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Development of Electrospun Fish Gelatin/Silver/Poly(lactic acid) Nanofibers

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In this work, the evaluation of fish scales (FS), which is an organic waste of the fishing industry and cannot be recycled, was studied. FS was cleaned and dried before being used as fish gelatin (FG) in the electrospinning process. A polymer solution of PLA was used as a carrier for FG and silver nanoparticles (Ag). The amount of PLA and Ag was kept constant for each electrospinning solution while FG was added in three different ratios (0.5 %, 1 % and 2 %). The electrospinning process was carried out at 0.5 mL/h feeding rate, 11 kV applied voltage and 10 cm collector distance. Obtained nanofibrous surfaces were characterized via X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). The results confirmed porous fiber formation which has potential use in many fields, such as filtration, biomedical and healthcare applications.
Keywords: electrospinning, fish gelatin, silver, polylactic acid.

1. INTRODUCTION

The uncontrolled increase in the world population leads to a decrease in the available natural resources and an increase in environmental pollution. In recent years, efforts have been made in many areas to prevent the increase in environmental pollution by waste management, waste recycling and waste processing and utilization in different areas and thus slow down the increase in the total amount of waste. Since Turkey is a peninsula surrounded by seas on three sides, a considerable variety of seafood is available and consumed in the country. Scales and bones left over from fish constitute an environmental waste. Therefore, it is important to utilize fish scales, skin and bones to reduce the total amount of waste. In the applications we have come across in this sense, it has been observed that fish gelatin is obtained from fish scales, skin and bones and can be used in different areas [1 – 3]. In the studies carried out for the utilization and reduction of wastes, the production of alternative gelatin from animal wastes provides both convenience in terms of process and creates an important opportunity in terms of the use of gelatin in different sectors from food to cosmetics and pharmaceutical industry [4]. Based on this, gelatin was recently obtained from freshwater and saltwater fish using six different methods, and its structural and functional properties were investigated [5].

One of the most common uses of gelatin in the world is as a viscosity enhancer in the food industry. The characteristic features of gelatin such as transparency, colorlessness and gel structure increase its usability. In addition, the ease of the production process and the lack of problems in terms of material supply have paved the way for its preference. The use of pork skin and bones in the production of gelatin is problematic for different religions, hence the search for alternative materials. Gelatin derived from fish has low proline (Pro) and hydroxyproline (Hyp) content compared to gelatin derived from mammals. Therefore, fish-derived gelatin retains its form at room temperature without showing gelling behavior. In the literature, it is stated that hydrogels are auxiliary materials for medical textiles, medicine and the health sector and can be used in many fields due to their insoluble, cross-linked hydrophilic structures. It was stated that biocompatible fish gelatins can be evaluated in this field [6]. Studies have shown that fish gelatin supports skin tissue and accelerates wound healing and strengthens the regeneration process in people with diabetes and bone diseases. Potential uses include antioxidant, antihypertensive, antimicrobial, tissue regeneration supportive, wound healing, bone formation accelerator and anticancer applications [1].

In a study by Kwak et al. aqueous solution of fish gelatin was prepared and nanofiber structures were produced. Electrospinning of fish gelatin did not require any additional temperature control, unlike mammalian gelatin. The concentration of aqueous fish gelatin was expressed as an important factor in determining the electrospinning and diameter of gelatin nanofibers. Cell viability assay showed that nanofibrous structures produced from fish gelatin showed better cell adhesion and proliferation rate compared to the film form; it was also reported that nanofibrous fish gelatin scaffolds had good cytocompatibility, similar to mammalian gelatin sources. Fish gelatin is a promising alternative to mammalian-derived conventional gelatin due to its environmental friendliness and ease to be manufactured [7]. Shi et al. investigated fish gelatins and composites containing fish gelatins and proposed various approaches. The mechanical and biological properties of fish gelatins improved after the addition of polysaccharides;

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the polymers obtained by using gelatin and chitosan obtained from fish skin for tissue engineering resembled hydrophilic and nonwoven fibrous structures after electrospinning and therefore their suitability for tissue engineering was evaluated. In most of the existing studies, there is an opinion that the studies on fish gelatin and polysaccharides should be deepened [8].

In addition to the use of PLA and fish gelatin (FG) together in tissue engineering, it has been stated that in addition to material selection, fabrication methods are also critical for the design of neural scaffolds and that structures produced by electrospinning can potentially mimic the structure of the natural extracellular matrix (ECM), allowing the production of nanofibrous scaffolds that offer optimal surface properties for cell attachment, proliferation and differentiation. In the literature, the production of biomaterials supporting peripheral nerve regeneration was aimed and surfaces were produced with PLA blended with fish gelatin at five different weight ratios using electroplating method. The produced surfaces were characterized in terms of peripheral nerve regeneration potential, physicochemical properties and in vitro cytotoxicity experiments were performed to characterize their biological properties. It was demonstrated that the nanofibrous membrane structures produced by the electroplating method were biocompatible and PG11 membranes were a good substrate for fibroblast adhesion and proliferation compared to pure PLA and FG as their morphology and structure were improved [9].

In this study, nanofibrous surfaces were fabricated by using fish gelatin (FG), silver nanoparticles (Ag) and poly(lactic acid) (PLA) carrier polymer obtained from fish scales (FS), which constitute environmental waste, and the characteristics of the produced surfaces were investigated. The novelty of this work was to use fish gelatin, silver and PLA together as three components to produce nanofiber surfaces. The characterization of the produced nanofiber surfaces was carried out by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX).

2. EXPERIMENTAL METHODS

2.1. Obtaining fish felatin from fish scales

The collected rock sea bass fish scales were first cleaned with soapy water, the coarsest dirt was removed from their surfaces and left to dry. 10 g of fish scales were weighed and added into 100 ml of water together with 0.8 ml of HCl and shaken in the beaker for 5 min and then rinsed with distilled water. After rinsing, filtration was performed. 2 g NaOH was added to 100 ml of water and filtered fish scales were placed into the flotte tubes. These tubes were processed at 100 °C for 60 minutes. When the process was over, fish scales were filtered and transferred to glass jars and then inserted in an oven to dry at 60 °C.

2.2. Fabrication of Nanofiber Surfaces Containing PLA, Fish Gelatin and Silver

Silver nitrate and fish scales are not easily soluble materials. Therefore, 0.5 % (0.5 g), 1 % (1 g), 2 % (2 g) fish scales and 0.35 g silver nitrate were added to 100 ml of water and stirred in an ultrasonic bath for 1 hour. After an hour, 1.2 g of sodium boron hydride was added as a binder and the process continued for another 30 min. At the end of the process, it was left on a filter paper for filtration. After filtration, the remaining materials were kept in the oven. The dried materials were removed from the filter paper. Dried fish scales and silver nitrate mixture was added in 10 ml DMF. The function of DMF was to reduce Ag ions into Ag nanoparticles as explained in the literature [10]. This process was done separately to obtain mixtures containing 3 different ratios of fish scales. They were processed in an ultrasonic mixer for 30 mins.

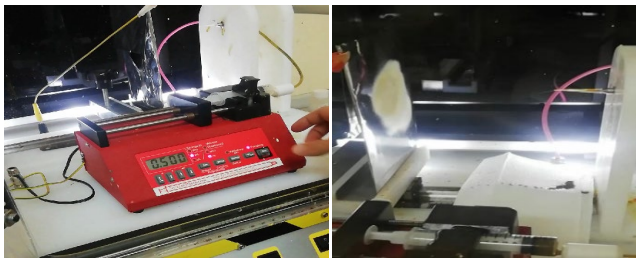


Fig. 1. Electrospinning device and fiber production moment visualization

To prepare the PLA solution, 0.8 g of PLA was added into 8 ml of chloroform and stirred in a magnetic stirrer for about an hour to dissolve the PLA. Then, 1 ml of the fish gelatin/Ag mixture was added to the prepared PLA solution and mixed further to prepare the electrospinning solution.

The prepared solution was transferred into a 10 ml syringe and fed into the electrospinning device as shown in Fig. 1. The production was carried out at 0.5 mL/h feed rate, 11 kV voltage and 10 cm collector distance. The obtained nanofiber surfaces were characterized and investigated by FTIR, EDX, SEM, XRD analysis.

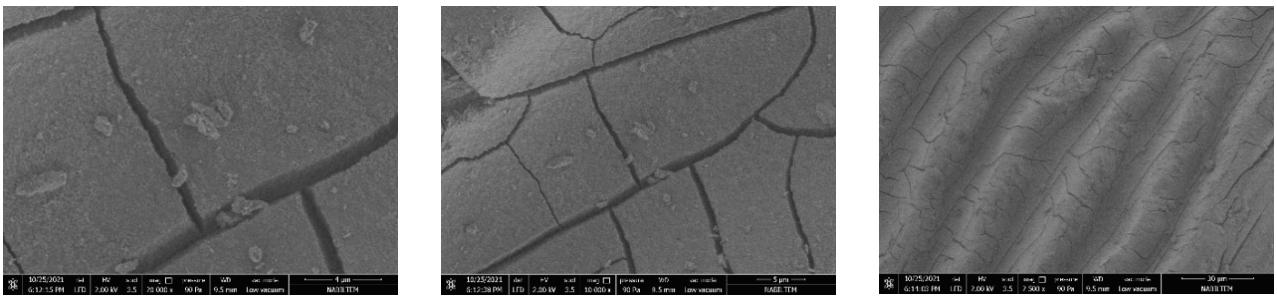
3. RESULTS AND DISCUSSION

The amount of PLA and Ag were kept constant in the electrospinning solution while fish gelatin was used in three different ratios, 0.5 %, 1 %, 2 %. Therefore, the electrospinning process was used to produce three sets of nanofibers each containing the same amount of PLA and Ag but different amount of fish gelatin. Table 1 shows the chemical composition of the prıduced nanofibrous surfaces while Fig. 2 – Fig. 5 show the SEM images of fish gelatin and produced FG/Ag/PLA nanofiber structures.

Table 1. The chemical composition of the newly developed fibres

	FG, %w/v	$AgNO_3$, %w/v	PLA, %w/v
0.5 % FG/Ag/PLA	0.5	0.038	8.88
1 % FG/Ag/PLA	1.1	0.038	8.88
2 % FG/Ag/PLA	2.2	0.038	8.88

Examination of SEM images of produced nanofibers showed that porous fiber structures were formed. It was detected that the fiber diameters increased due to the increased viscosity of the polymer solution as a result of the increasing amount of fish gelatin in the polymer solution. For comparison purposes, the images of the produced samples were recorded at the same magnifications.



a b c

Fig. 2. SEM images of fish gelatin: a – 20000x; b – 10000x; c – 2500x

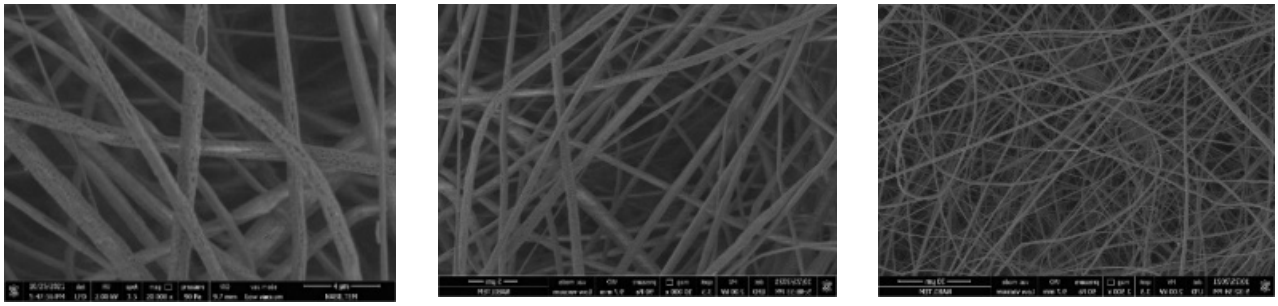


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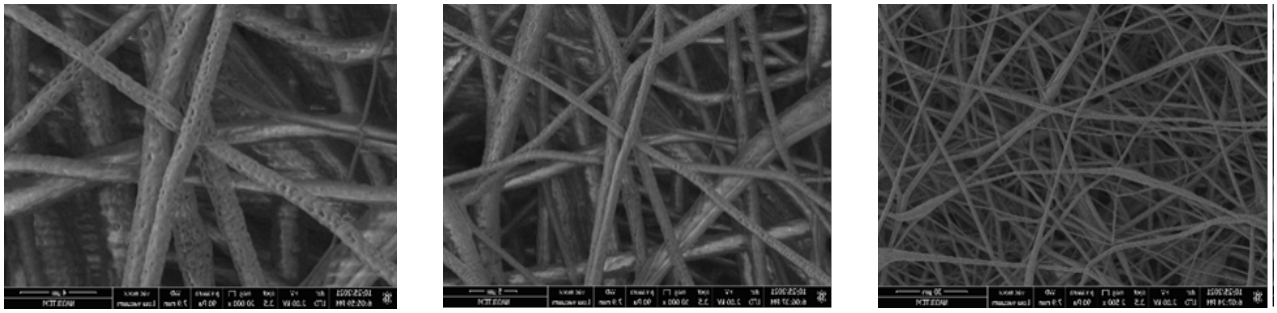
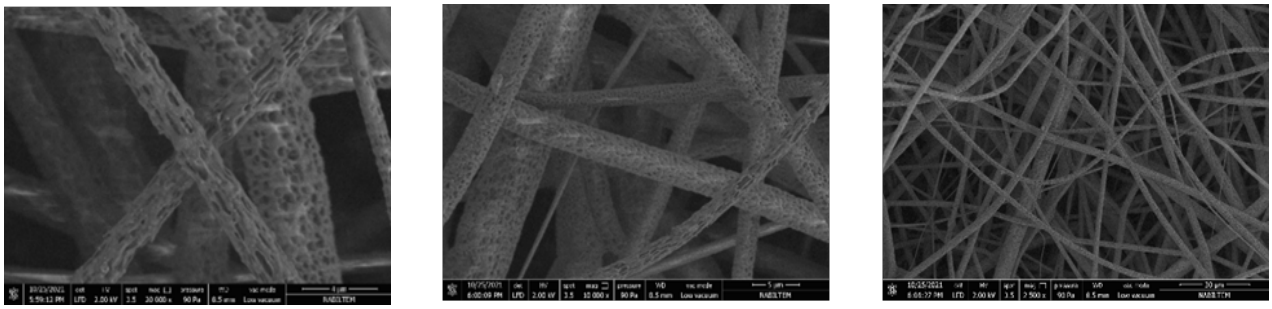
Fig. 3. SEM images of nanofibers produced with 0.5 % FG/Ag/PLA: a – 20000x; b – 10000x; c – 2500x



a b c

Fig. 4. SEM images of nanofibers produced with 1 % FG/Ag/PLA: a – 20000x; b – 10000x; c – 2500x



a b c

Fig. 5. SEM images of nanofibers produced with 2 % FG/Ag/PLA: a – 20000x; b – 10000x; c – 2500x

No obvious beat (bead) formations were observed in the images while there was an increase in pore formation with the increase in the ratio of fish gelatin in the structure. Thanks to this porous structure of the fibers produced, it will be possible to find usage in many areas such as ECM [11]. EDX results of the produced nanofiber surfaces are shown in Table 2. As it is known, the characteristic atoms in fish gelatin are phosphorus (P) and calcium (Ca) atoms. It is expected that phosphorus and calcium ratios will increase as the ratio of fish gelatin increases. In Table 2, it was determined that the amounts of Ca and P elements increase depending on the increase in FG in the solution used in nanofiber production as expected. The fact that Ca and P elements were not found in the fibers produced as a result of the shots made with the polymer solution to which 0.5 % of the solution containing FG was added, but were found in the production made with the polymer solution to which 1 % and 2 % of the solution containing FG was added: it can be

interpreted that the amount of FG in the total solution was small and the EDX analysis was performed in a small area, so FG was not found in the analyzed area.

Table 2. EDX results of nanofibers produced by FG and electrospinning

Element	FG	0.5 % FG/Ag/PLA	1 % FG/Ag/PLA	2 % FG/Ag/PLA
	wt.%			
C	15.77	57.7	52.85	51.37
O	48.87	42.3	46.99	48.07
Na	3.65	0	0	0
P	16.6	0	0.11	0.36
Ca	15.11	0	0.05	0.21
Total	100	100	100	100

There was an increase in the elemental amounts in the samples doped at different ratios. Especially the increase in the amount of P and Ca increased with the increase in fish gelatin content. In SEM images, it was determined that the increase in the amount of fish gelatin caused an increase in the amount and size of pores, which was reflected as an increase in the diameter of the fibers as seen in Table 3.

Table 3. Diameter values of the produced nanofibers measured by SEM analysis

Diameter values of fibers, nm		
0.5 % FG/Ag/PLA	1 % FG/Ag/PLA	2 % FG/Ag/PLA
765.2	860.0	1990.0
489.0	1182.8	2025.7
546.3	1195.1	2048.0
756.5	1263.0	2418.2
593.6	1306.0	1782.0
718.2	1405.0	1702.1
820.0	1480.0	2018.0
760.0	1032.0	2387.4
610.0	1370.0	2019.8
690.5	1199.0	2084.1
Standard deviations		
103.51	182.60	223.40

The FTIR results of the samples elementally analyzed within the scope of the study are given in Fig. 6.

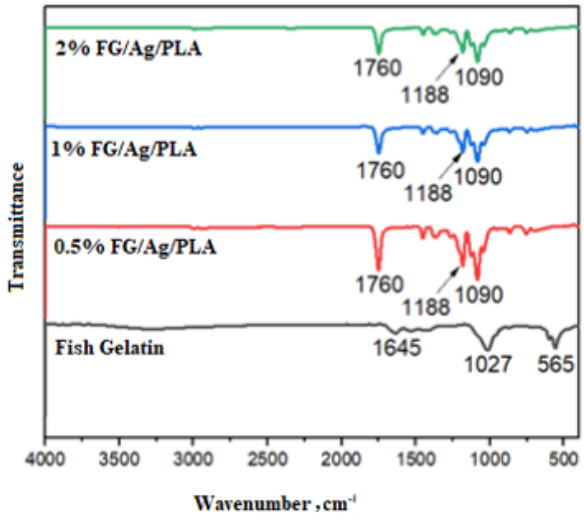


Fig. 6. FTIR spectra of fish gelatin and silver doped PLA nanofibers

Support was taken from the literature for the FTIR spectra of PLA. Harmaen et al. in their study on PLA composite production, listed the typical PLA peaks that can be encountered in FTIR analysis. According to this study, the peak in the range of 1767 – 1759 cm^{-1} indicates the carbonyl group of PLA and the peak around 1182 cm^{-1} indicates the C-O- bond in -CH-O [11]. In the present study, it was observed that the peaks at 1645 cm^{-1}, 1027 cm^{-1} and 565 cm^{-1} in fish gelatin were not found in nanofibers and the spectra of nanofibrous surfaces produced by electrospinning matched the peaks of PLA.

The peak recorded at 1090 cm^{-1} coincides with the peak in the FTIR spectrum of Ag nanoparticles given in the study by Thirunavoukkarasu et al. in which they produced silver nanoparticles by reduction of silver nitrate and was attributed to C-O stretching [12].

Fig. 7 shows the XRD patterns of PLA nanofibers doped with fish gelatin and different ratios of FG/Ag, and the peaks in the patterns of the nanofibers overlap with the XRD patterns of PLA nanofibers produced by the electrospinning method in the study by Oliveira et al.

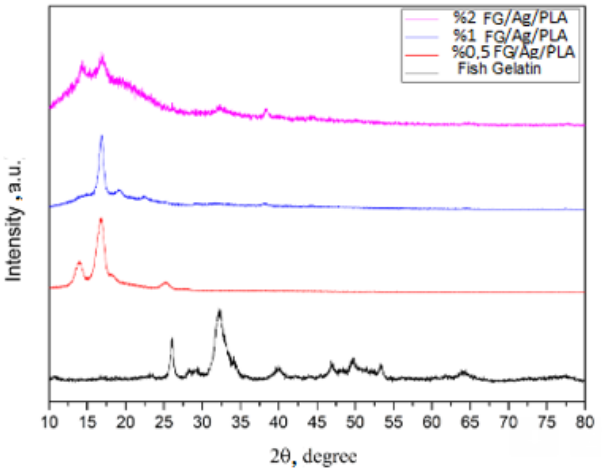


Fig. 7. XRD patterns of fish gelatin and silver doped PLA nanofibers

The characteristic spectra of PLA are those where 2θ is below 20º [13]. However, it is seen in Fig. 7 that the characteristic spectrum of FG is $2\theta > 20º$. The peaks at 26º, 33º and 40º in the XRD patterns of the nanofibers are attributed to the characteristic peaks of FG obtained from rock bass.

4. CONCLUSIONS

In this study, fish gelatin, silver and PLA were used together as three components to produce nanofibrous surfaces via the electrospinning method. Characteristic properties of the produced surfaces were investigated via XRD, FTIR, SEM-EDX analyses. XRD patterns, FTIR spectra and EDX elemental analysis showed that FG/Ag/PLA nanofiber surfaces were successfully produced. In SEM images, it was found that the pore formation on the nanofiber surfaces increased with increasing amount of FG. Thanks to the porous structure, the nanocomposite fiber produced has the potential to be used especially in the health and biomedical sector.

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