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Application of gas foaming technique for improving porous properties of chitosan-polyvinyl alcohol (CS/PVA) nanofiber-based biodegradable scaffolds

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The human body consists of systems, systems are organs, organs are cells and extracellular matrix. The extracellular matrix has an important role in the human body with the task of connecting cells to each other. Due to various reasons, diseases in which the extracellular matrix is damaged have been witnessed from the past to the present. Tissue engineers are in research to improve and prevent this damage. As a result of these researches, the production of tissue scaffolds has started. Tissue scaffolds are structures designed to mimic the extracellular matrix. These scaffolds can perform functions belonging to the extracellular matrix, such as providing mechanical strength, helping to establish communication with the surrounding tissue to respond to physiological and biological changes, as well as forming suitable adhesion surfaces for cells. They may also contribute to the regeneration of the true extracellular matrix. Tissue scaffolds can be produced by many methods and one of them is the gas foaming technique. In our study, tissue scaffolds produced by this method were used and it was aimed to increase the porosity of these scaffolds.

In the experiments, tissue scaffolds were obtained by electro-spinning method and $NaBH_4$-methanol solution was used for gas foaming method. As a result, it was observed that the porosity properties of the tissue scaffolds inflated in 0.1M solution were increased. These observations were determined and documented by the analysis results.

Keywords: Polymer chemistry; biotechnology; electro-spinning; gas foaming; nanofiber; material science

INTRODUCTION

Polymeric biomaterials consist of organic polymers with varying properties and topologies. They are adaptable, easy to process, and offer customizable mechanical properties. Polymeric biomaterials can be either synthetic or naturally occurring [1].

Synthetic polymers such as polyethylene, poly(lactic-co-glycolic acid) (PLGA), and polyurethane are commonly used in biomedical applications due to their tunable properties. These polymers offer advantages such as biocompatibility, biodegradability, and controlled drug release [2].

Natural polymers derived from sources such as collagen, chitosan, and alginate often mimic the extracellular matrix components found in tissues. These biomaterials create a favorable environment promoting cell anchorage, multiplication, and new tissue formation. Natural polymers are used in wound dressings, tissue engineering scaffolds, and drug delivery systems [3]. Examples of different applications of polymeric biomaterials include:

• In drug delivery systems, polymeric matrices can be used to encapsulate and release drugs slowly, reducing side effects while enhancing therapeutic efficacy [4].

• In tissue engineering, biologically degradable polymers are used as structural scaffolds to aid in cell growth, tissue regeneration, and wound healing.

• In contact lenses, hydrogels (a type of polymeric biomaterials), are used to create soft contact lenses due to their superior oxygen permeability, biocompatibility, and comfort [5].

Various processing techniques exist to synthesize polymers and then convert them into the desired biomaterial form. After producing the desired biomaterial, surface modification techniques are used to increase the bioactivity, biocompatibility and functionality of polymeric biomaterials. Techniques related to these steps can be listed as follows.

• *Step-growth polymerization*. This reaction of difunctional monomers produces linear or branching polymers. Two typical step-growth polymerization techniques are polycondensation and polyaddition. Polyesters, polyurethanes, and polycarbonates are examples of polymers produced using step-growth polymerization [6].

• *Chain growth polymerization*. Chain-growth polymerization is the sequential addition of monomers to an active polymer chain, resulting in polymer growth. Chain growth polymerization methods include radical polymerization, anionic polymerization, and controlled/ living

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polymerization. Polymers such as polyethylene, polystyrene and polyacrylates are produced by chain growth polymerization [7].

The originality of the project is that the application of gas foaming technique to increase the porous properties of chitosan/polyvinyl alcohol (CS/PVA) nanofiber scaffolds synthesized using electrospinning technique for scaffold applications and the parametric analysis of this technique are not presented in the literature.

No studies were found on the application of gas foaming technique to improve the porous properties of CS/PVA nanofiber-based biodegradable scaffolds for drug delivery systems or as an aid in cell growth, tissue regeneration, and wound healing. Our study addressed three research questions:

1. What are the optimum conditions under which CS/PVA structured nanofibers can be synthesized by electrospinning technique?

2. Can the porous structure of the scaffold be improved by gas foaming technique? Can porosity be increased with this technique?

3. What are the effects of the change in parametric values (time, amount, etc.) of $NaBH_4$ selected as the chemical to be used and the amount of methanol used as a solvent on the porosity of the scaffold?

EXPERIMENTAL

The application of gas foaming technique in this study has four main steps.

Step I: Preparation of electrospinning solutions

Preparation of suitable solutions for the electrospinning method As detailed in Coşkuner's publication [8], polyvinyl alcohol (PVA) will be dissolved into deionized water under continuous stirring at 90 °C for 2 h to obtain a homogeneous solution with a concentration of 10% by weight. Following dissolution, the PVA solution will be allowed to cool to room temperature under continuous stirring, and mixing will continue for 24 hours. Separately, CS powder will be dissolved in a 2% by volume solution of acetic acid in water at room temperature under moderate stirring to produce a homogeneous 2.5% by weight solution. The CS and PVA solutions will then be combined in a mass ratio of 1:4 to prepare the CS/PVA mixing. The mixing solution will be stirred continuously for 24 hours at room temperature.

Step II: Synthesis of CS/PVA nanofibers

The nanofibers will be created by electrospinning at standard conditions using a plate collector wrapped in aluminum foil. Both pure and composite polymer solutions will be delivered through 10 mL syringes using a pump that precisely regulates the flow rate. The pick-up distance between the tip and the pick-up will be modified in accordance with the characteristics of the solution used. The process parameters will be empirically adjusted based on the observed Taylor cone initiation. Nanofibers will collect on flat plate collectors, subsequently allowed to dry at ambient conditions, and then preserved in a vacuum desiccator prior to characterization analyses. As the optimum condition of electrospinning, 33 kV will be applied. Collection distance is 20 cm, flow rate is 1.2 mLh^{-1}.

Step III: Characterization of CS/PVA nanofibers

Morphological characterization of fresh and used nanofibers will be performed by scanning electron microscopy (SEM). The nanofibers will be embedded in the carbon band, and an Au-Pd coating will be applied to each sample prior to analysis. Fiber diameters will be measured using the equipment's software for the extraction of numerical averages and corresponding distribution curves. Representative diameter values will be obtained by capturing images from various regions of the samples and performing random measurements across the fibers. The chemical structure of each nanofiber will be investigated in terms of chemical bonds and functional groups using Fourier transform infrared spectroscopy (FT-IR). In the attenuated total reflection (ATR) mode, measurements will be conducted with a resolution of 1 cm^{-1} using a 532 nm laser, across the spectral region spanning 650-4000 cm^{-1}.

To evaluate the potential of nanofibers for biosensing applications, it is essential to assess their stability under thermal conditions and in aqueous environments. Measurements of contact angle, *in vitro* degradation behavior, swelling capacity in phosphate-buffered saline (PBS), and morphological analysis *via* scanning electron microscopy (SEM) will all be used to determine stability. Nanofiber biodegradation will be assessed *in vitro* by quantifying sample weight loss in a PBS solution, according to a well-known approach. Initially, each sample will be dissected and weighed (W_0). Subsequently, the samples will be immersed in a PBS solution with a pH of 7.4, maintained at 37 °C for a duration of 21 days. At predetermined intervals on days 7, 14, and 21, samples will be retrieved from the solution, gently rinsed, vacuum-dried, and reweighed (W_d). *In vitro* degradation will be calculated according to the following equation [8]:

$$\textit{In vitro}\ \text{degradation}\ (D, \%) = [(W_0 - W_d) / W_0] \times 100 \quad (1)$$

The degree of swelling of the nanofibers will be analyzed by evaluating their liquid uptake capacity in PBS. The nanofiber samples will be divided into uniform square segments, their initial dry weights (W_d) will be recorded. These samples will then be immersed in a PBS solution and maintained at 37 °C. The individual sample pieces will be retrieved and weighed by wiping off excess water (W_w). The degree of swelling will be calculated using the following equation, which represents PBS absorption [8]:

$$\text{Swelling degree (SD, \%)} = [(W_w - W_d) / W_d] \times 100 \quad (2)$$

Step IV: Application of gas foaming technique to CS/PVA scaffold

Gas foaming technique will be used to increase the porosity of the obtained CS/PVA nanofiber structured biodegradable scaffold. The basis of this technique is to increase the porosity by passing through the fiber structure during the release of the H_2 gas produced by the reaction of the $NaBH_4$ compound with methanol [9].

$$NaBH_4 + 4\ CH_3OH \rightarrow NaB(OCH_3)_4 + 4\ H_2 \quad (3)$$

Different $NaBH_4$ solutions in the range of 0.1-0.3 M will be prepared with 20 mL of methanol. These solutions will be added to the CS/PVA nanofiber scaffold obtained by the electrospinning method. Hydrogen gas output will be checked at different times determined between 0-24 h. Then, the tissue scaffolds prepared under different conditions will be dried in a vacuum oven at 50 °C for 24 h. The change of porous properties of the tissue scaffolds using gas foaming technique will be measured by mercury porosimetry. The obtained data will be compared. It is aimed to increase the porosity feature by at least 50%.

RESULTS AND DISCUSSION

Due to various reasons, tissue production has been targeted for the regeneration of damaged extracellular matrix, and increasing the porosity of this tissue is essential in our study to promote cell settlement and tissue regeneration within the artificial tissue. Electrospun tissue scaffolds were used in the experiments, and their porosity was increased by applying the gas foaming technique. Nanofibers obtained by the electrospinning method were inflated using a $NaBH_4$-methanol solution through the gas foaming technique. Solutions with different concentrations were prepared, and the best increase in porosity was observed with a 0.1M $NaBH_4$-methanol solution. Additionally, the nanofiber control sample, which was only soaked in methanol, showed no change in porosity. Consequently, it was observed that the porosity of the tissue scaffolds inflated in the 0.1M solution was increased. These observations were determined through the analysis results and documented as follows:

Under an optical microscope, only polymeric bonds were observed in the control sample, whereas in the inflated sample, polymeric bonds and bubble-like structures were also observed. These bubble-like structures indicate the porosity feature in the sample. As illustrated in Figure 1, the optical microscope images of the control sample at ×1000 and ×500 magnifications are presented, while Figure 2 shows the optical microscope image of the inflated sample at ×50 magnification.

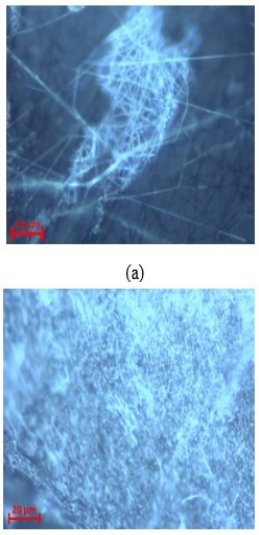

Figure 1. The image of the control sample under the optical microscope; (a) ×1000, (b) ×500

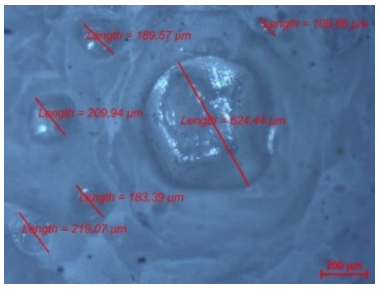

Figure 2. Measured image of the inflated sample under ×50 optical microscope

The increase in the porosity property of CS/PVA nanofibers in which gas foaming technique with $NaBH_4$-methanol was applied, was observed with an 8% increase in permeability as a result of FT-IR analysis, as shown in Figure 3.

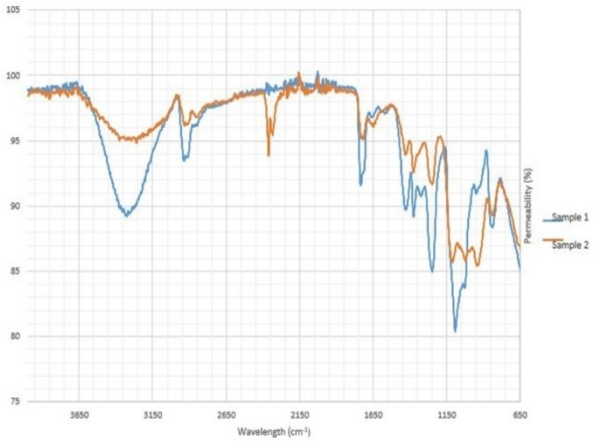

Figure 3. FT-IR spectroscopy result of CS/PVA nanofibers; (sample 1) control sample, (sample 2) swollen with $NaBH_4$–methanol solution

When the polymeric bond thicknesses of the swollen sample states (b) were observed compared to the control sample (a), it was concluded that there was an increase of 66.7% with the gas foaming technique which is depicted in Figure 4.

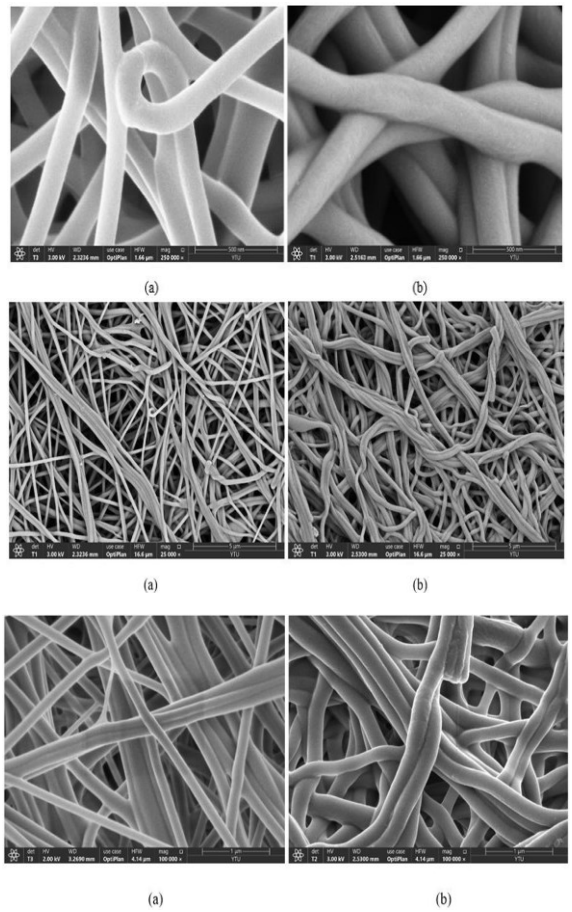

Figure 4. SEM images of the samples; (a) Control sample, (b) Sample swollen with $NaBH_4$-methanol solution

In the results of BET analysis, the surface area increased between 1.65 and 1.85 times. In addition, it was determined that the gas foaming technique increased the pore volume by 2 times compared to the control sample.

It was concluded that the nanofibers were not cross-linked as the cause of the fragmentation in the in vitro degradation and swelling degree tests.

It is suggested that nanofiber tissue that is broken down in PBS solution, which has a pH similar to body fluid, can be used in drug carrier applications. The nanofiber CS/PVA regenerated by cross-linking is predicted not to be broken down in the PBS solution, and is recommended for use in tissue regeneration or medical filters.

CONCLUSION

This study aims to produce tissue and enhance its porosity for use in the regeneration of extracellular matrix damaged due to various reasons. Porosity is crucial for cell attachment within the artificial tissue and for promoting tissue regeneration. This successfully conducted study highlights the potential of the gas foaming technique to enhance the porosity of nanofibers. In this way, nanofibers with high porosity are recommended to be used in supporting wound healing, creating artificial tissues and organs, implant materials used for bone repair, placement and growth of cartilage cells, production of biosensors used in biomedical applications and filters.

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