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Research Article

COATING OF ARTIFICIAL VASCULAR GRAFT WITH SILK FIBROIN AND CURCUMIN

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Abstract

This study aims to enhance the surface property of Dacron by coating with the silk fibroin (SF) solution. Surface modification with silk fibroin was done with the addition of turmeric extract in order to reduce the risk of blood clotting, which is the biggest challenge with artificial grafts. In this study, silk fibroin was prepared by two different methods to coat the surface of the graft to get a smooth surface to control the release of the anticoagulation agent. The first method was the Ajisawa method and the second one was the dissolution of silk fibroin in the formic acid. Both of the two methods were explained in detail in the materials and methods part. As anticoagulation agent, turmeric extract was preferred. Characterization of obtained grafts was done with Fourier-Transform Infrared (FTIR) spectroscopy. Release studies were performed. Obtained data showed that silk fibroin is a good coating material for reducing porosity and coating Dacron. By coating with silk fibroin, the controlled release of the turmeric extract can be achieved.

Keywords: Artificial graft, silk fibroin, curcumin, coating, anticoagulant properties.

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Araştırma Makalesi

YAPAY VASKÜLER GREFTİN İPEK FİBROİN VE KURKUMİN İLE KAPLANMASI

Özet

Bu çalışma, ipek fibroin (SF) çözeltisi ile kaplanarak Dacron yüzey özelliğini arttırmayı amaçlamaktadır. Yapay greftlerde en büyük zorluk olan kan pıhtılaşma riskini azaltmak için zerdeçal ekstraktının eklenmesiyle ipek fibroin ile yüzey modifikasyonu yapılmıştır. Bu çalışmada, antikoagülan ajanın salımını kontrol etmek ve pürüzsüz bir yüzey elde etmek için greftin yüzeyinin kaplamasında kullanılan ipek fibroin iki farklı yöntemle hazırlanmıştır. İlk yöntem Ajisawa yöntemi, ikincisi ise ipek fibroinin formik asitte çözünmesi yöntemidir. Bu iki yöntemin her ikisi de materyal ve yöntem bölümünde ayrıntılı olarak açıklanmıştır. Antikoagülan ajan olarak zerdeçal ektraktı tercih edilmştir. Elde edilen greftlerin karakterizasyonu Fourier dönüşümlü kızılötesi (FTIR) spektroskopisi ile yapılmıştır. Salım çalışmaları gerçekleştirilmiştir. Elde edilen veriler, ipek fibroinin gözenekliliği azaltmak ve Dacron'u kaplamak için iyi bir kaplama malzemesi olduğunu göstermiştir. İpek fibroin ile kaplanarak, zerdeçal ekstraktının kontrollü salımı elde edilebilmiştir.

Anahtar Kelimeler: yapay greft, ipek fibroin, kurkumin, kaplama, antikoagülan özellikler.

1. INTRODUCTION

In the last years, the number of patients who are suffering from vascular diseases increased. There is a limited option to treat vascular diseases one of the most useful ways is by-pass surgery. In these surgeries, the artificial vascular grafts which are made by textile fibers, replace or repair diseased arteries. Prosthetic materials like polyethylene terephthalate (Dacron), polytetrafluoroethylene (PTFE), expanded PTFE (ePTFE), and polyurethane are used for vascular grafts. There are various types of raw material to produce vascular grafts, such as Dacron (a special name for polyester), Teflon, or Nylon. The ideal vascular graft requires being biocompatible, non-thrombogenic, and fatigue resistant, flexible yet robust, readily available.

However, these prosthetic materials prove to have many shortcomings and problems, especially when the vessel diameter is less than 5mm. The most common problems are coagulation, porosity, and dilatation (Eren & Ulcay, 2010). To improve these shortcomings of grafts, surface modification is proposed. The few known methods are coating, hydrolysis or reduction, plasma treatment, crosslinking, etc. Natural polymers and compounds become more available for surface modification methods due to their biocompatibility (Pan, Tang, Weng, Wang, & Huang, 2006). For instance, coating with proteins (i.e. collagen, albumin, gelatin), by crosslinking, plasma treatment with reactive gases to create new functional groups on Dacron and, using bioactive materials which have an anticoagulant effect such as heparin (Pillai, Kumar, Houshyar, Padhye, & Bhattacharyya, 2020).

In this study, silk fibroin was chosen to coat the surface of the graft to get a smooth surface and control the release of the anticoagulation agent. The advantages of using the silk fibroin are better smooth surface on the wall of graft rather than using collagen and it is cheaper than collagen. In Table 1, mechanical strength properties of silk, fibroin was shown.

Table 1 Mechanical strength of silkworm silk along with other fibrous materials

Materials	**Young's modulus (GPa)**	**Ultimate strength (MPa)**	**Breaking strain (%)**	**Toughness (MJ m^{-3})**
Silkworm silk fibroin *(Bombyx mori)*	10-17	300-740	4-26	70-78
Kevlar	130	3600	2.7	50
Collagen	0.0018-0.046	0.9-7.4	24-68	-

As Table 1 shows silk fibroin has better mechanical properties than collagen.

Silk consists of two parallel fibroin fibers, which are attached to a layer of sericin. So silk has two main proteins: silk fibroin (fibrous protein) and sericin (globular protein) (Huang, Bailey, Wang, & Feng, 2017). In the literature, silk proteins have been used in various studies due to their versatile process abilities. Silk proteins are biocompatible, biodegradable, strong, and thermally stable (Cao & Zhang, 2016; Shao et al., 2016; Yin et al., 2017). These properties also increased its application areas. For many applications, especially biomedical applications, sericin must be removed from silk in order to prevent allergic reactions to the human body.
Natural bioactive compounds have been used for their biological activities (Dulak, 2005). Curcumin has shown potent activity against various ailments such as cardiovascular systems, liver, lungs, kidneys, eyes, and gastrointestinal and, as well as conditions such as fibrosis, wound healing problems, aging, asthma, and endometriosis. Low constitutional toxicity and a big range of pharmacological activity as anti-oxidation, anti-thrombus, and anti-proliferation properties of curcumin could be very efficient for drug-eluting stents (Gupta, Patchva, Koh, & Aggarwal, 2013; D. C. Kim, Ku, & Bae, 2012; W.-H. Lee et al., 2013; Pan et al., 2006). In the literature, many studies propose that curcumin can inhibit platelet aggregation induced by ADP, collagen, and epinephrine (H. S. Lee, 2006; Park et al., 2005; Srivastava, Puri, & Srimal, 1986). Curcumin can also prohibit of platelet aggregation by platelet-activated factors, and the formation of thromboxane A2 (TXA2) inhibit because of platelets (Shah et al., 1999).

Due to all these beneficiary properties of both silk fibroin and turmeric extract were used in the study. By using silk fibroin, both coatings of the Dacron and improving bioactivity of the turmeric extract were done (Luo et al., 2016).

2. MATERIALS AND METHODS

2.1. Materials

Sodium carbonate (0.05 %), formic acid, sulfuric acid, sodium sulfide, and calcium chloride were purchased from Merck, Germany. Dialysis tube (Snake Skin TM Dialysis Tubing, 10K MWCO, 22 mm) was purchased from Thermo Fischer, USA. For turmeric extract locally bought turmeric was used. Curcumin standard (99.5 % HPLC grade) was bought from Sigma Aldrich, USA.

2.2. Degumming of Raw Silk

The traditional method was used to remove sericin from the silk fiber. Raw silk treated in the boiling aqueous 0.05% Na_2CO_3 solution (v/w) for 30 minutes. The same steps were repeated 3 times. After treatment degummed silk was washed by deionized water several times. Washed degummed silk was left to dry (H. J. Kim et al., 2017).

2.3. Preparation of Silk Fibroin (SF) Solution

To obtain silk fibroin solution which was used to coat grafts, both Ajisawa solution and formic acid were used. Ajisawa solution was prepared as given in the literature ($CaCl_2$/ethanol/water; 111/92/144), and the mixture was stirred at 80 °C for 2 hours (Ajisawa, 1997). Then the solution was dialyzed with deionized water for at least 3 days at room temperature to remove the neutral salts, ethanol, and impurities at 4 °C. For the formic acid method, silk fibroin was dissolved in stirring formic acid, and the solution was prepared.

2.4. Preparation of Turmeric Extract

The turmeric extract was prepared with a 1:30 solid to liquid ratio is 70% ethanol solution for 21 hours. After extraction obtained extract was filtered to remove the insoluble parts. The obtained extract was kept in the sealed container in order to keep alcohol content constant.

2.5. Preparation of Silk Fibroin Coated Grafts

Two sets of experiments were carried out with two different preparation methods of silk fibroin which are Ajisawa solution and formic acid. Each set consists of graft coated with silk fibroin, silk fibroin and glycerol, silk fibroin and turmeric extract, and silk fibroin, glycerol, and turmeric extract. The effect of glycerol as plasticizer is also investigated in these experiments. In Table 2 prepared samples and their components are given.

Table 2 Prepared Samples and Their Components for Each Set-Up

Sample Name	**Components**	**Silk Fibroin Preparation Method/ Experimental Set-Up**
SF_1	Silk Fibroin	Ajisawa Solution/ Set-Up 1
SFG_2	Silk Fibroin and Glycerol	Ajisawa Solution/ Set-Up 1
SFC_3	Silk Fibroin, Turmeric Extract	Ajisawa Solution/ Set-Up 1
$SFGC_4$	Silk Fibroin, Glycerol, Turmeric Extract	Ajisawa Solution/ Set-Up 1

SF_5	Silk Fibroin	Formic Acid Solution/ Set-Up 2
SFG_6	Silk Fibroin and Glycerol	Formic Acid Solution/ Set-Up 2
SFC_7	Silk Fibroin, Turmeric Extract	Formic Acid Solution/ Set-Up 2
$SFGC_8$	Silk Fibroin, Glycerol, Turmeric Extract	Formic Acid Solution/ Set-Up 2

For each graft, 5 ml of SF solution was used, and 0.13 ml glycerol as a plasticizer, 0.05 mL turmeric extract, which has 0.004 mg turmeric extract as an anticoagulant was added. In order to observe the effect of coating, some grafts were dipped 20 times, and some grafts were dipped 40 times to the solutions in a sealed container.

2.6. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The samples' spectra were recorded in 650 to 4000 cm^{-1} by using Perkin Elmer Spectrum 100 FTIR spectrometer. Components were analyzed as a powder with ATR mode.

2.7. Release of Turmeric Extract from Grafts

For the release studies, the phosphate buffer solution was used in order to achieve physiological pH. For this purpose, 3 different types of samples were used, which were mentioned in the text as SFC, SFGC, and SFGC waited. SFC indicates Dacron coated with solution consists of silk fibroin, and turmeric extract by dipped 40 times into solution, SFGC indicates Dacron coated with solution consists of silk fibroin, turmeric extract, and glycerol by dipped 40 times into solution and SFGC waited indicates Dacron coated with solution consists of silk fibroin and turmeric extract and waited 1 week in these solutions. In Table 3 samples used in the release studies and components are given.

Table 3. Samples Used in the Release Studies and Their Components

Sample Name	**Components**	**Preparation Method**
SFC	Silk Fibroin, Turmeric Extract	40 times dipped
SFGC	Silk Fibroin, Glycerol, Turmeric Extract	40 times dipped
SFGC waited	Silk Fibroin, Glycerol, Turmeric Extract	40 times dipped and waited 1 week

For the release studies, each coated Dacron was immersed into phosphate buffer and initially, samples were taken every 5 minutes. Curcumin standard was used for calibration. All readings were done in UV-spectrophotometer at 427nm.

3. RESULTS AND DISCUSSION

3.1. Characterization of Coated Grafts

As explained in the materials and method section various samples were prepared for this study. The stated solutions applied to impregnate into the vascular graft with different methods. Three samples were cut from dacron for each solution. One sample was dipped 20 times, second sample was dipped 40 times and another one was dipped and waited in solution for 1 week. In Figure 1 picture of prepared samples is given.

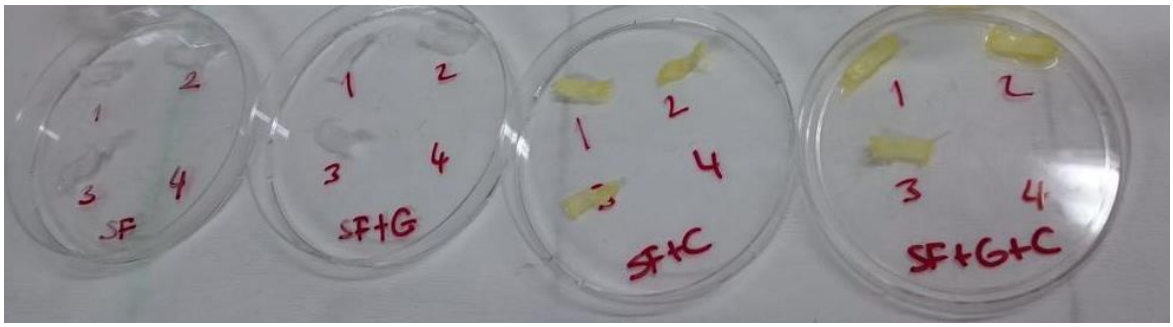

Figure 1. Coated Grafts from Experimental Setup 1

For the characterization of coated grafts Fourier-Transform Infrared (FTIR) spectroscopy analysis was used. In Figures 2 and 3 FTIR spectra of grafts coated with silk fibroin which was prepared with Ajisawa solution are given.

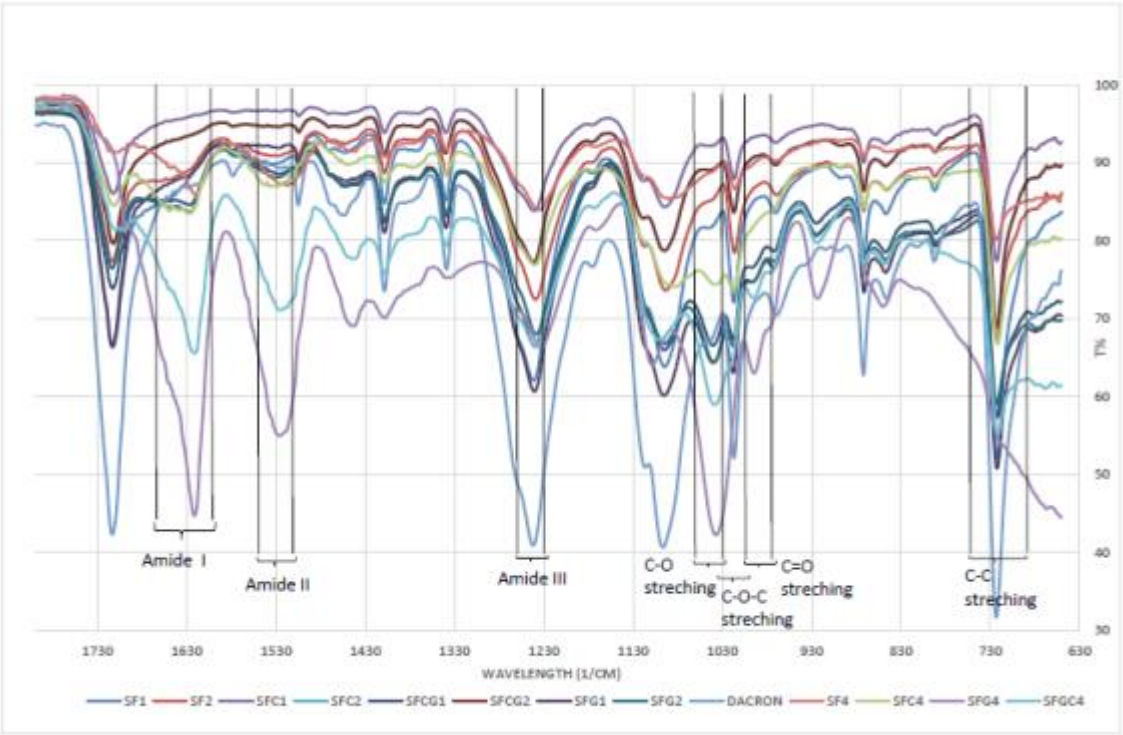

Figure 2. FTIR spectra of experimental set-up 1 between 630-1730 cm^{-1}

In Figure 3 rest of the FTIR spectra of grafts coated with silk fibroin which was prepared with Ajisawa solution were given.

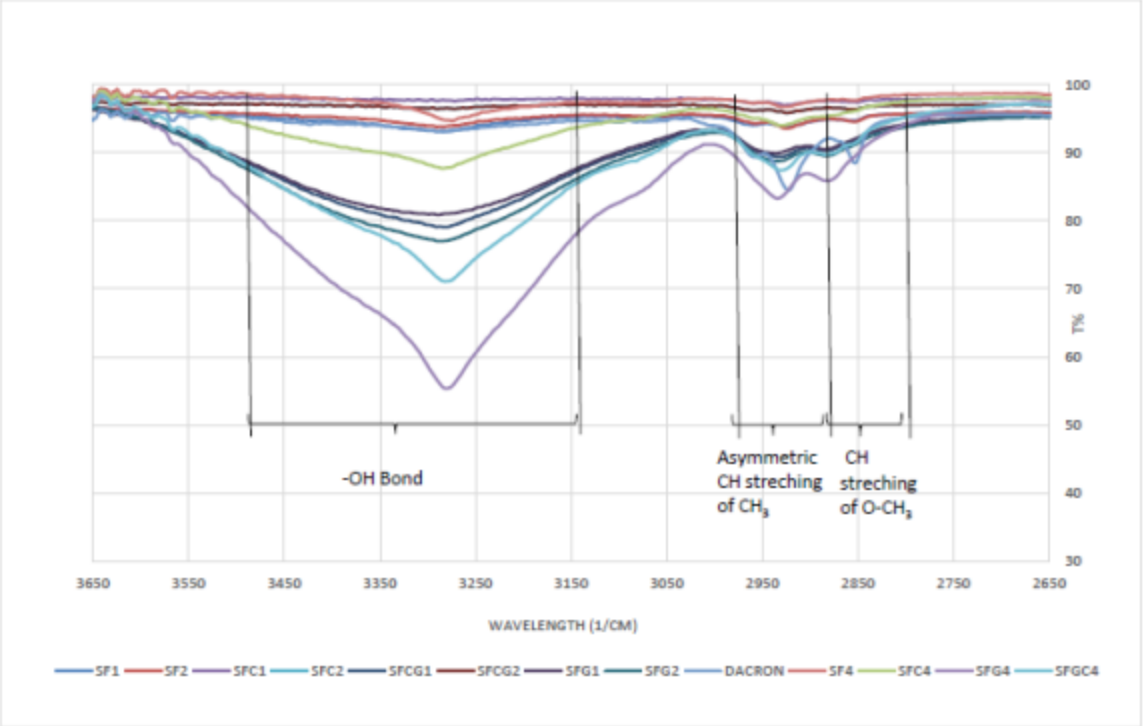

Figure 3. FTIR spectra of experimental set-up 1 between 2700-3700 cm^{-1}

The same analyses were done for experimental set up 2. In Figures 4 and 5 FTIR spectra of grafts coated with silk fibroin which was prepared with formic acid were given.

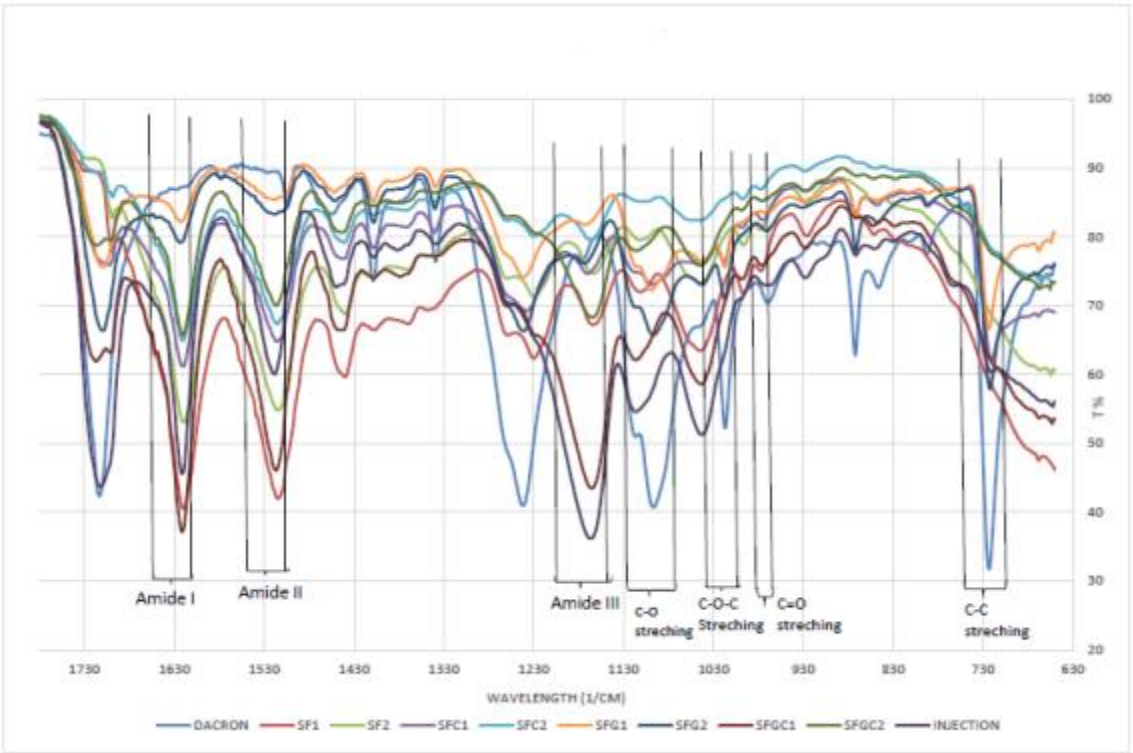

Figure 4. FTIR spectra of experimental set-up 2 between 630-1730 cm^{-1}

In Figure 5 rest of the FTIR spectra of grafts coated with silk fibroin which was prepared with formic acid are given.

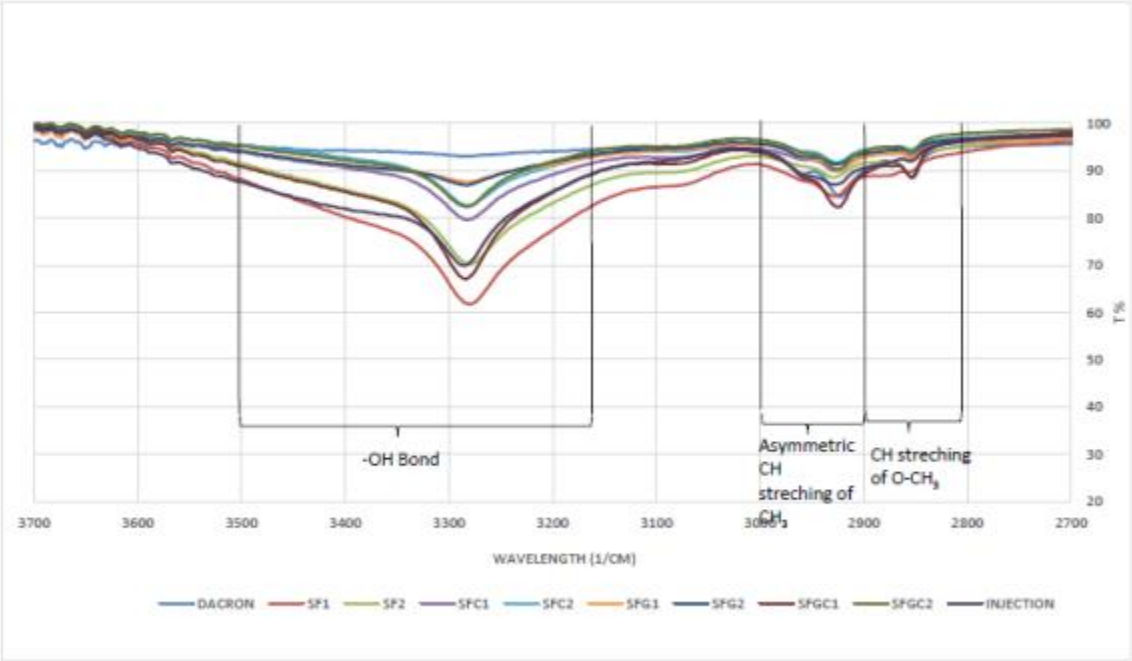

Figure 5. FTIR spectra of experimental set-up 2 between 2700-3700 cm^{-1}

For silk fibroin, structural characteristic peaks were observed at 1621 cm^{-1} (amide I) and 1515 cm^{-1} (amide II), and 1166 cm^{-1} (amide III). These amide bands were presented in the given FTIR spectra with exception of Dacron sample which was not coated with silk fibroin.
For the identification of curcumin; stretching band of O-H at 3500-3200 cm^{-1}, C-H stretching at 2800-3000 cm^{-1}, C-O bonding at 1098 $cm^{-1,}$ and C=O stretching at 968 cm^{-1} peaks were checked. The samples were immersed in turmeric extract matched with the FTIR spectrum reported in the literature. These results showed that the coating of turmeric extract into Dacron was done successfully. In the FTIR spectra, vibrations of C-C stretching were observed due to the presence of glycerol. As seen from FTIR results coating of SFGC waited samples are better due to the better coating of Dacron.

3.2. Release of Turmeric Extract

The samples were dipped 40 times and samples were dipped and waited in solution for 1 week are used for release studies. In Figure 6 release profiles of the turmeric extract from samples prepared with Ajisawa solution are given.

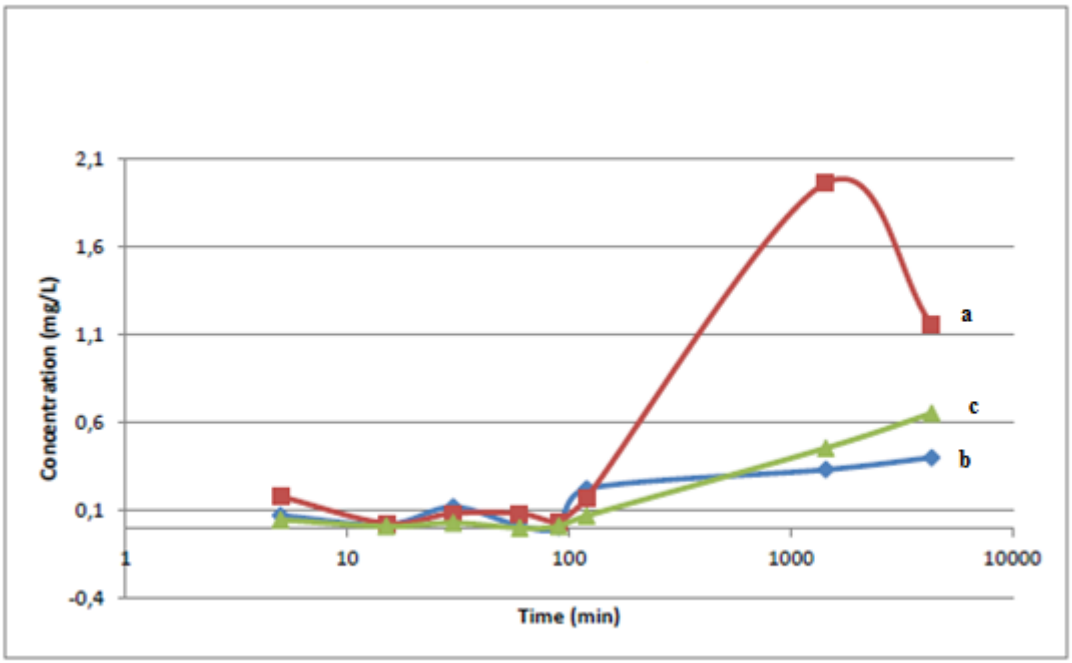

Figure 6. Release profiles of turmeric extract from 40 times dipped samples prepared with Ajisawa solution a) SFGC b) SFC c) SFGC waited

As seen from Figure 6, all three samples showed the same release profile for the first 100 min, and then release profiles were changed. Turmeric extract in the SFGC sample was released three times more than other samples. In Figure 7, concentrations of released turmeric extract at 1440 min are given.

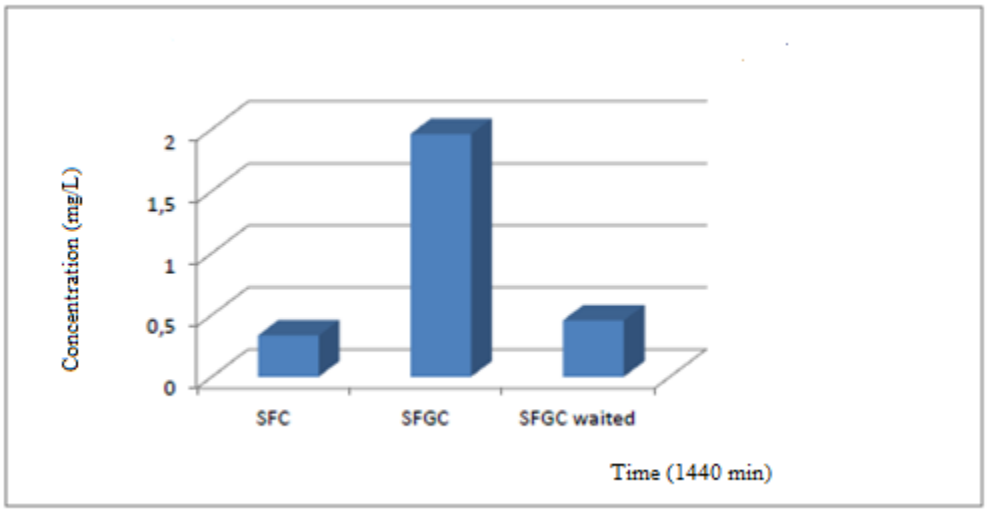

Figure 7. The concentration of turmeric extract at 1440 min for samples prepared with Ajisawa solution

As seen from Figure 7, the SFGC sample released turmeric extract more than other samples. We believe that these results can be explained by various reasons. First, without glycerol SFC grafts are too brittle when they are dried so this resulted in the smallest amount of release of turmeric extract. Secondly, FTIR results showed that SFGC waited sample is the best-coated

sample due to a long period of duration in the solution. So with this coating, turmeric extract can hold on to the surface of Dacron better due to hydrophobic interactions. So with SFGC waited sample, slow and sustained release of the turmeric extract is possible. In Figure 8 release profiles of the turmeric extract from samples prepared with formic acid were given.

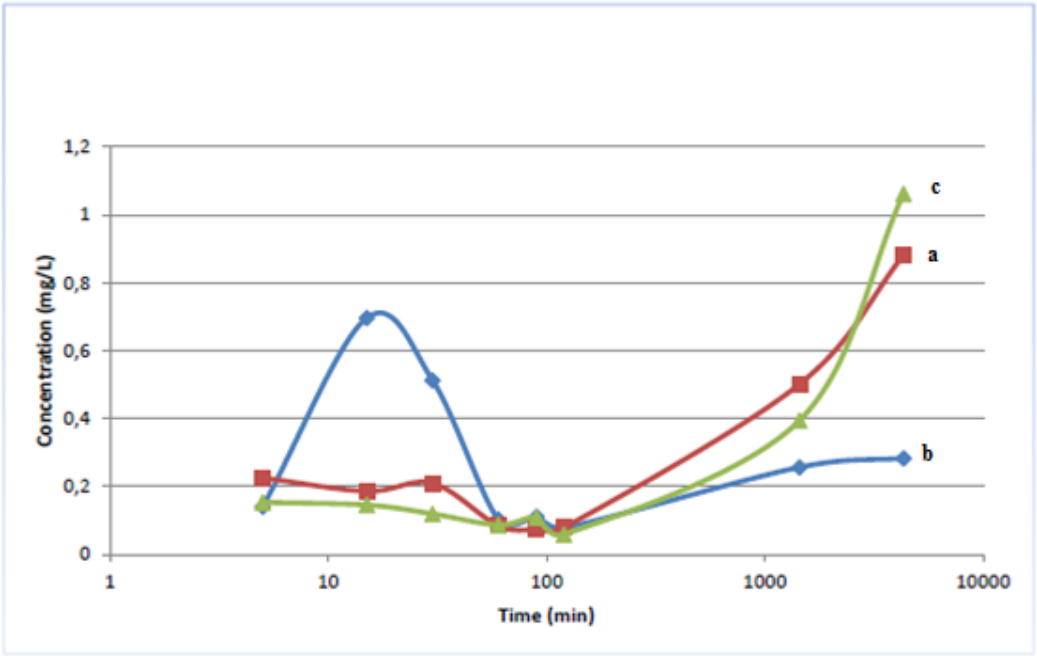

Figure 8. Release profiles of turmeric extract from 40 times dipped samples prepared with formic acid a) SFGC b) SFC c) SFGC waited

Different from samples prepared with Ajisawa in this experimental set-up SFGC and SFGC waited showed similar release profiles. This could be resulted due to lack of water in the medium due to the preparation of silk fibroin solution with formic acid. So lack of water in the medium might alter the hydrophobic interactions between Dacron and turmeric extract.

The obtained data in Figure 9 compared for two methods at the same time (at 1440 min), the sample prepared with Ajisawa solution was released a higher amount of curcumin. (1.7 vs 0.58 mg/L).

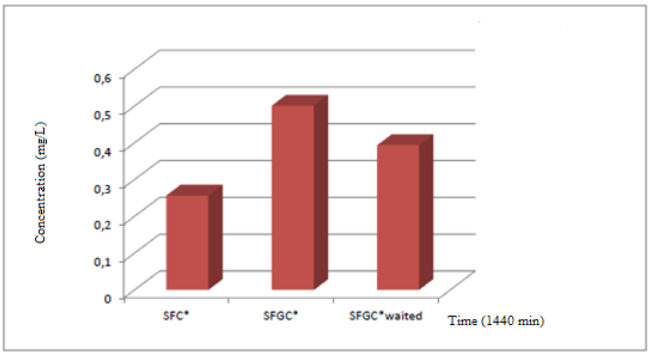

Figure 9. The concentration of turmeric extract at 1440 min for samples prepared with formic acid

4. CONCLUSIONS AND RECOMMENDATIONS

Teflon® (Polytetrafluoroethylene) and Dacron® (Polyethylene terephthalate) are widely used prosthetic materials for artificial vascular grafts. In this study, Dacron, which is widely used in industry, was chosen. Dacron is a polyester fabric (poly (ethylene terephthalate)), which is a hydrophobic polymer. For a coating material, silk fibroin (SF) is chosen; SF is a natural protein and well known as biocompatible, degradable, and nontoxic. The aim of using SF is to enhance the surface property of graft by reducing porosity.

According to the FTIR spectra and release profile of turmeric extract, it can be seen that SFGC waited sample (coated with SF solution prepared with Ajisawa solution, turmeric extract, and glycerol) is the most proper one for vascular diseases. Because of high coagulation risk at the beginning of the artificial graft transplanting process, the initial release of a high amount of turmeric extract is required and it is provided by SF solution with the Ajisawa method. The anticoagulant effect of turmeric extract and SF coating of vascular polyester graft will be studied further.

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CONFLICTS OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Ajisawa, A. (1997). Dissolution aqueous of silk fibroin with calciumchloride / ethanol solution. *The Journal of Sericultural Science of Japan*, 67(2), 91–94.

Cao, T. T., & Zhang, Y. Q. (2016). Processing and characterization of silk sericin from Bombyx mori and its application in biomaterials and biomedicines. *Materials Science and Engineering C*, 61, 940–952. Retrieved from https://doi.org/10.1016/j.msec.2015.12.082

Dulak, J. (2005). Nutraceuticals as anti-angiogenic agents: Hopes and reality. *Journal of Physiology and Pharmacology*, 56(SUPPL. 1), 51–67.

Eren, S., & Ulcay, Y. (2010). Yapay Tekstil Damarları. *Electronic Journal of Textile Technologies*, 2010(1), 35–47.

Gupta, S. C., Patchva, S., Koh, W., & Aggarwal, B. B. (2013). Discovery of Curcumin, a Component of the Golden Spice, and Its Miraculous Biological Activities. *Clin Exp Pharmacol Physiol*, 39(3), 283–299.

Huang, Y., Bailey, K., Wang, S., & Feng, X. (2017). Silk fibroin films for potential applications in controlled release. *Reactive and Functional Polymers*, 116(May), 57–68.

Kim, D. C., Ku, S. K., & Bae, J. S. (2012). Anticoagulant activities of curcumin and its derivative. *BMB Reports*, 45(4), 221–226.

Kim, H. J., Kim, M. K., Lee, K. H., Nho, S. K., Han, M. S., & Um, I. C. (2017). Effect of degumming methods on structural characteristics and properties of regenerated silk. *International Journal of Biological Macromolecules*, 104, 294–302.

Lee, H. S. (2006). Antiplatelet property of Curcuma longa L. rhizome-derived ar-turmerone. *Bioresource Technology*, 97(12), 1372–1376.

Lee, W.-H., Loo, C.-Y., Bebawy, M., Luk, F., Mason, R., & Rohanizadeh, R. (2013). Curcumin and its Derivatives: Their Application in Neuropharmacology and Neuroscience in the

21st Century. *Current Neuropharmacology*, 11(4), 338–378.

Luo, T., Yang, L., Wu, J., Zheng, Z., Li, G., Wang, X., & Kaplan, D. L. (2016). Stabilization of Natural Antioxidants by Silk Biomaterials. *ACS Applied Materials & Interfaces*, 8(21), 13573–13582.

Pan, C. J., Tang, J. J., Weng, Y. J., Wang, J., & Huang, N. (2006). Preparation, characterization and anticoagulation of curcumin-eluting controlled biodegradable coating stents. *Journal of Controlled Release*, 116(1), 42–49.

Park, S. D., Jung, J. H., Lee, H. W., Kwon, Y. M., Chung, K. H., Kim, M. G., & Kim, C. H. (2005). Zedoariae rhizoma and curcumin inhibits platelet-derived growth factor-induced proliferation of human hepatic myofibroblasts. *International Immunopharmacology*, 5(3), 555–569.

Pillai, M. M., Kumar, G. S., Houshyar, S., Padhye, R., & Bhattacharyya, A. (2020). Effect of nanocomposite coating and biomolecule functionalization on silk fibroin based conducting 3D braided scaffolds for peripheral nerve tissue engineering. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 24, 102131.

Shah, B. H., Nawaz, Z., Pertani, S. A., Roomi, A., Mahmood, H., Saeed, S. A., & Gilani, A. H. (1999). Inhibitory effect of curcumin, a food spice from turmeric, on platelet-activating factor- and arachidonic acid-mediated platelet aggregation through inhibition of thromboxane formation and Ca2+ signaling. *Biochemical Pharmacology*, 58(7), 1167–1172.

Shao, W., He, J., Sang, F., Ding, B., Chen, L., Cui, S., … Tan, W. (2016). Coaxial electrospun aligned tussah silk fibroin nanostructured fiber scaffolds embedded with hydroxyapatite-tussah silk fibroin nanoparticles for bone tissue engineering. *Materials Science and Engineering C*, 58, 342–351.

Srivastava, R., Puri, V., & Srimal, R. C. (1986). Effect of curcumin on platelet aggregation and vascular prostacyclin synthesis. *Arzneimittel-Forschung/Drug Research*, 36(4), 715–717.

Yin, Z., Kuang, D., Wang, S., Zheng, Z., Yadavalli, V. K., & Lu, S. (2017). Swellable silk fibroin microneedles for transdermal drug delivery. *International Journal of Biological Macromolecules*. yi