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ELECTROSPUN NANOFIBROUS SCAFFOLDS IN GREEN NANOTECHNOLOGY AND NANOMEDICINE

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Abstract- Electrospinning has been determined as an important technique for the production of several structured nanofibers. The fabrication and production of biocompatible scaffolds that can mimic the native extracellular matrix (ECM) for tissue engineering, has attracted significant attention in recent years. Mimicking the biomechanical and biological structure of ECM is an effective strategy to design and develop scaffolds in the field of tissue engineering. Nanofibrous scaffolds can ideally mimic the physical structure of ECM and the ECM can be fabricated by some methods such as self-assembly, phase separation, and electrospinning. Among them, electrospinning is the most widely used method because of its simple and cost-effectiveness [1-3]. Due to ECM-mimicking, electrospun nanofibers are highly favored as tissue-engineered scaffolds [4, 5]. In example, bone tissue that is originally composed of hydroxyapatite and collagen can be assembled by electrospinning. The electrospun nanofibrous scaffolds can be used as carriers for various types of drugs, active molecules and genes, whereby the release profile can be finally controlled by regulation of the scaffold's morphology, porosity and composition. The main advantage of these systems is that it suggests site-specific delivery of any number of therapeutics from the scaffold into the body [6]. In our study, silk/nylon-6 electrospun nanofiber membranes were obtained by electrospinning in order to protect the environment and the distancing of some heavy metals from wastewater [7, 8]. In another study, a natural polymer and nano hydroxyapatite particles (nHAp) were integrated into nanofibers that will also include an anticancer drug [1, 6].

Keywords: Electrospinning, Biomimetic, Tissue Engineering, Nanotechnology, Nanomedicine.

I. INTRODUCTION

Nanotechnology is the engineering of molecularly precise structures-typically 0.1 μm or smaller and, ultimately, molecular machines. The most common nanostructures are nanocarbons, dendrimers, fullerenes, hydrogels, aerogels, nanocomposites, nanowires, quantum dots, nanocages, nanoparticles, nanorods, nanoshells and nanofibres. Nanofibers can be processed by a number of techniques such as phase separation, self-assembly and electrospinning [9, 10].

Over the last decade, electrospinning has been recognized as a simple and versatile process to fabricate various polymeric and inorganic fiber mats with the high surface area-volume ratio and porosity. Electrospinning has attracted much attention not only because of its versatility in spinning a wide variety of polymeric fibers but also because of its consistency in producing fibers with submicron thickness. From a biological and medical viewpoint, almost all of the human tissues and organs are deposited in nanofibrous forms or structures. Bone, dentin, collagen, cartilage and skin are characterized by well-organized fibrous structures in nanometer scale [1, 11].

As such, nanofiber researches in medicine consist of tissue engineering, wound dressing, drug delivery and medical prostheses. For tissue engineering and wound dressing, electrospun polymer nanofibers are treated as tissue scaffolds, which enhance cell growth and proliferation. The nanofiber scaffolds with seeded cells can be implanted to patient's body to repair the damaged tissues. For drug delivery system, nanofibers are considered as a potential drug carrier [10-12].

Polymer nanofibers fabricated via electrospinning have been proposed for a number of soft tissue prostheses applications such as blood vessel, vascular, breast etc. In addition, electrospun biocompatible polymer nanofibers can also be deposited as a thin porous film onto a hard tissue prosthetic device designed to be implanted into the human body [1].

All those data in Figure 1 show the medical entities (public and private), biocompatible materials developers and medical suppliers will be very interested to cover this important market niche [13].

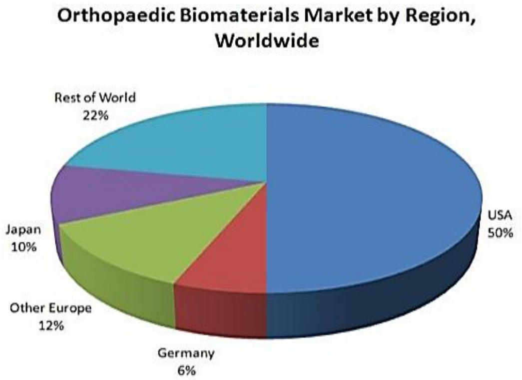


Figure 1. Market Countries Distribution [13]

II. MATERIAL AND METHODS

A. Chemicals and Equipment

In this study, the silk cocoons were obtained from Koza Birlik, Nylon-6 polymer was obtained from Aldrich, Calcium chloride ($CaCl_2$), Ammonium hydrogenphosphate $(NH_4)_2HPO_4$, Sodium carbonate ($NaCO_3$), Nitric acid (HNO_3), Sodium hydroxide (NaOH) and Sodium chloride (NaCl) were purchased from Merck, Trifloroacetic acid (TFA) and Copper (II) nitrate hemi (pentahydrate) ($Cu(NO_3)_2$. 2.5 H_2O) were from the firm ALFA AESAR acetic acid (CH_3COOH) Riedel-Dehaen, sodium hypochloride (NaOCl) from Birpa and Tetrazolium salt (MTT) used in cell culture studies was from the firm Serva.

B. A Natural Polymer and Nano Hydroxyapatite Particles (nHAp) Integrated Nanofibers

B.1. Scaffold Preparation

The natural polymer (Type 1 collagen)/hydroxyapatite scaffolds were prepared by using electrospinning technique. Natural polymer was dissolved with its specific solvent. Nano Hydroxyapatite particles were also added to obtain several ratio of solution. For the process of electrospinning, the polymer solution was placed in a 5 ml plastic syringe fitted to a needle with a tip diameter of 6.5-8 cm. Electrospinning voltage was applied to the needle at 12-15 kV using a high-voltage power supply at a spin distance of 12 cm. The flow rate of the injector pump was fixed at 0.1 mL/s. A syringe pump was used to feed the polymer solution into the needle tip at an obtained feed rate. The solution formed a Taylor cone upon exit and was collected onto glass slides or coverslips of different sizes placed on a wire-grid ground collector in the form of a nonwoven fabric (Figure 2a).

B.2. Scaffold Characterization

The morphological properties of obtained scaffolds were examined with Scanning Electron Microscobe (SEM) Analysis. The diameter of the fibers were also measured with SEM technique.

C. Silk/Nylon-6 Electrospun Nanofiber Membranes

C.1. Removal of Silk Fibroins from Sericin

Silk cocoons (Bombyx mori) were boiled in a beaker containing 0.02 M Na_2CO_3 solution. This process was repeated by replacing the solution until a clear supernatant is obtained. Then, the silk thread bobbins were washed with distilled water. Thus, the Sericin proteins placed on the silk polymer were decomposed. The washed silk was left for drying in a vacuum oven at 70 ºC for 24 h.

C.2. Preparation of Polymeric Nanofibril Structures

In this study, nylon-6 was selected in order to provide mechanical support to the prepared membranes. Electrospin technique was used during the preparation of nanofibril structures. According to this technique, about 15% silk and nylon-6, free from sericin, were dissolved in trifluoroacetic acid, separately. Prepared solutions were mixed at a ratio of 50:50 percentages and were used under a voltage of 20 kV at a spin distance of 12 cm. The flow rate of the injector pump was fixed at 0.1 mL/s. Liu et al., 2004 [7] has determined that the best mixing ratio among all for silk-nylon-6 solutions is 50:50 %.

C.3. Characterization of the Obtained Silk-Nylon-6 Nanofibril Structures

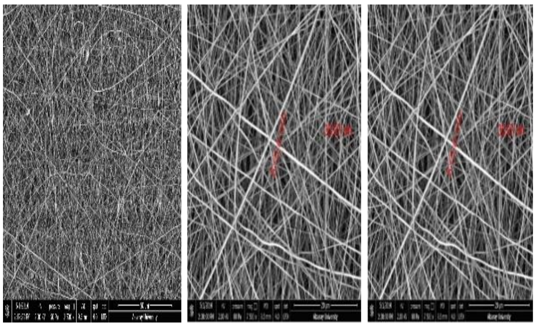
The structural property of silk-nylon-6 nanofibril array membranes obtained by electrospin technique was characterized by ATR-FTIR spectroscopy. For comparison purpose, virgin silk, nylon-6 polymer and silk-nylon-6 blend nanofibril array structures obtained from ATR-FTIR spectra were investigated individually. At the modification step of this work, prepared silk-nylon-6 nanofibril array membranes were modified by calcium-phosphate compounds, which are well known to have high affinity towards heavy metals. In order to achieve this process, calcium-phosphate compounds were precipitated onto nanofibril array membranes.

C.4. Adsorption Studies

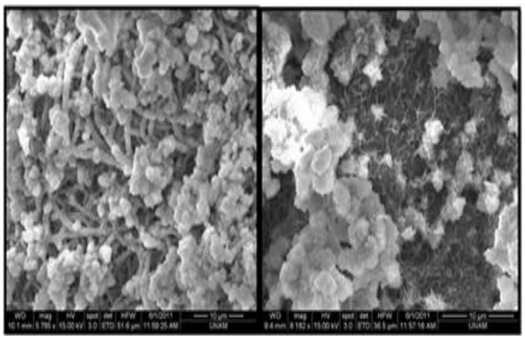
The characterized and calcium phosphate compound precipitated products of silk-nylon-6 nanofibril array membranes were cut into pieces having a surface area of 1.0 cm^2 and an average weight of 4.28 mg so as to enable their fitting into a column. Copper, cadmium, iron and lead metals were used for the adsorption studies of the above mentioned nanofibril array membranes, onto which calcium-phosphate compounds with high affinity towards heavy metals were precipitated.

C.5. Toxicity Studies

During toxicity studies, Microtox technique was employed through the luminescence induced by Vibrio fishery bacteria. When EC_{50} value is expressed in percentages, the higher value indicates the lower toxicity whereas the lower one displays the higher toxicity. The results are detected at each 5 and 15 min. The validity of the toxicity test is correlated by the activity versus time, using Bacillus sp originated β-galactosidase and Trichoderma viride.



(a)



(b)

Figure 2. (a) SEM morphologies of the electrospun nanofibers, (b) SEM micrographs of silk-nylon-6 nanofibril array membranes, obtained by the precipitation of calcium-phospate salts

III. RESULTS AND DISCUSSION

A. Natural Polymer and Nano-Hydroxyapatite Particles (nHAp) Integrated Nanofibers

Nanofibrous scaffolds constituted of a natural polymer were produced through the electrospinning technique. A well-developed fibrous morphology could be generated during the experiments. We characterized by using the SEM morphologies of the electrospun nanofibers of natural polymer.

The diameter of the fibers was in the range of 36 nm - 515 nm on average (Figure 2a). The experiment will be continued with various type of anticancer drugs. The nanofibers with these type and diameter have common usage in medical applications. This work was observed to provide matrix conditions for cells to adhere and populate and suggests the nanofibrous scaffolds may be useful as a novel bone regeneration matrix.

B. Silk/Nylon-6 Electrospin Nanofiber Membranes

Silk-nylon-6 nanofibril array membranes, obtained by the precipitation of calcium-phospate salts as a result of five set of experiments, were investigated by SEM studies (Figure 2b). The absorption studies with heavy metals employed in batch process have yielded the following results. Each heavy metal concentration decrease with respect to time is followed from the same initial value of 30 ppm, which gradually drops, to a value of 11.02 ± 1.64, 15.70 ± 1.44, 12.60 ± 1.71, 13.81 ± 1.02 for individual copper, cadmium, iron and lead metals, respectively. The most efficient removal is observed for the copper heavy metal as shown from Figure 3.

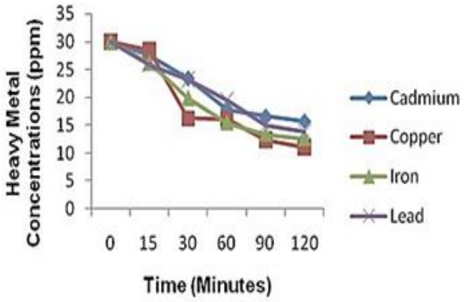


Figure 3. The absorption studies with heavy metals employed in batch process

EC_{50} values obtained at the end of 5 min and 15 min for the samples kept in batch system for a period of 120 min are displayed in Figure 3. It is clearly observed that the highest EC_{50} values are obtained for copper metal both for 5 and 15 min which are 148% and 124%, respectively (Figure 3).

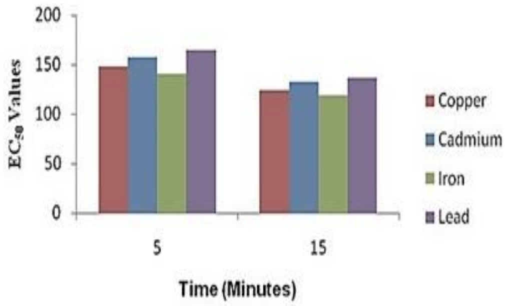


Figure 4. EC_{50} values recorded at 5 and 15 min intervals in batch system

IV. CONCLUSIONS

In this work, electrospin technique was employed for two different purposes in nanotechnological fields of green technology and nanomedicine. The first one involves the removal of heavy metal ions from industrial waste water. The second purpose is the application of this technique in nanomedicine.

For waste water treatment, the prepared electrospun silk-nylon-6 nanofibril array membranes followed by the precipitation of calcium-phosphate salts to remove copper, cadmium, iron and lead heavy metals, proved to be promising nanofilters with a fiber diameter of 35-500 nm (Figure 2b).

Five individual nanofibril scaffolds were placed in a column arranged with a surface area of 1.0 cm^2 and average weight of 4.28 g. Subsequently, they were observed to display an efficient removal of heavy metal ions, especially yielding from copper metal (Figure 3). In addition, toxicity studies were also observed to yield lower toxicity values reducing metal toxicity by 88 %, for higher EC_{50} data compared to other metals under study (Figure 4). This can be interpreted by the high ratio of the larger surface area to the volume content of the nanofibril material having a higher capacity for absorption of metal ions. As previously mentioned; this method shows a great effectiveness for heavy metal ions, especially for copper. Thus, this kind of nanofibrous filtration technique may also be used in many areas in green nanotechnology.

It can be considered that, this method may become a serious milestone for leaching/bioleaching. Consequently, this technique has proved to play an important role in wastewater treatment with regard to other conventional methods and it is a promising technique for a wide range of applications in nanotechnology.

Another application of this study in nanomedicine is related with the production and characterization of anticarcinogen containing nHAp/Type 1 collagen biocomposite polymeric nanofibers via electrospin technique. The nanofibril scaffold prepared for nanomedicinal application was obtained using nHAp/Type 1 collagen as shown in SEM photographs. The diameter of the fibers was in the range of 36 nm - 515 nm on average (Figure 2(a)). Our further studies will aim to produce an anticarcinogen integrated scaffolds. Hence, these studies enhance the usage of nanofibrous scaffolds as a drug release carriers for cancer treatment. The conditions should be improved to provide a better matrix for cells to adhere and populate. Thus, the collagen/HA nanocomposite nanofibrous scaffold may be employed as a novel bone regeneration matrix.

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BIOGRAPHIES

Necdet Saglam works for Department of Nanotechnology and Nanomedicine, Graduate School of Science and Engineering, Hacettepe University, Ankara, Turkey. He has joined a number of academic committees and duties such as chair, editor, referee; and was Vice Dean of Education Faculty, Hacettepe University form 1997 to 2006. During the years between 2007 and 2011, he has become Rector of Aksaray University, Aksaray, Turkey. He has supervised a number of graduate theses on science education and science fields. At present, he teaches Nanotechnology and Nanomedicine subjects. He has presented a number of papers at international conferences. His research interest is on "Green-Biotechnology and Nano-Technology", and in these subject areas, he has joined several research project teams.

Ezgi Emul received her B.Sc. degree from Department of Chemical Engineering, Ankara University, Ankara, Turkey in 2013. In addition to her principle courses, she also took Biochemical Engineering and Drug Delivery Systems during her Bachelor years. She is also an Erasmus Alumni, studied at Carlow Institute of Technology in Ireland at the Department of Analytical and Forensic Science in 2011. She is working on her Master's studies at Nanotechnology and Nanomedicine Division, Hacettepe University, Ankara, Turkey in field of Electrospinning Techniques.

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Feza Korkusuz graduated from Medical Faculty, Ankara University, Ankara, Turkey in 1986. He attended specification in Orthopedic Surgery in T0raumatology at Department of Orthopedic Surgery and Traumatology, Medical Faculty, Gazi University. Between 1989 and 1990, he studied at Department of Orthopaedic Surgery, Faculty of Medicine, Osaka University, Japan. His clinical and research studies focused on spine surgery, sports medicine and he graduated with a Postgraduate Diploma. He worked at Middle East Technical University, Ankara, Turkey for 20 years as an orthopedic surgeon at the Medical Center as the Director and Head of Department of Physical Education and Sports. In 1994 and in 2001 he became an Associate Professor and Professor, respectively. In the year 2000, he received the Scientific and Technological Research Council of Turkey Promotion Award in Medical Sciences. Since 1991, he is a Member of the Orthopaedic Research Society. In 2008, he became an active Member of the Association of Bone and Joint Surgeons, and since 2006, he has been a member of the corresponding and deputy editor of Clinical Orthopaedics and Related Research. He has published 94 manuscripts in journals and 6 chapters in international books.