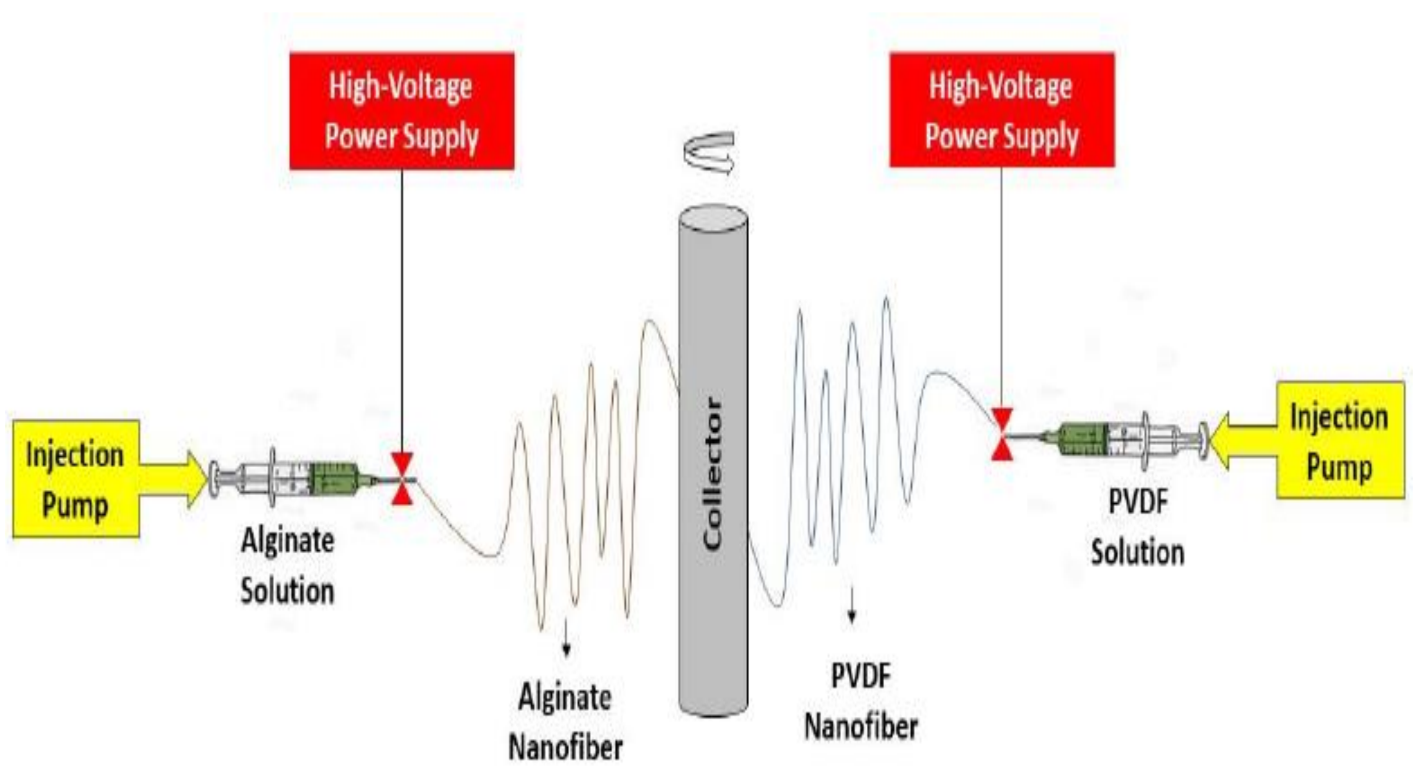
Piezoelectric Electrospun Nanofibrous Materials for Tissue Engineering Applications

Aryan Najjari , Rouhollah Mehdinavaz Aghdam , SA. Seyyed Ebrahimi

School of Metallurgy and Materials Engineering , College of Engineering , University of Tehran , Tehran , Iran

Abstract

Cardiovascular disease is known as the major cause of death all around the world today. In the majority of cases, cardiovascular disease leads to myocardial infarction, heart attack, which can trigger heart failure and death. In these cases, the damaged part of the heart will become scar tissue and the heart has poor regeneration ability; by this way, tissue engineering is a suitable way for regenerating the scar tissue. Electrospinning is a promising method for fabricating scaffolds for cardiac tissue engineering. In this research, an electrospun nanofibrous hybrid scaffold was fabricated. This scaffold was based on alginate. In order to increase the mechanical properties and piezoelectric properties of the cited scaffold, PVDF was added to alginate. To prepare this scaffold, alginate solution and PVDF solution were prepared separately and then they transferred into two different syringes; then by the help of dual electrospinning these two solutions, with their own suitable rates, made the nanofibrous scaffold. The fabricated nanocomposite scaffold was analyzed by scanning electron microscopy (SEM) to determine the fiber diameter and evaluate the fiber morphology. Also, fourier transform infrared (FTIR) spectroscopy and Water Contact Angle test were done in this project to evaluate the properties of the fabricated hybrid scaffold.



The schematic figure of the electrospinning process. The polymer solutions are poured in the syringes; in this method high-voltage electricity is used to draw charged threads of polymer solutions to fiber diameters.

Experimental Details

PVDF solution preparation

The solution was prepared by dissolving the powder of the PVDF polymer in the mixture of solvents (DMF/acetone 5:5) at 70 °C (the polymer concentration in the solvents was 18% wt). After 20 minutes, a homogeneous solution was obtained, and the degassing was done at room temperature for 5 minutes.

Alginate solution preparation

The solution was obtained by solving 0.3 grams of alginate in 10 ml of distilled water for 45 minutes. Meanwhile, for increasing the viscosity and preparing the alginate solution a PEO solution was added to the alginate solution. The PEO solution was prepared by solving 0.3 grams of PEO in 10 ml of distilled water (overnight) at 50 °C. The final solution contained 70% alginate solution and 30% PEO solution (these two solutions must be stirred at room temperature for about 30 minutes to make a homogenous solution).

The scaffold preparation

The Alginate/PVDF scaffold was prepared with the ratio of 70/30% wt.

Electrospinning preparation

The stable solutions at room temperature were transferred in two 5 ml syringes and fed to the electrospinning needles. 18 kV was applied at the tip of the needles. The flow rate of alginate solution was 0.3 ml/h and this parameter for PVDF solution was 1.00 ml/h. The distance between the needles and the collector was fixed at 15 cm. The electrospinning machine in this research was supplied by Nano Azma.

Procedure of crosslinking

A day after the electrospinning, the fabricated mats soaked in 2:1 wt ethanol/water solution with the addition of 3% wt CaCl2 for 2 purposes: elimination of the carrier PEO and alginate crosslinking at the same time. Then, the rinse of the mats was done, three times with distilled water, in order to eliminate the remaining substances which were used for crosslinking. Finally, the lyophilization of the mats was done, overnight, in order to take out the excess water amount.

Morphological study of the fabricated scaffolds

The fiber morphology of the fabricated scaffolds was analyzed by scanning electron microscopy (SEM). Before imaging, gold coating of the scaffolds was done by using a sputter coater. Image analysis software (Image J) was used to determine the average fiber diameter. At least 10 fibers in each image were considered for calculation.

Fourier Transform Infrared spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was carried out to understand the presence of PVDF in the scaffold. It is also used for determining the crosslinked scaffold.

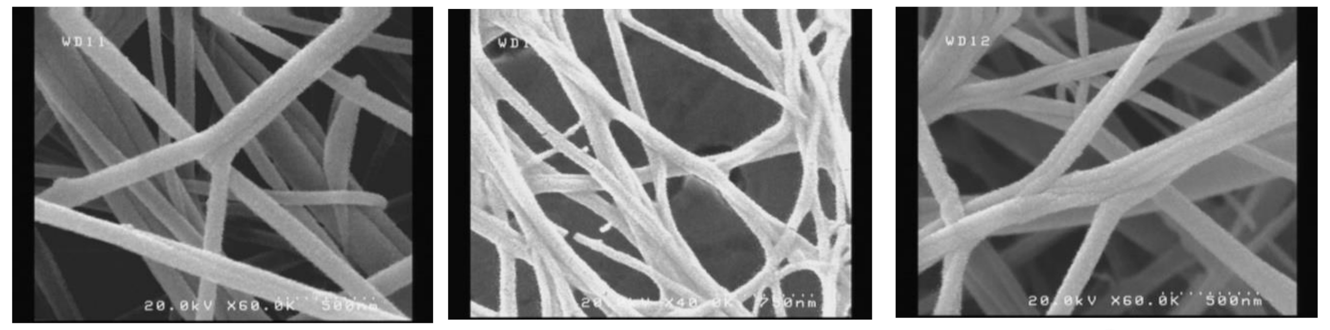
Water contact angle

Hydrophilicity was determined on a video-based optical contact angle meter. 3 × 1 cm samples were used for measuring the contact angle and ascertaining the hydrophilicity. These samples were fixed on a glass microscope slide. Then instillation of a drop of water was done on the samples which were situated on meter stage. The contact angle was measured in software and the average of 3 angles is reported for each sample.

Result and Discussion

Morphology of the fabricated scaffolds

SEM images as shown in figure 1, represent the fiber morphology of alginate/PVDF (70/30% wt) and PVDF scaffolds. The measured diameter of the fibers in crosslinked and non-crosslinked situations are almost equal. Consequently, the crosslinking does not cause any specific changes on fibers' diameter.

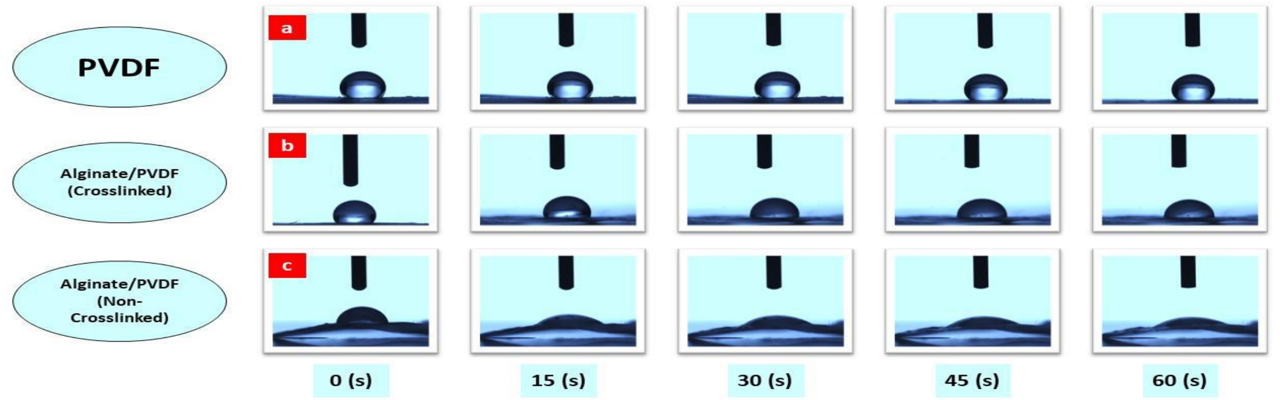


A B C

Figure 1: **A**: PVDF based scaffold. The average fiber diameter in this scaffold was 95.22 nm. **B**: Crosslinked Alginate/PVDF scaffold. The average Alginate fiber diameter in this scaffold was 59.93 nm and PVDF fiber diameter was 104.55 nm .**C**: Non-crosslinked Alginate/PVDF scaffold. The average Alginate fiber diameter in this scaffold was 51.4 nm and PVDF fiber diameter was 131.81 nm

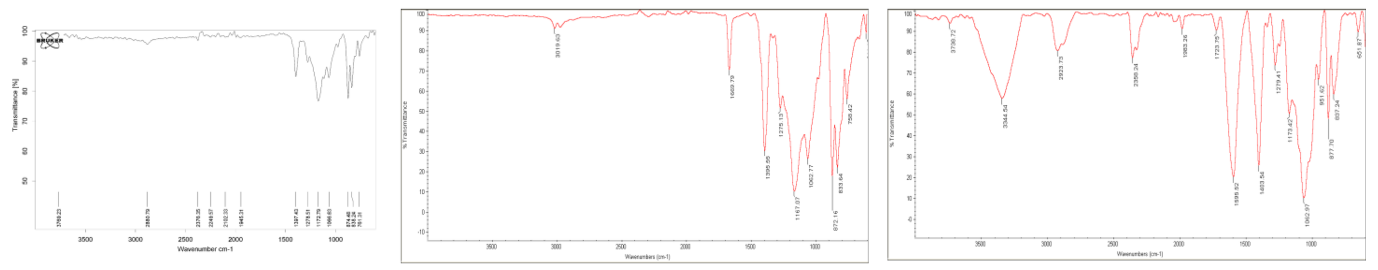
Hydrophilic properties

The hydrophilicity of a scaffold for regeneration of scarred heart is a decisive factor. It paves the way for better cell growth and attachment because cardiomyocytes favor hydrophilic surfaces. PVDF is completely hydrophobic; on the other hand, alginate is hydrophilic. Their combination has a contact angle between them. Both crosslinked and non-crosslinked alginate/PVDF scaffolds were tested and as we expected the non-crosslinked scaffold showed better hydrophilicity.



a: the PVDF based scaffold showed a hydrophobic characteristic and the shape of the water drop did not change during the test time. b: The crosslinked Alginate/PVDF scaffold showed better hydrophilicity and c: the non-crosslinked Alginate/PVDF scaffold had the highest hydrophilicity and the water drop had completely lost its drop shape.

FTIR Spectroscopy



A B C

A: PVDF. The main peaks are shown between 1000 and 1500 cm-1; so, sharp peaks in this period prove the presence of PVDF. **B**: Non-crosslinked Alginate/PVDF. The sharp peaks between 1000 cm-1 and 1500 cm-1 can be observed which prove the presence of PVDF; also, another sharp peak is shown in 3000 cm-1 which is related to presence of Alginate in the fabricated polymer scaffold .**C**: Crosslinked Alginate/PVDF. In this figure not only are sharp peaks related to PVDF and Alginate shown, but also other peaks can be observed which are related to crosslinking materials and it can demonstrate that the crosslinking procedure is done.

Conclusion

We have fabricated a scaffold which has the combination of two different polymers with different properties. In this project 3 different tests were done on the fabricated scaffold and the results were discussed. Due to the piezoelectric characteristic of PVDF, this polymer is able to speed up the regeneration process of the heart. But PVDF is hydrophobic and non-biodegradable; therefore, it is not allowed to be the predominant part of a scaffold. We chose alginate as the predominant and main polymer of the scaffold because of its suitable biocompatibility and hydrophilicity. In conclusion, the fabricated scaffold, based on the tests which are done in this project, is able to provide suitable characteristics and properties for cardiac tissue engineering.

References

[1] C. Estel and C. R. Conti, "Global Burden of Cardiovascular Disease," *Cardiovasc. Innov. Appl.*, vol. 1, no. 4, pp. 369–377, 2016, doi: 10.15212/cvia.2016.0029.

[2] S. Pok, J. D. Myers, S. V. Madihally, and J. G. Jacot, "A multilayered scaffold of a chitosan and gelatin hydrogel supported by a PCL core for cardiac tissue engineering," *Acta Biomater.*, vol. 9, no. 3, pp. 5630–5642, 2013, doi: 10.1016/j.actbio.2012.10.032.

[3] X. Wang, J. Chang, T. Tian, and B. Ma, "Preparation of calcium silicate/decellularized porcine myocardial matrix crosslinked by procyanidins for cardiac tissue engineering," *RSC Adv.*, vol. 6, no. 41, pp. 35091–35101, 2016, doi: 10.1039/c6ra02947g.

[4] C. Gálvez-Montón, C. Prat-Vidal, S. Roura, C. Soler-Botija, and A. Bayes-Genis, "Cardiac Tissue Engineering and the Bioartificial Heart," *Rev. Española Cardiol. (English Ed.*, vol. 66, no. 5, pp. 391–399, 2013, doi: 10.1016/j.rec.2012.11.012.

[5] P. B. Malafaya, G. A. Silva, and R. L. Reis, "Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications," *Adv. Drug Deliv. Rev.*, vol. 59, no. 4–5, pp. 207–233, 2007, doi: 10.1016/j.addr.2007.03.012.

[6] M. S. Kim and G. Kim, "Three-dimensional electrospun polycaprolactone (PCL)/alginate hybrid composite scaffolds," *Carbohydr. Polym.*, vol. 114, pp. 213–221, 2014, doi: 10.1016/j.carbpol.2014.08.008.

[7] R. Arumugam, E. S. Srinadhu, B. Subramanian, and S. Nallani, "β-PVDF based electrospun nanofibers – A promising material for developing cardiac patches," *Med. Hypotheses*, vol. 122, pp. 31–34, 2019, doi: 10.1016/j.mehy.2018.10.005.

[8] Y. Li, C. Liao, and S. C. Tjong, "Electrospun polyvinylidene fluoride-based fibrous scaffolds with piezoelectric characteristics for bone and neural tissue engineering," *Nanomaterials*, vol. 9, no. 7, 2019, doi: 10.3390/nano9070952.

[9] N. Adadi *et al.*, "Electrospun Fibrous PVDF-TrFe Scaffolds for Cardiac Tissue Engineering, Differentiation, and Maturation," *Adv. Mater. Technol.*, vol. 5, no. 3, pp. 1–11, 2020, doi: 10.1002/admt.201900820.

[10] F. P. W. Melchels, A. M. C. Barradas, C. A. Van Blitterswijk, J. De Boer, J. Feijen, and D. W. Grijpma, "Effects of the architecture of tissue engineering scaffolds on cell seeding and culturing," *Acta Biomater.*, vol. 6, no. 11, pp. 4208–4217, 2010, doi: 10.1016/j.actbio.2010.06.012.

[11] C. W. Macosko *et al.*, "A novel degradable polycarbonate networks for tissue engineering," *Biomaterials*, vol. 24, pp. 801–808, 2003.

[12] Y. Zhu, C. Gao, X. Liu, and J. Shen, "Surface modification of polycaprolactone membrane via aminolysis and biomacromolecule immobilization for promoting cytocompatibility of human endothelial cells," *Biomacromolecules*, vol. 3, no. 6, pp. 1312–1319, 2002, doi: 10.1021/bm020074y.

[13] C. P. Barnes, S. A. Sell, E. D. Boland, D. G. Simpson, and G. L. Bowlin, "Nanofiber technology: Designing the next generation of tissue engineering scaffolds," *Adv. Drug Deliv. Rev.*, vol. 59, no. 14, pp. 1413–1433, 2007, doi: 10.1016/j.addr.2007.04.022.

[14] Q. P. Pham, U. Sharma, and A. G. Mikos, "Electrospinning of polymeric nanofibers for tissue engineering applications: A review," *Tissue Eng.*, vol. 12, no. 5, pp. 1197–1211, 2006, doi: 10.1089/ten.2006.12.1197.

[15] W. Li, C. T. Laurencin, E. J. Caterson, R. S. Tuan, and F. K. Ko, "<Li_et_al-2002-Journal_of_Biomedical_Materials_Research.pdf>," 2001.

[16] N. T. Hiep and B. T. Lee, "Electro-spinning of PLGA/PCL blends for tissue engineering and their biocompatibility," *J. Mater. Sci. Mater. Med.*, vol. 21, no. 6, pp. 1969–1978, 2010, doi: 10.1007/s10856-010-4048-y.

[17] R. F. Pereira, A. Carvalho, M. H. Gil, A. Mendes, and P. J. Bártolo, "Influence of Aloe vera on water absorption and enzymatic in vitro degradation of alginate hydrogel films," *Carbohydr. Polym.*, vol. 98, no. 1, pp. 311–320, 2013, doi: 10.1016/j.carbpol.2013.05.076.

Acknowledgement

The authors would like to thank the Iran National Science Foundation (INSF) for the financial support of this research project.