ELECTRICALLY CONDUCTIVE SILK FIBROIN / GLYCERINE / POLYPYRROLE BIOFILMS FOR BIOMEDICAL APPLICATIONS

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Approval of Director of Graduate School of Applied Sciences

Prof. Dr. İlkay SALİHOĞLU

Director

We certify this thesis is satisfactory for the award of the Degree of Master of Science in Biomedical Engineering

Examing Committee In Charge :

Prof. Dr. Nedime SERAKINCI, Committee Member, Department of Medical Genetics, NEU.

Assoc. Prof. Dr. Terin ADALI, Supervisor, Committee Member, Department of Biomedical Engineering, NEU.

Dr. Rasime KALKAN, Committee Member, Department of Medical Genetics, NEU.

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last Name : Eyyüp Kavalcı

Signature :

Date : 02.05.2014

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ABSTRACT

The main aim of this work is to blend and characterize electrically conductive Silk Fibroin / Glycerine / Polypyrrole and Silk Fibroin / Glycerine / Iron (III) Oxide (Fe_2O_3) biofilms for biomedical applications. Biofilms were prepared via UV-irradiation and via casting method at 25 ^{o}C. Swelling, Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR) analyses and conductivity tests were applied to characterize biofilms.

SEM analysis indicated that as the amount of polypyrrole increases, the surface smoothness of the biofilms also increases. FTIR analysis showed that the chemical structure of the silk fibroin protein is not affected by the blending process. The electrical conductivity of the biofilms decreases as the amount of glycerine increases in the mixture. The elasticity of the Silk Fibroin / Polypyrrole biofilms has been enhanced by adding glycerine in the mixture.

The Silk Fibroin / Polypyrrole blended biofilms have been observed to exhibit as much as 116.1% swelling ratio within five minutes in PBS solution and 64.15% in acidic buffer solution. According to the test results, the most stable biofilms are Silk Fibroin / Glycerine biofilms. The degradation observed when Polypyrrole and Fe_2O_3 added into the blend films in both pH = 7.4 and pH = 1.2 buffer solutions.

The Silk Fibroin / Glycerine / Polypyrrole biofilms have great potential applications in gene delivery systems because of their electrical conductivity and biodegradability properties which allow them to carry the genes to the targeted cells.

These results demonstrated that the Silk Fibroin / Glycerine / Polypyrrole biofilms have potential applications in biomedical sciences and gene delivery systems.

Keywords: Electrically conductive biofilms, Silk Fibroin, Polypyrrole, Iron (III) Oxide, Swelling, Gene Therapy

ÖZET

Bu çalışmanın amacı, elektrik iletkenliğine sahip İpek Fibroin / Gliserin / Polipirol ve İpek Fibroin / Gliserin / Demir (III) Oksit (Fe_2O_3) biyofilmlerini çeşitli biyomedikal uygulamaları için hazırlamaktır.

UV-fotopolimerizasyon tekniği ile heterojen koşullarda ve döküm tekniği ile oda sıcaklığında oluşturulan filmler, Taramalı Elektron Mikroskobu (TEM), Fourier Dönüşümlü Kızılötesi Spektroskopisi (FTIR), şişme ve elektrik iletkenliği testleri ile karakterize edilmiştir.

TEM analizleri sonucunda, Polipirol miktarının artması ile biyofilmlerin yüzey morfolojisinde pürüzsüz yüzeyin arttığı gözlemlenmiştir. FTIR spektra sonuçlarının analizi sonucunda ise İpek Fibroin / Gliserin ve İpek Fibroin / Gliserin / Polipirol biyofilmlerinin ipek fibroine aşılanmadığı net olarak tespit edilmiştir.

Elektrik iletkenliği testleri sonucunda, gliserin miktarının artmasının elektrik iletkenliğini azalttığını, biyofilmin kırılganlığını azaltıp elastikiyetiğini artırdığı rapor edilmiştir.

Şişme testleri sonucunda, fosfat tampon çözeltisi içerinde ilk beş dakikada en yüksek şişme oranını %116.1 ve asidik tampon çözeltisi içerisinde %64.15 olarak gözlemlenmiştir. Test sonuçlarına göre en kararlı biyofilmlerin ipek fibroin / gliserin biyofilmleri olduğu gözlemlenmiştir. Polipirol ve demir (III) oksit ilavesi ile hem pH = 7.4 hem de pH = 1.2 içerisinde degradasyon tespit edilmiştir.

İpek Fibroin / Gliserin / Polipirol biyofilmlerin, elektriksel ve biyoçözünürlülük özelliklerinin, genleri hedeflenen hücrelere taşıma olanağından dolayı, gen iletim sistemlerinde büyük bir potansiyele sahip olmalarını sağlamaktadır.

Sonuçlar, İpek Fibroin / Gliserin / Polipirol biyofilmlerin, biyomedikal bilimlerinde ve gene terapisi uygulama alanlarında potansiyellerinin yüksek olduğunu göstermektedir.

Anahtar Kelimeler: Elektriksel İletken Biyofilmler, İpek Fibroin, Polipirol, Demir (III) Oksit, Şişme, Gen Terapisi

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LIST OF ABBREVIATIONS

SF	Silk Fibroin
B. Mori	Bombyx Mori
Gly	Glycine
Ala	Alanine
Ser	Serine
n	Number of Sequence Repetition
V	Potential Difference, Voltage
R	Electrical Resistance
i	Electrical Current
E_{str}	Electric Strength
ρ	Electrical Conductivity
FBR	Foreign Body Response
O/W	Oil-in-Water
W/O	Water-in-Oil
LbL	Layer by layer
PVA	Poly-vinyl alcohol
PPy	Polypyrrole
Gly	Glycerine
Fe_2O_3	Iron (III) Oxide
UV	Ultraviolet
SEM	Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
TEM	Taramalı Elektron Mikroskobu

CHAPTER 1

INTRODUCTION

1.1. Silk Fibroin

Silk Fibroin (SF) is a natural polymer which is obtained from silkworms like *Bombyx mori* and other insects. Silk Fibroin is a highly biocompatible polymer and it has ability to be combined with other biocompatible polymers. There is a long history of usage of silk fibroin in medicine in many different applications and it has attracted many scientists' interest from various disciplines, because of its structure and properties (Sah and Pramanik, 2010).

Silk fibroin which is derived from *Bombyx mori* cocoons is used to form tubes, hydrogels, composites, thin films, sponges, fibers and microspheres. In tissue engineering, in vitro disease models and drug delivery applications, these materials are used as biomaterials for implants (Rockwood et al., 2011).

Silk is a fibrous protein which is characterized by a highly repetitive primary sequence that leads to significant homogeneity in secondary structure, in example, β-sheets in the case of many of the silks. Generally, these kinds of proteins have very important mechanical properties, comparing with the molecular and catalytic recognition functions of globular proteins. In tissue engineering field, because of these important mechanical properties, these materials provides many useful opportunities to the scientists in the fields of controlled release, scaffolds and biomaterials (Altman et al., 2003).

In this thesis, it is aimed to present a biofilm formation which is a combination of pure silk fibroin with glycerine to improve its mechanical properties by using the chemical properties of glycerine and then, blending it with Polypyrrole to improve its electrical properties. Promising results will lead this biofilm able to use in different biomedical applications because of its strong, versatile and efficient properties.

1.2. Properties of Silk Fibroin

1.2.1. Chemical Properties

Silk fiber is produced by *Bombyx mori* (B. mori) larva and it is an excellent biological protein polymer. It has been used in textile industry and medical applications for thousands of years because of its unique textile properties such as mechanical properties, gloss, fineness and handle (Khan et al., 2009).

Fibroin and sericin are the two different proteins that are formed from silk. Fibroin is the structural protein of silk fiber, whereas sericin is the water soluble proteinaceous glue that serves to bond the fibers together. The majority of fibroin's composition is highly periodic, with simple repeating sections broken by more complex regions containing amino acids with bulkier side chains. The highly repetitive sections are composed of glycine (45%), alanine (30%) and serine (12%) in a roughly 3:2:1 ratio and dominated by [Gly-Ala-Gly-Ala-Gly-Ser]$_n$ sequences (Khan et al., 2009).

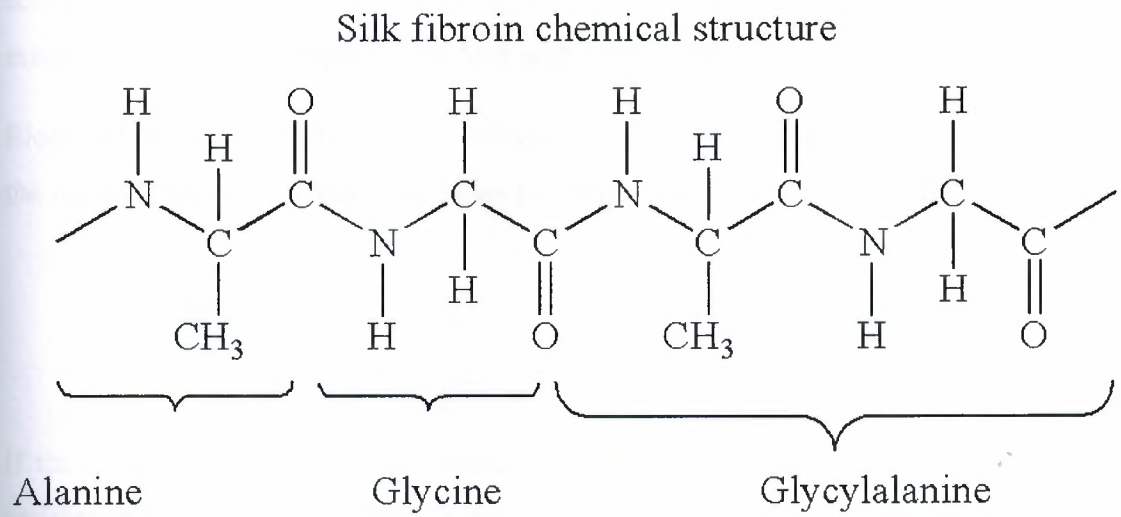

Figure 1.1: Primary Structure of Silk Fibroin (Valuzzi et al., 1999)

There are three known structures that fibroin mainly forms which are Silk I, II and III. Silk I is the original form of fibroin that is obtained from the *Bombyx mori* cocoons. Silk II is the chemically processed version of Silk I, which has higher mechanical properties. Silk III is the advanced version of Silk II that is formed in solutions of silk fibroin at an interface which has been discovered recently (Valluzzi et al., 1999).

1.2.2. Electrical Properties

Natural silk has wide applications in various branches of industry because of its high strength, hygroscopicity, elasticity and isolating properties. Experiments show that natural silk has more advantages comparing to artificial fibers in strength, radiation resistance, electric field resistance, mechanical loading and temperature (Ismaiilova and Alieva, 2007).

Electrical properties of materials are one of the most important criterias for the scientists determining their fields of use in the industry. Because, every material has a resistance and as the current passes through on it, the electrical energy becomes heat energy resulting with loss of power and even damaging the place where it is used for. Therefore, many scientific researches have been making in order to improve such properties of promising materials for the future uses (Ismaiilova and Alieva, 2007).

Electrical resistance, R, is the ratio between the potential difference (voltage) V applied to the material and the current i that flows through it and it can be shown as:

$$R = \frac{V}{i}$$

If the potential difference V is measured in volts (V) and the current i in amperes (A), the resistance R is shown in ohms, a capital Greek omega (Ω). The shape and origin of a material nature play an important role on the resistance of it. Therefore, the resistivity is directly related with the material itself. So, resistivity ρ_e is defined as the ratio of electric field E to current density J, which is current per cross-sectional area.

$$\rho_e = \frac{E}{J}$$

The electric field is the gradient in electric potential and the unit of resistivity is ohm-meter (Ω-m) (Park and Lakes, 2007, p.84).

Electric strength E_{str} and the electric conductivity ρ are the basic parameters that play important roles on the electrophysical properties of polymer dielectrics including natural silk. These parameters can change with the differences in the temperature and the supplied voltage frequency. In addition, the structure and composition of the polymer are directly related with these parameters (Ismaiilova and Alieva, 2007).

Structural influence on E_{str} of polymer dielectrics is very important in order to obtain new modified isolation material with specific properties. E_{str} is calculated by using the defects in the amorphous and crystalline parts of the polymer (Ismaiilova and Alieva, 2007).

If the polymer has large supermolecular formations, for example, spherulites, when the spherulite dimension R increases, the values of E_{str} decrease, when R≡h, (h is the thickness of the film) the electric strength takes a constant value. An increase in the crystallinity and the processes of internal stress relaxation affect the growth of the E_{str} values (Ismaiilova and Alieva, 2007).

The electric properties of polymers are used to classify them by their electric conductivity (or specific bulk and surface resistance) and electric strength. The electric conductivity of real polymers applied as dielectrics are directly relating with their composition, molecular structure and supermolecular structure. However, it is known that the mechanism of ionic electric conductivity has a fundamental importance on polymer dielectrics (Ismaiilova and Alieva, 2007).

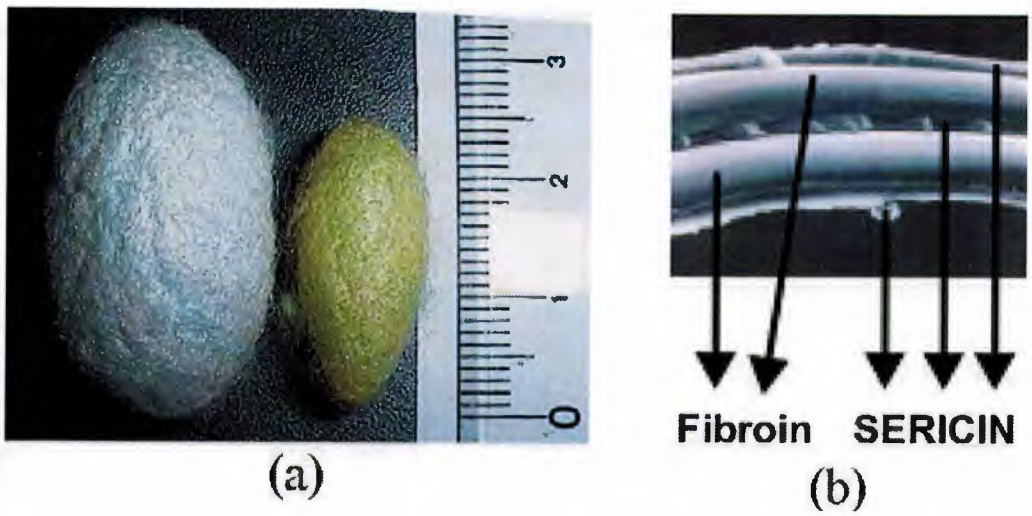

Figure 1.2: Silk Cocoons, a) and silk fibers, b) (Cheung et al., 2009)

1.2.3. Mechanical Properties

Silk is a mechanically strong biomaterial that contains a wide range of functional and mechanical properties for biomedical applications. It can be used in many medical applications because of its mechanical properties, environmental stability, biocompatibility and biodegradability (Sah and Pramanik, 2010).

Silk is a kind of biomaterial that has high elasticity, significant crystallinity, resistance to failure under compression and strength and toughness. The combination of the β-sheet crystals, the interphase between the crystals, the semi-crystalline regions and the shear alignment of the molecular chain are the basis for silk's unique mechanical properties (Altman et al., 2003).

Silk fibroin has unique mechanical properties, excellent biocompatibility, environmental stability, biodegradability and the capacity to support cell and tissue growth. Therefore, many studies have been developing rapidly in different biomedical fields such as scaffolds for tissue engineering, drug delivery systems, artificial skin, cartilage tissue, biosensors, artificial bone regeneration and wire ropes for the substitution of the anterior cruciate ligaments etc (Khan et al., 2009).

Table 1.1: Comparison of mechanical properties of common silks (silkworm and spider dragline) to several types of biomaterial fibers and tissues commonly used today (Altman et al., 2003)

Material	UTS (MPa)	Modulus (GPa)	% Strain at break	Authors
B. mori silk (w/ sericin)[a]	500	5–12	19	Perez-Rigueiro et al. [68]
B. mori silk (w/o sericin)[b]	610–690	15–17	4–16	Perez-Rigueiro et al. [68]
B. mori silk[c]	740	10	20	Cunniff et al. [13]
Spider silk[d]	875–972	11–13	17–18	Cunniff et al. [13]
Collagen[e]	0.9–7.4	0.0018–0.046	24–68	Pins et al. [69]
Collagen X-linked[f]	47–72	0.4–0.8	12–16	Pins et al. [69]
PLA[g]	28–50	1.2–3.0	2–6	Engelberg and Kohn [70]
Tendon (comprised of mainly collagen)	150	1.5	12	Gosline et al. [71]
Bone	160	20	3	Gosline et al. [71]
Kevlar (49 fiber)	3600	130	2.7	Gosline et al. [71]
Synthetic Rubber	50	0.001	850	Gosline et al. [71]

[a] *Bombyx mori* silkworm silk—determined from bave (multithread fibers naturally produced from the silk worm coated in sericin).
[b] *Bombyx mori* silkworm silk—determined from single brins (individual fibroin filaments following extraction of sericin).
[c] *Bombyx mori* silkworm silk—average calculated from data in Ref. [13].
[d] *Nephila clavipes* silk produced naturally and through controlled silking.
[e] Rat-tail collagen Type I extruded fibers tested after stretching from 0% to 50%.
[f] Rat-tail collagen dehydrothermally cross-linked and tested after stretching from 0% to 50%.
[g] Polylactic acid with molecular weights ranging from 50,000 to 300,000.

Silk-worm silk is an excellent material comparing with the commonly used polymeric biodegradable biomaterials such as collagen and poly (L-lactic acid) (PLA).

Silk fibroin has important mechanical properties. It is biodegradable and its highly crystallized structure degrades slowly, but the rate of biodegradation depends on the mechanical environment, implantation site and features of the prepared silk material (Rockwood et al., 2011).

1.2.4. Biodegradation

According to the results of US Pharmacopeia, a biomaterial is said to be an absorbable when it loses most of its tensile strength within 60 days after the implantation in vivo. Within this definition, silk is consired to be non-degradable. However, according to the proven studies, silk is degradable, but over longer time periods due to proteolytic degradation usually mediated by a foreign body response (Altman et al., 2003).

It is known that silk fibers lose the majority of their tensile strength within 1 year in vivo and fail to be recognized at the site within 2 years (Altman et al., 2003).

Generally, silk is absorbed slowly in the body. The implantation site, mechanical environment and variables related to the health and physiological status of the patient, the type (virgin silk versus extracted black braided fibroin) and the diameter of the silk fiber are the important criterias that can affect the rate of adsorption of the silk. Furthermore, various methods in silk processing may result different changes in the protein structure potentially increasing or decreasing susceptibility to degradation (Altman et al., 2003).

The variables that obtained in the studies have not been studied in detail. Therefore, it is difficult to make clear understanding of the relationships between structure, processing and degradability (Altman et al., 2003).

1.2.5. Solubility and Swelling Properties

Silk Fibroin is an insoluble material in most solvents including water, therefore, special aqueous solutions are needed in order to make it soluble and regenarate it into a desirable form to make it suitable for the biomedical applications (Sah and Pramanik, 2010).

By using concentrated neutral salts, such as calcium chloride, lithium bromide and similar ones, aqueous silk fibroin solution can be obtained by dissolving SF into their solution. From the researches it is known that silk fibroin becomes soluble in certain high ionic-strength aqueous solutions of chaotropic salts, which are able to destabilize the proteins in solution and increase their solubility (Sah and Pramanik, 2010).

Many researches have been made in order to find suitable solvents for preparing silk fibroin solutions, but only very few literatures are available which explains the whole processing of silk and characterization of silk solution (Sah and Pramanik, 2010).

The ionization of the network, its degree of crosslinking and its hydrophilic and hydrophobic balance changes the degree of swelling. Therefore, changes in the polymer compositions directly affects the degree of swelling. This situation can increase the cumulative amount and the rate of drug release potentially. The increase in SF concentration can decrease the swellling ratio of SF scaffolds. Combination of Silk Fibroin with other materials such as chitosan and hyaluronic acid can increase the swelling, when the results compared with the pure Silk Fibroin (Haider et al., 2005).

1.2.6. Biocompatibility

All researches and experiments that have been made up to now has shown that silk fibroin has an excellent biocompatibility and foreign body response after the implantation in vivo comparing with the other biomaterials used in today's applications (Meinel et al., 2005).

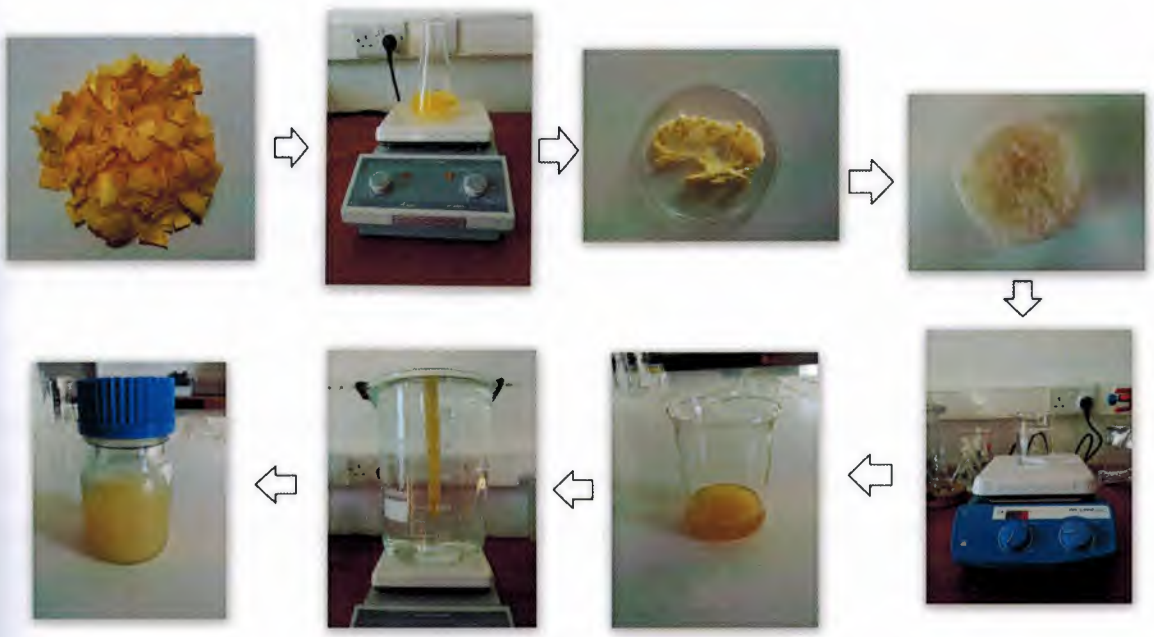

Figure 1.3: Step by step, extraction of fibroin protein from *Bombyx mori* silk cocoon

Silk has been used mostly as sutures for wound ligation and it became the most common natural suture surpassing the collagen, cross-linked used in the biomedical industry over the past 100 years because of its high biocompatibility (Altman et al., 2003).

1.2.7. Thrombogenic Properties

All biomaterials that are obtained from a non-autologous source will cause some level of foreign body response (FBR) following the implantation in vivo. This is because of the original nature of any living system in order to protect it from the foreign materials (Altman et al., 2003).

When a foreign material that is not biocompatible with the body is inserted into the body, circulating blood proteins and biomolecules adsorb to the foreign material and form a thrombus and this is called as blood response in terms of thrombogenic response (Altman et al., 2003).

When a thrombus becomes large enough and dislodges, it is called as an embolism which can travel through the blood veins to the heart or brain and cause an heart attack or a stroke (Altman et al., 2003).

Studies have found out that silk films and fibers are very promising for tissue engineering according to their strong physical properties and high biocompatibility, especially where high tensile forces or mechanical loads are applied or in cases where slow biodegradation is required (Panilaitis et al., 2003).

Many researches have been made in order to find out the inflammatory response of raw silk fibers with silk fibroin's extracts in an in vitro system. The results show that silk fibers are highly immunologically inert in long and short-term culture (Panilaitis et al., 2003).

1.3. Forms of Silk Fibroin

1.3.1. Silk Fibroin Microspheres

Silk Fibroin (SF) is a fibrous protein which is a biocompatible and biodegradable material which has been studied in the field of controlled-release drug delivery systems, enzyme immobilization and tissue engineering.

The regenerated Silk Fibroin devices such as films, microparticles, fibers and nanoparticles have been studied for these applications. *Bombyx mori*, which is domesticated silkworm is the most widely investigated SF and it has been used in many studies (Imsombut et al., 2010).

Biodegradable microspheres are often preferred for the use of controlled-release drug delivery systems because of their well-defined model for degradation and drug release. In order to prepare the Silk Fibroin microspheres, some methods have been reported which are water-in-oil (W/O) emulsion solvent evaporation, ball-milling and spray drying (Imsombut et al., 2010).

In order to change Silk Fibroin matrix from random coil (water-soluble) to β-sheet (water-insoluble) forms, the heat or the alcohol treatments are required for these method. However, an appropriate method for preparing the Silk Fibroin microspheres for drug encapsulation is left to be identified (Imsombut et al., 2010).

For the preparation of particles of water-insoluble or hydrophobic biodegradable polymers, the O/W emulsion solvent diffusion method has been used. Therefore, by using the W/O emulsion solvent diffusion method, the particles of water-soluble or hydrophilic polymers could be prepared (Imsombut et al., 2010).

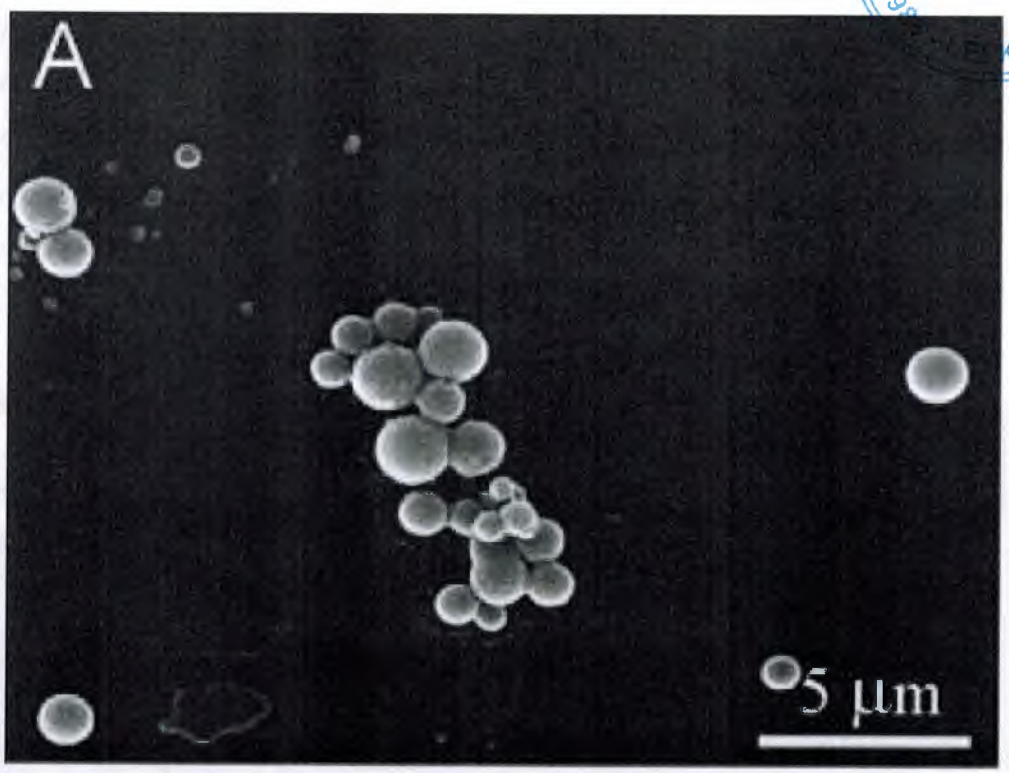

Figure 1.4: SEM (Scanning Electron Microscope) image of silk fibroin microspheres (Cao et al., 2007)

1.3.2. Silk Fibroin Nanoparticles

Nanoparticles have an ability to deliver many kinds of drugs to targeted areas of the body for sustained periods of time, because of that, they have attracted scientists' interest in the field of drug delivery (Cao et al., 2007).

These delivery systems has many advantages, such as reduced toxicity, improved efficacy, patient compliance and convenience compared to traditional dosage forms (Cao et al., 2007).

The system based on proteins is rather promising compared to other systems, because of it is biodegradable, non-antigenic and relatively easy to prepare. In addition, for further surface modification and covalent drug attachment, special amino acid sequences of protein are supposed to get such protein-based nanoparticles with different possibilities (Cao et al., 2007).

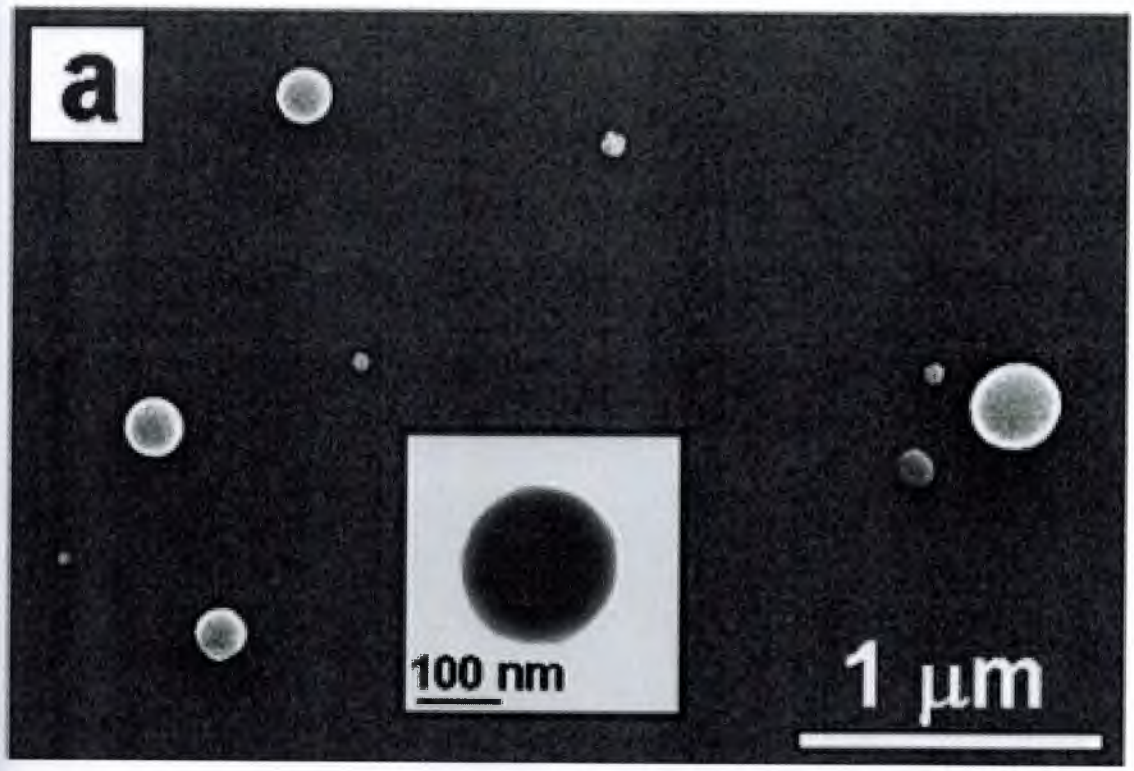

Figure 1.5: Silk fibroin nanoparticles (Myung et al., 2008)

Nanoparticles have been widely investigated in different fields of the life sciences such as histological studies, separation technologies, drug delivery systems, clinical diagnostic assays, and cosmetics. The results obtained from these studies offers great advantages such as sterilization and easy purification, sustained release action and drug targetting possibilities (Myung et al., 2008).

1.3.3. Silk Fibroin Scaffolds

The recent development of new three-dimensional biodegradable porous scaffolds have attracted scientists' interest in the field of regenerative medicine and tissue engineering. In order to design and prepare scaffolds, there are some critical requirements that must be known (Yan et al., 2011).

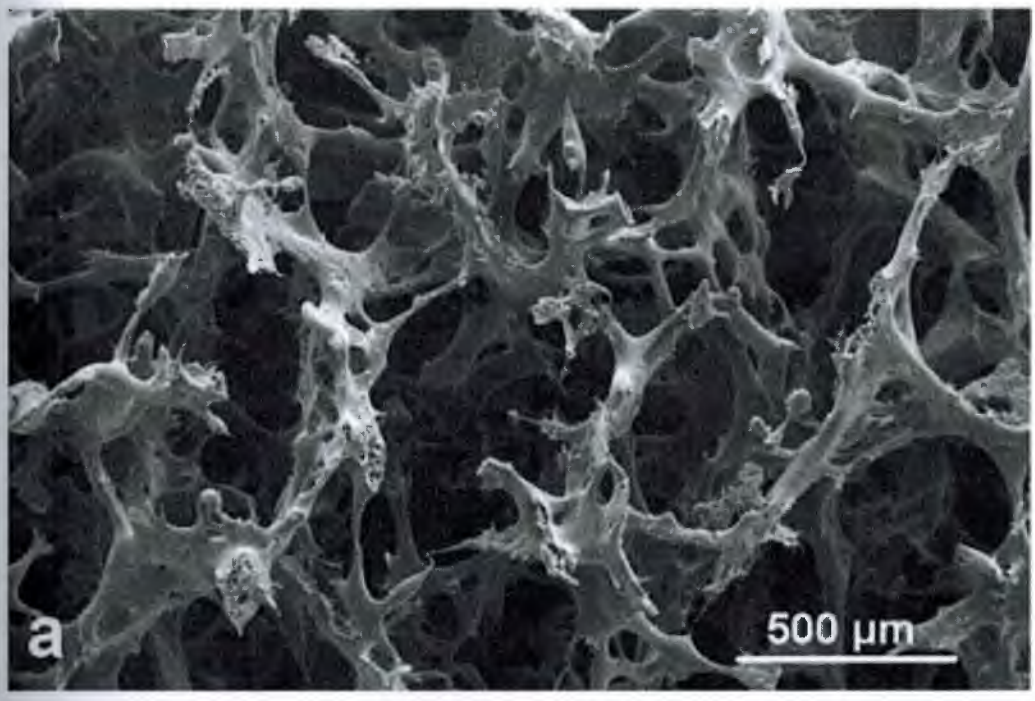

Figure 1.6: SEM (Scanning Electron Microscope) image of silk fibroin scaffolds (Yan et al., 2011)

Various types of biomaterial have been investigated by considering those requirements as matrices to be used in tissue-engineered scaffolding, like synthetic and naturally occuring polymers and bioactive calcium phosphate. Other than these, SF obtained from the silkworm *Bombyx mori* has proved that it is a promising candidate as a scaffolding material (Yan et al., 2011).

In vivo applications, the foreign body reponse of silk fibroin scaffolds is dependent on the implantation site and the model that is chosen and in most cases, the response is low and it decreases exponentially with time (Yan et al., 2011).

Silk fibroin is a versatile material for tissue engineering scaffolding because of it degradability and mechanical properties. It can be easily combined with different structures, such as fiber meshes, membranes, hydrogels, three-dimensional porous scaffolds and microspheres. Considering the above reasons, silk-based scaffolds have been successfully applied in the field of tissue engineering of skin, bone, cartilage, tendon and ligament (Yan et al., 2011).

Different types of methods have used to produce porous silk fibroin scaffolds, like salt leaching, gas foaming, freeze-drying and rapid prototyping. A new strategy has proposed in order to produce porous silk fibroin scaffolds by using aqueous-derived silk fibroin solutions and the salt-leaching method. The whole process was made in an aqueous environment and the produced scaffolds showed new features regarding the biodegradation and mechanical properties. (Kim et al., 2005).

In tissue engineering field, three-dimensional scaffolds has an important place because of they provide a place for attachment, increased surface area, support large cell mass and capable of shaping particular structures. Silk fibroin scaffolds have slow degradation in vivo (Minoura et al., 1998).

1.3.4. Silk Fibroin Biofilms

Silkworm silk fibroin has a wide usage in many different areas such as textile and biomedical applications for many years (Jiang et al., 2007).

Silk fibroin protein has an excellent biocompatibility in vivo and because of that, it is used in artificial tendons, blood vessels and skin grafts in biomedical applications. Studies with the silk fibroin protein have proven promising results both in vitro and in vivo (Jiang et al., 2007).

In the biofilm process, spin casting and layer-by-layer (LbL) grafting methods are used widely to produce uniform thin and ultrathin polymeric films. Spin casting is the easier method for the fabrication (100-1000nm thick films) of ultrathin silk films, where layer-by-layer method allows fabricating of ultrathin (1-100nm) multilayered films in a step-wise manner (Jiang et al., 2007).

Layer-by-layer method has different approaches and it is used widely in the fabrication of superhydrophobic surfaces, biosensors, electroluminescent devices, controlled drug delivery systems and fuel-cell membranes, but technological drawbacks limit their usage in different areas. However, in the recent studies spin casting and LbL methods have combined together as spin-assisted LbL (SA-LbL) method and it was found that it is more efficient in the production and it expanded the applications of ultrathin films (Jiang et al., 2007).

It is possible to produce silk fibroin films which has 2D or 3D nano- or micropatterns by using a soft-lithography-based simple casting technique. It is possible to form at least sub-30nm transverse silk fibroin films from an aqueous silk solution (Perry et al., 2008)..

This method is simple and the fabrication is completed without adding harsh chemicals, salts or high pressures that most micro- and nanofabrication techniques use. By using this simple casting method, high-quality films that have wide spectrum of nano- and micropatterns can be produced (Perry et al., 2008).

Studies and experiments that have been made in order to produce thin and ultrathin regenerated silk fibroin films and investigation of their mechanical properties have a great popularity because of their excellent strength and elasticity combined with tunable biodegradable properties. However, there are some drawbacks on the mechanical properties that show the difficulties of forming uniform ultrathin films and the limited approaches (Jiang et al., 2007).

1.4. Silk Fibroin Biofilms Blended with Glycerine

Film-shaped biomaterials have a wide range of applications in the field of tissue engineering. However, the cast dry films that are derived from silk cocoon sericin has fragile tensile properties and this causes to difficulties and inconvenience in the practical applications. In order to solve this problem, chemical cross-linkers are used to increase the tensile properties of sericin-based films. However, the chemical cross-linker reagents can result in toxicity problems and lower biocompatibility. Therefore, different materials and combinations are being observed in order to eliminate such problems and find out highly biocompatible and non-toxic materials and combinations. (Zhang et al., 2011).

Silk Fibroin is a great material that can be easily form into films and it is highly biocompatible with the human body. Similar to human skin, in the wet state, silk fibroin biofilms have good dissolved oxygen permeability which gives chances for the biomedical applications. However, pure silk fibroin films are soluble in water because of its random coil structures (Lu et al., 2009).

In order to use silk fibroin films in the human body, the structural features of the protein should be transformed from random coil to β-sheet form by treatment with mechanical stretching, heating, curing in water vapor and immersion in polar organic solvents. On the other hand, pure silk fibroin biofilms are brittle and stiff in dry state. Therefore, they need to modify in order to change their physical and mechanical properties in the use of flexible systems (Lu et al., 2009).

Glycerine (or glycerol) is a simple polyol (sugar alcohol) compound. It has been used in order to improve the mechanical and physical properties of silk fibroin biofilms. Glycerine has an ability to reduce phase seperation between PVA (Poly-vinyl alcohol) and silk in the blending or to accelerate silk gelation. The recent studies have shown that the blending of silk fibroin and glycerine provided important benefits to the film properties, such as silk fibroin crystallization behaviour and being flexible in the means of elasticity (Lu et al., 2009).

1.5. Silk Fibroin Biofilms Blended with Polypyrrole

Polypyrrole (PPy) is an electroactive polymer that consists of pyrrole monomers which held together by negatively charged dopants. One of the most useful advantage of Polypyrrole which makes it preferable from the other polymers is that by using the electrical stimulation, the release of incorporated molecules can be reduced and controlled (Richardson et al., 2009).

Polypyrrole (PPy) has been used in many studies as a conducting polymer because of its high electrical conductivity and straight-forward preparation methods. Polypyrrole materials are stable in air, has good electrochemical properties, high conductivity and thermal stability. In addition, they can be easily formed chemically and electrochemically (Kassim et al., 2006).

Polypyrrole conducting polymers have a wide range of surface conductivities depending on the field that they function. However, they have practical problems in the utilization because of the poor mechanical properties like low processibility and brittleness of Polypyrrole. Therefore, in order to solve this problem, polypyrrole must be blended with other polymers in order to improve the mechanical properties without losing the conductivity (Kassim et al., 2006).

1.6. Silk Fibroin Biofilms Blended with Iron (III) Oxide (Fe_2O_3)

Iron (III) Oxide is the inorganic compound with the formula Fe_2O_3. It is one of the three oxides of iron and it occurs naturally as the mineral magnetite. Iron (III) oxide is often called rust and when it is dissolved in a chemical it has good conducting properties.

In this thesis, during the experiments, Iron (III) oxide was dissolved in concentrated hydrochloric acid and blended with pure silk fibroin solution and glycerine in order to make a biofilm and compare its properties with the one made with polypyrrole.

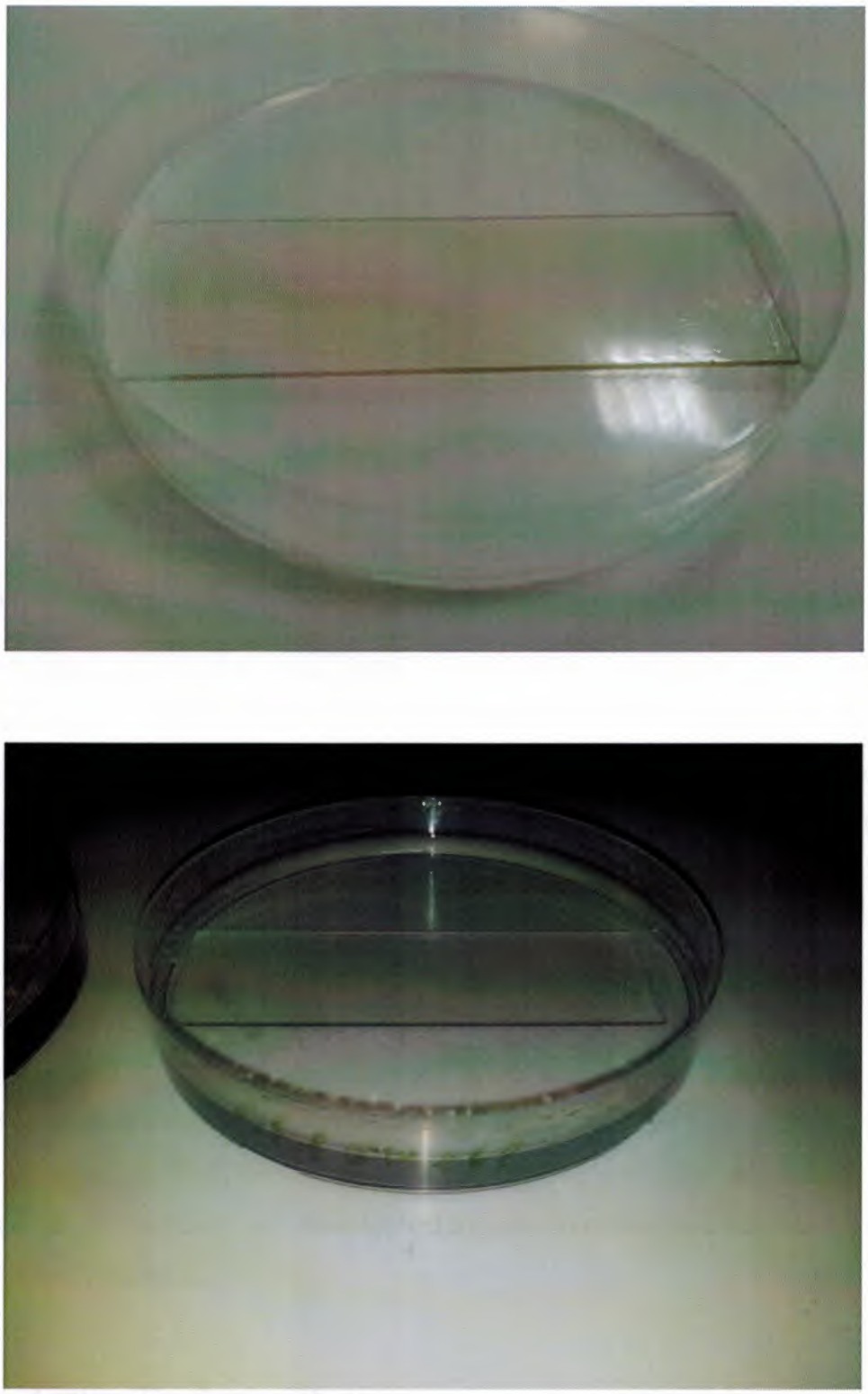

Figure 1.7: Different types of formed silk fibroin biofilms

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Bombyx Mori cocoons, some chemicals and materials were used according to special procedures in order to prepare pure silk fibroin protein. In order to obtain pure silk fibroin protein, all steps must be done carefullly and specific materials must be used with the correct amounts.

Sodium Carbonate Na_2CO_3, Iron (III) Oxide Fe_2O_3, Concentrated Nitric Acid, Concentrated Hydrochloric Acid were purchased from Merck and PolyPyrrole (PPy), Calcium Chloride, $CaCl_2$, Ethanol and dialysis membrane (cut off M.W. 12,400) were also purchased from Sigma-Aldrich and all of them were used during the processes which are defined in the following sections.

2.2. Methods

2.2.1. Silk Fibroin Purification Processes

In this section, all processes that were applied in order to obtain pure silk fibroin solution were explained in details.

2.2.1.1. Cutting The *Bombyx Mori* Cocoons

The whole purification process starts with the cutting of a bunch of domestic *Bombyx Mori* cocoons in the shape of small squares but not so small and then, making them ready for the degumming process.

Figure 2.1: The cut *Bombyx mori* cocoons

2.2.1.2. Degumming Process

Degumming is the process of removing the sericin from the *Bombyx mori* cocoons, which is a sticky substance produced by the silkworm that holds the strands of silk together (Sah and Pramanik, 2010).

In this process, cocoons were put into a beaker that contains 0.1M Sodium Carbonate (Na_2CO_3) solution 1g/100mL (w/v). Then, the beaker was placed on a hot plate stirrer at the speed of 1.5 rpm at 75 °C and let it to be stirred for three hours. This process was repeated 3 times with replacing the solution in each session.

Figure 2.2: *Bombyx mori* cocoons in the degumming process in 0.1M Sodium Carbonate (Na_2CO_3) solution at the speed of 1.5 rpm at 75 °C

After the third session of degumming process, the degummed silk was washed and rinsed in order to eliminate the remaining solution on them. Then, it was left to dry overnight or about 12 hours at the room temperature in order to obtain the silk fibers.

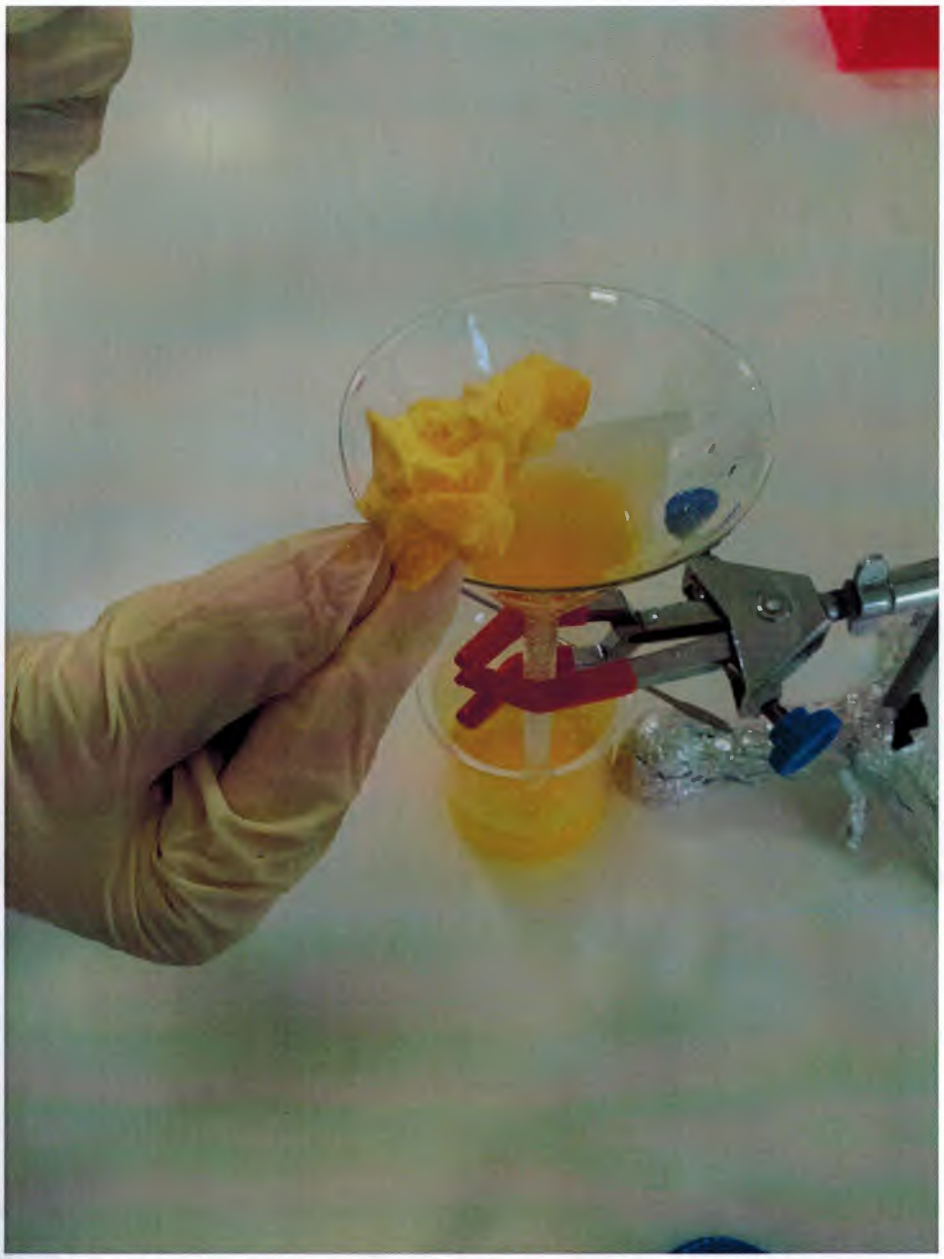

Figure 2.3: The first washing step of the degummed silk fibroin

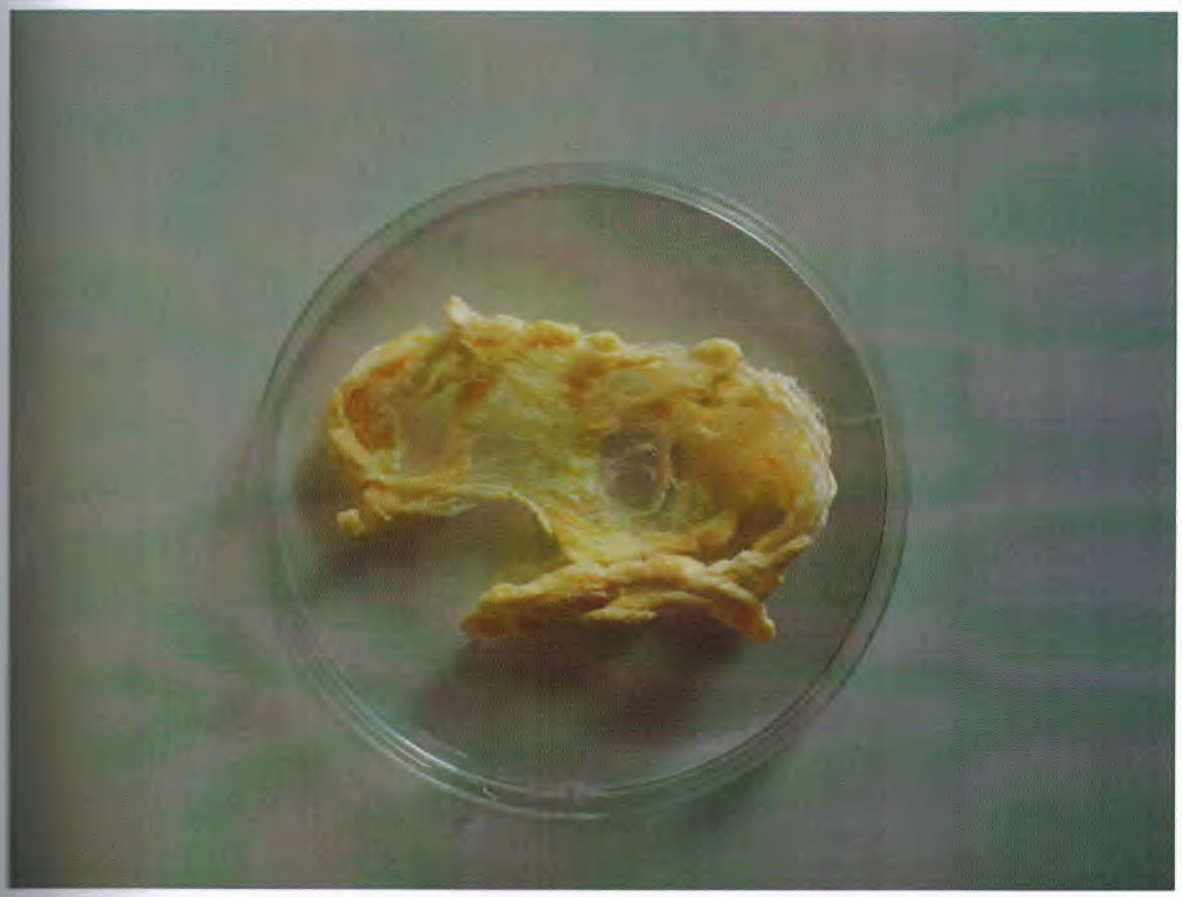

Figure 2.4: Degummed silk fibroin after the degumming process which was left to dry at the room temperature

When the degummed silk fibroin is dry after 12 hours of waiting, it is lint in order to make it ready for the dissolution process. This is done in order to make it easy to dissolve in the prepared solution. Otherwise, it does not dissolve totally and left precipitates.

Figure 2.5: Linted silk fibroin after the degumming process

2.2.1.3. Dissolution Process

Dissolution is the third step of silk fibroin purification process which is used to obtain an aqueous form of silk fibroin by breaking down the long silk fibroin polypeptide chains into shorter length chains. During the process, the silk fibroin was blended with $\mathbf{n}_{C2H5OH}$: $\mathbf{n}_{H2O}$: $\mathbf{n}_{CaCl2}$ (2:8:1) molar ratio at 75 °C with continuous stirring until the total dissolution in order to get the aqueous silk fibroin electrolyte solution.

Figure 2.6: The prepared $\mathbf{n}_{C2H5OH}$: $\mathbf{n}_{H2O}$: $\mathbf{n}_{CaCl2}$ (2:8:1) solution

Figure 2.7: Aqueous silk fibroin electrolyte solution after the dissolution process

2.2.1.4. Dialysis

Dialysis procedure was used to remove the ions within the solution that was obtained in the dissolution step in order to obtain the pure silk fibroin solution. Dialysis procedure was started by pouring the aqueous silk fibroin electrolyte solution into a carboxymethyl cellulose semi-permeable membrane tube and putting the membrane tube into a large beaker (5 Liters) which was filled with ultrapure distilled water.

The semi-permeable membrane tube allowed the ions to diffuse from the aqueous silk fibroin electrolyte solution to the water. This step was repeated 2 times (5 hours for each session) with continuous stirring.

At the end of the defined periods of stirring, the process ended up with the formation of pure aqueous silk fibroin solution with the concentration of 6%. After this process, the obtained pure silk fibroin solution was ready for biofilm preparations and other applications.

Figure 2.8: Aqueous silk fibroin electrolyte solution in the carboxymethyl cellulose semi-permeable membrane tube in the dialysis procedure

Figure 2.9: Pure aqueous silk fibroin solution

2.2.2. Biofilm Preparation Process

In the biofilm preparation process, 6 different types of biofilms was formed at the room temperature until the total biofilm formation and the process was carried out according to the defined ratios at table 2.1 for each biofilm.

Polypyrrole is a hard dissolving organic polymer and it is higly conductive. Therefore, it is hard to dissolve and use it as a liquid form in the biofilm preparation process. During the formation, 0.0001gr polyprrole was dissolved in 20mL concentrated hydrochloric acid and blended with silk fibroin and glycerine in that form.

Iron (III) Oxide Fe_2O_3 is a conducting material and it was dissolved in concentrated hydrochloric acid in order to use it in the formation process. During the formation, 0.050gr Fe_2O_3 was dissolved in 20mL concentrated hydrochloric acid and blended with silk fibroin and glycerine in that form.

The formation process was carried out carefully and for each biofilm formation clean and unused materials was used. All biofilms let for formation at the room temperature until the total biofilm formation occured which was 2 days.

The process was started by forming a pure silk fibroin biofilm which was made up of 2mL of pure silk fibroin solution and then, it was poured over a piece of glass which was put in a petri dish and let for formation. Later on, 2mL of pure silk fibroin solution and 0.050gr glycerine were blended and poured over a piece of glass like pure silk fibroin biofilm. Then, 2mL of pure silk fibroin solution and 50μL polpyrrole dissolved in concentrated hydrochloric acid were blended and poured over a piece of glass. After that, 2mL of pure silk fibroin solution and 50μL Fe_2O_3 dissolved in concentrated hydrochloric acid were blended and poured over a piece of glass. Finally, 2mL of pure silk fibroin solution, 0.050gr glycerine and 50μL polpyrrole dissolved in concentrated hydrochloric acid were blended and poured over a piece of glass. Similarly, 2mL of pure silk fibroin solution, 0.050gr glycerine and 50μL Fe_2O_3 dissolved in concentrated hydrochloric acid was blended and poured over a piece of glass.

All piece of glasses were put in petri dishes before pouring the blended solutions and all blended solutions let for biofilm formation at the room temperature.

Table 2.1: Prepared biofilms and their ratios

Prepared Biofilms	Amounts Used			
	Pure SF (6%)	**Gly**	**PPy**	**Fe_2O_3**
SF	2mL	-	-	-
SF + Gly	2mL	0.050gr	-	-
SF + PPy	2mL	-	50µL	-
SF + Fe_2O_3	2mL	-	-	50µL
SF + Gly + PPy	2mL	0.050gr	50µL	-
SF + Gly + Fe_2O_3	2mL	0.050gr	-	50µL

2.2.2.1. Methanol Treatment

After the biofilm formation process, methanol was poured on the surfaces of the formed biofilms which were put in petri dishes in the biofilm formation process. This procedure was done in order to fix the secondary structure and convert the random coils to β-sheet structure by hydrogen bonding. After waiting about ten minutes, the methanol applied biofilms were put in dry petri dishes.

Figure 2.10: Different types of formed biofilms

2.2.2.2. Protein Concentration Calculation

By taking 1mL of pure aqueous silk fibroin solution which was obtained after the dialysis process and pouring it on a glass surface and then, applying a continuous heat at 37 °C was resulted as a silk fibroin biofilm formation. By weighing the 1mL of pure silk fibroin solution and the resulted biofilm, the amount of protein which was extracted from *Bombyx mori* cocoons was calculated in 1mL content.

2.2.3. Swelling

The silk fibroin biofilms which were prepared in different types and amounts were tested for their swelling properties in Phosphate Buffer Saline and Acetic Buffer Saline solutions.

The swelling ratios were calculated by using the below formula:

$$Swelling\ \% = \frac{\text{weight(s)} - \text{weight(dry)}}{\text{weight(dry)}} * 100\ \%$$

where; weight(s) is the biofilm's weight during swelling at any given time and weight(dry) is the weight of biofilm when it is dry at the beginning of the swelling test.

2.2.4. Scanning Electron Microscope (SEM) Analysis

A Scanning Electron Microscope (SEM) is a type of electron microscope which is used to obtain images of a sample by scanning it with a beam of electrons. The electrons interact with atoms in the sample and they produce different types of signals that can be detected by the microscope which contain information about the sample's surface topography and composition (McMullan, 2006).

A Scanning Electron Microscope Analysis was carried out at TUBİTAK – Marmara Araştırma Merkezi at Gebze, İstanbul, Turkey by using a SEM JSM-6510 model microscope.

CHAPTER 3

RESULTS AND DISCUSSION

3.1.SEM Analysis

In order to investigate the morphological features of raw silk fibers, degummed silk fibers and some of the pure silk fibroin biofilms obtained in the experiments of this thesis which is defined at the Table 2.1 (SF + Glycerine and SF + Glycerine + Polypyrrole), Scanning Electron Microscope (SEM) analysis was done.

3.1.1. SEM Analysis of Raw Silk Fibers

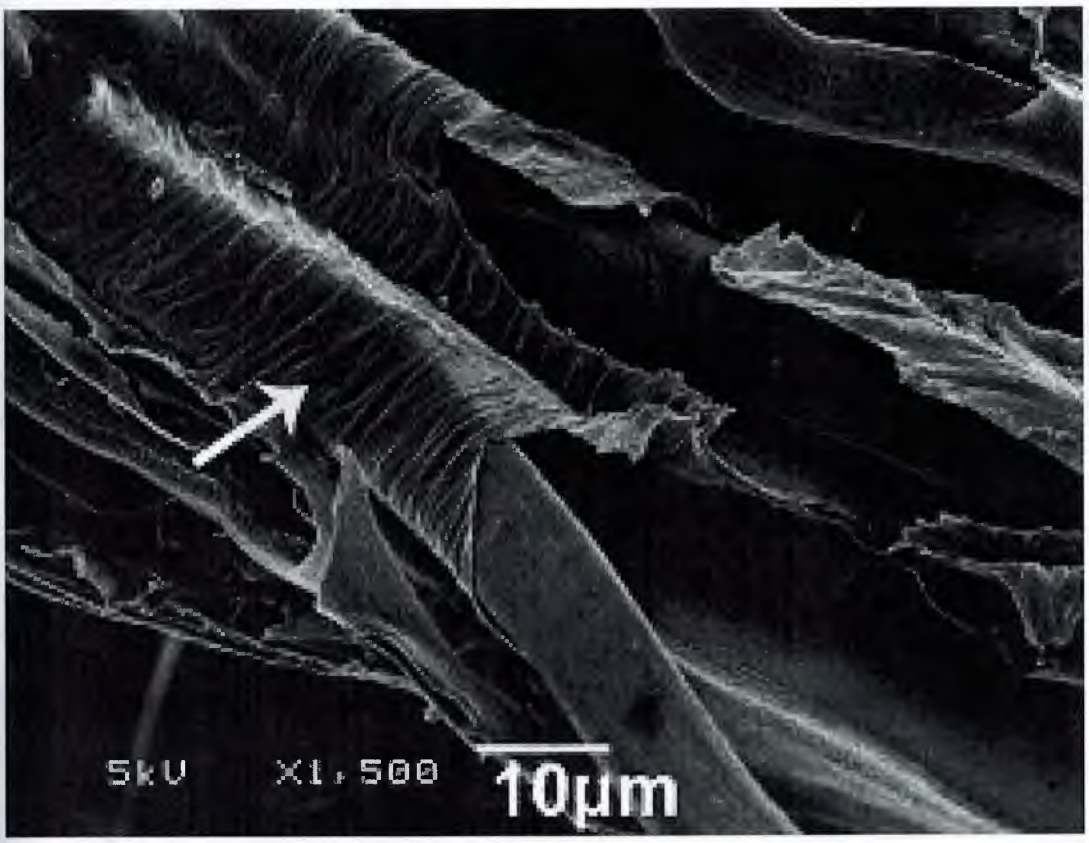

Figure 3.1: A SEM picture of raw silk fibers (Sahoo et al., 2010)

The raw silk fibers are coated with the sericin in the normal structure of the silk and with the SEM picture in the Figure 3.1, the sericin covering the fibers is shown with the white pointer.

3.1.2. SEM Analysis of Degummed Silk Fibers

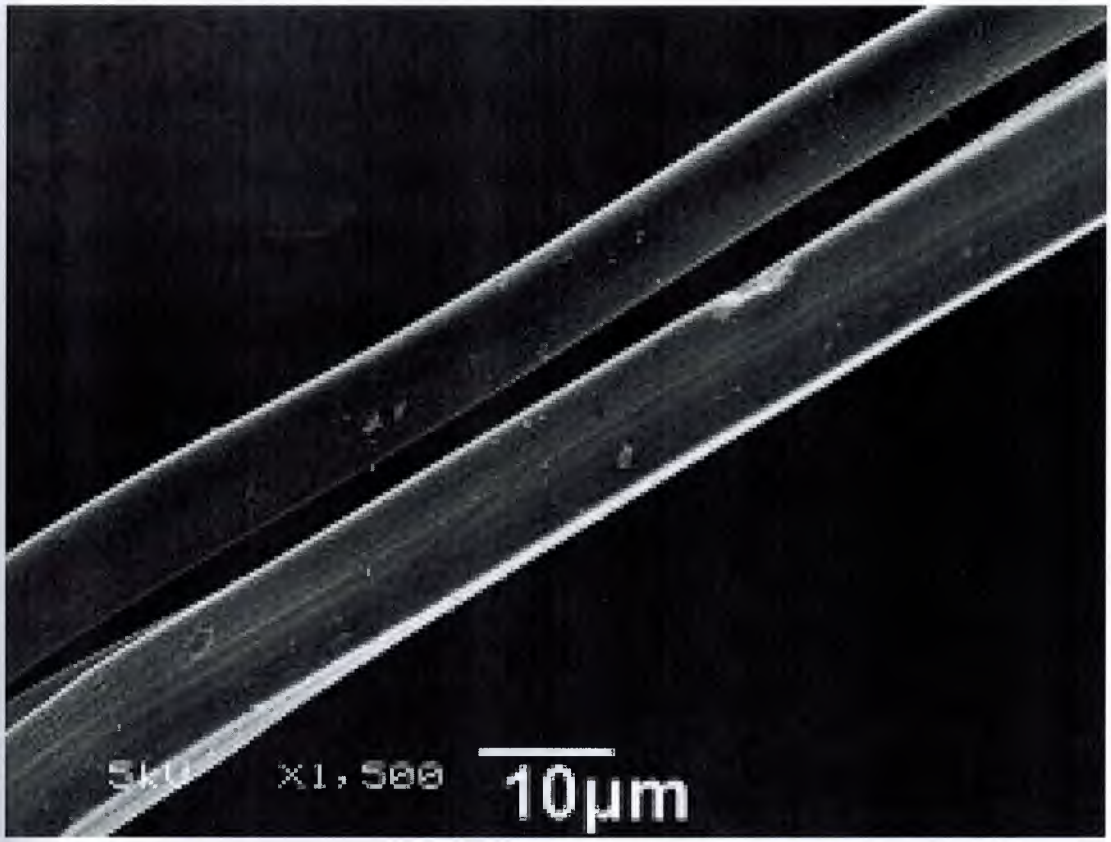

Figure 3.2: A SEM picture of degummed silk fibers (Jaworska et al., 2003)

After the degumming process, the sericin protein was clearly removed and a SEM picture in the Figure 3.2 shows the smooth surfaces of the silk fibers. This picture also shows the effectiveness of the degumming process of removal of the sericin protein.

3.1.3. SEM Analysis of Pure Silk Fibroin Biofilms

Figure 3.3: A SEM picture of a Pure SF Biofilm

The mechanical properties of the pure silk fibroin biofilm has been modified by using glycerine as an elesticity improvement agent. By using glycerine, the use of organic solvent has been avoided. To be able to form new and more flexible silk-based biofilms, the blending ability of glycerine has been used with hydrophobic silk fibroin protein and more stabilization and elasticity has been achieved. By processing pure water, the biocompatibility has been enhanced. The silk fibroin / glycerine biofilms exhibited altered mechanical properties. When compared with the pure silk fibroin biofilms, it has improved elongation time at break. Glycerine appears to replace water in silk fibroin chain hydration. This improves the initial stability of helical structures in the biofilms.

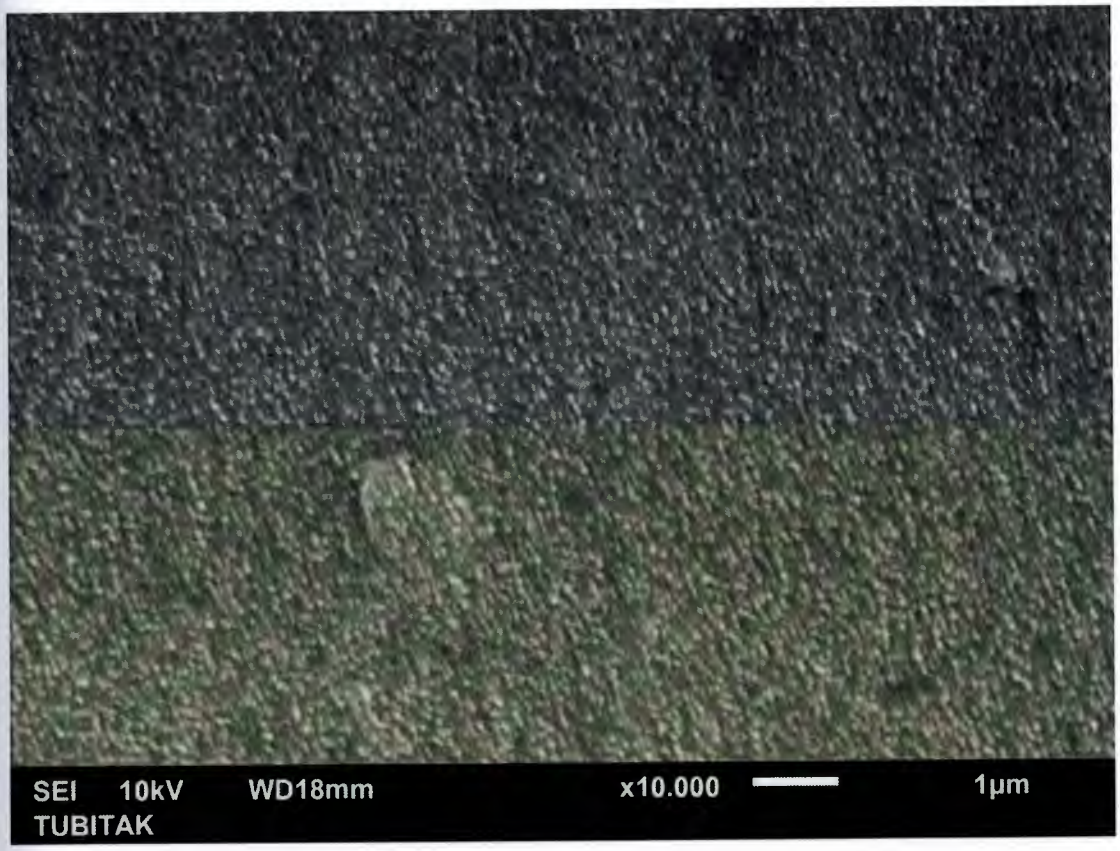

Figure 3.4: A SEM picture of a Pure SF + Glycerine Biofilm

In Figure 3.4 the porous structure of pure silk fibroin and glycerine biofilm can be seen easily when it is compared with pure silk fibroin structure on Figure 3.3. This SEM picture clearly shows the effect of glycerine to pure silk fibroin structure.

Figure 3.5: A SEM Picture of a Pure SF + Glycerine + Polypyrrole Biofilm

Silk fibroin / glycerine and polypyrrole biofilms with improved eletroactivity have a lot of applications in biomedical devices (Cervantes et al., 2012). Biofilms consisting of conductive polymers have applications in biosensing, controlled drug delivery and tissue engineering with improved cellular growth (Guimard et al., 2007); (Ravichandran et al., 2010). In figure 3.5, it is very clear that the crystallinity improved by adding polypyrrole into the system. The final form of the biofilm is more rigid, less elastic than the pure silk fibroin biofilm.

3.2.FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) is a kind of technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. A spectral data in a wide spectral range is collected with an FTIR spectrometer simultaneously (Griffiths and Hasseth, 2007).

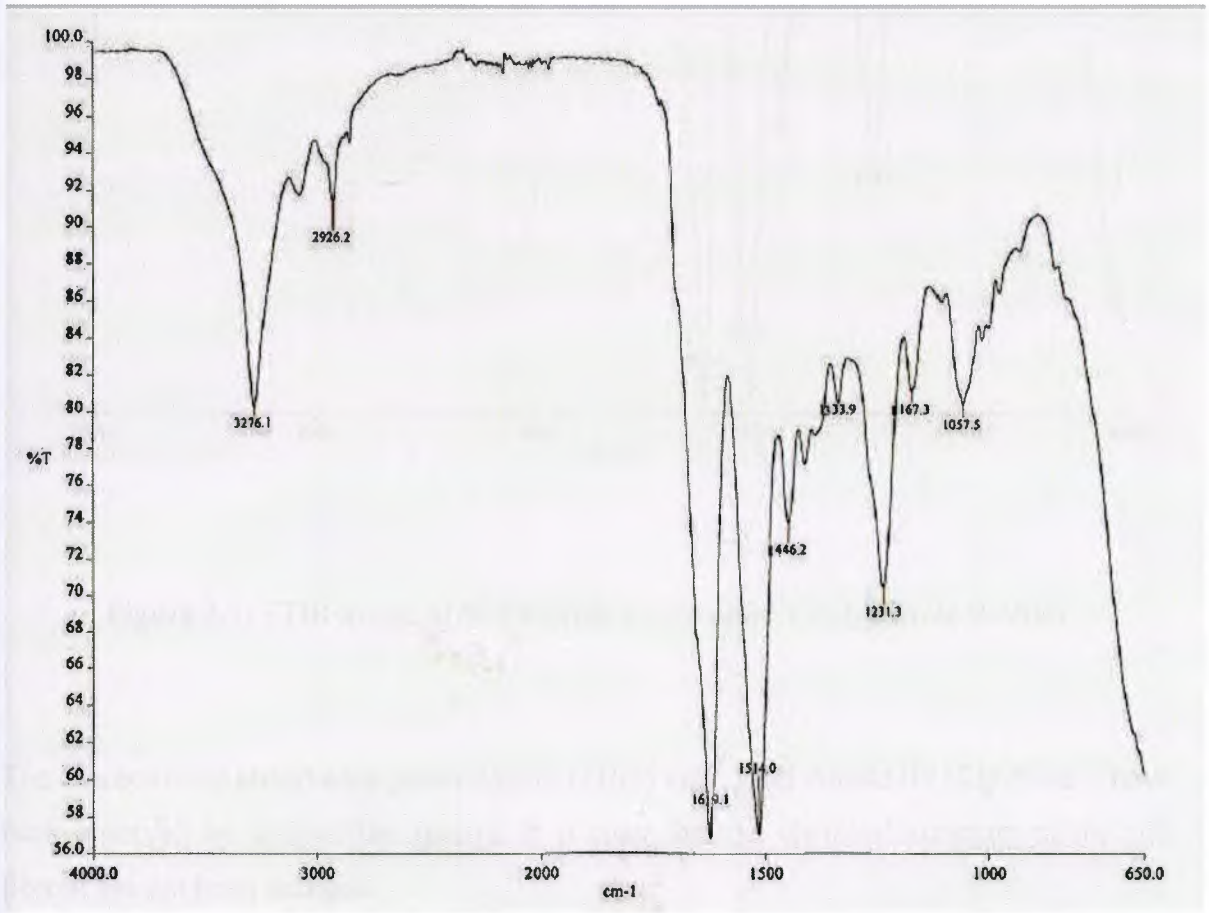

Figure 3.6: FTIR image of Silk fibroin + Glycerine Biofilm

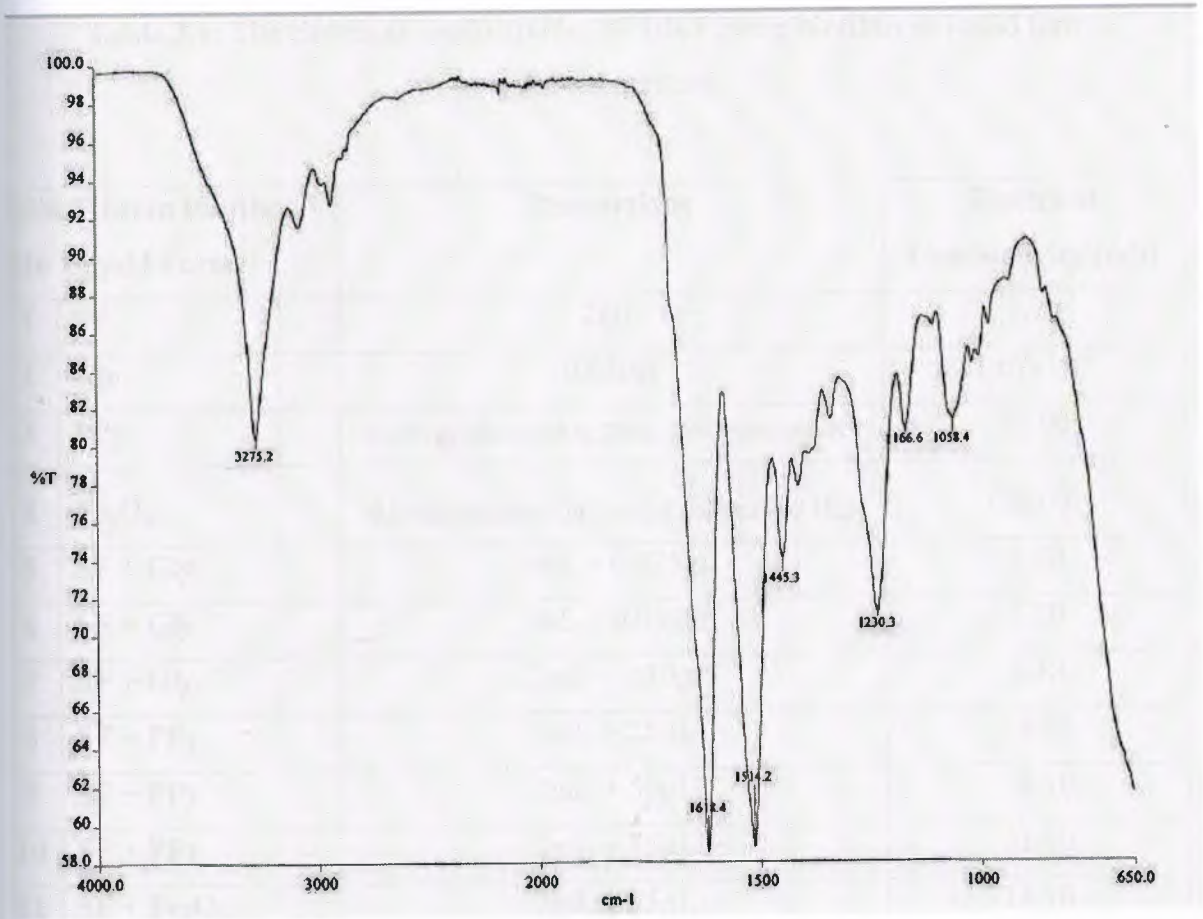

Figure 3.7: FTIR image of Silk Fibroin + Glycerine + Polypyrrole Biofilm

The characteristic absorbance peaks Amide I (1655 cm^{-1}) and Amide II (1537.5 cm^{-1}) have been observed on both of the spectra. It is clear that the chemical structure of the silk fibroin has not been changed.

3.3.Electrical Conductivity Analysis

Electrical conductivity analysis was made for the silk fibroin biofilms which are defined in Table 3.1 in order to observe the changes in the electrical conductivity of pure silk fibroin by blending it with glycerine, Fe_2O_3 and Polypyrrole in liquid form.

Table 3.1: The electrical conductivities of Silk Fibroin biofilms in liquid form at room temperature

Silk Fibroin Biofilms (In Liquid Form)		Proportions	Electrical Conductivity (mS)
1	**SF**	2mL	1.70
2	**Gly**	0.050gr	$1.67x10^{-4}$
3	**PPy**	0.0001gr dissolved in 20mL Concentrated HCl	33.00
4	**Fe_2O_3**	0.050gr dissolved in 20mL Concentrated HCl	20.00
5	**SF + Gly**	2mL + 0.025gr	1.60
6	**SF + Gly**	2mL + 0.050gr	1.50
7	**SF + Gly**	2mL + 0.10gr	1.40
8	**SF + PPy**	2mL + 25µL	18.50
9	**SF + PPy**	2mL + 50µL	38.10
10	**SF + PPy**	2mL + 75µL	51.60
11	**SF + Fe_2O_3**	2mL + 25µL	14.10
12	**SF + Fe_2O_3**	2mL + 50µL	27.30
13	**SF + Fe_2O_3**	2mL + 75µL	35.40
14	**SF + Gly + PPy**	2mL + 0.050gr + 25µL	16.80
15	**SF + Gly + PPy**	2mL + 0.050gr + 50µL	30.60
16	**SF + Gly + PPy**	2mL + 0.050gr + 75µL	42.70
17	**SF + Gly + PPy**	2mL + 0.10gr + 50µL	23.80
18	**SF + Gly + Fe_2O_3**	2mL + 0.050gr + 25µL	10.60
19	**SF + Gly + Fe_2O_3**	2mL + 0.050gr + 50µL	18.90
20	**SF + Gly + Fe_2O_3**	2mL + 0.050gr + 75µL	25.60
21	**SF + Gly + Fe_2O_3**	2mL + 0.10gr + 50µL	13.20

The obtained results showed that polypyrrole is a more conductive material than glycerine and Iron (III) oxide. When pure silk fibroin solution was blended with glycerine, it decreased the conductivity but increased the elasticity of the biofilm. On the other hand, the results showed that polypyrrole is more conductive than iron (III) oxide.

According to the electrical conductivity results on Table 3.1, SF + PPy biofilm has the highest electrical conductivity. However, the form of SF+PPy biofilm was very brittle and this made it difficult to use in a wide range of biomedical applications. Therefore, in order to increase the elasticity, glycerine was blended with pure silk fibroin solution and polypyrrole. As the amount of glycerine increased, the elasticity was also increased. Glycerine, decreased the electical conductivity of the blended biofilm and after a certain amount, it changed the morphological structure of pure silk fibroin biofilms.

The aim of this thesis is to make a biofilm which has high elasticity and electrical conductivity, therefore SF+Gly+PPy biofilm was the best obtained biofilm which corresponds the main properties of the aim.

3.4.Swelling Test

Swelling tests were made for the blended silk fibroin biofilms in order to observe their swelling ratios and weights in Phosphate Buffer Saline Solution at pH 7.4 which mimics the human blood and Acetic Buffer Solution at pH 1.2 which mimics the human urine and also gastric juice in order to estimate swelling behaviours of biofilms under in vitro conditions.

3.4.1. Swelling Test in Phosphate Buffer Saline Solution

Table 3.2: Properties of biofilms which were used in PBS swelling test

Biofilms	Proportions	Weight in dry state
SF + Gly	2mL + 0.0563gr	0.0073gr
SF + PPy	2mL + 50μL	0.0036gr
SF + Fe_2O_3	2mL + 50μL	0.0021gr
SF + Gly + PPy	2mL + 0.0563gr + 50μL	0.0019gr
SF + Gly + Fe_2O_3	2mL + 0.0563gr + 50μL	0.0058gr

Table 3.3: The weight results of different silk fibroin biofilms while swelling in Phosphate Buffer Saline Solution at pH 7.4 at room temperature

Time (Minutes)	SF+Gly Weight(g)	SF+PPy Weight(g)	SF+Fe_2O_3 Weight(g)	SF+Gly+PPy Weight(g)	SF+Gly+Fe_2O_3 Weight(g)
0	0.0073	0.0036	0.0021	0.0019	0.0058
5	0.0119	0.0094	0.0031	0.0040	0.0080
10	0.0119	0.0080	0.0031	0.0032	0.0075
15	0.0124	0.0072	0.0029	0.0029	0.0069
20	0.0131	0.0065	0.0026	0.0025	0.0063
25	0.0129	0.0056	0.0023	0.0021	0.0055
40	0.0128	0.0051	0.0025	0.0018	0.0046
55	0.0126	0.0043	0.0022	0.0013	0.0039
70	0.0127	0.0040	0.0019	0.0011	0.0031
85	0.0125	0.0039	0.0014	0.0008	0.0022
115	0.0123	0.0038	0.0011	0.0005	0.0016
175	0.0127	0.0039	0.0009	biodegraded	0.0012
235	0.0126	0.0037	0.0010	-	0.0010
295	0.0127	0.0036	0.0011	-	0.0009
1735	0.0126	0.0034	0.0008	-	0.0008
3175	0.0127	0.0033	0.0006	-	0.0006

The swelling ratio percentage results of the biofilms in phosphate buffer saline solution showed that, SF+Gly biofilm swelled within 40 minutes and reached to the equilibrium swelling ratio after this point. No degradation have been observed during the swelling process at pH = 7.4. The SF+PPy biofilm swelled up to 161.11% ratio within 5 minutes after that the swelling ratio has been slowly decreased and the initial state of the film has been observed at 3175 minutes.

$SF+Fe_2O_3$ and $SF+Gly+Fe_2O_3$ biofilms were compared according to their swelling ratios. The $SF+Fe_2O_3$ is more stable than the biofilm which was blended with glycerine ($SF+Gly+Fe_2O_3$). The degradation has been started at 25 minutes for the $SF+Gly+Fe_2O_3$ biofilm and at 55 minutes for $SF+Fe_2O_3$ biofilms, respectively. The SF+Gly+PPy biofilms swelled slightly at the beginning of the swelling test and the degradation has been started at 40 minutes.

Table 3.4: Swelling ratios of silk fibroin biofilms while swelling in Phosphate Buffer Saline Solution at pH 7.4

Time (Minutes)	Swelling Ratios (%)				
	SF+Gly	SF+PPy	$SF+Fe_2O_3$	SF+Gly+PPy	$SF+Gly+Fe_2O_3$
0	0	0	0	0	0
5	63.01	161.11	47.61	110.53	37.93
10	63.01	122.22	47.61	68.42	29.31
15	69.86	100.00	38.06	52.63	18.97
20	79.45	80.56	23.81	31.58	8.62
25	76.71	55.56	9.52	10.53	-5.17
40	75.34	41.67	19.05	-5.26	-20.69
55	72.60	19.44	4.76	-31.58	-32.76
70	73.97	11.11	-9.52	-42.11	-46.55
85	71.23	8.33	-33.33	-57.89	-62.07
115	68.49	5.56	-47.61	-73.68	-72.41
175	73.97	5.33	-57.14	-	-79.31
235	72.60	2.78	-52.38	-	-82.76
295	73.97	0	-47.61	-	-84.48
1735	72.60	-5.56	-61.90	-	-86.21
3175	73.97	-8.33	-71.43	-	-89.66

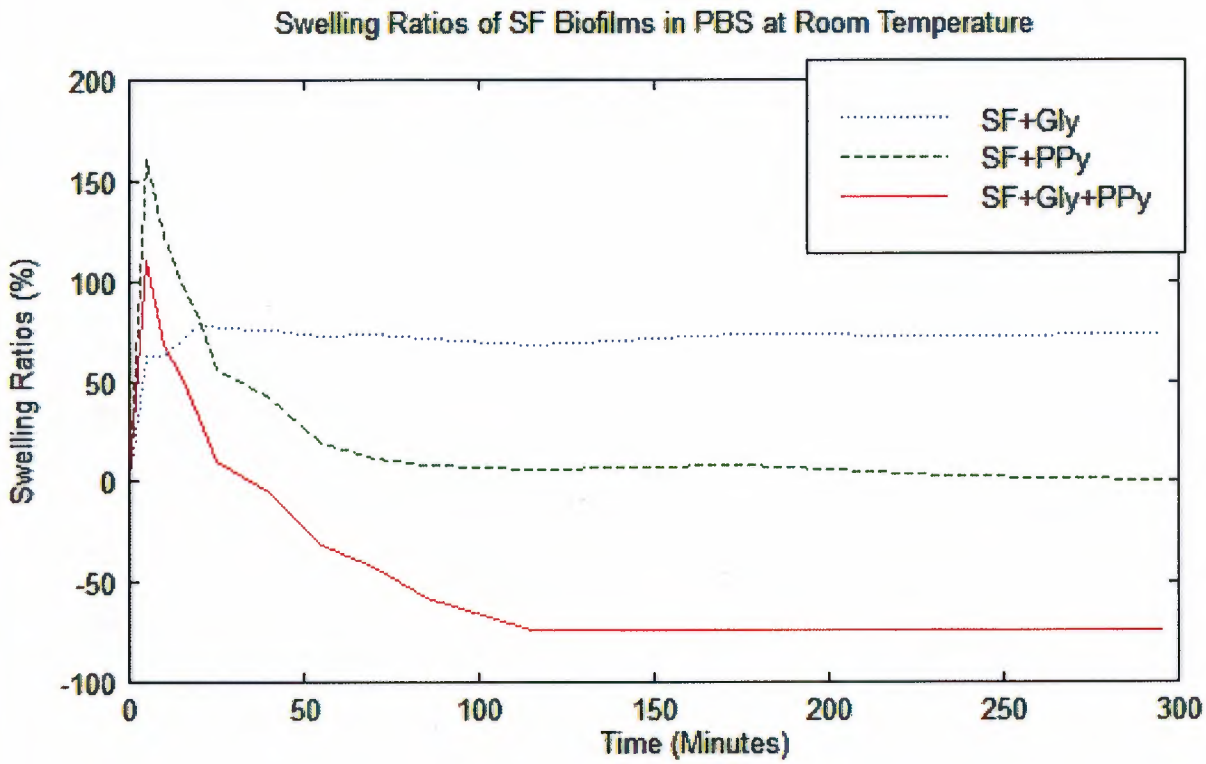

Figure 3.8: Swelling Ratios of SF+Gly, SF+PPy and SF+Gly+PPy biofilms in PBS solution at room temperature

According to the swelling ratios on Figure 3.8, it is obviously seen that glycerine increases the swelling ratio when it is blended with pure silk fibroin solution. Similar to this, when it is blended with pure silk fibroin solution and polypyrrole, it makes the biofilm biodegradable. At the beginning of the PBS test, the biofilm swelled a little and then, it started to biodegradate as the time passed. Glycerine made the biofilm biodegradable because SF+PPy biofilm swelled a little at the beginning and then came back to nearly its beginning weight and did not biodegrade.

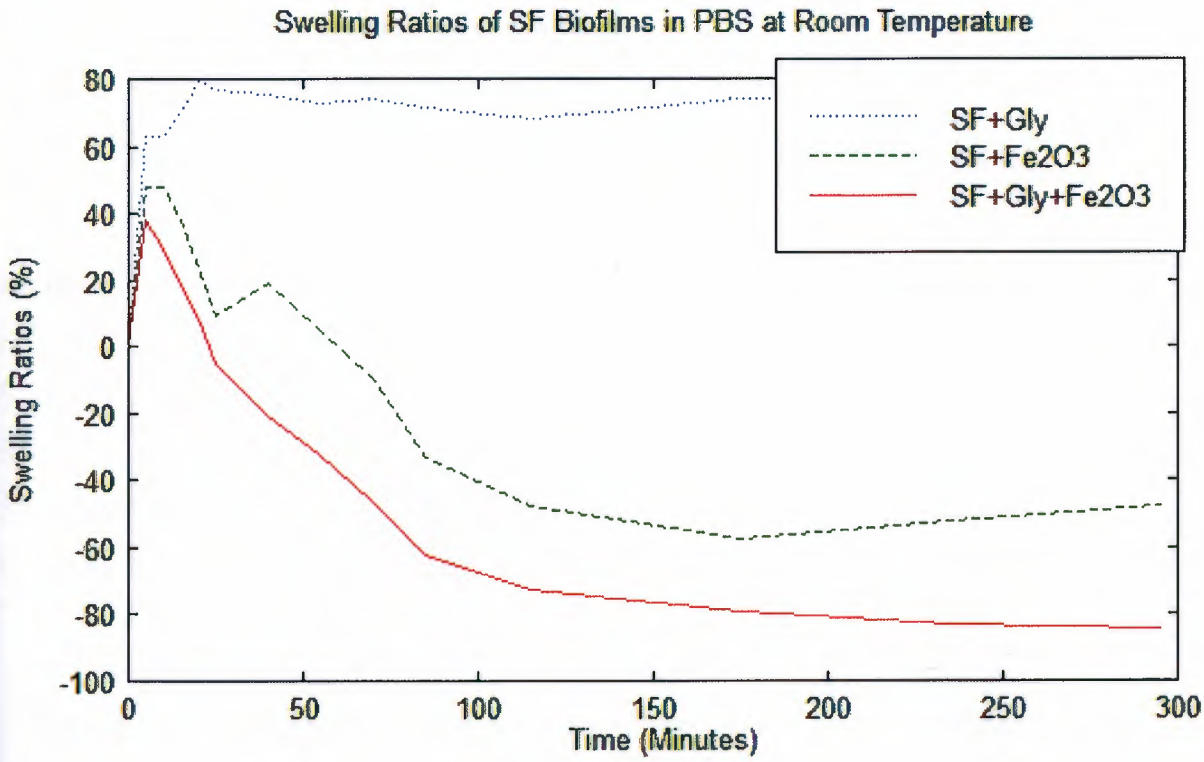

Figure 3.9: Swelling Ratios of SF+Gly, SF+Fe_2O_3 and SF+Gly+Fe_2O_3 biofilms in PBS solution at room temperature

The swelling ratios on Figure 3.9 shows that pure silk fibroin and glycerine biofilm swelled during the test. However, pure silk fibroin and Iron (III) oxide biofilm swelled a little at the beginning and then biodegraded slowly. This shows that iron (III) oxide makes the biofilm biodegradable like glycerine.

For the SF+Gly+Fe_2O_3 biofilm, similar to SF+Fe_2O_3 it swelled at the beginning but it biodegraded faster than SF+Fe_2O_3. Because, iron (III) oxide made the biofilm biodegradable and when it was blended with glycerine, it increased the level of biodegradation of the biofilm more than iron (III) oxide during the test.

3.4.2. Swelling Test in Acetic Buffer Solution

Table 3.5: Properties of biofilms which were used in ABS swelling test

Biofilms	Proportions	Weight in dry state
SF + Gly	2mL + 0.0563gr	0.0097gr
SF + PPy	2mL + 50μL	0.0053gr
SF + Fe_2O_3	2mL + 50μL	0.0011gr
SF + Gly + PPy	2mL + 0.0563gr + 50μL	0.0016gr
SF + Gly + Fe_2O_3	2mL + 0.0563gr + 50μL	0.0029gr

Table 3.6: The weight results of different silk fibroin biofilms while swelling in Acetic Buffer Solution at pH 1.2 at room temperature

Time (Minutes)	SF+Gly Weight(g)	SF+PPy Weight(g)	SF+Fe_2O_3 Weight(g)	SF+Gly+PPy Weight(g)	SF+Gly+Fe_2O_3 Weight(g)
0	0.0097	0.0053	0.0011	0.0016	0.0029
5	0.0154	0.0087	0.0017	0.0020	0.0045
10	0.0153	0.0089	0.0017	0.0022	0.0046
15	0.0167	0.0091	0.0014	0.0020	0.0032
20	0.0168	0.0086	0.0014	0.0016	0.0030
25	0.0174	0.0075	0.0016	0.0015	0.0025
40	0.0168	0.0073	0.0014	0.0010	0.0021
55	0.0166	0.0060	0.0012	0.0008	0.0017
70	0.0163	0.0058	0.0008	0.0006	0.0016
130	0.0166	0.0049	0.0006	0.0004	0.0010
190	0.0165	0.0045	0.0004	0.0003	0.0009
250	0.0166	0.0038	0.0004	0.0001	0.0009
1690	0.0148	0.0029	0.0003	Degraded	0.0009
3130	0.0140	0.0026	0.0003	-	0.0008

Table 3.7: Swelling ratios of silk fibroin biofilms while swelling in Acetic Buffer Solution at pH 1.2

Time (Minutes)	Swelling Ratios (%)				
	SF+Gly	**SF+PPy**	**SF+Fe_2O_3**	**SF+Gly+PPy**	**SF+Gly+Fe_2O_3**
0	0	0	0	0	0
5	58.76	64.15	54.54	25.00	55.17
10	57.73	67.92	54.54	37.50	58.62
15	72.16	71.70	27.27	25.00	10.34
20	73.20	62.26	27.27	0	3.45
25	79.38	41.50	27.30	-6.25	-13.79
40	73.20	37.74	27.27	-37.50	-27.59
55	71.13	13.20	9.09	-50.00	-41.38
70	68.04	9.43	-27.27	-62.50	-44.83
130	71.13	-7.55	-45.45	-75.00	-65.52
190	70.10	-15.09	-63.64	-81.25	-68.97
250	71.13	-28.30	-63.64	-93.75	-68.97
1690	52.58	-45.28	-72.73	-	-68.97
3130	44.33	-50.94	-72.73	-	-72.41

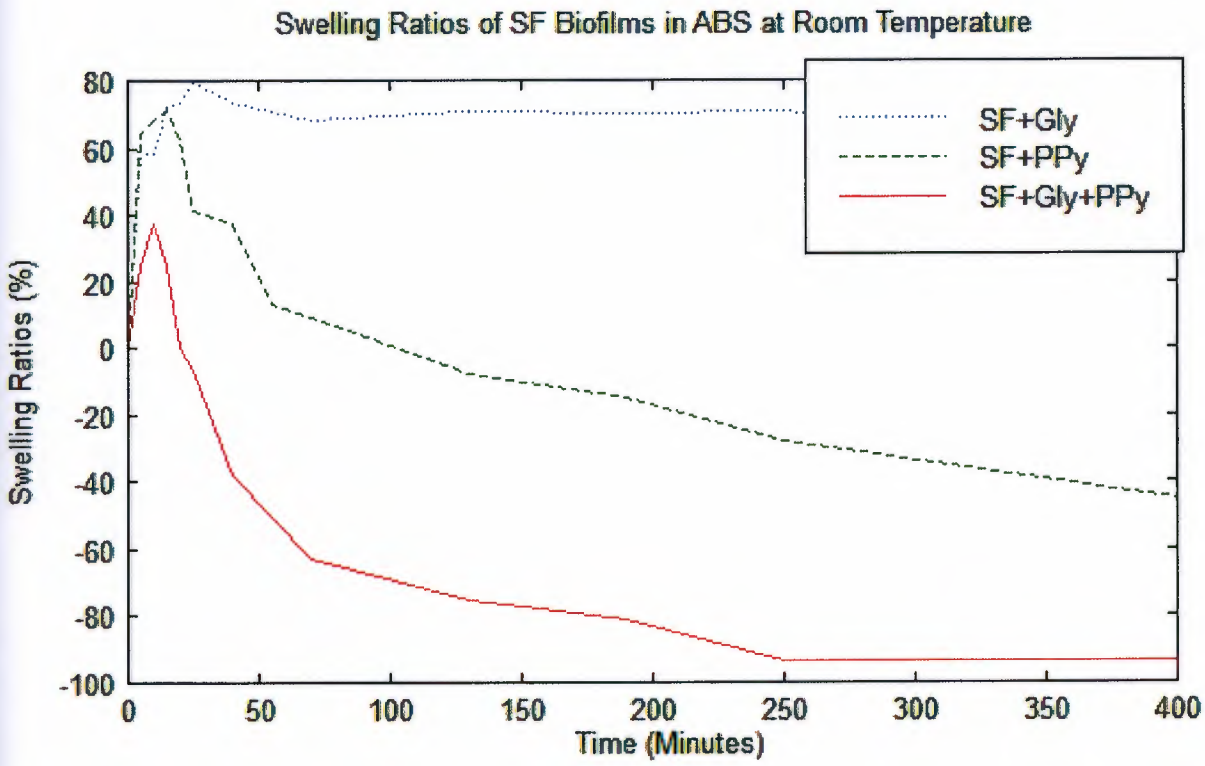

Figure 3.10: Swelling Ratios of SF+Gly, SF+PPy and SF+Gly+PPy biofilms in ABS solution at room temperature

The swelling ratios on Figure 3.10 shows that pure silk fibroin and glycerine biofilm swelled during the test and it nearly showed the same effect when it was tested in PBS test. SF+PPy biofilm swelled a little at the beginning of the test and then, it biodegraded slowly. However, it did not biodegrade in PBS test. Therefore, it is seen that, this biofilm does not have a resistance against biodegration in acidic mediums. Similar to this, SF+Gly+PPy biofilm swelled a little but it biodegraded faster than SF+PPy because of the effect of glycerine and acidic medium.

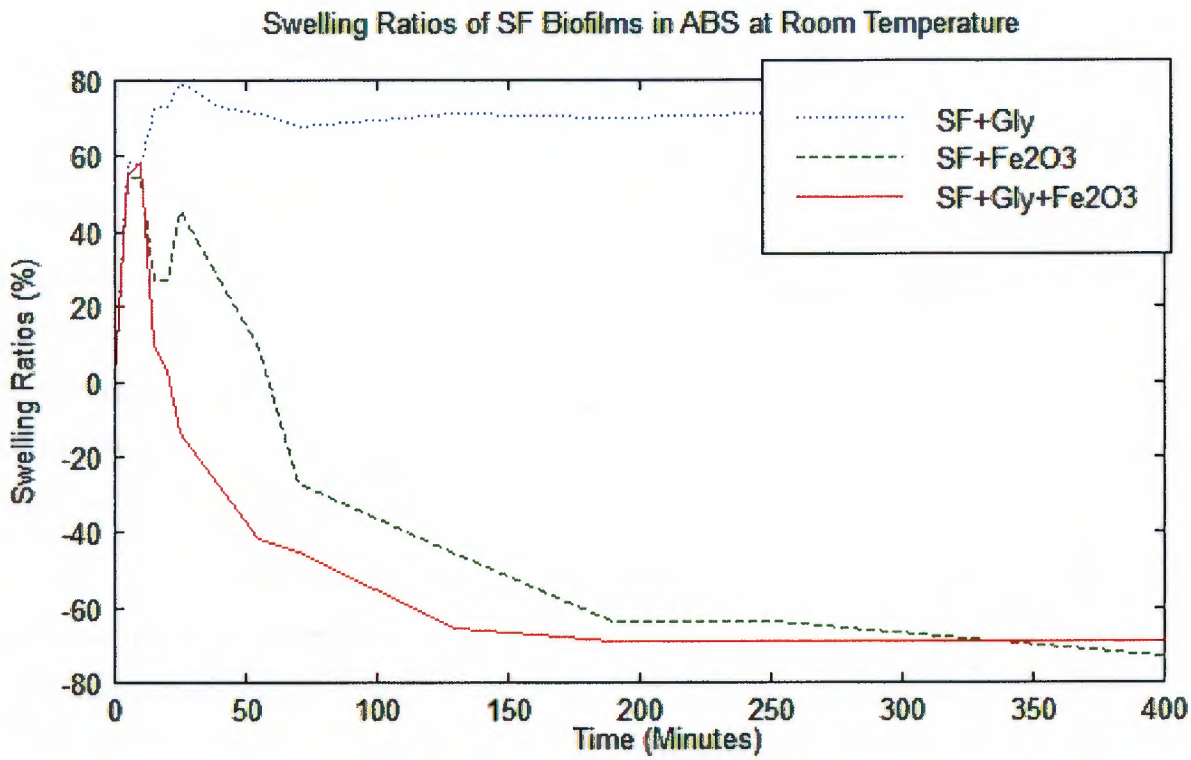

Figure 3.11: Swelling Ratios of SF+Gly, SF+Fe_2O_3 and SF+Gly+ Fe_2O_3 biofilms in ABS solution at room temperature

According to the results on Figure 3.11, it can be said that SF+Gly biofilm showed the same effect in both PBS and ABS tests. SF+Fe_2O_3 swelled a little at the beginning but it biodegraded faster in ABS solution than PBS solution. Iron (III) oxide made the biofilm biodegradable and acidic medium triggered the level of biodegradation.

Similar to this, SF+Gly+Fe_2O_3 biofilm was also swelled a little and biodegraded faster than SF+Fe_2O_3 because of the effect of glycerine and acidic medium that increases the level of biodegration.

CHAPTER 4

CONCLUSIONS

In this thesis it was aimed to prepare a silk fibroin biofilm which is highly biocompatible, good conducting and elastic in order to use it with an implantable electrode or in tissue engineering or in any kind of biomedical device. Therefore, in order to form such a biofilm polypyrrole and Iron (III) oxide were used to increase the conductivity and glycerine was used to increase the elasticity of the biofilm.

During the experiments, pure silk fibroin solution was blended with glycerine, polypyrrole and Iron (III) oxide and different types of biofilms in different proportions were formed at room temperature and by using UV induced photopolymerization technique. However, biofilms made by using UV induced photopolymerization technique were not successful because of the affects of UV to chemical structures of polypyrrole, Iron (III) oxide and glycerine blended silk fibroin biofilms. Therefore, all further tests were applied to the biofilms which were formed at room temperature.

The electrical conductivity test showed that silk fibroin and polypyrrole blended biofilm had the highest electrical conductivity. However, this biofilm was very brittle and it made it difficult in order to be used as a biomaterial in wide biomedical application fields. Therefore, the best obtained silk fibroin biofilm which had high electrical conductivity and elasticity was the combination of silk fibroin blended with glycerine and polypyrrole.

The swelling tests of silk fibroin biofilms both in PBS and ABS solutions showed that, glycerine made the biofilms biodegrable and similar to this iron (III) oxide was also made the biofilms biodegradable. In ABS solution, SF+PPy, SF+Gly+PPy, SF+Fe_2O_3 and SF+Gly+Fe_2O_3 biofilms biodegraded more and faster than in PBS solution. Therefore, it can be said that, acidic mediums also increase the level of biodegration of the biofilms comparing to the effect of glycerine and iron (III) oxide.

SEM micrographs showed that the morphology of SF / Gly / PPy blended biofilm was smoother than the SF / Gly biofilm and it has hishest crystallinity. Further characteriziation has been applied by using FTIR spectrum. It was concluded that, when pure silk fibroin was blended with glycerine and polypyrrole, it did not affect the chemical structure of silk fibroin.

As a conclusion, SF / Gly / PPy biofilms are good candidates for preparing elastic and electrically conductive biofilms for biomedical device design and applications.

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