Materials Letters 276 (2020) 128191

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/mlblue

A facile method to fabricate propolis enriched biomimetic PVA architectures by co-electrospinning

Rumeysa Bilginer, Ahu Arslan Yildiz *

Department of Bioengineering, Izmir Institute of Technology (IZTECH), 35430 Izmir, Turkey

ARTICLE INFO

Article history:
Received 7 April 2020
Received in revised form 27 May 2020
Accepted 19 June 2020
Available online 23 June 2020

Keywords:
Biomimetic
Polymeric composites
Propolis
Co-electrospinning
Tissue engineering

ABSTRACT

This study depicts easy process of propolis by co-electrospinning without using any toxic agent for biomedical applications. To achieve this, polyvinyl alcohol was utilized as co-spinning agent to fabricate biomimetic Propolis/PVA scaffold. Here, whilst PVA was used as a supportive material to accumulate propolis in scaffold, propolis was employed to enrich biologic aspect of scaffold. This strategy overcomes challenges of propolis processing originated from solubility problems and offers easy processability of propolis in order to use in biomedical applications. Electrospun Propolis/PVA scaffolds were crosslinked with glutaraldehyde and drop-cast model was utilized as a control. Formation of porous, bead-free nanofiber architectures was confirmed through surface morphology analysis, while drop-cast model shows non-porous morphology. Wettability results confirmed both crosslinking and integration of propolis into polyvinyl alcohol scaffold moved contact angle to hydrophobic region. Presence and amount of propolis in hybrid scaffolds were validated via absorbance spectrum results. Bioactivity and biocompatibility of propolis-enriched scaffolds were analyzed through protein adsorption capacity. Obtained findings are evidence that electrospinning methodology offers easy and biosafe process of propolis. Electrospun Propolis/PVA exhibits desired properties and could be potentially utilized as scaffold for tissue engineering or as a wound dressing graft in biomedical field.

1. Introduction

Propolis is a natural resinous material collected from honeybees and shows bioactive, antibacterial, antiviral, anaesthetic, antiseptic, and antioxidant properties due to presence of phenolic, terpenoid and alkaloids ingredients [1,2]. Beside its usage in traditional medicine, it has been also used in biomedical fields such as drug delivery [3–5], and wound dressing [6,7]. However, despite its use in wide range of applications, as like other natural materials [8], processing of propolis and maintaining its function without disrupting structure during process is tedious, which limits bio-based applications of propolis. Processing problem of propolis arises from its limited solubility. In most organic solvents the solubility of propolis is limited [9] and only small portion of propolis can be dissolved in water [10]. In addition to that, lipophilic content of propolis prevents to be solubilized in aqueous and biological media result in limiting clinical applications and minimizing bioavailability of propolis [11,12]. To date, propolis has been processed by several fabrication techniques; such as traditional casting to generate biofilm [13,14], spray drying [15,16] and encapsulation into micro or nanoparticles [17,18]. To the best of our knowledge, pure propolis has not been processed by electrospinning methodology due to aforementioned limitations and solubility problems.

To enrich synthetic polymers with natural materials via electrospinning, there are few obstacles that need to be overcome. Since synthetic polymer and natural material have different properties it is challenging to find the optimum co-spinning parameters and a suitable common solvent. Therefore, in this study we aimed to overcome processing problem of propolis that arises from its solubility. Here, propolis was processed via electrospinning with assistance of aqueous polyvinyl alcohol (PVA) to prevent bioactivity loss. PVA is utilized as co-spinning agent for easy spinning of propolis, so propolis is accumulated in PVA. After electrospinning, it was crosslinked via glutaraldehyde (GTA) to obtain non-soluble scaffold in aqueous environment. Also, propolis was drop-casted on electrospun PVA for comparison. Morphological characterization was done by SEM. Presence of propolis in scaffolds was validated by UV–Visible spectrophotometer. Wettability analysis was performed by static water contact angle. The main advantage of proposed strategy is to offer easy integration of propolis into synthetic materials. Result confirmed that Propolis/PVA nanofiber

* Corresponding author.
E-mail address: ahuarslan@iyte.edu.tr (A. Arslan Yildiz).

https://doi.org/10.1016/j.matlet.2020.128191
0167-577X/

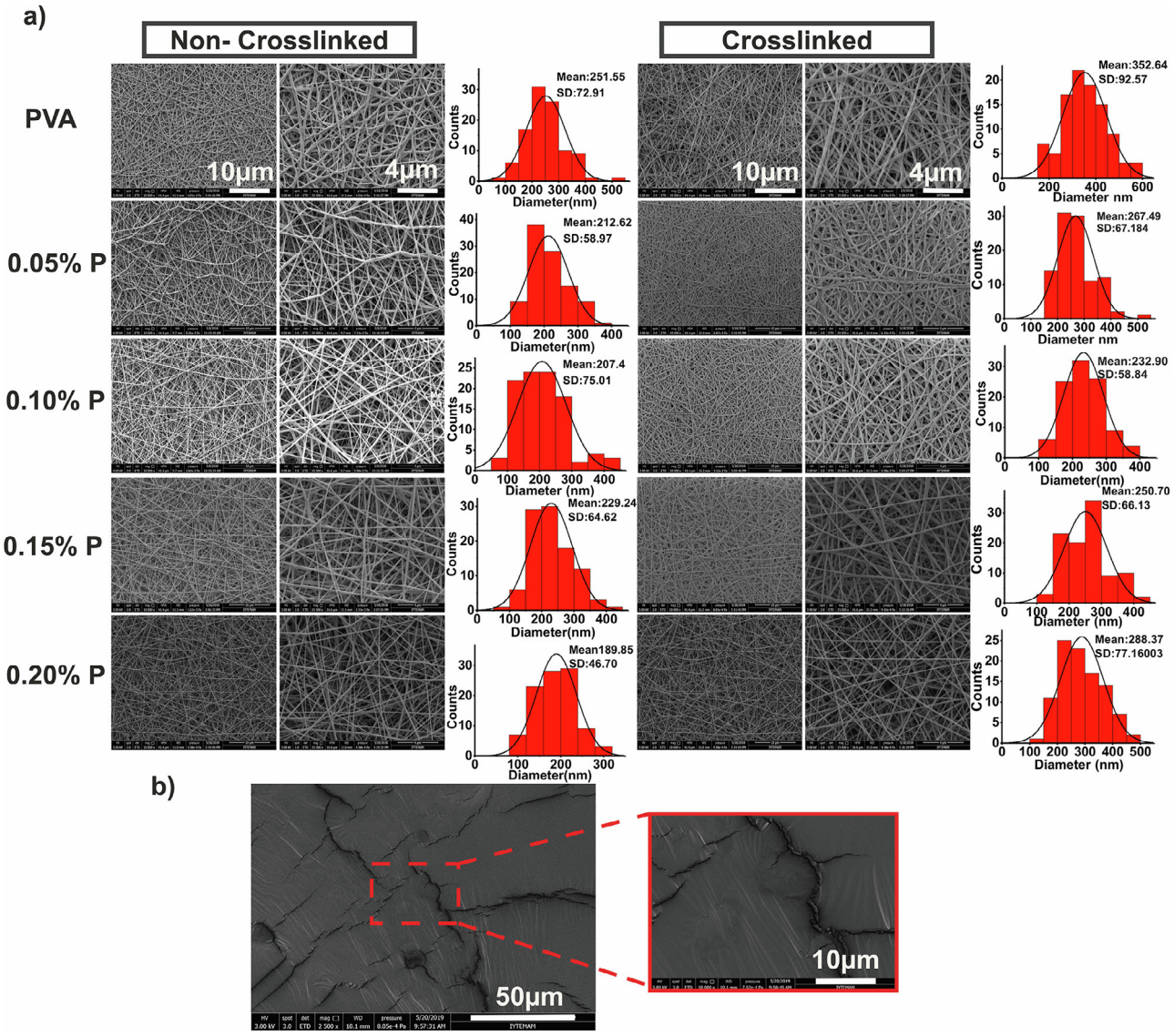

Fig. 1. SEM images of a) electrospun crosslinked and non-crosslinked PVA/propolis scaffolds; containing 0.05, 0.10% 0.15 and 0.20% propolis and b) Drop-cast Propolis/PVA.

possess promising scaffold material to be used in biomedical and tissue engineering applications.

2. Materials and methods

Raw Propolis was obtained as a gift from Onkafarma Natural Products Co. Ltd. (Turkey). Polyvinyl alcohol (PVA, wt 30,000–70,000), bovine serum albumin (BSA), GTA was purchased from Sigma Aldrich. Ethanol (99%) and hydrochloric acid (HCl) (37%) was purchased from Isolab. Sodium dodecyl sulfate (SDS) was purchased from Bioshop. Propolis/PVA hybrid scaffolds were prepared by using 20% PVA (w/v) with different propolis proportions; 0.05, 0.10, 0.15 and 0.20% (w/v) respectively. Corresponding amount of ethanolic propolis was added to aqueous PVA solution prior to electrospinning. Electrospinning of Propolis/PVA was performed at room temperature with 28 kV voltage, 1 ml/h flow rate and 180 mm working distance via Invenso (Ne300). Later, composite scaffolds were exposed to 0.01 M GTA containing acetone and HCl for crosslinking. As a control group propolis was drop-casted on crosslinked, electrospun PVA was incubated in liquid propolis overnight at room temperature. Morphological analysis of both crosslinked and non-crosslinked scaffolds was done by SEM analysis (SEM- Quanta FEG 250), and average diameters were analyzed through Image J software (NIH). The total propolis content was determined by solubilizing Propolis/PVA scaffolds after electrospinning process. Absorbance values of ethanol:water (1:1) solubilized Propolis/PVA scaffolds were obtained at 290 nm using ethanol:water solubilized propolis calibration curve ranging between 0 and 250 µg/ml. Wettability of hybrid scaffolds was analyzed via water contact angle (Attension) analysis. The protein adsorption capacity of scaffolds was examined by Bicinchoninic acid (BCA) assay kit (PierceTM, Thermo Scientific).

3. Results and discussion

Fig. 1a shows SEM images and diameter distribution of crosslinked and non-crosslinked Propolis/PVA scaffolds, and pristine PVA. Bead-free nanofibers have been generated successfully. The average diameter is varied between 189.85 nm ± 46.70 and 251.5 5 nm ± 72.91, and addition of propolis increased fiber diameter for non-crosslinked scaffolds, which originates from increasing adhesive properties after propolis addition [19–21]. Crosslinking

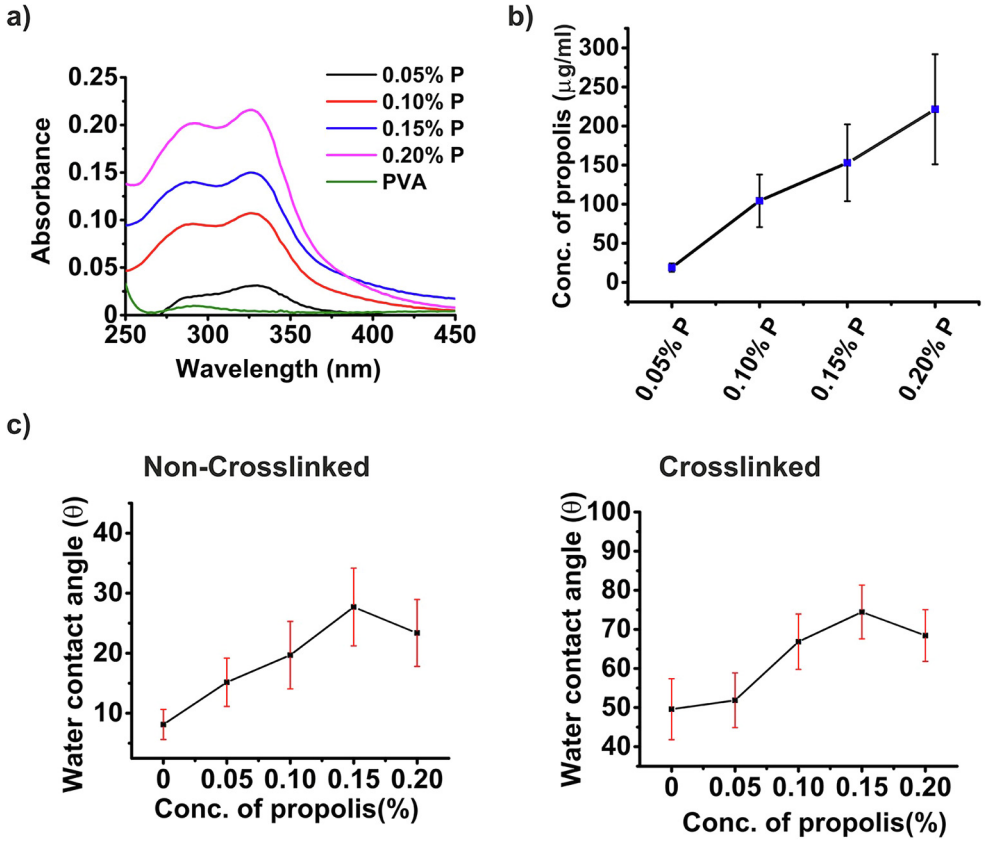

Fig. 2. a) UV–Vis spectra; b) Propolis concentration; c) Contact angle analysis; of Propolis/PVA scaffolds.

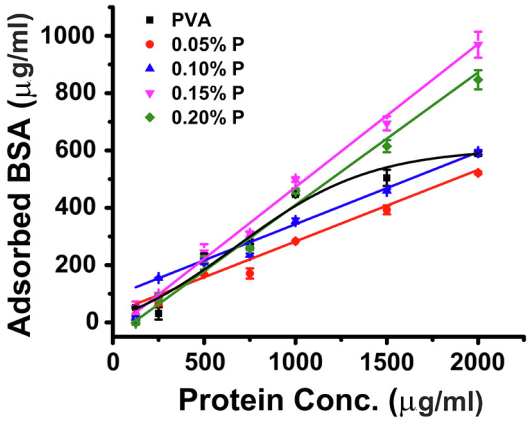

Fig. 3. Protein adsorption profiles of Propolis/PVA scaffolds (n = 3).

with GTA enhanced the diameter of PVA/propolis nanofibers, which ranged between 232.90 nm ± 66.13 and 352.64 nm ± 92.57. Moreover, the morphology of drop-cast Propolis/PVA scaffold was analyzed via SEM to compare with electrospun scaffolds. Fig. 1b showed non-porous, flat morphology of drop-cast Propolis/PVA owing to sticky nature of propolis. Both porosity and surface area-to-volume ratio diminished; as a result, binding of cells, which is crucial for biomedical applications, will decrease.

Propolis content of hybrid scaffolds was quantitively confirmed by UV–Vis spectroscopy. Fig. 2a demonstrates absorbance spectrum of hybrid Propolis/PVA scaffolds, where propolis-enriched scaffolds have characteristic absorbance maxima around 300–350 nm. As shown in Fig. 2b propolis concentration in hybrid scaffolds were given as a function of increasing propolis content. Max propolis concentration reached to 221.45 μg/ml for 0.20% Propolis/PVA. Results strongly supported that absorbance values are consistent with increasing amount of propolis, which signified electrospinning of propolis could be achieved with assistance of PVA co-spinning agent.

As depicted in Fig. 2c, wettability analysis showed both crosslinking and incorporation of propolis altered contact angle of highly hydrophilic PVA, and it moved to slightly hydrophobic region. Contact angle ranged between 8.13° ± 2.5 and 27.7° ± 6.48 for non-crosslinked, and 49.58° ± 7.8 and 74.46° ± 6.87 for crosslinked scaffolds confirming integration of propolis that has hydrophobic nature [21]. To investigate biocompatibility and biofunctionality of Propolis/PVA scaffolds, protein adsorption analysis was done. Fig. 3 illustrates adsorbed protein amount on five different scaffolds, where protein adsorption profile showed linear increase in all. The highest amount of adsorbed protein obtained for 0.15 and 0.20% Propolis/PVA, which can be attributed to high amino acid content of propolis. It is known that increment in fiber diameter with propolis addition affects adhesive properties of fibers that can also increase protein adsorption [21]. Results confirmed that increased protein adsorption occurs for increased propolis amount.

4. Conclusion

In this project, in order to circumvent obstacles related with solubility and processing of propolis, we aimed to process propolis with electrospinning technique without using any toxic solvent while using aqueous PVA as carrier. We have also demonstrated possibility of propolis integration via electrospinning into synthetic polymers such as PVA, where hybrid scaffold can be utilized

for varied biomedical applications. Evidences indicated that drop-casting process is not appropriate for propolis integration, especially for tissue engineering applications where porosity is highly required. Therefore, electrospinning methodology is more satisfying technique to integrate propolis in porous architectures.

CRediT authorship contribution statement

Rumeysa Bilginer: Methodology, Investigation, Visualization, Writing - original draft. **Ahu Arslan Yildiz:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] G.A. Burdock, Food Chem. Toxicol. 36 (4) (1998) 347–363.
[2] D.C. Zancanela, R.D. Herculano, C.S. Funari, C.M. Marcos, A.M.F. Almeida, A.C. Guastaldi, Mater. Lett. 209 (2017) 39–42.
[3] M.G. Arafa, D. Ghalwash, D.M. El-Kersh, M.M. Elmazar, Sci. Rep. 8 (1) (2018) 18056.
[4] T. Nascimento, P. Silva, L. Azevedo, L. Rocha, I. Porto, T. Flávio, T.F.A.L. Moura, I. Basílio-Júnior, L. Aparecido, M. Grillo, C. Dornelas, E. Jorge, S. Fonseca, E. De, J. Oliveira, T. Zhang, D. Watson, Nanoscale Res. Lett. 11 (2016) 1–16.
[5] H.C. Rosseto, L.d.A.S.d. Toledo, L.M.B.d. Francisco, E. Esposito, Y. Lim, G. Valacchi, R. Cortesi, M.L. Bruschi, Colloids Surf. B: Biointerfaces 158 (2017) 441–452.
[6] S. Sharaf, M.E. El-Naggar, Int. J. Biol. Macromol. 133 (2019) 583–591.
[7] S. Cavalu, P.M. Pasca, M. Brocks, Materiale Plastice 56 (2019) 479–483.
[8] E. Mele, J. Mater. Chem. B 4 (28) (2016) 4801–4812.
[9] E.L. Ghisalberti, Bee World 60 (2) (2015) 59–84.
[10] J.M. Sforcin, V. Bankova, J. Ethnopharmacol. 133 (2) (2011) 253–260.
[11] D.M. Kim, G.D. Lee, S.H. Aum, H.J. Kim, Biol. Pharm. Bull. 31 (9) (2008) 1704–1710.
[12] P.D. Petrov, C.B. Tsvetanov, P. Mokreva, K. Yoncheva, S. Konstantinov, B. Trusheva, M. Popova, V. Bankova, RSC Adv. 6 (36) (2016) 30728–30731.
[13] P.M. Matei, P. Martin-Ramos, M. Sanchez-Bascones, S. Hernandez-Navarro, A. Correa-Guimaraes, L.M. Navas-Gracia, C.A. Rufino, M.C. Ramos-Sanchez, J. Martin-Gil, Int. J. Polym. Sci. 2015 (2015) 11.
[14] A.J. Silva, J.R. Silva, N.C. de Souza, P.C.S. Souto, Mater. Lett. 116 (2014) 235–238.
[15] C. Jansen-Alves, F.D. Krumreich, G.P. Zandona, M.A. Gularte, C.D. Borges, R.C. Zambiazi, Food Bioprocess Technol. 12 (5) (2019) 729–740.
[16] D.K. Pratami, A. Munim, M. Yohda, H. Hermansyah, M. Gozan, Y.R.P. Putri, M. Sahlan, AIP Conf. Proc. 2085 (1) (2019) 020040.
[17] S. El-Guendouz, B. Lyoussi, J.P. Lourenço, A.M. Rosa da Costa, M.G. Miguel, C. Barrocas Dias, A. Manhita, L. Jordao, I. Nogueira, M.L. Faleiro, J. Taiwan Inst. Chem. Eng. 102 (2019) 25–33.
[18] S.-J. Yang, J.-J. Wu, Y.-C. Wang, C.-F. Huang, T.-M. Wu, C.-J. Shieh, C.-M.J. Chang, J. Supercrit. Fluids 94 (2014) 138–146.
[19] C. Asawahame, K. Sutjarittangtham, S. Eitssayeam, Y. Tragoolpua, B. Sirithunyalug, J. Sirithunyalug, AAPS PharmSciTech 16 (1) (2015) 182.
[20] C. Asawahame, K. Sutjarittangtham, S. Eitssayeam, Y. Tragoolpua, B. Sirithunyalug, J. Sirithunyalug, Chiang Mai J. Sci. 42 (2015) 469–480.
[21] J.I. Kim, H.R. Pant, H.J. Sim, K.M. Lee, C.S. Kim, Mater. Sci. Eng. C Mater. Biol. Appl. 44 (2014) 52.