CHARACTERIZATION, SOLUBILITY, HEMOCOMPATIBILITY TESTS OF POLYETHYLENE GLYCOL DIMETHACRYLATE COATED SILK FIBERS

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY

By
FATMA ZOR

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Biomedical Engineering

NICOSIA, 2017

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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 materials and results that are not original to this work.

Name, Last name: FATMA ZOR

Signature:

Date:

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To my family…

ABSTRACT

The aim of this study is to test silk fibers' and Polyethylene-glycol dimethacrylate's (PEGDMA) hemocompatibility, to test their anti-coagulant property and capability of PEGDMA to coat silk fibers. Also to check if silk fiber had more success on blood for anti-coagulation alone or after coated with PEGDMA. The coagulation of blood on biomaterials is very dangerous and it can cause serious illnesses, even death, so the effect of uncoated and PEGDMA coated silk fibers' hemocompatibility and blood coagulation have been tested in this study. Characterization of silk fiber coated with PEGDMA have been analyzed by inverted light microscope, XRD, FTIR and SEM analysis.

Hemocompatibility tests for uncoated and coated silk fibers have been done with 3 different lengths of silk fibers to see the effect of silk fiber and PEGDMA on the human blood. The aim was to test when the length of silk fiber has increased, the anti-coagulation effect will increase or not. Hemocompatibility tests indicated that silk fibers have anti-coagulant property on blood but after coating with PEGDMA, they were more effective. APTT, INR and PT levels have been tested to see the anti-coagulant effect.

For the success of coating procedure, the diameters of PEGDMA coated and uncoated silk fibers have been measured by inverted light microscope and results showed that coating process with dipping technique has been done successfully. The swelling and solubility of PEGDMA coated and uncoated silk fibers have been tested.

Keywords: Silk Fiber; Polyethylene Glycol Dimethacrylate; Coating; Anti-coagulation; Hemocompatibility

ÖZET

Bu çalışmanın amacı ipek liflerinin ve polietilen glikol dimetakrilatın (PEGDMA) kan uyumluluğunu, kan pıhtılaşmasını önleyici özelliklerini ve PEGDMA'nın ipek liflerini kaplama kabiliyetini test etmektir. Ayrıca kan pıhtılaşması önleyici özelliğini ipek lifleri tek başına mı yoksa PEGDMA ile kaplandığı zaman mı daha etkili olduğunu test etmektir. Biyomateryallerde kan pıhtılaşması çok tehlikelidir ve ciddi rahatsızlıklara hatta ölüme sebep olabilir, dolayısıyle PEGDMA ile kaplanmış ve kaplanmamış ipek liflerinin kan uyumluluğu ve pıhtılaşma önleyici özelliği üzerindeki etkileri test edilmiştir. PEGDMA ile kaplanmış ipek liflerinin tanımlanması ters elektron mikroskopu, XRD, FTIR ve SEM analizi ile yapılmıştır.

İpek fiberi ve PEGDMA'nın kan üzerindeki etkisini görebilmek için kaplanmamış ve kaplanmış ipek fiberlerinin 3 farklı uzunluğu ile kan uyumluluk testi yapılmıştır. Amaç ipek fiberinin uzunluğu arttığı zaman kan pıhtılaşma önleyici özelliğininde artıp artmadığını test etmektir. Kan uyumluluğu testi sonuçları ipek fiberlerinin pıhtılaşmayı önleyici özelliğinin olduğunu fakat PEGDMA ile kaplandığı zaman daha etkili olduğunu göstermiştir. Pıhtılaşmayı önleyici özelliğinin etkisini görebilmek için APTT, INR ve PT değerleri ölçülmüştür.

Kaplama yönteminin başarılı olup olmadığını görebilmek için kaplanmış ve kaplanmamış ipek fiberlerinin çapları ters elektron mikroskopuyla ölçüldü ve bu yöntemin başarılı olduğu görüldü. Kaplanmış ve kaplanmamış fiberlere şişme ve çözünürlük testi yapılmıştır.

Anahtar Kelimeler: İpek Lifi; Polietilen Glikol Dimetakrilat; Kaplama; Pıhtılaşma Önleyici; Kan Uyumluluğu

TABLE OF CONTENTS

CHAPTER THREE: RESULTS AND DISCUSSIONS

CHAPTER FOUR: CONCLUSION

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SF:	Silk Fiber
PEGDMA:	Polyethylene Glycol Dimethacrylate
B.mori:	Bombyx Mori
APTT:	Activated Partial Thromboplastin Time
PT:	Prothrombin time
INR:	International Normalized Ratio
RCF:	Relative centrifugal force
HCl:	Hydrochloric acid
NaOH:	Sodium Hydroxide
PBS:	Phosphate buffered saline
SW:	Swell
PD :	Partially Degraded
DG:	Degraded
NC:	No change
BF:	Bubble formation
SFL:	Started to float
FL:	Floating
XRD:	X-Ray Diffraction
SEM:	Scanning Electron Microscopy
FTIR:	Fourier Transform Infrared Spectroscopy

CHAPTER 1

INTRODUCTION

1.1 General Introduction

A biomaterial is a substance which is engineered into a form, alone or as a part of a complex system, to interact with a desired biological system. These biomaterials are often fabricated or designed into medical devices to help in tissue regeneration, to augment the function of a tissue and, in some cases, to even replace the tissue. They are also used in the development of biomolecule delivery. Cells attach and migrate along the biomaterial platform. These biomaterials can be broadly classified into natural and synthetic biomaterials. Silk fibers are also used as biomaterials (Vepari and Kaplan, 2007). Silk fibers offer a wide range of properties such as biocompatibility, biodegradability, mechanically superior, inhibit cell attachment, cell proliferation and anticoagulant properties (Li et al., 2015).

Poly ethylene glycol dimethacrylate (PEGDMA) has been used for coating of silk fibers in this study. There have been different researches that PEGDMA has been used as coating material with combination of different polymers (Boodagh et al., 2016). The aim of using PEGDMA as a coating material was to test the blood compatibility and anti-coagulant property when coated on silk fiber and also to test the adhesion property and mechanical strength. In this study swelling and solubility experiments have been done with coated and uncoated silk fibers. SEM, XRD and FTIR analysis has been done in this study.

1.2 Information about Silk Fibroin and Silk Fiber

Silks are fibrous proteins which produced by spiders (e.g., *Nephila clavipes*) and silkworms (e.g., *Bombyx mori*) in the nature [Figure 1.1]. Silk fibroin is derived from silkworm which is an important protein and polymer in biomedical applications. Silk fibroin has plenty of properties such as; good mechanical properties, high strength, high biocompatibility, biodegradability, hemocompatibility and non-toxicity (Zhao et al., 2007). Silk fibroin is the most important renewable natural polymer.

Figure 1.1: Silkworms and silk cocoons (adapted from http://whyfiles.org/2010/)

Silk is a natural polypeptide composite material consisting of two major proteins, fibroin, and sericin. The raw silk has a structure of two parallel fibroin fibers linked by disulfide bonds and held together with successive sticky layers of sericin on their surfaces that help in the formation of a cocoon [Fig. 1.2]. Fibroin has been used in textile manufacturing and for several biomaterial applications, whereas sericin is considered a waste material in the textile industry (Li et al., 2015).

Figure 1.2: Silk Cocoon (adapted from http://fiber429.rssing.com/chan-8389728/all_p2.html)

Silk Fibroin has several different forms such as silk sutures and silk fibroin biomaterials as well as its fiber [Fig. 1.3] and yarn form (Kancevicha and Lukyanchikovs, 2012). In this study, silk fiber form has been used. As a biomaterial, silk fiber plays a key role in the design and synthesis of nanocomposite materials with controllable loading with nanoparticles dispersed on the surface of the chains.

Figure1.3: Silk Fibers (adaptedfrom http://textilewiki.blogspot.com.cy/2015/11/ production-process-of-silk-fiber.html)

1.2.1 Different Applications of Silk

In one of the research, silk has been used in yarn form for the woven bio-resistant blood vessel prosthesis (Kancevicha and Lukyanchikovs, 2012). According to the research of Kancevicha and Lukyanchikovs, 2012, several characteristics of silk which include; biocompatibility, good flexibility, high strength in low thickness causes chosen of silk. It has special physical property and in surgeries it is very preferable. Also the implants which made of natural silk, helps in reducing the toxin production after implantation.

In another research, silk fibroin has been used as a treatment for the enhancement of corneal epithelial cell growth (Suzuki et al., 2015). According to Suzuki et al (2015), that research has been aimed for enhance the attachment and proliferation of corneal epithelial cells by increasing the permeability of silk fibroin membranes. The fibroin solution has been mixed with poly ethylene glycol (PEG). The elastic modulus has been increased in PEG-treated silk membranes than the non-treated ones. As a result, the cell attachment and proliferation has been influenced in a positive way after using these membranes.

In one of the research electrspun silk-based scaffolds has been used for bone tissue engineering which found as useful in the research (Li et al., 2006). Ni et al. (2008) showed that treatment for rabbits in tracheal defects by using silk-based stent was successfully applied.

1.3 Information about Polyethylene Glycol Dimethacrylate (PEGDMA)

Polyethylene glycol is known as condensation polymers of water and ethylene oxide which the general chemical formula is $H(OCH_2CH_2)_nOH$.

PEGDMA has different properties such as protein resistance, low toxicity, immunogenicity, and good solubility in water and organic solvents (Cheng et al., 2014). PEGDMA also has blood coagulation property and when tested in blood, the APTT levels increased (Zheng et al., 2016). But a polymer alone cannot be very effective and when combining different biocompatible materials with each other, it showed that the combination were more effective than the material itself (Pacelli et al., 2014).

In this study PEGDMA used as a coat material on silk fiber, the combination and the silk fiber alone has been tested for blood compatibility in Coagulation Analyzer.

1.4 Blood Compatibility and Coagulation

Biocompatibility of devices and materials which contacts with blood, relates mainly to the thrombotic response which induced by the materials. Biocompatibility plays a key role to decide the application of implantable biomaterials, for example, orthopedic and artificial blood vessel implants (Elahi et al., 2014).

The APTT (Activated Partial Thromboplastin Time), measures the activity of coagulation from the natural and common pathways. The division of the clotting cascade into the natural, external and common pathways has little *in vivo* validity but remains a useful concept. APTT reflects duration in which the intrinsic pathway is activated over the contact with blood (Elahi et al., 2014).

Prothrombin time (PT) and INR (international normalized ratio) are blood clotting test which measures the clotting factors.

The levels of APTT, INR and PT can be measured by Coagulation Analyzer which uses blood plasma.

1.5 Aims of this Study

Aims of this study are;

- Coating silk fibers with PEGDMA by using dipping technique,
- Testing uncoated and coated silk fibers for hemocompatibility and anti-coagulation ability
- Testing uncoated and coated silk fibers' swelling and solubility ability.

1.6 Limiting Factors of this Study

The numbers of blood samples were the limiting factors of this study for hemocompatibility tests. In hematology laboratory blood samples that used in this study, belong to patients which their samples have been used in several tests before the experimental work done. This means that the plasma of some of the patients have been taken from the sample and used. The plasma amount should be enough for the experimental work of this study, but most of the patients had small amount of plasma because of the tests done before the experiments. This is the reason why the number of blood sample were 5 or 6.

Silk fibers which have been measured under inverted light microscope should be cut into small pieces, so the coated fibers could be damaged because of the scissors during cut.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

In this study silk fibers were bought from Bursa-Turkey in fiber form. Human blood has been obtained from Near East University (NEU) Hospital and STA Compact Stago Coagulation Analyzer device has been used for the coagulation test in NEU Hospital’s hematology laboratory. Polyethylene glycol dimethacrylate (PEGDMA) has been purchased from Sigma-Aldrich and used for coating silk fibers. Near East University Pharmacy Department’s Tissue and Cell Culture Laboratory's Inverted Light Microscope has been used for viewing and measuring the diameters of coated and uncoated silk fibers. Pure water, Phosphate Buffered Saline (PBS) solution, Hydrochloric Acid (HCl) solution and Sodium Hydroxide (NaOH) solution have been taken and used in Near East University Faculty of Engineering’s Biomedical Laboratory. X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) analysis have been done by TÜBİTAK- Marmara Research Centre at Gebze, Istanbul, Turkey.

2.2 Coating Process of Silk Fibers with Dipping Technique

Silk Fibers which obtained from Electrospinning Technique were coated with Polyethylene Glycol Dimethacrylate (PEGDMA) with Dipping Technique [Figure 2.1].

Before starting the coating procedure, silk fibers have been sterilized by Near East University Hospital’s Sterilization Department. Silk fibers’ diameters have been measured with the Inverted Light Microscope in NEU Pharmacy Faculty's Tissue and Cell Culture Laboratory to be able to see the difference of coated and uncoated silk fibers and understand if the coating procedure is successfully applied or not.

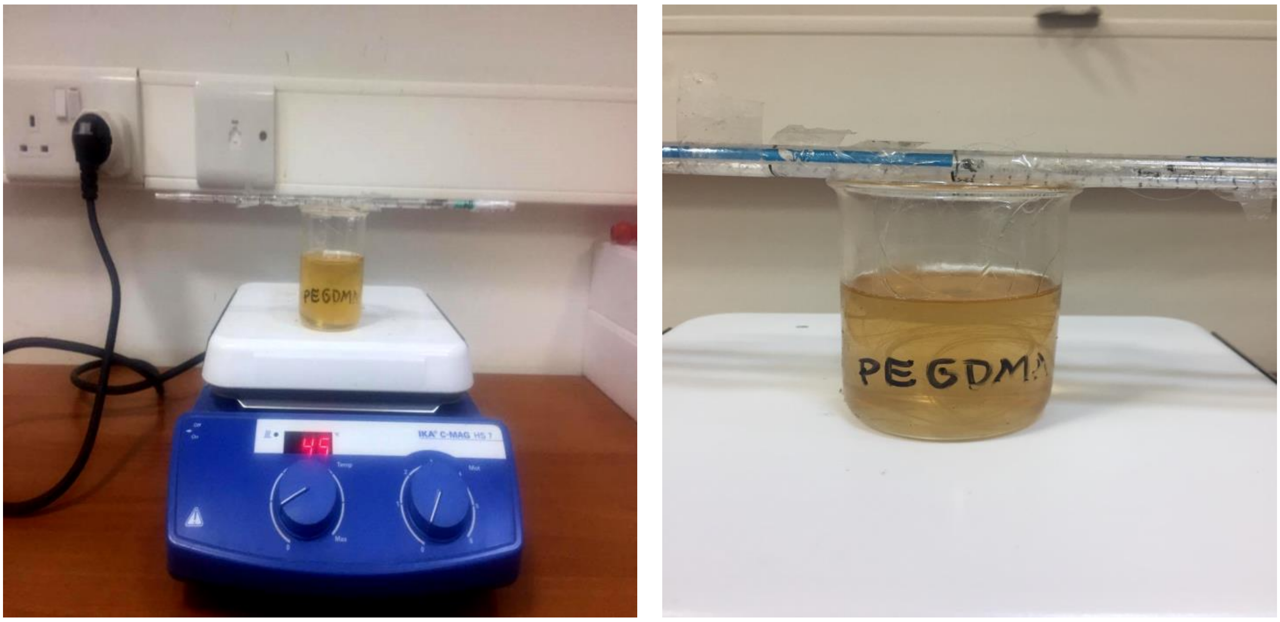


Figure 2.1: Silk fibers coating with PEGDMA by dipping technique in 45°C on hot plate.

The silk fibers which have been used were single fibers and 4 fibers which twisted together. This method has been used to see the effects and differences between single silk fibers and 4 twisted silk fibers. The coating procedure has been applied to both the single and 4 twisted silk fibers.

Firstly, PEGDMA have been poured in 100 ml beaker as 70 ml. Silk fibers were attached on pipettes and silk fibers have been dipped in the PEGDMA solution [Figure 2.1].

This technique has been done in two different methods;

In the first method, the silk fibers have been kept for 30 minutes that have been dipped in PEGDMA without heat. After 30 minutes, silk fibers have been taken out to let them to dry in room temperature. After drying, the same dipping procedure has been repeated. After 5 times of dipping procedure, some of the coated silk fiber samples have been brought to Pharmacy Faculty's Tissue and Cell Culture Laboratory and measure the diameters of coated silk fibers if the coating procedure has been done successfully or not. When the uncoated and coated silk fibers were compared, it can be assumed that the coating procedure was not successfully made. The reason was, there was not much difference in diameters between uncoated and coated silk fibers. To be able to tell the procedure is successful, coated silk fibers' diameters should be higher than the uncoated silk fibers. The first procedure was not successful so the procedure has been changed.

In this second method, rather than dipping the silk fibers in PEGDMA solution and leaving them for 30 minutes, the fibers have been dipped to PEGDMA for 2 hours and placed the dipped silk fibers' beaker on the hot plate which was 65℃. After 2 hours of dipping, the silk fibers have been taken out of the PEGDMA solution and dry them with blow dry without heat and from a distance not to harm silk fibers.

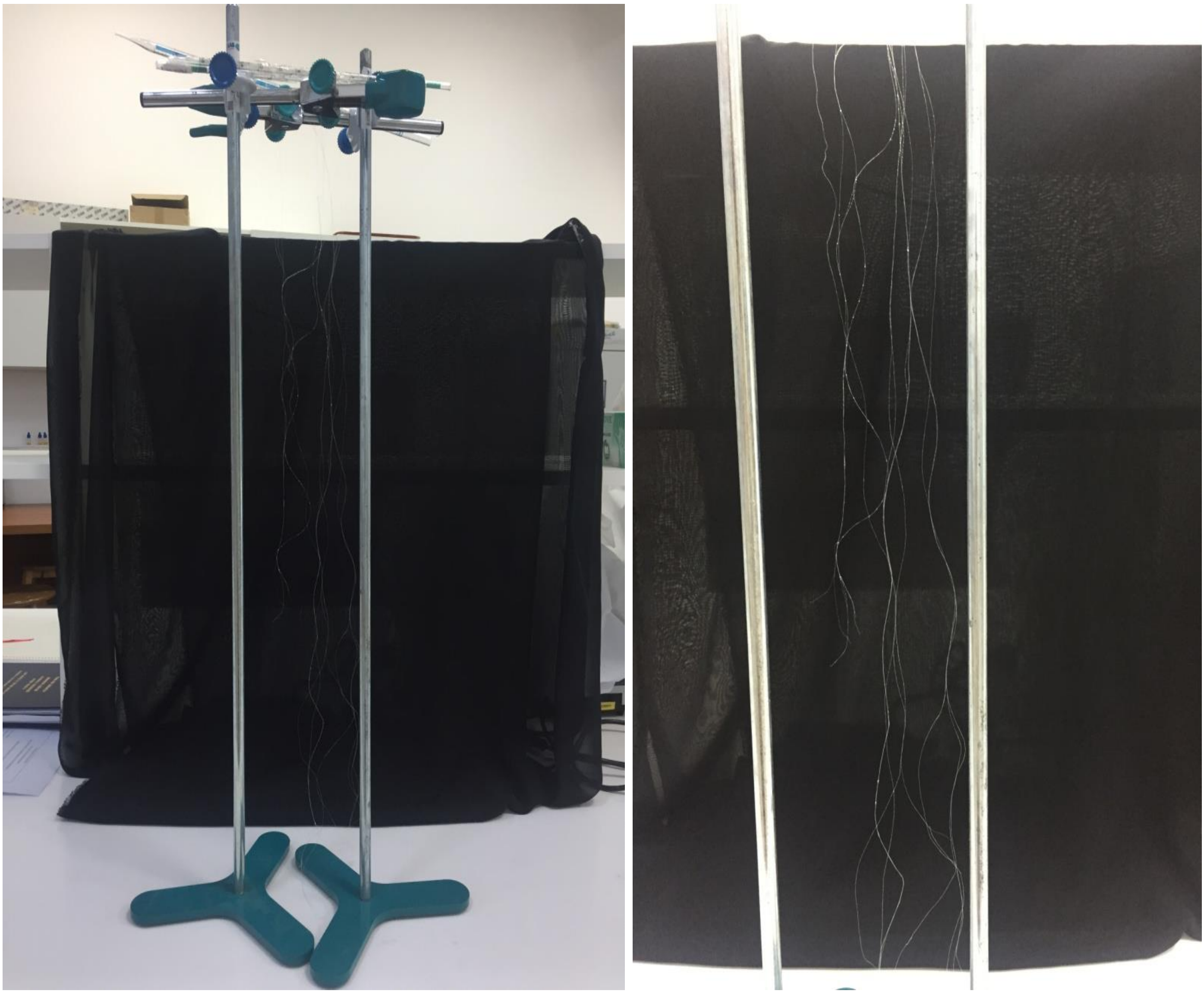


Figure 2.2: Drying of PEGDMA coated silk fibers

After applying this procedure several times it was observed that, because of the heat, the silk fibers started to degrade, so the temperature has been changed from 65℃ to 45℃. The same procedure has been repeated for 10 times and samples' diameters have been measured by Pharmacy Faculty's Laboratory's Inverted Light Microscope. When the diameters of second coated and uncoated silk fibers compared with each other, we can state that the coating procedure was done successfully.

2.3 Measurement of Uncoated Silk Fibers

2.3.1 Measurement of Uncoated Single Silk Fibers

Diameters of silk fibers were measured by Near East University Pharmacy Faculty’s Cell and Tissue Culture Laboratory’s Inverted Light Microscope. The aim of the measurement before starting the procedure is that to see if coating procedure will be done successfully or not. If diameters increase, it can be assumed that the coating of silk fibers is successful. If it is not increasing, it means that more coating should be done to make sure the procedure has been made successfully.

Uncoated single silk fibers have been viewed by Inverted Light Microscope [Figure 2.3].

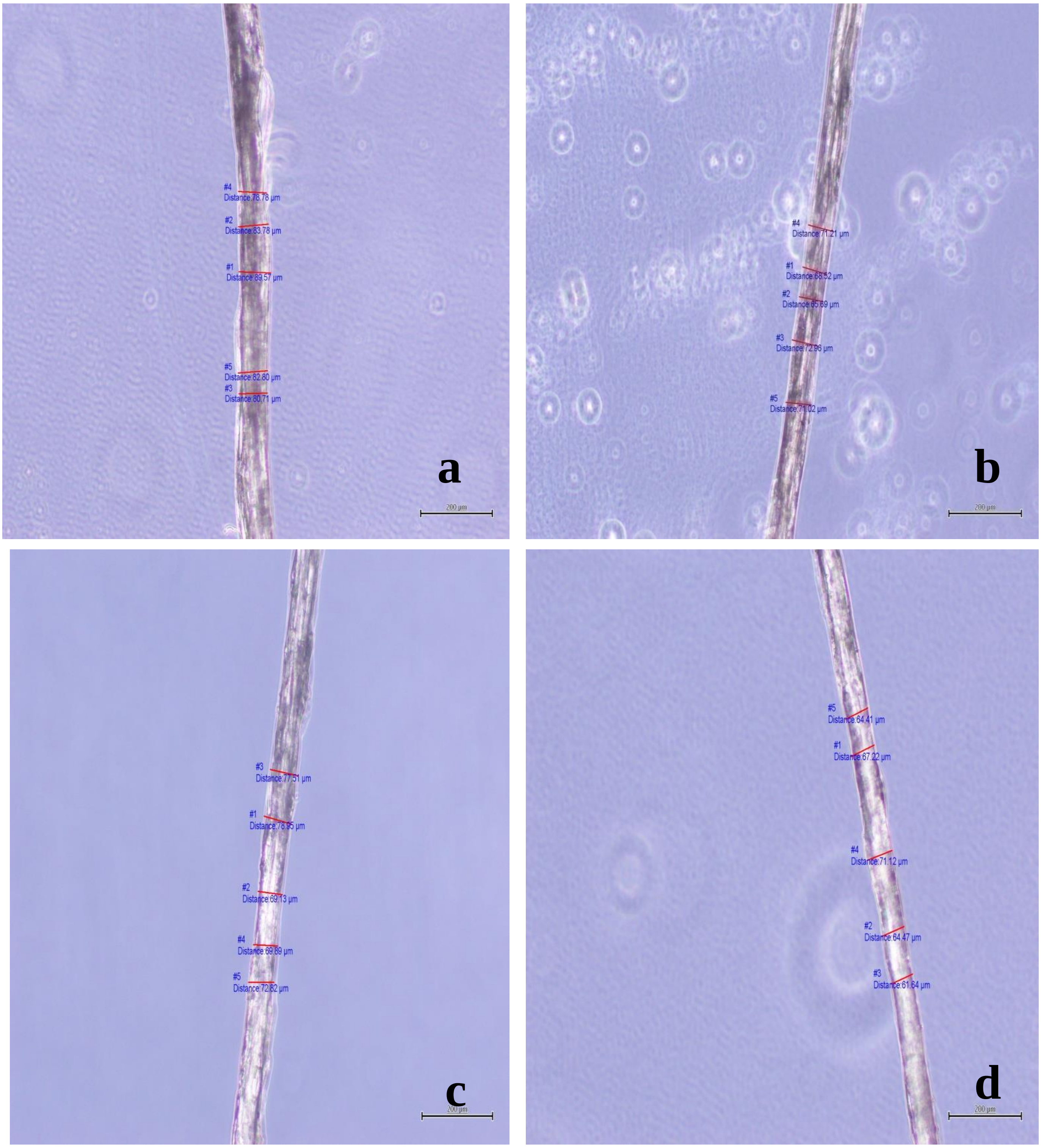


Figure 2.3: Uncoated single silk fiber view under Inverted Light Microscope with different silk fibers' diameter measurements.

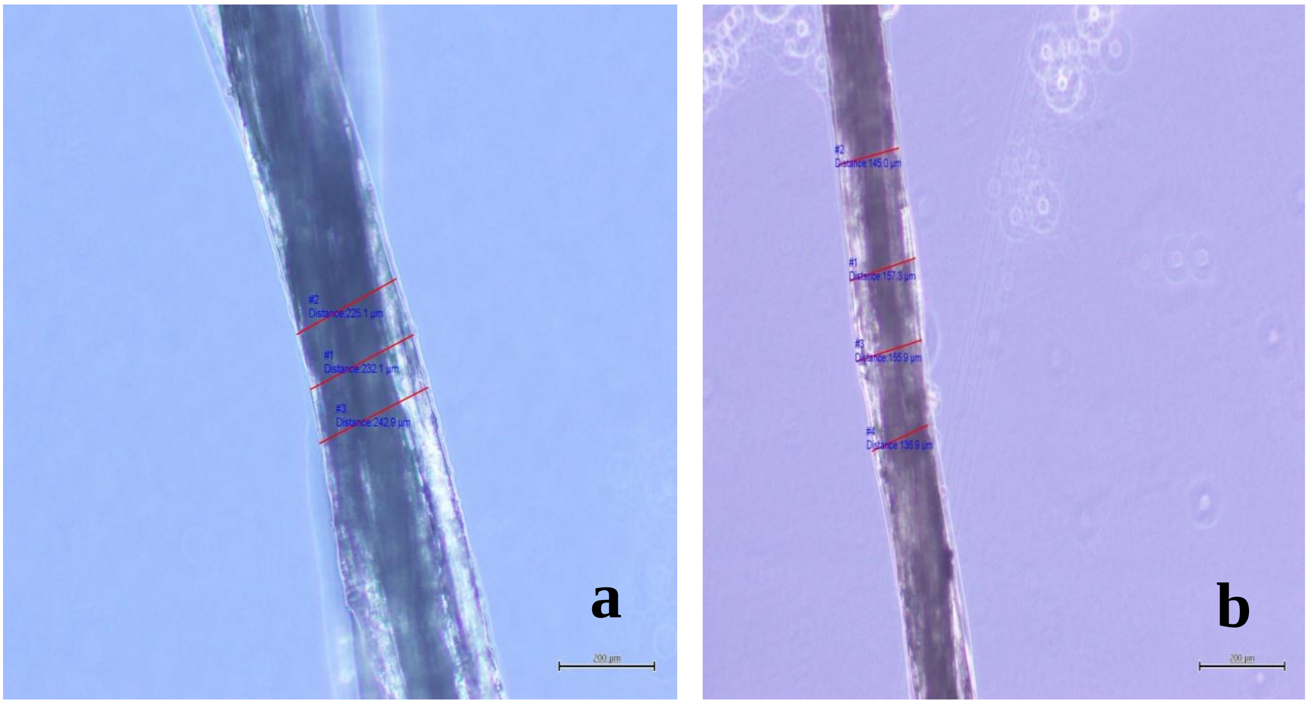
As shown in Figure 2.3 after viewing the silk fibers under the inverted light microscope, 5 different places on each of the silk fiber that has been cut from different part have been measured.

The diameters of silk fiber in Figure 2.3a is; 78.78 µm, 83.78 µm, 89.57 µm, 82.80 µm and 80.71 µm. The average diameter for the silk fiber in Figure 2.3a is 83.13µm. In Figure 2.3b diameters are; 71.21 µm, 68.52 µm, 65.69µm, 72.96 µm and 71.02 µm. The average diameter for the silk fiber in Figure 2.3b is 69.88 µm. In Figure 2.3c diameters are; 77.51µm, 78.95µm, 69.13µm, 69.89µm and 72.82µm. The average diameter for the silk fiber in Figure 2.3c is 73.66 µm. In Figure 2.3d diameters are; 64.41µm, 67.22 µm, 71.12µm, 64.47 µm and 61.64 µm. The average diameter for the silk fiber in Figure 2.3d is 65.77 µm.

The total average for all of the silk fibers which have been measured is 73.11 µm. So by these measurements, it can be assumed that the uncoated single silk fiber's diameter is approximately 73.11 µm.

2.3.2 Measurement of Uncoated 4-twisted Silk Fibers

4 silk fibers have been taken and twisted tightly with each other and then measured under Inverted Light Microscope [Figure 2.4].



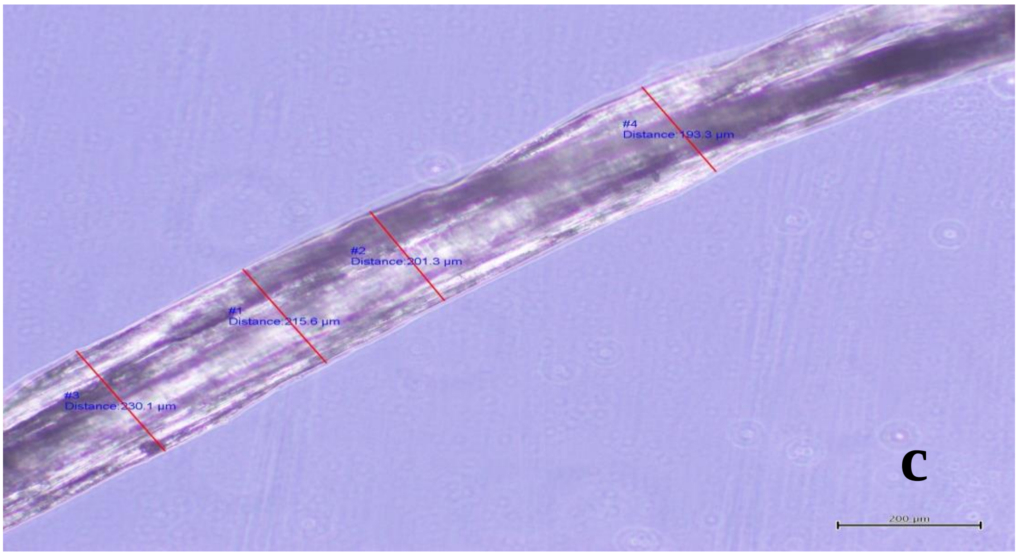


Figure 2.4: 4 twisted uncoated SF view under inverted light microscope and diameters.

In Figure 2.4a; diameters are shown as 242.9 µm, 232.1 µm and 225.1 µm. The average of diameters for this 4 twisted silk fiber is 233.37 µm. In Figure 2.4b; diameters are shown as 145.0 µm, 157.3 µm, 155.9 µm and 139.9 µm. The average diameter for the silk fiber in Figure 2.4b is 149.53 µm. In Figure 2.4c; diameters are shown as 193.3 µm, 201.3 µm, 215.6 µm and 230.1 µm. The average diameter for the silk fiber in Figure 2.4c is 210.08 µm.

The total average for these uncoated 4 twisted different silk fibers is 197.66 µm. So it can be assumed that the diameter of 4 twisted silk fibers before coating procedure is approximately 197.66 µm.

2.4 Measurement of PEGDMA Coated Silk Fibers

2.4.1 Measurement of PEGDMA Coated Single Silk Fibers

After finishing the coating process (Chapter 2.2), coated silk fibers have been measured under inverted light microscope to see the differences after coating. The measurements have been done after 5 times coating, 10 times coating and 15 times coating. The aim of measuring after several coatings and then continuing to coat was that to see the improvement between each coating. If the procedure is successful, after each coating, the diameters of silk fibers should be increased.

1st Coating of Single Silk Fibers

Single SF diameters have been measured after 5 times PEGDMA coating [Figure 2.5].

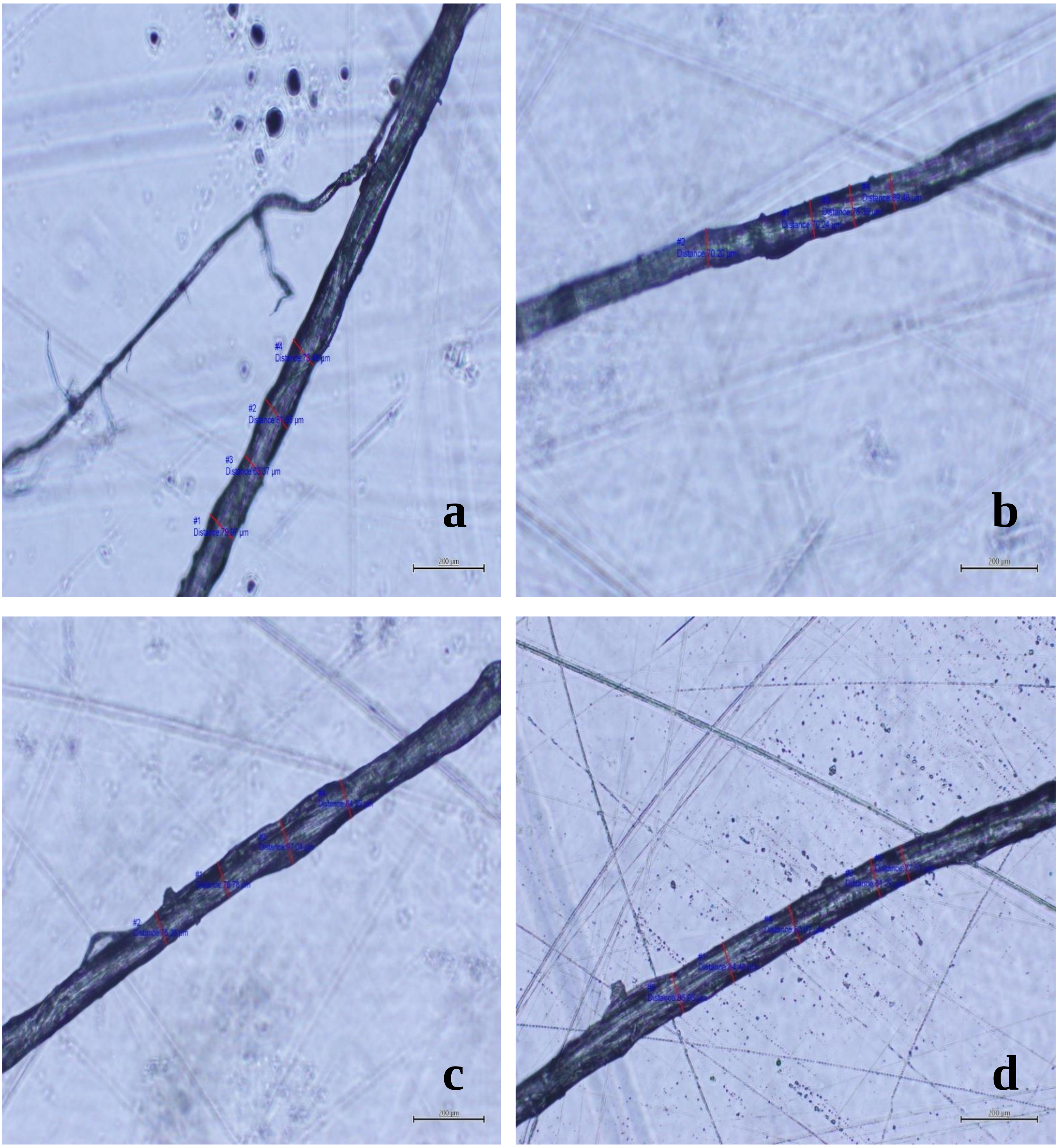


Figure 2.5: Single silk fiber view under inverted light microscope after 5 times coating with PEGDMA and diameter measurements.

In this first 5 times coating, the first method has been used which explained in Chapter 2.2.

In Figure 2.5a; diameters are shown as 73.46µm, 81.65µm, 63.37µm and 79.97µm. The average diameter for the silk fiber in Figure 2.5a is 74.61µm. In Figure 2.5b; diameters are shown as 69.48µm, 78.35µm, 70.24µm and 70.20µm. The average diameter for the silk fiber in Figure 2.5b is 72.07µm. In Figure 2.5c; the diameters are shown as 84.25µm, 97.04µm, 78.78µm and 75.26µm. The average diameter for the silk fiber in Figure 2.5c is 83.83µm. In Figure 2.5d; the diameters are shown as 77.51µm, 81.22µm, 83.87µm, 84.42µm and 86.98µm. The average diameter for the silk fiber in Figure 2.5d is 82.7µm.

The total average for these single silk fibers, which have been coated with 5 times dipping in PEGDMA, is 78.30µm. So it can be assumed that the average diameter of single silk fibers after 5 times coating with PEGDMA is approximately 78.30µm.

2nd Coating of Single Silk Fibers

After measuring 5 times coated single silk fibers' diameters with Inverted Light Microscope, coated single silk fibers have been continued coating with PEGDMA by changing the method. After 5 times coating, there was not enough difference between uncoated and 5 times coated single silk fibers, so the method has been changed. The second method has been used in this 2nd coating procedure which explained in Chapter 2.2.

In this 2nd coating, single silk fibers have been coated 10 times, (dipped in PEGDMA for 2 hours and then blow dry without heat to dry) their diameters have been measured by Inverted Light Microscope.

10 times coated single silk fibers have been viewed under Inverted Light Microscope [Figure 2.6].

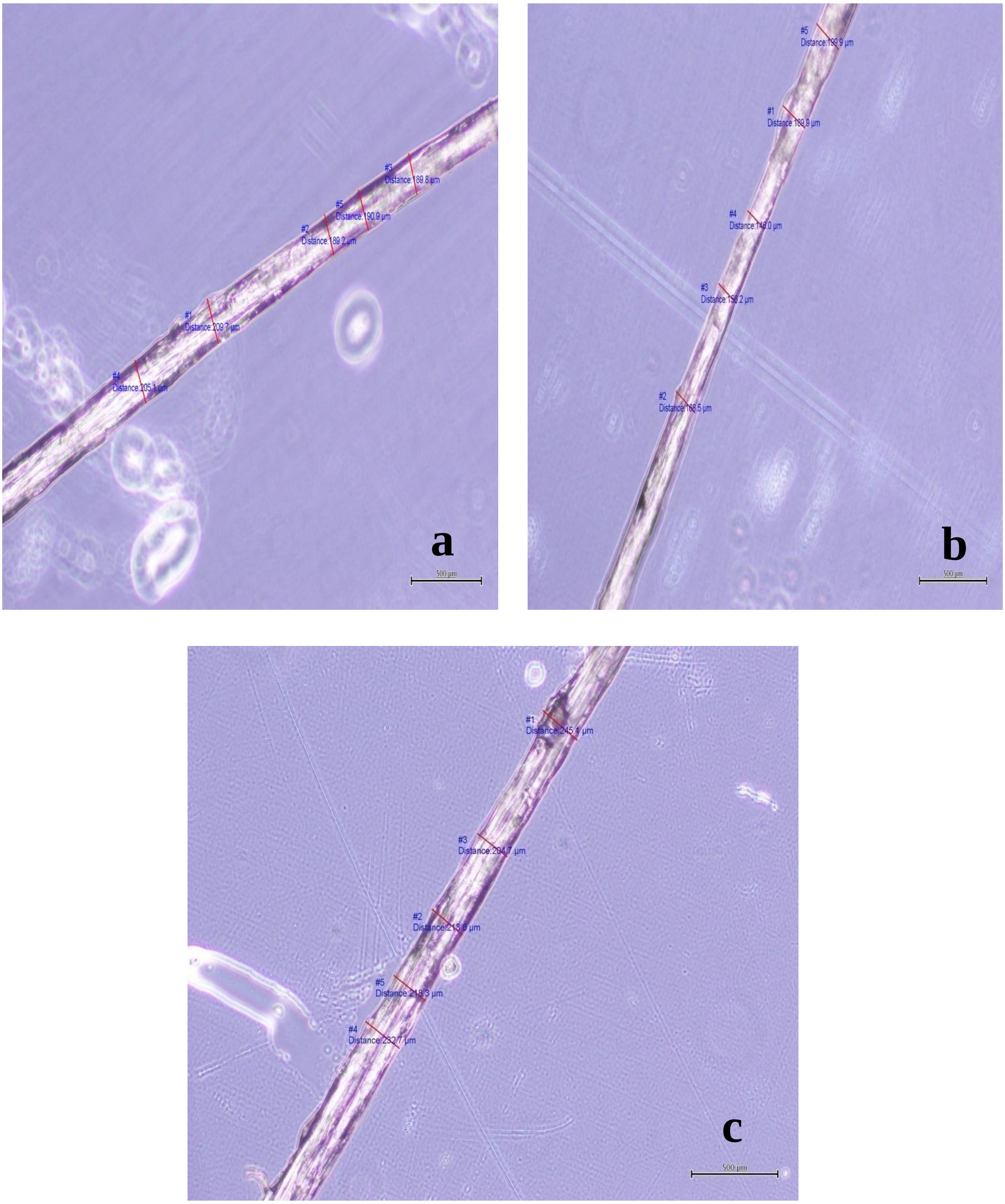


Figure 2.6: Single silk fiber view under inverted light microscope after 10 times coating with PEGDMA and diameter measurements.

Silk fibers diameters after 10 times coating with PEGDMA have been measured.

In Figure 2.6a; diameters are shown as 189.8µm, 190.9µm, 189.2µm, 209.7µm and 205.1µm . The average diameter for the silk fiber in Figure 2.6a is 196.94µm. In Figure 2.6b; diameters are shown as 199.9µm, 189.9µm, 146.0µm, 156.2µm and 168.5µm. The average diameter for the silk fiber in Figure 2.6b is 172.1µm. In Figure 2.6c; the diameters are shown as 245.1µm, 204.7µm, 213.6µm, 218.3µm and 232.7µm. The average diameter for the silk fiber in Figure 2.6c is 222.88µm.

The total average for these single silk fibers, which have been coated with 10 times dipping in PEGDMA, is 197.31µm. So it can be assumed that the average diameter of single silk fibers after 10 times coating with PEGDMA is approximately 197.31µm.

3rd Coating of Single Silk Fibers

After obtaining the results from 10 times coated single silk fibers, coated single silk fibers have been continued coating with PEGDMA by the second method. After obtaining the results of 10 times coated fibers, the difference between 5 times coated and 10 times coated single silk fibers have been noticed.

In the first method, the procedure was not successful, but in this second method the improvement could be easily noticed. The second method has been used in this 3rd coating procedure which explained in Chapter 2.2.

For 3rd coating, silk fibers have been coated for 15 times with the second method and the fibers have been viewed under Inverted Light Microscope to measure their diameters [Figure 2.7].

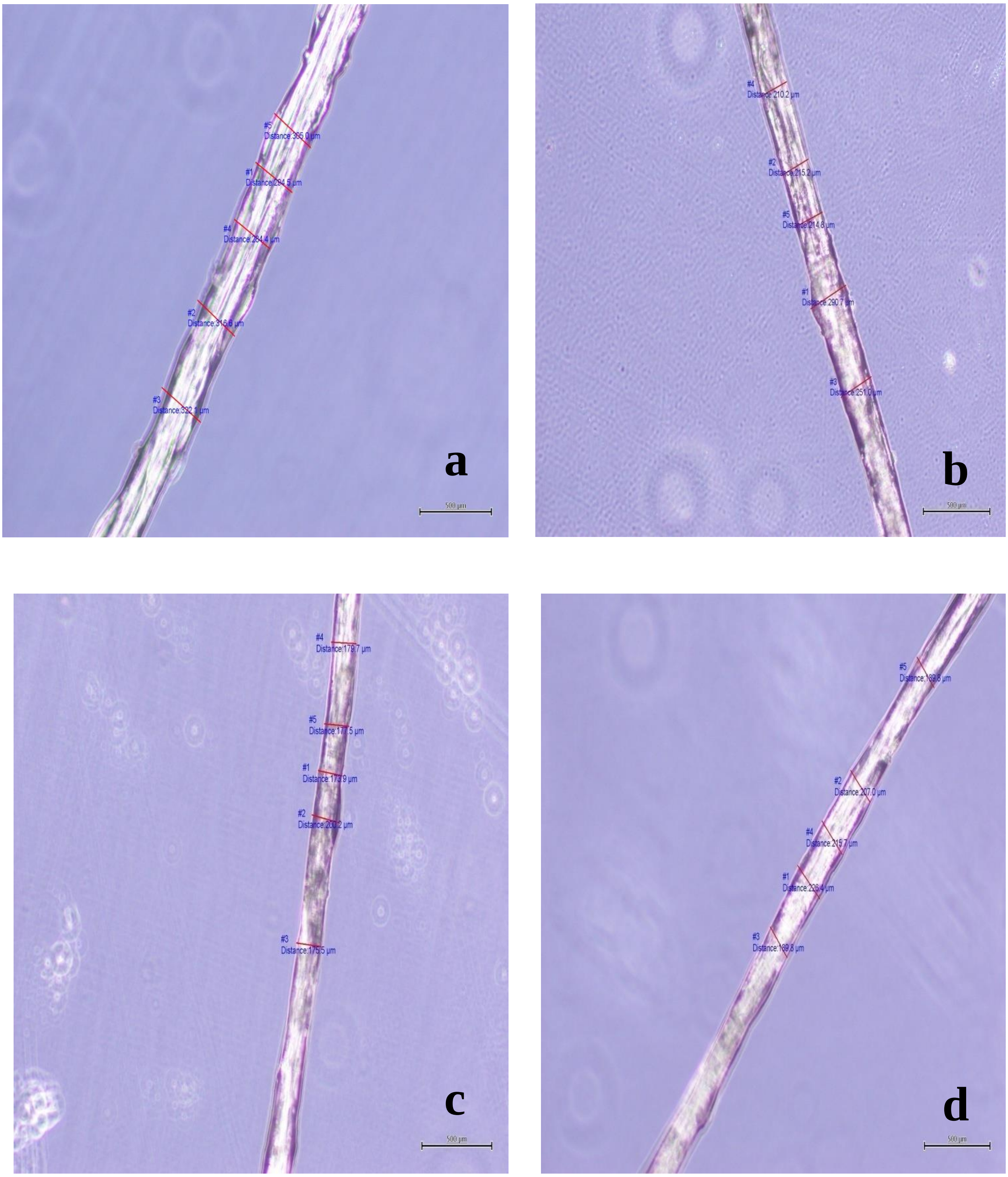


Figure 2.7: Single silk fiber view under inverted light microscope after 15 times coating with PEGDMA and diameter measurements.

Silk fibers diameters after 15 times coating with PEGDMA have been measured.

In Figure 2.7a; diameters are shown as 305.0µm, 294.5µm, 284.4µm, 316.6µm and 322.1µm. The average diameter for the silk fiber in Figure 2.7a is 304.52µm. In Figure 2.7b; diameters are shown as 210.2 µm, 215.2µm, 214.8µm, 290.7µm and 251.0µm. The average diameter for the silk fiber in Figure 2.7b is 236.38µm. In Figure 2.7c; the diameters are shown as 179.7µm, 177.5µm, 173.9µm, 200.2µm and 175.5µm. The average diameter for the silk fiber in Figure 2.7c is 181.36 µm. In Figure 2.7d; the diameters are shown as 189.8µm, 207.0µm, 215.7µm, 226.4µm and 189.8µm. The average diameter for the silk fiber in Figure 2.7d is 205.74µm.

The total average for these single silk fibers, which have been coated with 15 times dipping in PEGDMA, is 232.0µm. So it can be assumed that the average diameter of single silk fibers after 15 times coating with PEGDMA is approximately 232.0µm.

2.4.2 Measurement of PEGDMA Coated 4-twisted Silk Fibers

In Chapter 2.3.2, the method of 4 twisted silk fibers has been explained. After measuring diameters of 4 twisted uncoated silk fibers, the coating process has been started. 4 twisted coated silk fibers have been measured 2 times. First measurement has been done after 5 coating, and the second measurement has been done after 15 coating.

1st Coating of 4 Twisted Silk Fibers

The first 5 times coating has been done with the first method which had explained in Chapter 2.2. After 5 times coating, fibers have been viewed and their measurements have been done with the same Inverted Light Microscope.

In Figure 2.8, the diameters and view of coated 4-twisted silk fiber after 5 times coating has shown.

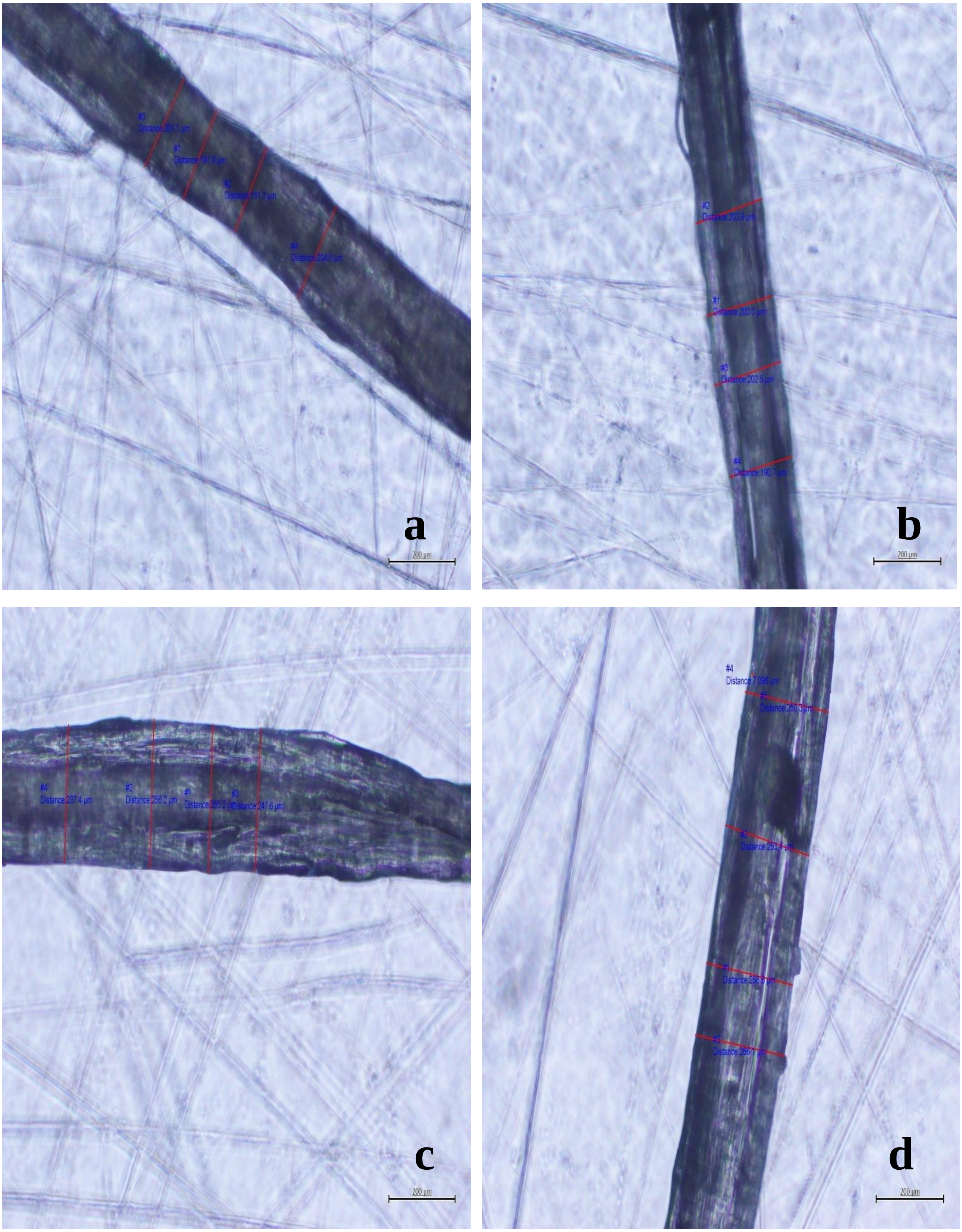


Figure 2.8: 4 twisted silk fiber view under inverted light microscope after 5 times coating with PEGDMA and diameter measurements.

4 twisted silk fibers' diameters, after 5 times coating with PEGDMA, have been measured.

In Figure 2.8a; diameters are shown as 201.1µm, 191.9µm, 181.3µm and 204.8µm. The average diameter for the silk fiber in Figure 2.8a 194.78µm. In Figure 2.8b; diameters are shown as 203.9 µm, 200.0µm, 202.5µm and 190.7µm. The average diameter for the silk fiber in Figure 2.8b is 199.28µm. In Figure 2.8c; diameters are shown as 237.4µm, 256.2µm, 255.2µm and 247.6µm. The average diameter for the silk fiber in Figure 2.8c is 249.1µm. In Figure 2.8d; diameters are shown as 250.3µm, 253.4µm, 258.6µm and 266.1µm. The average diameter for the silk fiber in Figure 2.8d is 257.1µm.

The total average for these 4 twisted silk fibers, which have been coated with 5 times dipping in PEGDMA, is 225.1µm. So it can be assumed that the average diameter of 4 twisted silk fibers after 5 times coating with PEGDMA is approximately 225.1µm.

2nd Coating of 4 Twisted Silk Fibers

After 5 times coating and measuring 4 twisted silk fibers diameters, the method of the process has been changed from the first method to the second method. The reason of this change was the method being unsuccessful.

For this second coating, the second method has been used which had explained in Chapter 2.2. The coating procedure has been applied 15 times to fibers. After 15 times of coating, 4 twisted fibers have been viewed and their diameters have been measured by the same Inverted Light Microscope [Figure 2.9].

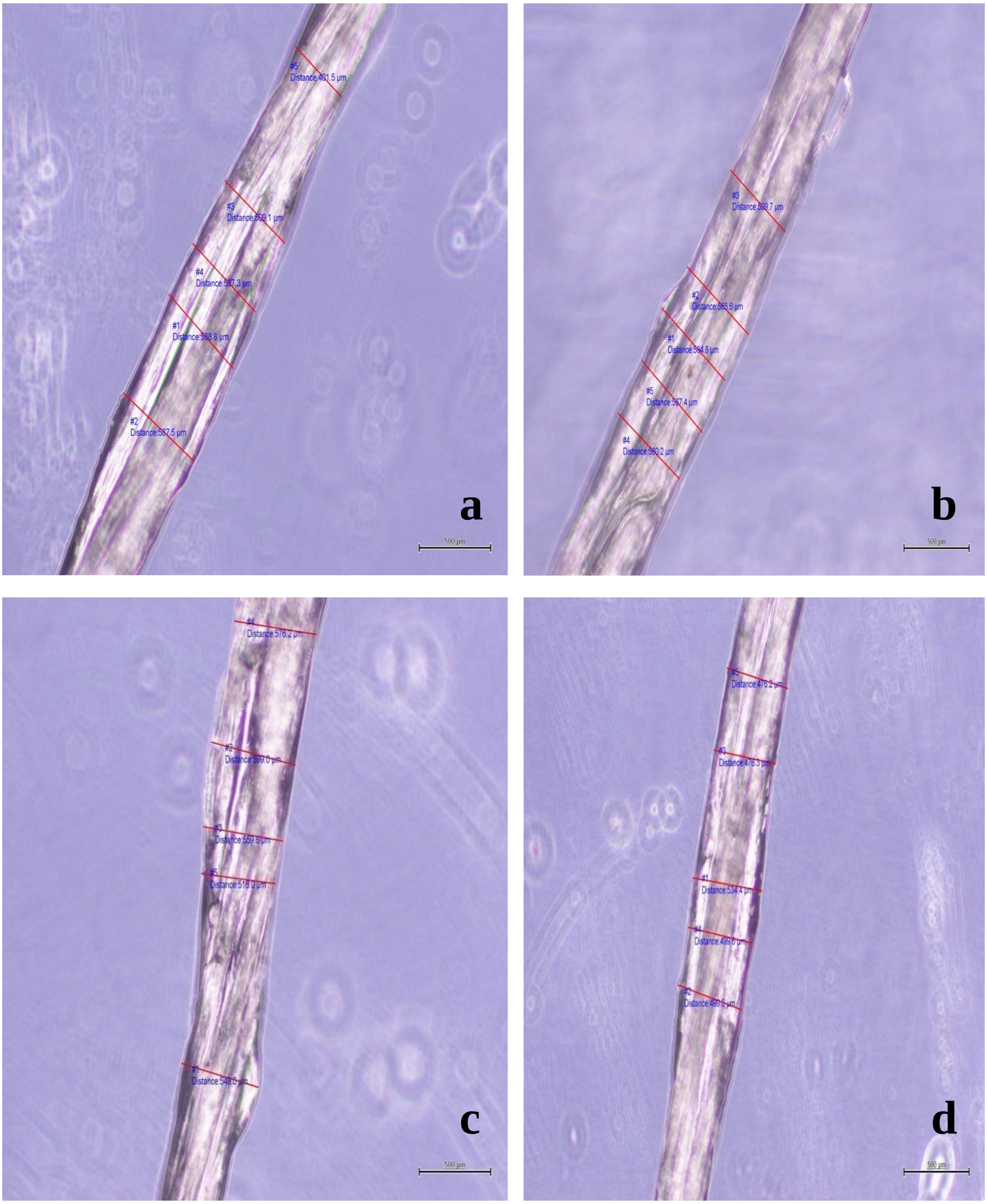


Figure 2.9: 4 twisted silk fiber view under inverted light microscope after 15 times coating with PEGDMA and diameter measurements.

4 twisted silk fibers' diameters, after 15 times coating with PEGDMA, have been measured.

In Figure 2.9a; diameters are shown as 401.5µm, 509.1µm, 537.3µm, 568.8µm and 587.5µm. The average diameter for the silk fiber in Figure 2.9a is 520.8µm. In Figure 2.9b; diameters are shown as 509.7µm, 565.6µm, 584.8µm, 567.4µm and 560.2µm. The average diameter for the silk fiber in Figure 2.9b is 557.5µm. In Figure 2.9c; diameters are shown as 576.2µm, 599.0µm, 559.6µm, 516.0µm and 549.0µm. The average diameter for the silk fiber in Figure 2.9c is 560.0µm. In Figure 2.9d; diameters are shown as 476.2µm, 476.3µm, 534.4µm, 499.6µm and 499.0µm. The average diameter for the silk fiber in Figure 2.9d is 497.1µm.

The total average for these 4 twisted silk fibers, which have been coated with 5 times dipping in PEGDMA, is 533.9µm. So it can be assumed that the average diameter of 4 twisted silk fibers after 15 times coating with PEGDMA is approximately 533.9µm.

2.5 Hemocompatibility Test for Coated and Uncoated Silk Fibers

In this study, one of the aims was to test if silk fibers play a crucial role in anticoagulation of blood and if it is compatible with blood and also to see the effects of silk fibers on the blood. By putting sterilized uncoated and coated silk fibers in different blood samples, the effects of silk fibers on blood samples of different patients were considered. Different lengths of silk fibers were used to see what the effects are when fibers are longer and shorter.

The hemocompatibility tests are done in two different forms of silk fibers. The first test has been done with uncoated silk fibers and the second test has been done with polyethylene glycol dimethacrylate (PEGDMA) coated silk fibers.

Hemocompatibility tests for uncoated and coated silk fibers have been done with 3 different lengths of silk fibers to see the effect of coated and uncoated silk fiber on the human blood. The aim was to see when we increase the length of silk fiber would it affect the anti-coagulation more than the shorter fiber or not. Also, to see the difference between the coated and uncoated silk fiber effect on blood. So; 10cm, 13cm and 15cm of coated and uncoated silk fibers have been used in the blood compatibility test.

The general method of the hemocompatibility test for both coated and uncoated silk fibers have been done with these steps; 5-6 different patients' blood samples have been taken [Figure 2.10] and the PT sec, INR, and APTT values were noted initially before starting to do tests.

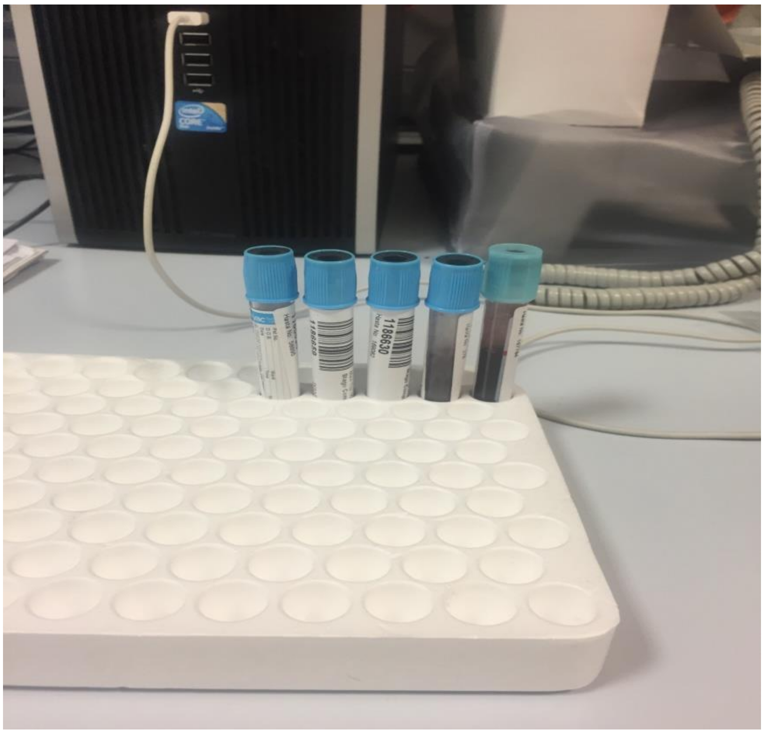


Figure 2.10: Blood samples which contain silk fibers.

For 10cm 13cm and 15cm silk fiber; after noting down the normal values for the blood, silk fibers has been added to each blood sample and waited for different time intervals which are 10 min, 20 min, 45min and 90min. For each length of silk fibers, tables are shown in Chapter 3.1. After each time interval has finished, blood samples have been centrifuged for 5 minutes in 3200 RCF, which contain silk fibers in it [Figure 2.11]. After then blood samples have been put in Coagulation Analyzer device to measure the PT sec, INR, and APTT values [Figure 2.12]. The reason of centrifuging blood samples is to separate the plasma and erythrocytes (red blood cells) because, the Coagulation Analyzer device uses only the plasma of the blood, not the red blood cells, to analyze. After the measurement, blood samples have been mixed gently again and this procedure has been repeated for each time interval and for 3 different lengths of silk fibers.

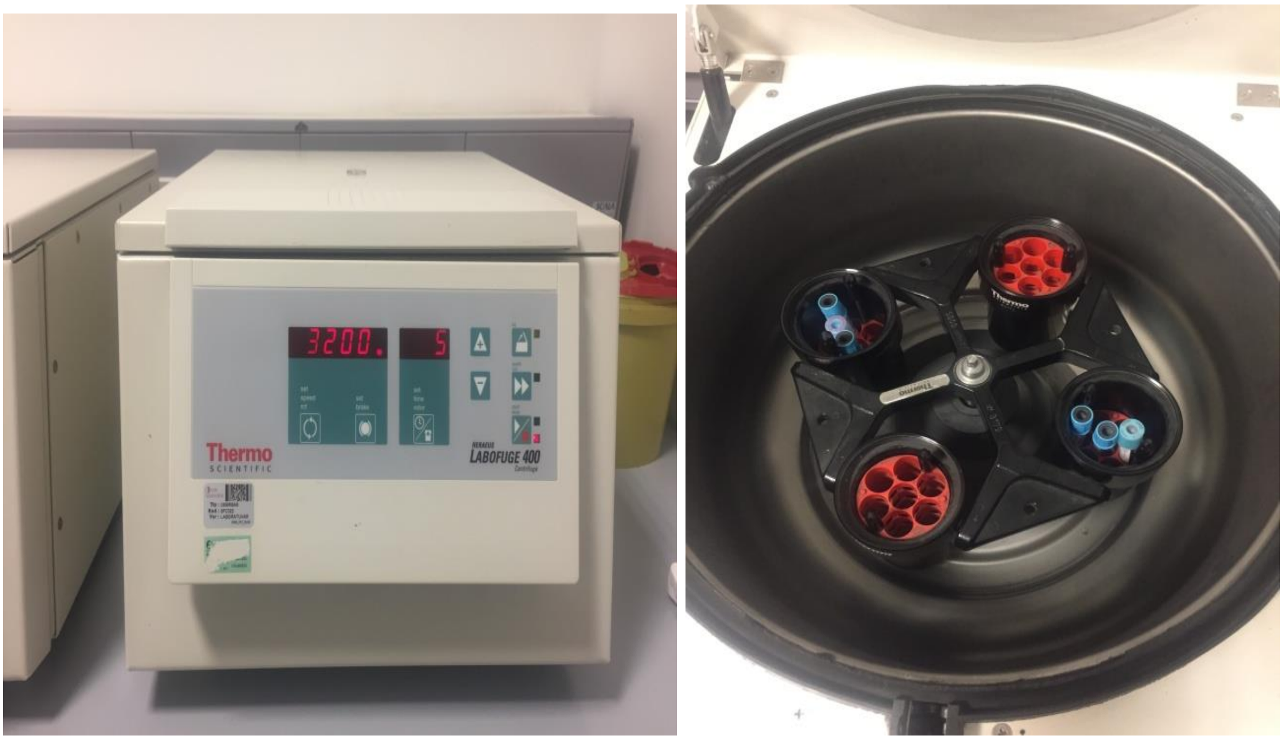


Figure 2.11: Thermo Scientific Labofuge 400 Centrifuge device in Near East University Hospital Laboratory

Figure 2.12: Stago Compact Coagulation Analyzer Device in Near East University Hospital Laboratory

2.6 Scanning Electron Microscopy (SEM)

SEM device's working principle is to use electron beams to obtain images from materials. The electrons interact with atoms in the sample and they produce different type of signals that can be detected by the microscope. This analysis was carried out at TUBITAK-Marmara Research Center at Gebze, Istanbul, Turkey using SEM JEOL/JSM-6335F model which acceleration voltage is 10kV. The device produces images of the samples by focusing a beam of electrons on it and samples were sputter-coated with gold.

2.7 X-Ray Diffraction (XRD)

X-ray diffraction analysis has been done at TUBITAK-Marmara Research Center at Gebze, Istanbul, Turkey using Shimadzu XRD-6000 model diffractometer with a Cu- X-ray tube (λ=1.5405 Angstrom). The diffractometer scans and obtain diffraction intensity curves.

2.8 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) analysis has been used to obtain an infrared spectrum of emission, photoconductivity, absorption or Raman scattering of materials. This analysis has been done using the FTIR Perkin Elmer Spectrum One Spectrometry device at TUBITAK-Marmara Research Center at Gebze, Istanbul, Turkey.

2.9 Swelling and Solubility Experiments of Uncoated and Coated Silk Fibers

Uncoated, 8 times coated and 15 times coated SF have been used in swelling and solubility experiments. Each SF were 20 cm long. In this experiment 1M NaOH (pH=13.0), 0.1M HCl (pH=1), Phosphate Buffer Saline (PBS)(pH=7.1) and pure water have been used. The aim of this experiment was to observe changes and response of coated and uncoated silk fibers in acid, base, buffer solution and pure water in different time intervals. First of all, each of the solution has been put inside beakers as 20 ml. Then, uncoated, 8 times coated and 15 times coated SF have been put inside the solutions mentioned above. Photos of each fiber have been taken when they have been put inside each solution without time passing. After that, the specific time intervals have been noted down as 10 min, 15min, 30min, 60min and 90 min. After each time interval, changes in each beaker have been observed and photos have been taken for each fiber.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Measurement of Uncoated and Coated Silk Fibers

3.1.1 Measurement of Uncoated and Coated Single Silk Fibers

Uncoated and coated single silk fibers' inverted light microscope images have been taken and their diameters have been measured. All of the figures have been shown in Chapter 2. As shown in Table 3.1 below, all of the single silk fiber's average diameters have been listed. Uncoated silk fibers' average diameter is 73.10 µm while 5 times coated single silk fibers' is 78.30 µm. Between uncoated and 5 times coated silk fibers, there have been very small change in diameter as 5.20µm.

Table 3.1: Uncoated and coated single silk fibers' diameters and total average diameters

Silk Fibers	Figures	Diameters of Silk Fibers
Uncoated Single Silk Fiber	Figure 2.3a	83.13 µm
	Figure 2.3b	69.88 µm
	Figure 2.3c	73.66 µm
	Figure 2.3d	65.77 µm
	Total Average	**73.10 µm**
5 times Coated Single Silk Fibers	Figure 2.5a	74.61 µm
	Figure 2.5b	72.07 µm
	Figure 2.5c	83.83 µm
	Figure 2.5d	82.7 µm
	Total Average	**78.30 µm**
10 times Coated Single Silk Fibers	Figure 2.6a	196.94 µm
	Figure 2.6b	172.1 µm
	Figure 2.6c	222.88 µm
	Total Average	**197.31 µm**
15 times Coated Single Silk Fibers	Figure 2.7a	304.52 µm
	Figure 2.7b	236.38 µm
	Figure 2.7c	181.36 µm
	Figure 2.7d	205.74 µm
	Total Average	**232.00 µm**

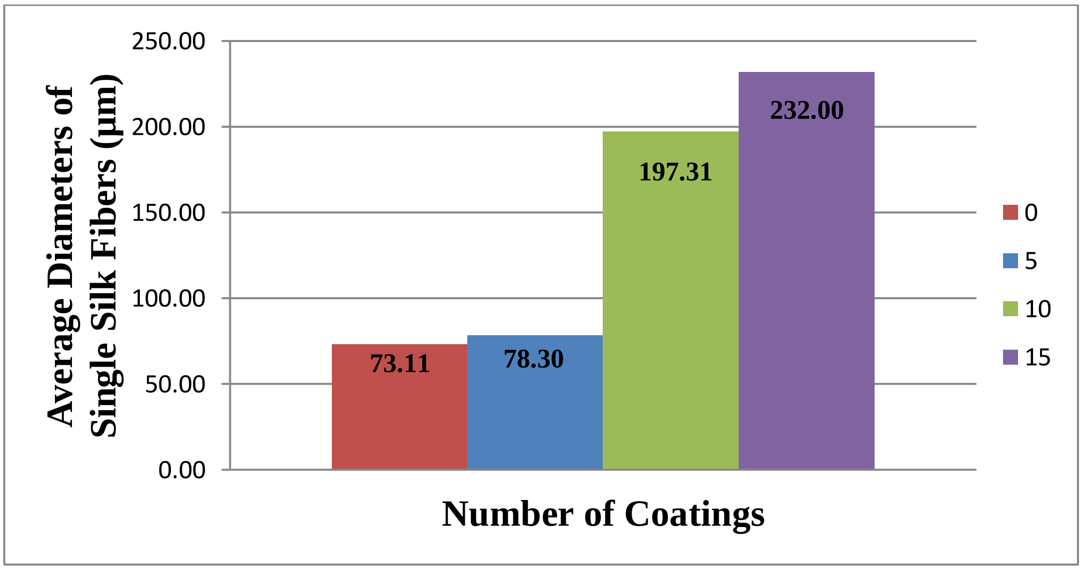
The first 5 times coating has been done with first method which has explained in Chapter 2.2. The first method's success rate was very low and it affected the results which we can understand from very small change. So, after 5 times coating and considering that the method was not successful, because of the small change in diameter of silk fibers, the method has been changed and the coating procedure has been continued with the second method which has been explained in Chapter 2.2.

As we can see in Table 3.1, after 10 times coating, the average diameter has been reached to approximately 197.31µm. So between 5 times and 10 times coating the change is approximately 119.01µm which can be accepted as successful. The change in method has been affected the results.

Also first method caused degradation of silk fibers because of the heat amount used in coating process which was 65°C. So it can be stated that 65°C was too much for silk fiber coating procedure and it causes degradation of silk fiber which it is not preferable.

After 15 times total coating of single silk fibers, the average was 232.0 µm. The difference between 10 and 15 times coating is approximately 34.7µm. The biggest difference in this single silk fiber coating has been between after 5 times to 10 times coating. Table 3.2 below shows the difference in diameters before coating and after each coating for single silk fibers.

Table 3.2: Average diameters of single silk fibers before coating and after each coating



So as we can see in both Table 3.1 and Table 3.2, there has been great change in diameters of silk fibers after coating procedure. The diameter change shows us that the PEGDMA solution coated the silk fibers and results of measurements after specific coatings shows us that PEGDMA solution coated fibers layer by layer and resulted in diameter increase.

3.1.2 Measurement of Uncoated and Coated 4-twisted Silk Fibers

Uncoated and coated 4-twisted silk fibers' inverted light microscope images have been taken and their diameters have been measured. All of the figures have been shown in Chapter 2. As shown in Table 3.3 below, all of the 4-twisted silk fiber's average diameters have been listed. Uncoated 4 twisted silk fibers' average diameter is 197.66µm while 5 times coated 4-twisted silk fibers' is 225.07µm. Between uncoated and 5 times coated silk fibers, there have been a small change in diameter approximately 27.41µm.

Table 3.3: Uncoated and coated 4-twisted silk fibers' diameters and total average diameters

Silk Fibers	**Figures**	**Diameters of Silk Fibers**
Uncoated 4 twisted Silk Fibers	Figure 2.4a	233.37 µm
	Figure2.4b	149.53 µm
	Figure 2.4c	210.08 µm
	Total Average	**197.66 µm**
5 times Coated 4 twisted Silk Fibers	Figure 2.8a	194.78 µm
	Figure 2.8b	199.28 µm
	Figure 2.8c	249.1 µm
	Figure 2.8d	257.1 µm
	Total Average	**225.07 µm**
15 times Coated 4 twisted Silk Fibers	Figure 2.9a	520.84 µm
	Figure 2.9b	557.54 µm
	Figure 2.9c	560.0 µm
	Figure 2.9d	497.1 µm
	Total Average	**533.9 µm**

The first 5 times coating has been done with first method which has explained in Chapter 2.2. The first method's success rate was very low in single silk fibers and it can also be considered as low for 4-twisted silk fibers as well. As shown in Table 3.4, there is not much difference between uncoated and 5 times coated.

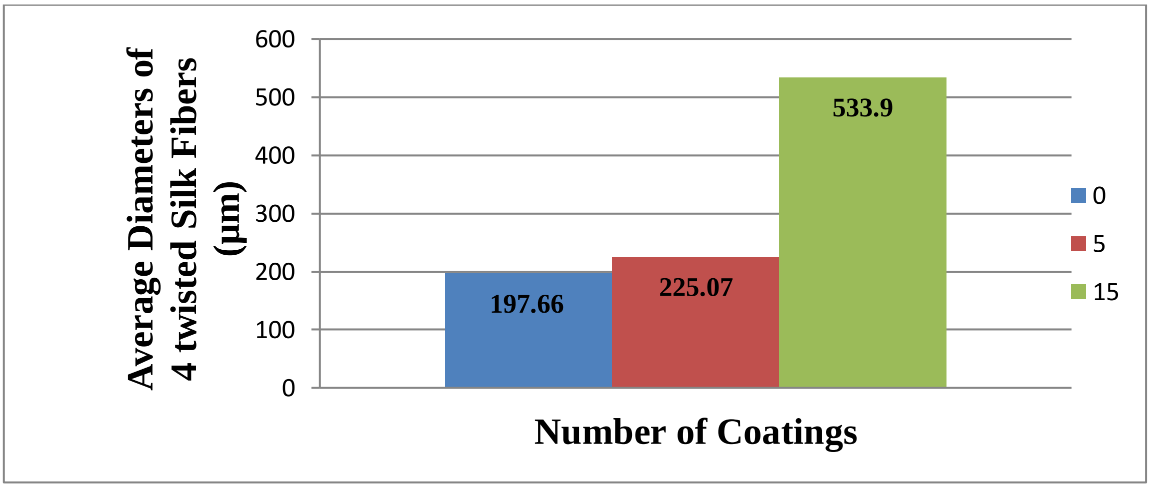
The first method have been affected the results which we can understand from the small change in diameter after 5 coating. So, after 5 times coating and considering that the method was not successful, because of the small change in diameter of silk fibers, the method has been changed and the coating procedure has been continued with the second method which has been explained in Chapter 2.2.

Also first method caused degradation of silk fibers because of the heat amount used in coating process which was 65°C. So it can be stated that 65°C was too much for silk fiber coating procedure and it causes degradation of silk fiber which it is not preferable.

As we can see in Table 3.3, after 15 times coating, the average diameter has been reached to approximately 533.9µm. So between 5 times and 15 times coating the change is approximately 308.83µm which can be considered as successful. The change in method has been affected the results in a positive way.

The biggest difference in this 4-twisted silk fiber coating has been between after 5 times to 15 times coating. The differences in diameters before coating and after each coating for 4-twisted silk fibers are shown in Table 3.4.

Table 3.4: Average diameters of single silk fibers before coating and after each coating



So, as shown in Table 3.3 and Table 3.4, there has been great change in diameters of silk fibers after coating procedure. The diameter change shows us that the PEGDMA solution coated the silk fibers and results of measurements after specific coatings shows us that PEGDMA solution coated fibers layer by layer and resulted in diameter increase. This means that the experiment has been done successfully.

3.2 Hemocompatibility Tests

3.2.1 Hemocompatibility Test for Uncoated Silk Fibers

In Table 3.5 normal values of APTT, PT (sec) and INR have shown. As shown in three tables; Table 3.6, Table 3.7 and Table 3.8, as the length of silk fibers increases, the APTT level increases more. It means that coagulation level is decreasing. For every patient, the degrees of change in values changes because the patients' blood's which has been used as blood samples, have different illnesses.

Table 3.5: Reference values for tests

Test	Reference Values
APTT	23,6-35,2
PT(sec)	11,5-15
INR	0,80-1,20

As shown in Table 3.6, when the silk fiber's length was shorter, some values of some patient's increase while the other's decrease. This could be because of several errors like the illnesses of patients. During the experiment, when the silk fibers have been mixed with blood samples, after specific time passed, the centrifuge has been used to separate plasma and red blood cells. After centrifuging, it has been seen that some of the silk fibers started to come out of the blood. This caused some errors and this is one of the reasons which caused unexpected results.

The time intervals also affected the coagulation formation, it can be assumed that as time interval increases while the fibers kept inside the blood, coagulation is not occurring successfully. This was one of the main aims of doing hemocompatibility test for silk fibers.

Table 3.6: 10 cm uncoated silk fiber added to 6 different blood samples in different time intervals which tested by Coagulation Analyzer.

Blood Samples	**Tests**	**Normal Blood Values**	**10 min**	**20 min**	**45 min**	**90 min**
Sample 1	PT sec:	12.8	13.1	12.8	13.1	13.3
	INR:	1	1.03	1	1.03	1.05
	APTT:	26.5	25.7	26	26	26.7
Sample 2	PT sec:	13.4	13.5	13.6	14	14.5
	INR:	1.06	1.07	1.08	1.12	1.18
	APTT:	31.7	32.9	33.5	34.7	36.3
Sample 3	PT sec:	21.6	22.1	21.9	21.4	22.6
	INR:	1.97	2.03	2.01	1.95	2.09
	APTT:	31.7	52.9	50.8	48.7	47.6
Sample 4	PT sec:	17.4	17.8	17.6	17.6	17.9
	INR:	1.49	1.54	1.51	1.51	1.55
	APTT:	35.6	36	35.5	35.6	35.5
Sample 5	PT sec:	25.8	26.9	26.3	26.8	28.4
	INR:	2.49	2.63	2.55	2.61	2.82
	APTT:	51.5	51.3	51.6	52.3	52.3
Sample 6	PT sec:	15.7	15.7	15.7	15.8	16.2
	INR:	1.3	1.3	1.3	1.31	1.36
	APTT:	37.5	38.5	38.4	37.9	38.3

In this test, except from sample 4, all of the patients APTT levels increased but there is not so much difference between their normal values and the last values. All of the patients' INR and PT (sec) values have been increased but there is not so much difference for these tests as well. This means that 10 cm for uncoated silk fibers were not enough to increase the values and increase the anti-coagulation. For sample 3 after 10 min, the APTT value increased from 31.7 to 52.9 which is a big difference. But if we look at the values after 20 min, 45 min and 90 min, value decreased to 50.8, 48.7 and 47.6 respectively. But between patient's normal value and the last value, there is increment. So the rapid rise after 10 min then decrease to lower values can be considered as practical error.

For 13 cm uncoated silk fibers, the same method has been repeated which mentioned in Chapter 2.5 with different blood samples of different patients. The table is shown below.

Table 3.7: 13 cm Uncoated Silk Fiber added to 6 different Blood Samples in different time intervals which tested by Coagulation Analyzer.

Blood Samples	Tests	Normal Blood Values	10 min	20 min	45 min	90 min
Sample 1	PT sec:	12.2	12.2	12.4	12.6	13.1
	INR:	0.94	0.94	0.96	0.98	1.03
	APTT:	25.8	26.8	27.9	28.9	29.7
Sample 2	PT sec:	13.4	13.5	13.6	14.1	13.8
	INR:	1.86	1.07	1.08	1.13	1.1
	APTT:	25.8	28.7	29.5	30	30.6
Sample 3	PT sec:	13.8	13.9	13.5	13.7	13.9
	INR:	1.1	1.11	1.07	1.09	1.11
	APTT:	27.5	29.1	29.6	30.6	31
Sample 4	PT sec:	19	18.9	18.9	18.4	18.3
	INR:	1.67	1.66	1.65	1.6	1.59
	APTT:	45.4	44.2	43	42.9	42.7
Sample 5	PT sec:	12.6	14.2	14.9	15.3	15.4
	INR:	0.98	1.14	1.22	1.26	1.27
	APTT:	35.6	36.5	37.8	40.1	40.4

There is more improvement in this 13 cm uncoated silk fiber blood compatibility test than 10 cm test. As shown in Table 3.7 above, all of the samples' APTT level increased, except from sample 4. It can be assumed as practical error. As sample 1 and 5's INR level have increased, sample 2 and 4's have decreased while sample 3 has no change. There is not much difference in PT (sec) levels, so it can be assumed that 13 cm silk fiber is not enough to have anti-coagulant property.

For 15 cm uncoated silk fibers, the same method has been repeated which mentioned in Chapter 2.5 with different blood samples of different patients. The table is shown below.

Table 3.8: 15 cm Uncoated Silk Fiber added to 6 different Blood Samples in different time intervals which tested by Coagulation Analyzer

Blood Samples	Tests	Normal Blood Values	10 min	20 min	45 min	90 min
Sample 1	PT sec:	11.9	12.1	10.4	15.3	12.7
	INR:	0.91	0.93	0.76	1.26	0.99
	APTT:	27.4	27.5	28.4	29	29.7
Sample 2	PT sec:	13.9	13.5	13.6	14.6	14.6
	INR:	1.11	1.07	1.08	1.19	1.19
	APTT:	31	31.8	32.6	33.6	34.1
Sample 3	PT sec:	16.1	16.1	16.2	14.9	17.2
	INR:	1.35	1.35	1.36	1.22	1.47
	APTT:	30.1	31.7	32.5	33.2	33.7
Sample 4	PT sec:	25.8	24.9	25	26.1	25.7
	INR:	2.49	2.38	2.39	2.52	2.47
	APTT:	41.4	42	42.3	42.9	42.9
Sample 5	PT sec:	12	12.1	11.7	12.6	13
	INR:	0.92	0.93	0.89	0.98	1.02
	APTT:	28.2	28.9	29	29.7	30.3

For this test, the APTT levels increased more than 10cm and 13cm silk fiber. For INR levels, there was not big difference and for PT (sec) there is more increment than 10cm and 13cm except from sample 4. It can be assumed as practical error.

So it can be assumed that silk fiber has anti-coagulant effect on blood but more silk fibers should be used in order to have greater effect.

3.2.2 Hemocompatibility Test for Coated Silk Fibers

Coating procedure of silk fibers has been explained in Chapter 2.2 and method of hemocompatibility test has been explained in Chapter 2.5.

Silk fibers have been coated and their blood compatibility have been tested. As mentioned in Chapter 3.2.1, uncoated silk fibers have very small anti-coagulant effect on blood. So they are coated with PEGDMA and tested if PEGDMA will have more anti-coagulant effect when combining with silk fibers.

Table 3.9: 10 cm PEGDMA coated silk fiber added to 5 different Blood Samples in different time intervals which tested by Coagulation Analyzer

Blood Samples	Tests	Normal Blood Values	10 min	20 min	45 min	90 min
Sample 1	PT Sec:	12.3	12	12.2	12.4	13.3
	INR:	0.91	0.88	0.9	0.92	1
	APTT:	28.6	28.6	30.2	30.8	32.2
Sample 2	PT Sec:	12.2	12	11.8	11.8	12.2
	INR:	0.9	0.88	0.86	0.86	0.9
	APTT:	20.6	27.5	28	28	29.1
Sample 3	PT Sec:	21.4	20.9	21.1	21.5	20.6
	INR:	1.81	1.76	1.78	1.82	1.72
	APTT:	31.9	33	33.1	34.5	36.4
Sample 4	PT Sec:	12	12.1	12.1	12.4	13.2
	INR:	0.88	0.89	0.89	0.92	0.99
	APTT:	29.4	30	31.2	32	33.6
Sample 5	PT Sec:	16.2	16.0	16.0	16.1	16.5
	INR:	1.28	1.26	1.26	1.27	1.31
	APTT:	39.7	39.8	41.2	42.4	44.8

According to the results of 10 cm coated silk fibers coagulation analyzer results, APTT levels increased a lot more than 15cm of uncoated silk fibers. This showed that coated 10 cm silk fiber has more anti-coagulant effect on blood then uncoated silk fibers. INR and PT levels didn’t change a lot in this test.

Table 3.10: 13 cm PEGDMA coated silk fiber added to 5 different Blood Samples in different time intervals which tested by Coagulation Analyzer

Blood Samples	Tests	Normal Blood Values	10 min	20 min	45 min	90 min
Sample 1	PT Sec:	12.6	12.5	12.3	12.9	12.7
	INR:	0.94	0.93	0.91	0.96	0.95
	APTT:	24.6	25.2	26.1	26.4	26.3
Sample 2	PT Sec:	13.4	13.4	13.1	13.9	13.6
	INR:	1.01	1.01	0.98	1.06	1.03
	APTT:	29.3	29.8	30	30.5	31.5
Sample 3	PT Sec:	12.1	11.7	11.9	12.8	12.4
	INR:	0.89	0.85	0.87	0.96	0.92
	APTT:	24.9	25.5	26.1	26.6	27.4
Sample 4	PT Sec:	12.8	12.4	12.3	13.1	12.9
	INR:	0.96	0.92	0.91	0.98	0.96
	APTT:	28	28.4	29.2	29.7	30.1
Sample 5	PT Sec:	12.6	12.1	12.2	12.7	12.6
	INR:	0.94	0.89	0.9	0.95	0.94
	APTT:	26.5	26.7	27.5	27.8	28.2

In this test APTT levels increased but the change is less than 10cm coated fibers test. For 10 cm coated silk fibers test there has been bigger effects than in this test. So it can be assumed as combination of silk fiber and PEGDMA has more anti-coagulant effect than silk fiber alone, but when the length increases, difference start to decrease. There is nearly no change in INR and PT (sec) levels.

Table 3.11: 15 cm PEGDMA coated silk fiber added to 5 different Blood Samples in different time intervals which tested by Coagulation Analyzer

Blood Samples	Tests	Normal Blood Values	10 min	20 min	45 min	90 min
Sample 1	PT Sec:	35.6	35.2	33.1	32.8	32.6
	INR:	3.40	3.40	3.10	3.07	3.05
	APTT:	50.3	59.9	60.9	55.4	56
Sample 2	PT Sec:	15.9	15.3	15.0	15	15.2
	INR:	1.25	1.19	1.16	1.16	1.18
	APTT:	34.3	36.2	36.7	34.2	33.7
Sample 3	PT Sec:	13.8	13.7	14.0	14.0	13.9
	INR:	1.05	1.04	1.07	1.07	1.06
	APTT:	34.8	36.6	36.1	35.2	35.2
Sample 4	PT Sec:	20	19.4	19.5	19.9	19.9
	INR:	1.66	1.60	1.61	1.65	1.65
	APTT:	30.9	33.3	34.4	33.1	34.5
Sample 5	PT Sec:	12.7	12.5	12.7	12.5	12.5
	INR:	0.95	0.93	0.95	0.93	0.93
	APTT:	30.5	31.8	31.5	30.2	30.1

Except from sample 1's results, other APTT levels has very small increase in this 15 cm coated silk fiber test. There has been more increment in 10 cm coated silk fiber than in 13 cm and 15 cm coated silk fibers. As the length increased, the levels started to decrease. But in uncoated silk fiber hemocompatibility tests, it was vice versa. As the length of uncoated silk fibers increased, the anti-coagulation occurs increased.

This can be assumed as PEGDMA has anti-coagulant effect when combined with silk fibers but if we increase the length, the anti-coagulant effect starts to decrease. So the amount of coated silk fibers is important for the anti-coagulation.

3.3 Scanning Electron Microscopy

The Scanning Electron Microscopy analysis was done at TÜBİTAK- Marmara Research Centre at Gebze, Istanbul, Turkey by using a SEM JEOL/JSM-6335F model. SEM is used for imaging and comparing very small surface characteristics.

SEM images have been taken with different magnifications for uncoated silk fiber (Fig. 3.1) and 15 times coated 4 twisted silk fibers (Fig. 3.2).

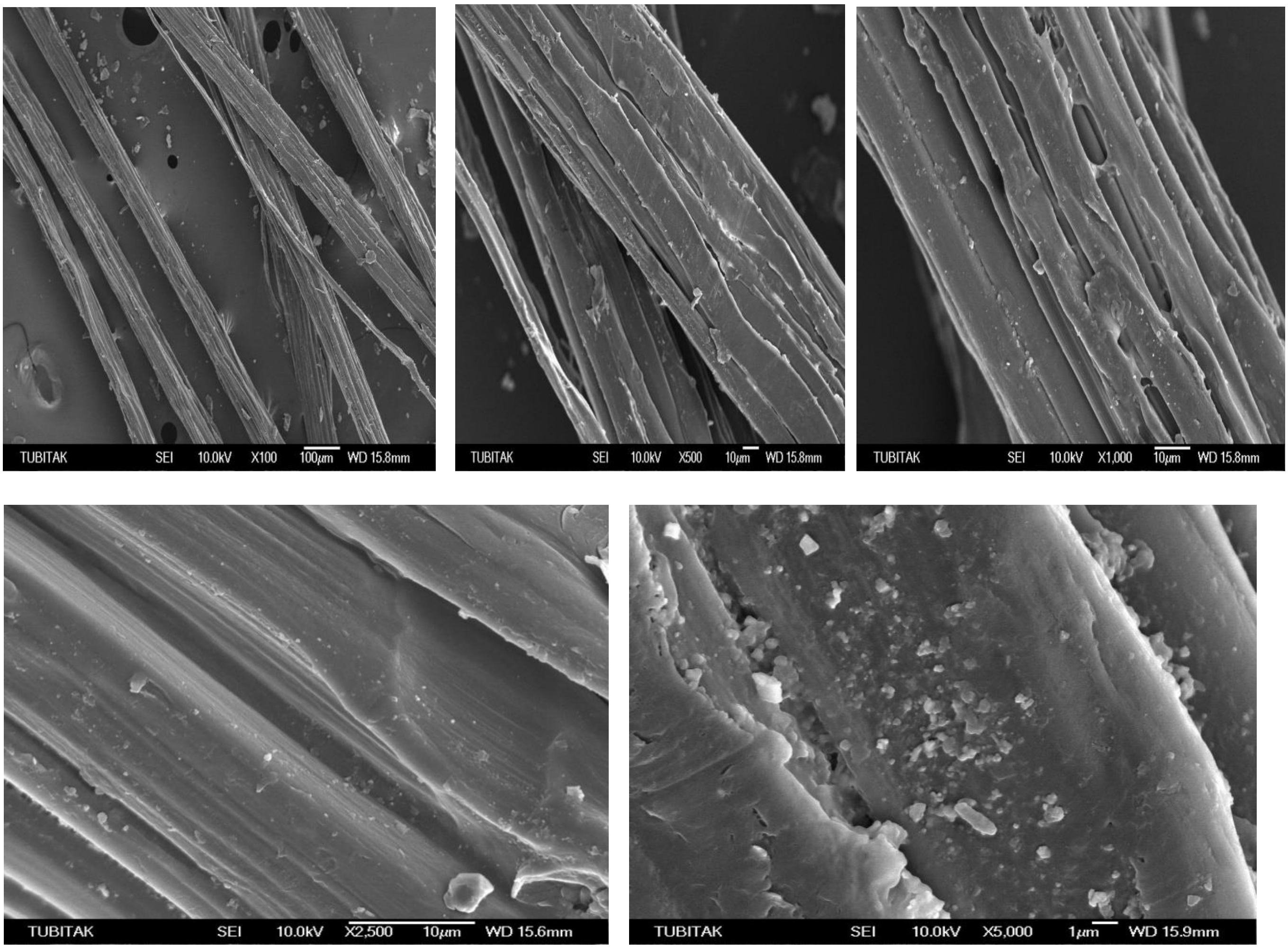


Figure 3.1: Scanning Electron Microscopy images of uncoated single silk fiber with different magnifications

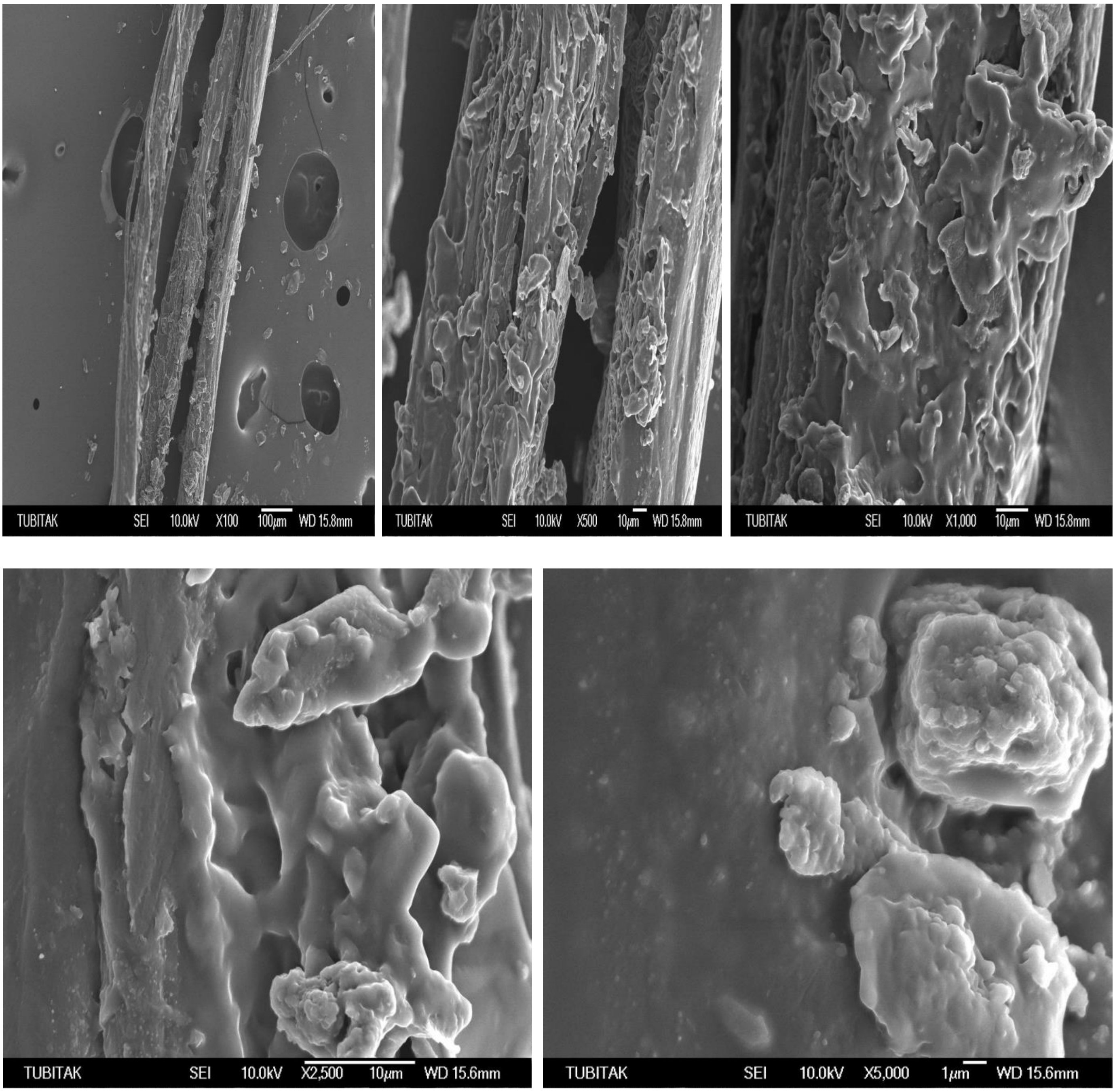


Figure 3.2: Scanning Electron Microscopy images of 15 times coated 4 twisted silk fiber with different magnifications

As shown in Figure 3.1, uncoated single silk fiber has smoother surface than 15 times coated 4 twisted silk fiber in Figure 3.2. In Figure 3.2, the image of coated 4 twisted silk fiber has rough surface because of PEGDMA coating. PEGDMA solution stuck on the surface and layer-by-layer, the solution hardened and became a coat. That roughness shows us the hardened coated PEGDMA. Also we can say that the coating procedure has been done successfully. The aim in increasing the diameter by coating was to increase the mechanical strength of silk fibers.

3.4 X-Ray Diffraction Analysis (XRD)

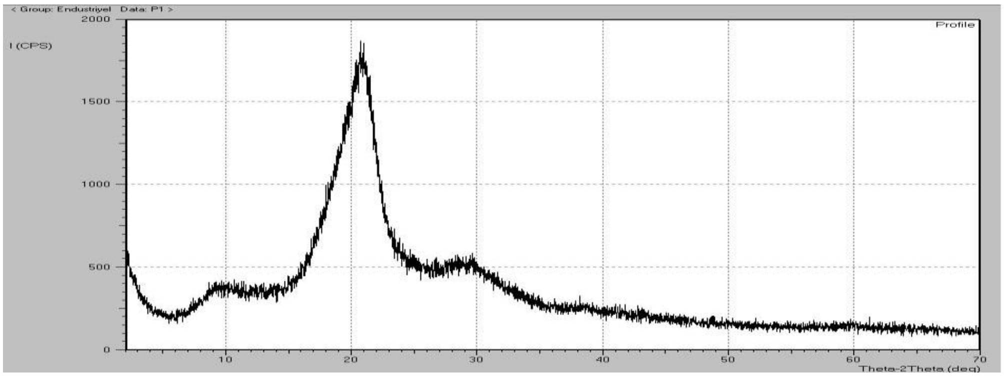


Figure 3.3: XRD analysis of uncoated single silk fiber

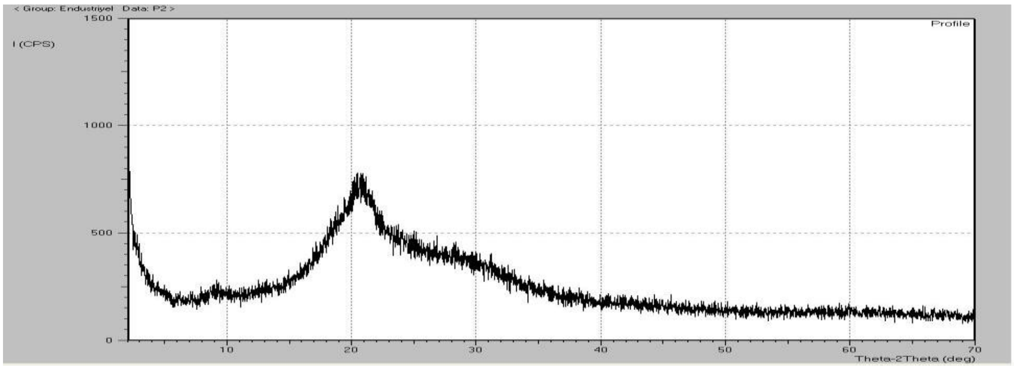


Figure 3.4: XRD analysis of coated 4-twisted silk fiber

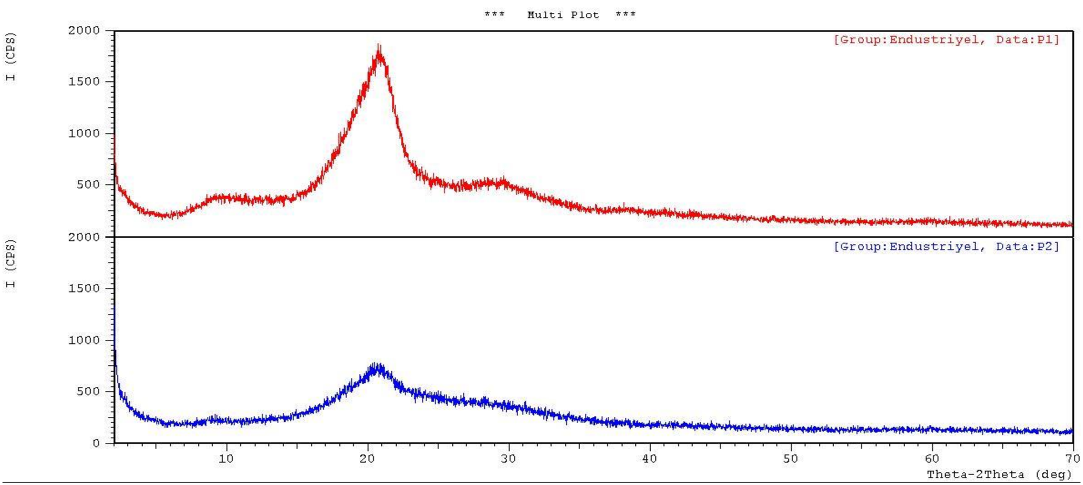


Figure 3.5: XRD analysis overlay for both uncoated single silk fiber and coated 4-twisted silk fiber

X-ray diffraction of uncoated silk fiber gives two characteristic crystallinity peaks at 2ϴ = 11.62°, 10.56° and 10.46°. In the coated silk fiber sample new weaker crystalline peaks appear at 12.58°and 20.50°.

So, both the crystalline structure and the degree of crytallinity are affected as a result of coating and crosslinking.

3.5 Fourier Transform Infrared Spectroscopy (FTIR)

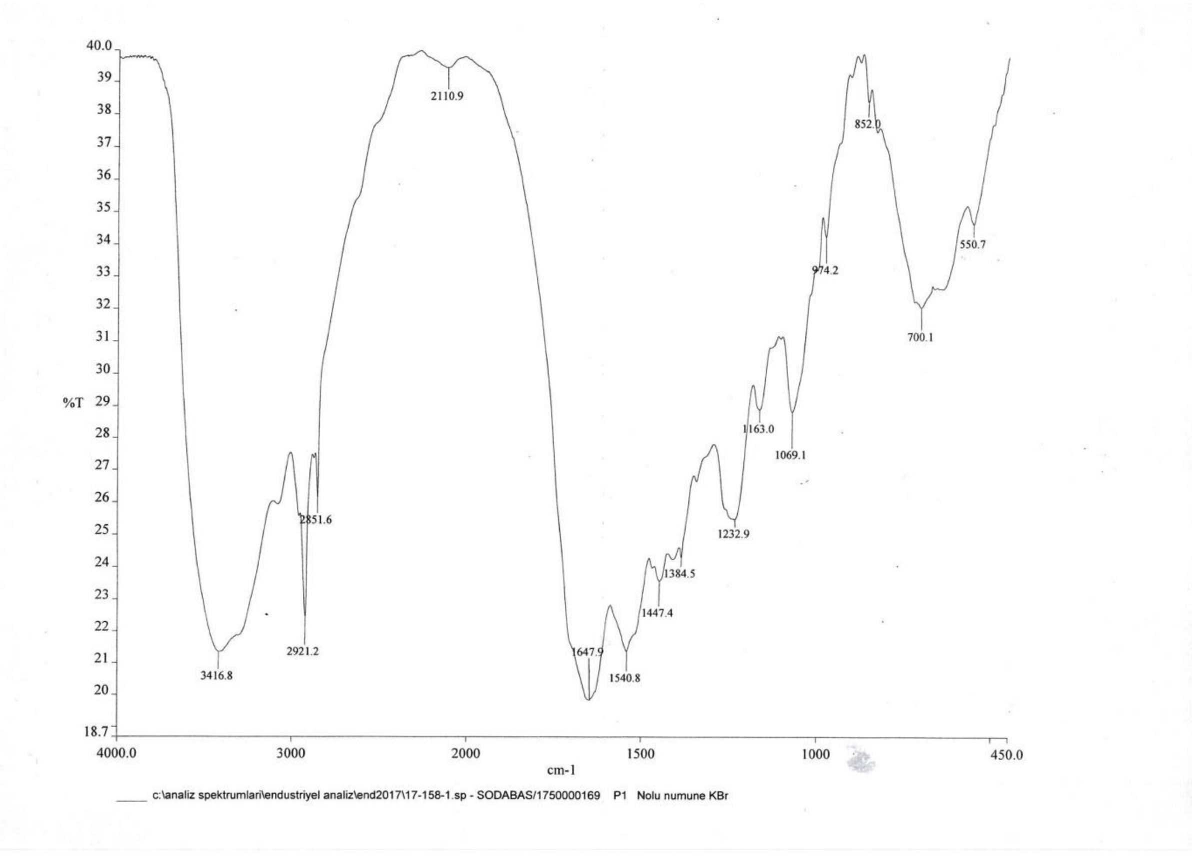


Figure 3.6: FTIR analysis for uncoated single silk fiber

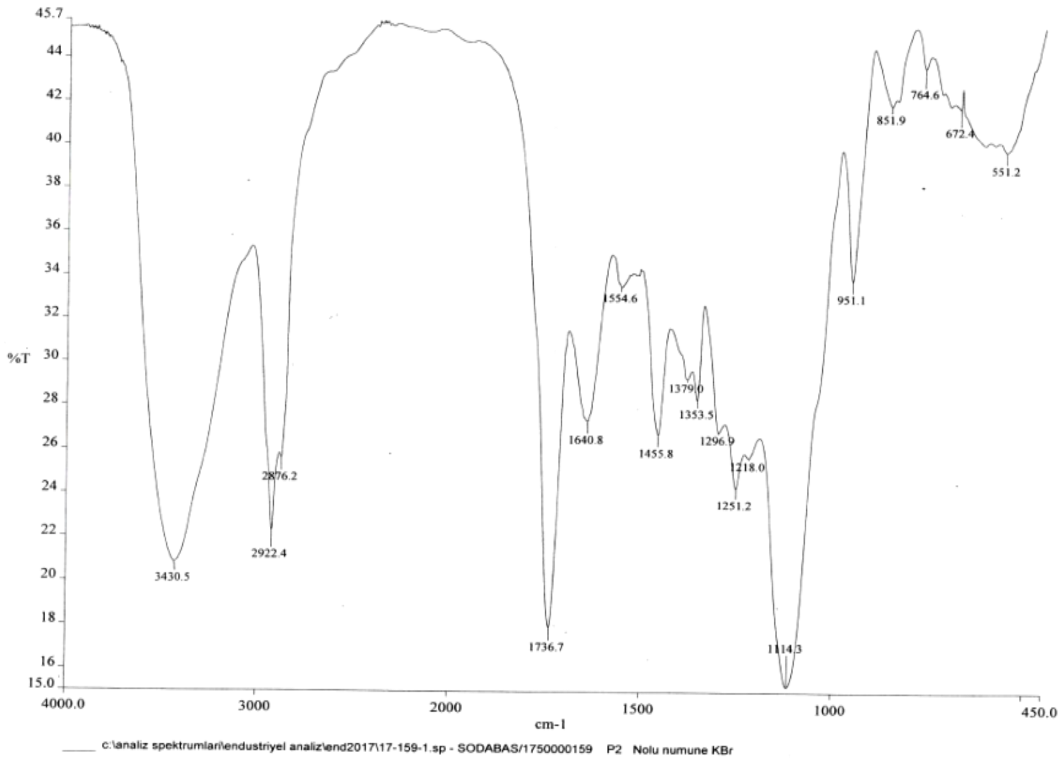


Figure 3.7: FTIR analysis for coated 4-twisted silk fiber

The characteristic absorbance peaks Amide I (1655 cm^{-1}) and Amide II (1537 cm^{-1}) have been observed on both of the spectra. It is clear that the chemical structure of the silk fibers has not been changed.

3.6 Swelling and Solubility Experiments of Coated and Uncoated Silk Fibers

Observations of this experiment have been shown in Table 3.12.

Table 3.12: Observations for swelling and solubility experiment.

		Observations for different time intervals				
Uncoated Single SF	**Tests**	**10 min**	**15 min**	**30 min**	**60 min**	**90 min**
	1M NaOH pH=13.0	SW	SW	SW	PD	DG
	0.1 M HCl pH=1	NC	NC	NC	NC	NC
	PBS pH=7.1	NC	NC	NC	NC	NC
	Pure Water	BF	BF	BF	SFL	SFL
8 times coated Single SF	**Tests**	**10 min**	**15 min**	**30 min**	**60 min**	**90 min**
	1M NaOH pH=13.0	SW	SW	SW	PD	DG
	0.1 M HCl pH=1	NC	NC	NC	NC	NC
	PBS pH=7.1	NC	NC	NC	NC	NC
	Pure Water	BF	BF	BF	SFL	FL
15 times coated Single SF	**Tests**	**10 min**	**15 min**	**30 min**	**60 min**	**90 min**
	1M NaOH pH=13.0	SW	SW	SW	PD	DG
	0.1 M HCl pH=1	NC	NC	NC	NC	NC
	PBS pH=7.1	NC	NC	NC	NC	NC
	Pure Water	BF	BF	BF	SFL	FL

*SW: swell, PD: partially degraded, DG: degraded , NC: no change, BF: bubble formation, SFL: started to float, FL: floating

As shown in Table 3.11, in NaOH all of the coated and uncoated silk fibers has been degraded. Firstly, they started swelling after 5 min so it can be assumed that uncoated and coated silk fibers have swelling and degrading property in NaOH alkali solution.

For PBS and HCl acid solution, there were no changes in states of all fibers. This showed that HCl and PBS didn't change uncoated and PEGDMA coated silk fibers character. For pure water, all of the fibers started to have bubbles inside the solution and on the surface of fibers. For uncoated fibers, fibers just started to float but in 8 times coated and 15 times coated silk fibers, fibers were floating on the surface of pure water. 15 times coated silk fiber were floating more than the other fibers. This can be assumed as PEGDMA coated silk fibers had floating effect when put in pure water.

CHAPTER 4

CONCLUSION

4.1 Conclusion

To conclude, hemocompatibility and coagulation test were done with 10cm 13cm and 15cm uncoated and coated silk fibers which can be resulted that silk fibers are biocompatible with blood but after coating, the ability increased. Silk fibers caused APTT, PT and INR levels to increase which means that it has an anti-coagulant effect on blood which was the main aim in this study.

The diameters of PEGDMA coated and uncoated silk fibers have been measured and it can be assumed that coating process with dipping technique is done successfully. There are big changes in before coating and after coating so it can be assumed that silk fibers have ability to hold PEGDMA material and together, they have a better hemocompatibility and anti-coagulant effect.

Swelling and solubility tests have showed that coated and uncoated fibers can be dissolve in NaOH alkali solution. For PBS and HCl acid solution, there were no changes in states of all fibers so it can be assumed that acidic and buffer solutions does not affect their character.

For pure water, all of the fibers started to have bubbles inside the solution and on the surface of fibers this means that it started to have reaction.

4.2 Recommendations

As a future work, first of all, the fibers which were measured can be increased and coated more to see the effect on blood when coating material amount has been increased.

Coating material can be changed and other materials can be tested by combining silk fibers. In this way researchers can be able to compare the success of different biocompatible materials and decide which has more anti-coagulant effect.

Silk fibers can be used to make scaffolds and human cells can be put on those scaffolds so real human cells can grown with silk fibers *in vitro*.

Different biomaterials can be produced by using silk fibers; for ex: stents. By focusing on improvement of silk fibers, it can help researchers to improve biomaterials. This will help science to improve.

REFERENCES

Adalı, T., & Uncu, M. (2016). Silk fibroin as a non-thrombogenic biomaterial. *International journal of biological macromolecules*, *90*, 11-19.

Adali, T., & Yilmaz, E. (2009). Synthesis, characterization and biocompatibility studies on chitosan-graft-poly (EGDMA). *Carbohydrate Polymers*, *77*(1), 136-141.

Boodagh, P., Guo, D. J., Nagiah, N., & Tan, W. (2016). Evaluation of electrospun PLLA/PEGDMA polymer coatings for vascular stent material. Journal of Biomaterials science, Polymer edition, 27(11), 1086-1099.

Cheng, C. C., Mi, F. L., Hsu, S. H., & Don, T. M. (2014). Structure characterizations and protein resistance of chitosan membranes selectively crosslinked by poly (ethylene glycol) dimethacrylate. *Cellulose*, *21*(3).

Elahi, F. M., Guan, G., & Wang, L. (2014). Hemocompatibility of Surface Modified Silk Fibroin Materials: A Review. *Reviews on advanced materials science*, *38*(2).

Gorbet, M. B., & Sefton, M. V. (2004). Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. *Biomaterials*, *25*(26), 5681-5703.

Hao, Y., Sun, D., Wang, Q., Dong, F., Zhang, G., & Wang, J. (2015). In vitro blood compatibility evaluation of silk fibroin by chemical crosslinking. *Materials Technology*, *30*(6), 327-331.

Kancevica, V., & Lukjancikovs, A. (2013). Vascular Implants Reinforced with Natural Silk Yarns. *Rigas Tehniskas Universitates Zinatniskie Raksti*, *8*, 16.

Kancevicha, V., & Lukyanchikovs, A. (2012). Silk Fibroin Bombyx Mori Yarns for Bio-Resistant Woven Aortic Prostheses. *Material Science (1691-3132)*, (7).

Li, C., Vepari, C., Jin, H. J., Kim, H. J., & Kaplan, D. L. (2006). Electrospun silk-BMP-2 scaffolds for bone tissue engineering. Biomaterials, 27(16), 3115-3124.

Li, G., Li, Y., Chen, G., He, J., Han, Y., Wang, X., & Kaplan, D. L. (2015). Silk-Based Biomaterials in Biomedical Textiles and Fiber-Based Implants. *Advanced healthcare materials*, *4*(8), 1134-1151.

Pacelli, S., Paolicelli, P., Pepi, F., Garzoli, S., Polini, A., Tita, B., & Casadei, M. A. (2014). Gellan gum and polyethylene glycol dimethacrylate double network hydrogels with improved mechanical properties. *Journal of Polymer Research*, *21*(5), 1.

Suzuki, S., Dawson, R. A., Chirila, T. V., Shadforth, A., Hogerheyde, T. A., Edwards, G. A., & Harkin, D. G. (2015). Treatment of silk fibroin with poly (ethylene glycol) for the enhancement of corneal epithelial cell growth. *Journal of functional biomaterials*, *6*(2), 345-366.

Vepari, C., & Kaplan, D. L. (2007). Silk as a biomaterial. Progress in polymer science, 32(8), 991-1007.

Zhao, H., Feng, X., & Shi, H., (2007). Untangling a Sticky Problem: The Tensile Properties of Natural Silks. *Mater. Sci. Eng.*, C 27 675–683.

Zheng, Z., Wang, W., Huang, X., Lv, Q., Fan, W., Yu, W., & Zhang, Z. (2016). Fabrication, characterization, and hemocompatibility investigation of polysulfone grafted with polyethylene glycol and heparin used in membrane oxygenators. *Artificial Organs*, *40*(11).