Research Article

Ömer Fırat Turşucular*, Yusuf Ulcay

An experimental study for chemical characterization of artificial anterior cruciate ligament with coated chitosan as biomaterial

https://doi.org/10.1515/chem-2022-0324
received February 17, 2023; accepted April 8, 2023

Abstract: The importance and aim of this experimental study is that raw artificial anterior cruciate ligament samples were produced with various 3-D braiding constructions with various technical yarns using the 3-D braiding method. Later, it is aimed to determine the chemical bond changes between raw samples with ethylene oxide (EtO) sterilization and bio-chemical finishing samples by applying padding process and EtO sterilization processes for all samples with 3-D braiding structures, due to the cross-linking of biocompatible chitosan (CHI) with biological cross-linker glutaraldehyde (GA). The importance of this experimental study is that it is the first experimental chemical analysis in this field in the world scientific study. Padding and EtO sterilization processes were applied on all samples and compared to various technical yarns with 3-D braiding structures thanks to biocompatible CHI. Chemical analysis was interpreted for all samples. It was determined that the applied temperature, concentration, pH, yarn types, characteristic bonds in the chemical structure of the technical yarns, applied bio-chemical finishing process and EtO sterilization had effect on the formation, shifting and breaking of chemical bonds. It was determined that the yarn number, braiding geometry, braiding angle (°) and braid construction had no effect on the formation or shifting of chemical bonds. New bonds were formed thanks to CHI and GA due to their extremely reactive between 5 and 5.5 pH. They reacted quickly with Schiff base bond in all samples. CHI was ionized in all samples. It was determined that new bonds were formed in UHMWPE, PPD-T and HT PET structures. The most common bond formations were HT PET > PPD-T > UHMWPE. The reasons for these chemical structure changes in all samples depended on their chemical structures, bond types, molecular weights, reactivities, ease and speed of diffusions, crystallinities of technical yarns and all chemicals used. In order to increase the formation of new chemical bonds the pH should be between 5 and 5.5, GA concentration should be a minimum of 25% or higher. The dissolution time of CHI should be minimum 3 h or more. The dissolution process temperature of CHI should be minimum of 70°C or higher. The absorption, adsorption and chelation properties of CHI on all samples will also be evident successfully as in this experimental chemical study.

Keywords: biomaterials, artificial ACL ligament, braiding technology, bio-chemical finishing, FT-IR chemical characterization

1 Introduction

Around 100,000–250,000 anterior cruciate ligament (ACL) injuries occur each year. In the USA, the cost of surgery for female athletes involved in athletics reaches US$650 million each year [1]. Ligaments are solid and fibrous band structures that cover the joint and are parallel to each other [2]. They have fibroblast cells and extracellular matrix, which form the most common collagen I and collagen III types in the structure of the ligaments [2,3]. Ligaments vary in length from 25 to 35 mm. The cross-sections have a triangular cross-section from the ends to the midpoint and have a diameter ranging from 4 to 10 mm [3,4]. In young people aged 16–26 years, the percent elongation at break is 44.3 ± 8.5 (%) and the maximum breaking force is 1,730 ± 660 N. In the elderly aged 48–85 years, the percent elongation at break is 30 ± 10 (%) and the maximum breaking force is 734 ± 266 N [5]. The design criteria of artificial ACL ligaments are it should be biocompatible, high tensile strength, high radial strength, high dimensional stability, easy to process, similar to the natural ACL

* Corresponding author: Ömer Fırat Turşucular, Department of Textile Engineering, Graduate School of Natural and Applied Sciences, Bursa Uludağ University, 16059, Bursa, Turkey, e-mail: omerfrattursucular@gmail.com
Yusuf Ulcay: Department of Textile Engineering, Graduate School of Natural and Applied Sciences, Bursa Uludağ University, 16059, Bursa, Turkey
ORCID: Ömer Fırat Turşucular 0000-0003-1162-0742; Yusuf Ulcay 0000-0001-6685-8278

Open Access. © 2023 the author(s), published by De Gruyter.
ligament tension–strain graph, lumen diameter between 9 and 11 mm. They should also have low hysteresis and dense structures [5].

Ultra-high molecular weight polyethylene (UHMWPE) is a semi-crystalline polymer composed of large number of transvinylene monomers. It also has a very high molecular weight of 3.1 million g/mol, very high orientation, very high crystallinity and polymerization degree of 110,000. In a biomedical study, the cross-linking density of UHMWPE increases depending on the large number of vinylidene monomers. As the radiation dose increases, the number and cross-linking of transvinylene monomers increases [6].

Para aramid (PPD-T) is a lyotropic liquid crystalline organic polymer. It has unique structure with very high crystallinity connected to each other in the para position with 85% amide bonds between the two aromatic benzene rings. It also has low density, high specific modulus of elasticity, high specific strength and high thermal resistance. Surface modifications such as chemical grafting, plasma treatment, coating method and surface construction of nanostructures can be applied. Its reactivity and surface roughness increase due to these surface modifications [7]. It has been determined that –OH, C=O and –NH2 groups are rapidly hydrolyzed by increasing the reactivity in acid or alkaline environment in some chemical study on PPD-T. The interfacial bond strength between the fiber and matrix elements increases significantly due to the diffusion of polar groups. It improved its UV resistance, surface activity, thermal and mechanical properties by reducing its number of –NH2 groups because of its chemical treatment with hyperbranched polysiloxane [7].

Polyester (PET) is a highly linear macromolecule with high crystalline synthetic chemical structure containing more than 85% ester diol and benzene-1,4-dicarboxylic acid (terephthalic acid) or dimethyl terephthalate. It contains methylene, carbonyl and ester groups in its chemical structure. It is hydrophobic due to its high crystallinity [8]. PET has been widely used in biomedical applications. It is successfully coated with biological chemicals such as collagen or fibronectin to improve cell–polymer interaction [8,9].

Chitosan (CHI, poly-β-(1 → 4)-2-amino-2-deoxy-d-glucose) is a nitrogenous (–NH2 based) polysaccharide. It is produced by N-deacetylation process in large quantities, thanks to chitin. It is the second most abundant natural biopolymer in the world. It is produced by passing chitin, which is extracted from shellfish, through various chemical processes (by N-deacetylation). Many properties of these biopolymers are similar to each other. It is a potential alternative biopolymer to other natural and synthetic biopolymers for environmental applications due to its ease of processing, non-toxicity, natural antibacterial and biodegradable properties. It enables to form various new compounds with various organic and inorganic compounds by chemical processes due to its highly reactive groups such as –OH and –NH2. It has a very high, tight and effective chelation feature with organic and inorganic compounds. Films, membranes, beads and composite structures can be also created [10]. It has poor adsorption due to its non-porous structure and low surface area. Particularly, chemicals with large molecular sizes cannot easily diffuse into it, which has a reticulated structure. It is easily hydrolyzed in an acidic environment and has a high swelling tendency. It has low adsorption capacity below pH 5. Due to this disadvantage, various physical or chemical surface modifications are applied. The cross-linking method increases its chemical resistance in both acidic and alkaline environments. Cross-linked CHI has higher mechanical properties, dimensional stability, hydrophilicity and larger pore size. The longer the polymer chain the greater the surface area and the number of pores. This reduces its crystallinity and increases the adsorption of its functional groups. Solvent or solvent-free solution concentrations affect its bead adsorption behavior [11].

Glutaraldehyde (GA) is widely applied as a cross-linking agent due to its low cost, high reactivity and high solubility in water solution. Schiff bases, which are formed as a result of the reaction between GA and the free –NH2 groups of lysine or hydroxylysine, are formed easily and quickly at the beginning of cross-linking. In the next stage these Schiff bases are stabilized by further reactions leading to the formation of different products. Biomechanical tests showed also that GA bi-treated collagen is suitable for in vivo studies and can be used for tissue engineering applications. GA provides homogeneous distribution on forms such as film, microcapsule, granule and fiber [12]. In an experimental study aldehyde, mono, dihydrate, cyclic cis and trans isomer structures were observed in free and equal amounts in coated CHI with GA due to the presence of 25 and 50% aqueous solution GA [12]. However, its structures in different forms were not much affected by the concentration of GA but temperature is a very important factor and increases the amount of aldehyde. According to pH dependence the cross-linking of proteins in GA was high due to a decrease in the reactivity of protonated –NH2 groups in CHI to react with C=O groups. Reaction did not occur practically in a very high acidic environment. Cross-linking was just observed at pH > pI due to the –NH2 groups of the deprotonated proteins in CHI. Degree of protonation of –NH2 groups of CHI, which had a polysaccharide, also determined the water solubility [12].
Its pH values ranged from 3.5 to 4.5 depending on its molecular mass, degree of deacetylation and concentration of the solution for its pH value in acetic acid solution. Moreover, cross-linking reaction proceeded at a high speed [12]. An imine group (–RC≡N) from Schiff base bond occurred by the condensation reaction between primary –NH₂ from CHI and an active aldehyde from salicylaldehyde as their fourier-transform infrared spectroscopy (FT-IR) results [13]. CHI and GA were cross-linked at a rate of 80.8% in another experimental study. High antibacterial activity was detected. The degree of cross-linking was important as the degree of deacetylation on its features [14]. Aldehyde groups in GA solutions diffused easily and reacted with the –NH₂ groups in the wet CHI microspheres. –NH₂ groups in CHI can be formed by cross-linking mechanism with GA, which had aldehyde groups. –N–H and O–H bending vibrations shifted from 3,439 to 3,417 cm⁻¹, –CH₃ symmetric stretch shifted from 2,925 to 2,937 cm⁻¹, C==O bending vibration shifted from 1,666 to 1,645 cm⁻¹, C–N bending vibration shifted from 1,438 to 1,406 cm⁻¹, C–OH bending vibration shifted from 1,073 to 1,037 cm⁻¹, –CH₃ bending vibration was not at 1,363 cm⁻¹ and C–O–C bending vibration is not observed at 1,155 cm⁻¹ but amide(II) bonding was observed at 1,559 cm⁻¹ due to the detailed FT-IR test results. The chemical bonding mechanism included –NH₂ groups in CHI and abundant amount of aldehyde groups in GA as a cross-linking, and were experimentally investigated for a long time. pH, ionic strength, temperature, concentration of CHI and degree of cross-linking of CHI were some of the important chemical parameters on its chemical gelation rate with coated GA [14].

The applications of braiding structures are generally used in the biomedical field such as surgical yarns and artificial veins. It can be also used as electromagnetic shielding and cables in industrial field because of its high mechanical strength and flexibility [15]. Ethylene oxide (EtO) sterilization is the most widely used sterilization method in biomaterials due to its high penetration capacity and high efficiency at low temperatures. The relative humidity of EtO can vary between 40 and 80%, its gas concentration is between 450 and 1,200 mg/L, its temperature is between 40 and 65°C and its exposure time varies from few hours to few days [16,17]. The chemical structures of all technical yarns and their main chemicals as a single image are presented in Figure 1 [10,18–21].

### 2 Experimental

#### 2.1 Materials

T1 and T2 samples were produced from UHMWPE technical yarn with 445 dtex yarn number, 45° braid angle, 1 center and 16 braid yarns. The only difference between T1
and T2 samples was the braiding construction. T1 was produced with diamond and T2 was produced with double braided in braiding constructions. T3 and T4 samples were produced from PPD-T technical yarn with 1670 dtex yarn number, 45° braid angle, 1 center and 16 braid yarns. The only difference between T3 and T4 samples was the braiding construction. T3 was produced with diamond and T4 was produced with double braided in braiding constructions. T5 and T6 samples were produced from HT PET technical yarn with 1670 dtex yarn number, 45° braid angle, 1 center and 16 braid yarns. The only difference between T5 and T6 samples was the braiding construction. T5 was produced with diamond and T6 was produced with double braided in braiding constructions. EtO sterilization was applied on all samples. The meanings of the samples with the R and T symbols on the FT-IR charts are as follows: R1 was the raw sample form of T1. F1 was the form of T1 in which bio-chemical finishing process has been applied. R2 was the raw sample form of T2. F2 was the form of T2 with bio-chemical finishing process applied. R3 was the raw sample form of T3. F3 was the form of T3 with bio-chemical finishing process applied. R4 was the raw sample form of T4. F4 was the form of T4 with bio-chemical finishing process applied. R5 was the raw sample form of T5. F5 was the form of T5 in which bio-chemical finishing process has been applied. R6 was the raw sample form of T6. F6 was the form of T6 with bio-chemical finishing process applied. UHMWPE and PPD-T technical yarns were purchased from Durak Tekstil A.Ş in Bursa, Turkey. HT PET technical yarn was purchased from KordSA Technical Textile A.Ş in Kocaeli, Turkey. The bio-chemical finishing process was applied based on the following bio-finishing recipe and the liquid (flotte) ratio was 1:10, CHI (85%) with powder form at 2%, N-acetyl d-glucosamine (2%) with liquid form at 7%, GA (100%) with liquid form at 25% and acetic acid (80%) with liquid form at 1%. CHI (85%) and N-acetyl d-glucosamine (2%) were purchased from ADAGA A.Ş in Antalya, Turkey. GA (100%) was purchased from Kimbiotek Kimyevi Mad. San. and Tic. A.Ş in Istanbul, Turkey. Acetic acid (80%) was purchased from Bursa Teknik Kimya A.Ş in Bursa, Turkey.

2.2 Methods

2.2.1 Braiding production process

In the braiding production preparation, some technical yarns such as UHMWPE, PPD-T and HT PET bobbins in the creel were transferred to the braid bobbins by passing the control of the yarn tension meter. PPD-T, UHMWPE and HT PET technical yarns were also used as core and braid yarns with different yarn counts (dtex) in its manufacturing process. Braiding production preparation process and braiding production process were actualized in Bursa Bağcı Elyaf and Apparel Materials Construction Industry Trade Limited Company in Bursa. They are shown in Figure 2.

2.2.2 Bio-chemical finishing process

The pH values of the solution were measured by P-510 portable pH meter of Peak Instruments INC. Solution ambient temperature was also measured with the helping of a mercury thermometer (maximum operating temperature was 115°C). All in one method was applied for the preparation of bio-chemical solution. All biological

Figure 2: (a) Yarn preparation for braiding, (b) braiding production process, and (c) 3-D braiding yarn product.
chemicals that have chemicals in liquid forms were prepared in accordance with the bath (flotte) recipe with the help of beakers. Weight measurements were taken with the help of a weight device for chemicals in powder forms and then added to the solution with the help of beakers where the liquid volume was 1 L. All chemicals were prepared in accordance with the bio-chemical finishing process recipe. Time (t) at 3 h, temperature (T₀rt) at 70°C and pH at 5–5.5 were applied as process parameters of the bio-chemical finishing chemical recipe. Bio-chemical finishing process was carried out in Bursa Uludağ University in Bursa. Color change state of the bio-chemical solution was observed from light yellow to dark brown in the solution depending on time. Impregnation of the braiding samples with a continuous method in a foulard made of high density polyethylene material, which was a chemically inert and light polymeric material, was applied as post-process. It was applied as impregnation process in ten passages for 30 min for bio-chemical bonding of the braiding textile structures and the biological chemical solution.

2.2.3 After operations are PBS bio-chemical (in vitro condition) process and EtO sterilization process

PBS bio-chemical was used to provide pH 7.2 for the in vitro condition. Twenty tablets were used for a 2 L solution volume with one tablet equivalent to 100 mL of water. This post-treatment process was applied on all samples. In totally, EtO sterilization process was applied separately on all samples for six raw samples and six bio-chemical finishing samples. It was also subjected to thermal drying and fixation in an oven with temperature as 115°C for 10 minutes at Bursa Uludağ University. As a last operation EtO sterilization was applied with 1 atm as pressure, 50°C as temperature for 16 hours on all samples at the Çekirge State Hospital in Bursa.

2.2.4 FT-IR chemical analysis

In order to determine the comparative chemical analyses of all samples, FT-IR chemical analysis was applied to all samples. The ambient conditions of the test were 20 ± 2°C, rH 65%, under 1 atm pressure after conditioning for 24 h. FT-IR chemical analysis was performed for all samples on a Thermo Scientific Nicolet IS50 FT-IR branded device in Bursa Technical University Fiber and Polymer Engineering-2 laboratory.

3 Results and discussion

The results of FT-IR chemical graphics were presented in Figures 3–5. Results of FT-IR chemical graphics were
determined for all bio-composite materials with applied EtO process. They had as raw materials from R1 to R6 and had as bio-chemical finishing processes from F1 to F6. FT-IR chemical analysis had y-axis was transmittance from 0 to 100% and x-axis was wavenumbers from 500 to 4,000 cm$^{-1}$ was used for all bio-composite materials (artificial ACL ligaments).

### 3.1 Results of FT-IR chemical analyses

Chemical analysis results of UHMWPE structured T1 and T2 samples were presented in Table 1. Chemical analysis results of PPD-T structured T3 and T4 samples were presented in Table 2. Chemical analysis results of HT PET
4 Conclusions

General chemical analysis was interpreted for all samples as follows.

Table 2: Chemical analysis results of PPD-T structured T3 and T4 samples

<table>
<thead>
<tr>
<th>Bond status</th>
<th>Bond types</th>
<th>Wavelength (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shifted</td>
<td>-N–H amine(n)</td>
<td>3,305</td>
</tr>
<tr>
<td>Shifted</td>
<td>Aliphatic C–H and C–H₂</td>
<td>Between 2,920 and 2,930</td>
</tr>
<tr>
<td>Broken</td>
<td>-O–H and C–H in GA</td>
<td>2,870</td>
</tr>
<tr>
<td>Broken</td>
<td>-N=C=S</td>
<td>2,075</td>
</tr>
<tr>
<td>Shifted</td>
<td>-C=O in ester or ketone</td>
<td>Between 1,715 and 1,740</td>
</tr>
<tr>
<td>Shifted</td>
<td>-O–H in water</td>
<td>1,640</td>
</tr>
<tr>
<td>Shifted</td>
<td>-NH₃, -C–NO₂, -C=N=O, -CS–NH₂, -N–H and conjugated cyclic -C≡N</td>
<td>Between 1,510 and 1,540</td>
</tr>
<tr>
<td>Formed</td>
<td>-N=N=O and -CH₃ alkane</td>
<td>1,460</td>
</tr>
<tr>
<td>Shifted</td>
<td>-O–H</td>
<td>Between 1,300 and 1,400</td>
</tr>
<tr>
<td>Shifted</td>
<td>-C=O</td>
<td>1,015</td>
</tr>
<tr>
<td>Shifted</td>
<td>-C=Cl</td>
<td>940</td>
</tr>
<tr>
<td>Formed</td>
<td>-C=C–H alkene</td>
<td>820</td>
</tr>
<tr>
<td>Shifted</td>
<td>-C=C–H alkene and 1 or 2 neighbor aromatic -C–H</td>
<td>Between 820 and 890</td>
</tr>
<tr>
<td>Formed</td>
<td>-NH₂</td>
<td>720</td>
</tr>
<tr>
<td>Shifted</td>
<td>-C=Cl</td>
<td>620</td>
</tr>
<tr>
<td>Shifted</td>
<td>-NH₂</td>
<td>515</td>
</tr>
</tbody>
</table>

The general summary for T3 and T4 was that the aliphatic CH₂, N–H, C=O and aromatic benzene ring groups of PPD-T and the aliphatic O–H and NH₂ groups of CHI reacted with each other to form new and complex bonds such as N–N=O, CH₃ alkane and C=C–H. N”–O” in CHI was ionization. PPD-T’s and CHI’s bonds such as NH₃, -C=NO₂, -C=N=O, CS–NH₂, -N–H, conjugated cyclic -C≡N, aromatic C–H with 1 and 2 neighbors, aliphatic C–H and C=H₂ bonds were vibrated and shifted. The aromatic structure of PPD-T was preserved. -N=C=S in PPD-T and -O–H and C–H bonds in GA were broken. Bond breaks were detected.
Cross-section analysis was performed in all samples. The cross-section analysis showed that the crystallinity of the samples was decreased in all cases. This was due to the increase in the number of pores and the number of cracks. The aliphatic structures turned into aromatic and complex structures. The aromatic structure of HT PET was not preserved and was partially transformed into aliphatic structure. No bond breaks were detected in the UHMWPE structure. It was determined that new bonds were formed in UHMWPE, PPD-T and HT PET structures. The most common bond formations were HT PET > PPD-T > UHMWPE.

In order to increase the formation of new chemical bonds in all samples the following should be actualized:

The pH should be 5–5.5. GA concentration should be a minimum of 25% or higher. The dissolution time of CHI should be a minimum of 3 h or more. The process temperature should be a minimum of 70°C or higher. In this way, the molecular size of CHI will be reduced so that it will facilitate easier and faster diffusion and adsorption. The chelation property of CHI on all samples will be also evident successfully.

In the future studies about various artificial ACL ligament experimental studies to be actualized, the chemical analyses of CHI cross-linked with GA on technical yarns used in this experimental study can be based. In addition, in future experimental studies on artificial ACL ligament it is thought that more comprehensive in vitro or in vivo experimental studies will be actualized by using different biocompatible biopolymers by changing and comparing various process parameters such as pH, temperature, time, concentration, textile form, production
method, construction, yarn type, yarn count and bio-finishing process.

Acknowledgements: Thanks to Mr. İnål Kaan Duygun, assistant of Department of Fiber and Polymer Engineering at T.C Bursa Technical University for assistance in using the FT-IR device. All raw samples were collected from Bursa Bağçı Elyaf and Apparel Materials Construction Industry Trade Limited Company in Bursa with the help of Mr. Fedahı KILIÇAY who is the head of manufacturing department.

Funding information: This work was supported by the T.C Bursa Uludağ University registered under the number: T.C B.U.Ü BAP OUAP (MH) 2020/9.

Author contributions: Ömer Furat Turşucular – conceptualization, manuscript writing, data acquisition, technical data analysis and manuscript spell check. Yusuf Ulcay – conceptualization and manuscript spell check.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Ethical approval: This experimental study was not related to human or animal use.

Data availability statement: All data generated or analyzed during this study are included in this published article (and supplementary information files).

References


