Hashemi et al., *BioImpacts*, 2023, 13(4), 275-287
doi: 10.34172/bi.2023.26317
http://bi.tbzmed.ac.ir/

Preparation and evaluation of a polycaprolactone/chitosan/propolis fibrous nanocomposite scaffold as a tissue engineering skin substitute

Seyedeh-Sara Hashemi[1,2], Ali-Akbar Mohammadi[1,3], Seyedeh-Somayeh Rajabi[4], Parisa Sanati[1,5], Alireza Rafati[6], Mehdi Kian[2,7*], Zahra Zarei[3*]

[1]Burn and Wound Healing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Comparative Biomedical Sciences, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Fars, Iran
[3]Division of Plastic and Reconstructive Surgery, Department of Surgery, Shiraz University of Medical Sciences, Shiraz, Fars, Iran
[4]Department of Tissue Engineering, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran
[5]Iran National Elite Foundation, Tehran, Iran
[6]Division of Pharmacology and Pharmaceutical Chemistry, Sarvestan Branch, Islamic Azad University, Sarvestan, Fars, Iran
[7]Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Fars, Iran

Article Info

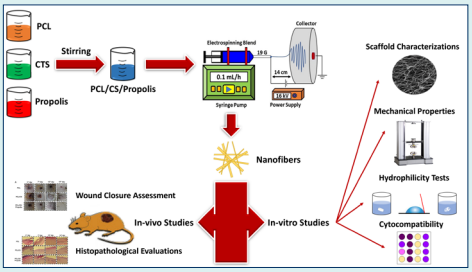
Article Type:
Original Article

Article History:
Received: 5 Apr. 2022
Revised: 5 Sep. 2022
Accepted: 12 Sep. 2022
ePublished: 11 June 2023

Keywords:
Skin substitutes
Polycaprolactone
Chitosan
Propolis
Nanofibers
Wound healing

Abstract

Introduction: Recently, the application of nanofibrous mats for dressing skin wounds has received great attention. In this study, we aimed to fabricate and characterize an electrospun nanofibrous mat containing polycaprolactone (PCL), chitosan (CTS), and propolis for use as a tissue-engineered skin substitute.



Methods: Raw propolis was extracted, and its phenolic and flavonoid contents were measured. The physiochemical and biological properties of the fabricated mats, including PCL, PCL/CTS, and PCL/CTS/Propolis were evaluated by scanning electron microscopy (SEM), atomic force microscopy (AFM), mechanical analysis, swelling and degradation behaviors, contact angle measurement, cell attachment, DAPI staining, and MTT assay. On the other hand, the drug release pattern of propolis from the PCL/CTS/Propolis scaffold was determined. A deep second-degree burn wound model was induced in rats to investigate wound healing using macroscopical and histopathological evaluations.

Results: The results revealed that the propolis extract contained high amounts of phenolic and flavonoid compounds. The fabricated scaffold had suitable physicochemical and mechanical properties. Uniform, bead-free, and well-branched fibers were observed in SEM images of mats. AFM analysis indicated that the addition of CTS and propolis to PCL elevated the surface roughness. MTT results revealed that the electrospun PCL/CTS/Propolis mat was biocompatible. The presence of fibroblast cells on the PCL/CTS/Propolis mats was confirmed by DAPI staining and SEM images. Also, propolis was sustainably released from the PCL/CTS/Propolis mat. The animal study revealed that addition of propolis significantly improved wound healing.

Conclusion: The nanofibrous PCL/CTS/Propolis mat can be applied as a tissue-engineered skin substitute for healing cutaneous wounds, such as burn wounds.

Introduction

Skin is an important organ of the body that has various essential vital functions, including the sensation of physical and chemical stimuli; thermoregulation; maintenance of underlying tissues moisture; elimination of excess fluids, ions, and biological byproducts; synthesis and storage of numerous bio-compounds (e.g., pigments, vitamin D, and keratins); and especially a protective barrier agent against adverse environmental agents. Skin can undergo severe or chronic injuries by trauma, pressure sores, burns, as well as some diseases such as diabetes that result in impairment or even inability of the skin functions. Hence, regeneration

*Corresponding authors: Mehdi Kian, Email: mehdi.kian@live.com; Zahra Zarei, Email: zarei2218@gmail.com

mats is a good practical strategy for controlling the drug delivery.[44] Their high surface-to-volume ratio can cause improvement in drug loading and various bioactive molecules with different bioactivities; for example, anti-inflammatory and anti-bacterial agents are capable to encapsulate into nanofibers.[44, 45] This method is especially advantageous for enhancing the delivery of low soluble drugs.[44] Our findings indicated that electrospinning a polymeric PCL/CTS blend containing propolis provided a sustainable means for consecutive release of propolis, which is a low soluble bioactive compound. Similarly, in two other studies conducted by Eskandarnia et al, the results indicated that nanofibrous mats created by the electrospinning technique provided sustainable platforms for the continuous release of propolis.[40,41]

Macroscopical evaluations showed that grafting PCL/CTS/Propolis nanocomposite fibrous mat accelerated the percentage of wound closure in the rat models of burn-induced skin injury. In line with our results, Eskandarnia et al[41] have reported that mixing ethanolic extract of propolis with polyurethane and hyaluronic acid decreased the wound area in a rat model of the excisional wound. Similarly, in another study, Eskandarnia et al[40] revealed that a bilayer wound dressing containing ethanolic extract of propolis remarkably reduced the wound area in rats. Altogether, it seems that wound dressing grafts containing propolis accelerate the process of wound healing in experimental animal models.

Researchers believe that propolis can reduce the amount of excessive free radicals at the wound site, which impair the wound healing process.[16,46] Our findings indicated that propolis possesses Gallic acid and quercetin, which are both potent phenolic antioxidants.[46,47] Therefore, the wound healing accelerating effects of PCL/CTS/Propolis observed in this study could be due to the antioxidant activity of propolis.

On the other hand, there is some evidence of a modulatory role of propolis in promoting TGFβ/Smad signaling that up-regulates expression and deposition of collagen type I, elevates proliferation of the fibroblasts and keratinocytes, and induces angiogenesis in a cutaneous wound.[48] In line with these findings, histopathological evaluation in the present study revealed that grafting nanocomposite fibrous mat containing propolis on cutaneous wound induced by burn injury reduced inflammation and enhanced skin tissue angiogenesis, granulation, and re-epithelialization in the rat model. Hence, the accelerating effects of PCL/CTS/Propolis could also be owing to the anti-inflammatory activity of propolis.

Conclusion

In summary, the PCL/CTS/Propolis mat was successfully fabricated with appropriate morphological, physiochemical, and biological properties. PCL/CTS/Propolis mat prepared in this study had proper hydrophilicity and water uptake capacity, as well as good mechanical characteristics and a beadless nanofibrous shape. In addition, findings from the present study revealed that PCL/CTS/Propolis electrospun mat showed biocompatibility and accelerated wound healing in the rat model of burn injury.

Research Highlights

What is the current knowledge?

√ Fabricated mats composed of PCL, CTS, and natural bioactive compounds have shown promising effects in dressing skin wounds.

√ Propolis is a natural resinous compound that possesses antioxidant, anti-inflammatory, and wound healing properties.

What is new here?

√ The fabricated nanofibrous mat composed of PCL, CTS, and propolis exhibited acceptable physical, chemical, and biocompatible characteristics.

√ The PCL/CTS/Propolis mat had the potential for dressing the skin wounds.

Acknowledgment

We appreciate the Burn and Wound Healing Research Center of Shiraz University of Medical Sciences, which helped us in this project. The authors would like to thank Shiraz University of Medical Sciences, Shiraz, Iran and also Center for Development of Clinical Research of Nemazi hospital and Dr. Nasrin Shokrpour for editorial assistance.

Authors' Contribution

Conceptualization: Seyedeh-Sara Hashemi, Ali Akbar Mohammadi.
Data curation: Seyedeh-Sara Hashemi, Seyedeh-Somayeh Rajabi, Mehdi Kian.
Formal analysis: Seyedeh-Sara Hashemi, Mehdi Kian.
Funding acquisition: Seyedeh-Sara Hashemi, Ali Akbar Mohammadi, Zahra Zarei.
Investigation: Seyedeh-Somayeh Rajabi, Alireza Rafati, Mehdi Kian.
Methodology: Seyedeh-Sara Hashemi, Seyedeh-Somayeh Rajabi, Mehdi Kian.
Project administration: Seyedeh-Sara Hashemi, Ali Akbar Mohammadi, Zahra Zarei.
Resources: Seyedeh-Sara Hashemi, Zahra Zarei.
Supervision: Ali Akbar Mohammadi, Zahra Zarei.
Validation: Seyedeh-Sara Hashemi, Ali Akbar Mohammadi.
Visualization: Seyedeh-Sara Hashemi, Parisa Sanati, Mehdi Kian.
Writing–original draft: Seyedeh-Sara Hashemi, Parisa Sanati, Mehdi Kian.
Writing–review editing: Seyedeh-Sara Hashemi, Mehdi Kian.

Competing Interests

Authors declare all relevant interests that could be perceived as conflicting.

Data Availability Statement

Respectful readers may contact the corresponding author to reach all the data.

Ethical Statement

All the tests were carried out under the rules and regulations of the Iran Veterinary Organization for working with laboratory animals. The study protocol was approved by the ethics committee of Shiraz University of Medical Sciences (Code: IR.SUMS.REC. 1395.S716).