

Preparation and Characterization of Silk/Diopside Composite Nanofibers via Electrospinning for Tissue Engineering Application

Abbas Teimouri, Leila Ghorbanian, Iren Dabirian

Abstract—This work focused on preparation and characterizations of silk fibroin (SF)/nanodiopside nanoceramic via electrospinning process. Nanofibrous scaffolds were characterized by combined techniques of scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD). The results confirmed that fabricated SF/diopside scaffolds improved cell attachment and proliferation. The results indicated that the electrospun of SF/nanodiopside nanofibrous scaffolds could be considered as ideal candidates for tissue engineering.

Keywords—Electrospinning, nanofibers, silk fibroin, diopside, composite scaffold.

I. INTRODUCTION

SF is a kind of natural polymers with a great potential in biomedical application. [1] Due to its good biocompatibility, biodegradability, high tensile strength, hemostatic properties, non-cytotoxicity, low antigenicity and minimal inflammatory reaction, SF is an excellent candidate for generating tissue engineering scaffolds [2]-[5].

Based on previous findings, diopside ($\text{CaMgSi}_2\text{O}_6$) is advised as an excellent bioactive material for artificial bone and dental root, since it shows more potential of apatite formation ability and higher mechanical strength than hydroxyapatite. Moreover, it has been confirmed that the diopside has a fairly high mechanical strength, good bioactivity, excellent bending strength and a good biocompatibility [6]-[12].

Electrospinning is a new technique to fabricate nanofibrous scaffolds for tissue engineering due to the large surface area to volume ratio, that influences the adhesion, migration, and growth of cells [13]. In the past few years, there has been significant growth in research on exploring electrospun nanofibrous scaffold for tissue engineering applications [14]-[16].

In this report, we extend our recent study on silk composites [17], [18]. SF/nanodiopside were fabricated via electrospinning. Herein, the effect of nanodiopside on the surface morphology of electrospun SF/nanodiopside nanofibers were investigated. Finally, the cytocompatibility of

Abbas Teimouri and Iren Dabirian are with the Department of Chemistry, Payame Noor University, 19395-4697, Tehran, Iran (e-mail: a_teimouri@pnu.ac.ir, iren.dabirian.id@gmail.com).

Leila Ghorbanian was with Torabinejad Dental Research center and Department of Oral and Maxillofacial Pathology, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran (e-mail: le_ghorbanian@yahoo.com).

the SF/nanodiopside composite nanofibrous scaffold was studied by using MTT test.

II. MATERIALS AND METHODS

A. Materials

High quality raw cocoons of silkworm, *Bombyx mori* were purchased from a silk company (South Korea). Cellulose dialysis cassettes (Slide-A-lyzer, MWCO 12000 Da (Sigma)) were used to remove solvent impurities from SF solution. Calcium nitrate, Sodium carbonate, LiBr, magnesium chloride, magnesium nitrate, TEOS and all other solvent and chemicals were analytical grade from Sigma Chemical (St. Louis, MO, USA).

B. Preparation of Regenerated SF

SF was extracted from silk cocoons according to the previously described protocol with some modifications [18]. Briefly, boiling undamaged cocoons in sodium carbonate (0.02 M) for 30 minutes, resulted in the removal of unwanted parts (mainly sericin) from fibroin, followed by dissolving the degummed silk in LiBr (9.3 M) and dialyzing against water for 3 days. It yielded an aqueous silk solution with the concentration of 3.5% (wt./vol). This purified SF was kept in the temperature of 4 °C before use.

C. Nanodiopside Synthesis

The diopside ceramic was prepared through a modified sol-gel method described somewhere else [18]. Briefly, we dissolved 0.125 mol of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in ethanol and stirred vigorously for 30 min at 80° C to dissolve in the solvent. $\text{Si}(\text{OC}_2\text{H}_5)_4$ (TEOS) was added to the homogenous solution and was slowly stirred to obtain a wet gel. Being dried in an oven at 100 °C for 24 h resulted in a dried powder which was grounded in a hand-mortar, followed by calcinations at 700 °C for 2 h and at 1100 °C for further 24 h.

D. Electrospinning of SF Nanofiber

SF was dissolved in trifluoro acetic acid (TFA) to form a uniform polymer solution. The solution was stirred at room temperature for 3 h. The concentrations of silk/TFA solution were 8, 10 and 12 wt%.

An electrospinning apparatus manufactured was used, and was operated at room temperature.

All solutions were electrospun in the same processing conditions. These solutions were directly electrospun using a

typical electrospinning equipment [19]. The solution was fed to the glass syringe fitted with the stainless-steel-ended needle using an infusion pump. The applied voltage was kept at 18-30 kV, aluminum foil was used as a static collector and grounded. The electrospinning was performed at room temperature. The distance between the needle tip and the metal sheet was 12-15 cm and the flow rate of the feedstock was 15 $\mu\text{l}/\text{min}$. The resulting nanofibers on the foil were dried overnight at room temperature.

E. Fabrication of SF / Nano Diopside Composite

To form a composite of SF nanofibers and nano diopside, SF nanofibers were immersed in pure methanol for 15 min, and were then dried at room temperature for 24 h. Through methanol treatment, the SF nanofibers were transformed from a random coil structure into a β -sheet conformation [20]. The SF nanofibers were dipped in an nano diopside aqueous suspension at concentrations of 0.1, 0.5 and 1 wt%, and the products were dried at room temperature for 12 h to produce SF / nano diopside composites.

F. Characterization

The morphologies of the composite scaffolds were evaluated by scanning electron microscope (SEM, JEOL, JSM-6300, Tokyo, Japan), at an acceleration voltage of 20 kV. The samples were analyzed X-ray diffraction (XRD) (Philips X'PERT MPD X-ray Diffractometer), with Cu K α radiation ($\lambda=0.154056$ nm) in the 2θ range of $10-100^\circ$ (a step size of 0.04° and a time per step of 1 s). For FT-IR analysis, a JASCO FT/IR-680 PLUS spectrometer in KBr matrix in the range between $4000-400$ cm^{-1} was used to record the spectra.

III. RESULTS AND DISCUSSION

A. SEM Analysis

Fig. 1 shows SEM micrographs of the pure SF, nano diopside and SF/nanodiopside composite scaffolds containing 20wt. % of nano diopside.

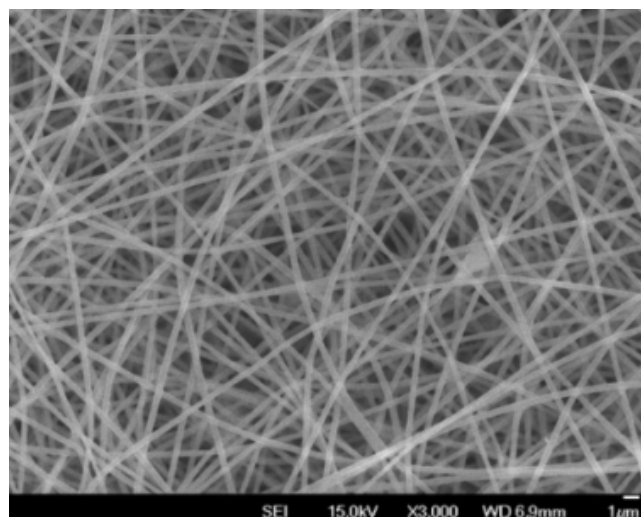
The average diameter of pure SF nanofibers was measured 108.20 ± 53 nm. When the nano diopside content in hybrids increased up to 20%, the average diameter of nanofibers gradually increased from 108.20 ± 53 nm to 364.44 ± 42 nm.

B. FT-IR Analysis

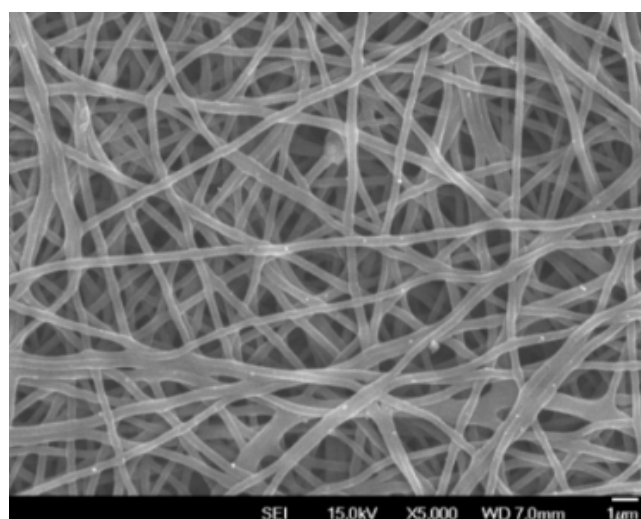
Fig. 2 shows the FTIR spectra of pure SF, pure nano diopside and the prepared SF/nanodiopside composite scaffolds in the $400-4000$ cm^{-1} range. peak in the region $600-700$ cm^{-1} which can be attributed to the bending vibrations, and bands in the region $850-1100$ cm^{-1} which are correspond to the stretching vibrations of the silicate structure. The sharp peak at 3533 cm^{-1} due to OH stretching vibrations.

The FT-IR spectra confirmed the transformation of SF from a random coil structure to a β -sheet conformation in the amide I and II regions.

The amide I peak, which reflects the stretching of C=O group along the SF backbone, is shifted from 1655 to 1630 cm^{-1} . The amide II, which originates from N-H deformation, is shifted from 1544 to 1536 cm^{-1} .



(a)



(b)

Fig. 1 The morphology of electrospun fibers with different nanodiopside contents: (a) 0%, (b) 20%, respectively

C. XRD Analysis

The XRD patterns of the nano diopside powder, pure SF and SF /nanodiopside composite scaffolds containing 20wt. % of nano diopside are shown in Fig. 3. The XRD spectrum of nano diopside showed peaks at 29.9° which are important peak nano diopside. The XRD spectrum nano diopside reveals had amorphous with little crystallinity. Diffraction peaks at about $2\theta=20^\circ-30^\circ$ could be attributed to β -sheet (silk II) structure. The broad peak corresponding to the random coil structure in both the pure SF and the composite scaffolds superimposed on the silk II peaks indicated the co-existence of random coil and silk II structure in the SF scaffold. The XRD patterns of the composites simultaneously exhibited characteristic peaks of forsterite, random coil and Silk II structure.

D. In vitro Evaluation of Cytotoxicity

The proliferation of MC3T3-E1 cells in contact with nanofibers was assayed after 1, 3 and 7 days of culture period by means of MTT test (Fig. 4). The cell number increased with culture time but exhibits a material dependence or an effect of nanofiber composition on cell proliferation rate. Upon cell growth, from day 1, a significant difference ($p < 0.05$) in cell number was observed between SF and SF/nano diopside nanofibers. Nonetheless, no significant difference between SF and SF/ nano diopside nanofibers was found. The maximum cell number was attained at 7 d where cells exhibited growth arrest thereafter.

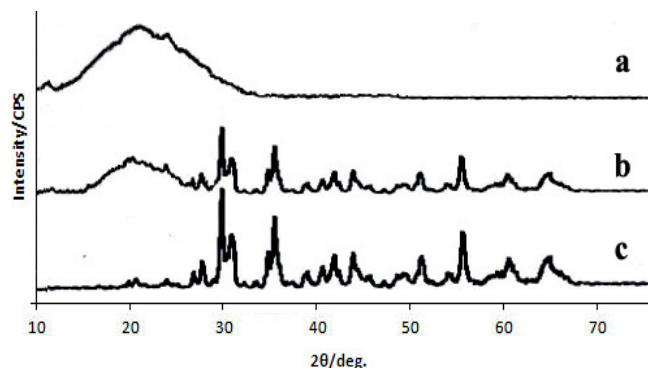


Fig. 2 XRD patterns of pure SF (a), 20% diopside/SF nanofibrous (b) and diopside nanopowders (c)

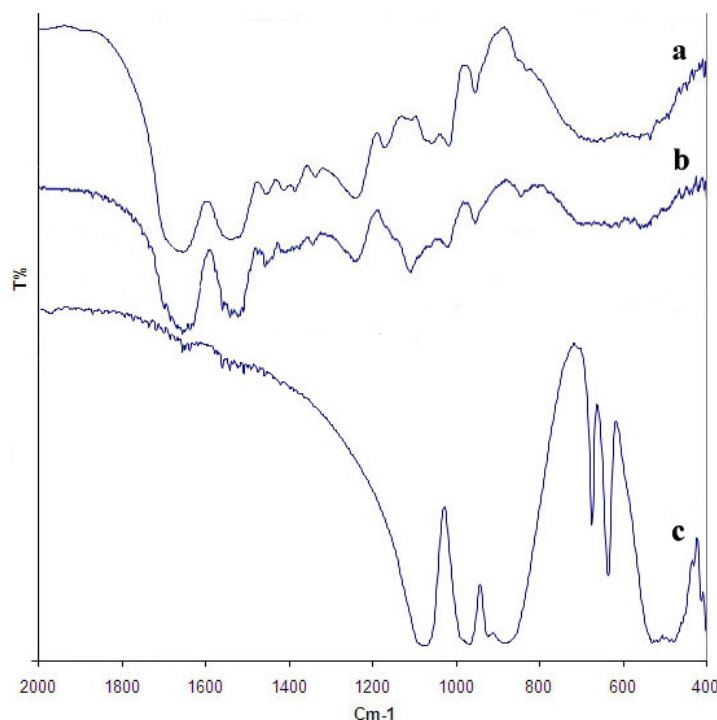


Fig. 3 FT-IR spectra of pure SF (a), 20% diopside/SF nanofibrous (b) and diopside nanopowders (c)

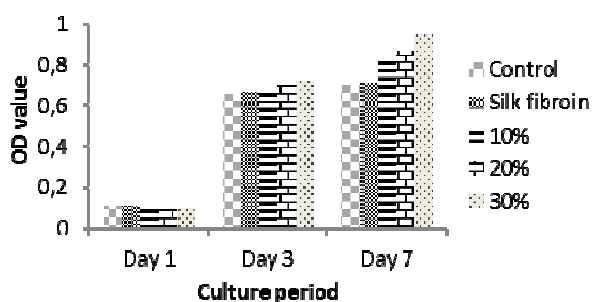


Fig. 4 *In vitro* cytotoxicity evaluation of MC3T3 cells in contact with scaffold for different periods of time. Data are presented as the mean \pm SD. significant difference ($p \leq 0.05$)

IV. CONCLUSION

Composite silk/nanodiopside nanofibrous scaffold was successfully fabricated by electrospinning method. An optimal

electrospinning condition was obtained for producing uniform cylindrical fibers. The diopside silk scaffolds supported the growth and expansion of cells based on cell adhesion, and morphology *in vitro*. Thus, the silk/nanodiopside composite scaffold, would be a good system for tissue engineering applications.

REFERENCES

- [1] Q. Zhang, S. Yan, M. Li, *Materials*. Vol. 2 (2009), p. 2276.
- [2] T. C. Holmes, *Trends in Biotechnology*. 20 (2002), p. 16.
- [3] E. Wenk, H. P. Merkle, L. Meinel, *Journal of Controlled Release*. Vol. 150 (2011), p. 128.
- [4] N. Bhardwaj, S.C. Kundu, *Carbohydrate Polymers*. Vol. 85 (2011), p. 325.
- [5] R.L. Horan, K. Antle, A.L. Collette, Y.Z. Wang, J. Huang, J.E. Moreau, V. Volloch, D.L. Kaplan, G.H. Altman, *Biomaterials*. Vol. 26 (2005), p. 3385.
- [6] M. Peter, P.T.S. Kumar, N.S. Binulal, S.V. Nair, H. Tamura, R. Jayakumar, *Carbohydrate Polymers*, Vol. 78 (2009), p. 926.

- [7] R. Jayakumar, D. Menon, K. Manzoor, S. Nair, H. Tamura, *Carbohydrate Polymers*, Vol. 82 (2010), p. 227.
- [8] S. Nakajima, Shika gakuho. *Dental science reports*, Vol. 90 (1990), p. 525-553.
- [9] M. Ashizuka, Y. Aimoto, T. Okuno, *Journal of the Ceramic Society of Japan*, Vol. 97 (1989), p. 544.
- [10] T. Nonami, S. Tsutsumi, *Materials in Medicine*, Vol. 10 (1999), p. 475.
- [11] T. Nonami, *MRS Proceedings*, Cambridge Univ Press, 1991, pp. 87.
- [12] T. Nonami, S. Tsutsumi, M. Fukuma, T. Urabe, *Jpn. Soc. Biomater*, Vol. 12 (1994), p. 274.
- [13] Venugopal J, Ramakrishna S. *Appl Biochem Biotechnol*. Vol. 125 (2005), p. 147.
- [14] K.T. Shalumon, N.S. Binulal, N. Selvamurugan, S.V. Nair, D. Menon, T. Furuike, H. Tamura, R. Jayakumar, *Carbohydr. Polym.* Vol. 77 (2009), p. 863.
- [15] R. Jayakumar, M. Prabakaran, S.V. Nair, H. Tamura, *Biotechnol. Adv.* Vol. 28 (2010), p. 142.
- [16] A. Greiner, J.H. Wendorff, *Angew. Chem. Int. Ed.* Vol. 46 (2007), p. 5670–5703.
- [17] A. Teimouri, L. Ghorbanian, A.N. Chermahini, R. Emadi, *Ceramics International*, Vol. 40 (2014), p. 6405.
- [18] L. Ghorbanian, R. Emadi, S.M. Razavi, H. Shin, A. Teimouri, *International journal of biological macromolecules*, Vol. 58 (2013), p. 275.
- [19] Li G, Li P, Yang XP. *Compos Sci Tech.* Vol. 68(3-4) (2008), p. 987.
- [20] S.H. Kim, Y.S. Nam, T.S. Lee, W.H. Park, *Polymer Journal* Vol. 35 (2003), p. 185.