PART I
BIOMEDICAL MATERIALS
Application of the Collagen as Biomaterials

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Abstract
Collagen is the protein of connective tissue in mammals. The content of collagen in the total protein is approximately 30% of the mammalian tissues. Due to its good cytocompatibility, researchers use this material for the biomedical research application. However, the control of its physical and biological properties is difficult. There are two obstacles in collagen application: 1) difficulty in regeneration of the collagen properties, and 2) difficulties in controlling the properties of the collagen products. The collagen is easily denatured and affected by the environment, which leads to unexpected results. On the other hand, the crosslinker to suppress the denaturation may cause the stiffness of the collagen product. So the researchers are investigating new ways to prepare a collagen product which can be used as a biomaterial for biomedical research application. An important component of the research is the structure and the function of extracellular matrix (ECM). That is, there is biorelevant structure-function-property relationship, which alters its function as an ECM. Recent studies on decellularized tissue is also based on the fact that the native structure of the ECM can be preserved, and therefore may perform the function of the original tissue. So, by replicating its microstructure and producing a collagen fiber complex, it is expected that the function of ECM can be replicated. In this chapter, we will be introducing recent studies on the preparation of a collagen matrix based on fibrillogenesis, orientation, complex formation and layered structure, and how these structures alter the physical and biological properties.

Keywords: Collagen, decellularization, extracellular matrix, fibrillogenesis, microenvironment, regenerative medicine

1.1 Introduction

Collagen is an extracellular-matrix (ECM) protein that plays an important role in the formation of tissues and organs and is involved in various functional expressions of cells [1]. A native ECM is a complex fiber-composite material in which collagen fibrils are a major component [2]. The function of an ECM is to provide support, tensile strength, and scaffolding for the tissue and cells. In addition, it should serve as a three-dimensional structure for cell adhesion
and movement and as a storage depot for growth factors, chemokines, and cytokines; and it should provide signals for morphogenesis and differentiation [3]. Approximately 30% of all vertebrate body protein is composed of collagen. Among these, the highest collagen composition can be found for the tendon, bone and cornea where 90% of ECM is collagen. Mainly, the collagen can be distinguished into two types; fibrillar and non-fibrillar. There are 28 types of collagen and the collagen types I, II and III are the classical fibril-forming collagens and account for 80–90% of all collagens in the human body. Collagen fibril is very important from the aspect that its properties and the morphology provide the key to the scaffolding structures in the body according to the location.

It has been shown that the collagen possesses non-immunogenicity and good cell compatibility, and can be obtained from various sources. These make collagen popular among biomaterials researchers, and diverse methods have been adopted for its application in the biomedical fields. The collagen is purified after being treated with pH adjustment or pepsin digestion. Either way, the collagen should be water soluble in order to process it for use as a collagen matrix for biomaterial applications. There are several kinds of collagen matrix: gel, film, microparticles, conjugates, minipellets or sponge [1, 4]. However, there are still many problems to overcome. For example, the collagen which is available in the marketplace is hydrophilic, which absorbs water at a high rate. So, the uncross-linked collagen matrix possesses low mechanical strength and fast degradation rate in aqueous solution. The collagen matrix degrades by the collagenase, so this makes the collagen applicable in some biomedical products where the biodegradation in the living body is required. However, control of the biodegradation is not easy. The properties of the collagen matrix can be controlled by cross-linking. The cross-linking is executed chemically or physically. Furthermore, using the same cross-linking process, the collagen matrix can be functionalized by immobilization or, blend of a second component. The collagen is composed of amino acid groups where the chemical reaction can be executed. Mainly, the cross-linking is executed using ε-amino groups of lysine or hydrolysine, and aspartic acid or glutamic acid residues. These residues are highly reactive and can be easily functionalized. The cross-linking can change physical and biological properties of the collagen matrix and can be applied for the loading of the drugs. For the chemical cross-link, glutaraldehyde, formaldehyde, hexamethylenediisocynate, polyepoxy compounds, carbodiimides, and acyl azides are commonly used [1, 4-14]. These show a good result in vivo, such as suppressing the inflammatory response and promoting the healing response. However, there are still several problems to be overcome. Although the collagen gels, sponges or films that have been cross-linked show an increase in the mechanical strength, the cross-link which consumes the functional groups are consumed for the cross-linking site, which may affect the biological properties. Moreover, the stiffness of the ECM is also a very important parameter, but it is not easy to control the stiffness of the collagen gel by cross-linking or a change in the collagen solution. That is, a stiff collagen gel can be prepared, but a gel with viscoelasticity cannot be prepared.
This is important because most of the native tissue possesses viscoelasticity which contributes to the toughness [15, 16]. A number of collagen matrices were reported, but the collagen products available in the marketplace is still scarce because of the problems mentioned above, so a new approach was required to move to the next step.

1.2 Structural Aspect of Native Tissue

1.2.1 Microenvironment

Before designing a biomaterial, it is necessary to understand the environment of the living body. When the biomaterial is designed for application in tissue engineering and regenerative medicine, the objective is the repair and remodeling of the damaged ECM and tissue, ultimately regenerating its function. The function of the ECMs is deeply related to the behavior of the cells which is affected by the cell-materials interaction. That is, the control of the cells behavior is very important in the aspect of regenerating the function of the ECMs. The cell is immersed in a dynamic landscape composed of insoluble macromolecules of the ECM, soluble bioactive factors and neighboring cells [17]. The environment which controls the fate of the cell inside the living body is called cellular microenvironment. It is very important in the aspect that the ultimate tissue structure and its function are decided by factors contributing to the cellular microenvironment. For this, there needs to be a fundamental understanding on the cellular microenvironment for the materials design. The cellular microenvironment is the environment in the living body which controls the fate of the cells. The microenvironment is composed of signals from the neighboring cells, physical stimuli, soluble factors such as growth factors, and insoluble factors such as ECM. The ECM has been shown to influence cell mitogenesis and chemotaxis [18, 19], direct cell differentiation [20-23], and to induce constructive host tissue remodeling responses [24-26]. The cells from the ECM sense, integrate and proceed the signals to determine behavior and functions, and the information is passed bidirectionally as the microenvironment is remodeled by the cells.

Development of biomaterials for tissue engineering and regenerative medicine has been approached mainly from the aspect of controlling the soluble and insoluble factors. As for the insoluble factor, diverse materials – natural or synthetic – are being investigated. The main goal of using these materials is to replicate the function of the ECM temporarily or permanently. By loading soluble factors in the materials, researchers tried to control the fate of the cells or stimulate the regeneration of the damaged tissues. On the other hand, manipulation of the morphology, microphase, surface physical properties and chemical properties of the material is a major approach for the control of the insoluble factors. These methods show good results and some of them are actually used for clinical practice.

Since the ECM is mainly composed of collagen, use of collagen to replicate its function is actively executed. It should be noted that the function of the
ECM is different according to the type of tissue such as cornea, brain, skin, tendon, or blood vessel, where they need to perform a certain function. So the design of the ECM using collagen should be different according to the targeted tissue. However, although the tissues perform different functions, the common aspect of the tissue is that all are made up of collagen fibrils. That is, in order to design a material which may replicate the function of ECMs, fibrillized structure should be considered. Furthermore, it should be acknowledged that the design should include a nanometer to centimeter scale. The schematic structural images of respective tissue from the nanometer to centimeter scale are shown in Figure 1.1. Yip discussed the importance of careful consideration of biorelevant structure-function-property relationships in the design of biomaterials [27]. That is, the regeneration of the physical properties of native ECM is important for the regeneration of biological properties. The importance of the structure can be seen in research related to the decellularized tissue which is discussed in the next section.

1.2.2 Decellularization

The decellularized tissue is a native tissue in which the cells are eliminated by certain treatment. Decellularization of tissue is based on the fact that preservation of the native ultrastructure and composition of ECM is possible [26]. The methods for the decellularization include use of chemical agents (ionic detergents, non-ionic detergents, acids and bases, hypotonic and hypertonic solution, and solvents), biological agents (enzymes and chelating agents), and physical treatment (temperature, pressure and electroporation). It should be understood that every cell removal agent and method will alter ECM composition and cause some degree of ultrastructure disruption. For example, the use of some
chemical agents such as sodium dodecyl sulfate (SDS) may cleave the collagen fibrils, but use of physical treatment such as high pressurization would not affect the main structure [26, 28–30]. Furthermore, incomplete rinsing of chemical agents or the cell debris after decellularization process may cause toxicity. However, the minimization of these undesirable effects, rather than complete avoidance by the living body, is the objective of decellularization. So, the focus is set on complete removal of the cells and preservation of the ultrastructure. The methods for the decellularization should be carefully considered according to the density of the fibers, the thickness and the lipid contents. Moreover, the complete washing of the cell debris or the chemical agents after decellularization should be executed because this could cause toxicity.

The mechanical strength after the elimination of the cells is maintained and the regeneration around the implanted decellularized tissue occurs without serious inflammatory response. So, the decellularization can be executed for the partial or full organs. It should be noted that the native tissue possesses complex structure and the whole structure – either macro or micro – is maintained after the appropriate decellularization process. Furthermore, the degradation of the decellularized tissue is slow, and the remodeling of the damaged tissue occurs without any problems. The regeneration within the living body occurs on the implanted decellularized tissue and starts to function as a replacement. Furthermore, the high mechanical strength of the decellularized tissue would endure the physical stress inside the living body [29, 31–32]. So many decellularized tissue products such as dermis, heart valve, blood vessel, bone and so on, have been introduced to the markets and are enjoying success.

1.2.3 Strategy for Designing Collagen-based Biomaterials

The key for the success of the biomaterials for regenerative medicine is control of the cells' fate which depends on the materials characteristics; three-dimensional ultrastructure, surface topology and composition of the ECM [17]. The successful point for decellularized tissue is that the three-dimensional ultrastructure, surface topology and composition of the ECM is maintained after the process. So, in order to reproduce the physical and biological properties of the ECM, we should first mimic its three factors as written above. The key points are the fibril formation, orientation, complex formation with second component such as GAG or elastin, and multiple layers. Since the structure of ECM differs according to the tissue, the mimicking of the structure should also be different according to what kind of tissue the researchers want to make. This is because the key function is different according to the tissue. For example, the tendon should have fibrillar structure with high orientation, the cornea should have fibrillar lattice structure, blood vessels should possess elastin-complex fibril structure with multiple layers and high orientation of collagen fibers, and skin should have elastin-complex fibril structure disregarding the orientation. Such ECM structures allow the various tissues to possess certain physical and biological properties adequate for functional performance. So, the structural consideration for replicating the function of tissue is very important.
Many articles consider this point and try to create an ECM resembling collagen structure. The ECM structure consists of collagen fibrils with a second component such as GAG or elastin forming collagen fiber complex. The collagen fiber complex is usually aligned and multi-layered. Such structure is very important from the aspect not only of the fate of the cells, but also for mechanical endurance performance against the stress given by the living body. This distinguishes the ECM from the other monolithic structure where single performance is expected. In most cases, a gain of physical properties would result in a loss of desired biological properties. The ECM possesses highly complex structure which is not easy to replicate. However, many trials for the creation of complex structure with controlled physical and biological properties that have been reported on during past decades mainly focused on controlling the ultrastructure, surface topology and composition of the collagen matrix. The next section is divided into four parts: preparation of collagen matrix based on fibrillogenesis, orientation, complex formation and layered structure. We will be discussing the most recent methods for the preparation of collagen matrix focused on these four subjects.

1.3 Processing of Collagen Matrix

1.3.1 Fibrillogenesis

The change of the ionic strength, pH, or hydrophobicity by additives in the aqueous solution may drive the alteration of collagen molecule alignment with certain regularity as shown in Figure 1.2. The alignment of the collagen molecules results in the formation of the fibrils which causes the precipitation (Fig. 1.2b). This is called fibrillogenesis or collagen reconstitution. The Fibrillogenesis is an aggregation of the collagen molecules which is an entropy driven process. The loss of solvent molecules from the surface of protein molecules results in assemblies with a circular cross-section, which minimize the surface area against the volume ratio of final assembly [33]. Hydrophobic residues of collagen (Leu, Ile, Val, Phe and Trp) play the main role in lateral aggregation [34, 35]. The fibrillogenesis occurs in an aqueous condition with a certain amount of salt. It is thought that formation of salt bridges by the salt is also a major driving force for the formation of fibril with certain periodicity [36]. However, it is also argued collagen fibrillogenesis is driven primarily by the formation of hydrogen-bonded water clusters bridging recognition sites on opposing helices, and that and hydrophobic interactions between opposing non-polar amino acid side chains is not a major driving force of collagen self-assembly [37]. However, the fact is that the physiological ionic strength and neutral pH and increasing temperature, induces spontaneous assembly of type I collagen into native-like fibers and hydrophobic interactions, salt bridge and hydrogen-bonded water clusters cannot be ignored.

The preparation of the collagen matrix is based on mixing the collagen aqueous solution with a certain amount of salt to adjust the physiological condition.
The mixture depends on how the collagen matrix is going to be used. For example, when the collagen and cell are mixed together, cell culture medium can be directly put into the container. The resulting material is mainly gel or sponge which has random fibrillized structure. The temperature for the fibrillogenesis is generally 37°C, but diverse temperature can be used if kept under the denaturation temperature. The thickness of collagen fibers can be controlled where the thickness increases in lower temperature [33]. The drawback of producing a gel or sponge with fibrils at 37°C is that the collagen may denature [38, 39]. The cross-linking usually executed for the gel or sponge is mechanically
too weak to support cell growth, proliferation and migration. The most usual phenomenon is the contraction of the matrix caused by the strong cell and matrix surface interaction. However, the cross-linking makes the matrix too brittle [10, 40]. Furthermore, the cross-linked collagen matrix does not show, or at least shows very slow degradation by collagenase [41, 42], which also implies that the possible capsulation in vivo might occur. However, a matrix which does not show contraction upon cell culture and slow degradation in vivo despite the use of cross-linker was successfully created. By trapping the collagen aqueous solution in the dialysis cassette and letting the NaCl diffuse into the collagen dialysis, the collagen aqueous solution turned into fibrillized gel [43]. This gel can be processed into a thin membrane, where the collagen membrane showed a much tougher mechanical strength and slower biodegradation rate in vivo. What is interesting is that this matrix showed suppressed inflammatory response in vivo. The behavior was almost the same as that of decellularized tissue which makes it possible to claim that the structural aspect is very important.

1.3.2 Orientation

The fibril formation can be obtained by the methods described earlier. But the orientation of the collagen fibrils is another problem. The orientation of the collagen molecules or fibrils is reported to be achieved by applying certain force. That is, if there is a certain driving force which allows the collagen fibrils to align, the collagen matrix with orientation can be obtained. The most commonly used method is flow chamber, which allows the collagen molecules to flow into the chamber and precipitate along the axis of the flow. Lanfer et al., have reported on how shear flow deposition would affect the orientation and density of the fibrils [44]. They have concluded that the degree of collagen fibril orientation increased with increasing flow rates of the solution, while the matrix density increased at higher collagen solution concentrations. The rearrangement of the collagen molecules is also reported to be achieved by controlling the concentration of the collagen solution. This is because the collagen behaves much like a liquid crystal and tends to reorganize in high concentration. The Giraud-Guille research group defined the collagen molecules as spontaneously self-organizing in vitro as cholesteric liquid crystal [45, 46]. The fibrillogenesis makes the liquid crystalline phase stable, inducing sol-gel transition. This alignment is especially advantageous for dense collagen, where the direct application for the dermal substitute is expected [47].

An alternative method for collagen alignment is the application of uniaxial elongational strain. This method is advantageous from the aspect that a high orientation percent is obtainable, and direct application on ligaments or tendons is possible. A good example has been reported by Falini et al. They reported on applying uniaxial elongational strain and then dehydrating it for 24 hrs [48]. They concluded that the strain of the collagen film or gel would cause the rearrangement of the collagen molecules when higher than 12% of elongational strain is applied and dehydrated. The orientation percent was
approximately 83%, which is very close to the Achilles tendon. However, this method is only applicable to collagen molecules and not to the collagen fibrils. Ross and his group also used an approach for the mechanical strain, but this time they repeated the strain procedure for a longer period of time (2.5% cyclic strain for 2 h per day for 4 days) after seeding the cells [49]. They showed that the alignment of cells along the collagen matrix reflects a response of the cellular environment to the applied strain, concluding that manipulating signal transduction pathways by engineering implantable anterior cruciate ligament grafts or modifying ACL healing response is possible.

Another promising approach is the use of electromagnetic field [50–52]. The collagen molecules tend to align perpendicular to the electromagnetic field upon gelling at above 1T. One great advantage of this method is that a high electromagnetic field can be applied to the collagen aqueous solution containing cells and culture condition. The major target is the regeneration of ECM which requires high tensile strength or the neuron tissue [50, 51]. For these, osteoblast, Schwann cell, glioblastoma cell and erythrocyte were cultured upon the collagen gel in which an electromagnetic field above 8T was applied. Y. Eguchi et al. reported that in the mixture of Schwann cells and collagen, Schwann cells oriented in the direction perpendicular to the magnetic field after 2 h of magnetic field exposure. In this case, Schwann cells aligned along the collagen fiber oriented by magnetic fields [52]. This means that the cells and the collagen orientation can be achieved contemporarily. The only problem that remains is how many collagen fibrils would align, because 100% of collagen can not align according to the applied electromagnetic field. Nonetheless, this method remains very promising for tissue engineering.

1.3.3 Complex Formation and Blending

Complex formation of collagen fibrils with a second component is one of the most difficult parts. Complex is mainly executed for the purpose of functionalization. It is generally known that complex formation can be achieved by mixing collagen solution with the second component and cross-link. However, there are some limitations which involve difficulties in collagen molecular control. This is because of the limitation of collagen complex formation with another component. It is known that collagen molecules require hydrogen bond when forming complex with a second component in aqueous solution [53]. Basically, the fibril formation and the complex formation does not occur contemporarily because the second component added to the collagen aqueous solution would function as a defect and prohibit the molecules to aggregate for fibrillogenesis (Figure 1.3). So the complex formation is commonly executed after fibrillogenesis. The most well-known material for collagen complex formation by far is hydroxyapatite, which is designed for bone regeneration. The bone mainly consists of collagen and hydroxyapatite composite where the hydroxyapatite molecules exist between the collagen fibers, providing stiff mechanical strength [54, 55]. The process of collagen-hydroxyapatite composite also targets the creation of such composite structure by diffusing calcium and phosphate ions into
the collagen fibrils. The process, also called mineralization, is advantageous in the aspect that the collagen orientation may be achieved at the same time. It should be noted that not all collagen-hydroxyapatite composite is composed of collagen fibrils, but it is still one of the most advanced field.

Collagen complex is also executed with synthetic polymers, GAG, proteins or oligo-(poly-) saccharides [9, 14, 56–63]. Fibrillized collagen-hydroxyapatite complex involves the diffusion of calcium and phosphate ions. However, this is not easy when the second component is oligomer or polymer. So, the collagen is not necessarily fibrillized through fibrillogenesis for the complex formation in these cases. Instead, the collagen molecules and the second components are mixed together in certain conditions and chemically cross-linked. This method usually involves a freeze-saw process after mixing two solutions or slurry from a sponge, or absorption of polymer solution into the collagen gel or film. These processes allow the formation of large pores suitable for cell migration and develop into the three-dimensional cell culture. Most often hyaluronic acid is used for the collagen complex, for it is known to enhance the cell migration. Furthermore, the existence of hyaluronic acid may induce the moistening affect, which allows the complex matrix to be applicable for the artificial skin. An alternative method for the collagen complex formation is electrospinning. The electrospinning allows the collagen and second component to form fibril blend [61, 64]. The fibrillized structure provides relatively higher mechanical strength than collagen gel or sponge. Furthermore, various polymers can be applied for the blending and can also be produced in a highly aligned state. However, several researchers argue that although electrospinning is advantageous from the aspect that nanometer scale collagen fibrils can be formed,
the collagen denatures, making it electrospun gelatin fibrils instead [65]. The importance of collagen fibrils possessing regulated D-periodicity is also pointed out by some researchers. This is because the lack of D-periodicity in the electrospun collagen fibrils may cause diseases such as osteogenesis imperfecta and induce cardiovascular disease [2, 66]. The question of using electrospinning for collagen fibrillation is still debated, but diverse blending ability between collagen and the second components, as well as good results of electrospun collagen products in vivo cannot be denied, and many products can be found in the market.

One of the most active research areas is collagen-biodegradable polymer complex. Designed to functionalize the collagen matrix, the polymer eventually degrades together with collagen. The favorite polymers are PLLA and PCL which are known for their nontoxicity and good biodegradability [65, 67–70]. The biodegradability is not a mandatory requirement. This sounds like an oxymoron, but the nondegradable polymers of the collagen complex are usually destined for the high mechanical strength. Sionkowska group uses hydrogen bond inducing polymers such as PVA, PVP or PEG to form complex with collagen molecules [53, 71–73]. Biodegradable polymers are usually applied in electrospinning with collagen in organic solvent. Good blending and altering the fibril diameter can be obtained via this method. So far, the electrospinning process is the only confirmed method for producing collagen fibril-polymer complex. The most interesting blend is a collagen-elastin blend designed for blood vessels which showed very good viscoelasticity [74, 75]. However, it should be noted that this is a blend, not a complex, which implies that there is no chemical or physical interaction between the collagen and the elastin. The necessity for the collagen to form complex instead of blend remains a question to be answered in the future.

1.3.4 Layered Structure

One of the most important parts of the native tissue is the layer. The idea of multiple layers was brought up for multi-functionalization. For example, the collagen side possessing different functions on each side can be prepared if such a matrix can be prepared. The best way to prepare a multilayered collagen matrix is to adjoin the collagen matrices using adhesives. However, the adhesive would alter the properties of collagen matrices at the interface of the layers and would consist of pure collagen. So a method for the collagen-collagen integration was investigated. The collagen does not integrate with other collagen once they are in solid form. Furthermore, the collagen matrix with fibrils does not normally absorb the polymer into its matrix. As a result, the collagen-collagen interface with entangled layers with polymer as the intermediate do not form. The immobilization technique is the only method that was actually possible for the collagen layer. The immobilization technique involves cross-linking the collagen.

One simple method is the slow drying process. Nam et al. showed that once the collagen matrix with fibrillized structure was formed, the microlayers
formed along an axis perpendicular to the surface by the slow evaporation of water [76]. Unlike the lyophilization or dehydrothermal processes, water is not completely eliminated by dehydration. The fibril rearrangement of collagen fibers by dehydration may induce the stability of the collagen matrix against heat as well as its dimensional stability in water. The dehydration causes the chain rearrangement of the collagen molecules, in which the collagen molecules are brought closer to each other [77, 78]. The stripping of the water bridge, which is connected via hydrogen bonds, occurs contemporarily, but the water bridge itself plays a minor role in the stability of the collagen triple helix, indicating that the air drying process does not cause the denaturation of the collagen matrix [79]. Upon rehydration of water, the water content is approximately 80% and it is no more a matrix with jelly-like property. Similarly, the deprivation of water molecules by compression to form a thick, multilayered structure can be obtained [80]. Both cases result in a collagen matrix with much denser collagen concentration, which possesses viscoelasticity.

The alternative method is the layer-by-layer deposition. This method is advantageous because the collagen aqueous solution is viscose and requires a long time to become a confluent solution when 2 collagen aqueous solutions of different concentrations are deposited on top of one another. The interface between the layers functions as a membrane, allowing water molecules from the sparse collagen layer to move to the dense layer, and the collagen concentration decreases near the interface. Then, the NaCl/Na_{2}HPO_{4} salts diffuse into the collagen layers from the bottom, causing fibrillation toward the upper part of the matrix solution [81]. The resulting collagen matrix is one with a multilayered structure without clear boundaries. The cell infiltration differed according to the layers, where the cell infiltration was shown for the less dense side. This method is related to the complex formation as shown in Section 1.3.3 in Figure 1.3.

The integration of collagen matrices with different components was also developed. Tampieri et al. have reported that the collagen matrix with different hydroxyapatite concentration at each layer and hyaluronic acid on a specific layer can be prepared [82]. They have developed the layer compatible to cartilage on one side and bone on the other side. This implies that the collagen with different functionality at each side can be prepared. Similarly, Gillette et al. have reported the integration of the collagen-based fibril matrix. By increasing the temperature, the collagen solution and the collagen solution with alginate integrate to form a fibrillized collagen matrix with thick interface [83, 84]. This method does not require the layer-by-layer deposition and it shows the importance of collagen—collagen interface, where the actual bonding between the collagen matrices is controlled.

1.4 Conclusions and Future Perspectives

A lot of literature introduces various applications of collagen designed for biomaterials. However, there are not very many successful collagen-based
products that can actually be found in the market. Moreover, scientific information on the physical, chemical and biological aspects of collagen and its behavior upon diverse treatment is still limited. The most difficult problem is lack of control of its physical, chemical and biological behavior. There are many reasons for these problems, but the most critical one is its special structure which is poorly understood. However, collagen still is an important material which challenges researchers. The knowledge about collagen is growing, and there were some very important breakthroughs over the last 10 years. We cannot ignore the good aspects of collagen such as its good biocompatibility and low antigenicity. Also, collagen is soluble in water and possesses functional groups which are relatively easy to chemically or physically modify in an aqueous condition. These benefits will encourage future developments and uses as indicated by the intensification of studies on the utilization of collagen in the growing fields of tissue engineering, regenerative medicine and drug delivery.

References
