PRESERVATION OF THE COLLAGEN STRUCTURE BY COAXIAL ELECTROSPINNING METHOD

by

Efe Cuma Yavuzsoy

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APPROVED BY:

Assoc. Prof. Dr.Bora Garipcan .....
(Thesis Advisor)

Prof. Dr. Murat Kazanci .....
(Thesis Co-advisor)

Assoc.Prof. Dr. Duygu Ege .....

Assist.Prof. Dr. Banu İyisan .....

Prof. Dr. Kadriye Tuzlakoğlu .....

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This thesis is dedicated to my beloved grandmother.

ACADEMIC ETHICS AND INTEGRITY STATEMENT

I, Efe Cuma Yavuzsoy, hereby certify that I am aware of the Academic Ethics and Integrity Policy issued by the Council of Higher Education (YÖK) and I fully acknowledge all the consequences due to its violation by plagiarism or any other way.

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ABSTRACT

PRESERVATION OF THE COLLAGEN STRUCTURE BY COAXIAL ELECTROSPINNING METHOD

Collagen is one of the most important material for biomedical technologies. Both its biological and mechanical properties enable collagen to be used in many fields such as biomaterials, tissue engineering etc. The use of collagen in the production of electrospun nanofibers reveals promising results for both research and clinical based areas such as tissue scaffolding. However, collagen can lose its natural structure by being affected by many parameters during the formation of nanofibers with desired physical properties. In this thesis, coating of collagen nanofibers with a biodegradable polymer, Poly Vinyl Alcohol (PVA), using coaxial electrospinning method is demonstrated. It was hypothesized that the structure could be protected by coating the collagen nanofibers with PVA. Thermal and spectroscopic analyzes show that collagen and PVA are present in the obtained nanofiber structures. With optical and scanning electron microscope images, the difference between the fibers produced by the coaxial electrospinning system and those produced by the conventional electrospinning system was demonstrated. This work is considered as a preliminary study, it is hypothesized that the nanofibers to be produced by the coaxial electrospinning method as used in this thesis may show biological activity-enhancing properties *in vivo* and *in vitro* experiments as tissue scaffolds in future research.

Keywords: Coaxial Electrospinning, Collagen Nanofibers, Collagen Tissue Scaffold, PVA

ÖZET

KOAKSİYAL (ÇİFT EKSENLİ) ELEKTROEĞİRME YÖNTEMİYLE KOLAJEN YAPISININ KORUMASI

Kolajen, biyomedikal teknolojiler için en önemli malzemelerdendir. Hem biyolojik kaynaklı olması hem de mekanik özellikleri kolajenin biyomalzeme, doku mühendisliği vb. pek çok alanda kullanılmasını sağlamaktadır. Elektrospun nanofiberlerin üretiminde kolajen kullanılması doku iskelesi gibi alanlarda araştırmada ve klinikte gelecek vadedici sonuçlar ortaya koymaktadır. Fakat kolajen, üretim esnasında pek çok dış etmenden etkilenerek doğal yapısını kaybedebilmektedir ve istenilen fiziksel özellikteki nanofiberlerin oluşması engellenebilmektedir. Bu tezde, çift eksenli elektroeğirme yöntemi kullanılarak biyobozunur bir polimer olan Poli Vinil Alkol (PVA) ile kolajen nanofiberlerin kaplanması çalışılmıştır. PVA'nın kolajen nanofiberleri kaplaması ile yapının korunabileceği düşünülmüştür. Termal ve spektroskopik analizler nanofiber yapısında kollajen ve PVA'nın bulunduğunu göstermektedir. Optik ve taramalı elektron mikroskop görüntüleriyle çift eksenli elektroeğirme sistemi ile üretilen fiberlerin geleneksel elektroeğirme sistemiyle üretilenlerle olan farkı ortaya konmuştur. Bir ön çalışma olarak değerlendirildiğinde bu tezde kullanılan çift eksenli elektroeğirme yöntemiyle üretilecek nanofiberlerin gelecekte doku iskelesi olarak *in vivo* ve *in vitro* çalışmalarda biyolojik aktiviteyi arttırıcı özellik gösterebileceği düşünülmektedir.

Anahtar Sözcükler: Koasiyal Elektroeğirme, Kolajen Nanofiber, Kolajen Doku İskelesi, PVA

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LIST OF ABBREVIATIONS

CMC	Caboxymethyl Chitin
ECM	Extracellular Matrix
FT-IR	Fourier Transform- Ifrared Spectroscoy
HA	Hydroxy Apatite
hMSCs	Human Mesenchymal Stem Cells
PCL	Polycaprolactone
PVA	Poly Vinyl Alcoholl
SEM	Scanning Electron Microscope
TGA	Thermogravimetric Analysis

1. INTRODUCTION

1.1 Motivation

Collagen, one of the natural polymers, is a major extracellular protein found in many tissues and organs [1],[2]. For example, on average 70% of the extracellular matrix (ECM) of the human skin consists of collagen [3]. Collagen provides more structural stability by forming long fibers in the skin. There are 29 different types of collagen. These different types of collagens are the result of assembly of 46 different polypeptide chains [4]. Collagen, which is used for different tasks such as growth and development inside the cell, is thus frequently used in various fields of medicine and biotechnology [5],[6],[7]. In addition, collagen nanofibers have a wide range of uses in biomedical technologies, from 3D tissue scaffolds [8] to drug-carrier systems [9]. This widespread use has enabled many researchers to do more research on collagen, as a result, the number of articles published in this area is increasing.

Collagen type I, which is found in almost every tissue as a structural support, is the most common protein in mammals. Collagen, which has a wide range of functions in tissues, provides the transmission of the force applied from the muscles to the bones as well as storing elastic energy in the tendon and ligament structures. In addition, collagen increases mechanical strength in dentin, and bone tissue. Collagen is not only found in stiff tissues. It is found as a matrix around the contractile cells. In the absence of this matrix, structures such as skin, blood vessels, etc. cannot perform their functions. Collagen type II is an important structure mostly found in cartilage tissue. It would be an understatement to say that collagen is a substance that only provides mechanical support. It also increases the transparency in collagen tissues (E.g., cornea)[10].

Collagen is a frequently used material in various fields of medicine. Different collagen-based structures are used as a drug delivery system. (Film [11], shields[12],

sponges[13], gel/hydrogel[14], pellets [15], nanoparticles [16]). Because of its characteristics like low immunogenicity or mechanical behaviours, collagen is a nice material to use in bone tissue engineering [17]. Especially the dense collagen content of the bone and the structural properties of collagen have increased the importance of using collagen for bone tissue engineering.

Electrospinning method, which is frequently used in the production of nanofibers, has been the main method in collagen nanofiber production for years [8]. Like scaffold, biocompatible surgical thread, etc. collagen nanofiber production has great advantages for its use in different fields of biomedicine. However, publications showing that the structure of collagen is degraded during the electrospinning process has led to the need for modification of the method [18]. In this thesis, coaxial electrospinning was used in order to increase the quality of collagen nanofiber production. Decreasing the damage to the 3-dimensional structure of collagen nanofibers can increase their use in both industrial and research based applications.

1.2 Objectives

In this thesis, it is aimed to coat the collagen nanofibers with PVA by modifying the traditional electrospinning method with coaxial tip. For this purpose, collagen isolated from calf skin and industrial grade PVA were used. Fiber samples synthesized by the coaxial electrospun technique were analyzed using Fourier-Transform Infrared Spectroscopy (FT-IR) and Thermogravimetric Analysis (TGA) and visualized using Scanning Electron Microscope (SEM).

PVA was preferred because of its high biocompatibility and low toxicity [19]. In addition, the elasticity of PVA was aimed to add mechanical strength to the nanofiber structure. On the other hand, it was aimed to reveal the potential of biomedical material by creating collagen-based nanofiber, which constitutes the majority of the extracellular matrix in almost all tissues, especially bone tissue. The main objectives of this thesis are:

1. Demonstration of coating collagen nanofibers by a synthetic polymer, called PVA.
2. Increasing the use of collagen as a biomaterial in a wider area.
3. Characterization of physical and chemical properties of coaxial nanofibers.
4. Comparison of traditional electrospinning and coaxial electrospinning methods.

2. BACKGROUND

2.1 Collagen

When examined in origin, the word "collagen" is derived from the word glue in ancient Greek. It was first used to define the structure that constitutes the connective tissue. At the beginning, what was isolated was actually gelatin because it was isolated from various animals using heat [20]. Later, as the 3-dimensional folded structure of collagen structure began to be discovered, it was understood that it could not be isolated by heating. Almost a quarter of the protein content of all animals is collagen. Collagen is the basic component of many tissues such as bones, tendons, cartilage, teeth, skin, etc. in the body [21]. Collagen is the essential component of ECM. Self-assembled collagen structure improves the mechanical properties of the tissues. In addition, collagen provides a molecular response to external stresses applied to the tissue by interacting with cells. It has not been fully answered how collagen, which is usually in the matrix structure, protects the tissue against the applied pressure [22].

2.1.1 Structure of Collagen

The collagen molecule was first described in 1940 as a single-chain polypeptide with amide bonds [23]. In the study conducted in 1951, α -helix and β sheet structures within the collagen molecule were shown [24]. Based on this study, 3-dimensional structure of collagen was modeled after 3 years [25],[26]. In 1994, the collagen triple helical structure was shown for the first time by using a high resolution microscope [27]. In this work, N-H and O==C bonds were clearly shown in Figure 2.1.

Most of Type I Collagen is produced by packing 50-200nm diameter fibrils together (Figure 2.2). Collagen molecules adjacent to these fibrils are interchangeable. This three-dimensional motion determines the subtypes of fibrous collagen (type I, II,

III, IV). Short segments at the ends of collagen chains allow collagen fibrils to form. These short segments do not interfere with the three-dimensional structure. The cross-linked fibrillar structure formed by the segments creates a strong structure by arranging the collagen molecules side by side [28].

2.1.2 Sources of Collagen

Since it is the main component of ECM, many different living things can be used as collagen resources for the industry and research purposes Figure 2.3. There are

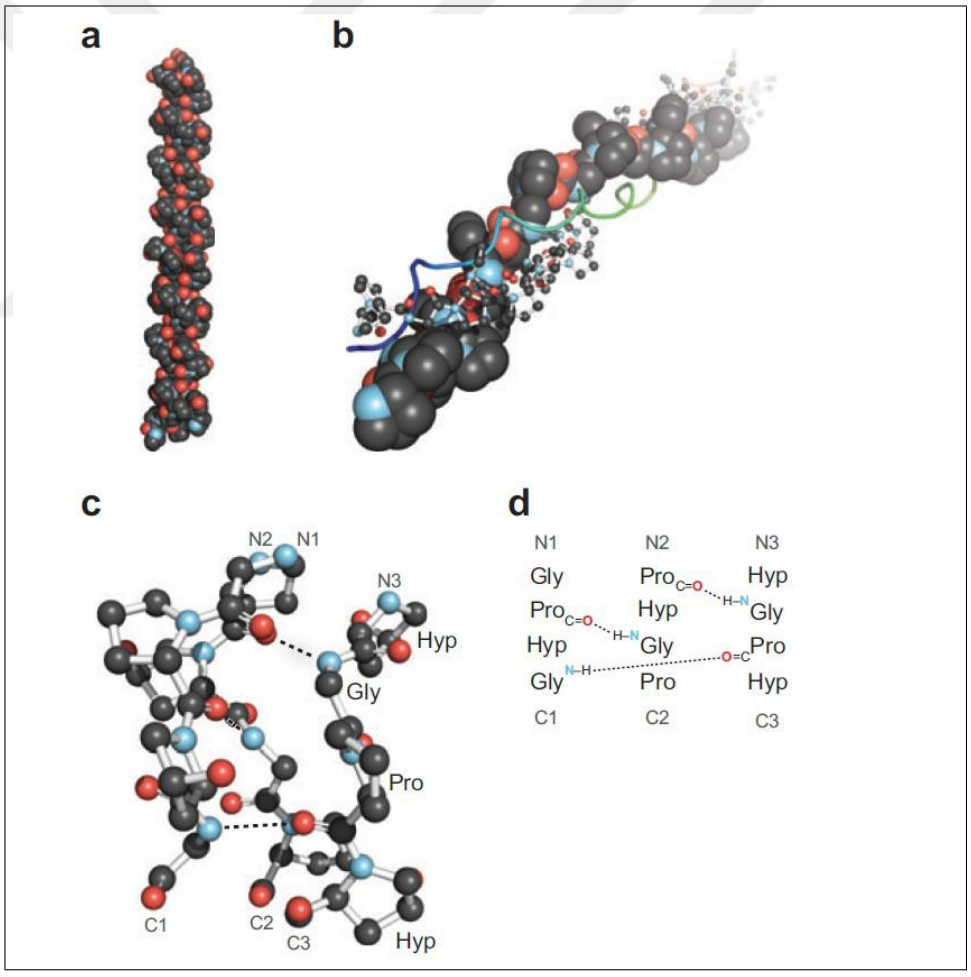


Figure 2.1 Triple Helical Structure of Collagen a-High Resolution Triple Helix Structure of the Crystallized Collagen, b-Ball-and-stick Representation of Triple Helical Structure of Collagen, c-Hydrogen Bonds are Emphasized Ball-and-Stick Representation, d-Staggering the Three Strands of c Representation [4].

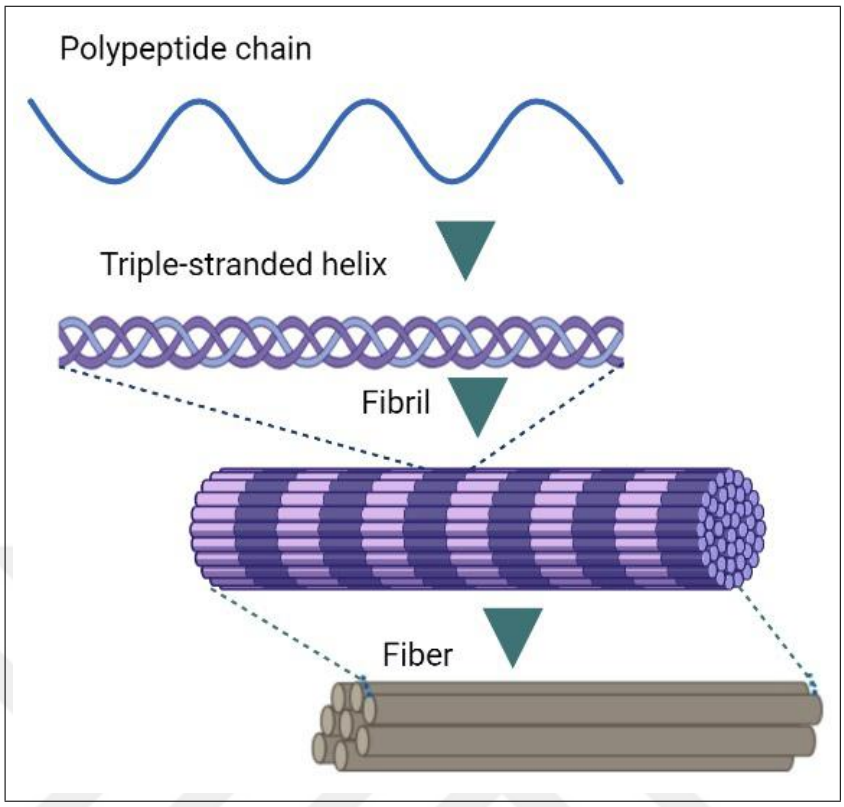


Figure 2.2 Structure of Collagen.

29 different types of collagen formed by the combination of 46 different polypeptide chains. All of these structures have a triple helix structure, but non-helical parts and sizes vary. The sources of the 5 most known types of collagen are [29]:

1. Collagen I: Mainly found in bone, tooth, ligament, vascular ligature, skin, teeth.
2. Collagen II: Mainly found in cartilage, eyes.
3. Collagen lll: Mainly found in fibers, blood vessels, muscle, skin.
4. Collagen IV: Basal lamina in the basement membrane.
5. Collagen V: Placenta, cell surfaces and hair.

One of the most well-known industrial sources of collagen is calfskin and bone. Although alternative sources are sought due to some animal diseases such as mad cow,

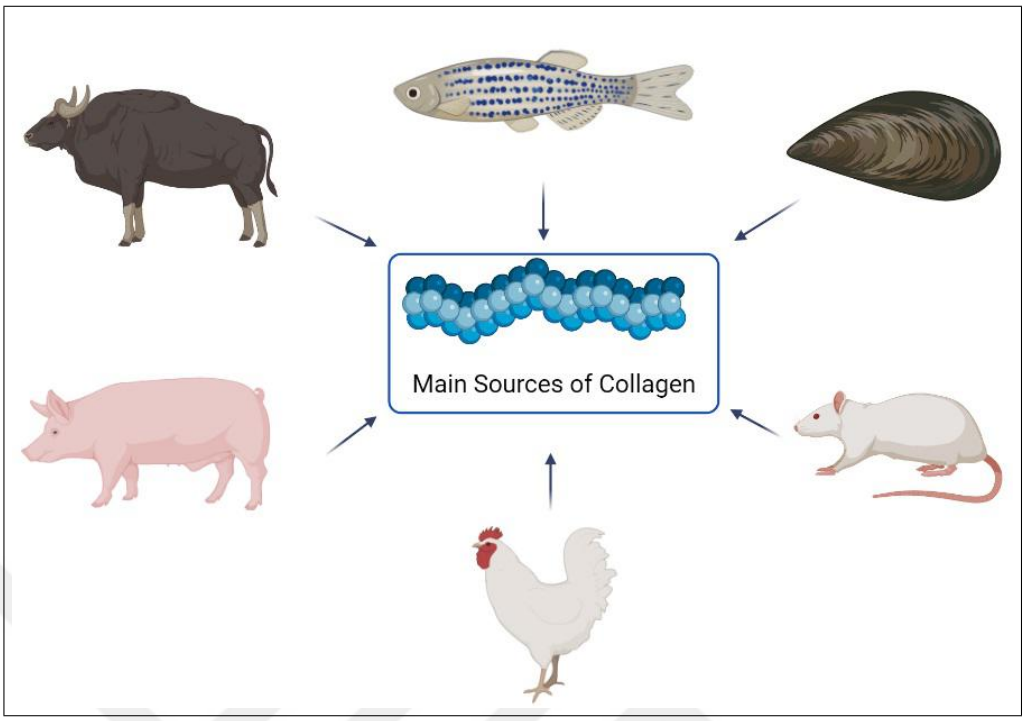


Figure 2.3 Different Collagen Sources.

the calf is still frequently used for collagen isolation. Another problem with calf-based products is that an average of 3% of people have allergies. Collagen isolated from bovine Achilles tendon, cartilage, skin, and bone structure are still used in industry, from biomedical products to food supplements. The skin and bones of pigs can also be used as a source of collagen on an industrial scale. The probability of an allergic reaction to pig collagen is much less. Collagen isolated from adult pigs are used in areas such as hernia repair, wound healing, plastic surgery [30].

Although industrial isolation methods are still being developed, marine source is considered as the most reliable source for collagen isolation. In general, collagen isolation from animal sources is a tiring, long and expensive method. The low yield (average 1 kg of raw material to 12 g collagen) has led researchers to marine resources. It is seen as an advantage that marine-based creatures do not contain many zoonotic diseases. Studies on allergy are still ongoing. The main advantages of marine-based collagen isolation can be listed as: high collagen content, more environmentally friendly, low molecular weight, high absorption, few ethical restrictions, and metabolic compatibil-

ity. Bone, fin, scales, skin, etc. structures of most fish used in the food industry are important sources for collagen isolation. Using the residues of fish produced for different purposes for collagen isolation is an environmentally friendly isolation method. Almost all vertebrate or invertebrate creatures can be used in marine-based collagen isolation [30]. Although marine-based collagen has many advantages, its high cost is a major limiting factor.

In addition to collagen sources isolated on an industrial scale, it has been shown that collagen can be successfully isolated from kangaroos, rats, crocodiles, birds, sheep, ducks, horses, frogs and humans. Since the isolation of recombinantly produced human collagen will have the lowest immune response, it is being investigated. Collagen produced from most of these animals remained at research scale and could not be switched to industrial scale. Collagen isolated from rat tail tendon is frequently used in laboratory scale. In addition, collagen can be isolated from invertebrates such as Neritacrepidularia or Archaeogastropod [30].

2.1.3 Mechanical Properties of Collagen

Mechanical properties of collagen are unique because it is found in many tissues such as tendons, bones, and ligaments. Collagen carries the tensile stress in a tissue. Collagen fibrils provide mechanical strength by bonding to tissues. It is thought that the mechanical strength property of the collagen molecule is highly dependent on its hierarchical structure [31].

In studies conducted on collagen-based tendons, it has been revealed that the structures mostly exhibit viscoelastic properties. Viscoelastic structure is thought to be effective in functionality, especially for tendon structure. In the literature, the Young's modulus of collagen fibers and dry tendon structures has been shown to vary between 1-8 GPa. On the other hand, The Young's moduli values of hydrated ones vary between 0.15-1 GPa. It was reported that macroscopic damage occurred in the tendon and collagen structure over 8-10% strain [31].

When shear stress was examined, it was reported that dehydrated type I collagen G = 33MPa, which is isolated from bovine Achilles tendon. In this study, it has been concluded that cross-linking increases shear modulus. However, it has been stated that excessive increase in cross-linking can make the tissue more fragile. This condition is known to be a common aging symptom [32].

2.1.4 Functional Properties of Collagen

Gel formation is one of the unique functional properties of collagen. Gel formation cannot be evaluated separately from viscosity because it is related to the system temperature and properties and size of the molecules. When gel formation starts collagen aggregates. This aggregation mainly depends on pH and temperature change and directly affects the fibril formation process. During gel formation, collagen dimer and trimer aggregate first. After the micro-fibrils begin to form, it spreads sideways. Type I collagen gel formation occurs at 28°C. Collagen gelation can be reversed by heat, but it is important not to reach a temperature that would disrupt the structure [33].

The surface property of collagen is determined by the presence of charged groups, hydrophilic or hydrophobic amino groups in the protein chain. The tendency of hydrophilic and hydrophobic molecules to move to the same surface reduces the surface tension in aqueous systems. This can be a factor that enhances gel formation [34].

2.1.5 Usage of Collagen for Biomedical Materials

Collagen is one of the most important materials used and researched in biomedical material technology. Collagen-based biomaterials can be examined in two groups: using the collagen-based structure remaining after decellularization of a tissue as a biomaterial or using the isolated collagen to create a different structure. The first group of biomaterials can be originated from animals or human body like grafts. The second group of materials, on the other hand, is important in how they are formed into a

functional structure rather than where they originate from [35].

Various forms of collagen are used as biomaterials in many studies, both in vivo and in vitro. Collagen scaffolds can be used to observe cellular responses (differentiation, migration, etc.) in research. Collagen is a widely used material for 3-dimensional cell culture experiments. In addition, collagen tissue scaffolds are frequently used in organ on chips in cases such as cancer research, drug release mechanisms, extracellular matrix imitation etc. [35].

As a biomedical solution, collagen is mostly used for the treatment of damage in parts such as bones, tendons, ligaments, skin, connective, and vascular tissues. There are many different methods for the treatment of these tissues. Collagen-based nanoparticle synthesis or collagen coating of various nanoparticles is one of these methods. Although there are many studies using collagen-based porous hydroxy-apatite (HA) tissue scaffold, this method is not preferred due to its cost. Modifying 3D collagen scaffold with HA nanoparticles can be considered as a solution [36].

Collagen gel form has various unique characteristics. Its characteristics such as biocompatibility, fluidity and injectability make it a strong candidate for delivery systems. The fact that non-fibrillar collagen has a small pore structure enables controlled release. Collagen-based controlled drug release can be used to against the infection in the bone and soft tissue [36].

Collagen can also be used in gene delivery by covalent attachment of plasmid DNA to the collagen matrix. Bone cell therapy can be provided, especially by the transport of proteins such as erythropoietin in the bone tissue. It has been demonstrated that DNA-loaded collagen sponges provide successful and stable gene transport [37].

2.2 Poly(vinyl Alcohol)

Poly (vinyl alcohol) (PVA), a water-soluble synthetic polymer, has a basic skeleton consisting of carbon atoms. It was discovered in 1924 by the saponification reaction between poly (vinyl alcohol) and poly (vinyl ester) [38]. PVA, which has a linear structure, is formed by hydrolyzing polyvinyl acetate and removing acetate groups from the structure as shown in Figure 2.4. Some of the properties of PVA may differ depending on hydrolyzation degree: physical, chemical, or mechanical properties. PVA is highly soluble in water. On the other hand, it does not have good solubility in most organic solvents. Crosslink is required for the formation of PVA hydrogels produced for use in various application areas. The degree of crosslink in the structure affects the physical, chemical, and diffusion properties of the hydrogel. For PVA hydrogels used in biological systems, all of these properties affect the behavior of the PVA in biological systems [39].

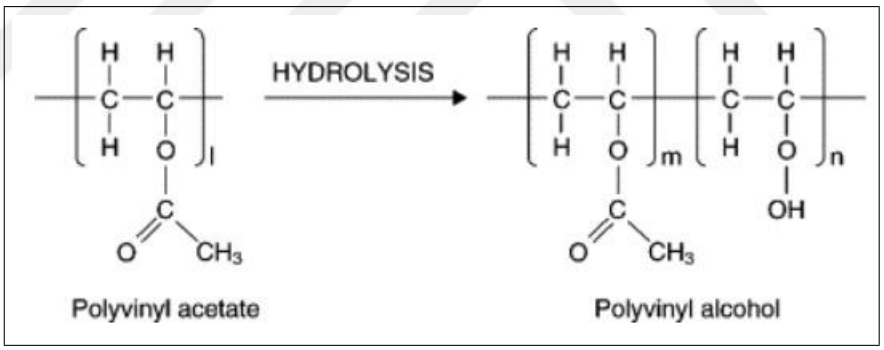


Figure 2.4 Formation of PVA [40].

Although it is a synthetic polymer, PVA shows nontoxic and noncarcinogenic characteristics. In addition, PVA has useful properties such as easy gel formation and good bio-adhesive properties. All these features enable PVA to be used frequently in industries such as cosmetics, pharmaceuticals, food, and paper. Additionally, the fact that it is a cheap material makes the use of PVA widespread. Its biodegradability and water solubility properties make PVA a successful candidate for degradable food packaging applications. The good interaction of PVA with different molecules (starch, chitosan, etc.) also makes it easy to modify for different purposes [41].

2.2.1 Usage of PVA as a Biomedical Material

Factors such as biodegradability, low toxicity and low cost have attracted the attention of researchers to use PVA as a biomedical material. PVA can be used in many areas from immobilization to antibacterial coating. PVA hydrogels, on the other hand, have many biomedical uses such as drug delivery, biosensors, implant surface coatings, etc. PVA hydrogels have also been used as contact lenses by their transparent structure and high moisture retention [38].

It is known that various PVA hydrogels and membranes are used in hemodialysis and artificial pancreas research. In addition, PVA can be used for biomedical applications such as synthetic vitreous humor. When examined in general, it is seen that PVA has a promising future in the synthesis of materials that can be implanted for the treatment of damage to cartilage and meniscus tissues. The main features of PVA that allow it to be used in medical products such as contact lenses are transparency, high tensile strength, and high elongation characteristics. These properties enable contact lenses made of PVA hydrogels to be used longer [39].

Implantable materials are not only use of PVA for medical industry. A mixture of PVA, polyethylene glycol and hydroxypropyl methylcellulose can be used as artificial tears. PVA can also be used in the coating of various medical implants. PVA coating is used in implantation to increase neurological regeneration [42]. Additionally, PVA can be used when mimicking natural tissues such as cartilage [43].

2.2.2 Usage of PVA in Tissue Engineering

The use of PVA in tissue engineering has become widespread, especially in recent years. Due to the fact that it is a cheap and accessible material, many research groups around the world conduct research on expanding the usage areas of various PVA forms in tissue engineering. It has been shown in the literature that pure PVA hydrogels have microarchitectures resembling healthy tissues [44]. In addition, the mechanical

properties of these hydrogels are similar to many tissues. This research has enabled the use of PVA in biomimicking research. Another research on biocompatibility has been carried out on freeze-thawed PVA hydrogels. It has been reported that PVA hydrogels form a suitable microenvironment for the growth and regeneration of human fibroblast cells [45]. This preliminary research attracted the attention of many researchers to tissue engineering usage of PVA. In another study, CO_2 solution was used to increase the pores of PVA hydrogels. In this study, it has been reported that the diffusivity of PVA hydrogels hydrolyzed with CO_2 increases [46].

2.2.3 Usage of PVA Nanofibers in Biomaterials Technology

Using PVA nanofibers as a tissue scaffold, many tissues (bone, skin, vascular, cornea, cartilage, neural etc.) have shown positive results in the controlled release of drugs, growth factors, DNA, etc. [47]. PVA nanofibers have often been used, especially for bone tissue repair and regeneration. In a study, the use of nanofibers produced by PVA and carboxymethyl chitin (CMC) as a tissue scaffold was investigated. It has been reported that Human Mesenchymal Stem Cells (hMSCs) show high adhesion and proliferation on these fibers [48]. In another study, nanofibers produced by mixing Polycaprolactone (PCL) and PVA polymers showed 70% higher cell attachment than fibers produced separately [49].

To mimic human bone structure, PVA and collagen mixture nanofibers were modified with nano-Hydroxyapatite. While the collagen structure added flexibility and resistance, Hydroxyapatite enabled the fibers to be formed more regularly. The resulting bone structure mimic nanofibers exhibited very close morphology to the bone in terms of mechanical and porosity properties [50].

PVA-based scaffolds are widely used in skin treatments. In the literature, it has been shown that nanofibers consisting of a mixture of PVA and gellan are used for skin tissue healing. It has been reported that nanofibers cross-linking between Gellan and PVA increase cell adhesion and proliferation on human dermal fibroblast cells [51].

Although not very common, nanofibers made of PVA and collagen combination are used as tissue scaffolding. Researchers looking to combine a controlled drug release with a tissue scaffold have produced PVA-Collagen nanofibers modified with zeolite and silica. When using Zeolites as drug carriers, the final product has been shown to prolong in vitro degradation and provide high cell adhesion in fibroblasts [52]. In a study using PVA-Chitosan nanofibers as a tissue scaffold, it was reported that the adhesion, proliferation, and migration of PC12 cells increased [53].

2.3 Electrospinning

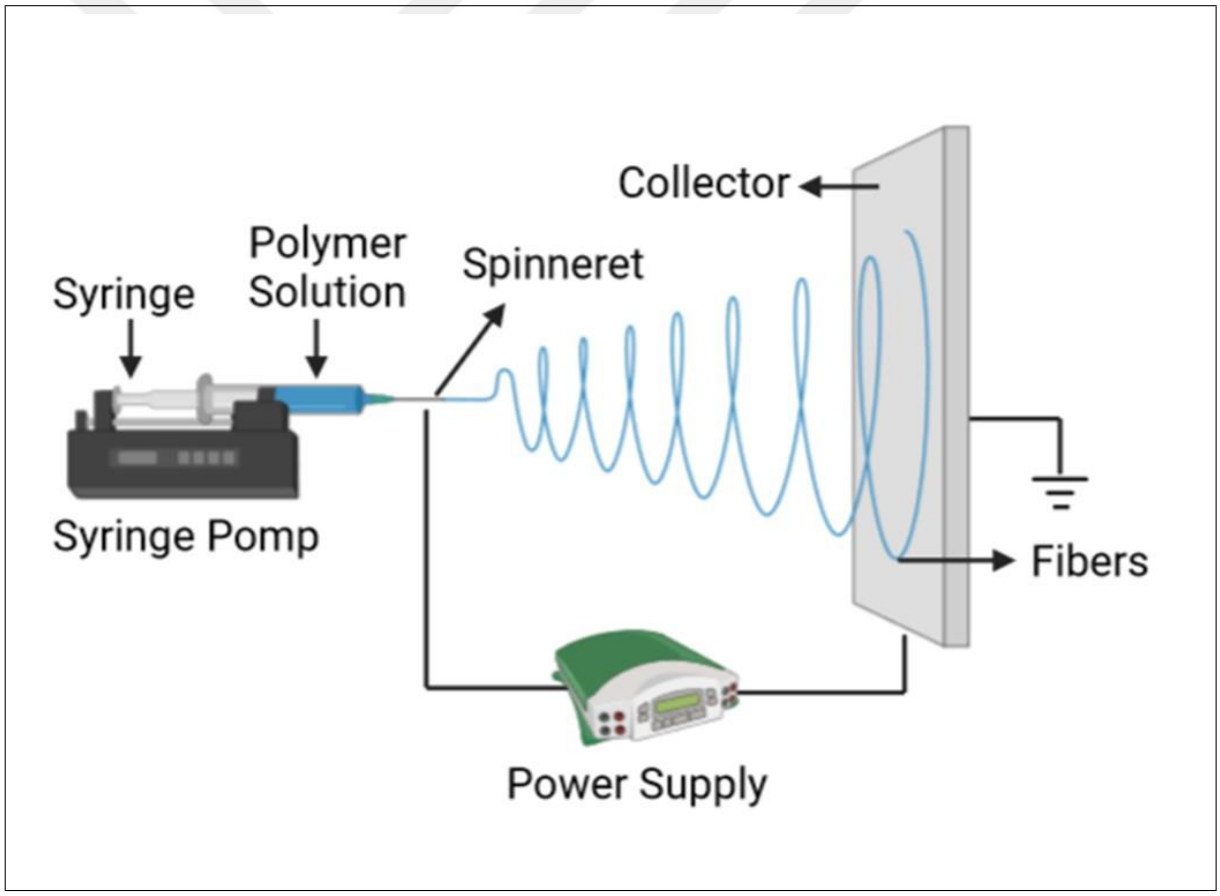


Figure 2.5 Principles of Electrospinning.

Electrospinning is a technique that enables continuous nanometer-diameter fiber production. Nanofiber can be formed from ceramics, polymers, metals, or composite materials with the electrospinning method [54]. With electrospinning, it is possible

to produce fibers of micron to nanometer diameter, depending on the materials and method used. The length of these very small-scale fibers can be several meters. One of the most important features of the method is that it has the ability to interfere with the fiber structure by changing its various parameters [55].

The electrospinning device consists of a high-voltage power supply, earthing unit, syringe pump and syringe as shown in Figure 2.5 This method, which is widely used in nanofiber production, is based on the principle of fiber formation from solution with high voltage electrical field. The solution coming from the syringe at a certain speed through the pump forms a conical structure at the tip of a needle. This structure, called the Taylor cone, forms fibers in a small diameter until the solution reaches the collector (Figure 2.6) [56]. After the liquid leaves the pump as a drop, the fluctuation in the electric field causes the fiber to spiral. During spiraling, the diameter of the fiber becomes smaller. Grounded substrate is usually made of aluminum [55].

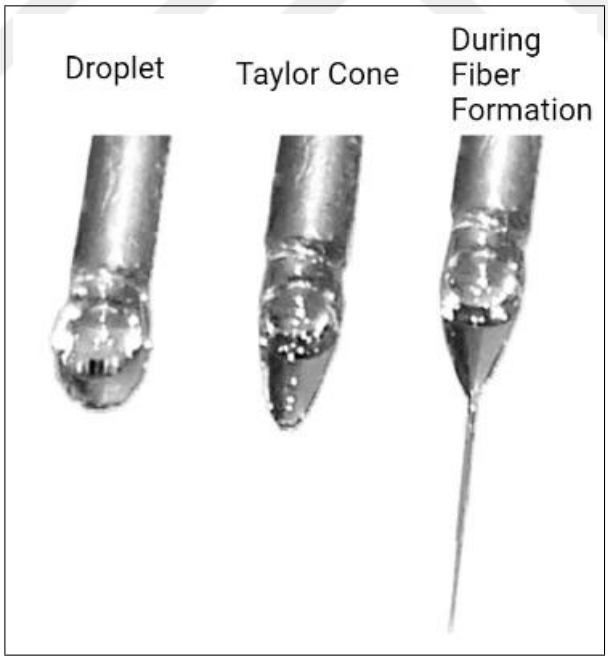


Figure 2.6 Tip of the Needle while Electrospinning Occurs [55].

Depending on the properties of the fiber to be synthesized, the electrospinning can be set up vertically or horizontally. In vertical electrospinning, the effect of gravity can also be utilized while the fiber is being collected, but it is also an undesirable factor

that unwanted drops reach the collector. In horizontal electrospinning, the fibers are more difficult to reach the collector and the distance between the collector and the needle tip is very important. The horizontal electrospinning setup preferred to be used in this thesis is shown in Figure 2.7.

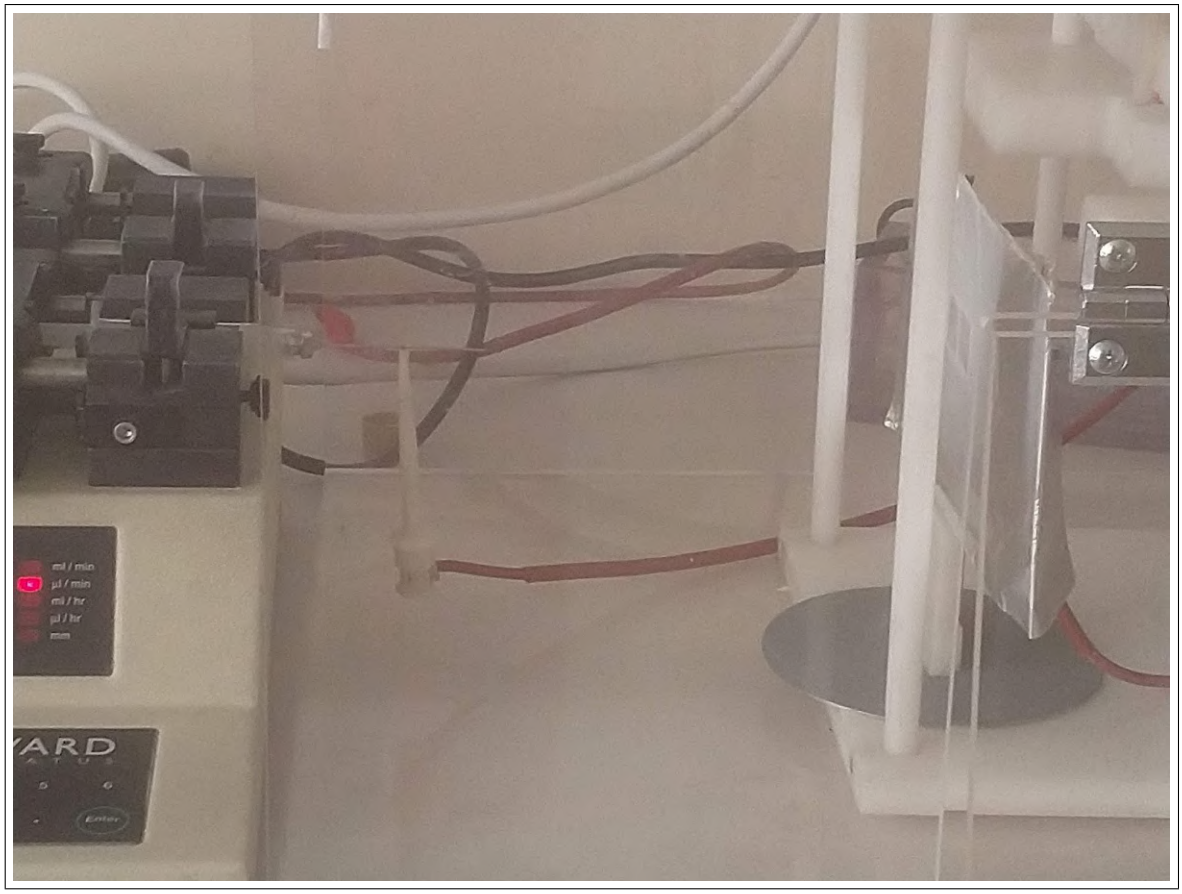


Figure 2.7 Horizontal Electrospinning Setup Used in this Thesis.

2.3.1 Electrospinning Parameters

There are many parameters that are important to pay attention to and to set the optimal in the electrospinning process. Electrospinning device parameters consist of electric field, distance between needle tip-collector, needle diameter, flow rate. Solution-dependent parameters are viscosity, concentration, and conductivity. In addition to all these parameters, there are also environmental parameters consisting of humidity and temperature. All of the parameters are very important in the quality of the fiber to be formed [56].

Voltage

The voltage delivered from a high-voltage power source is critical to turn the spherical droplet at the tip of the needle into a Taylor cone and initiate fiber formation. It is known that an increase in applied voltage generally lowers fiber diameters. However, each solution has optimal voltage values that can be applied [57]. For example, it has been shown that when nanofiber is formed by electrospinning from poly (ethylene oxide), the cone disappears and turns into bead form at high voltage values [58].

Solution Flow Rate

The flow of solution from the needle tip plays a primary role in determining the morphological properties of nanofibers. The flow rate must be optimal for the formation of a drop-free nanofiber. In the literature, it was observed that polymer droplets were formed on the fibers after the solution was pumped at a speed above a threshold in polystyrene nanofiber formation. In the same study, it was revealed that fiber diameters are also dependent on the flow rate of the polymer solution [59].

Distance Between Tip and Collector

The distance between the tip of the syringe needle and the collector plays a key role in the morphology of the fibers. Different distances can be used for each material depending on the deterioration, precipitation and evaporation times of the material used [60]. In order to ensure that the fibers are produced with similar morphology, the distance should not be changed during synthesis. In general, it has been observed that when the distance between the needle tip and the collector is kept short, a lot of drops can be produced and the fiber diameters are larger [61].

Polymer Concentration and Solution Viscosity

In electrospinning, a charged solution is stretched in one direction to create a

fiber. The concentration of the solution to which the electric charge is applied can affect whether or not fiber is formed. At low concentrations, the fiber structure cannot be formed and the solution can reach the collector in parts [62]. Even if fiber formation occurs at low concentrations, it can occur as short fiber fragments rather than as a whole. Generally, the increase in concentration increases the viscosity, resulting in a more uniform fiber. However, excessive increase in concentration and viscosity may cause the solution not to flow out of the needle tip. The solution whose concentration increases too much can clog and stop the fiber formation. There is a certain threshold for the concentration and viscosity of each solution [63].

Solution Conductivity

The conductivity of the solution used to produce nanofiber is effective in Taylor cone formation. In addition, the conductivity of the solution is also important for controlling the diameter of the nanofibers. A solution with very low conductivity remains as a droplet at the tip of the syringe and cannot become conical. Increasing conductivity up to a certain threshold provides a reduction in fiber diameter [64].

Solvent

In the electrospinning method, the liquid in which the polymer dissolves is also very important. Although the polymer dissolves well in a liquid, that solvent may not be suitable for electrospinning. The boiling point of the solvent is important for electrospinning because the volatility of the solution during fiber production is also an important factor [63].

Humidity and Temperature

Researchers producing nanofibers with electrospinning generally conducted research on setup and materials used. However, a factor that has been emphasized lately

is environmental conditions. There are studies showing that factors such as temperature and humidity of environment have effects on the diameter and morphology of nanofibers [65]. Humidity can cause the solution to solidify, changing its viscosity. As a result, the diameters of the fibers may vary and even the fiber may not be formed.

2.4 Coaxial Electrospinning

The traditional electrospinning method is a long-used nanofiber production method. Its long-term use has led to various research to improve the method. Coaxial electrospinning, which was introduced in the early 2000s, is a technique that continues to be developed. Coaxial electrospinning, which is based on passing more than one solution through the capillaries and forming a single fiber, has emerged with the modification of traditional electrospinning [66].

The most important feature of coaxial electrospinning is that the solutions used not only preserve their characteristics in traditional electrospinning, but also allow interactions between polymer-polymer, inorganic material-polymer, inorganic material-inorganic material. The possibility of these interactions has made coaxial electrospinning a promising method for the use of fibers in biomedical technology, nanoelectronics, drug carrier systems, etc. [67].

Coaxial electrospinning method can enable fiber formation by highlighting the best features of different solutions that can be used. For example, nano-topography of materials such as metal salts, enzymes or oligomers is very unique. However, their chemical properties are a limiting factor in fiber production. With the support of a polymer with coaxial electrospinning, it may be possible to produce a uniform fiber from these materials [68]. While it is not possible to produce nanofibers from Teflon, it has been achieved to produce Teflon nanofibers on inner PCL layer with coaxial electrospinning [69].

The coaxial electrospinning setup includes all the parts that traditional electro-

spinning contains, plus a core-shell nozzle and an additional syringe and pump (Figure 2.8). As in traditional electrospinning, in the presence of the electric field, the drop at the needle tip switches to Taylor cone and starts fiber formation [70].

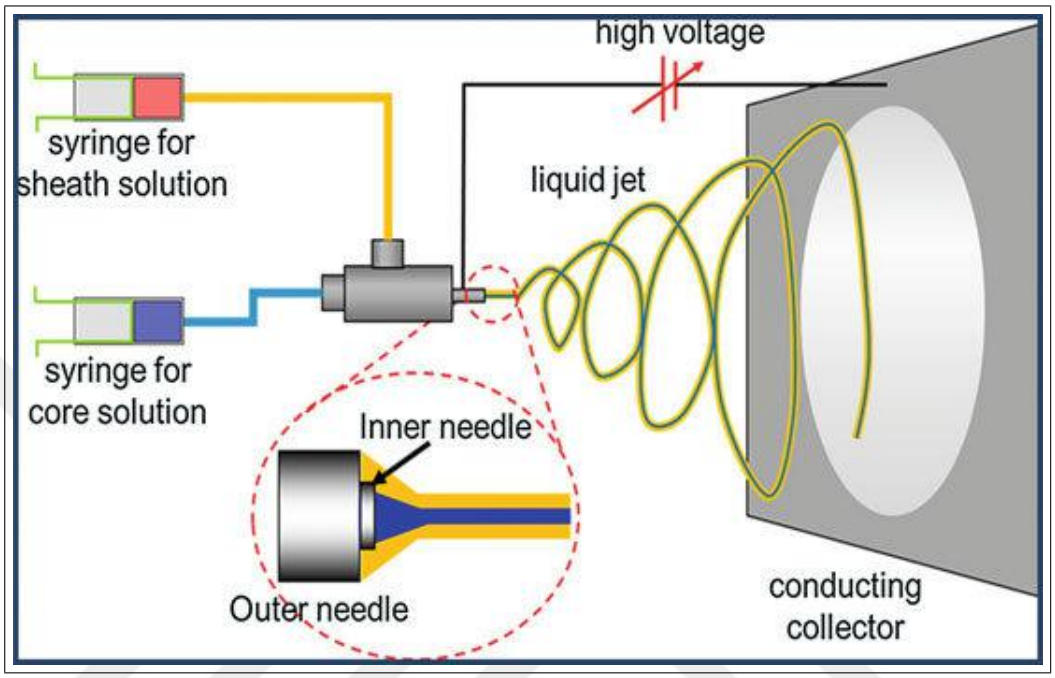


Figure 2.8 Setup of a Coaxial Electrospinning [71].

2.4.1 Drug Delivery and Tissue Engineering Applications

It was mentioned in the previous chapter that fibers produced by traditional electrospinning are frequently used in biomedical technology. Coaxial electrospinning, a modified version of the traditional method, has a wide range of uses. In biomedical technologies, core-shell fibers have application areas such as tissue/organ regeneration, DNA/drug/biomolecule carrier systems [72].

In core-shell fibers conducted in-vitro studies, it was shown that the fibers could release the drug in a controlled and continuous manner in wound healing [73]. In another study, it was shown that the lysosome preserves its biological activity by producing fiber from lysosome (inner) and PCL (outer) within coaxial electrospinning method [74]. Although having two or more layers is a great advantage in drug release systems,

studies have shown that at least 20% of the drug is released in the first stage (as soon as application occurs) [72]. Further research on coaxial electrospinning is needed to optimize this release profile.

Fiber structures are very useful in tissue scaffold forming in tissue engineering applications. Core-shell nanofibers are an advantageous tissue scaffold method since it is possible to dope various molecules into it. In one study, a growth factor (Platelet-Derived Growth Factor BB (PDGF-BB)) loaded into the coaxial fiber and significantly increased cell adhesion and growth [75]. In another study, core-shell fiber scaffold containing PCL/gelatin was reported to release dopped growth factor for more than 15 days [76]. Coaxial electrospinning is very promising in terms of allowing the use of materials that cannot be used due to their toxic properties, although they have excellent mechanical properties as tissue scaffolding. In the near future, it will be possible to use the mechanical advantages of the toxic material by using a non-toxic or even biocompatible polymer as the outer layer.

3. MATERIALS AND METHODS

3.1 Collagen Isolation from Calf Skin

The most important raw material of this thesis is collagen. For its use, collagen was extracted from one of the sources which was mentioned previously (Chapter 2.1.2 Sources of Collagen). Since it is widely used molecule in both biomedical and food industries, there are many different procedures in collagen extraction. In this thesis, a procedure was chosen from the Kazanci laboratory where collagen was extracted with high yield [77]. The calf skin used in our study was provided by my co-advisor Prof. Dr. Murat Kazanci.



Figure 3.1 Collagen Solution in Dialysis.

Frozen calf skin was cleaned of hair and adipose tissue with a lancet. After cleaning, the skin was cut into small pieces and removed to stock ($-20°C$). 20 g cleaned

skin was taken from the stock and minced smaller pieces. Approximately 300 mL (coverthe skin in the beaker) 0.1M NaOH was prepared for 20 g skin tissue and mixed together for 48h at 4°C. NaOH helps the skin to be cleaned from the remaining hair and adipose tissue. The skin pieces were washed 3 times with dH_2O and stored in 4°C. The skin pieces added into the solution containing 300 mL of 10 mM HCl and 1 g of Pepsin and mixed for 20 hours with a mechanical stirrer (All mixing was performed in ice 4°C, pH: 2 (pH was checked every hour)). Then, the skin pieces were removed from the solution and placed in stock (−20°C) to reuse. The solution was centrifuged at 10000 g for 20 minutes at 4°C. 2M Urea and 50mM NaCl were added to the supernatant and mixed. Tris-Edta was added until the pH:7 to stop the enzyme activity (All mixing was performed in ice). After the solution was filled into dialysis tubes (Figure 3.1), it was dialyzed in dH_2O at 4°C for 18 hours (Water was changed with clean dH_2O 3 times periodically). Collagen was taken out from the dialysis tubes in Figure 3.2 and frozen at −80°C for 24 hours. For dehydration, lyophilization was performed for 3 days at −65°C and collagen was obtained in sponge forms.

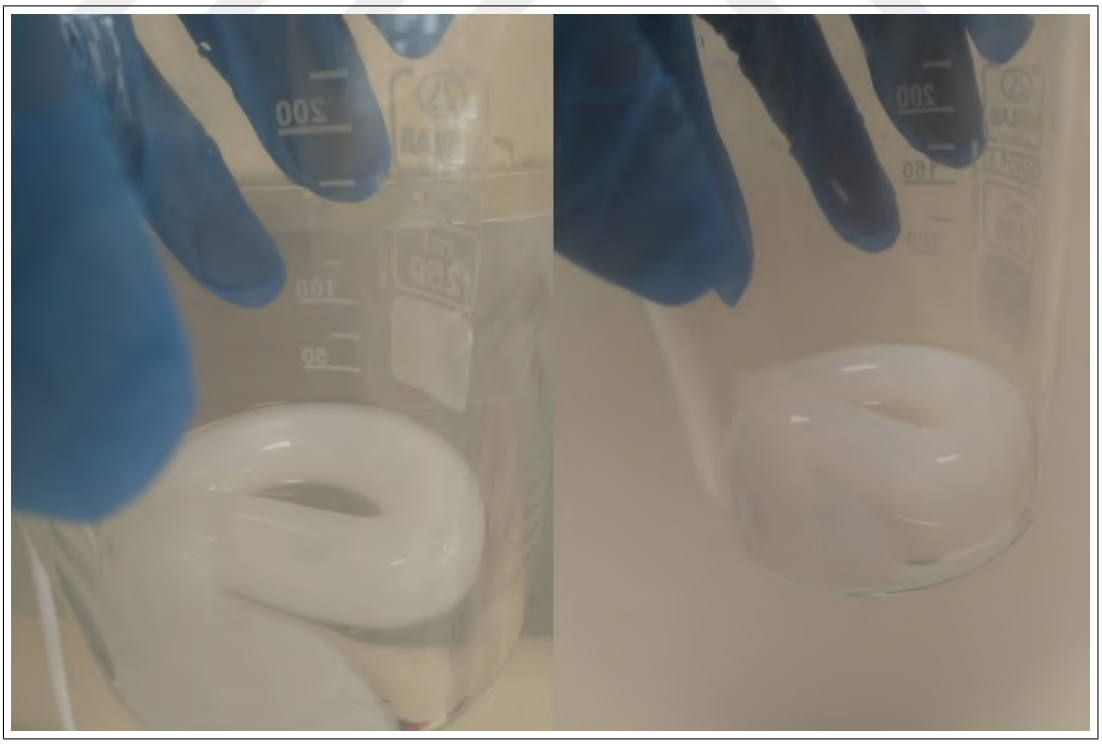


Figure 3.2 Collagen After Dialysis.

3.2 Electrospinning Process

3.2.1 Preparation of the Electrospinning Solutions

The importance of the properties of the solvents in electrospinning was mentioned in section 4.6. There is a possibility that the natural structure of collagen may deteriorate during preparation of solutions and electrospinning. In my thesis, Acetic Acid (AA), which was previously used in our research group and has been shown to protect the collagen structure better, comparing with organic solvents was employed [56],[77]. Collagen solution was prepared by dissolving 15% by weight (w/v) collagen in 90% AA.

PVA is a water-soluble polymer that has been used in nanofiber production for a long time. In the literature, nanofiber production at different PVA concentrations has been previously shown, and the most smooth and robust structure was produced by 10% PVA in dH_2O [78]. For PVA concentration, which I used as the outer layer of coaxial electrospinning, 8 % and 10 % PVA concentrations have been used and it has been decided to use 10 % PVA.

3.2.2 Production of Nanofibers

We employed three different nanofiber production techniques:

1. Collagen and PVA nanofibers were separately dissolved and nanofibers were obtained
2. Collagen and PVA were mixed in a solution
3. PVA coated collagen nanofibers were obtained by coaxial method

Production of Collagen Nanofibers and PVA Nanofibers

Collagen nanofibers were produced using traditional electrospinning setup were used as control group. Collagen nanofiber production method has been taken from the previous papers of our laboratory [77]. The collagen solution specified in the previous step was transferred into a 3 mL syringe. Specially produced needle for electrospinning was attached to the tip of the syringe. The syringe was placed on a pump with adjustable pumping speed rate per unit time and its speed was set at 0.5 uL/min. A power supply was connected to the middle of the needle with an electrode and a voltage of 17-18 kW was applied in fiber production. During the process, the outer temperature was kept in the range of 23°C($\pm$1). Collector was covered with aluminum foil and fibers were collected on the foil. Collector- needle tip distance was set as 10 cm. At the end, collagen nanofibers were successfully produced.

PVA nanofibers were produced using traditional electrospinning setup and used as the control group. 10 % PVA solution was used for nanofiber production. Collagen nanofiber production parameters (Voltage, temperature, speed, and distance) were kept same for PVA nanofiber production. As a result, PVA nanofiber production has been successfully performed.

Production of Collagen-PVA Mixed Nanofibers

Collagen-PVA mixed nanofibers were produced by traditional electrospinning setup as the third comparison set. Mixed nanofibers were produced, as it was decided to compare them with the nanofibers that were produced by coaxial electrospinning method. The Collagen and PVA solutions were mixed 1: 1 volume ratio for 2 hours. Parameters (Voltage, temperature, speed, and distance) were kept same for Collagen-PVA mixed nanofiber production. As a result, Collagen-PVA nanofiber production has been successfully performed.

PVA Coated Collagen Nanofibers by Coaxial Method

Collagen-PVA coaxial nanofibers were produced by using the coaxial electro-

spinning setup. After filling the collagen solution into the inner part syringe and the PVA solution into the outer part syringe, both syringes were placed in the syringe pump. Figure 3.3 A power supply was connected to the middle of the coaxial needle with an electrode and a voltage of 17 kW was supplied during the fiber production process. Temperature and distance were kept same as in the other methods. 2 different pumping speed were employed. First, the speed of inner and outer solution was set 0.1 uL/min. The new pumping speed was determined due to the excessive droplet formation during electrospinning and the ratio of 1: 4 between the inner-outer coaxial needle tips. The pumping speed of the inner solution (Collagen) was set 0.4 uL/min and outer solution (PVA) 0.1 uL/min. Collagen-PVA coaxial nanofiber production was achieved by using these parameters.

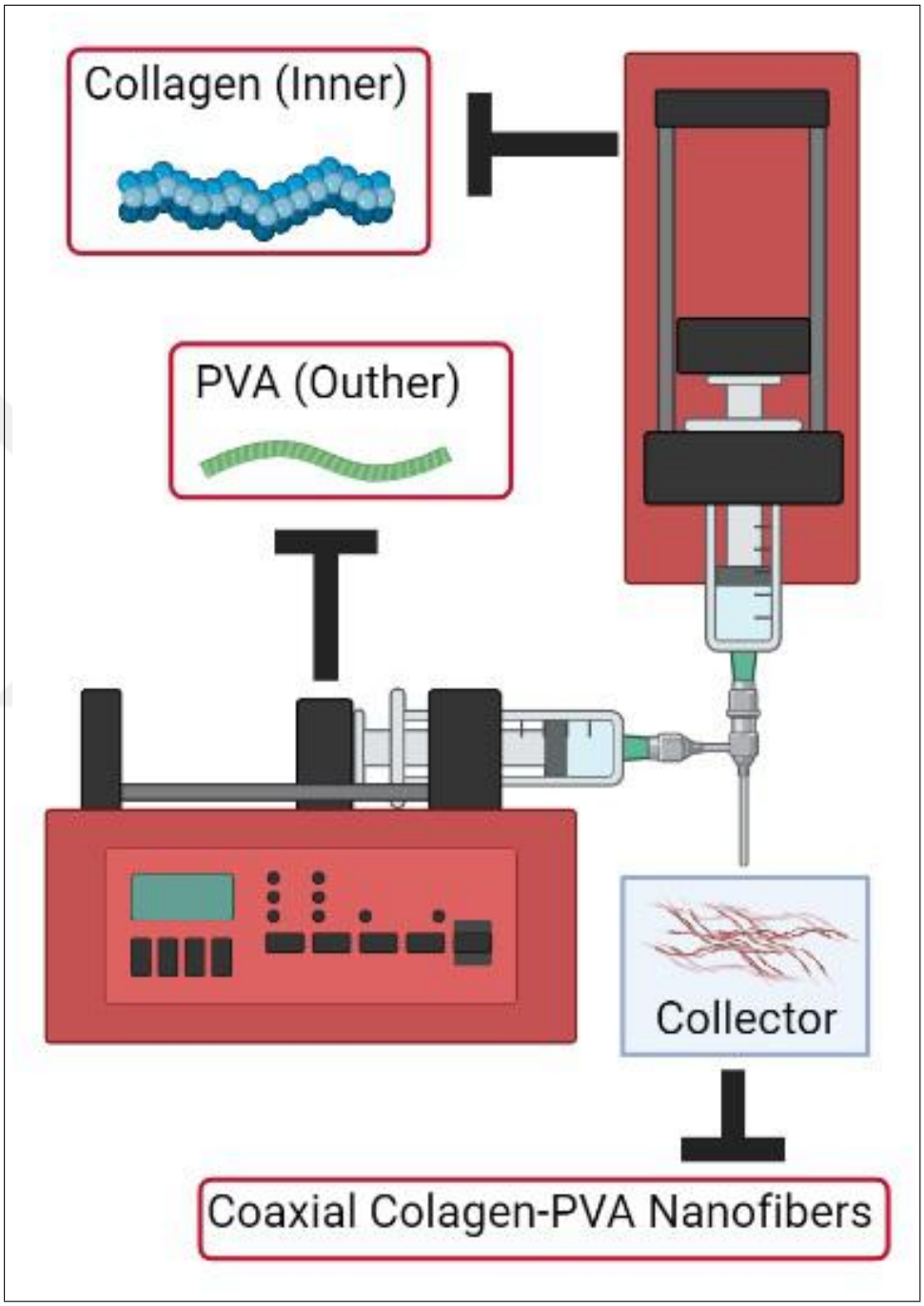


Figure 3.3 Experimental Setup of Coaxial Nanofibers Production.

3.2.3 Characterization Techniques

Nanofibers were characterized by various techniques such as optical (Light Microscopy), morphological (Scanning Electron Microscopy (SEM)) and thermal (Thermogravimetric analysis).

Fourier Transform Infrared Spectrophotometer (FT-IR)

Infrared (IR) spectroscopy is a test technique used in the characterization of substances that are organic or inorganic compounds. FT-IR can determine the chemical structure of a molecule by measuring the absorbance of light at different frequencies. An IR spectrum represents the fingerprint of a sample, with absorption peaks corresponding to the frequencies of vibrations between the bonds of the atoms that make up the material. Because each material is a unique combination of atoms, it is impossible to produce same IR spectrum for two different compounds. It can be applied to the analysis of solids, liquids, and gases [56].

In this study, the FT-IR (Perkin Elmer Spectrum Two) was used to observe the ingredients in nanofibers and to show that electrospun nanofibers contain PVA and collagen molecules. Spectra was performed in the range of 4000-400 cm^{-1} wave number and taken the average of 6 scanning. For the sample preparation, nanofibers collected on aluminum foil were taken by forceps.

Scanning Electron Microscopy(SEM) Imaging

SEM is a device used to view the surface down to the size of sub-micrometers. After the nanofibers were produced in this study, SEM (Thermo Scientific Quattro S) was used to visually reveal the differences between the morphologies of all 4 groups of fibers collected on the aluminum foil(Collagen, PVA, Collagen-PVA mixed, Coaxial Collagen-PVA).

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is an analysis method that shows the temperature-related change in material weight. In this thesis, TGA (Perkin Elmer STA 8000) has been used to demonstrate the temperature dependent degradation of nanofibers in order to compare different fiber groups. Samples were prepared by isolating nanofibers on the aluminum foil. Each sample was first weighed to determine the initial weight. Data for each group was normalized according to initial weight.

4. RESULTS

4.1 FT-IR Analysis

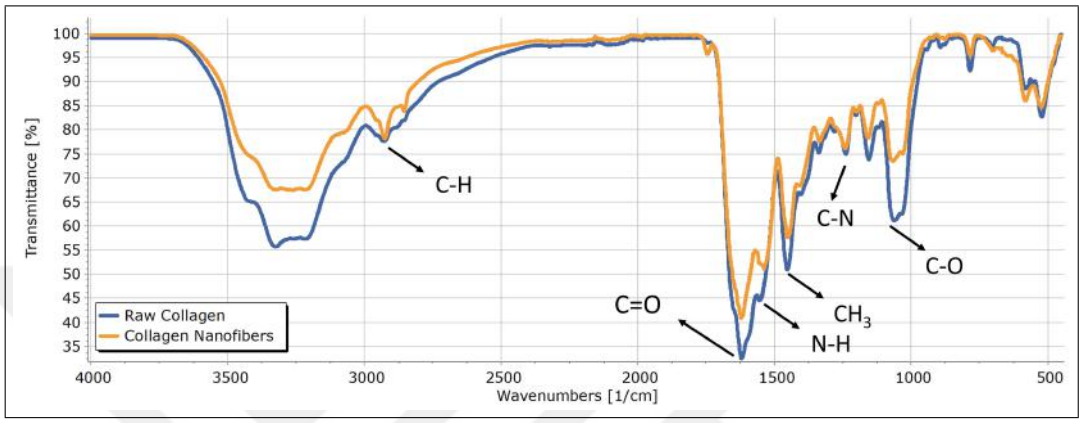


Figure 4.1 FT-IR Spectra of Raw Collagen and Collagen Nanofiber.

The Figure 4.1 shows the comparison of spectra results of raw collagen isolated from calf skin(blue) and collagen nanofibers (orange). The peaks shown here provide the information about the different physical state of collagen molecules. The most decisive peak for collagen is the C=O point at 1620 cm $^{-1}$ which is the finger print region for collagen proteins [56]. This point is called Amide I and is the most prominent peak in the FT-IR spectra of collagen structures. This point can be seen in both raw collagen and electrospun collagen nanofibers. The N-H interaction at the 1530 cm^{-1} called as Amide II band, which is characteristic for collagen. C-N at 1280 cm^{-1} and N-H at 1160 cm^{-1} are among the characteristic peaks of collagen called Amide III band. The peak is caused by the C-H vibration is observed at 2900 cm^{-1}. In 1440 and 1530 cm^{-1}, it is seen that there are extra C and H vibrations. The triple-helix structure of collagen is established by Hydrogen bonds. The severity of the peaks where C-H interactions occur can give an idea about the deterioration of the structure [79]. If the intensity of the C-H peaks decreases, the structure can be said to be largely denatured. In the C-H interaction found in 2900 cm^{-1}, the peak of the nanofiber and the peak of the raw collagen are almost the same, but it is observed that the intensity of the peak decreases in the other C-H interactions, 1440 cm^{-1} and 1530 cm^{-1}.

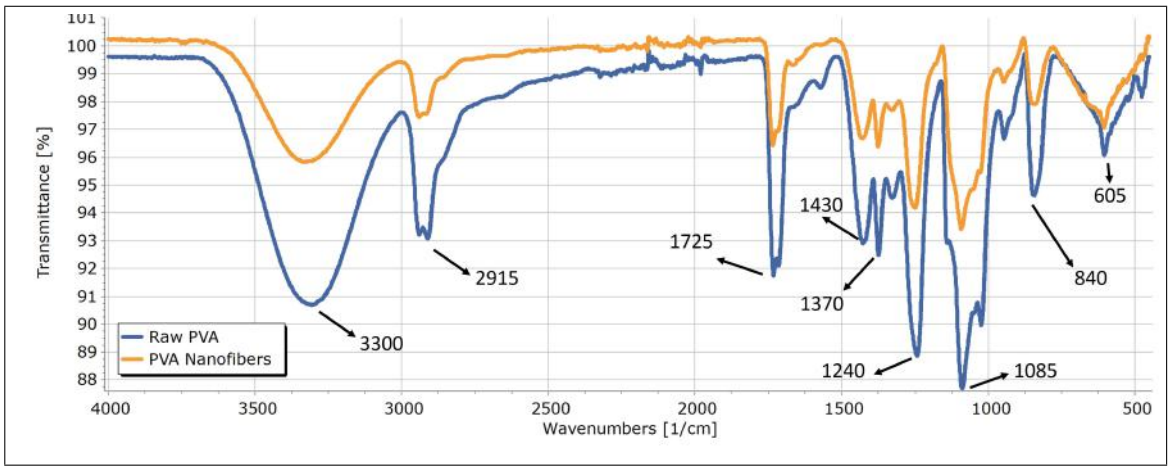


Figure 4.2 FT-IR Spectra of Raw PVA and PVA Nanofiber.

An industrial grade PVA (Akbel Kimya) was used in this thesis for the production of nanofibers. Figure 4.2 shows the FT-IR spectra results of raw PVA and PVA nanofiber. It shows similar peaks when compared to other PVA FT-IR results used in the literature [80]. At 3300 cm^{-1} point, both raw PVA and PVA nanofibers showed a strong peak. The peak at this point is due to the vibration of the hydroxyl groups in the O-H interaction. The peak at 1725 cm^{-1}, which is characteristic for PVA [80], is the C=C vibration. The strong peak seen in 1085 cm^{-1} indicates C-O acetyl group interaction. This sharp peak is characteristic for PVA and can be observed in both raw and nanofiber forms. CH_2 vibration is seen at 1430 and 1370 cm^{-1}.

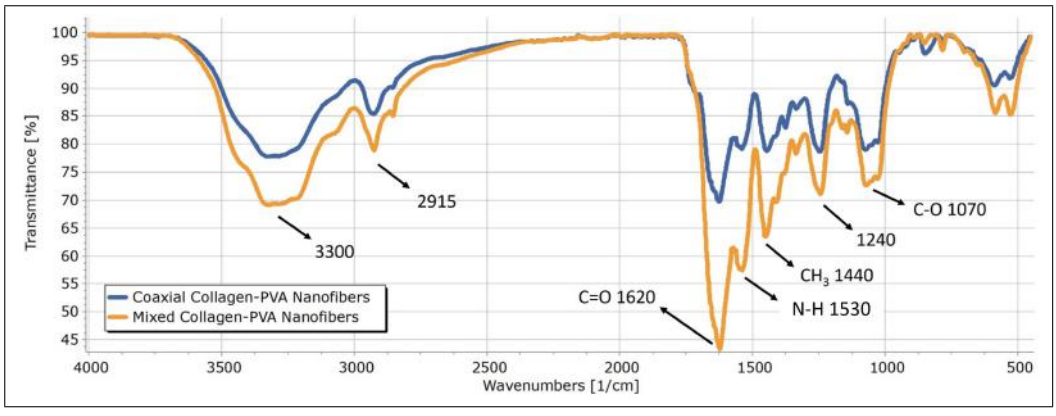


Figure 4.3 FT-IR Spectra of Coaxial Nanofiber and Mixed Nanofiber.

In Figure 4.3, coaxial Collagen-PVA nanofibers are compared with mixed Collagen-PVA nanofibers as a control group. It is possible to see the characteristic peaks of

collagen and PVA in two nanofiber structures (coaxial and mixed). In addition to the characteristic Collagen peaks: 1620 cm^{-1}, 1530 cm^{-1}, 1440 cm^{-1}, and 1070 cm^{-1}, characteristic PVA peaks: 3300 cm^{-1}, 2915 cm^{-1}, and 1240 cm^{-1} can be observed. In the experimental design, we were not sure whether both materials equally are contributed to fiber formation or the structures would be dominated by one of them. However, FT-IR result shows the peaks of collagen and PVA, so it can be said that both are present in the nanofiber structures. FT-IR analyses does not show the differences between mixed collagen-PVA nanofibers and coaxial collagen-PVA nanofibers.

4.2 Optical Microscopy Images

Figure 4.4 shows optical microscope images of collagen, PVA, mixed collagen-PVA and coaxial collagen-PVA nanofibers. Although the diameters and lengths of collagen nanofibers in Figure 4.4, A and B are of sufficient, they do not provide clear images. There are problems in illustrating collagen fiber structures. On the other hand, it is seen that nanofibers produced from PVA (Figure 4.4, C and D), have a smooth, long, and rigid structure. Fibers formed by mixing Collagen and PVA in equal proportions (Figure 4.4, E and F) are not successful in forming a uniform structure. As a result of the low chemical interactions of Collagen and PVA, it is difficult to create a standard nanofiber surface by mixing both. Coaxial nanofibers synthesized with collagen in the inner and PVA in the outer layer (Figure 4.4, G and H) are seen as a smooth structure. According to the optical microscope images (Especially Figure 4.4, H), the outer layer and inner layer of the fiber shine differently. This light difference can be interpreted as the coaxial structure has been successfully formed. In addition, when we compare coaxial collagen-PVA nanofibers to collagen nanofibers and mixed collagen-PVA nanofibers, it is seen that they have a much more uniform structure.

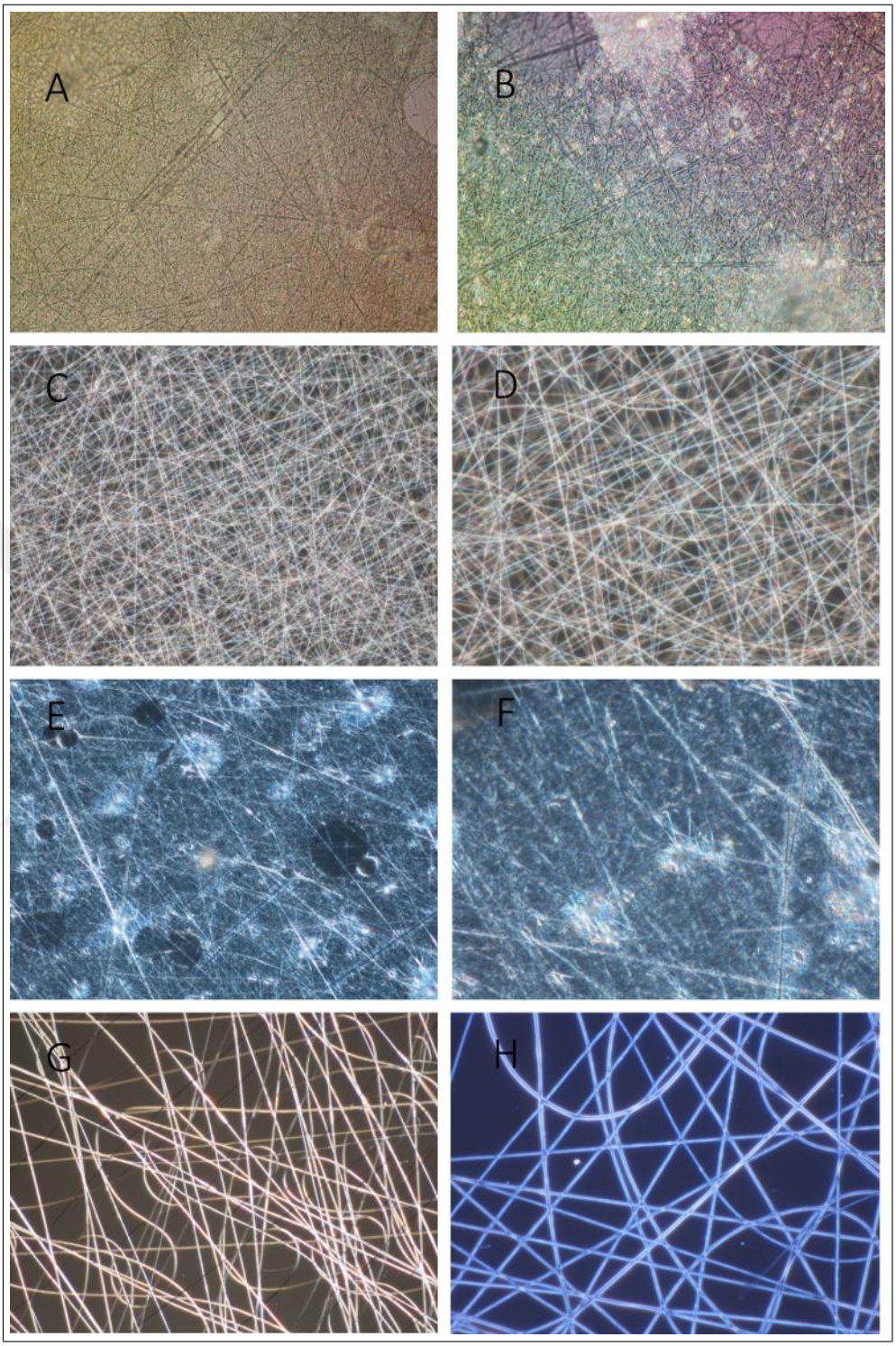


Figure 4.4 Optical Images of Nanofibers A) Collagen Nanofiber 50X (brigthfield), B) Collagen Nanofiber 100X (brigthfield), C) PVA Nanofiber 50X (brigthfield), D) PVA Nanofiber 100X (brigthfield), E) Mixed Collagen-PVA Nanofiber 50X (darkfield), F) Mixed Collagen-PVA Nanofiber 100X (darkfield), G) Coaxial Collagen-PVA Nanofiber 50X (darkfield), H) Coaxial Collagen-PVA Nanofiber 100X (darkfield).

4.3 Scanning Electron Microscopy (SEM) Images

Uniform collagen nanofibers (Figure 4.8) are not easy to produce even in the same bunch. We obtained long continues nanofibers. The diameters of the fibers range from 150 nm to 700 nm. Diameter range is very wide. We have demonstrated that we can produce short diameter collagen fibers. However, large diameter nanofibers can also occur in the same group. PVA is a very useful polymer to syntheses standard fibers. Industrial PVA also revealed a smooth nanofibers (Figure 4.6) that have similar morphologies as in the literature. The nanofibers formed in the mixture of Collagen and PVA (Figure 4.7) were produced in order to compare them with the coaxial nanofibers. The synthesized mixed fiber structures are not uniform (different diameters in the same fiber bunch). Collagen-PVA coaxial nanofibers were able to maintain a smooth nanofiber structure even in long time production. The diameters of the synthesized nanofibers are generally in closer range the other samples. Nanofibers with diameters in the 250 nm-540 nm range are shown in Figure 4.8.

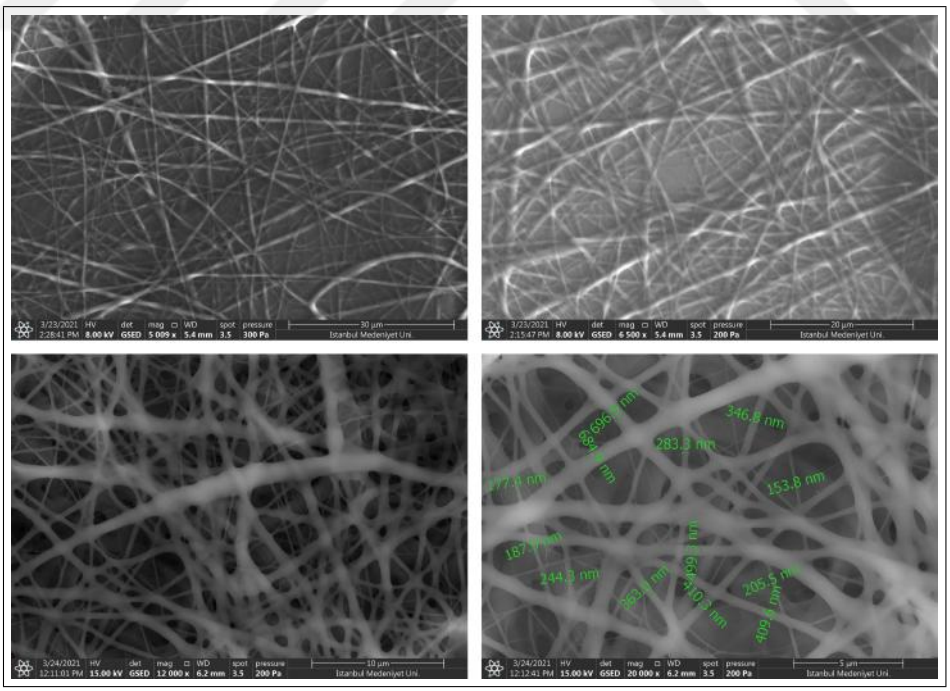


Figure 4.5 SEM Images of Collagen Nanofibers.

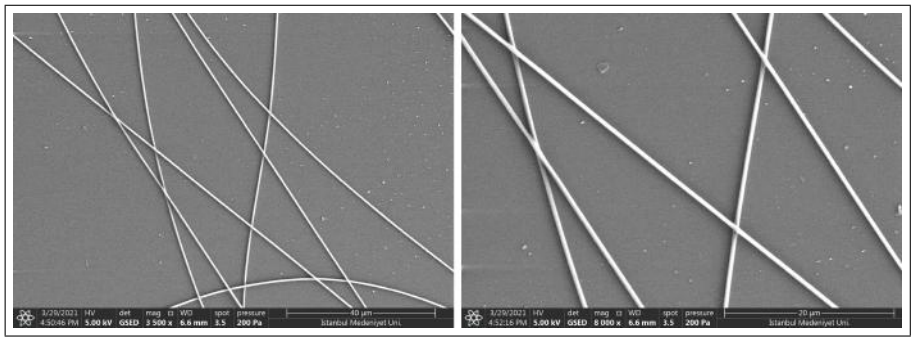


Figure 4.6 SEM Images of PVA Nanofibers.

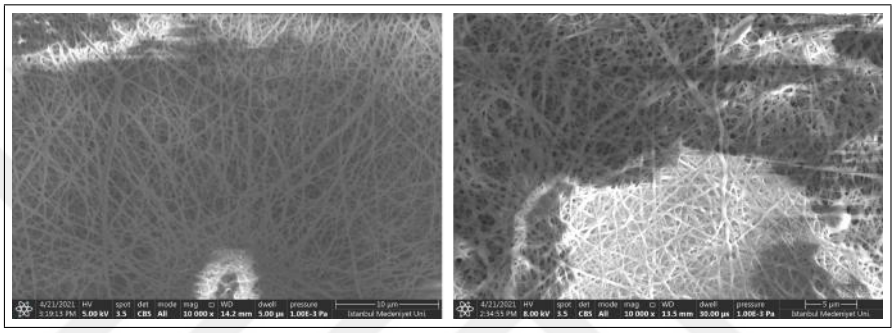


Figure 4.7 SEM Images of Mixed Collagen-PVA Nanofibers.

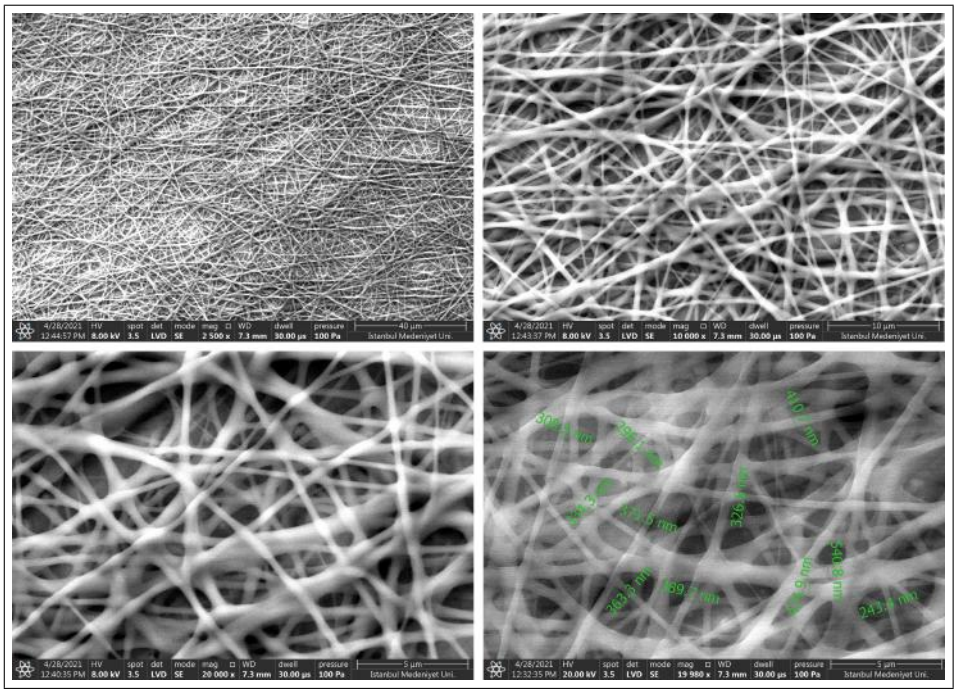


Figure 4.8 SEM Images of Coaxial Collagen-PVA Nanofibers.

4.3.1 Diameter Analysis

Diameter measurements were made for each nanofiber types (Collagen, PVA, Mixed, and Coaxial) from SEM images. (Figure 4.9) It was observed that the mean diameter of coaxial collagen-PVA nanofibers was between collagen nanofibers and PVA nanofibers. Unpaired t test was performed to understand the level of differences between all groups using GraphPad Prism 9.1.2. software. There is a significant difference between coaxial nanofibers and collagen nanofibers (P value <0.0001). There is a significant difference between PVA nanofibers, which have the largest mean diameter, and coaxial nanofibers (P value <0.0001). There is a significant difference between mixed nanofibers with Collagen and PVA solutions at a ratio of 1:1 and coaxial nanofibers (P value <0.05). Since they are PVA coated fibers, it was expected that the coaxial nanofibers would be thicker than the collagen nanofibers. However, the mean diameters of coaxial nanofibers (367.5 nm) are not as thick as PVA nanofibers (648.9 nm).

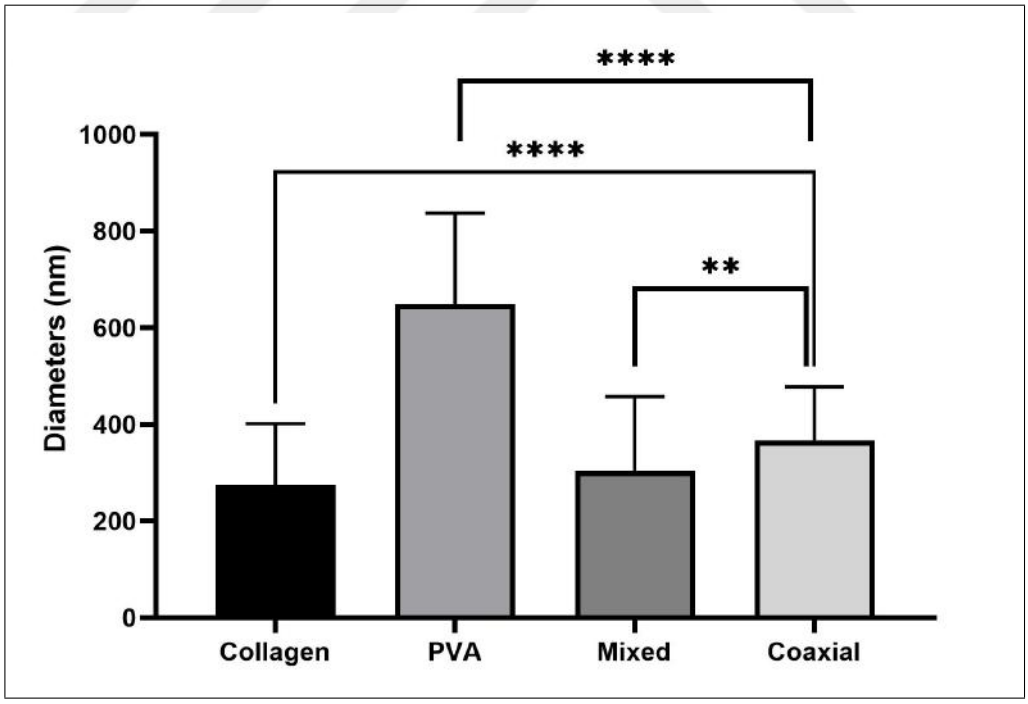


Figure 4.9 Comparison of Mean Diameters of Nanofibers.

4.4 Thermogravimetric Analysis (TGA)

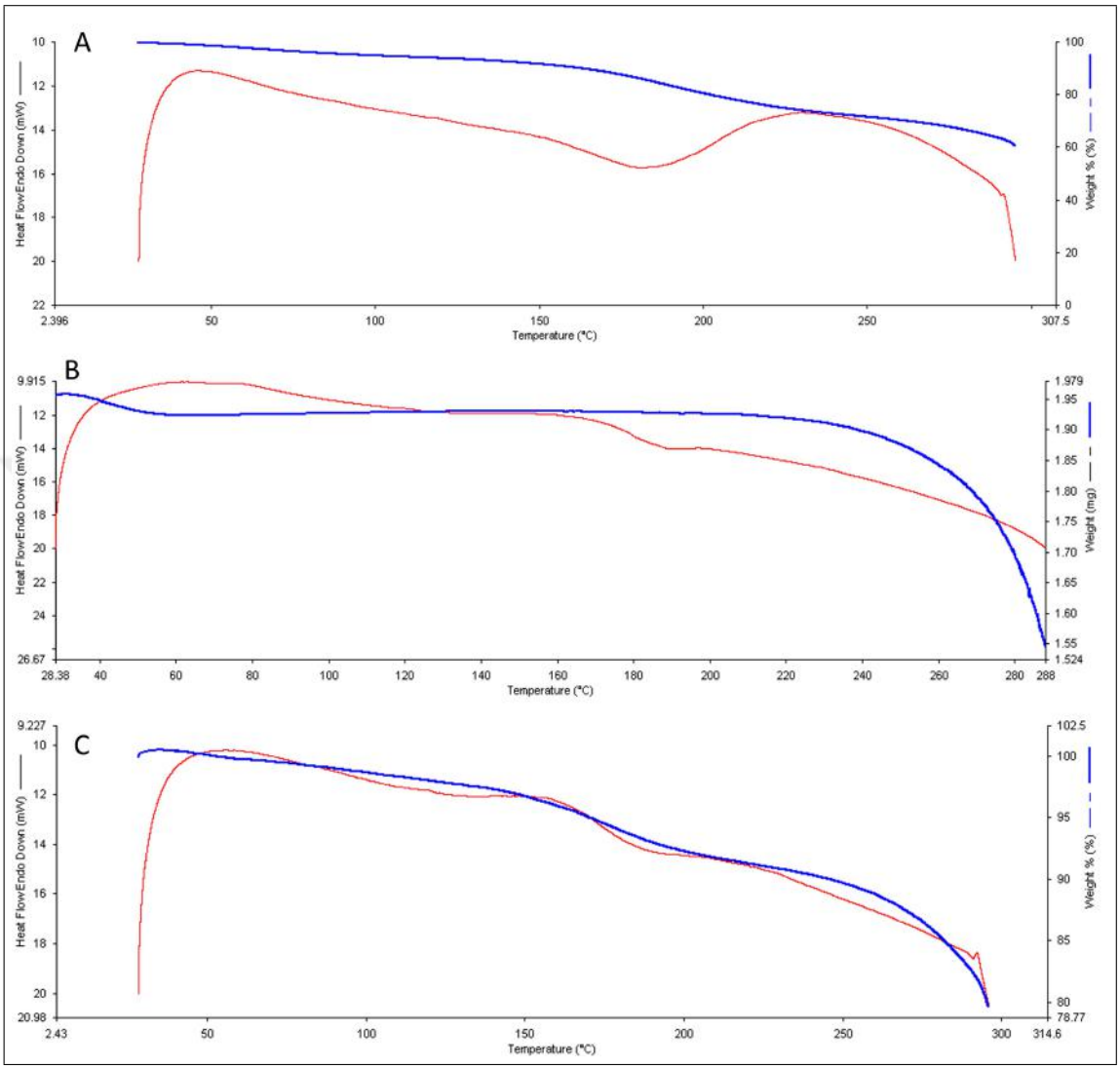


Figure 4.10 TGA Results of Nanofibers A) Collagen nanofibers B) PVA nanofibers C) Collagen-PVA coaxial nanofibers.

Collagen nanofiber, PVA nanofiber and collagen-PVA coaxial nanofiber TGA graphs are given separately (Figure 4.10). In the literature [79], it has been shown that the weight of collagen decreases from 75-80°C to 190-200°C on the TGA graph. The weight change of the collagen nanofiber synthesized as the control group between these temperatures is consistent with the literature (Figure 4.10, A). Some of the water molecules in the structure were lost in the range of 50-200°C in collagen nanofibers. However, the marginal water loss in the structure occurred after 230°C. When the thermal change of PVA is examined, it is seen that it is not affected by the temper-

ature up to 230-240 C. At temperatures above 240°C, there is rapid deterioration in the structure. The TGA plot of PVA nanofibers (Figure 4.10, B) shows results consistent with the literature [81]. The marginal decrease in the graph after 230°C can be explained by the loss of water content in PVA nanofibers. In the Collagen-PVA coaxial nanofiber TGA graph (Figure 4.10, C), it is interpreted that the weight loss up to 190°C is caused by collagen. It is interpreted that the marginal weight loss after the peak at 190°C is due to the more heat resistant polymer, PVA. The thermal behavior of the two polymers, which are not very far from each other, made inferences difficult. During the production of Collagen-PVA coaxial nanofiber, two polymers are coated by physical interaction. Since there was no chemical interaction during this coating [82], it was interpreted that the two polymers showed separate characteristics in the TGA results.

5. DISCUSSION AND CONCLUSIONS

Although the production of collagen, which has a wide range of biomedical use, has been studied for years, many different sources and methods have been used. Today, different methods continue to be used and there is no fixed procedure. Each method and source have different advantages and disadvantages. It has been shown that the collagen we need can be obtained with sufficient purity by the procedure that has been used in our laboratory [18]. This thesis also provides the recycling of animal skin residue. During recycling, the collagen in its structure was isolated to use as a biomaterial.

FT-IR analysis was performed in order to compare the characteristic features of the isolated collagen with those in the literature and to make a positive control. According to the FT-IR analysis data, it was revealed that the isolated calf skin-derived collagen in this thesis showed the same characteristic vibrations as the other isolated collagens in the literature [56]. Collagen nanofiber and bulk collagen were compared by FT-IR analysis to determine how the structure of collagen nanofibers changed during nanofibers synthesis. It was observed that there was no major change in the structure since both structures gives very similar spectra. However, the low intensity of vibrations of collagen nanofibers at the FT-IR peak points indicates that the 3D structure of collagen is disrupted while the fibers are formed due to applied electrical field.

Similarly, FT-IR was used for structural comparison of industrial grade PVA and PVA nanofibers. Although the peak points did not change as expected, the weaker peaks of PVA nanofibers can be interpreted as obtaining PVA in elongated fiber forms during electrospinning. It is seen that both collagen nanofibers and PVA nanofibers produced using conventional electrospinning cause physical changes in the 3D structure.

It was aimed to coat collagen nanofibers with PVA by using the coaxial electro-

spinning method, which was created by modifying the traditional electrospinning tip. FT-IR was used to determine the contents of coaxial nanofibers. It was determined that the mixed collagen PVA nanofibers used as the control group and the coaxial collagen PVA nanofibers had the same content that was indicated by the same band position. Thesis results prove that both collagen and PVA are present in the coaxial nanofiber structure. Although it does not provide any information that the fiber coating has taken place, FT-IR analysis is necessary to learn the contents of the structure.

One of the characteristics of polymers is their thermal properties. TGA measurement was carried out to compare the thermal properties of nanofibers. When the TGA graphs of collagen nanofibers were compared with the literature, similar results were observed. Compared to the literature, it was observed that the temperature-dependent weight peaks were not very clear [79]. It has been shown that the triple helical structure of collagen is disrupted during nanofiber production. The reason for the absence of sharp peaks as a result of the thermal measurement can be interpreted as the deterioration of the structure.

TGA measurement graph of the other control group, PVA nanofibers, was taken. PVA, which can show very different characteristics according to the way it is synthesized and its purity, could not be expected to fully comply with the results in the other research. However, it is promising that the industrial grade PVA used in the experiments reveals similar results to the graphics in the literature [81]. PVA, TGA graphs are examined in 3 sections in the literature. 50-200°C range, 200-450°C range and 450-700°C range. Since the measurements could not exceed 300°C, the focus was on the first two intervals. PVA is generally a heat resistant polymer. In the first part, a major weight loss was not observed in the range of 50-200°C. However, a radical decrease was observed after 200°C.

After comparing the two different polymers separately, the thermal properties of coaxial collagen PVA nanofibers were measured. In the Figure 4.10 C, it is seen that the weight loss is slower up to 200°C, but there is a rapid decrease after 200°C. It is known that PVA is a more heat resistant polymer. In this case, more collagen loss

occurred in the first part. The slow acceleration of collagen loss can be interpreted as the presence of more PVA as a protective layer around collagen. When examined in general terms, the fact that the total weight does not decrease marginally, although most of the collagen is lost up to 200°C, and there is a decrease similar to the TGA graph of PVA after 200°C, is an evidence that most of the structure contains PVA [81].

The most important of the main objectives of the thesis research is to ensure that the collagen fibers are coated with PVA. Microscopic characterization is essential to visualize whether this goal could be achieved. Optical microscope and SEM were used for detailed visualization of the structure.

It is observed that the structure is not uniform in SEM images of collagen nanofibers. It is known that the stability of collagen does not last very long. There are many factors affecting the structure of collagen nanofibers (ambient temperature, humidity, etc.). As a result, it has been shown by SEM images that collagen nanofibers cannot maintain a proper fiber form. In addition, the fluctuation in the structure also affects the average diameter length. The diameter size range of collagen nanofibers is wide.

PVA has been shown many times to form successful nanofibers. Although it has different characteristics in different solvents and different concentrations, generally PVA can form high quality nanofibers. As the SEM images show, net, and uniform PVA nanofiber structures could be formed.

As a control, a mixed nanofiber containing both collagen and PVA was produced. A uniform structure cannot be seen in the SEM images of the nanofibers produced by mixing the solutions at a 1:1 concentration. No chemical interaction is expected between the collagen solution and the PVA solution. In this case, the two solutions form a suspension solution. Collagen-PVA mixed nanofibers are formed when the suspension solution comes out of the electrospinning tip. The biggest reason why the structure cannot be a uniform is the possibility that every solution coming out of the needle in unit time may not be the same. While the PVA solution is pumped from the syringe

for a while in the suspension solution, the collagen solution may be pumped after a while. As a result, it has been shown that a uniform fiber form cannot be obtained by mixing PVA and collagen.

PVA, is used to coat the outer surface of collagen nanofibers, in coaxial electrospinning method. When the SEM images are examined, it is seen that the structure is a uniform. In addition, it can be seen that the fiber diameters increase. Since SEM presents a superficial image, it is very difficult to distinguish the coaxial layers of the structure. However, the presence of collagen in the structure is certain in FT-IR analysis. In addition, the structure we see in the SEM image does not resemble the collagen nanofibers at all and is very similar to the PVA structure. As a result, the fibers we see in SEM can be interpreted as PVA nanofibers containing collagen.

Collagen isolated from a bench may exhibit similar diameter characteristics within itself. However, even though collagen isolated from a same source, it can result nanofibers with different properties. In a study, electrospinning was performed using two different brands of collagen isolated from the same source, and it was shown that both samples had very different diameters from each other [83]. In the literature, the mean diameter of collagen nanofibers formed by solution preparation with acetic acid is similar to the mean diameter of the ones produced in this thesis [84].In a study, it was shown that the diameters of PVA nanofibers produced by 10% PVA:dH_2O solution were in the range of 650-700nm [85]. The mean diameter of the PVA nanofibers produced in this thesis is in the same range in the literature [85].

Chen et al. demonstrated the production of coaxial nanofibers using collagen (shell) and thermoplastic polyurethane (core) [86]. It has been shown that the mean diameter of the produced core shell nanofibers is between the mean diameter of collagen nanofibers and the mean diameter of thermoplastic polyurethane nanofibers. In this study, although the collagen was used as a shell layer, it has been shown that the mean diameter of the core shell nanofibers is between the nanofibers of the two materials used separately. Considering this research, it is expected that the mean diameter of collagen-PVA coaxial nanofibers produced in this thesis is between mean diameter of

collagen nanofibers and mean diameter of PVA nanofibers. In the results shown in the Figure 4.9 are consistent of the expectation.

Although the optical microscope does not provide as detailed images as SEM, it still provides quality images. Optical microscope images of collagen nanofibers are consistent with SEM images. When the fibers are examined from a wide angle, it is seen that there are no uniform structures. PVA nanofibers, on the other hand, have a very flat appearance, with average diameters close to each other. Optical microscope images confirms the SEM results that electrospinning from the mixture and coaxial spinning provide completely two different fiber structures.

Optical microscope images of coaxial collagen PVA nanofibers reveal promising results. When the reflections of the light are examined carefully, a two-layered structure is seen. In the optical microscope image, there is a visible contrast difference between the outside and inside of the fiber structures. This difference shows that the coaxial fiber has been successfully formed. The optical microscope images of the nanofibers, which we are sure to be in the structure of both collagen and PVA with other analyzes, show the presence of PVA-coated collagen nanofibers.

The studies carried out in this thesis focused on improving the structural integrity and uniformity of collagen nanofiber. It is known that collagen is used in many biotechnological products today. It can be deduced that this study could open the way for different applications of those two molecules in different fields. Conventional electrospinning has certain shortage in collagen nanofibers production. With this research, it is thought that the production of collagen nanofibers by coating with various polymers may be more effective for different application.

As both nanofiber technology and the use of collagen in biotechnology are increasing day by day, many new steps can be taken on this pioneering work. First of all, different characterization methods can be added to prove the coating of collagen with a different polymer by coaxial electrospinning. This study suggested that PVA coating could reduce the collagen nanofibers structural degradation. As future steps,

in vivo and in vitro studies of PVA-coated collagen nanofibers can be performed and their direct effects on living organisms can be examined. By using the coaxial electrospinning method, the outer surface of the collagen can be coated with other polymers. By testing different polymers, the most effective coating material for the target can be determined. Polymer-coated collagen nanofibers with proven effectiveness can be used for the functionalization of different industrial products such as wound dressing cloths. Or vice verse synthetic polymers could be also coated by collagen to make them biologically more appealing for biomedical applications.

REFERENCES

1. Choi, S. M., H. Y. Kang, H.-J. Min, *et al.*, "Bioactive fish collagen/polycaprolactone composite nanofibrous scaffolds fabricated by electrospinning for 3d cell culture," *Journal of Biotechnology*, Vol. 205, pp. 47–58, 2015.

2. Law, J. X., F. Musa, B. H. I. Ruszymah, *et al.*, "A comparative study of skin cell activities in collagen and fibrin constructs," *Medical Engineering & Physics*, Vol. 38, no. 9, pp. 854–861, 2016.

3. Gelse, K., E. Pöschl, and T. Aigner, "Collagens structure, function, and biosynthesis," *Advanced Drug Delivery Reviews*, Vol. 55, no. 12, pp. 1531–1546, 2003.

4. Shoulders, M. D., and R. T. Raines, "Collagen structure and stability," *Annual Review of Biochemistry*, Vol. 78, pp. 929–958, 2009.

5. Arslan, Y. E., T. S. Arslan, B. Derkus, *et al.*, "Fabrication of human hair keratin/jellyfish collagen/eggshell-derived hydroxyapatite osteoinductive biocomposite scaffolds for bone tissue engineering: From waste to regenerative medicine products," *Colloids and Surfaces B: Biointerfaces*, Vol. 154, pp. 160–170, 2017.

6. Song, W., D. C. Markel, S. Wang, *et al.*, "Electrospun polyvinyl alcohol–collagen–hydroxyapatite nanofibers: a biomimetic extracellular matrix for osteoblastic cells," *Nanotechnology*, Vol. 23, no. 11, p. 115101, 2012.

7. Zulkifli, F. H., F. S. J. Hussain, M. S. B. A. Rasad, *et al.*, "In vitro degradation study of novel hec/pva/collagen nanofibrous scaffold for skin tissue engineering applications," *Polymer Degradation and Stability*, Vol. 110, pp. 473–481, 2014.

8. Law, J. X., L. L. Liau, A. Saim, *et al.*, "Electrospun collagen nanofibers and their applications in skin tissue engineering," *Tissue Engineering and Regenerative Medicine*, Vol. 14, no. 6, pp. 699–718, 2017.

9. Aras, O., and M. Kazanci, "Production of collagen micro-and nanofibers for potential drug-carrier systems," *Journal of Enzyme Inhibition and Medicinal Chemistry*, Vol. 30, no. 6, pp. 1013–1016, 2015.

10. Fratzl, P., "Collagen: structure and mechanics, an introduction," in *Collagen* (Fratzl, P., ed.), ch. 1, pp. 1–13, Boston, MA: Springer, 2008.

11. Minabe, M., A. Uematsu, K. Nishijima, *et al.*, "Application of a local drug delivery system to periodontal therapy: I. development of collagen preparations with immobilized tetracycline," *Journal of Periodontology*, Vol. 60, no. 2, pp. 113–117, 1989.

12. Lee, V. H., "New directions in the optimization of ocular drug delivery," *Journal of Ocular Pharmacology and Therapeutics*, Vol. 6, no. 2, pp. 157–164, 1990.

13. Doillon, C. J., and F. H. Silver, "Collagen-based wound dressing: Effects of hyaluronic acid and firponectin on wound healing," *Biomaterials*, Vol. 7, no. 1, pp. 3–8, 1986.

14. Albu, M. G., I. Titorencu, and M. V. Ghica, "Collagen-based drug delivery systems for tissue engineering," in *Biomaterials* (Pignatello, R., ed.), ch. 17, pp. 333–360, IntechOpen, 2011.

15. Nakagawa, T., and T. Tagawa, "Ultrastructural study of direct bone formation induced by bmps-collagen complex implanted into an ectopic site," *Oral Diseases*, Vol. 6, no. 3, pp. 172–179, 2000.

16. Bender, A. R., H. Von Briesen, J. Kreuter, *et al.*, "Efficiency of nanoparticles as a carrier system for antiviral agents in human immunodeficiency virus-infected human monocytes/macrophages in vitro.," *Antimicrobial Agents and Chemotherapy*, Vol. 40, no. 6, pp. 1467–1471, 1996.

17. Patino, M. G., M. E. Neiders, S. Andreana, *et al.*, "Collagen as an implantable material in medicine and dentistry," *Journal of Oral Implantology*, Vol. 28, no. 5, pp. 220–225, 2002.

18. Bürck, J., O. Aras, L. Bertinetti, *et al.*, "Observation of triple helix motif on electrospun collagen nanofibers and its effect on the physical and structural properties," *Journal of Molecular Structure*, Vol. 1151, pp. 73–80, 2018.

19. Kamarul, T., G. Krishnamurithy, N. D. Salih, *et al.*, "Biocompatibility and toxicity of poly (vinyl alcohol)/n, o-carboxymethyl chitosan scaffold," *The Scientific World Journal*, Vol. 2014, 2014.

20. Wandelt, K., *Encyclopedia of Interfacial Chemistry: Surface Science and Electrochemistry*, Bonn, Germany: Elsevier 1st edition, 2018.

21. Bhattacharjee, A., and M. Bansal, "Collagen structure: the madras triple helix and the current scenario," *IUBMB Life*, Vol. 57, no. 3, pp. 161–172, 2005.

22. Goldberga, I., R. Li, and M. J. Duer, "Collagen structure–function relationships from solid-state nmr spectroscopy," *Accounts of Chemical Research*, Vol. 51, no. 7, pp. 1621–1629, 2018.

23. Astbury, W., and F. O. Bell, "Molecular structure of the collagen fibres," *Nature*, Vol. 145, no. 3672, pp. 421–422, 1940.

24. Pauling, L., and R. B. Corey, "The structure of fibrous proteins of the collagen-gelatin group," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 37, no. 5, p. 272, 1951.

25. Ramachandran, G., and G. Kartha, "Structure of collagen," *Nature*, Vol. 174, no. 4423, pp. 269–270, 1954.

26. Ramachandran, G., and G. Kartha, "Structure of collagen," *Nature*, Vol. 176, no. 4482, pp. 593–595, 1955.

27. Bella, J., M. Eaton, B. Brodsky, *et al.*, "Crystal and molecular structure of a collagen-like peptide at 1.9 a resolution," *Science*, Vol. 266, no. 5182, pp. 75–81, 1994.

28. Lodish, H., A. Berk, S. L. Zipursky, *et al.*, *Collagen: The Fibrous Proteins of the Matrix*. New York: W. H. Freeman, 4th ed., 2000.

29. Cheah, K., "Collagen genes and inherited connective tissue disease.," *Biochemical Journal*, Vol. 229, no. 2, p. 287, 1985.

30. Silvipriya, K., K. K. Kumar, A. Bhat, *et al.*, "Collagen: Animal sources and biomedical application," *Journal of Applied Pharmaceutical Science*, Vol. 5, no. 3, pp. 123–127, 2015.

31. Yang, L., *Mechanical properties of collagen fibrils and elastic fibers explored by AFM*. PhD thesis, University of Twente, Enschede, The Netherlands, 2008.

32. Yang, L., K. O. Van der Werf, C. F. Fitié, *et al.*, "Mechanical properties of native and cross-linked type i collagen fibrils," *Biophysical Journal*, Vol. 94, no. 6, pp. 2204–2211, 2008.

33. Gómez-Guillén, M., B. Giménez, M. a. López-Caballero, *et al.*, "Functional and bioactive properties of collagen and gelatin from alternative sources: A review," *Food Hydrocolloids*, Vol. 25, no. 8, pp. 1813–1827, 2011.

34. Schrieber, R., and H. Gareis, "Gelatine handbook: Theory and industrial practice," pp. I–XII, 04, 2007.

35. Parenteau-Bareil, R., R. Gauvin, and F. Berthod, "Collagen-based biomaterials for tissue engineering applications," *Materials*, Vol. 3, no. 3, pp. 1863–1887, 2010.

36. Muthukumar, T., G. Sreekumar, T. Sastry, and M. Chamundeeswari, "Collagen as a potential biomaterial in biomedical applications," *Reviews on Advanced Materials Science*, Vol. 53, no. 1, pp. 29–39, 2018.

37. Scherer, F., U. Schillinger, U. Putz, *et al.*, "Nonviral vector loaded collagen sponges for sustained gene delivery in vitro and in vivo," *The Journal of Gene Medicine: A Cross-Disciplinary Journal for Research on the Science of Gene Transfer and Its Clinical Applications*, Vol. 4, no. 6, pp. 634–643, 2002.

38. Halima, N. B., "Poly (vinyl alcohol): review of its promising applications and insights into biodegradation," *RSC Advances*, Vol. 6, no. 46, pp. 39823–39832, 2016.

39. Baker, M. I., S. P. Walsh, Z. Schwartz, *et al.*, "A review of polyvinyl alcohol and its uses in cartilage and orthopedic applications," *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, Vol. 100, no. 5, pp. 1451–1457, 2012.

40. Farris, S., and L. Piergiovanni, "Emerging coating technologies for food and beverage packaging materials," in *Emerging Food Packaging Technologies*, pp. 274–302, Elsevier, 2012.

41. Ibarra, V. G., R. Sendón, and A. R.-B. de Quirós, "Antimicrobial food packaging based on biodegradable materials," in *Antimicrobial Food Packaging*, pp. 363–384, Elsevier, 2016.

42. Weis, C., E. K. Odermatt, J. Kressler, *et al.*, "Poly (vinyl alcohol) membranes for adhesion prevention," *Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*, Vol. 70, no. 2, pp. 191–202, 2004.

43. Kobayashi, M., Y.-S. Chang, and M. Oka, "A two year in vivo study of polyvinyl alcohol-hydrogel (pva-h) artificial meniscus," *Biomaterials*, Vol. 26, no. 16, pp. 3243–3248, 2005.

44. Jiang, S., S. Liu, and W. Feng, "Pva hydrogel properties for biomedical application," *Journal of the Mechanical Behavior of Biomedical Materials*, Vol. 4, no. 7, pp. 1228–1233, 2011.

45. Gupta, S., T. J. Webster, and A. Sinha, "Evolution of pva gels prepared without crosslinking agents as a cell adhesive surface," *Journal of Materials Science: Materials in Medicine*, Vol. 22, no. 7, pp. 1763–1772, 2011.

46. Luo, W., S. Zhang, P. Li, *et al.*, "Surfactant-free co2-in-water emulsion-templated poly (vinyl alcohol)(pva) hydrogels," *Polymer*, Vol. 61, pp. 183–191, 2015.

47. Teixeira, M. A., M. T. P. Amorim, and H. P. Felgueiras, "Poly (vinyl alcohol)-based nanofibrous electrospun scaffolds for tissue engineering applications," *Polymers*, Vol. 12, no. 1, p. 7, 2020.

48. Shalumon, K., N. Binulal, N. Selvamurugan, *et al.*, "Electrospinning of carboxymethyl chitin/poly (vinyl alcohol) nanofibrous scaffolds for tissue engineering applications," *Carbohydrate Polymers*, Vol. 77, no. 4, pp. 863–869, 2009.

49. Prabha, R. D., D. C. E. Kraft, L. Harkness, *et al.*, "Bioactive nano-fibrous scaffold for vascularized craniofacial bone regeneration," *Journal of Tissue Engineering and Regenerative Medicine*, Vol. 12, no. 3, pp. e1537–e1548, 2018.

50. Asran, A. S., S. Henning, and G. H. Michler, "Polyvinyl alcohol–collagen–hydroxyapatite biocomposite nanofibrous scaffold: Mimicking the key features of natural bone at the nanoscale level," *Polymer*, Vol. 51, no. 4, pp. 868–876, 2010.

51. Vashisth, P., K. Nikhil, P. Roy, *et al.*, "A novel gellan–pva nanofibrous scaffold for skin tissue regeneration: Fabrication and characterization," *Carbohydrate Polymers*, Vol. 136, pp. 851–859, 2016.

52. Mehrasa, M., A. O. Anarkoli, M. Rafienia, *et al.*, "Incorporation of zeolite and silica nanoparticles into electrospun pva/collagen nanofibrous scaffolds: the influence on the physical, chemical properties and cell behavior," *International Journal of Polymeric Materials and Polymeric Biomaterials*, Vol. 65, no. 9, pp. 457–465, 2016.

53. Alhosseini, S. N., F. Moztarzadeh, M. Mozafari, *et al.*, "Synthesis and characterization of electrospun polyvinyl alcohol nanofibrous scaffolds modified by blending with chitosan for neural tissue engineering," *International Journal of Nanomedicine*, Vol. 7, p. 25, 2012.

54. Varghese, R. J., S. Parani, S. Thomas, *et al.*, "Introduction to nanomaterials: synthesis and applications," in *Nanomaterials for Solar Cell Applications*, pp. 75–95, Elsevier, 2019.

55. Laudenslager, M., and W. Sigmund, "Electrospinning," *Encyclopedia of Nanotechnology; Bhushan, B., Ed.; Springer: Dordrecht, The Netherlands*, pp. 769–775, 2012.

56. Hacıosmanoğlu, S. K., "Elektro-eğirme yöntemiyle elde edilen kollajen nanofiberlerin alternatif yöntemler kullanılarak dayanıklılığının arttırılması," Master's thesis, İstanbul Medeniyet University, 2018.

57. Sill, T. J., and H. A. Von Recum, "Electrospinning: applications in drug delivery and tissue engineering," *Biomaterials*, Vol. 29, no. 13, pp. 1989–2006, 2008.

58. Deitzel, J. M., J. Kleinmeyer, D. Harris, and N. B. Tan, "The effect of processing variables on the morphology of electrospun nanofibers and textiles," *Polymer*, Vol. 42, no. 1, pp. 261–272, 2001.

59. Megelski, S., J. S. Stephens, D. B. Chase, *et al.*, "Micro and nanostructured surface morphology on electrospun polymer fibers," *Macromolecules*, Vol. 35, no. 22, pp. 8456–8466, 2002.

60. Matabola, K., and R. Moutloali, "The influence of electrospinning parameters on the morphology and diameter of poly (vinyledene fluoride) nanofibers-effect of sodium chloride," *Journal of Materials Science*, Vol. 48, no. 16, pp. 5475–5482, 2013.

61. Wang, T., and S. Kumar, "Electrospinning of polyacrylonitrile nanofibers," *Journal of Applied Polymer Science*, Vol. 102, no. 2, pp. 1023–1029, 2006.

62. Haider, S., Y. Al-Zeghayer, F. A. A. Ali, *et al.*, "Highly aligned narrow diameter chitosan electrospun nanofibers," *Journal of Polymer Research*, Vol. 20, no. 4, pp. 1–11, 2013.

63. Haider, A., S. Haider, and I.-K. Kang, "A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology," *Arabian Journal of Chemistry*, Vol. 11, no. 8, pp. 1165–1188, 2018.

64. Sun, B., Y. Long, H. Zhang, *et al.*, "Advances in three-dimensional nanofibrous macrostructures via electrospinning," *Progress in Polymer Science*, Vol. 39, no. 5, pp. 862–890, 2014.

65. Pelipenko, J., J. Kristl, B. Janković, *et al.*, "The impact of relative humidity during electrospinning on the morphology and mechanical properties of nanofibers," *International Journal of Pharmaceutics*, Vol. 456, no. 1, pp. 125–134, 2013.

66. Qin, X., "Coaxial electrospinning of nanofibers," in *Electrospun Nanofibers*, pp. 41–71, Elsevier, 2017.

67. Greiner, A., and J. H. Wendorff, "Electrospinning: a fascinating method for the preparation of ultrathin fibers," *Angewandte Chemie International Edition*, Vol. 46, no. 30, pp. 5670–5703, 2007.

68. Li, D., J. T. McCann, and Y. Xia, "Use of electrospinning to directly fabricate hollow nanofibers with functionalized inner and outer surfaces," *Small*, Vol. 1, no. 1, pp. 83–86, 2005.

69. Han, D., and A. J. Steckl, "Superhydrophobic and oleophobic fibers by coaxial electrospinning," *Langmuir*, Vol. 25, no. 16, pp. 9454–9462, 2009.

70. Yarin, A., "Coaxial electrospinning and emulsion electrospinning of core–shell fibers," *Polymers for Advanced Technologies*, Vol. 22, no. 3, pp. 310–317, 2011.

71. Khan, M. Q., D. Kharaghani, I. S. Kim, *et al.*, "Nanofibers for medical textiles," 2019.

72. Elahi, M. F., W. Lu, G. Guoping, and F. Khan, "Core-shell fibers for biomedical applications-a review," *J. Bioeng. Biomed. Sci*, Vol. 3, no. 1, pp. 1–14, 2013.

73. Díaz, J. E., A. Barrero, M. Márquez, *et al.*, "Controlled encapsulation of hydrophobic liquids in hydrophilic polymer nanofibers by co-electrospinning," *Advanced Functional Materials*, Vol. 16, no. 16, pp. 2110–2116, 2006.

74. Jiang, H., Y. Hu, Y. Li, *et al.*, "A facile technique to prepare biodegradable coaxial electrospun nanofibers for controlled release of bioactive agents," *Journal of Controlled Release*, Vol. 108, no. 2-3, pp. 237–243, 2005.

75. Li, H., C. Zhao, Z. Wang, *et al.*, "Controlled release of pdgf-bb by coaxial electrospun dextran/poly (l-lactide-co-ε-caprolactone) fibers with an ultrafine core/shell structure," *Journal of Biomaterials Science, Polymer Edition*, Vol. 21, no. 6-7, pp. 803–819, 2010.

76. Lu, Y., H. Jiang, K. Tu, *et al.*, "Mild immobilization of diverse macromolecular bioactive agents onto multifunctional fibrous membranes prepared by coaxial electrospinning," *Acta Biomaterialia*, Vol. 5, no. 5, pp. 1562–1574, 2009.

77. Kazanci, M., S. K. Haciosmanoglu, and G. Kamel, "Synchrotron fourier transform infrared microspectroscopy (sftirm) analysis of unfolding behavior of electrospun collagen nanofibers," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, Vol. 251, p. 119420, 2021.

78. Abd El-aziz, A., A. El-Maghraby, and N. A. Taha, "Comparison between polyvinyl alcohol (pva) nanofiber and polyvinyl alcohol (pva) nanofiber/hydroxyapatite (ha) for removal of zn2+ ions from wastewater," *Arabian Journal of Chemistry*, Vol. 10, no. 8, pp. 1052–1060, 2017.

79. León-Mancilla, B., M. Araiza-Téllez, J. Flores-Flores, *et al.*, "Physico-chemical characterization of collagen scaffolds for tissue engineering," *Journal of Applied Research and Technology*, Vol. 14, no. 1, pp. 77–85, 2016.

80. Jabbar, W., N. Habubi, and S. Chiad, "Optical characterization of silver doped poly (vinyl alcohol) films," *Journal of the Arkansas Academy of Science*, Vol. 64, no. 1, pp. 101–105, 2010.

81. Gomaa, M. M., C. Hugenschmidt, M. Dickmann, *et al.*, "Crosslinked pva/ssa proton exchange membranes: correlation between physiochemical properties and free volume determined by positron annihilation spectroscopy," *Physical Chemistry Chemical Physics*, Vol. 20, no. 44, pp. 28287–28299, 2018.

82. Marin, È., M. G. Albu Kaya, M. V. Ghica, *et al.*, "Collagen-polyvinyl alcohol-indomethacin biohybrid matrices as wound dressings," *Pharmaceutics*, Vol. 10, no. 4, p. 224, 2018.

83. Zeugolis, D., B. Li, R. R. Lareu, *et al.*, "Collagen solubility testing, a quality assurance step for reproducible electro-spun nano-fibre fabrication. a technical note," *Journal of Biomaterials Science, Polymer Edition*, Vol. 19, no. 10, pp. 1307–1317, 2008.

84. Huang, Y., Y. Wang, L. Chen, *et al.*, "Facile construction of mechanically tough collagen fibers reinforced by chitin nanofibers as cell alignment templates," *Journal of Materials Chemistry B*, Vol. 6, no. 6, pp. 918–929, 2018.

85. Aljehani, A. K., M. A. Hussaini, M. A. Hussain, *et al.*, "Effect of electrospinning parameters on nanofiber diameter made of poly (vinyl alcohol) as determined by atomic force microscopy," in *2nd Middle East Conference on Biomedical Engineering*, pp. 379–381, IEEE, 2014.

86. Chen, R., C. Huang, Q. Ke, *et al.*, "Preparation and characterization of coaxial electrospun thermoplastic polyurethane/collagen compound nanofibers for tissue engineering applications," *Colloids and Surfaces B: Biointerfaces*, Vol. 79, no. 2, pp. 315–325, 2010.