IMPROVEMENT OF BIOCOMPATIBILITY OF THE CARDIOVASCULAR SYSTEM STENTS BY MODIFICATION

KARDİYOVASKÜLER SİSTEM STENTLERİNİN MODİFİYE EDİLEREK BİYOUYUMLULUKLARININ ARTTIRILMASI

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Submitted to Institute of Science of Hacettepe University
as a a partial fulfillment to the Requirements for the degree of
MASTER OF SCIENCE
in
CHEMISTRY

2007

ANKARA

To the Institute for Graduate Studies in Pure and Applied Sciences,

This study has been accepted as a thesis for the degree of MASTER OF SCIENCE in CHEMISTRY by our Examining Committee.

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ÖZ

Kardiyovasküler sistem rahatsızlıklarında kullanılan intrakoroner stentler yığın yapısı olarak iyi birer materyal olsa da yüzey canlı sistem için oldukça önemli dezavantajlara sahiptir. Doku – biyomalzeme etkileşimi yüzeyde gerçekleşen bir olay olduğundan, zamanla yüzeyden salınacak toksik iyonlar, metal yüzeyinin immün sistem tarafından yabancı cisim cevabına maruz kalması, metal yüzeyin koagulasyon mekanizmasındaki proteinleri tetikleyebilmesi, trombosit adezyonu ve stent yerleştirilirken ortaya çıkan enflamasyon bu dezavantajların başlıcalarıdır. Çalışmada, stent materyalinin yapımında kullanılan 316L tipi paslanmaz çelik plakalar kullanılmış olup yüzeylerinin biyouyumluluğunu arttırmak üzere immunolojik olarak inaktif polietilenglikol, 2-hidroksietilmetakrilat, etilendiamin, hegzametildisilan ve hegzametildisiloksan polimerik yapılarla plazma boşalım tekniğiyle kaplanması uygulanıp enerji dispersif x-ışınları spektroskopisi, taramalı elektron mikroskopisi ve durağan damla temas açısı yöntemleri ile karakterize edilmiştir. Karakterizasyon sonucu kaplama materyallerinde bulunan O, N, C ve Si atomlarının yüzdelerinin kaplama olmayan plakalardaki atom yüzdelerine göre bağıl değerleri yükselmiş ve kaplama materyaline göre değişen su temas açısı ile ıslanabilirlik değerleri ölçülmüştür. Polietilenglikol, 2-hidroksietilmetakrilat ve etilendiamin kaplamalar 44.8° ve 66.5° arasında değişen temas açıları ile hidrofilik karakter gösterirken silikon bazlı kaplamaların 88.5° - 103.7° arasında ölçülen su temas açısı değerleri hidrofobik karakter ve düşük yüzey ıslanabilme özelliklerine işaret etmektedir. Karakterize edilen plakaların daha sonra canlı sistemler için herhangi bir toksik etki taşıyıp taşımadığını anlamak için hücre çoğalım testine tutulmuş ve test sonucunda plaklarla etkileştirilen hücrelerin bağıl proliferasyonu 81.3% - 93.8% arasında gözlenmiştir. Kaplama materyalinde kullanılan polimerik malzemelerin stentler kan pıhtılaşma mekanizmasında görev alan proteinleri ne yönde etkilediğini incelemek üzere ise kan uyum testlerine tutulmuş olup burada da pıhtı oluşum süresinde protrombin zamanı, aktif kısmi tromboplastin zamanı ve fibrinojen zamanı testleri uygulanmıştır. 2-

hidroksietilmetakrilat, etilendiamin ve hegzametildisiloksan kaplamaların ektrinsik koagulasyon mekanizmasına çok az etki ettiği görülmüştür.

Anahtar Sözcükler: 316L paslanmaz çelik, koroner stent, restenoz, koagulasyon, plazma yüzey modifikasyonu.

Danışman: Prof. Dr. Emir Baki DENKBAŞ, Hacettepe Üniversitesi, Kimya Bölümü, Biyokimya Anabilim Dalı

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ABSTRACT

Although the bulk structures of intracoronary stents which are used in the cardiovascular disease have excellent properties on bulk structures, the surfaces have many disadvantages for the living system. The tissue – biomaterial interaction occurs on the surface and the release of toxic ions, the foreign material host response to the metallic surfaces, triggering the blood proteins in the coagulation cascade by the surface and platelet adhesion, inflammation during the stent placement are the main complications. In this study, 316L stainless steel plaques which are the raw materials of the coronary stents were used and the surfaces of the plaques are coated with plasma glow discharge assisted technique with immunulogically inactive polyethyleneglycol, 2-hydroxyethylmethacrylate, ethylenediamine, hexamethyldisilane and hexamethyldisiloxane polymeric materials. The coatings are characterized with energy dispersive x-ray spectroscopy, scanning electron microscopy and sessile drop contact angle techniques. Relative existence of atoms like O, N, C and Si that exist in polymeric coating materials to bare stainless steel samples are calculated and water contact angle measurements showed the surface wettability changes with the monomers used. Polyethyleneglycol, ethylendiamine and 2-hydroxyethylmethacrylate coatings display hydrophilic character with a contact angle values between 44.8° and 66.5° and silicon based coatings generated hydrophobic surface character with contact angles in a range of 88.5° - 103.7°, and have poor surface wettability properties. Characterized plaques are tested for cell proliferation in order to evaluate any cytotoxic effect and the results show a relative cell proliferation ratio between 81.3% - 93.8%. The evaluation of blood plasma proteins that interact with the coating material show the effect of monomer used on prothrombin time, activated partial thromboplastin time and fibrinogen time and the results show that 2-hydroxyethylemethacrylate, ethylenediamine and hexamethyldisiloxane coatings hardly affect the extrinsic coagulation mechanism.

Key Words: 316L Stainless Steel, coronary stent, restenosis, coagulation, plasma surface modification

Advisor: Prof. Dr. Emir Baki DENKBAŞ, Hacettepe University, Department of Chemistry, Biochemistry Division

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ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my advisor, Prof. Dr. Emir Baki Denkbaş, for his guideness, undying encouragement and expertise during the process of creating my thesis. It was the best opportunity to work with him during the very beginning of my academic carrier.

I am very much indebted to Assist. Prof. Dr. Selçuk Aktürk for his great support in Scanning Electron Microsopy studies, and valuable advices.

I am also very gratefully indebted to the Hacettepe University Plasma Aided Bioengineering and Biotechnology Research Group, Prof. Dr. Mehmet Mutlu, Ebru Akdoğan, Eren Tur, Yalçın Öksüz and Başak Beyhan, for their excellent help during the thesis studies.

I appreciate the assistance and support I also received from Assoc. Prof. Dr. Hürkan Kurşaklıoğlu, Assoc. Prof. Dr. Atila İyisoy, Assist Prof. Dr. Ahmet İfran and Assist. Prof. Çetin Kocaefe.

I gratefully thank to the members of Biopolymeric Systems Research Group, Eylem Öztürk, Ph.D., Yasemin Mısırlı, Tamer Çırak, Doğa Kavaz, Banu Erdem Çetin, Arzu Çalışkan, Melek Uçar, Berna Berberoğlu, Osman Kazak, Bernas Özkan, Funda Budak, Merve Gültekinoğlu and Burcu Aslan for their help, friendship and encouragement.

I would like to thank all my colleagues at Hacettepe University, Chemistry Department for their academic and non-academic friendship.

Finally, I would like to thank my family for their neverending support and encouragement during the preparation of the thesis studies...

CEM BAYRAM

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1.INTRODUCTION

Coronary artery disease (CAD), in other say, atherosclerosis is the leading cause of cardiovascular mortality worldwide, with > 4.5 million deaths occurring in the developing world. Coronary artery disease is a condition characterized by the development of atherosclerotic plaques (fibro-fatty deposits) in the coronary arteries. Atherosclerosis is a chronic and widespread immunoinflammatory disease of large and medium-sized arteries fueled by atherogenic lipoproteins, in particular modified LDL. Atherosclerosis consists of the formation of fibro-fatty and fibrous lesions, preceded and accompanied by inflammation.

Atherosclerosis is a very important health problem that can cause serious results even mortality that makes the treatment unavoidable. One of the most common non-surgical treatment for opening obstructed coronary arteries is Percutanueous Transluminal Coronary Angioplasty (PTCA). In this procedure, a catheter is placed in the circulation system from the femoral artery and directed to coronary arteries. Then a baloon is inflated at the site of plaque formation. In many times, the baloon angioplasty is not sufficient to open the obliterated site, so a small device called stent is placed in there to keep the lumen open.

Metallic stent is typically a hollow cylindrical tube [d = 2–4 mm; l = 15–20 mm] with a patterned slit structure in two or three segments. It is usually made of a mesh type structure patterned in a metallic or polymer tube. It allows the flow of blood in the damaged vessel, thus avoiding it from being obliterated.

The treatment of baloon angioplasty with intravenous metallic stents for cardiovascular system diseases, has been the method of choice in the world with by-pass surgery; but some complications that threatens the human health and clashes the solutions provided by these metallic stents can be seen.

Some of these complications are; abrupt vessel closure during the deployment of stents and the restenosis during the follow-up period. The restenosis is still one of the major clinical problem of stent implantations with a 30% percent incidence, for the first 3 months following the operation. Secondary important complications are coagulation in metallic surface, inflammation of tissues and release of toxic metal ions and complexes by corrosion of metal surface. To minimize these

complications and to provide maximum biocompatibility of metallic stents for human health has been a major goal since the foundation of the implantation method by biocompatible material sciences and medicine. The starting point of these complications are originated from material surface – tissue interactions. The aim of the researches is to make these interactions in optimum conditions in cardiovascular system and to reach the maximum level of compability. The cost and complexibility of this problem, leads the scientists to eliminate complications and inhibition of these processes with the discovery of alternative and more effective surface modification techniques.

There are numerous examples in the literature and many commercial products related with the disadvantages of metallic stents. The modification of stent surfaces with many techniques like plasma surface modification, plasma ion immersing, covalent immobilizing of bioagents or drug eluting stents for the inhibition of cell proliferation to avoid restenosis are the main research topics in laboratories.

In this presented study, The aim is to carry out alternative surface modification techniques of biocompatible materials with plasma aid for the elimination of problems in stent implantation and to test the efficiency of modifications.

2. GENERAL INFORMATION

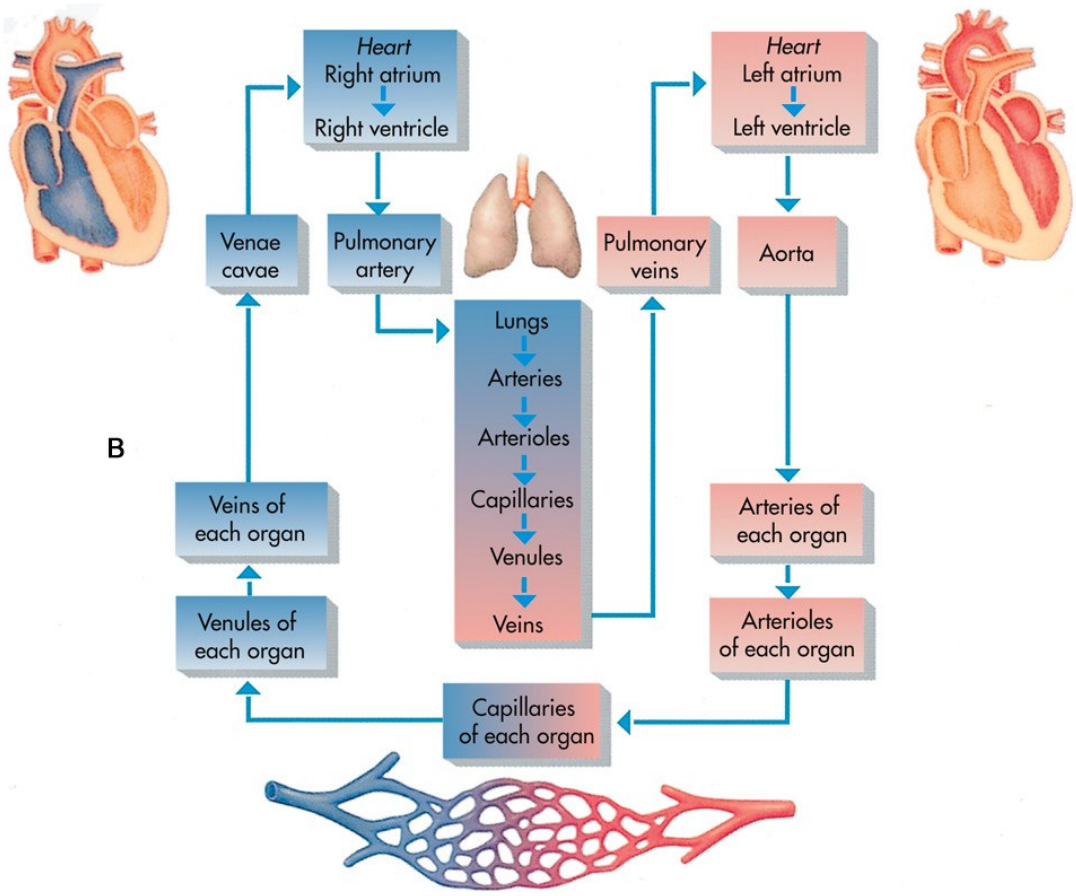
2.1. Vascular System

The vascular system, also called the circulatory system, is made up of the vessels that carry blood through the body. The arteries carry blood throughout the body, delivering oxygen and nutrients to the body tissues and the veins take away tissue waste matter.

The vessels of the blood circulatory system are:

- arteries - blood vessels that carry oxygenated blood away from the heart to the body.
- veins - blood vessels that carry blood from the body back into the heart.
- capillaries - tiny blood vessels between arteries and veins that distribute oxygen-rich blood to the body.

Blood moves through the circulatory system as a result of being pumped out by the heart. Blood leaving the heart through the arteries is saturated with oxygen. The arteries break down into smaller and smaller branches in order to bring oxygen and other nutrients to the cells of the body's tissues and organs. As blood moves through the capillaries, the oxygen and other nutrients move out into the cells, and waste matter from the cells moves into the capillaries. As the blood leaves the capillaries, it moves through the veins, which become larger and larger to carry the blood back to the heart (Iskandar A.Z., 2005).



From Thibodeau GA, Patton KT: *Anatomy & physiology*, ed 5, St Louis, 2003, Mosby.

Figure 2.1 Human Circulatory System

2.1.1. Coronary Circulation

The coronary circulation consists of the blood vessels that supply blood to and from the heart muscle itself. Although blood fills the chambers of the heart, the muscle tissue of the heart, or myocardium, is so thick that it requires coronary blood vessels to deliver blood deep into the myocardium. The vessels that supply blood with a high concentration of oxygen to the myocardium are known as coronary arteries. The vessels that remove the deoxygenated blood from the heart muscle are known as cardiac veins.

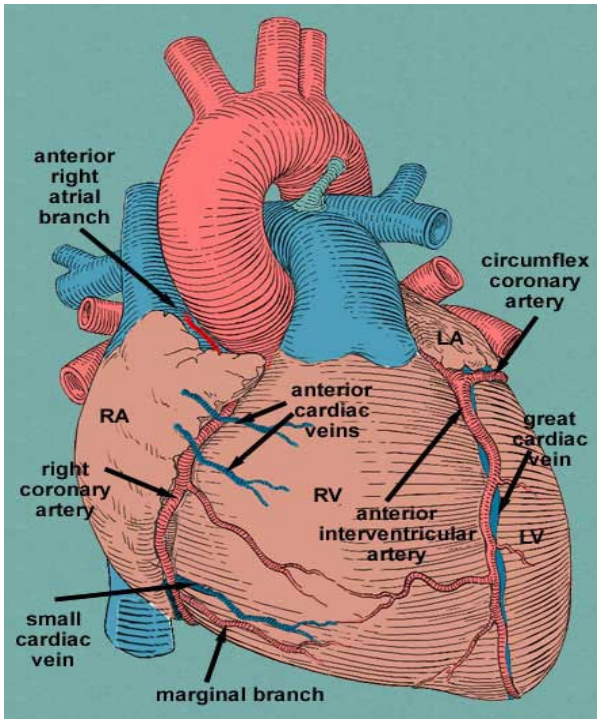


Figure 2.2. Coronary Circulatory System

The coronary arteries that run on the surface of the heart are called epicardial coronary arteries. These arteries, when healthy, are capable of autoregulation to maintain coronary blood flow at levels appropriate to the needs of the heart muscle.

The right coronary artery (RCA) originates above the right cusp of the aortic valve. It travels down the right atrioventricular groove, towards the crux of the heart.

The left coronary artery, abbreviated LCA arises from the aorta above the left cusp of the aortic valve.

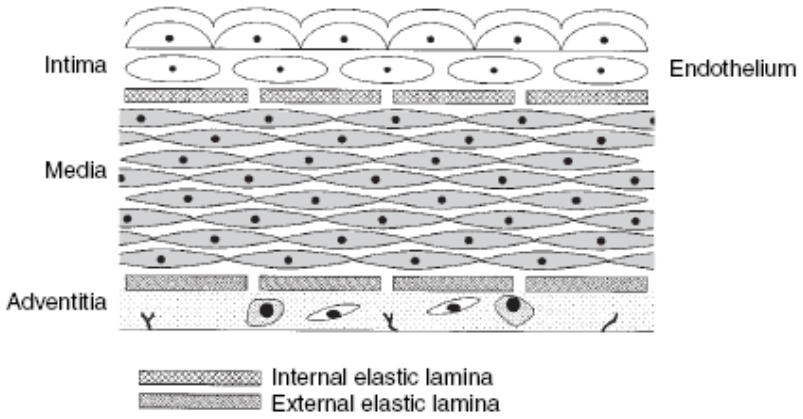


Figure 2.3. Schematic Diagram of the Arterial Wall (*Coronary Artery Disease in Clinical Practice, Satish Mittal*)

2.2. Coronary Artery Disease

Coronary artery disease (CAD) is the leading cause of cardiovascular mortality worldwide, with > 4.5 million deaths occurring in the developing world. Despite a recent decline in developed countries, both CAD mortality and the prevalence of CAD risk factors continue to rise rapidly in developing countries (Okrainec et. al., 2004).

Coronary artery disease (CAD) is a condition characterized by the development of atherosclerotic plaques (fibro-fatty deposits) in the coronary arteries. Atherosclerosis is a chronic and widespread immunoinflammatory disease of large and medium-sized arteries fueled by atherogenic lipoproteins, in particular modified LDL. Atherosclerosis consists of the formation of fibro-fatty and fibrous lesions, preceded and accompanied by inflammation. (Ross, 1993, Stary et. al., 1995)

As the plaque increases in size, the insides of the coronary arteries get narrower and less blood can flow through them. Eventually, blood flow to the heart muscle is reduced, and, because blood carries much-needed oxygen, the heart muscle is not able to receive the amount of oxygen it needs. Reduced or cutoff blood flow and oxygen supply to the heart muscle can result in:

- **Angina**. Angina that occurs regularly with activity, upon awakening, or at other predictable times is termed stable angina and is associated with high grade narrowings of the heart arteries is chest pain or discomfort that occurs when the heart does not get enough blood.

- **Myocardial Infarction**. A myocardial infarction (heart attack) happens when a blood clot develops at the site of plaque in a coronary artery and suddenly cuts off most or all blood supply to that part of the heart muscle. Cells in the heart muscle begin to die if they do not receive enough oxygen-rich blood. This can cause permanent damage to the heart muscle. Limitation of blood flow to the heart causes ischemia (cell starvation secondary to a lack of oxygen) of the myocardial cells. When myocardial cells die from lack of oxygen, this is called a myocardial infarction (commonly called a heart attack). It leads to heart muscle damage, heart muscle death and later scarring without heart muscle regrowth.

2.2.1. Risk Factors for Atherosclerosis

- **Family History.** It is estimated that about 40% of the risk of developing ischemic heart disease is controlled by genetic factors, and 60% by environmental factors. Hyperlipidemia, hyperfibrinogenemia, and abnormalities of other coagulation factors are often genetically determined. A positive family history increases the risk by a factor of approximately 1.5 and should also be taken into account in assessing individual risk. Risk rises further if more than one family member is affected. A history of heart attack in two or more first-degree relatives triples the risk of CAD (Ajjan and Grant, 2006).

- **Age and Sex.** Most new CAD events and most coronary deaths occur in older people (≥65 years). Middle-aged men from 35 to 65 have a higher CAD risk than women, because they have a high prevalence of risk factors, and are predisposed to abdominal obesity and the metabolic syndrome. In women between the ages of 45 to 75, the onset of CAD is delayed by some 10 to 15 years compared to men; thus most CAD in women occurs after age 65 years. Most deaths due to CAD in women under the age of 65 years occur due to multiple risk factors and the metabolic syndrome. (Engstrom et. al., 2004)

- **Hemostatic and Thrombogenic Factors.** Hemostatic factors associated with increased risk of coronary events include increased level of fibrinogen, activator factor VII, PAI-1, tPA, von Willebrand factor, factor V Leiden, and decreased, antithrombin III. A low fibrinogen level indicates reduced risk. An elevated plasma level of tPA is apparently associated with increased MI and stroke in healthy men. Fibrinogen increases blood viscosity and platelet aggregation, leading to a hypercoagulable state, promoting thrombosis. It also plays an important role in atherogenesis by fibrin deposition in vessel walls and may promote smooth muscle cells (SMC) migration and proliferation. Fibrinogen levels are increased in smoking, sedentary lifestyle, increased triglycerides, hypercholesterolemia, obesity, advancing age, oral contraceptive pills use, and stress. (Tousilis et. al., 2002)

- **Low-Density Lipoprotein (LDL).** Because LDLs transport cholesterol to the arteries and can be retained there by arterial proteoglycans starting the formation of plaques, increased levels are associated with atherosclerosis, and thus heart attack, stroke and peripheral vascular disease. This is why cholesterol inside LDL lipoproteins is called bad cholesterol. Still, it is not the cholesterol that is bad; it is instead how and where it is being transported, and in what amounts over time. (Carmena et. al., 2004)

2.2.2. Treatment Methods for Atherosclerosis

Atherosclerosis is very important health problem that can cause serious results even mortality that makes the treatment unavoidable. There are many kinds of methods for the early treatments like quitting smoking, avoiding stress, going on a proper diet, exercising and getting into fit. By the means of these actions, the plaque formation can be prevented in coronary arteries with collateral drug treatments sometimes like, anticoagulants or cholesterol lowering ones.

If the plaque formation is at extremely high levels that threatens the life, angioplasty method can be applied to the patient.

2.2.2.1. Percutaneous Transluminal Coronary Angioplasty

One of the most common non-surgical treatment for opening obstructed coronary arteries is Percutanueous Transluminal Coronary Angioplasty (PTCA). The name itself says a lot about the procedure:

Percutaneous means access to the blood vessel is made through the skin

Transluminal means the procedure is performed within the blood vessel

Coronary specifies that the coronary artery is being treated

Angioplasty means "to reshape" the blood vessel (with balloon inflation)Also referred to as "balloon treatment" because special balloons are used to open up obstructed arteries, illustrated on the left, this procedure sometimes also involves the use of devices known as "stents" to help keep the arteries open (Michaels, A. D. and Chatterjee K., 2002)

The angioplasty procedure usually consists of most of the following steps:

1. Access into the femoral artery in the leg is created by a device called an "introducer needle". This procedure is often termed percutaneous access.
2. Once access into the artery is gained, a "sheath introducer" is placed in the opening to keep the artery open and control bleeding.
3. Through this sheath, a long, flexible, soft plastic tube called a "guiding catheter" is pushed. The tip of the guiding catheter is placed at the mouth of the coronary artery. The guiding catheter also allows for radiopaque dyes (usually iodine based) to be injected into the coronary artery, so that the disease state and location can be readily assessed using real time x-ray visualization.
4. During the x-ray visualization, the cardiologist estimates the size of the coronary artery and selects the type of balloon catheter and coronary guidewire that will be used during the case. Heparin or other type of blood thinner is given to maintain blood flow.
5. The coronary guidewire which is an extremely thin wire with a radiopauqe flexible tip that is inserted into through the guiding catheter and into the coronary artery. While visualizing again by real-time x-ray imaging, the

cardiologist guides the wire through the coronary artery to the site of the stenosis or blockage. The tip of the wire is then passed across the blockage. The cardiologist controls the movement and direction of the guide wire by gently manipulating the end that sits outside the patient through twisting of the guidewire.

6. While the guidewire is in place, it now acts as the pathway to the stenosis. The tip of the angioplasty or balloon catheter is hollow and is then inserted at the back of the guidewire--thus the guidewire is now inside of the angioplasty catheter. The angioplasty catheter is gently pushed forward, until the deflated balloon is inside of the blockage.
7. The balloon is then inflated, and it compresses the atheromatous plaque and stretches the artery wall to expand.
8. If an expandable wire mesh tube (stent) was on the balloon, then the stent will be implanted (left behind) to support the new stretched open position of the artery from the inside.

2.3. Coronary Stents

Metallic stent is typically a hollow cylindrical tube [d = 2–4 mm; l = 15–20 mm] with a patterned slit structure in two or three segments. It is usually made of a mesh type structure patterned in a metallic or polymer tube. It controls the flow of blood in the damaged vessel, thus avoiding it from being ruptured (Kathuria, 2005).

Various biomaterials such as stainless steel, nitinol, platinum, titanium and tantalum alloys and gold or polymer coated stents have been used for such applications. Improving the surface as well as the cut roughness of the stent is

crucial to blood compatibility, because the first-layer cell formation on the surface is essential to minimize the blood clotting (Duerig, 2001).

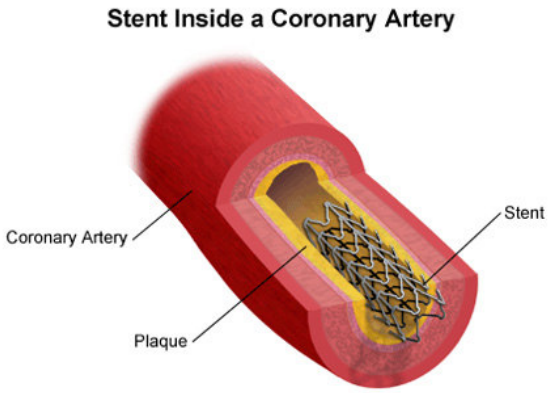


Figure 2.4 Stent Inside a Coronary Artery

In the year 1977, the balloon angioplasty technique was developed for the heart cardiovascular surgery (Gruentzig et. al., 1977). In some cases to overcome the re-collapse of vessel, stent therapy was introduced during 1980s (Palmaz, 1988). For insertion it is crimped onto a plastic balloon, which is transported to the lesion site by the catheter. Thereby after the expansion of the balloon with high pressure, the stent is unfolded and pressed against the wall. The balloon is deflated, the catheter is withdrawn and stent remains placed in the opening in order to prevent it from further collapse, so that the functioning of the blood vessel becomes normal again.

2.3.1. Adverse Effects of Coronary Stents

The metallic stents is the most used type of intracoronary devices of percutaneous transluminal coronary angioplasty operations. Although the bulk structure of these metallic devices has excellent mechanical properties, surface of the device may result very important health threatening problems. Tissue – biomaterial interaction occurs in the surface of implanted devices, and these surface properties must been altered for a specific biocompatibility criteria.

Percutaneous transluminal coronary angioplasty (PTCA) has become the main method of coronary revascularisation, accounting for more than 1 500 000 procedures worldwide every year.

Stent implantation, however, tends to cause injury to the blood vessel resulting in neointimal proliferation, known as in-stent restenosis (Bennett and O'Sullivan, 2001).

Restenosis is the reduction of the luminal size due to loss of gain in lumen size after intravascular interventional procedure. Several cellular and molecular events occur

sequentially after a vascular injury. The initial response of the elastic fibres of the vascular wall to overstretching by balloon catheter is elastic recoil, responsible for the loss of gain, which characterises the early phase of restenosis. The endothelial denudation and the exposure of subintimal components cause platelet adherence and aggregation, fibrinogen binding, and thrombus formation. Thrombus formation can also act as a scaffold into which vascular smooth-muscle cells can migrate, synthesise matrix and collagen, and reorganise the thrombus, providing the substrate for neointimal formation. Activated platelets release several mitogens and chemotactic factors, which stimulate smooth-muscle-cell migration and proliferation into the injury site. Inflammatory mediators and cellular elements contribute to trigger a complex array of events that modulate matrix production and cellular proliferation. Finally, remodelling, a gradual dynamic process mediated by adventitial myofibroblasts that leads to a change in vessel size by constrictive remodelling without an overall change in tissue volume, contributes to the loss of lumen at later times. Stenting reduces elastic recoil and negative remodelling, the mechanical component of restenosis, but also stimulates the cellular mechanisms yielding to in-stent restenosis (Fattori and Piva, 2003).

Stent restenosis rates are reported to be 15–20% in ideal coronary lesions, but may occur in over 30–60% of patients with complex lesions (Joung et. al., 2003).

Intracoronary metallic stents can also cause long term problems due to its thrombogenic nature. Platelet interaction with a material that has thrombogenic nature is the primary factor in thrombosis (Gottsauner-Wolf et. al., 1996). Restenosis can occur acutely either from thrombosis or elastic recoil (Suggs et. al., 1999). The activation of platelets in the blood flow is the main trigger effect of thrombosis. The metallic ions acting as a foreign material induce the activation of platelets (Ruygrok and Serruys, 1996).

To avoid from the complications above, metallic stents were exposed to surface modifications like hydrocarbon, fluorocarbon or diamond like carbon coatings (Haidopulos et. al., 2005, Joung et. al, 2003, Gutensohn et. al.,2000).

The coated materials can act as suitable and biocompatible barrier between the material surface and biologic area, altering or reducing the thrombogenic factors,

metallic ion release. By coating the surface with suitable materials, biological agents like heparin can be covalently attached to the stent surface for the prevention of thrombogenic disadvantages (Yoshioka et. al., 2003 , Tan et. al., 2003).

2.4. Plasma Polymerization

Plasma polymerization technique is widely used in modifying the surface properties of almost every type of materials. Plasma modification is applied with the different methods such as radio frequency glow discharge, microwave, atmospheric pressure, low pressure, low temperature. With the technique of radio frequency glow discharge, it is possible that the change the parameters of a surface such as adhesion, wettability, membrane permeability, hardness, resistance to corrosion and biocompatibility (Inagaki et.al., 1982; Tusek et al., 2001; Bae et. al, 2001)

The surfaces are etched or deposited, oxidated, crosslinked with various chemical molecules. In this technique, it is possible that to maintain the surface and the bulk properties completely different desired to any criteria, without changing the bulk properties such as stress and hardness which are very important especially in biomedical applications of biomaterials. The modification occurs at the surface and the bulk properties remains the same. (Kaminska et. al., 2002)

The advantages of radio frequency glow discharge are; being safe for environmental health, reproducubility, appliable with various types and monomers and selective modification.(Bae et. al., 2001)

2.4.1. Principles of Plasma Polymerization

Plasma is defined as the fourth state of the material. The plasma is generated by high temperature, electrical or magnetic field (Akman, 1993). Also a strong electric discharge is used in generating a plasma. Free electrons or radicals gain high energies and transfer their energies to other atoms and molecules. At this time emission is occured. The new molecules and radicals in various types are generated

during these reactions. The plasma is categorized mainly in two titles depend on the temperature of gas (Li et. al., 1997; Tusek et. al., 2001).

In high temperature plasmas, the temperature of gas exceeds 10^6 K. The nuclear explosions and controlled fusion reactions can be examples to high temperature plasmas. In the high temperature plasmas, many types of ions are present.

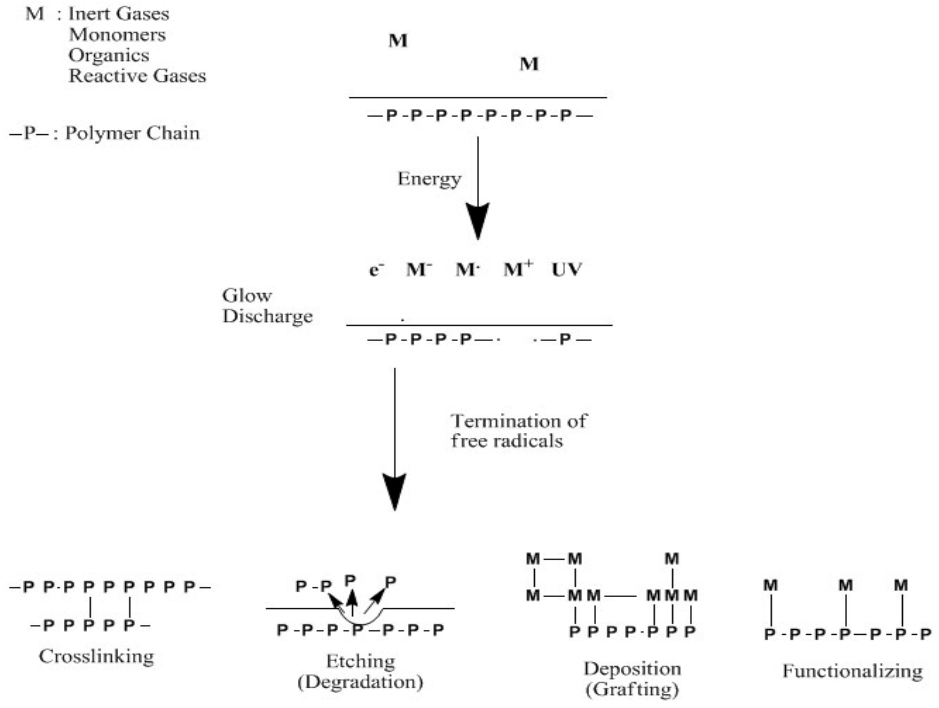


Figure 2.5. General Mechanisms in Plasma Polymerization

In low temperature plasmas, the temperature of gas lower than 10^6 K. Apart from ions, also the other types of components like radicals are present in low temperature plasmas.

(i) Hot plasma; the temperature of gas is over 1000 K, generally at 10^4 K. The emission in electrical bulb, electric arc and the other high glow discharge are examples of hot plasma.

(ii) Cold plasma; The temperature of gas is lower than 1000 K, in generally at 10^2 K. The glow discharge plasmas that occured at low temperatures are the examples of cold plasma.

A material subjected to a plasma medium can be exposed to mainly two different phenomenon as etching from surface or deposition onto the surface (Yasuda, 1984; Flosch et. al, 1992). It is possible that to select the one of these phenomenons by adjusting the plasma parameters. The plasma instruments gives this chance to get the most appropriate plasma applications by adjusting the properties of time, discharge power, distance between electrodes and pressure.

The most important major advantages of plasma deposition are; very high uniformity and very thin coating. The plasma deposition does not affect the bulk properties. The coating occurs at the surface, leaving the bulk structure the same.

Cold plasma is applied when the polymerization of organic substances. The main cause of this application is the degradation of the coating monomer at high temperatures.

In plasma polymerization it is a very big advantage that almost every type of organic compounds can be used in modifying materials. The coating occurs rapidly and uniformly on the material surface. The product is very pure because of not using of any initiator, solvent, stabiliser etc... This high purity is very important in medical areas. The plasma polymerization is a very short process compared to conventional coating processes. In conventional processes, prepolymer synthesis, preparing the coating solution, coating and drying processes can exceed a day, but in plasma polymerization these steps are all achieved in less than a hour (Mutlu et. al.,1997; Inagaki et. al., 1982)

2.4.2. Kinetics of Plasma Polymerization

The rate of the plasma depends on the structure of monomer used (Yasuda, 1985). In addition, the reactor design, monomer flow rate, pressure, discharge power, plasma duration, frequency and electrode wideness are also controllable parameters that affect the polymerization rate (Kaminska et. al., 2002).

The unsaturation level of the monomer is one of the very important factors that affect the polymerization rate. At the same conditions acethylene that have triple bonds is pollymerized more rapidly than ethylene which have double bonds.

Reactor geometry indicates the homogenity of the coating and the thickness. There are many types of reactors used in the plasma systems. Tubular, rectangular or other types of designes affect the monomer flow through the reactor. The retention time of monomer in the reactor before evacuation by the pump depends on the geometry.

The flow rate of the monomer is reported as an effective parameter on the deposition thickness. As the flow rate increases, the thickness decreases if the other parameters is kept same. In low flow rates, the polymerization occurs with more monomers and so the thickness of deposition increases.

The pressure inside the reactor is also an effective parameter on polymerization rate and deposition composition. The ionization of the gaseous residues in the reactor affects the mean free path of the monomers used by decreasing their kinetic energies. The impurities of gaseous residues can also be ionized and deposited onto the material, resulting differences in the composition of coating.

Generally as the discharge power increases, the deposition increases.

When the polymerization reaction is stable, the duration effects only the deposition amount and coating thickness. There is no any important changes in the bulk structure of polymer deposited or in the chemical composition.

Electrode wideness is also an effective parameter on kinetics of plasma polymerization. The short electrode wideness results high electron density which produces high polymerization rates.

2.4.3. Mechanism of Plasma Polymerization

Generally, the reactions between the material surface and low pressure glow discharge plasma is summarized into three titles. (Tusek et. al., 2001)

(1) Surface Reactions: The reactions between the species in gas phase and in the surface species, and the species in material surfaces only, generates functional groups at first and then cross-linked structures. Argon, ammonia, carbon monoxide, carbon dioxide, flor, hydrogen, nitrogen, water, oxygen plasmas are the examples of these types of reactions.

(2) Plasma Polymerization: Any type of organic monomer used in the plasma polymerization results in a thin layer on the material surface by the polymerization of monomer used. These type of reactions also include the self reactions of gas species inside the plasma reactor. The exact mechanism of plasma polymerization reactions are not clearly understood. The species of ions, free radicals, reactive groups contribute the plasma polymerization.

(3) Cleaning and etching: The substances that is present in the surface material are removed by chemical reactions and physical breakages. Oxygen plasma is widely used to clean surfaces from organic impurities.

2.4.4. Plasma Surface Modification In Biomedical Applications

Although many synthetic biomaterials have physical properties that meet and even exceed those of natural body tissue, they can often cause adverse physiological reactions such as infection, inflammation and thrombosis formation. Through surface modification, biocompatibility as well as biofunctionality can be achieved without changing the bulk properties of the material. There are many ways by which to alter the interaction of biomaterials with their physiological environments, of these; plasma surface modification provides device manufacturers with a flexible, safe and environmentally friendly process that is extremely effective.

- **Bioseparation**

Polymer membranes with selective permeation are widely used for applications such as, haemodialysis, protein purification, artificial organs and biosensors. Membrane materials must exhibit high selectivity of permeation to achieve high product purity while retaining high permeation rates to maximize productivity. High selectivity can be enhanced by incorporating chemical functionality (grafting) or physical restriction (controlled pore size) into the membranes.

- **Sterilization**

Plasma technology has also been considered for disinfection and sterilization of medical devices. The most convenient aspect of plasma technology is the potential for simultaneous surface modification and sterilization in biomedical device fabrication. Plasma sterilization may be suitable for medical implants and devices that are sensitive to temperature, radiation and chemicals.

- **Ocular prostheses**

Products such as contact lenses and intraocular lenses have been successfully modified by plasma treatment to impart protein- and cell-repelling characteristics, decrease bacterial adhesion, improve wettability and enhance patient comfort. Also, an ammonia plasma treatment has been applied to artificial corneas to enhance corneal epithelial cell attachment and growth.

- **Orthopaedic applications**

Plasma has also been employed to modify the surface of metal implants for adhesion promotion to bone cements or to enhance cell attachment and growth. Plasma crosslinking of a bioabsorbable polymer surface has been shown to regulate the material's degradation rate. Plasma technology can also improve the mechanical properties of totally absorbable composite bone plates strengthening the interfacial bonding between reinforced absorbable fibers and the absorbable matrix, and by retarding water penetration into the reinforced fibers and matrix to slow the rate of degradation.

- **Tissue culturing**

The surfaces of culture substrates, such as petri dishes, roller bottles, microcarriers and membranes can be modified by plasma treatment to improve cell growth, protein

binding or non-binding and cell-specific attachment by controlling surface chemical structures, surface energies and/or surface charge states.

2.4.5. Plasma Surface Modification of Vascular Stents

Metallic endovascular stents are used to scaffold biological lumen, most often in diseased arteries. They are mainly made of 316L stainless steel and the chemical composition is still controversial in the long term although they are largely implanted. (Huang et. al., 2006)

By applying a thin layer of coating to metallic surface, the disadvantages of stent use like metallic ion release, adhesion properties, thrombosis can be altered or reduced. Numerous examples can be found in the literature as plasma coating of metallic implants like silane , fluorocarbon films, metallic ions. (Kathuria, 2005).

To avoid the metallic ions release in the body, for the complete isolation of these metallic surfaces from the body liquid, a thin homogenous film coating can be applied to metallic structures without changing its bulk structure. In 2005, Haidopoulos et. al., succesfully covered the SS 316L metallic surface with hexafluoroethane film. With this process, the corrosion resistance of metallic material is increased (Haidopulos et. al., 2005).

The metallic surfaces can be exposed to many types of polymeric plasmas. Silane plasmas can activate the metallic surfaces for the further modification of surfaces. The activated sites can be used to covalent attachment of other chemical compounds or useful drug molecules onto the surfaces. Joung activated SS 316L surface by radio frequency HMDS plasma. The active sites of Si-Si groups were then treated with acrylic acid plasma. Metallic surface – Silane – Acrylic acid (SS – Si-Si – AAc) composition can directly covalently attached with estrogen and investigated the properties against restenosis (Joung et. al, 2003).

2.5. The Coagulation Cascade

Trauma to tissues and blood vessels initiates blood clotting, by setting one of the coagulation pathways into motion. Coagulation is a result of cascading chemical reactions of plasma proteins (clotting factors). These normally inactive factors circulate through the blood and are activated through enzymatic cleavage and/or surface contact (with, for example, the membranes of activated platelets or the surface of a biomaterial).

The extrinsic pathway is a coagulation cascade that begins with trauma to vascular walls and surrounding tissues. Tissue trauma causes the release of tissue thromboplastin, a collection of several factors including phospholipids from damaged cell membranes as well as a lipoprotein complex. The lipoprotein complex can function as a proteolytic (protein digesting) enzyme. Tissue thromboplastin combines with and activates factor VII, and this complex is then able to enzymatically activate factor X. Factor Xa combines with phospholipids (from the tissue thromboplastin or from platelets) and with factor Va to form prothrombin activator.

At this point in the extrinsic cascade occur with the same positive feedback loop due to thrombin and with the same outcome: the formation of fibrin.

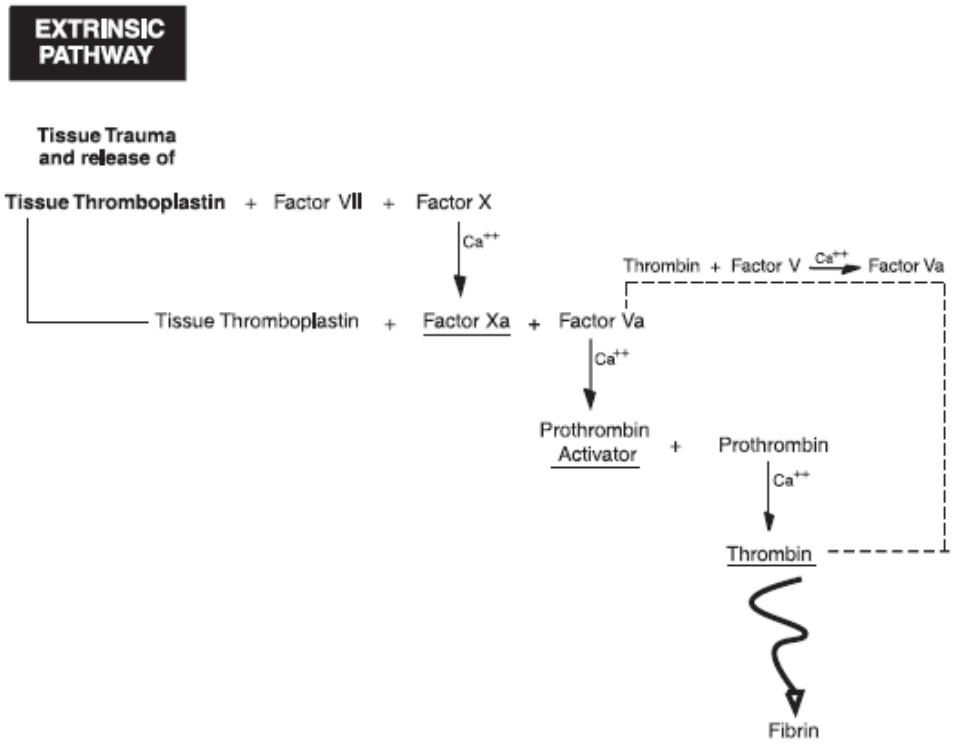


Figure 2.6. Extrinsic Pathway of Coagulation Cascade

The intrinsic pathway is a coagulation cascade that begins with the exposure of blood to a foreign surface (i.e., a surface that is not the membrane of a normal, healthy endothelial cell) or with trauma to platelets within the blood (via, for example, interactions with a foreign surface).

The intrinsic pathway can be initiated on blood contact with collagen in the subendothelium or on blood contact with a biomaterial. This is an important point to emphasize from a biomaterials perspective: Blood coagulation is automatically initiated upon implantation of a biomaterial or a device.

To start the intrinsic cascade, factor XII binds to the foreign surface and is activated, starting a chain of reactions. Platelets that adhere to the foreign surface (which is defined as a surface other than the membrane of a healthy endothelial cell) or that are damaged release phospholipids from their membranes, as well as a compound called platelet factor 3. These platelet-derived compounds can participate in the intrinsic cascade at two points: during the activation of factor X and in the formation of prothrombin activator (just as the tissue thromboplastin was necessary for the formation of prothrombin activator in the extrinsic pathway, Hoffmann A.S., 1997).

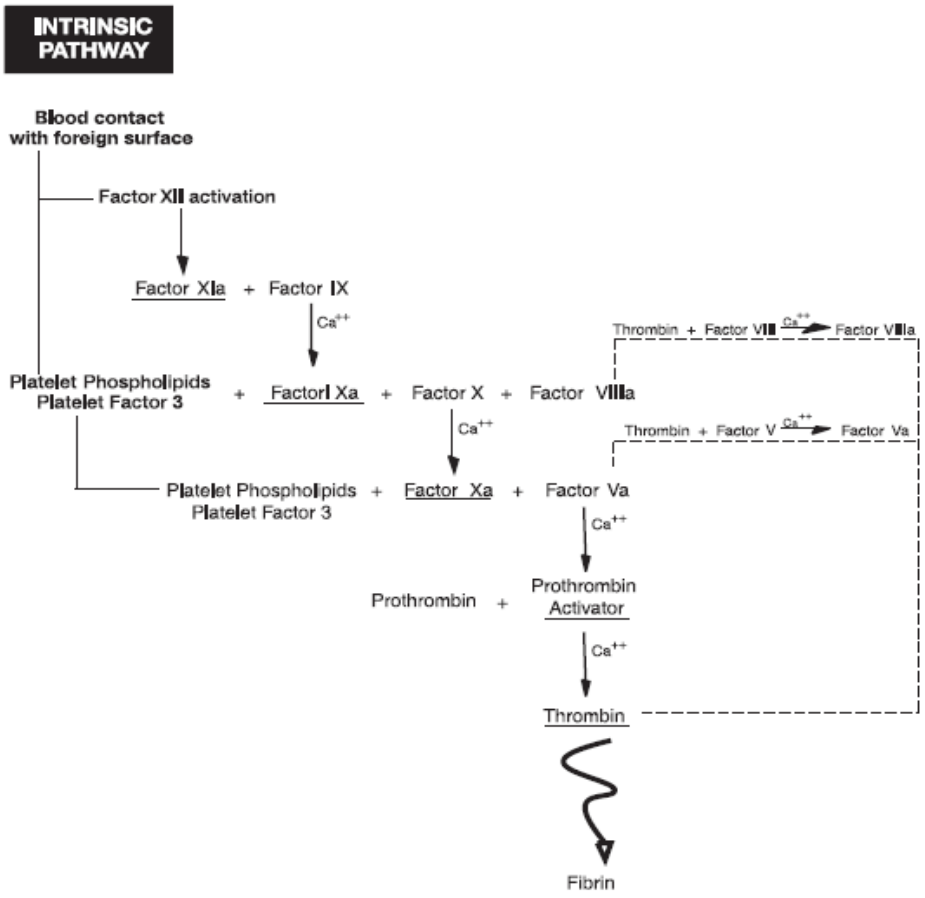


Figure 2.7. Intrinsic Pathway of Coagulation Cascade

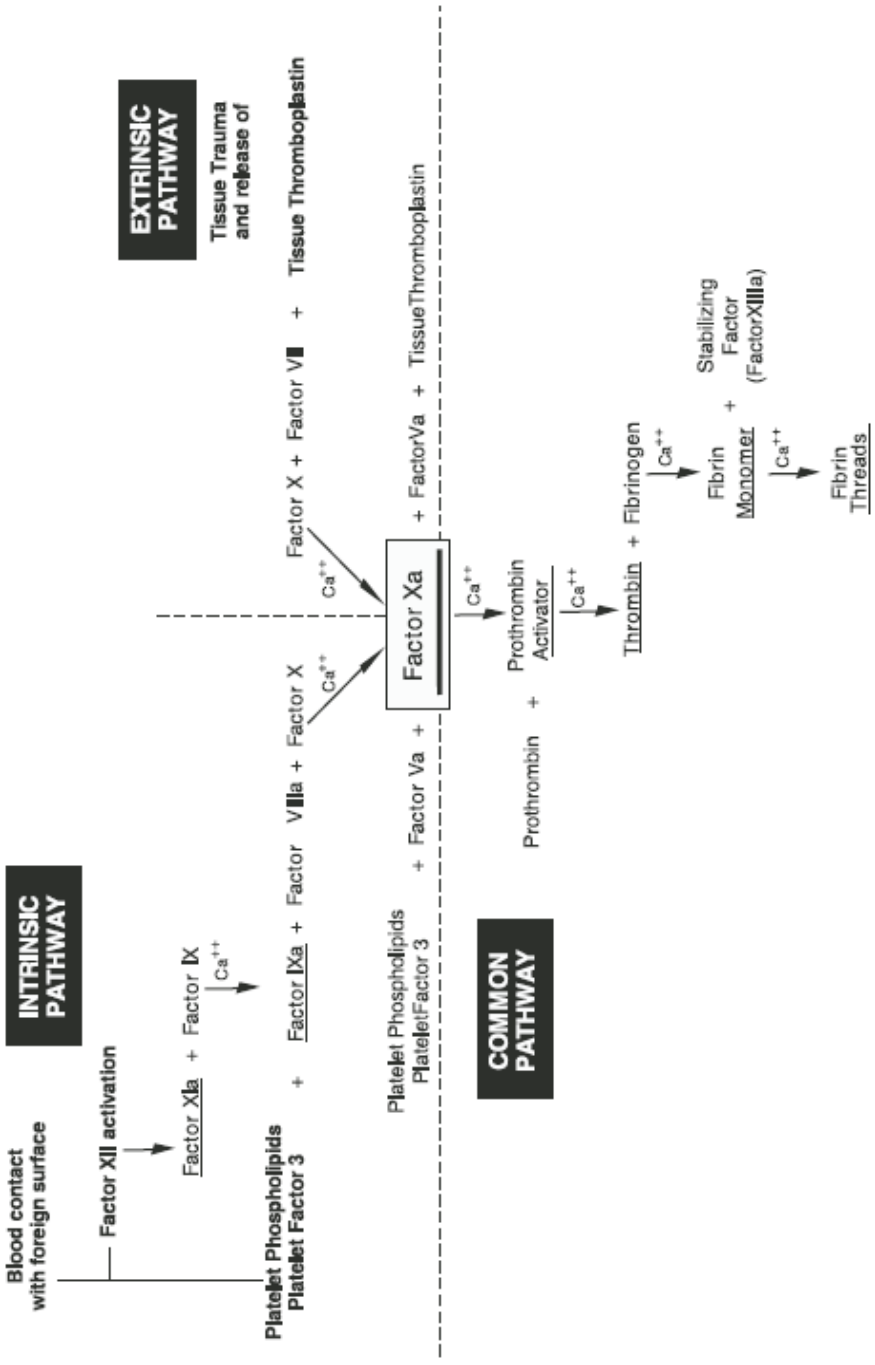


Figure 2.8. Common Pathway of Coagulation Cascade

2.5.1. Coagulation Test Parameters

2.5.1.1. Activated Partial Thromboplastin Time

The activated partial thromboplastin time (APTT) is a common screening test done to evaluate function of the intrinsic clotting system.

- It has largely replaced the older PT, which was unable to incorporate variables in surface/contact time.
- The APTT now measures the clotting time of plasma, from the activation of factor XII by a reagent (a negatively charged activator such as silica and a phospholipid) through the formation of a fibrin clot.
- If a patient's APTT is abnormal, additional tests will be done to determine the exact cause of the coagulation problem.

2.5.1.2. Prothrombin Time

Prothrombin time (PT) is a blood test that measures how long it takes blood to clot. A prothrombin time test can be used to screen for bleeding abnormalities. PT is also used to monitor treatment with medication that prevents the formation of blood clots.

At least a dozen blood proteins, or blood clotting factors, are needed to clot blood and stop bleeding (coagulation). Prothrombin, or factor II, is one of several clotting factors produced by the liver. Adequate amounts of vitamin K are needed to produce prothrombin. Prothrombin time is an important coagulation test because it measures the presence and activity of five different blood clotting factors (factors I, II, V, VII, and X). The prothrombin time is lengthened by:

- Low levels of blood proteins (blood clotting factors).
- A decrease in activity of any of the factors.
- The absence of any of the factors.
- The presence of a substance that blocks the activity of any of the factors.

2.5.1.3. Fibrinogen Time

Fibrinogen levels can be measured in venous blood. Normal levels are about 150-300mg/dL. Higher levels are, amongst others, associated with cardiovascular disease (>460mg/dL). It may be elevated in any form of inflammation, as it is an acute phase protein.

It is used in veterinary medicine as an inflammatory marker: in horses a level above the normal range of 1.0-4.0 g/L suggests some degree of systemic inflammatory response.

Low levels of fibrinogen can indicate a systemic activation of the clotting system, with consumption of clotting factors faster than synthesis. This excessive clotting factor consumption condition is known as Disseminated Intravascular Coagulation or "DIC." DIC can be difficult to diagnose, but a strong clue is low fibrinogen levels in the setting of prolonged clotting times (PT or PTT), in the context of acute critical illness such as sepsis or trauma.

3. EXPERIMENTAL

In the experimental part of the study 1cm x 2 cm Stainless Steel 316L plaques, which have the same chemical composition like stents, were used. SS 316L metal is the raw material of many types of bare cardiovascular stents. Working with the plaque form of this material provides ease in contact angle, energy – dispersive x-ray spectroscopy (EDX) studies and low cost.

3.1. Surface Pre-Cleaning of SS 316L Plaques

The surface of the SS 316L plaques may contain organic or inorganic impurities. To avoid any interfering ion or radical that will come from these impurites during the plasma surface modifying, the surfaces were pre-cleaned with piranha solution.

The piranha solution is consists of hydrogen peroxide and sulphuric acid. A typical mixture of piranha solution is 3:1 ratio of sulphuric acid to hydrogen peroxide solution. Both hydrogen peroxide and sulphuric acid are taken from its stock solutions which are 30% and 95 – 97% respectively (Wei et. al., 2003).

To create the piranha bath, one typically starts with a bath of sulphuric acid, to which is added the peroxide. One must always add the peroxide to the acid, not the other way around. The mixture reaction is exothermic, hence the solution will become hot. Once the mixture has stabilized, it can be further heated to sustain its reactivity.

The SS 316L plaques were interacted with piranha solution for 5 minutes. Pre-cleaned plaques were then rinsed with distilled water 2 times for 10 minutes in water sonication bath.

Pre-cleaned SS 316L plaques were dried in vacuum oven and kept in closed glassware.

3.2. Plasma Surface Modification of SS 316L Plaques

3.2.1. Plasma Modification System

The surface of SS 316L plaques were modified with the radio frequency glow discharge (RFGD) plasma deposition technique. Plasma modification system has a 13.56 MHz radio frequency generator, and inside the reactor there are two copper electrodes. One is attached via the impedance unit to the generator and the other one is grounded. The plasma reactor is attached with $1x10^{-4}$ mbar vacuum pump for evacuation of reactor gas. The reactor is fed with monomer tank and argon gas during the process.

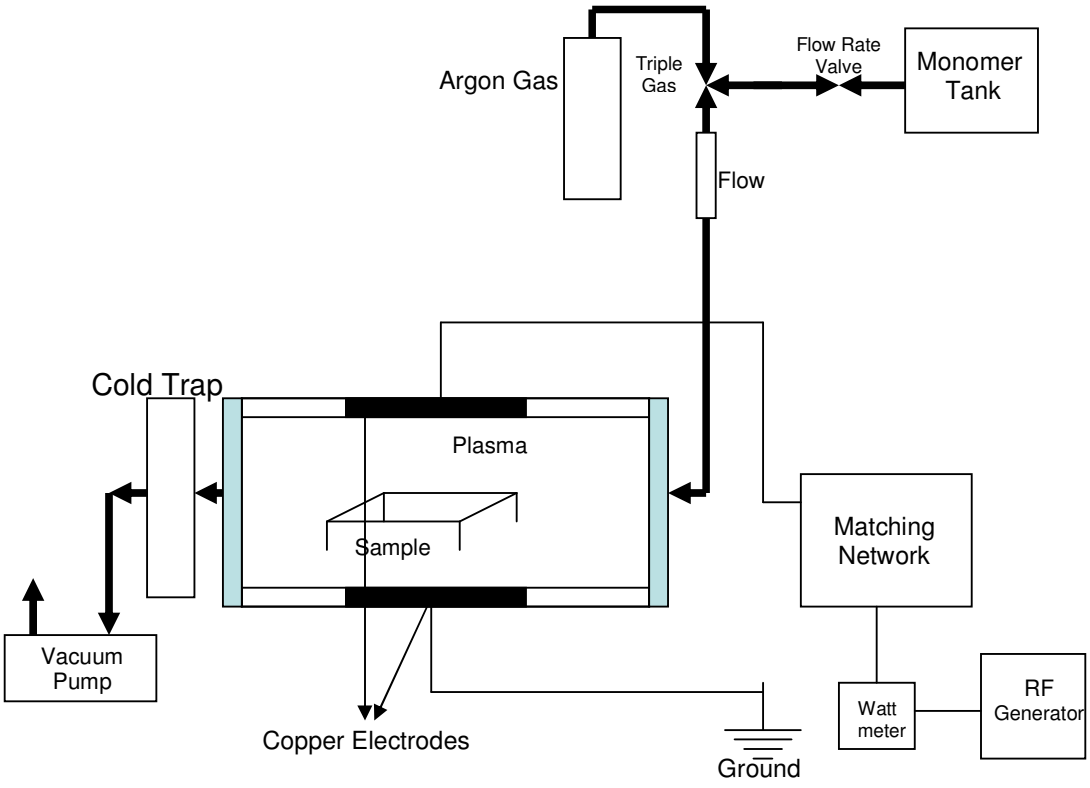


Figure 3.1 Plasma Polymerization System

3.2.2. Surface Modification Method

In the surface modification method 5 different monomers and prepolymers polyethyleneglycol (Acros, Belgium, Mw=300), 2-hydroxyethylmethacrylate (Aldrich, USA), ethylenediamine (Fluka,USA), hexamethyldisilane and hexamethyldisiloxane (Merck, Germany) were used in two different power levels (25 W and 50W).

In a typical procedure;

- The plaques were placed perpendicularly onto a stereofoam support that deployed in the middle of the electrodes, and the lid was closed well.

- The pump was activated and the pressure inside the reactor decreased to 0.14 – 0.16 mbar.

- The inert argon gas was passed through to system for 10 minutes to sweep away any reactive species like oxygen and nitrogen atoms left in the reactor.

- The pressure was again decreased to 0.16 mbar, which is enough to obtain a homogenous plasma.

- The monomer was fed up into the reactor and the process was continued for 10 minutes to ensure the existence of monomer presence only.

- RF generator was activated at the desired power and discharge lasts for 10 minutes. During the process modification was occured.

- After the process, the generator was deactivated and argon gas was supplied into the reactor for 10 minutes for the sweeping the monomer

and radical residues that can be adsorbed onto the plaques surfaces away.

- The samples were kept in vacuum for 10 minutes.

The samples were collected and kept in closed vessels for further analysis (Akdogan et. al., 2005).

3.3. Characterization of Plaques

The bare metal plaques and surface modifed plaques were characterized with sessile drop contact angle measurement for the evaluation of hydrophilicity, with SEM imaging and energy dispersive X-ray spectroscopy (EDX) for the characterization of chemical surface composition.

3.3.1. Morphological Evaluations

Morphological investigations of SS 316L plaques were carried out with scanning electron microscopy (LEO, Germany) technique at 10.00 kV energy and 1000 – 3000 X magnifying.

3.3.2. Chemical Characterization of Surfaces

The chemical surface characterization of the bare and modified SS 316L plaques were carried out with energy dispersive X-ray spectroscopy (EDX) device attached to scanning electron microscopy. The spectra were taken during the SEM imaging.

Energy dispersive X-ray spectroscopy is an analytical tool predominantly used for chemical characterization. Being a type of spectroscopy, it relies on the investigation of a sample through interactions between light and matter, analyzing X-rays in its particular case. Its characterization capabilities are due in large part to the fundamental principle that each element of the periodic table has a unique electronic structure and, thus, a unique response to electromagnetic waves (Stefaniak et. al., 2006).

3.3.3. Contact Angle Measurements

Contact angles of plaque surfaces were measured by sessile drop technique. The measurement apparatus consists of a camera and camera lens (Hitachi, Japan), sample platform and water dropping syringe. In the measurements deionized water was used. The shape of the drop was captured by “Open Video Capture” software via a video card (AsusTek Computer Inc., Taiwan) attached to the camera. The resulting images were analyzed in the ImagePro Software (USA). The contact angles (Θ) of the surfaces were calculated with the formula;

$$\Theta/2 = \arctan 2h/d \qquad (3.1)$$

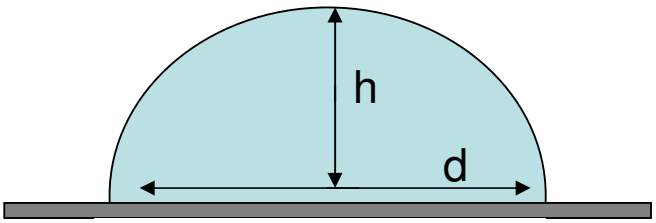


Figure 3.2. Sessile drop contact angle measurement

Measurements were repeated at least 10 times, and the results were given as averages.

3.4. *In Vitro* Cytotoxicity Assay

The prepared surface modified SS 316L plaques were subjected to an *in vitro* cytotoxicity test whether they effect the cell proliferation or not. The cell proliferation was investigated for 2 days and the results were compared with control groups.

In the procedure 1cm x 2cm plaques were incubated with equal amounts of precultured mouse connective tissue fibroblast cells (L-929 cell line, Foot-and-Mouth Disease Institute (Ankara) of Ministry of Agriculture & Rural Affairs of Turkey) in growth media consist of 90% RPMI 1640 with L-Glutamine + 10% FBS (Bio-Industries, USA) in incubator (Revco, USA) supplied with 5 % CO_2 at 37℃ for 2 days. Then the monolayer growing cells were harvested by using Trypsin-EDTA solution (Bio-Industries, USA) and immediately suspended in fresh cell growth media. 0.5 ml of cell suspension was mixed with 0.5 ml of 0.4% Trypan Blue solution (Bio-Industries, USA). The mixture was vortexed to obtain a homogenous solution and both viable (colorless) and non viable (blue) cells were counted using a Thoma Haemocytometer.

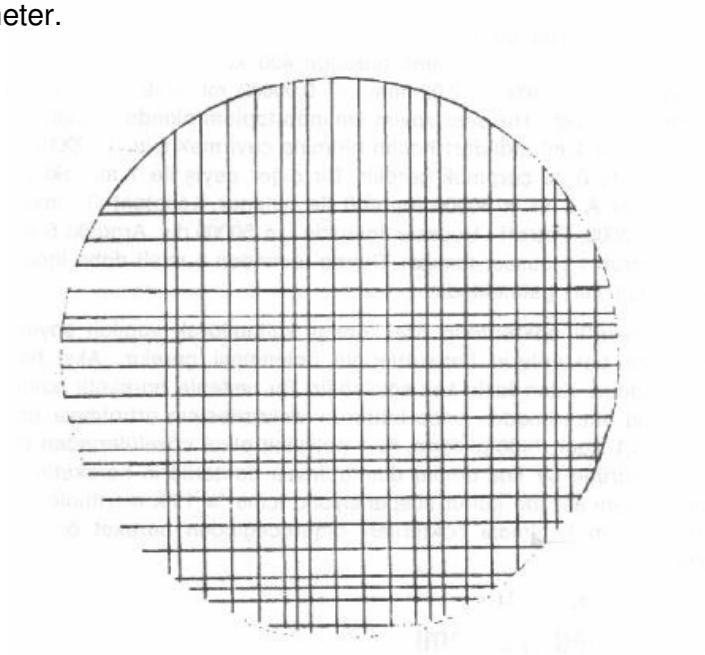


Figure 3.3. Thoma Microscope Slide.

In Thoma slide there are 400 small squares, of which dimensions are 50 μm x 50 μm, in the counting area. The counting area is basically a square prisma with a depth of 100 μm.

The volume of a one small square is; 0,05 mm x 0,05 mm x 0,1 = 0,00025 mm^3

In the counting area, there are 400 of these squares, and so:

0,00025mm^3 x 400 = 0,1 mm^3

The conversion factor is; 1ml = 1 cm^3 = 10000 x 0,1 mm^3

Then finally the calculation formula for the counting area of Thoma slide is:

Cells/ml = Counted Cells X Diluting Factor X 10000 (3.2)

3.5. *In Vitro* Haemocompatibility Tests

In this part of the study, the plasma surface modified materials were exposed to some coagulation tests to investigate whether they affect the coagulation cascade or not. The clotting characteristics of the plasma were taken as the blank values of parameters APTT, PT and Fibrinogen, and were compared with the plasma in contact with the samples.

3.5.1. *In Vitro* Haemocompatibility Test Procedure

The citrated whole blood taken from a healthy donor was centrifuged at 4000 rpm for 7 min at room temperature to obtain platelet poor plasma (PPP). The samples placed in a polystyrene 24-well plate dish were in contact with 1ml of PPP for 30 min 37℃ (Yoshioka et. al., 2003). The plasma samples were tested with the following procedure:

- The clotting balls were put in each test tubes.

- The automatic pipette was filled with test reagent, Neoplastine for PT, $CaCl_2$ for APTT and Fibri Prest for Fibrinogen.

- Plasma samples were added to clotting ball containing test cuvettes (50µl plasma for PT, 50µl plasma and 50 µl APTT reagent for APTT, and 1/20 diluted 100 µl plasma for fibrinogen test).

- The samples were incubated for definite time (50 seconds for PT and fibrinogen and 170 seconds for APTT) at 37℃.

- After incubation the sample containing cuvettes were transferred immediately to testing part of the analyzer and the specific test reagents were added (100 µl for PT and 50 µl for APTT and fibrinogen).

- The results were taken after the measuring time.

All analyzes were triplicated.

3.5.2. *In Vitro* Haemocompatibility Test Device

The *in vitro* haemocompatibility tests of SS 316L plaques were done with STA 4 Compact Blood Coagulation Analyzer, (Diagnostica Stago, France) using the required test kits such as PTT, APTT, Fibrinogen and D – Dimer. (Diagnostica Stago, France).

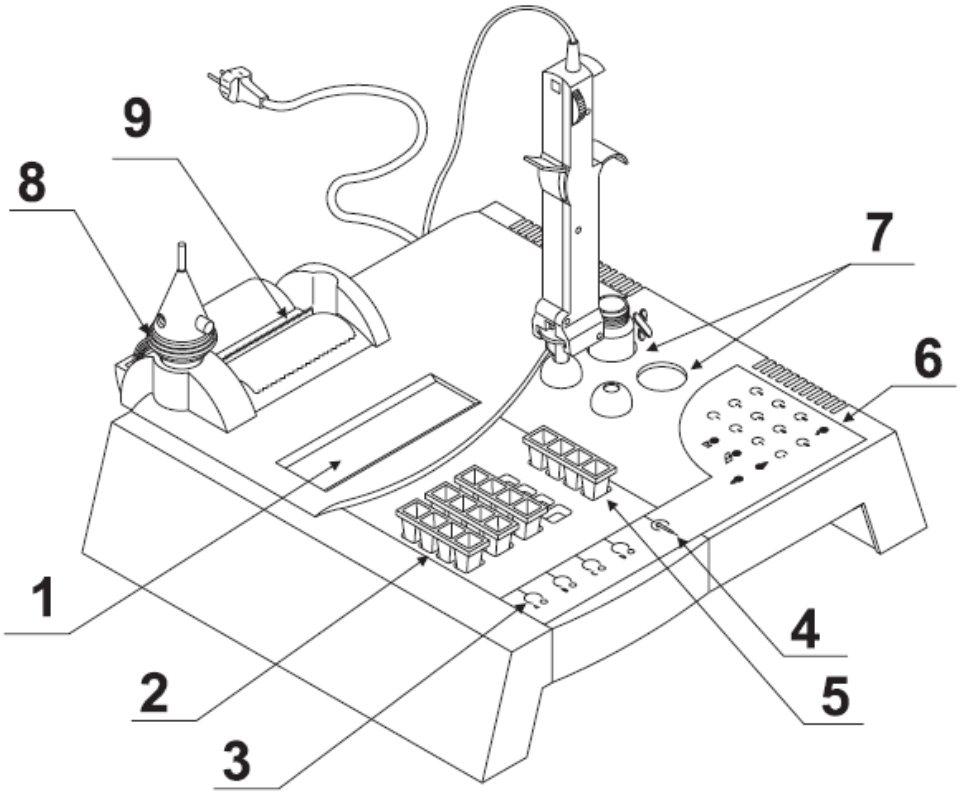


Figure 3.4. Blood Coagulation Test Device

1 - Back light liquid crystal display

2 - Incubation area (4 columns of 4 cells) thermostated at 37 ℃

3 - Control keys for incubation timers A to D:

4 - Control key for pipette

5 - Measurement area thermostated at 37 ℃

6 - Numerical keyboard, see following description

7 - Two 37 ℃ thermostated reagent and multipette storage positions

8 - Single storage position for the ball dispenser

9 - Thermal printer

3.5.3. Principle of Clot Determination

The principle consists of measuring the variations of the ball oscillation amplitude through inductive sensors.
The ball has a pendular movement obtained

- thanks to the two curvated rail tracks of the cuvettes
- and an alternate electro-magnetic field created by two independent coils

The oscillation amplitude is constant when the environment has a constant viscosity.
The oscillation amplitude decreases when the environment viscosity increases.

Constant pendular swing of the ball at constant medium viscosity is achieved on through application of an electro-magnetic field created alternately at opposite sides of each measurement well .

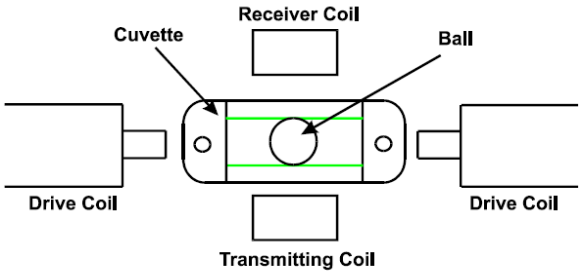


Figure 3.5. Clot Sensing System

The intensity of magnetic field can be varied depending on the tests to be carried out (PT, APTT...) and on the expected clot.

The detection system of the oscillation amplitude variations is based on two measurement coils.

The transmitting coil emits an electro-magnetic field. The signal received by the receiver coil depends on the ball position in the cuvette.

An algorithm uses these oscillation amplitude variations to determine the clotting time.

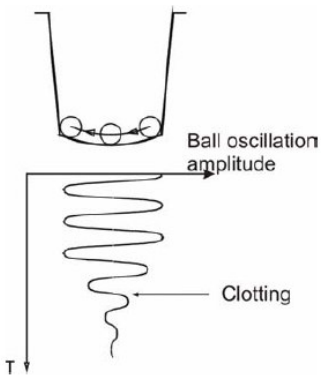


Figure 3.6. Clot Forming

4. RESULTS AND DISCUSSION

The main purpose of this study is to change the surface characteristics of 316L stainless steel plaques which are used to manufacture intracoronary stents. The tissue – biomaterial interaction is a case that occurs in the surface of the material, so; different surface characteristics generates different host responses. 5 different monomers were used during the modification and the surface properties were investigated as morphologic, chemical composition, surface wettability, cytotoxicity and haemocompatibility.

The results of the study were explained in following subsections.

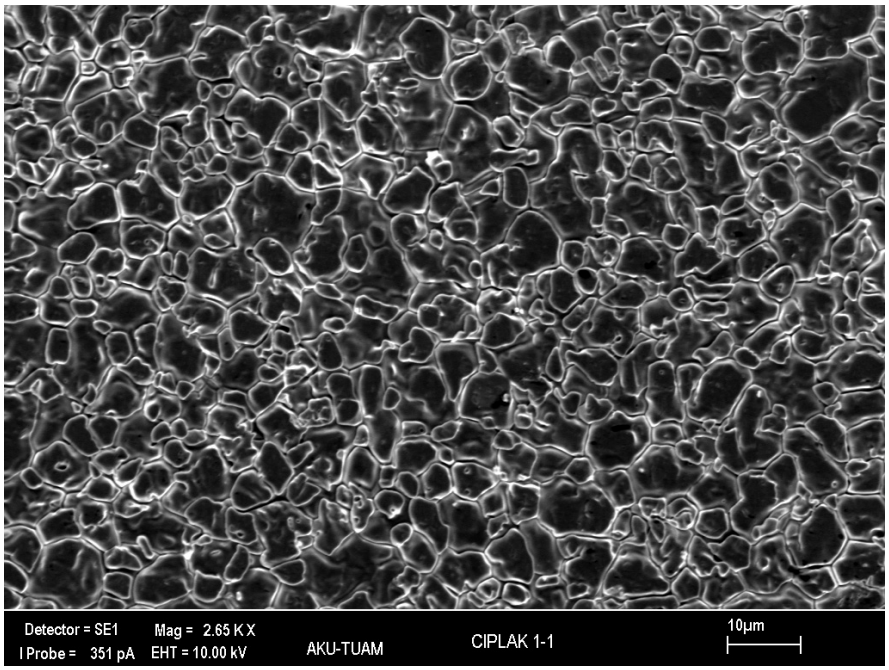
4.1. Characterization of SS 316L Plaques

The SS 316L plaques of which surfaces plasma modified were characterized by Scanning Electron Microscopy imaging for morphological evaluation, energy dispersive x-ray spectroscopy for chemical composition of the surface area and sessile drop contact angle methods for the evaluation of hydrophilicity character.

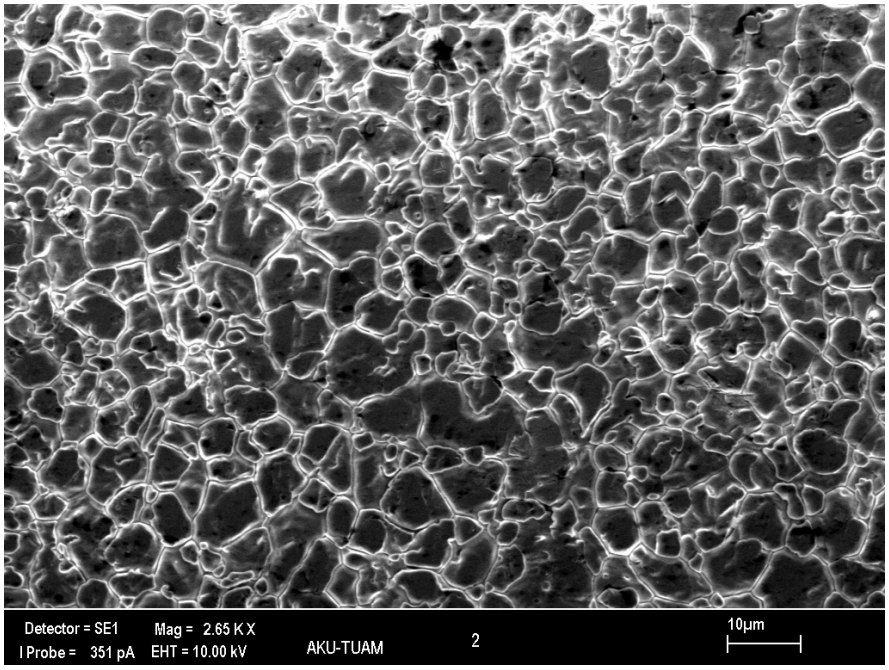
4.1.1. Morphological Evaluations

The surface of the 316L stainless steel material are shown in Figure 4. The figures from 4A to 4F, belong to bare steel, poly ethyleneglycol coated steel, ethylenediamine coated steel, 2-hydroxyethyl methacrylate coated steel, hexamethyldisilane coated steel, hexamethyldisiloxane coated steel, respectively. All the metals were pre-cleaned with piranha solution.

The images show that there is no changes between the plaques due to the thickness of the coating.

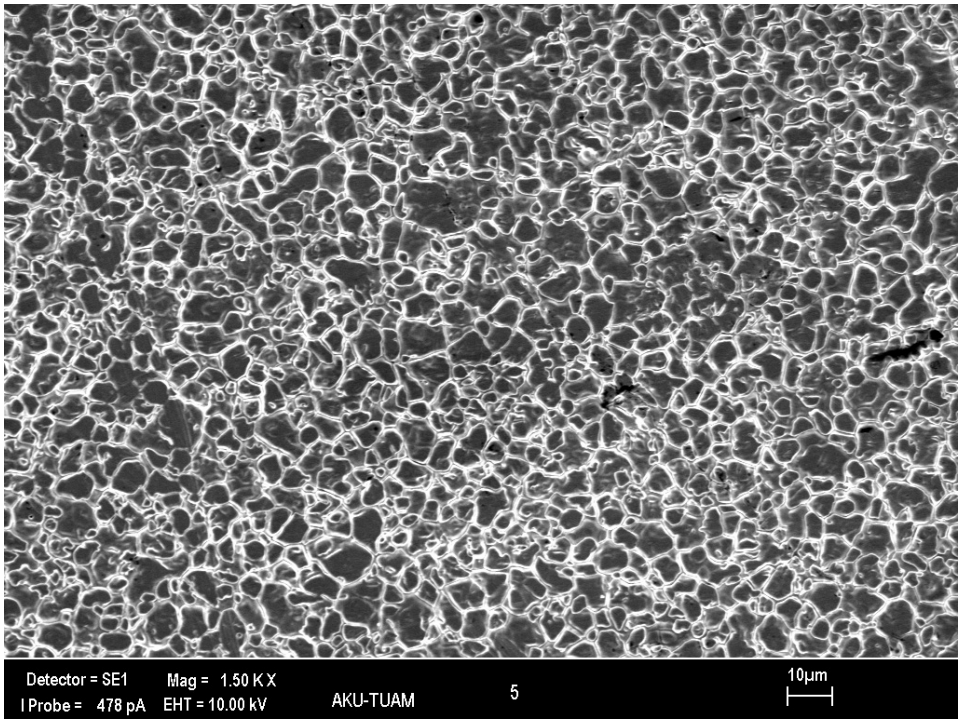


A

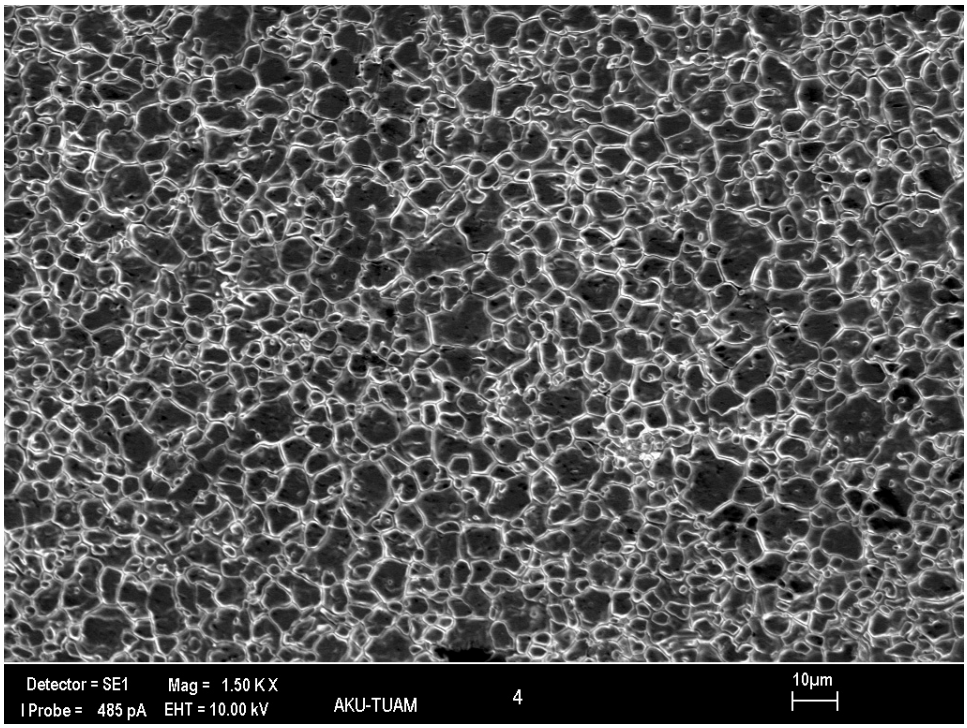


B

Figure 4.1A and 4.1B. SEM microimages of SS 316L plaques, (A) bare steel, (B) poly ethyleneglycol coated steel.

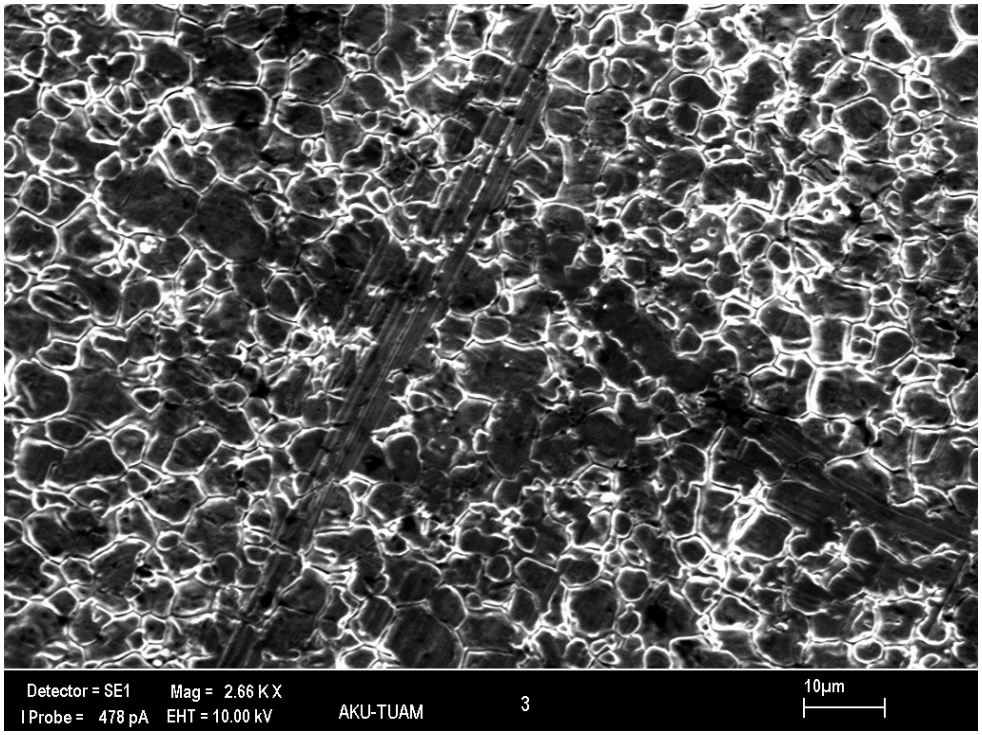


C

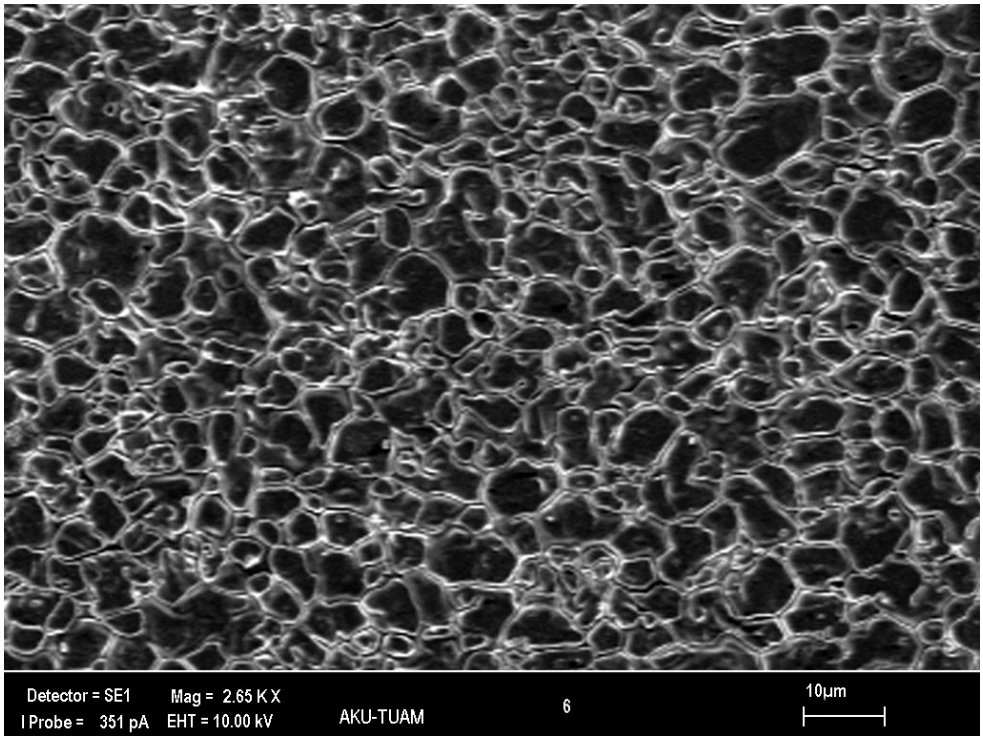


D

Figure 4.1C and 4.1D. SEM microimages of SS 316L plaques, (C) 2-hydroxyethylmethacrylate coated steel, (D) hexamethyldisilane coated steel.



E



F

Figure 4.1E and 4.1F. SEM microimages of SS 316L plaques, (E) ethylenediamine coated steel, (B) hexamethyldisiloxane coated steel.

4.1.2. Energy Dispersive X-Ray Spectroscopic Measurements

Energy dispersive X-Ray (EDX) spectra of the SS 316L plaques are shown in Figures 4.2A. – 4.7A. In the spectra, Gaussian-shaped peaks of most abundant elements of Fe, Cr, Ni in unmodified SS 316L are shown at 0.76 and 6.4, 0.52 and 5.2, 0.83 and 7.4 keV, respectively. The increase in C peaks at 0.27 keV can be seen in modified SS 316L and the results of relative abundance ratios are given in figures 4.2B. – 4.7B. Bare SS 316L material has C element with a ratio of 0.03% maximum in the composition. All modifying compounds contains C atom and the abundance ratio of C is in the range of 3.5% - 14.1%. C element is most abundant in 2-hydroxyethylmethacrylate coating with a ratio of 14.1%.

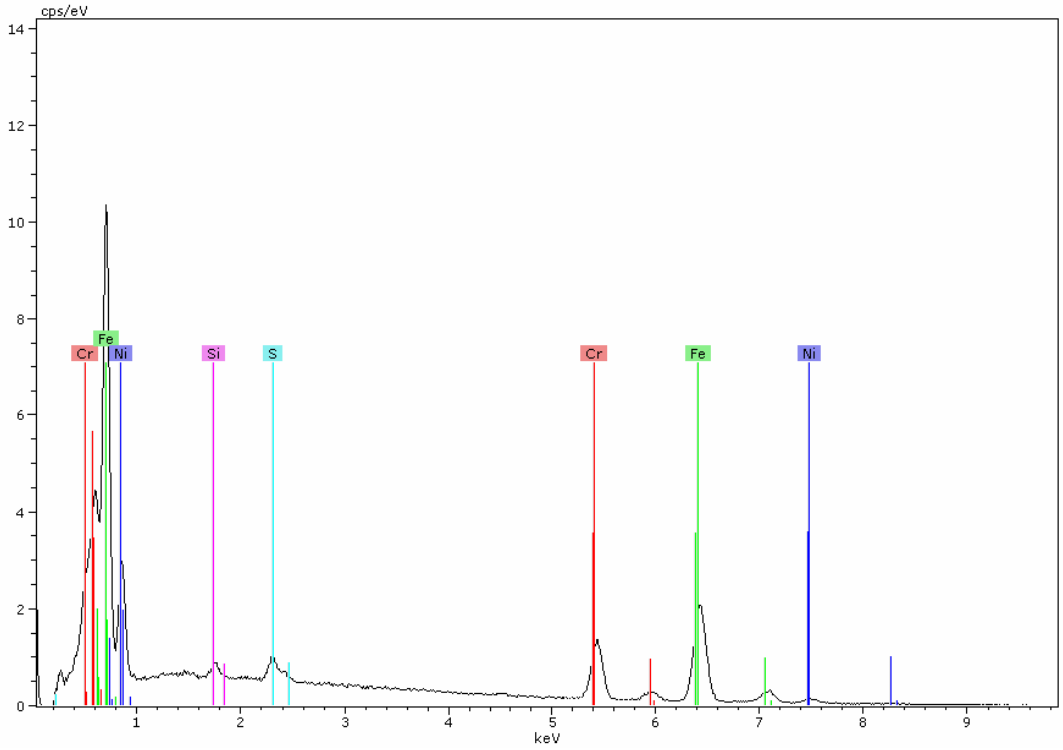


Figure 4.2A. EDX Spectrum of bare SS 316L plaque surface.

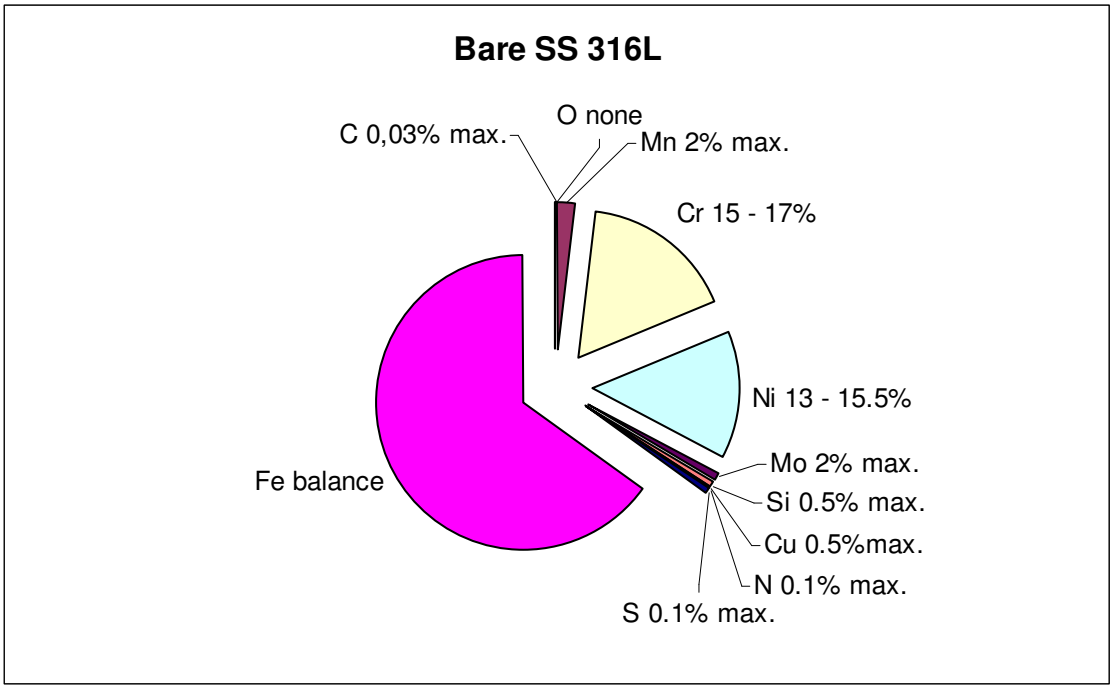


Figure 4.2B Element % of bare SS 316L plaque surface.

Polyethylene glycol and 2-hydroxyethyl methacrylate coated SS 316L spectra have oxygen peaks at 0.55 keV as can be seen in figures 4.3A and 4.4A, respectively. Relative abundance ratios of O atom in the polyethylene glycol and 2-hydroxyethyl methacrylate modifications were found as 1.9% and 0,11%, respectively (Figure 4.3B and 4.4B). Unmodified bare SS 316L plaques have no O peak in its spectra (Figure 4.2A) and the existence of peaks at 0.55 keV indicates that the surfaces have O containing compound. Also C atom peaks at 0.27 keV with the relative abundance ratio of 9% (Figure 4.3B) and 14.1% (Figure 4.4B) n polyethylene glycol and 2-hydroxyethyl methacrylate coatings are also another data that proves the modifications with the selected materials.

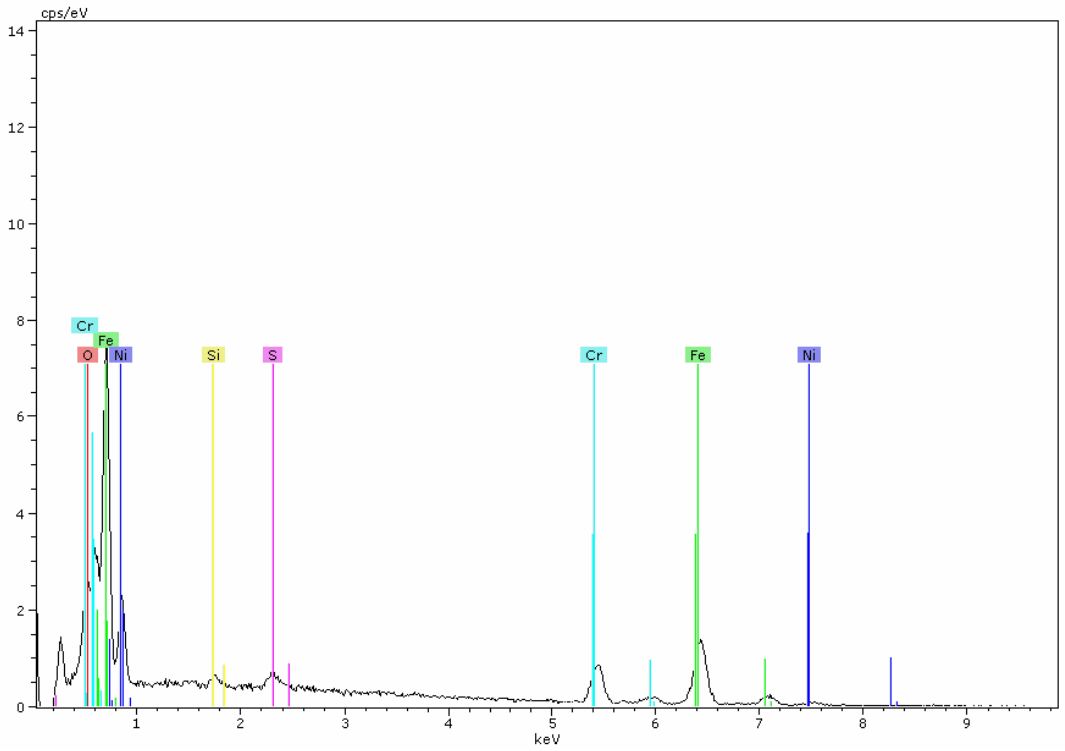


Figure 4.3A. EDX Spectrum of pls – PEG modified SS 316L plaque surface.

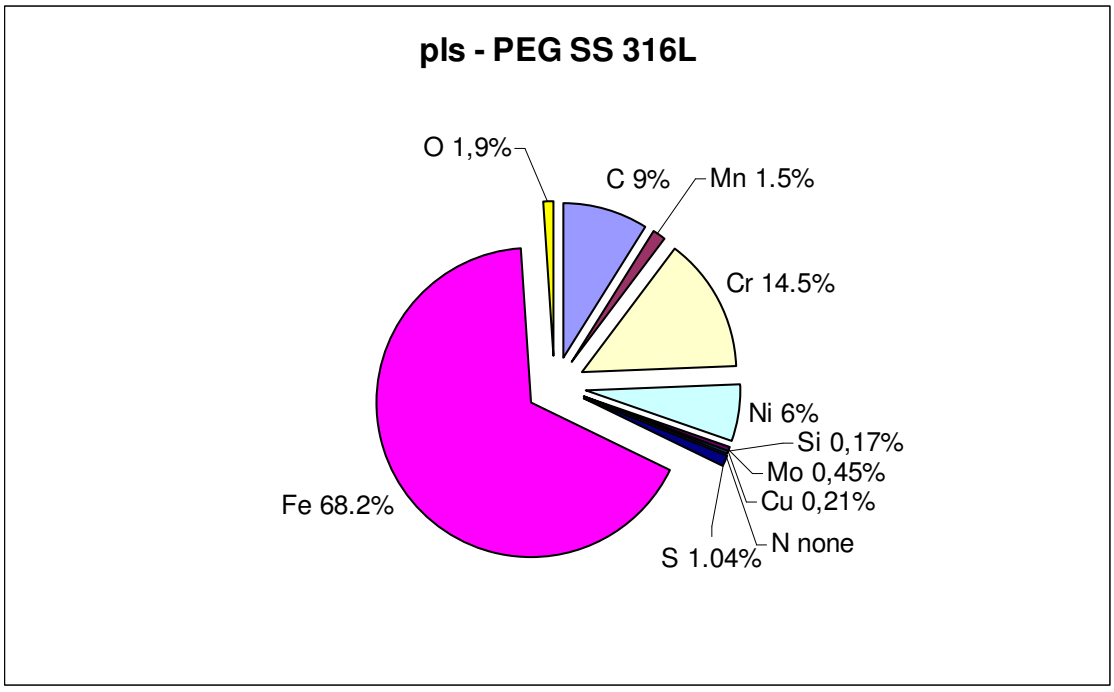


Figure 4.4B. Element % of pls-PEG modified SS 316L plaque surface.

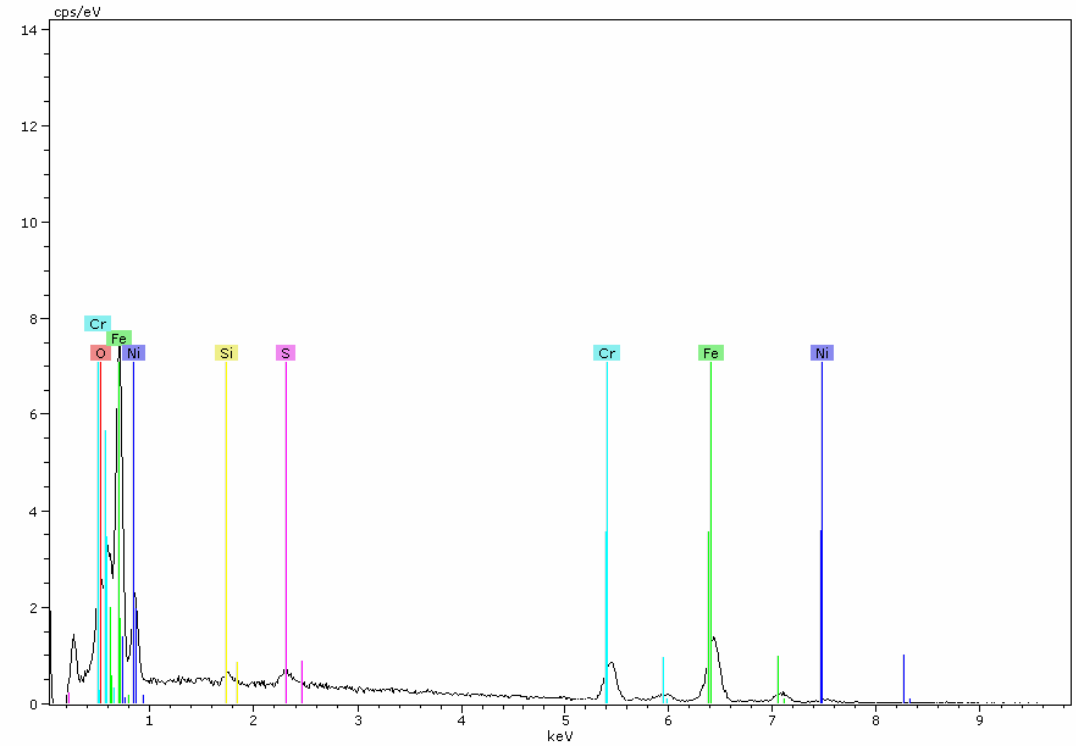


Figure 4.4A. EDX Spectrum of pls – HEMA modified SS 316L plaque surface.

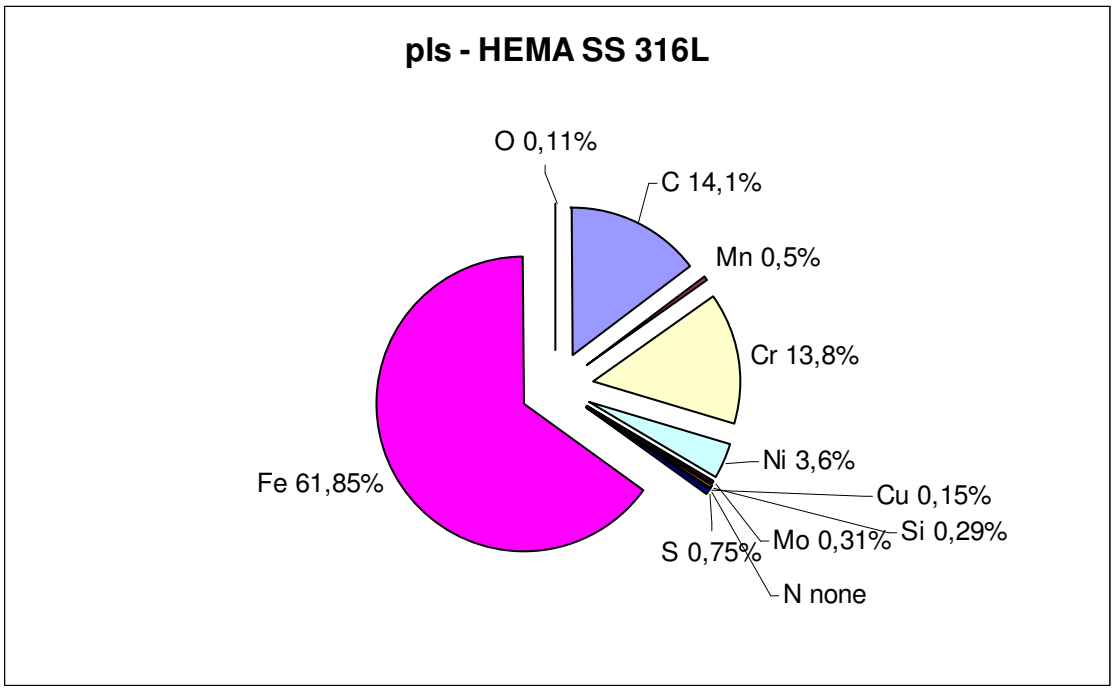


Figure 4.4B. Element % of pls-HEMA modified SS 316L plaque surface.

The spectra of SS 316L which are coated with silicon based materials, hexamethyldisilane and hexamethyldisiloxane have distinct Si peaks at 1.76keV (Figure 4.5A and Figure 4.6A). The pie charts showing the relative abundance ratios of Si element as 1.28% in hexamethyldisilane and 1.17% in hexamethyldisiloxane coatings, which are relatively more abundant than unmodified bare SS 316L plaques. In figure 4.6B, the ratio of relative abundance of oxygen is 0.45%, which refers to the O atom in siloxane compound. Also both spectra have C peaks at 0.27 keV and the relative abundance ratio of C element in the surface was calculated as 5.8% in each modifications (Figures 4.5B and 4.6B).

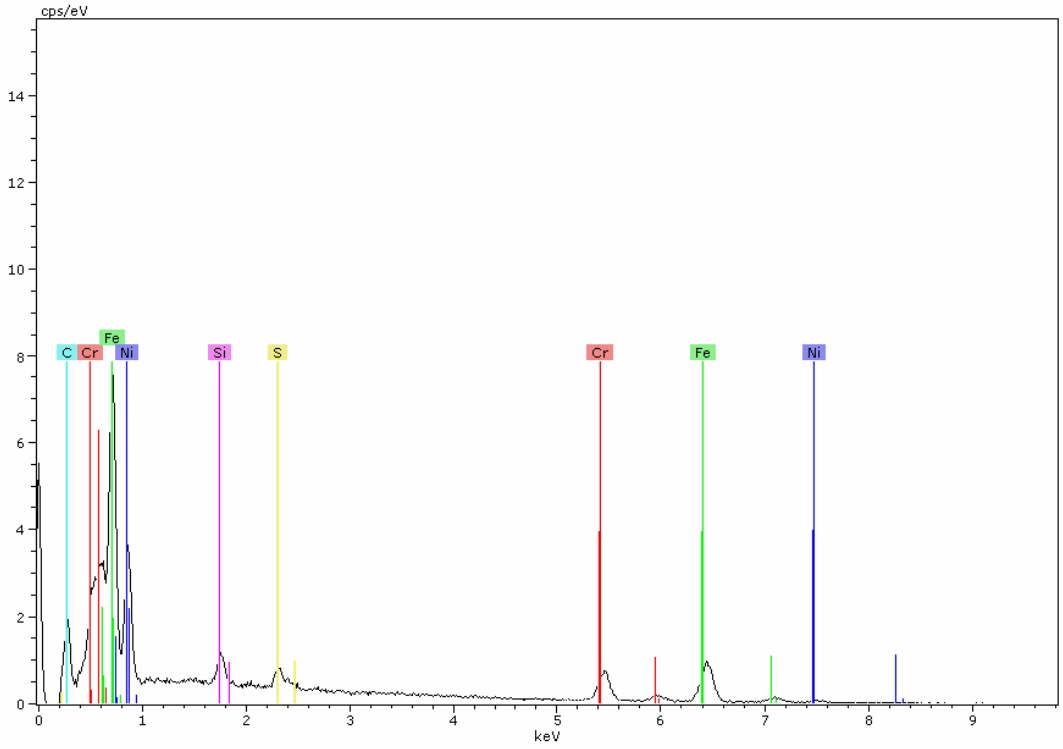


Figure 4.5A. EDX Spectrum of pls – HMDS modified SS 316L plaque surface.

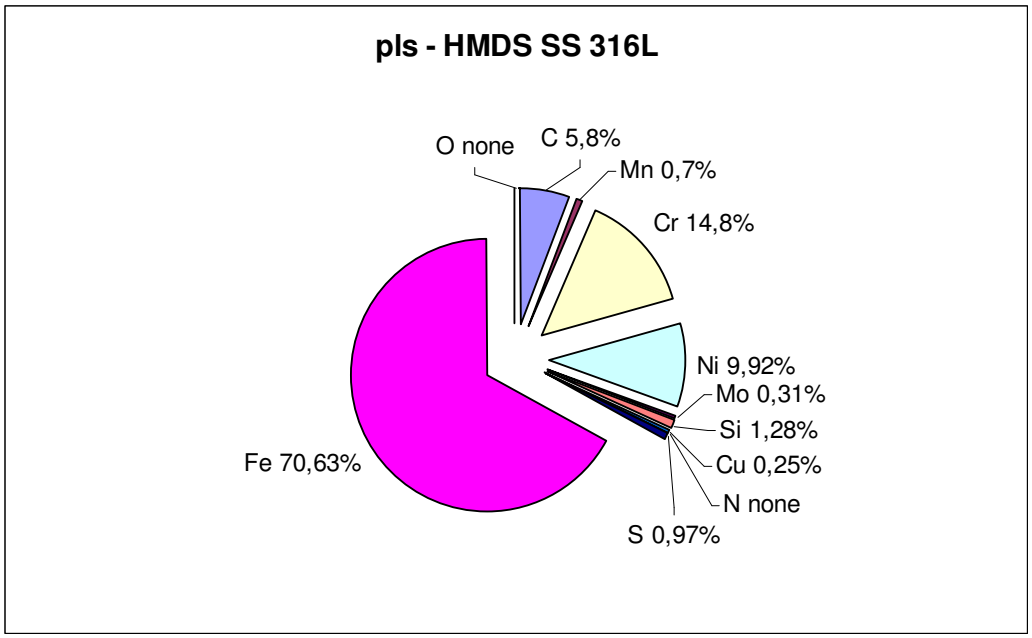


Figure 4.5B. Element % of pls-HMDS modified SS 316L plaque surface.

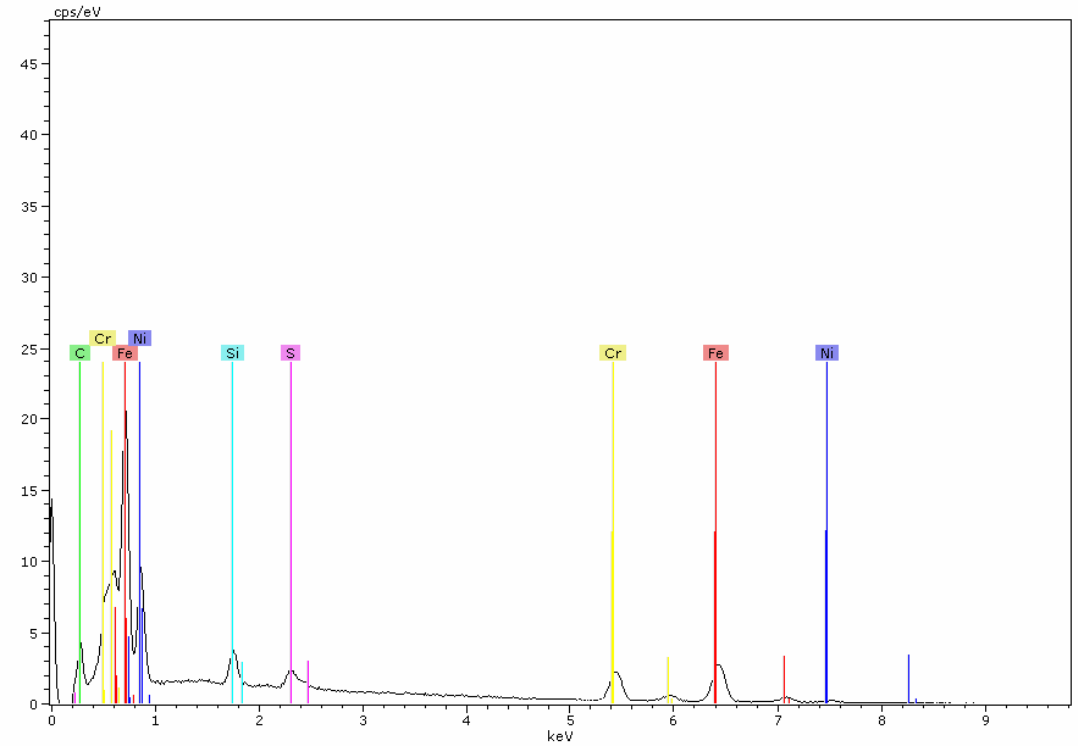


Figure 4.6A. EDX Spectrum of pls – HMDSO modified SS 316L plaque surface.

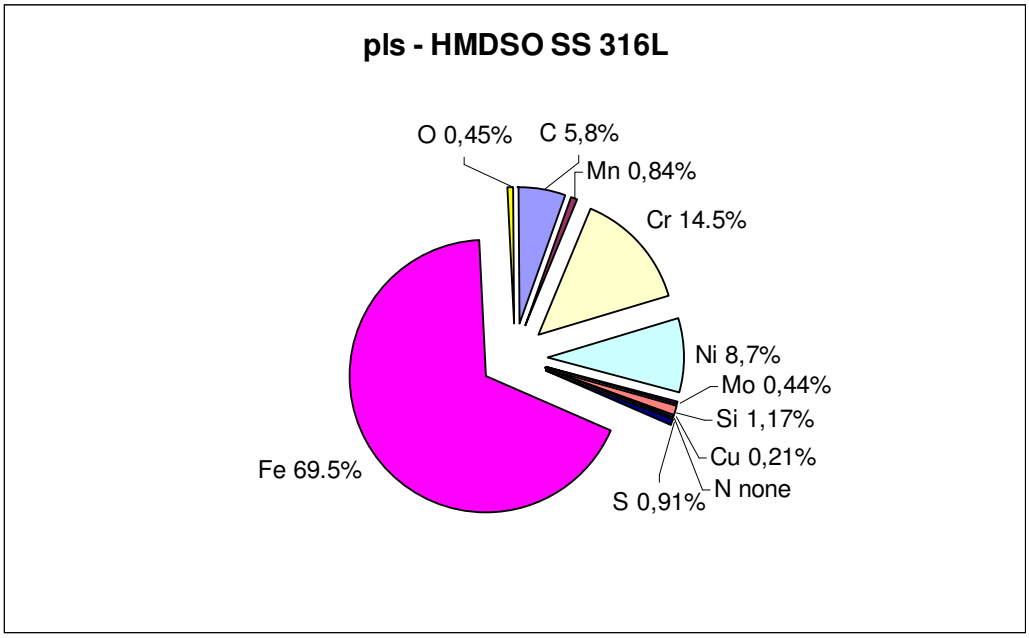


Figure 4.6B. Element % of pls-HMDSO modified SS 316L plaque surface.

Ethylenediamine coated SS316L surface contains high ratio of N element with a peak at 0.40 keV in the EDX spectrum of pls-EDA modified SS 316L plaque. The relative abundance ratio of nitrogen element were calculated as 8.95% in the figure 4.7B. Also the increase of C atom peak at 0.27 keV respect to the unmodified bare SS 316L spectrum indicates a compound containing C and N element is present in the surface. (Figure 4.7A)

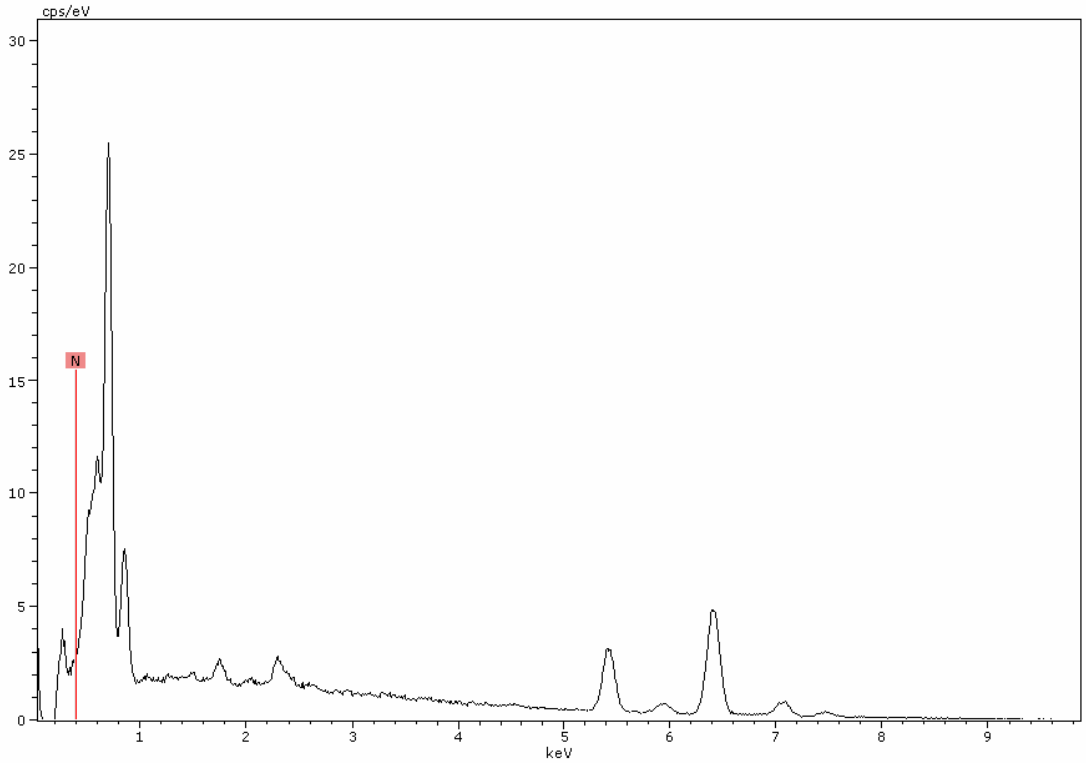


Figure 4.7A. EDX Spectrum of pls – EDA modified SS 316L plaque surface.

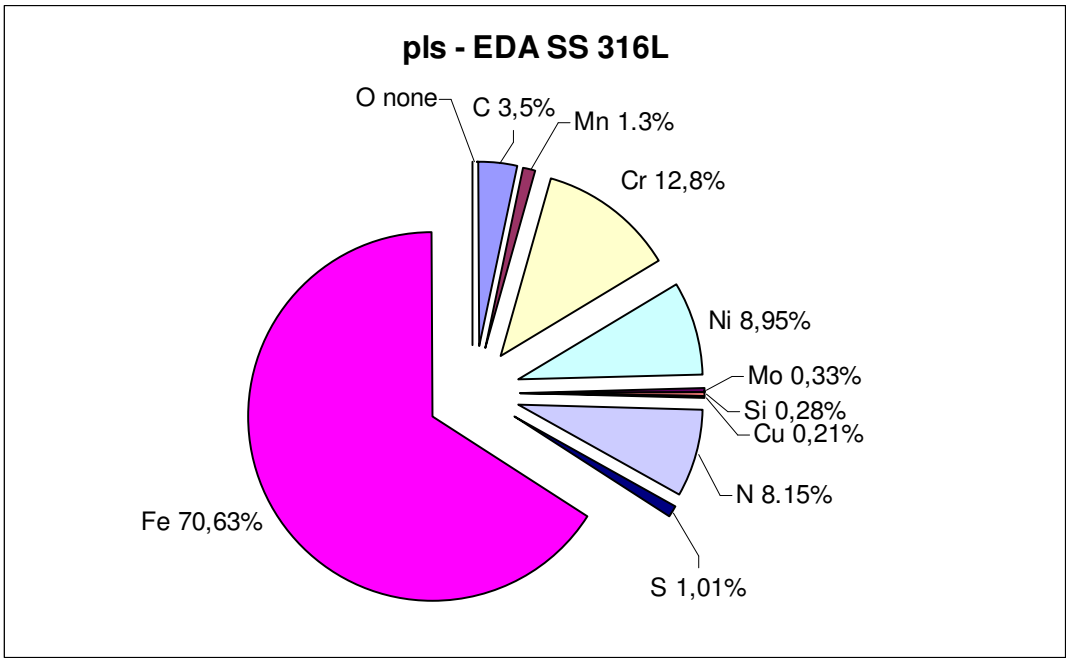


Figure 4.7B. Element % of pls-EDA modified SS 316L plaque surface.

4.1.3. Contact Angle Measurements of SS 316L Plaques

Figure 4.8A to F shows the contact angles of the surfaces of SS 316L plaques, which were determined by the sessile drop technique. The spreading drop shapes of water indicates the hydrophilicity of the surface.

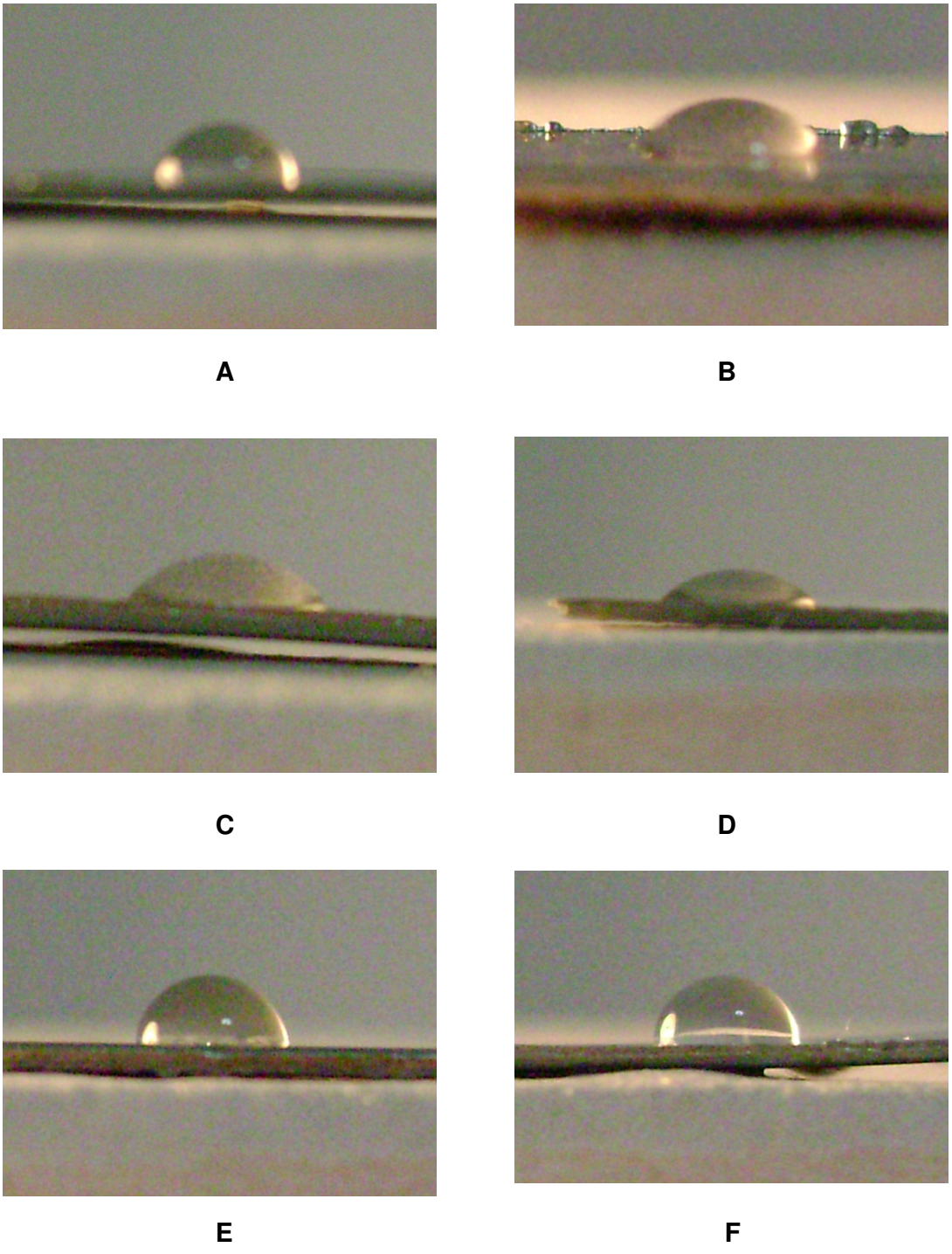
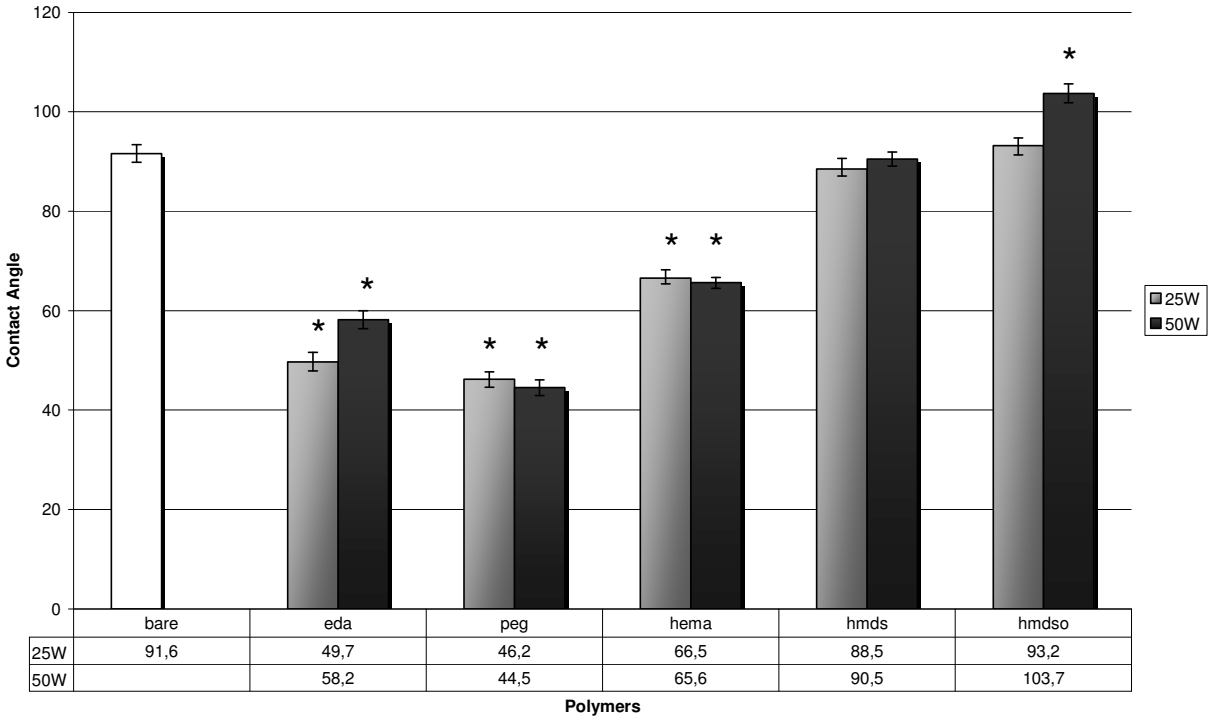


Figure 4.8 A.,B.,C.,D, E. and F. Water contact angle images of (A) bare SS 316L (B) plsHEMA SS 316L (C) plsEDA SS 316L (D) plsPEG SS 316L (E) plsHMDS SS 316L (F) plsHMDSO SS 316L

Students one-tailed T-test has been employed to test the statistical significance of the contact angle measurements. The individual measurments for each modification have been compared to the measurements of the bare SS 316L. 10 independent measurements have been utilized for the assessment of significance. The p cut-off value has been selected as 0.01 for significance.

The bare SS 316L plaque has the 91.6° water contact angle and has poor wettability properties. The used monomers have changed the surface wettability with both increasing and decreasing way with respect to the different functional groups. The results shows that the monomers which have polar and hydrophyl groups like –OH, -NH_2, C=O decreased the contact angle. –CH_3, functional group containing plasma modification generated hydrophobic surfaces. The surface wettability increases with the decreasing contact angle (Chen et. al., 2005).

The most wettable surface is pls – PEG SS 316L with a discharge power of 50 W as can be seen with the water drop shape in Figure 4.9D. The contact angle is 44.5° 50 W pls-PEG SS 316L which means that it has a hydrophilic character. The functional group of –OH is the most hydrophilic group amongst the used polymers. Other coatings which have decreased the water contact angle of SS 316L are ethylenediamine and 2-hydroxyethyl methacrylate as, 58.2° and 65.6° respectively at 50 W discharge power. –NH_2 in ethylene diamine and –OH, C=O in 2-hydroxyethyl methacrylate play a role in increasing surface wettability. Surface wettability was decreased with the increasing discharge power in ethlenediamine modification as can be seen in figure 4.9.



	bare	eda	peg	hema	hmds	hmdso
25W	91,6	49,7	46,2	66,5	88,5	93,2
50W		58,2	44,5	65,6	90,5	103,7

Figure 4.9. Contact Angle measurements of SS 316L plaques.

* indicates $p < 0.01$

The lowest wettable surface modification occured with hexamethyldisiloxane plasma at 50W discharge power. The contact angle of this modified surface is 103.7° and it is more hydrophobic than the bare SS 316L itself. Also plasma assisted hexamethyldisilane surface modifications generated hydrophobic surfaces with contact angles of 88.5° and 90.5° at 25 W and 50 W discharge powers, respectively. The results show that the silicone based plasma assisted modifications generates hydrophobic surfaces. Water drop shapes in figure 4.8E and figure 4.8.F refer to a hydrophobic surfaces. The surface wettability order occured as:

Pls-PEG SS 316L > Pls-EDA SS 316L > Pls-HEMA SS 316L > Pls- HMDS SS 316L > Unmodified bare SS 316L > Pls – HMDSO SS 316L.

.

4.2. Biocompatibility of SS 316L Plaques

4.2.1. *In Vitro* Cytotoxicity Assay

In vitro cell proliferation test that applied to plasma modified SS 316L plaques showed that there is no any important effect on the cell proliferation. The relative cell proliferation to control group is in the range of 81.3 % - 93.8%. The presence of SS 316L and plasma assisted surface modificated SS 316L plaques have no cytotoxic effect to cell proliferation.

Table 4.2. Cell proliferation assay results.

Sample	Initial Cell Concentration ($x10^6$cells/ml)	Cell Concentration 48 hours ($x10^6$cells/ml)	Relative Proliferation (%)
Control	0.4	1.6	-
SS 316L	0.4	1.4	87.5
Pls – EDA SS 316L	0.4	1.3	81.3
Pls – PEG SS 316L	0.4	1.5	93.8
Pls – HEMA SS 316L	0.4	1.5	93.8
Pls – HMDS SS 316L	0.4	1.5	93.8
Pls – HMDSO SS 316L	0.4	1.4	87.5

4.3. Haemocompatibility of SS 316L Plaques

Prothrombin Time, Activated Partial Thromboplastin Time and Fibrinogen Time measurements are essential haemocompatibility test for the behaviour of a biomaterial that in contact with blood. (Yoshioka et. al., 2003 and Tan et. al, 2003). These tests were applied to the materials to evaluate the effect of modifications in coagulation cascade.

Students one-tailed T-test has been employed to test the statistical significance of the Prothrombin Time, Activated Partial Thromboplastin Time and Fibrinogen Time measurements. The individual measurements for each modification have been compared to the measurements of the bare SS 316L. 3 independent measurements have been utilized for the assessment of significance. The p cut-off value has been selected as 0.05 for significance.

Figures 4.10., 4.11., 4.12. show the values of PT, APTT and Fibrinogen time of the plasma in contact with the samples.

The values measured for the materials were close to the blank in Figure 4.10, but pls – HEMA SS 316L have an increase effect respect to bare SS 316L on prothrombin time which belongs to the extrinsic coagulation pathway about 0.5 second ($p<0.05$). Pls – EDA SS 316L have a decrease effect in phrothrombin time about 0.4 second respect to SS 316L, which means the surface has some activating properties on extrinsic coagulation pathway ($p<0.05$).

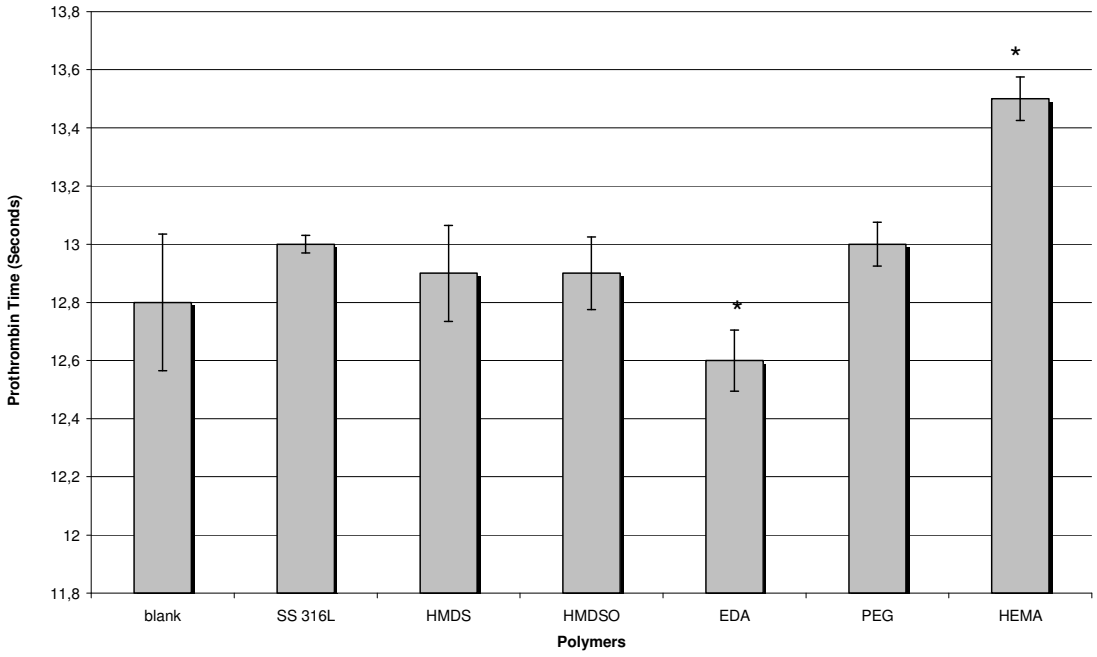


Figure 4.10. Prothrombin time results of blood plasma interacted with SS 316L plaques.

* indicates $p< 0.05$

In the values of APTT pls-HMDS SS 316L, pls-HMDSO SS 316L and pls-HEMA SS 316L, there are increasings in clotting time respect to the values of unmoddified SS 316L plaques as, 1.3, 1.4 and 2.6 seconds, respectively ($p<0.05$). The results indicate to an adsorption of proteins related with intrinsic coagulation pathway (Yoshioka et. al., 2003).

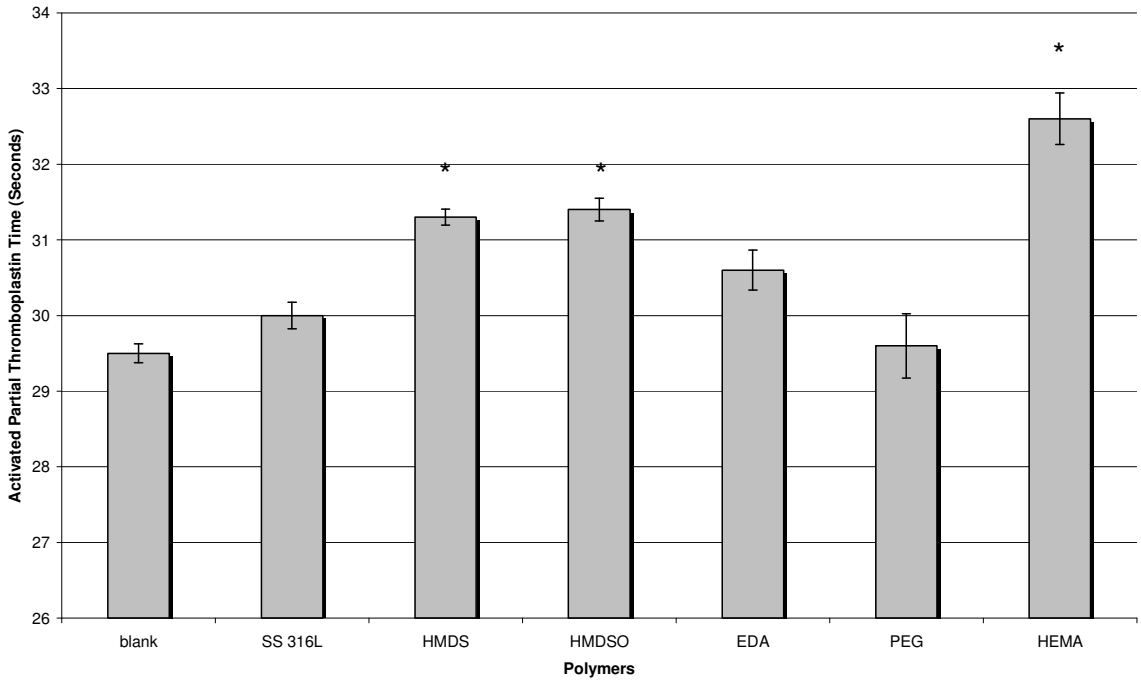


Figure 4.11. Activated partial thromboplastin time results of blood plasma interacted with SS 316L plaques.

* indicates $p< 0.05$

In Figure 4.12, there are some differences in clotting time in the measurements fibrinogen test. Pls-HEMA SS 316L with pls-EDA SS 316L, which again refer to an adsorption of plasma proteins that are involved with the intrinsic coagulation pathway (Yoshioka et. al., 2003), clotting time increaases by 2.1 and 1.1 seconds respect to unmodified bare SS 316L plaques ($p<0.05$). Contradictory to this, clotting time shortened in pls-HMDSO and pls-PEG modifications by 0.5 and 0.8 second, respectively, which indicates the surface characteristic of these material have inducing properties of fibrin formation ($p<0.05$).

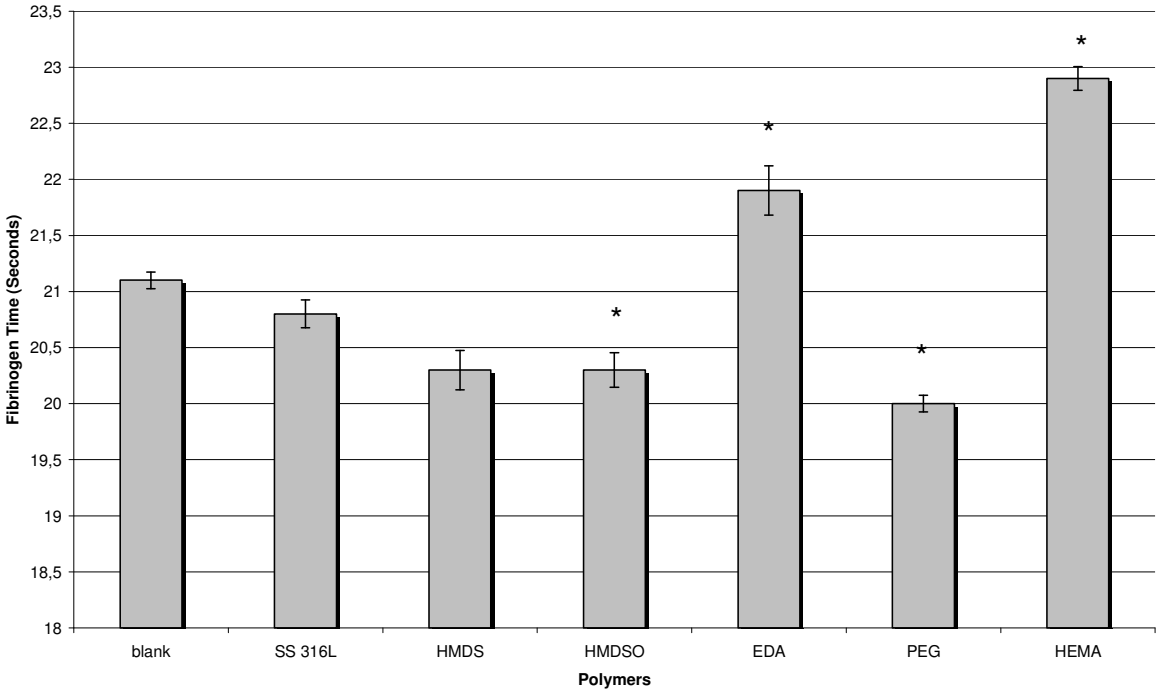


Figure 4.12. Fibrinogen time results of blood plasma interacted with SS 316L plaques.

* indicates $p< 0.05$

Therefore, it is concluded from figures that the samples hardly affect the blood-clotting system. HEMA modifications showed latency on blood clotting time in all tests. The polymeric surface modifications adsorbed plasma proteins due to its porous structure (Mutlu et. al, 2007).

5. CONCLUSION

In the study, the surfaces of SS 316L plaques were succesfully modified with 5 different organic monomers as, polyethyleneglycol, 2-hydroxyethylmethacrylate, ethylenediamine, hexamethyldisilane and hexamethyldisiloxane. The coated surfaces were characterized morphologically and chemically with SEM – EDX. The contact angle measurements evaluated the hydrophilic character of the coated surfaces. The haemocompatibility tests were applied to surfaces which revealed the behaviours of coating compounds against to plasma proteins involved with coagulation mechanism.

The surface morphology of SS 316L plaques both bare and modified ones have no significant difference in shape. Chemical surface characterization with SEM – EDX method showed that the coating had occured and the atoms that exist in the monomers were evaluated quantitavely with the obtained spectra.

Hydrophilic characters were measured with sessile drop contact angle method, and the hydrophilicity increased with the polar and hydrophilic groups that are present in the monomer type. The most hydrophilic coatings had occured with the PEG because of having –OH functional group which was the most hydrophilic one amongst the other types of functional groups.

The coatings also had no significant effect on cell proliferations, with respect to the data obtained with *in vitro* cell proliferation assay.

Blood protein values that are involved with the coagulation mechanism and clot forming times were almost at the same level with respect to the blank solution that had not interacted with SS 316L plaques. The pls HEMA SS 316L interacted blood plasma samples showed increase in clot forming times in all tests and pls – HMDSO SS 316L in APTT tests, which inidicates that the plasma proteins that are involved with the coagulation mechanism were adsorbed onto the surface.

The coating of the SS 316L plaques with biocompatible imunologically inactive polymeric materials was carried out in the study. The new surface properties showed good compatibility with the plasma proteins that are involved with the blood coagulation mechanism. With the functional groups that exist in the surface of the coating, it will be possible to bind any suitable bioagents related with the

cardiovascular disease and have an opportunity to develop intracoronary stents in an alternative way.

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