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Production and Characterization of Natural Sourced Hydroxyapatite Added Polystyrene Tissue Scaffolds by Electrospinning

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In this study, polystyrene (PS) solution was prepared with the help of ethylacetate (EA) solvent. Composite tissue scaffolds were produced by electrospinning technique by adding 1%, 5% and 8% natural source hydroxyapatite (NSHA), respectively. These produced scaffolds were characterized by performing fourier transformation infrared spectroscopy-FTIR, scanning electron microscopy-SEM, tensile test and cell culture tests. It is aimed to use the obtained tissue scaffolds as ideal materials in tissue engineering applications

Keywords: *Polystyrene, natural source hydroxyapatite, electrospinning, composite, tissue scaffolds*

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1. Introduction

For new tissues both in vitro and in vivo, formation of a three-dimensional (3D) matrix is an important challenge in the tissue field. In engineering, nutrients and waste materials must be removed for seed cells to survive, as they must be designed as a suitable environment for supply. Tissue scaffolds are synthetic and naturally produced three-dimensional matrices. Polymers called biomaterials are the structural, mechanical and biological properties they offer for tissue engineering approaches. Cell adhesion, proliferation, differentiation and extracellular matrix formation are highly similar to natural tissue, with these tissue scaffolds not only having the appropriate pore size, but also providing high surface area to provide a framework. Compared to synthetic polymers, the produced scaffolds provide non-toxic, good cell attachment and minimal undesirable core biocompatibility in natural polymers. Both the chemical and architectural properties of tissue scaffolds have a special effect on the formation of new tissue: an excellent surface

chemistry enables cell attachment, proliferation and differentiation, while adequate mechanical properties preserve the structure and functions of the scaffolds. Therefore, manufacturing methods such as freeze drying, salt washing, electrospinning are used for the regeneration of various tissues [1-3].

The electrospinning method, which requires a high electric field, was invented by Formhals in 1930. This technique enables the production of nanometer fibers and interconnected pores. It mimics the natural extracellular matrix for cell adhesion. When the applied electric field overcomes the surface, the tension is thrown as jets towards the polymer solution collector system and collects as fibers. The properties of these nanofibers are determined by changing the process parameters such as the viscosity and conductivity of the polymer solution, the voltage in the electric field, the average molecular weight of the polymer, the distance between the needles and collectors [4-6]. Electrospinning working principle is shown in Figure 1.1.

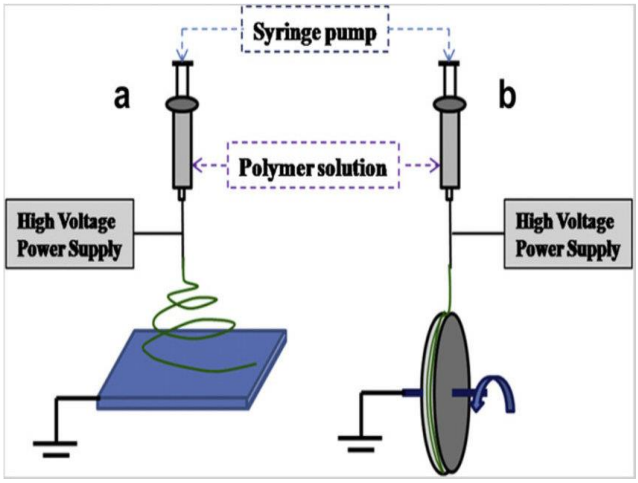

Figure 1.1. *Electrospinning working principle [7]*

It has started to be used as a biomaterial in ceramics with newly developed production techniques. The characteristic features of ceramics are that they are hard, porous, brittle, resistant to corrosion and body fluids, high compressive strength and biocompatible. Thanks to these properties, it is used in dental, orthopedic and cardiac applications. Calcium-phosphates (Ca-P) form the basis of the inorganic part of the bone. Ca-P is used in a similar way to synthetic bones.

Hydroxyapatite (HA) is widely used in the formation of hard tissues with its high osteoconductivity, low degradation rate and high mechanical strength with very good biocompatibility with hard tissues [8-10]. The chemical structure of HA is included in Figure 1.2.

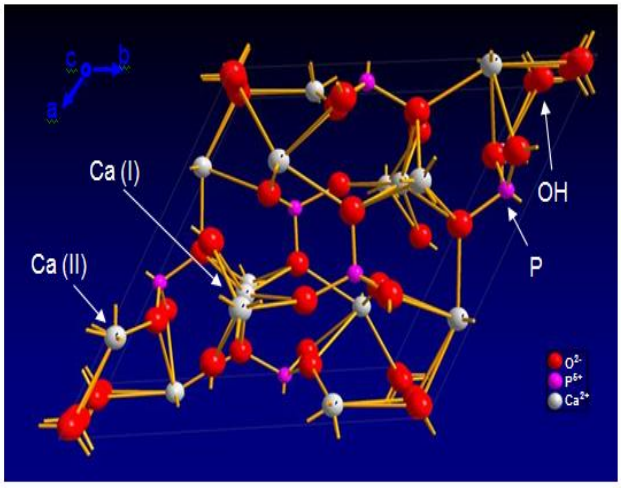

Figure 1.2. *Chemical structure of HA [11]*

Polystyrene (PS) can repair damage to bone tissue and regenerate bone tissue. It allows the cultured cells planted on it to adhere, proliferate and differentiate. Cell combination and proliferation create tissue [12-15]. The chemical structure of PS is shown in the Figure 1.3.

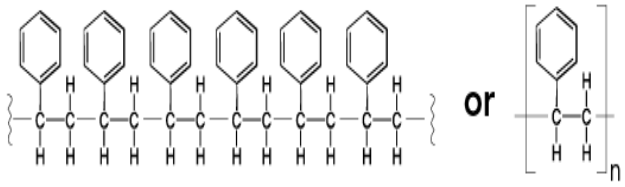

Figure 1.3. *Chemical structure of PS [16]*

In this study, the production of NSHA-added PS composite fabric scaffolds will be carried out by electrospinning technique. Characterization studies of the tissue scaffolds to be obtained will be carried out, and it is aimed to produce ideal tissue scaffolds that can appeal to tissue engineering applications.

2. Material and Method

2.1. Material

NSHA was obtained from African *Melongena* shells by chemical precipitation technique. anhydrous, 99.8% ethyl acetate, average M_w ~192,000 polystyrene, wax paper are used. Sigma-Aldrich brand was preferred as reference.

2.2. Method

2.2.1. Synthesis of NSHA

After the African *Melongena* sea shells were collected, they were sterilized in an ultrasonic bath with washing and distilled water. The NSHA material was synthesized by grinding in a ball mill and sieving through a 63 micrometer sieve and treated with orthophosphoric acid [1-3]. The NSHA synthesis steps are shown in the Figure 2.1.

Figure 2.1. *NSHA synthesis steps*

2.2.2. Nanofiber membrane production with electrospinning system

10 gr of PS polymer by weight was made into solution in EA solvent with a heated magnetic stirrer at 60 °C mixing temperature and 50 minutes mixing time. By adding 1%, 5%, 8% NSHA to the formed PS solution, a solution in four different compositions was obtained [4-8]. Table 2.1. shows the preparation values of composite solutions. Electrospinning parameters applied for composite nanofiber membrane production are shown in the Table 2.2. The production stages of high performance tissue scaffolds using the electrospinning technique are shown in the Figure 2.2.

Table 2.1.: Preparation values of composite solutions

Sample Name	Solution Mixing Time (min.)	Solution Mixing Temperature (°C)
10% PS	50	30
10% PS-1% NSHA	50	30
10% PS-5% NSHA	55	45
10% PS-8% NSHA	60	45

Table 2.2.: Electrospinning parameters applied for composite nanofiber membrane production

Sample Name	Flow Rate (ml/hr.)	High Voltage (kV)	Working Distance (cm)
10% PS	1.5	27.0	15
10% PS-1% NSHA	1.5	27.0	15
10% PS-5% NSHA	1.5	35	15
10% PS-8% NSHA	1.5	35	15

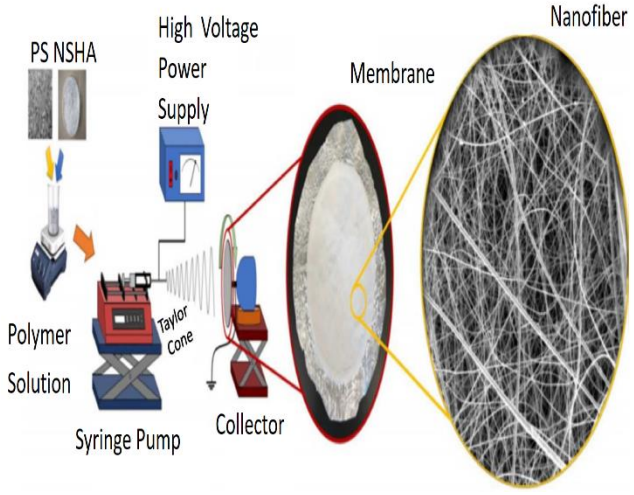

Figure 2.2. *Production stages of high performance tissue scaffolds using electrospinning technique*

2.3. Characterization studies

Structural (FTIR) analyzes of the membranes were performed on the Jasco 6600 model analyzer at wavelength ranges of 400 to 4400 cm^{-1}. The bonds in the structures of the samples were determined depending on the permeability percentage (% T) determined in the wavelength range of

400-4400 cm^{-1}. Tissues placed in the holders were examined and photographed with a ZEISS EVO SEM microscope. During the examination of the diameter and dimensions of the produced composite nanofibers, images magnified at x6000 times were examined for SEM analysis at 7 kV potential. The average diameter thickness of the nanofibers was measured on high resolution SEM photographs using Image j (National Health Organization) software. The mechanical (tensile) test was carried out by cutting the electrospun mats 1x5 cm in length according to the ASTM standard and repeating them 3 times under 500 Newton load. Membranes were placed in 96 plates and mesenchymal stem cells were added. Cell viability values were determined.

3. Result and Discussion

3.1. FTIR analysis

The severity of the PS peaks increases as the NSHA contribution in the PS structure increases. NSHA peaks are located at 800 cm^{-1} in the composite structure. However, since PS polymer has a similar peak in the same region, it is not obvious that it fully supports composite formation, while the increase in the intensity of PS peaks gives us information on composite formation [17-20]. FTIR analyzes of all composites have been performed and are shown in the Figure 3.1. The Table 3.1. contains the specific peaks of PS.

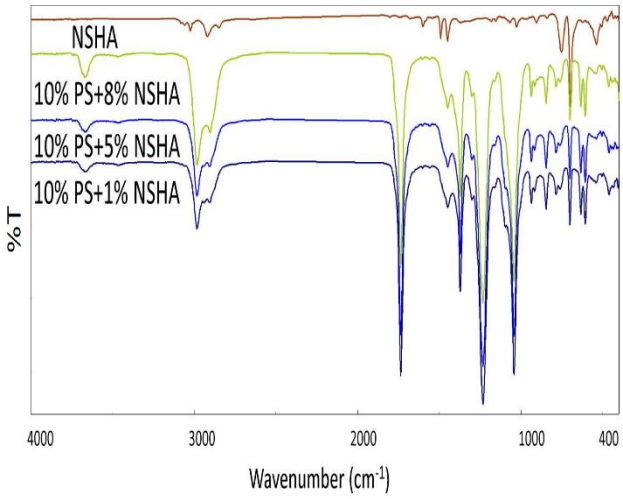

Figure 3.1. *FTIR plot of composite tissue scaffolds*

Table 3.1.: Specific peaks of PS

Sample name	C–H out phase bend (625–970) cm^{-1}	C–O stretch (880–1000) cm^{-1}	CH_2 bending (1300–1380) cm^{-1}	C=C stretch (1550–1610) cm^{-1}	C=O (1550–1750) cm^{-1}	C–H stretch aliphatic (2800–3000) cm^{-1}	C–H stretch aromatic (2800–3060) cm^{-1}	Hydroxyl (3610–3645) cm^{-1}
PS	667.39	906.57	1311.64	1583.61	1583.61	2850.88	2850.88	3647.51
	696.33	943.22	1329.00	1600.97	1600.97	2918.40	2918.40	
	702.11	964.44	1373.36		1670.41	2931.90	2931.90	
	750.33	979.87			1747.57		3005.20	
	758.05						3026.41	
	842.92						3059.20	
	906.57							

3.2. SEM analysis

Nanofiber formation was observed in all samples. Nanofibers were formed in a homogeneous form in all of the composites, and clumping and dispersion problems of NSHA additives did not occur. As the NSHA additive ratio increased, the viscosity of the polymer solution decreased and the electrical conductivity increased, so the nanofibers became thin. The thinnest nanofibers in the study were found to be in 10% PS + 8% NSHA composite [18,19]. Morphological images of composite tissue scaffolds are shown in the Figure 3.2.

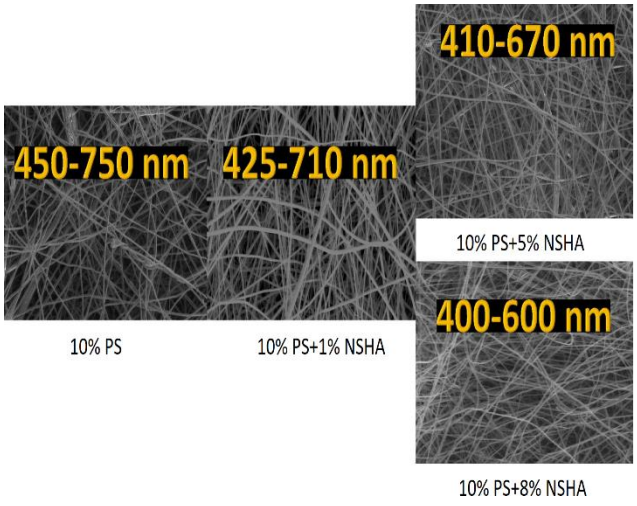

Figure 3.2. *Morphological images of composite tissue scaffolds*

3.3. Tensile test

The tensile strength values of the composite tissue scaffolds were determined in a three-center repetitive manner and their mechanical properties were determined by arithmetic averages. As a result of the tests performed in ASTM standards, the strongest composite tissue scaffold was

obtained in 10% PS + 8% NSHA sample. Tensile strength value is determined as 36 MPa. The lowest tensile strength value was obtained in 10% PS sample and was determined as 12 MPa [20]. The tensile strength values of composites are shown in the Figure 3.3.

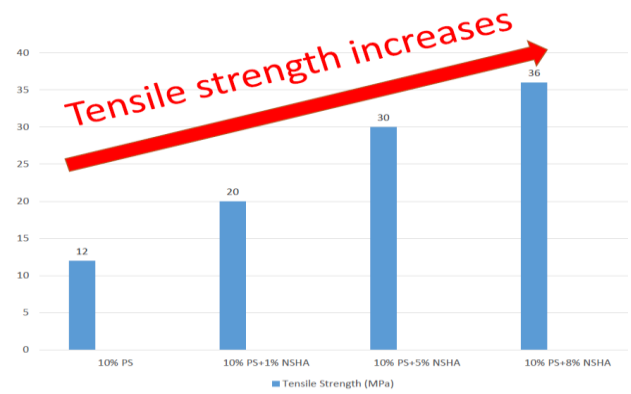

Figure 3.3. *The tensile strength values of composites*

3.4. Cell culture test

Mesenchymal stem cell transplantation was carried out with an interval of 24, 48, 96 hours to the membranes placed on 96 plates. Live and dead cell values of composites were determined after cell cultivation. While the most viable composite tissue scaffold was in 10% PS + 8% NSHA sample, cell viability was determined as 98%. As the NSHA contribution increased, the cell viability value gradually increased. It has been supported by structural, morphological and tensile tests that NSHA contribution has a positive contribution to the PS structure [20-25]. Cell viability values of tissue scaffolds are shown in the Figure 3.4.

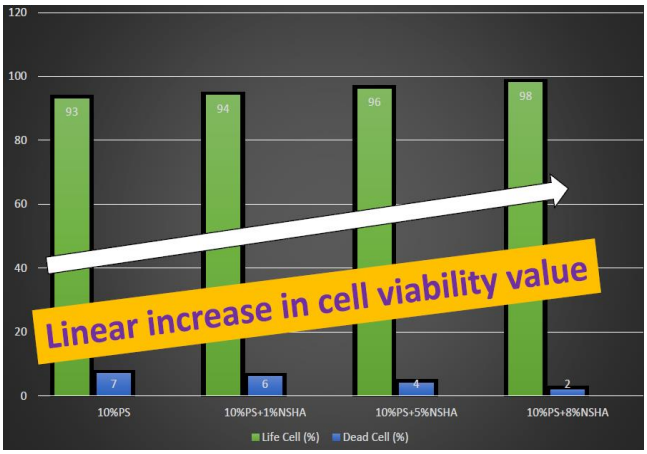

Figure 3.4. *Cell viability values of tissue scaffolds*

4. Conclusion

In this study, nanofiber membranes were successfully obtained by using PS and NSHA materials with the help of electrospinning technique. According to the results of the structural analysis of nanofiber membranes, it was determined that the peaks of PS and NSHA materials are included in the composite nanofiber membrane structure and support the composite formation. According to the results of the morphological analysis, when the PS membrane was examined, the diameters of the nanofibers were thick, but as the NSHA ratio increased in the composite structure, the nanofibers gradually became thinner. The thinnest nanofiber average diameter was measured at 10% PS + 8% NSHA membrane. Fiber diameters were found to be in the range of 400-600 nm. According to the mechanical analysis results applied to nanofiber membranes, the tensile strength value of PS nanofiber membrane was measured as 12 MPa, while the tensile strength value increased as the amount of NSHA additive increased. The highest mechanical properties were in 10% PS + 8% NSHA membrane and 36 MPa tensile strength value was obtained. When the biological characterization studies of nanofiber membranes are examined, as the NSHA contribution rate increases, the cell viability increases. Cell viability was determined as 98% at 10% PS + 8% NSHA membrane. As a result of the obtained findings, it will be able to show the ideal tissue scaffold material properties for tissue engineering applications

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