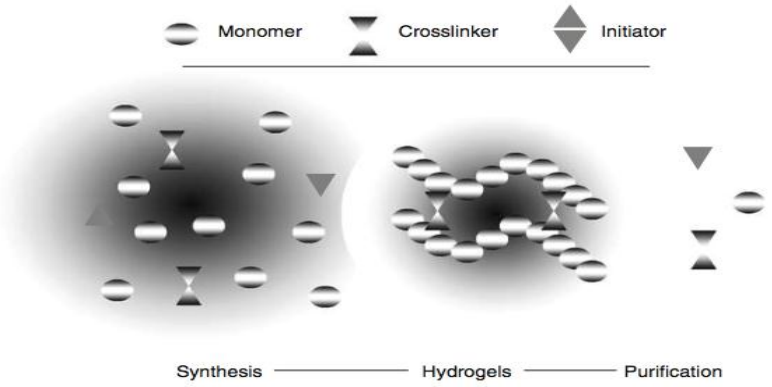

Figure 2.3.2.1 : Hydrogel Synthesis (49)

There are three ways of hydrogels synthesis; bulk, solution, and inverse dispersion methods. Most selected method is the solution technique which provides better control of polymerization heat and thus the polymer properties. Hydrogels that have high swelling properties usually produced by using this way. Monomer(s), initiator(s) and crosslinker(s) are able to dissolve in water (49).

2.3.3. Application areas of hydrogels

2.3.3.1. Tissue engineering and regeneration

Increasing need of developing new methods to improve tissue regeneration and replacement result in an increase of hydrogel studies in tissue engineering. Extra cellular membrane (ECM) has a very important role in tissue engineering, because it gives stability and shape to the tissues and organs it also performs in migration, profileration, differentiation, adhesion and apoptosis (50). Given to the importance of them, the main purpose of tissue engineering is to develop ECM like and biocompatible materials. Due to their capability of behaving like ECMs, hydrogels attract attention of many scientists (51). Hydrogels acting as three dimensional polymer scaffolds have an important role in tissue engineering. For example; cells of patients and polymer can be combined together, then it can be implanted to patient`s body when it is ready. After the implementation, hydrogel provides tissue re-growth and proliferation. In addition high porosity of hydrogels allow them to response osmotic pressure differences, elastic restoring forces and electrostatic forces through swelling or shrinking. They also can transfer nutrients and waste products. In addition, they are usually biocompatible and can be designed to dissolve and degrade away (52). Hydrogels employed in tissue engineering can be made from both natural and synthetic polymers. Synthetic materials that can form tissue engineering hydrogels are polypeptides, poly(ethylene oxide), poly(vinyl alcohol), poly(acrylic acid) and poly(propylene fumarate-co-ethylene glycol). Natural polymers that are

suitable for tissue engineering are agarose, alginate, collagen, fibrin, gelatin chitosan, collagen, and hyaluronic acid (53, 5)

There are numerous examples of hydrogels used in tissue engineering. For example; Biodegradable poly(vinyl alcohol) hydrogels intracated with polymers of phenylboronate able to tissue growth and support cell. Cell culture takes 5-20 days, after that the cells of interest can be taken by hydrogel scaffold dissolution with solution of saccharide (54,5).

Another study belongs to Blanchard et al (55) which is about keratin based hydrogels which can be used for cell growth. Keratin can be extracted from the patient`s own nail or hair. Hydrogels can be formed after the purification and oxidization of keratin. Because after these processes, sulfonic acid remnants in protein make disulfide crosslinks between backbones and due to their hydrophilic nature they bind water.

Beta-glucan hydrogels can be formulated via radiation fusion technology. Beta-glucan is a natural component and can be extracted from various fungi. Irradition of aqueous solutions of beta-glucan can be accomplished by using electron, gamma or UV beam. Formed hydrogels are biocompatible (56).

2.3.3.2. Drug delivery

There are many research papers that are published about using hydrogels in targeted drug transport, but only a few are took place in commercial markets. Drugs can be loaded and released through the pores of hydrogels and pore size of hydrogels can be modified easily by modifying the density of crosslinks in their structure. Additionally, using hydrogels can be an interesting method to provide sustained and

targeted drug release. By this way, desired effects of the drug can be increased and side effects can be decreased simultaneously. (57, 5).

Poly (ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly (ethylene oxide) triblock copolymers (PEO–PPO–PEO) (known as Pluronic or Poloxamer) can be given as an example of hydrogel drug delivery systems that are used in pharmacy (58). Paavola and co-workers formulated an injectable poloaxamer based gel for controlled delivery of the anesthetic agent lidocaine (59).

Another example is a supramolecular based hydrogel to carry out doxorubicin (DOX). Zhu and co-workers designed a supramolecular polymeric prodrug through host-guest relation between cyclodextrin-functionalized polyaldehyde and DOX-incorporated adamantine. Then, they used carboxymethyl chitosan as crosslinker agent and they formed an injectable DOX-loaded hydrogel which releases DOX according to change of pH level (60).

2.3.3.3. Contact lenses

The earliest and most well known biomedical application of hydrogels is the use of hydrogels in contact lenses (40). Due to their mechnanical stability and refractive index, they are appropriate for contact lenses (61).

Contact lenses are categorized as hard and soft. Hard contact lenses are not favorable because it takes a long time for users to adapt them. Hard contact lenses are usually made from hydrophobic materials such as or poly(hexa-fluoroisopropyl methacrylate) (HFIM), poly(methyl methacrylate) (PMMA), while soft contact lenses are relied on hydrogels (62).

2.3.3.4. Wound dressings

While conventional dressings are inadequate in mechanical strength, flexibility, porosity and actimicrobial activity, hydrogels have a promising potential due to their unique characteristics. (63). They can supply moisture and gas exchange through their pores. They can be replaced easily due to their flexibility. In addition, they have higher biodegradability and biocompatibility (64) Besides these traits, hydrogel dressings are faster in healing wounds and it is easier to monitor wound healing because of their transparency (65).

Kumar et al. (64) produced chitosan hydrogel/nano zinc oxide composite bandages to recover burns, diabetic foot ulcers and chronic wounds. Faster healing, enhanced water retention, absorption of drainage caused by wound, antimicrobial activity and blood clotting were observed. No cellular toxicity was reported.

An assymmetric chitosan membrane was reported by Mi et al. (66) and their study about this membrane had promising outcomes such as prevention of bacteria penetration and dehydration of wound. Through these advantages, it enhanced tissue regeneration.

Recently, Li et al. (67) demonstrated a hydrogel based on a natural polysaccharide, namely pullulan. Up to 4000% water intake is observed. Additionally, it had rapid hemostatic capacity which led prevention of drainage accumulation in wound bed without any cytotoxicity.

2.3.3.5. Hygiene products

Superabsorbent polymers (SAP) have an important role in agriculture and diaper industry since 70`s. They were firstly commercially created in Japan in 1978 to be used in feminine sanitary napkins. This compound was formulated based on a crosslinked starch-g-polyacrylate (68). In 90`s superporous hydrogel (SPH), which is a kind of superabsorbent polymers, was introduced as a new water absorbent system made up from covalently crosslinked hydrophilic polymers. SPHs show kinetics of size independent fast swelling dissimilar to SAPs. The early examples of superporous hydrogels were produced from highly hyrophilic acrylamide, sulfopropyl acrylate and salts of acrylic acid. Later, hybrid SPHs that were produced by adding a crosslinking agent to the previously made SPHs were introduced to the markets. For instance, when sodium alginate is added to acrylamide based SPH preparation, sodium alginate works as a crosslinking agent and crosslinks are created between alginate chains and calcium ions. Hybrid SPHs have numerous advantages such as high elasticity and high mechanical strength. (69). SAPs can improve skin health by absorbing fluids. They can be used comfortably without causing any diaper rash. In addition, they can also decrease the risk of fetal contaminations and gastrointestinal ifections by hindering germ colonization (5).

A feminine sanitary napkin which consists an absorbent polymer core positioned between two air-laid tissue sheets was introduced by Osborn. The core was formed from acidic monomers such as methacrylic acid, acrylic acid or 2-acrylamido-2-methyl propane sulfonic acid. This core was capable of absorbing medium to high menstrual flows and it gave comfort to users with low risk of staining and leakage (70).

2.3.4. Crosslinking by free radical polymerization

Chemically crosslinked hydrogels can be formulated through radical polymerization of low molecular weight monomers, macromers or water soluble polymers by utilizing crosslinking agents. Most well known example of this method is Poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel system. It can be formed by polymerization of HEMA in the presence of appropriate crosslinking agent. Apart from vinyl monomers, hydrogels also can be formed via radical polymerization of water-soluble polymers containing polymerizable groups. Natural, semi-synthetic and synthetic polymers can be utilized for the design of hydrogels through this method such as UV induced polymerization of PEG-based hydrogels. Synthesis of hydrogels through free radical polymerization reactions and cross-linking reactions can be seen in Figure 2.3.4.1 (44).

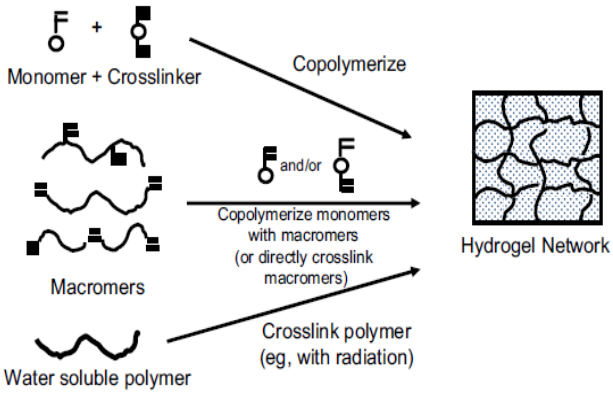

Figure 2.3.4.1 : Cross-linking reactions and free radical polymerization reactions (42)

Hydrophilic monomers that have double bonds such as acrylamide, hydroxyethyl methacrylate and acrylic acid can polymerized and can generate chemical bonds with crosslinkers that also have double bonds. These kind of polymers have permanent characteristics due to the covalent crosslinks.

2.4. DNA Hydrogels

There are numerous unique properties of DNA such as biocompability and adaptability for chemical modification. Therefore, DNA can be considered as the "smartest" natural component for biomedical applications. In addition, it is easy to design DNA sequences and it is inexpensive (71).

DNA-based hydrogels have significant features. (i) Stability. DNA can maintain its establishment under radical amounts of pressure, heat, and numerous chemical processes. (ii) Flexibility. The changeable nature of DNA hybridization yields many possibilities of building units for hydrogels. (iii) Specific programmability. DNA strands can be arranged according to nucleotide pairing rules and DNA secondary structures. Therefore, DNA hydrogels that are self assembled can be accomplished via specific sequence construction, and DNA integration into the polymers of synthetic nature can be made for producing DNA hybrid hydrogels. (iv) External stimulation can lead to the changes in the arrangement of some functional DNAs, thus leading reversible and convertible alterations to DNA-based hydrogels (72). (v) Easy synthesis and modification. Large quantities of DNA can be produced through an automated solid-phase method and various functional groups of acrydite, amino, carboxyl and thiol can be integrated to their structure which can make them able to respond to other functional groups. These customized DNAs can be easily manufactured via phosphoramidite chemistry (73).

First study about DNA-polymer hybrid hydrogel was reported by Nagahara and Matsuda in 1996. In this study, there were acrydite-modified ssDNAs at two different types and acrylamide monomers copolymerized with them individually which made them incorporate in the chains of polyacrylamide. As a result, two types of hydrogels were produced as can be seen in Figure 2.4.1. In Type I, oligoA serves as a crosslinker and gets hybridized with an oligoT-derivatized copolymer. In Type

II, oligoA gets hybridized with oligoT and attach to the side of chains of complementary copolymers. Resulted hydrogels still have the skill of specific recongnition of DNA sequences and they are biocompatible in nature. Usage of DNA polyacrylamide copolymers has increased to construct different hdyrogels which have gained attention of many scientists from different areas of studies (75).

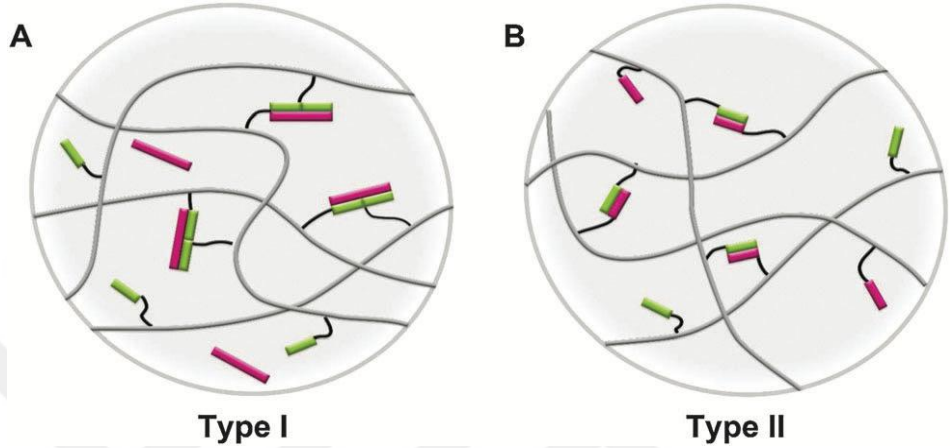

Figure 2.4.1: Formation of hydrogel via DNA-hybrid formation (76)

Rather than utilizing polyacrylamide, DNA-hybrid structures can also be manufactured with different kinds of polymers which respond to different kinds of stimuli.Temperature-responsive polymers are important members of these polymers and their gelation can be reversed by controlling the temperature (77, 78). Poly(N-isopropylacrylamide) (pNIPAM) is the most well known thermosensitive polymer and due to its low critical solution temperature (LCST) at 32°C, it is suitable to be used in biomedical applications. The volume of pNIPAM hydrogels changes based on temperature differences. At low temperature, the hydrogel is highly swollen due to the strong interaction between water and polymer. Whereas, at temperatures higher than the LCST, hydrogel disintegrates for interacting with itself via interactions in hydrophobic nature (79). In addition to the thermosensitive polymers, photoresponsive DNA-hybrid hydrogels were designed via photo-responsive crosslinkers from azobenzene groups (80) Through UV-vis light irradiation, cis-trans isomerization of azobenzene make azobenzene- integrated DNA hybridized with its

complementary strand, that resulted in gelation. This method is easy and hydrogels in different size and structures can be formed by this way.

2.4.1. Acrydite modified DNA hydrogel polymerization and thiol surface modification

A nucleic acid can be converted into a compound that is able to polymerize with acrylic monomers to produce polyacrylamides based on free-radical polymerization methods and can react with sulfhydryl (-SH) groups via thiol-ene chemistry. Therefore, in this thesis 5'-acrydite modified ssDNA was used to prepare DNA-polyacrylamide hydrogels. So 5'-acrydite modified ssDNA firstly incorparated into the hydrogel via its acrydite unit at one end, and then during detection of the target molecule on QCM sensor, DNA fragment of this functionalized monomer is expected to serve as a crosslinker in this project. The chemical structure of 5'-acrydite modified DNA and its reactions with thiol and other acryl groups can be seen in Figure No 2.4.1.1 (81).

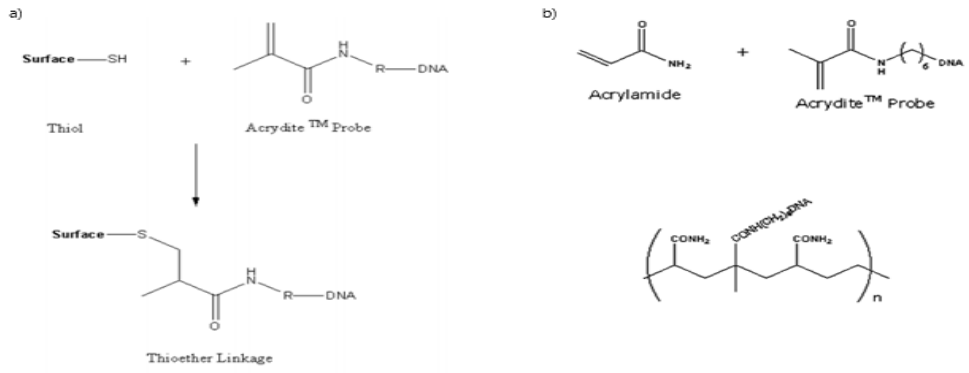

Figure 2.4.1.1: Reactions of 5'-acrydite modified DNA with thiol (a) and acrylamide (b) groups (81)

Acrydite chemistry has many advantages. This modification provides scientists to work with a wide scope of pH and temperature. It is efficient and so versatile that acrydite modified oligonucleotides can be coupled onto the polymer and glass surfaces (82).

Another strategy that was used in this thesis was thiol-ene chemistry. This chemistry was utilized to modify the surface of quartz crystal. That`s why 1,6-hexanedithiol was used as a crosslinker. Since it had 2 functional thiol groups in its structure, one of the thiol group attached to the gold surface by forming covalent bond and the other one reacted with acrylate group and became a crosslinker in the hydrogel.

There are three main pathways of thiol-ene surface modification which are named as grafting from, grafting to, and a combination of grafting to and grafting from methods. In this study, grafting from method was used to alter the surface of the crystal (83).

Grafting from method uses thiol-ene substrates which include initiators to activate reactions of grafting. Figure 2.5.2 shows illustration of surface modification via grafting from method. Grafting from method is simply based on the interaction between the monomers from the reactive surfaces. In this method, backbone is a part of the polymerization, because it is chemically altered to generate active sites. Thus, other unactivated monomers can be activated and get polymerized. By this method, the number of active sites is related to the number of grafted polymer chains, and the functionality and density of grafts can be controlled better and more robust grafts can be obtained (84).

The polymerization reaction between 5`-acrydite modified Poly-A,

bisacrylamide and acrylamide that took place in this thesis can be seen in Figure 2.4.1.2.

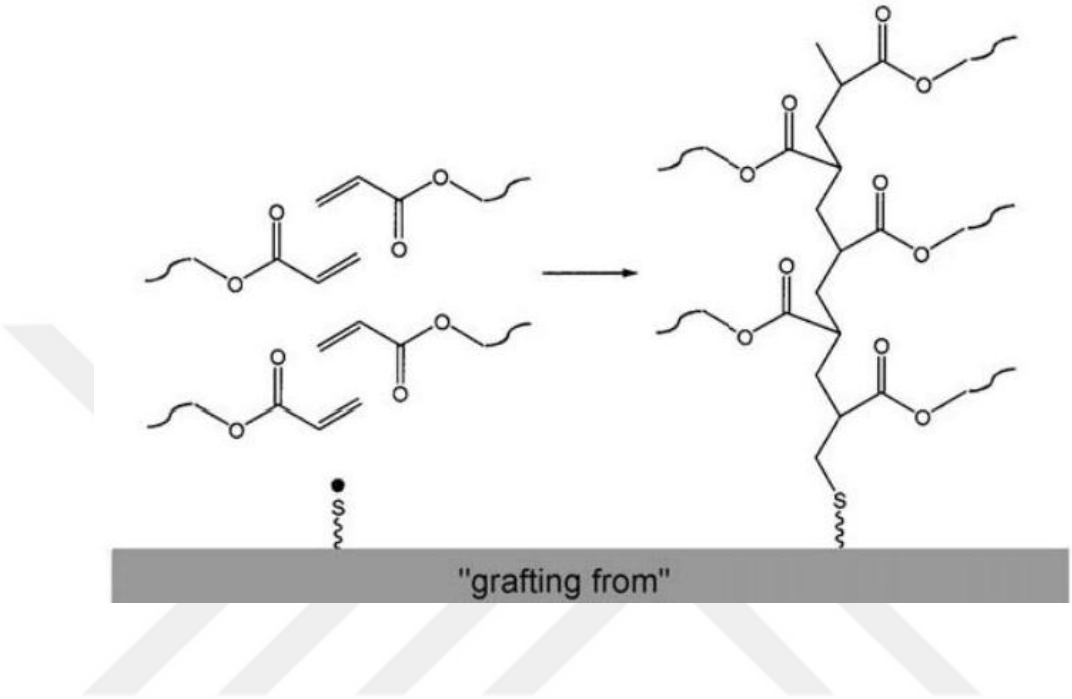

Figure 2.4.1.2: Surface Modification via Grafting from Method (83)

The polymerization reaction between 5`-acrydite modified Poly-A, bisacrylamide, acrylamide can be seen in Figure 2.5.3. Another chemical that was used was 1,6-hexanedithiol served as a crosslinker which also formed a covalent bond between gold surface and hydrogel. Bond between thiol and gold surface can also be seen in Figure 2.4.1.3.

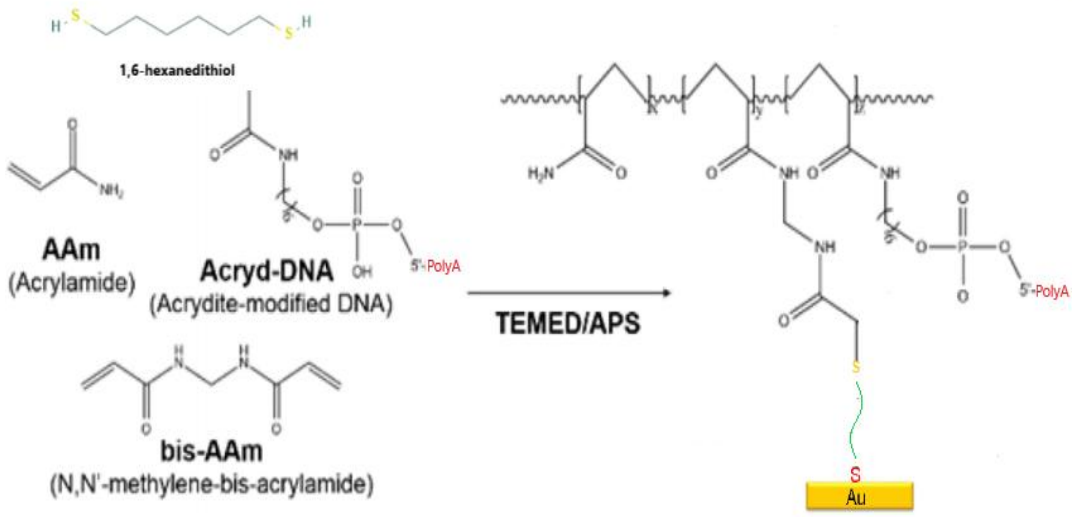

Figure 2.4.1.3: General Scheme for Radicallic Polymerization Reaction and Thiol –Au Surface Modification (85)

3. MATERIALS AND METHODS

3.1.Materials

3.1.1. Preparation of non-thiolated hydrogel

For non-thiolated polyacrylamide gel preparation another stock solution was prepared. 2900 mg of acrylamide, 1000 mg of N,N'-methylbisacrylamide were taken and they were dissolved in a 60 ml of distilled water in a beaker. The volume of the solution was completed to 100 ml by adding distilled water to it. To make 10% ammonium persulfate solution 1000 mg of ammonium persulfate was taken and dissolved in 10 ml of distilled water. To prepare Tris-HCl solution, 121.14 g of Tris base was added in a 800 ml distilled water. The pH was stabilized to 7.4 by adding HCl to it.

For a 3 ml 10% gel preparation, eppendorf tube was washed with distilled water for a 2-3 times. 1 ml from the stock solution was taken to the tube. Secondly, 1.25 ml of Tris HCl was added. Then 0.05 ml of 10% APS solution and 0.0025 ml of TEMED were added under the hood. Finally, 0.6975 ml of distilled water was put in to the tube quickly. The hydrogels can be kept in store by sealing at the room temperature.

3.1.2. Preparation of thiolated hydrogel

In this study, 1,6-hexanedithiol containing polyacrylamide hydrogels were used. For the preparation of 1,6-hexanedithiol acrylamide stock solution; 2900 mg of acrylamide, 900 mg of N,N'-methylbisacrylamide were taken and they were dissolved in a 60 ml of distilled water in a beaker. Then 0.06881 ml of 1,6-hexanedithiol was taken and added to the beaker. As the last step, the volume of the

solution was completed to 100 ml by adding distilled water in to it. The stock solution was mixed.

For a 3 ml 10% gel preparation, eppendorf tube was washed with distilled water for a 2-3 times. 1 mL from the stock solution was taken to the tube. Secondly, 1.25 Ml of Tris HCl was added. Then 0.05 mL of 10% APS solution and 0.0025 mL of TEMED were added under the hood. Finally, 0.6975 mL of distilled water was added to the tube quickly. The hydrogels can be kept in store by sealing at room temperature.

3.1.3. Stock solutions

First of all, stock solutions of 5`acrydite modified Poly-T, TEMED, hexanedithiol, bisacrylamide and acrylamide were prepared. Moles, volumes and masses used for 100 μL were tabulated for different DNA:bisacrylamide:hexanedithiol mole ratios by the help of Microsoft Excel (Table 3.1.3.1) After that, the required masses of chemicals for the preparation of 5 times diluted 10 μL hydrogel was calculated for each concentration and, the hydrogels were produced according to them.

3.1.3.1. Preparation of DNA stock solutions

A fresh solution of PBS was prepared with a PBS tablet and distilled water. 2788.02 μL of PBS was added the tube that contains 5`acrydite modified Poly-T and it was shaken and vortexed for a few seconds. The solution was taken into a falcon tube, sealed and stored at 4 °C.

3.1.3.2. Preparation of bisacrylamide stock solutions

For different ratios of bisacrylamide, the required masses of bisacrylamide were weighed and they were completed to 1000 μL by addition of tris solution. The solutions were taken into falcon tubes, sealed and stored at 4 °C.

3.1.3.3. Preparation of acrylamide stock solution

The ratio of acrylamide to other chemicals were never changed. So, 58 mg of acrylamide were weighed and it was completed to 1000 μL by addition of tris solution onto it. The solution was taken into a falcon tube, sealed and stored at 4 °C.

3.1.3.4. Preparation of hexanedithiol stock solution

To prepare 0.25% by mole stock solution, 4.46 μL of hexanedithiol was taken into an eppendorf tube and it was completed to 300 μL with distilled waterTubes were stored at 4 °C.

3.1.3.5. Preparation of TEMED stock solution

8.33 μL of TEMED was taken into an eppendorf tube and it was completed to 1000 μL by adding distilled water. Tube was stored at 4 °C.

Table 3.1.3.1. Moles and Masses Used in the Preparation of 100 μL DNA Hydrogel 0.25 equivalent hexaneditihiol

Mole of X-Linker*	Chemicals	Eq.	Mwt (g/mole)	n (mmol)	Mass (mg)	Volume (μL)
	Acrylamide	70.00	71.08	0.04080	2.90000	
	DNA-acrydite	0.20	7017.00	0.00012	0.81796	
	Bisacrylamide	0.55	200.19	0.00321	0.64174	
20	Hexanedithiol	0.25	150.31	0.00146	0.21902	0.22281
	Acrylamide	70.00	71.08	0.04080	2.90000	
	DNA-acrydite	0.40	7017.00	0.00023	1.63593	
40	Bisacrylamide	0.35	200.19	0.00204	0.40838	

	Hexanedithiol	0.25	150.31	0.00146	0.21902	0.22281
	Acrylamide	70.00	71.08	0.04080	2.90000	
	DNA-acrydite	0.50	7017.00	0.00029	2.04491	
50	Bisacrylamide	0.25	200.19	0.00146	0.29170	
	Hexanedithiol	0.25	150.31	0.00146	0.21902	0.22281
	Acrylamide	70.00	71.08	0.04080	2.90000	
	DNA-acrydite	0.60	7017.00	0.00035	2.45389	
60	Bisacrylamide	0.15	200.19	0.00087	0.17502	
	Hexanedithiol	0.25	150.31	0.00146	0.21902	0.22281

*Percent ratio of DNA-acrydite moles to total moles of all crosslinkers in hydrogel

3.1.4. Preperation of DNA hydrogels

DNA hydrogels were prepared according to the calculations as follows: Steps for preparation of 10 µL solution for 0.6: 0.15: 0.25 (DNA-acrydite: Bisacrylamide: Hexanedithiol) hydrogel were explained below. The amounts for the preparation of the DNA-containing soutions were the same but with different masses and volumes of chemicals.

3.1.4.1. Preparation of 10 µL of 0.6:0.15:0.25 (DNA-acrydite:Bisacrylamide:Hexanedithiol) hydrogel

97.5 µL from the acrydite-Poly T stock solution was taken into an eppendorf tube. 3.5 mg of bisacrylamide was weighed and it was completed to 1000 µL by adding tris solution onto it. From this stock solution, 1 µL from this bisacrylamide solution was added to the eppendorf tube which contains DNA-solution. To prepare acrylamide stock solution, 58 mg of acrylamide was weighed and it was completed to 1000 uL by adding tris solution onto it. 1 µL from this acrylamide solution was added to the eppendorf tube. After that, 0.3 µL of 0.25 hexanedithiol stock solution was added to the eppendorf tube. Then, 2.16 µL tris solution was added to the eppendorf tube. This eppendorf tube was centrifuged at 14000 rpm for 1 hour. After the process of centrifuge, tube was put into the -80 °C refrigerator and it was left for

3-4 hours for complete freezing. After freezing, the tube was put into the lyophilizer to remove the solvent (namely water) and obtain the residual solid of acrydite DNA+tris-buffer+hexanedithiol+bisacrylamide+acrylamide mixture. The tube was left in the lyophilizer overnight. Tube was taken from the lyophilizer and 7.46 μL of distilled water was added onto it. Then 0.17 μL of APS solution was added into the tube. 1 μL from TEMED stock solution was taken and added to the tube. Finally, 1.33 μL of distilled water was added to the tube immediately and the solution was vortexed for a few seconds.

3.1.5. Preparation of DNA pools

As mentioned above, open lid system was used in the experiments. Therefore, DNA pools of 50 μL was put on quartz crystals after the gelation. DNA pools were made from complimentary sequence of the DNA that was used in the hydrogel. Masses and volumes of Poly-A were calculated and tabulated in Table 3.1.5.1.

Table 3.1.5.1: Masses and Volumes Used in the Preparation Poly-A DNA Pool

	Mole (mmol)	Mass (mg)	Volume (μL)
For 60% 100 μLDNA gel	0.000350		
For 60% 10 μLDNA gel	0.000035		
Dilution 01:05	0.000007	0.087189	25.004020
For 50% 100 μL gel	0.000291		
For 50% 10 μL gel	0.000029		
Dilution 01:05	0.000006	0.072657	20.836683
For 40% 100 μL gel	0.000233		
For 40% 10 μL gel	0.000023		
Dilution 01:05	0.000005	0.058126	16.669346
For 20% 100 μL gel	0.000117		
For 20% 10 μL gel	0.000012		
Dilution 01:05	0.000002	0.029063	8.334673

To prepare a 60% Poly-A pool, 200 nmol of Poly-A was dissolved in 715 µL of PBS solution. After that, 25 µL of this solution was taken into eppendorf tube and it was completed to 50 µL with PBS solution. The same process was repeated for each concentration.

3.1.6. Modification of measurement chamber lid

The experiments were conducted by using a open lid system to be able to get more reliable results. The required modifications was done by Fusion software.

3.2. Methods

3.2.1. Cleaning of quartz crystals

After each experiment, crystals were washed with 70% of ethanol solution (in water), were incubated in piranha solution (sulfuric acid: hydrogen peroxide 7:3 by volume) for at least 10 minutes. Then they were rinsed with distilled water. Finally, they were dried with N_2 gas.

3.2.2. Hydrogel immobilization on gold surface

To compare the stabilization of the thiolated gel with other components on gold surface; 0.01 ml of thiolated gel, 0.01 ml of non-thiolated polyacrylamide gel and 0.01 ml of acrylamide and 1,6 hexanedithiol solution were taken onto the crystal surface and on a microscope slide. After the gelation, the crystal was placed in a beaker which contains distilled water which was enough to cover the surface of the crystal and the microscope slide. Crystal and slide were left overnight in distilled water.

3.2.3. Quartz crystal microbalance experiments

All measurements were taken with open QCM Q-1 by using crystals with 10 MHz resonant frequency. The device and the crystals were purchased from Novaetech S.r.l.

Freshly cleaned quartz crystal was put into measurement chamber and sealed with the lid of the QCM. 10 μL of gel solution was put in the middle of the quartz crystal before gelation started. The data recording was started. When gelation completed, 450 μL of distilled water was added onto the hydrogel on crystal for maximum swelling of the obtained hydrogel. By observing the frequency and dissipation shifts, time of target DNA injection was decided and 50 μL of pre-determined concentration of DNA injected.

Another experiment was done by preparing a hydrogel with 0.2: 0.55: 0.25 (DNA: Bisacrylamide: Hexanedithiol). 10 μL of gel solution was put in the middle of the quartz crystal before gelation started. The data recording was started. When gelation completed, 450 μL of distilled water was added onto the hydrogel on crystal for maximum swelling of the obtained hydrogel. At the time of stable frequency and dissipation change, 50 μL of PBS solution without DNA was injected to the pool of distilled water and the data was recorded.

4. RESULTS

4.1. Immobilization of Hydrogel on Gold

The results of immobilization can be seen in the figures below. In Figure 4.1.1 non-thiolated polyacrylamide pregel solution is at the upper side near to the electrode, at lower right of it there is thiolated pregel solution and at lower left of it there is acrylamide-thiol solution. On the leftmost at the microscopic slide, there is acrylamide-thiol solution, in the middle there is thiolated polyacrylamide pregel, and on the rightmost there is non-thiolated polyacrylamide pregel.

Figure 4.1.1: Gold Surface and Microscope Slide Before Gelation

After the gelation process finished. Another photograph of microscopic slide and crystal was taken (Figure 4.1.2)

Figure 4.1.2: Crsytal and Microscope Slide After Gelation

Figure 4.1.3 and 4.1.4 shows the microscope slide and quartz crystal after being left overnight in distilled water.

Figure 4.1.3: Microscope Slide After Being left overnight in Distilled water

Figure 4.1.4: Crystal after being left overnight in distilled water

Lastly, the quartz crystal was turned upside down to check whether thiolated gel has bound onto the gold surface (Figure 4.1.5)

Figure 4.1.5 : Crystal turned upside down

The same experiment was done in the PBS solution instead of distilled water. The gels and the acrylamide-thiol solution on gold surface can be seen in Figure 4.1.6. Acrylamide-thiol solution is in the middle, whereas non-thiolated polyacrylamide gel is on the leftmost and the thiolated gel is on the rightmost of the crystal.

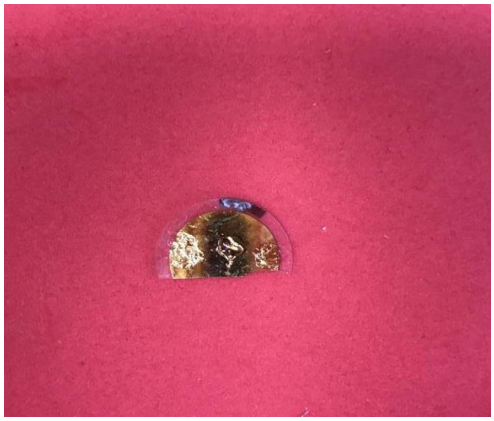

Figure 4.1.6: Quartz Crsytal After Gelation

The results of the experiment can be seen in Figure 4.1.7.

Figure 4.1.7: Crystal After being left overnight in PBS Solution

Lastly, the quartz crystal was turned upside down to check whether thiolated gel has bound onto the gold surface after being left overnight in PBS solution in Figure 4.1.8.

Figure 4.1.8: Crystal turned upside down after being left overnight in PBS

4.2. DNA Hydrogel Experiments

Freshly cleaned quartz crystal was placed in the measurement chamber. Then it was sealed with open lid that was printed before. Before starting the experiment calibration was conducted by openQCM Q-1 software and 10 µL of DNA pre-hydrogel solution was put on the crystal. Data was recorded during gelation. After the gelation completed 450 µL of distilled water was injected onto the hydrogel. After the stabilization in dissipation and resonance frequency was observed, 50 µL of

DNA-PBS solution that contained 2 times more DNA concentration which the DNA hydrogel was injected. Data was recorded during the whole experiment. The dissipation change vs relative time graph and frequency change vs relative time graph were drawn by Microsoft Excel. The same experiments was conducted for different DNA hydrogels. The mole ratios of DNA:Bisacrylamide:Hexanedithiol (DNA:Bis: Thi) were specified for each hydrogels and their dissipation changes over time can be seen in Figure 4.2.1.

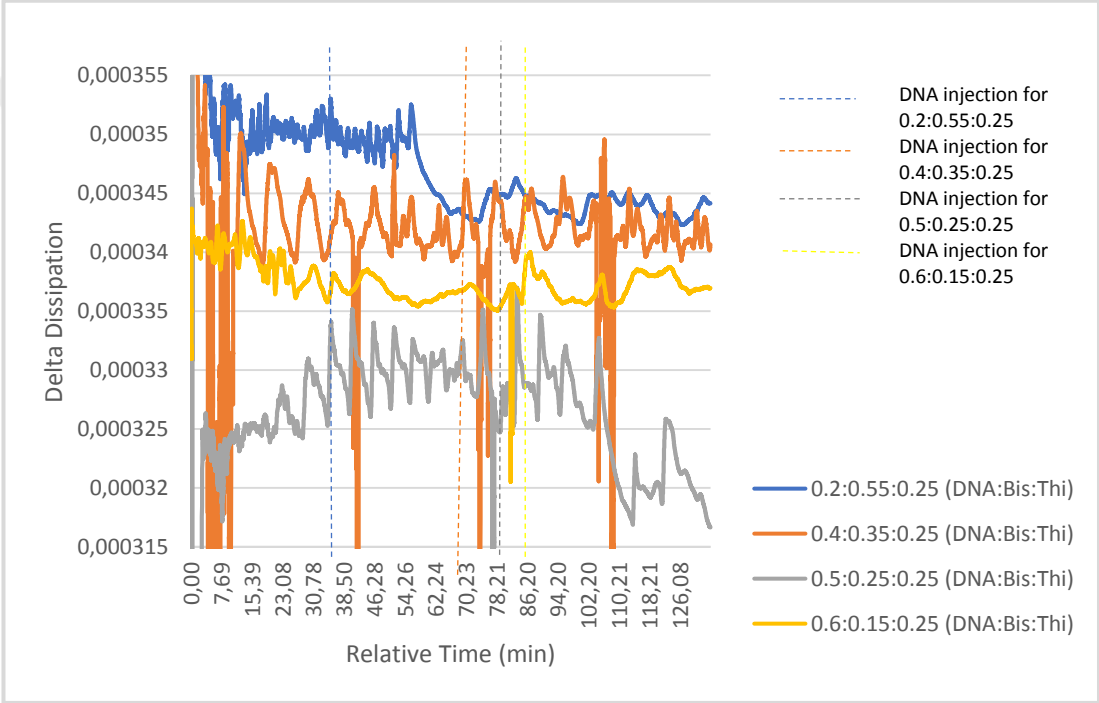

Figure 4.2.1: Comparison of Dissipation Changes over Relative Time

Figure 4.2.1 contains the data that was recorded right after the 450 µL of distilled water addition. There are 4 different hydrogels and each of them are represented with different colors in the graph. There are blue, orange, gray, yellow curves representing the molar ratios of DNA: Bis: Hexanedithiol of 0.2: 0.55: 0.25, 0.4: 0.35: 0.25, 0.5: 0.25: 0.25 and 0.6: 0.15: 0.25, respectively. In addition, DNA pool injection times are showed with specifically colored dashed lines for each

hydrogel. Graph shows a time range of 130 minutes. It can be seen that the hydrogels that had lower DNA concentrations showed higher dissipation changes. But the hydrogel with the highest DNA concentration behaved exceptionally and showed the second highest dissipation change values.

To get focused on the data after DNA injection onto the gel, another graph that was drawn (Figure 4.2.2). This figure shows dissipation changes over a time range of about 50 minutes.

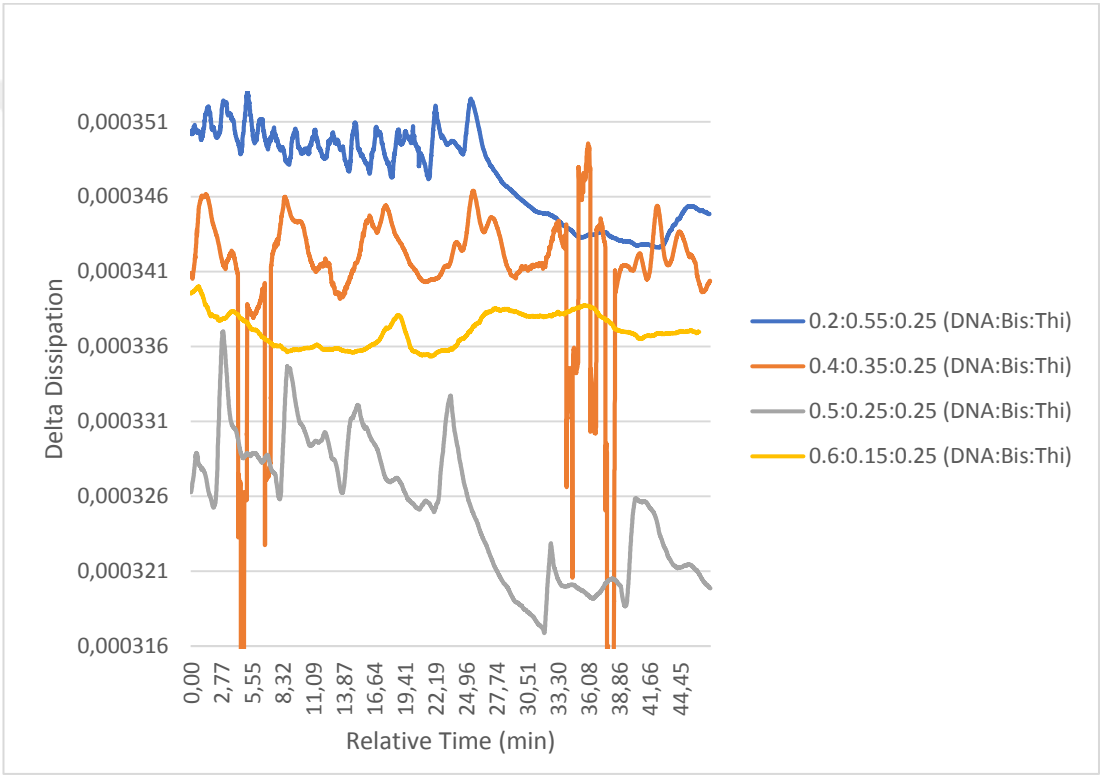

Figure 4.2.2: Dissipation Change vs Relative Time after DNA Injection

It can be seen that while the hydrogels of 0.2:0.55:0.25 (DNA:Bis:Thi) and 0.5:0.25:0.25 (DNA:Bis:Thi) shows similar behaviour patterns that their dissipation change values sharply decreases at about 24-26 minutes. The hydrogel 0.4:0.35:0.25 (DNA:Bis:Thi) has many abrupt increases and decreases. Dissipation change values

of hydrogel 0.6:0.15:0.25 (DNA:Bis:Thi) is relatively stable when it is compared with other hydrogels.

The frequency changes of these hydrogels were also compared in Figure 4.2.3. The same notations was used as in the Figure 4.2.1.

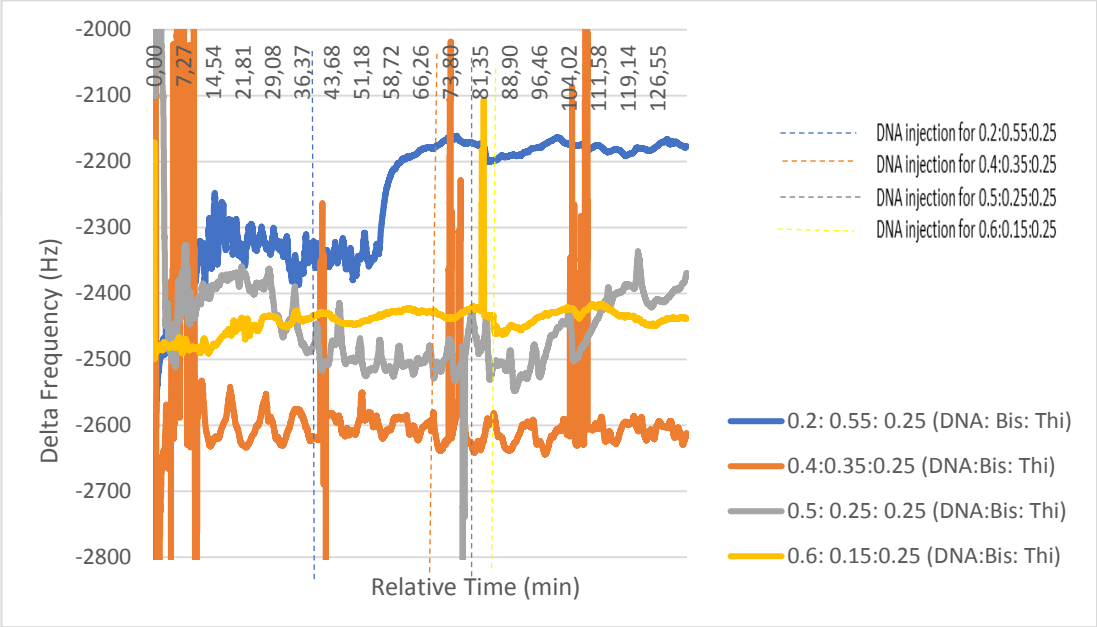

Figure 4.2.3: Comparison of Frequency Changes over Relative Time

Figure 4.2.3 covers the time right after the distilled water addition. In overall, it can be said that frequency decreases for all of the hydrogels. Besides that, when Figure 4.2.3 was compared with Figure 4.2.1, the frequency changes as the dissipation changes and their correlation is inverse.

Figure 4.2.4 shows frequency change after DNA injection. This figure shows frequency changes over a time range of about 50 minutes.

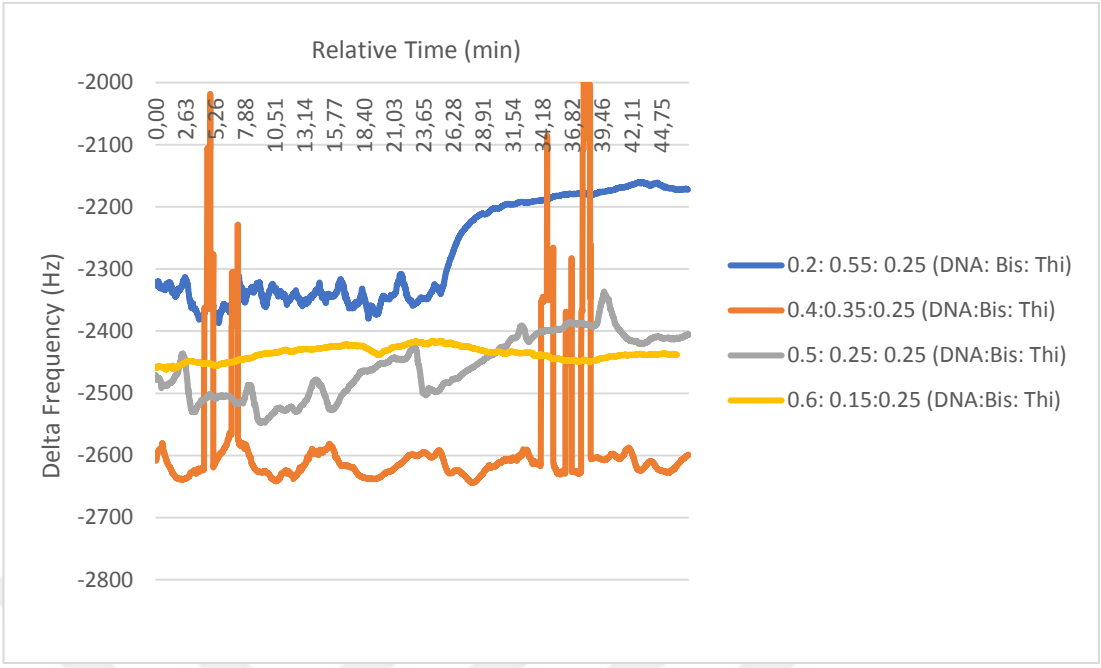

Figure 4.2.4: Frequency Change vs Relative Time after DNA injection

In Figure 4.2.4, it can be seen that the hydrogel of 0.6:0.15: 0.25 (DNA: Bis: Thi) molar ratio shows an almost stable behaviour. Frequency change values of the hydrogel 0.4:0.35:0.25 (DNA: Bis: Thi) changes abruptly. Frequencies of the 0.2:0.55:0.25 (DNA: Bis: Thi) hydrogel and 0.5: 0.25: 0.25 (DNA: Bis: Thi) hydrogel decreased at about 24-26 minutes.

Another experiment was done by preparing a 10 µL with 0.2:0.55:0.25 (DNA: Bis: Thi) molar ratios. Then it was swelled with 450 µL distilled water, after dissipation and frequency changes became relatively stable, 50 µL of PBS without DNA was added to the distilled water pool on the crystal. The data of this experiment were compared with the data of the experiment with 0.2: 0.55: 0.25 (DNA: Bis: Thi) hydrogel on which had target DNA. Comparison of frequency change and dissipation change was shown in Figure 4.2.5 and Figure 4.2.6. In both of them, brown lines represent the data of experiment without DNA addition and pink lines represent experiment with DNA injection. In addition, DNA injection is represented with a blue dashed line.

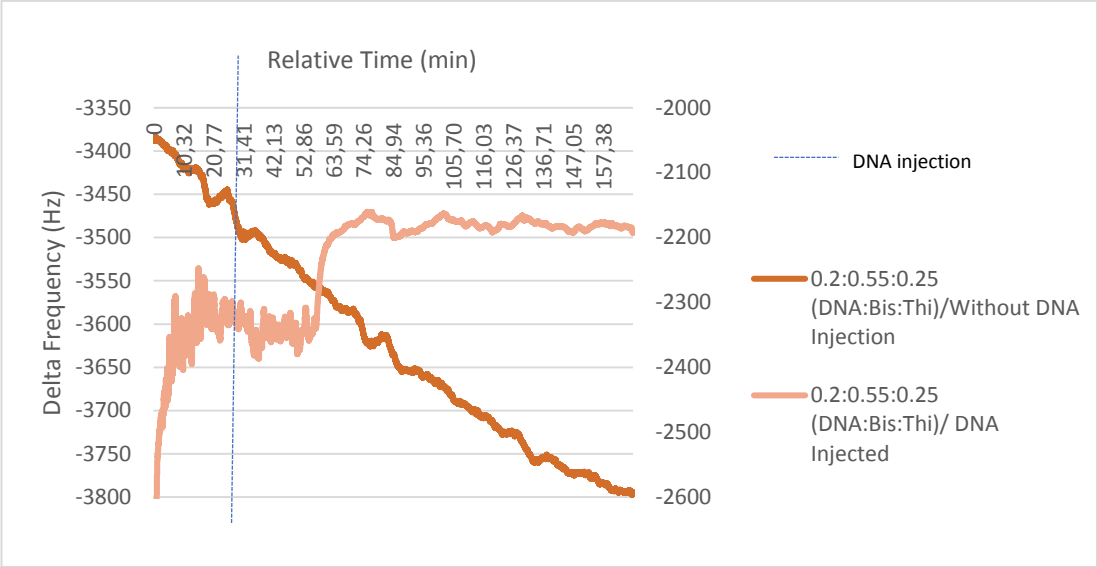

Figure 4.2.5: Frequency Comparison of 0.2: 0.55:0.25 (DNA:Bis:Thi) Hydrogels

Target DNA was injected to the distilled water pool at about 30 minutes. In Figure 4.2.5, it can be seen that two hydrogels behaved very differently. For the hydrogel without any target DNA on it, delta frequency decreased, while for the experiment with target DNA injection, delta frequency increased after 22 minutes.

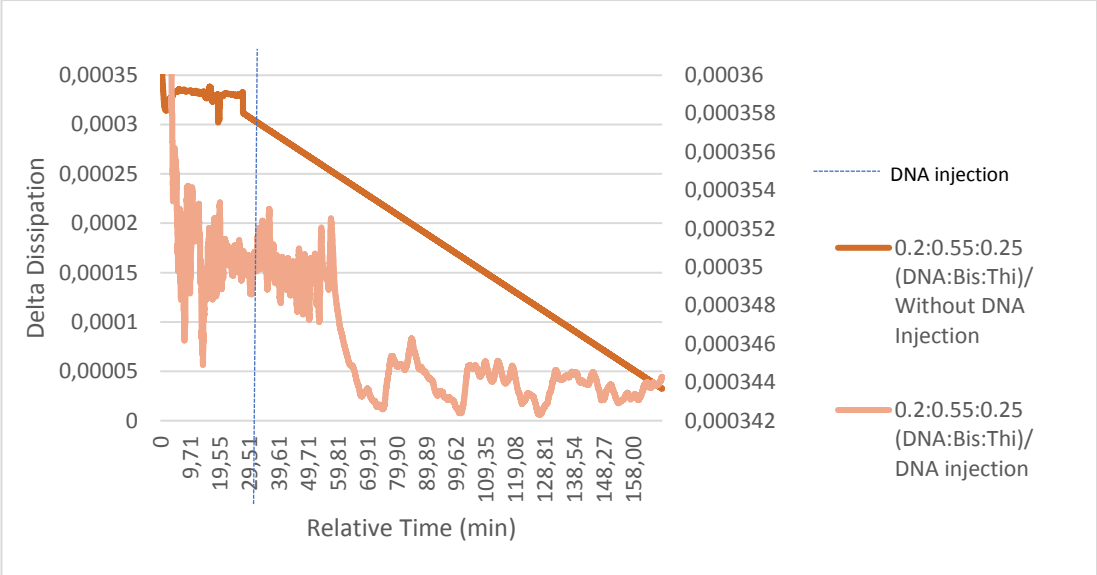

Figure 4.2.6: Dissipation Comparison of 0.2: 0.55:0.25 (DNA:Bis:Thi) Hydrogels

In Figure 4.2.6 it can be seen that, for both of the hydrogels delta dissipation decreases. But for the experiment with target DNA injection, abrupt delta dissipation decrease was observed 22 minutes after the DNA injection.

5. DISCUSSION AND CONCLUSION

5.1. Discussion

Polyacrylamide gels are the commonly used materials in the areas such as forensic chemistry, biotechnology and genetics. Polyacrylamide gel preparation is based on the free radical polymerization. General ingredients of these gels include acrylamide, bisacrylamide, a buffer solution with a predetermined pH which was Tris HCl buffer solution with a pH value between 7.3-7.5. The pH was adjusted to be in this range, because it is in the physiologically important spectrum. In other words, it is the range that is compatible with biological fluids. To initiate the free radical polymerization, APS and TEMED were used. APS served as a free radical source, whereas TEMED was used as an accelerator. The crosslinker agent was bisacrylamide which creates crosslinks between two acrylamide molecules. Polyacrylamide gels are stable and physically strong. Besides that, the physical characteristics of these gels such as pore size and elasticity can be modified by changing the ratio of acrylamide to bisacrylamide. Because of their easily modified traits, polyacrylamide gels are widely used in biomedical field (86).

In this study, polyacrylamide hydrogels were modified with 1,6-hexanedithiol. Free radical polymerization and thiol-ene click chemistry took place in the preparation of crosslinked hydrogels. 1,6-hexanedithiol was used because of the two available thiol functional groups (-SH) in its structure. The thiol groups show a strong affinity to noble metals that results in establishment of strong bonds of sulfur-gold atoms. One of the thiol groups in 1,6-hexanedithiol was expected to bind to the crystals gold surface, whereas the other thiol group could react with acrylamide groups in hydrogel mixture and became a part of the hydrogel. To check the bond between gold surface and the thiolated hydrogel; 0.01 mL of non-thiolated polyacrylamide gel, thiolated polyacrylamide gel and a solution of acrylamide and thiol were dropped onto the gold surface and left overnight for complete gellation in distilled water. As a result, only the thiolated polyacrylamide gel was immobilized

on the surface. There was no gelation between acrylamide and thiol, because there was no bisacrylamide which works as a crosslinker. Non-thiolated polyacrylamide gel could not bind onto the gold surface. The same experiment was done with the phosphate-buffered saline (PBS) solution instead of distilled water. Because pH value of PBS solution is approximately 7.4, which is in the physiologically important spectrum. Besides that, it has the same ion concentrations and osmolarity with the human body fluids. Doing the experiment within the PBS, obtained results made us to understand the behaviour of the hydrogel in biological fluids. The same results were observed. By comparing the results of the distilled water experiment, it can be said that thiolated gel behaved the same in PBS solution and the distilled water and onto the gold surface of the crystal. The results of the experiments can be seen on figures 4.1.1 to 4.1.8.

In this study, 10 μL of DNA hydrogels were prepared when the capacity of measurement chamber and swelling characteristics were taken into consideration. Because of the very small volumes of DNA hydrogels, some of the volumes such as TEMED and hexanedithiol volumes couldn`t be taken with pipettes. Therefore, stock solutions of the chemicals were prepared and required volumes of acrydited modified Poly T, hexaneditihol, acrylamide, bisacrylamide were mixed in large amounts of solvent, then freezed and were put into the lyophilizer for 3-4 hours to evaporate the liquid and obtaining in solid form. By this way they were stored more stable and small volumes of hydrogels were managed to be prepared with correct amounts.

During the experiments, pools of 450 μL of distilled water and 50 μL of DNA-PBS solution were poured to the gels. Because 500 μL of liquid totally covered the surface of the crystal and the lid preventing the QCM to measure evaporation. Firstly, 450 μL of distilled water was added, because the it was wanted to reach to an equilibrium of dissipation and frequency shift. After observing the equilibrium, 50 μL of DNA-PBS solution was injected with a pipette to record the changes in dissipation and frequency. Concentration of Poly A in the pool was 2 times higher than the Poly T concentration in the hydrogel. The motion was to obtain faster

hybridization and faster responses, because hydrogel could capture the complementary sequence more easily.

In this study open lid system was used by printing a new open lid that is compatible with the measurement chamber of the QCM-D. The reason for this modification was to eliminate the disadvantages of small volume of measurement chamber. Measurement chamber of QCM-D was about 30-50 μL, whereas the hydrogel volume was 10 μL. So, as the hydrogel got swollen, closed lid system caused leakage which is very threatening to the hardware of the QCM-D.

Information of how dissipation and frequency changes over time provides insight about the viscoelasiticity of the layer which is formed on the crystal surface. As starting point, alternating voltage leads crystal oscillation. If the adsorbed material creates a layer of rigid nature on the surface, a frequency diminishment is observed, meanwhile there isn`t any important change in dissipation. In the case of soft layer presence, dissipation shows a noticeable increase. To be more exact, the frequency decreases when there is a material bonding on the crystal because there is an increase in total mass. Rigid layer decreases the dissipation slowly, while soft layer causes higher loss of energy which leads to faster dissipation decrease than the rigid layer causes. Many previous QCM studies shows material adsorption onto the surface causes a frequency decrease (87, 88, 89, 90, 91, 92, 93, 94, 95). Likewise, in this study it is obvious that when distilled water was added onto the gels on the crystal, frequency decreased which is a proof that there was a mass increase on the surface of the crystal which means that hdyrogel got swollen. The very noisy nature of the resonance data could be related with the open lid system. All of the previous studies that were entioned were done by closed lid system that provided much more isolated system which gives healthier results. Because when the crystal and the solution are in contact with the air, the oscillaton of the crystal can be easily affected and that can lead noise in data.

In the case of dissipation, when the layer on the crystal is swollen and soft, QCM-D responses with higher dissipation factor, while in the case of rigid film

QCM-D responses with smaller dissipation change values. One of the most interesting thing that can be observed in Figure 4.2.1 that dissipation values show non-monotonic increments and decrements. It can be the result of the changes in the conformational structure of the gel. It is possible to say that as the gel swells, the distance between the molecules of the gel changes and it can cause this dissipation changes. Another possibilities might be the unfinished process of gelation or gel loss. The previous studies also confirm that during the layer formation these kind of structural changes can be accompanied with these dissipation patterns (95, 96). Figure 4.2.2 and Figure 4.2.4 show the hydrogel behaviours after the target DNA (Poly A) addition. When these graphs were analysed, it can be seen that hydrogel 0.6:0.15:0.25 (DNA:Bis:Thi) shows no significant decrease or increase in delta frequency and delta dissipation which can be concluded as there was no hybridization between acrydite modified Poly T in hydrogel and Poly A in the solution. The hydrogel 0.4: 0.35: 0.25 (DNA: Bis:Thi) also didn`t show any important change, so it can be said that there were no hybridization. But for hydrogel 0.2: 0.55: 0.25 (DNA: Bis: Thi) and hydrogel 0.5: 0.25: 0.25 (DNA: Bis: Thi) both of their delta frequency and delta dissipation started to change 24-26 minutes after the DNA addition. In both of them, frequency increases. If the delta dissipation values of these hydrogels are investigated, then it can be seen that delta dissipation of hydrogel 0.2: 0.55: 0.25 (DNA: Bis: Thi) and hydrogel 0.5: 0.25: 0.25 (DNA: Bis: Thi) decreased which confirms that there was a soft viscoelastic layer on the surface.

As the DNA concentration of the hydrogel increased, crosslinking amounts increased that created more dense hydrogel. As seen in Figure 4.2.1 dissipation changes are higher for softer hydrogels with smaller DNA concentrations except for the hydrogel of 0.5:0.25 :0:25 (DNA: Bis: Thi).

In Figure 4.2.5 and Figure 4.2.6 there were two hydrogels with the same molar ratios of 0.2:0.55:0.25 (DNA:Bis:Thi), for both of them 450 μL was used for swelling after delta dissipation and delta frequency have reached equilibrium. 50 μL of DNA-PBS solution was added to the distilled water, whereas to the second

hydrogel, 50 μL of PBS without DNA was added. Both of the dissipation and frequency of the second hydrogel decreased abruptly. Frequency decrease confirmed that there was a mass increase, but sudden dissipation decrement could be a reason of rigid layer presence or gel loss. The first hydrogel that was exposed to the target DNA showed a frequency increase after DNA injection onto it. The mass of DNA present in DNA pool is much smaller than the mass of the hydrogel and this caused a frequency decrease.

Finally, for 0.2:0.55:0.25 (DNA:Bis:Thi) hydrogel it can be said that it gave responses to DNA injection. Its frequency increased while its dissipation decreased which is expected. The focus point of this project was to interpret the changes in dissipation, therefore viscosity of hydrogel. Acrydite modified Poly-T recognized Poly A in DNA pool and hybridization occurred in hydrogel structure. As a result, hydrogel got denser because hybridization increased crosslinking amount in the hydrogel and made it more rigid. More rigid layer decreased the dissipation value as expected.

5.2. Conclusion

Main goal of this study was to provide a nanogram sensitive biosensor which is an integrated system of DNA hydrogel and the QCM. The steps that were finished in this dissertation are the design of hydrogel and its immobilization on gold surface. Hydrogel was modified with 1,6-hexanedithiol which contains two free thiol groups which show a great affinity towards to gold surface of the crystal. By this method, hydrogel could be immobilized onto the gold surface. After being sure that thiolated hydrogel was bound to the crystal, the DNA hydrogels were designed with the required amounts of necessary chemicals. Acrydite-modified Poly T sequence was used to conduct the QCM-D experiments. By this way, Poly T became a part of the polyacrylamide hydrogels and could react with thiol groups. Afterwards, an open lid was designed and 3D printed. Finally, QCM-D experiments were conducted for 4 different concentrations of DNA sequences inside the hydrogels. These hydrogels

give the expected results to target DNA over them by showing a decrement in dissipation. In conclusion, a sensitive system of DNA detection was obtained.

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7. CURRICULUM VITAE

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Highschool	Kartal Anatolian Highschool	2008

Work Experience

Title	**Employer**	**Date**
Intern	Drexel University	2017-2017
Intern	AEM Environmental Labs	2014-2014

Languages	**Reading**	**Speaking**	**Writing**
English	Very good	Very good	Very good

Language Proficiency Certificates

TOEFL IBT	**IELTS**
92	7.5

Computer Skills

Program	Level
MS Office	Very good