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Research Letter

Berberine and its derivatives in collagen matrices as antimicrobial agents

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Abstract

The combination of collagen with natural plant compounds confers anti-inflammatory, antimicrobial, and antiviral activities to the polymeric material. These favorable properties enable broad-spectrum application of traditional, natural polymers in biomedicine. In the present study, natural fish collagen was combined with commercially available berberine (BBR) and naturally occurring protoberberine alkaloids obtained from the medicinal herb *Chelidonium majus* L (BBR-F). The incorporation of plant constituents into collagen matrices was confirmed by Raman spectroscopy. The antimicrobial properties of the plant-polymeric composites were assessed against typical pathogenic microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Candida albicans)*. The plant-based collagen matrices inhibited the growth of all the studied pathogens.

Introduction

Wound infections are considered as one of the most common factors that complicate the wound-healing process. Most wound infections are caused by bacterial contamination. Gram-negative bacteria such as *Escherichia coli* and gram-positive bacteria such as *Staphylococcus aureus* are the main causative agents of chronic wound infections.[1] *Candida* spp. are also the most commonly identified fungi in wound infections.[2] The wound-healing process can be accelerated using the natural polymer collagen crosslinked with natural antimicrobial agents. Collagen is a major structural connective tissue protein found in skin, tendon, ligaments, and bone and is most abundant in the native extracellular matrix (ECM). This natural polymer has a fibrous structure and appropriate mechanical properties, is highly biocompatible and biodegradable, and has low immunogenicity. Therefore, it is a valuable biopolymer for application as a biomaterial in regenerative medicine and tissue engineering, and it is widely used in pharmaceutical industry as a drug carrier.[3] Collagen type I is also used as an ingredient in cosmetics, dental composites, and skin regeneration templates and as a nutritional supplement for bone and cartilage regeneration. Collagen can be extracted from a variety of sources such as porcine, bovine, avian, and marine tissues.[4] Despite many useful properties, crude natural collagen can be easily degraded by bacterial and fungal contamination.[5] Moreover, the treatment of polymicrobial, chronic infections is complicated and is limited/inefficient due to the rapid development of multidrug-resistant microorganisms. Thus, the incorporation of antimicrobial agents into native collagen can improve their biological properties, help to stabilize collagen biomaterial, and decrease contamination during its application.[6] Several approaches such as mechanical, physical, chemical, and biological methods are available for functionalizing collagen.[7]

Plants are also a natural source of antimicrobial agents.[8] One of the pharmacologically active plant biomolecules with strong antibacterial properties is berberine and its protoberberine derivatives.[9] Several studies have demonstrated the antimicrobial activity of protoberberine alkaloids.[10] The medicinal herb *Chelidonium majus* L. (*C. majus*) is one of the natural sources of berberine and other protoberberine active compounds. Natural extracts from *C. majus* show many biological activities, including antibacterial,[11] antioxidant,[12] anti-inflammatory,[13] anticancer,[14] antifungal, antiprotozoal,[15] and antiviral effects.[16] Furthermore, berberine can be incorporated into nanofibrous collagen membranes or alginate-based microspheres to prevent bacterial infection.[17]

The present study aimed to evaluate the ability of berberine (BBR) and its natural fraction isolated from *C. majus* (BBR-F) to protect fish collagen against bacterial and fungal infection.

Results

Physicochemical characterization of collagen and its composites with alkaloids

Polymer-based biocompatible materials were prepared by mixing technique that blends BBR and BBR-F with collagen (see "Preparation of collagen and berberine composite" section). The Raman spectra revealed that BBR forms complexes/composites with collagen. It is extremely difficult to obtain

good-quality Raman spectra data from the collagen-BBR composite because of high fluorescence of pure BBR and its low concentration in the composite. Moreover, the vibrational spectra of collagen consist of a complex set of overlapping bands as the collagen protein contains large polypeptides. However, because Raman spectroscopy can provide structural information about complex solid systems, it is used as a versatile tool in protein science and biotechnology. Figure 1(a) shows the Raman spectra of collagen and collagen-BBR and collagen-BBR-F composites in the range of 60 to 4200 cm^{-1}. In the collagen-BBR composite, the dominating peaks were associated with the ring deformation vibrations mixed with CH bending and scissoring modes. Spectra for the most intense vibrations in the Raman spectrum correspond to system-wide ring deformations. The strong breathing mode of the aromatic carbon ring of alkaloids in BBR-F appeared from 500 to 1000 cm^{-1}. Figure 1(b) shows a peak at 1519 cm^{-1} corresponding to a semicircle stretch of the N–C8–$C13_a$ ring coupled with CH bending at C8 and C13, and CH scissoring at C6 and in the dioxolane-type ring. The intense peak at 1394 cm^{-1} corresponded to ring deformations coupled with the in-plane bending of the C12H and C13H groups. Lower intensity vibrations at lower wavenumbers indicate a ring breathing mode at 716 cm^{-1} and the CH twist of the C6 and C5 groups at 689 cm^{-1}. At higher wavenumbers, the CH2 and CH3 stretching vibrations appeared at 2935 cm^{-1} and 3026 cm^{-1}, respectively. Moreover, the broad band assigned to the O–H stretching (hydrogen bonds) appeared at 3219.9 cm^{-1} and 3404 cm^{-1}. The fluorescence background decreased for the composite spectra.

Antimicrobial effect of collagen materials doped with BBR and BBR-F

To test whether collagen matrices doped with BBR and natural BBR-F can be used as an antimicrobial material, the antimicrobial activity of the obtained matrices was analyzed. The antibacterial effect of collagen matrices doped with BBR was evaluated against *S. aureus* and *E. coli*. The antimicrobial activity was tested by the bacteriostatic disk diffusion assay and measurement of zone of bacterial growth inhibition. TSA agar plates were inoculated with the above-mentioned bacteria. Next, paper disks impregnated with collagen-BBR and collagen-BBR-F composites were placed on the agar surface. Then, the agar plates were incubated under suitable conditions (the bacteria were cultured at 37°C for 24 h). After incubation, the inhibition zone was determined. Likewise, the disk diffusion test was also carried out for *C. albicans*. As shown in Fig. 2, the highest zone of growth inhibition for *S. aureus* and *E. coli* was observed at BBR concentrations of 17 mg/ml and 10 mg/ml. We did not observe a zone of growth inhibition with clear boundaries for the collagen composite with lower BBR concentrations (5 mg/ml and 1 mg/ml) [Figs. 2(a) and 4]. Interestingly, the zone of growth inhibition for *E. coli* was detected at the concentrations of 9.33 mg/ml and 5 mg/ml for collagen blended with natural BBR-F [Figs. 2(b) and S2]. The zone of growth inhibition was also detected in the *C. albicans* assay [Figs. 2 and S1(C)]. We noted that the size of the inhibition zone (mm) depended on BBR and BBR-F concentrations. Both *S. aureus* and *E. coli* were found to be sensitive to collagen matrices doped with BBR at the concentration of 17 mg/ml and 10 mg/ml and to matrices

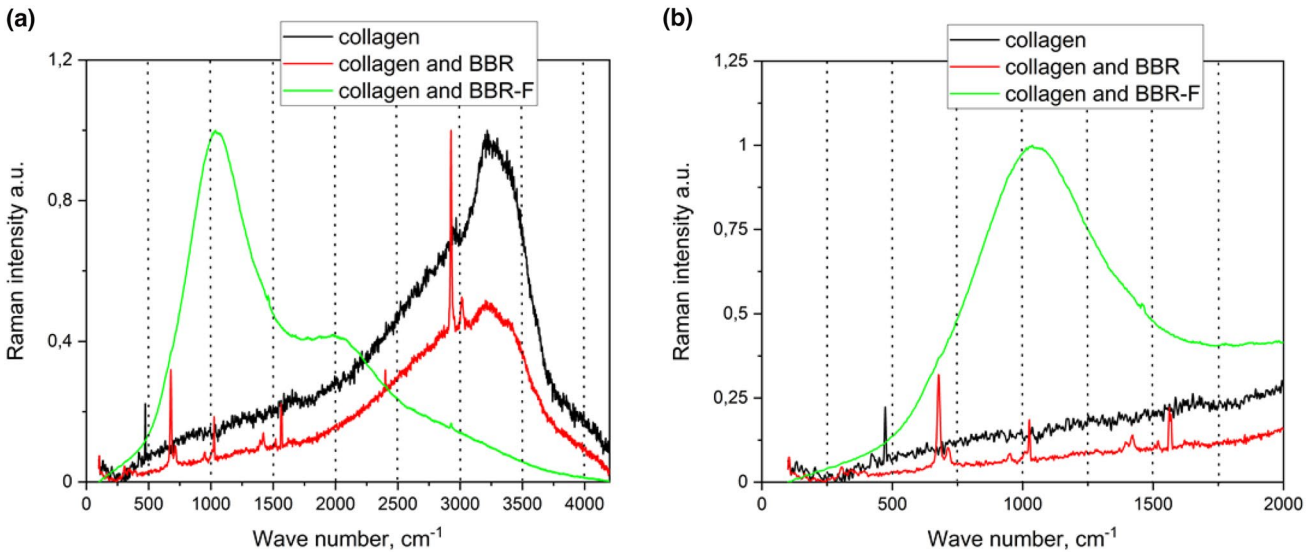


Figure 1. Raman spectra: (a) Raman spectra of collagen and collagen-berberine composite in the range of 60 to 4200 cm^{-1}; (b) Raman spectra of collagen and collagen-berberine composite in the range of 60 to 2000 cm^{-1}.

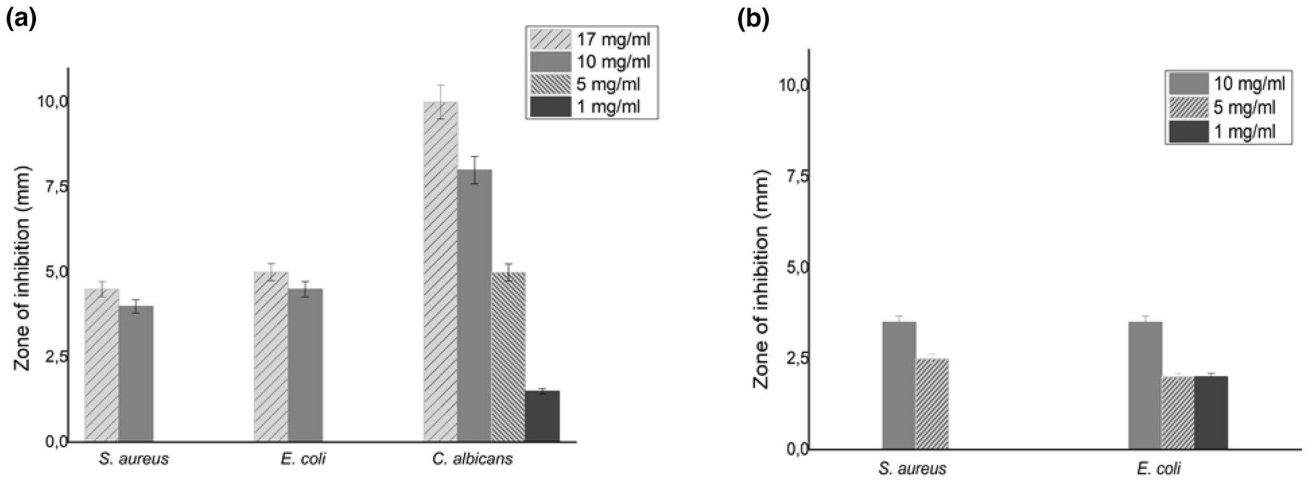


Figure 2. Antimicrobial activity of collagen (bio)composites: (a) Antimicrobial activity of collagen-BBR composite and (b) Antimicrobial activity of collagen-natural BBR-F composite.

doped with BBR-F at the concentration of 9 mg/ml and 5 mg/ml. These results demonstrated that the bioactive plant alkaloid-collagen composites could inhibit the growth of microorganisms. The results obtained from the disk diffusion assay are presented in Tables S1 and S2 and illustrated in Figs. 2, S1 and S2.

The results revealed that the collagen-BBR composite and the collagen-natural BBR-F composite could inhibit microbial growth. The collagen-BBR composite was the most effective matrices that suppressed microbial growth (*S. aureus, E. coli*, and *C. albicans*) at the concentrations of 17 mg/ml and 10 mg/ml for *S. aureus* and *E. coli* and at 17 mg/ml, 10 mg/ml, and 5 mg/ml concentrations for *C. albicans*.

Antimicrobial effect of collagen materials doped with BBR and BBR-F – OD600

To determine the inhibitory effect of the collagen-BBR-F matrices on the growth of *S. aureus*, *E. coli*, and *C. albicans*, spectrophotometric measurements with optical density 600 nm were performed. The inhibitory effect was detected using a 96-well plate. The plate was placed on a microplate reader (BioTek), and OD600 readings were recorded after 1, 2, 4, and 12 h of incubation. Briefly, collagen-BBR-F matrices containing different concentrations of active compounds were added to the 96-well microtiter plate. Diluted cultures of *S. aureus*, *E. coli*, and *C. albicans* were then added to the respective wells. The bacterial suspensions were incubated with different concentrations of BBR-Coll (17, 10, 5, and 1 mg/ml) and BBR-F-Coll (9, 5, 1, and 0.5 mg/ml), while the *C. albicans* suspension was incubated with BBR-Coll at 17, 10, 5, and 1 mg/ml concentrations. The wells with the diluted culture of pathogens and antibiotic (rifampicin) were used as a positive control. The wells with only culture broth were considered as a negative control.

The study showed that the natural plant compound-collagen composites inhibited the growth of microorganisms at various concentrations. The collagen composites were found to be most effective at the concentrations of 17 mg/ml and 10 mg/ml against *S. aureus*, *E. coli*, and *C. albicans* (BBR-Coll) and at 9 mg/ml and 5 mg/ml concentrations against *S. aureus* and *E. coli* (BBR-F-Coll) (Figs. 3 and 4).

Discussion

Among natural polymers, collagen has gained significant interest as an excellent biomaterial for various applications, including wound healing, dermal regeneration, tissue engineering, membranes in dermal cosmetics, and food supplements.[18] However, it is difficult to use unmodified native collagen for these applications as several microorganisms can bind to and degrade collagen by using proteases such as elastase, alkaline proteases, and collagenases. Moreover, several studies have shown that the majority of collagen products exposed to the environment contain potentially harmful bacteria and fungi that can cause illnesses.[19]

Recently, fish collagen has attracted the attention of many researchers and has been used in various applications in pharmaceutical and biomedical industry, for example, in medical devices, scaffold for tissue regeneration, and drug delivery system.[20] It is also used in cosmetic field and in food supplements.[21] Fish collagen-based matrices (compress) were reported to accelerate the wound-healing process. Fish collagen-based thin films can also be used as drug delivery systems for biomolecules that play a significant role in the tissue regeneration process, e.g., epidermal growth factors. Pure fish collagen has low mechanical stability, which can be improved by crosslinking techniques such as gamma irradiation and carbodiimide and by functionalization with an antimicrobial agent, such as coating with antibiotics, silver

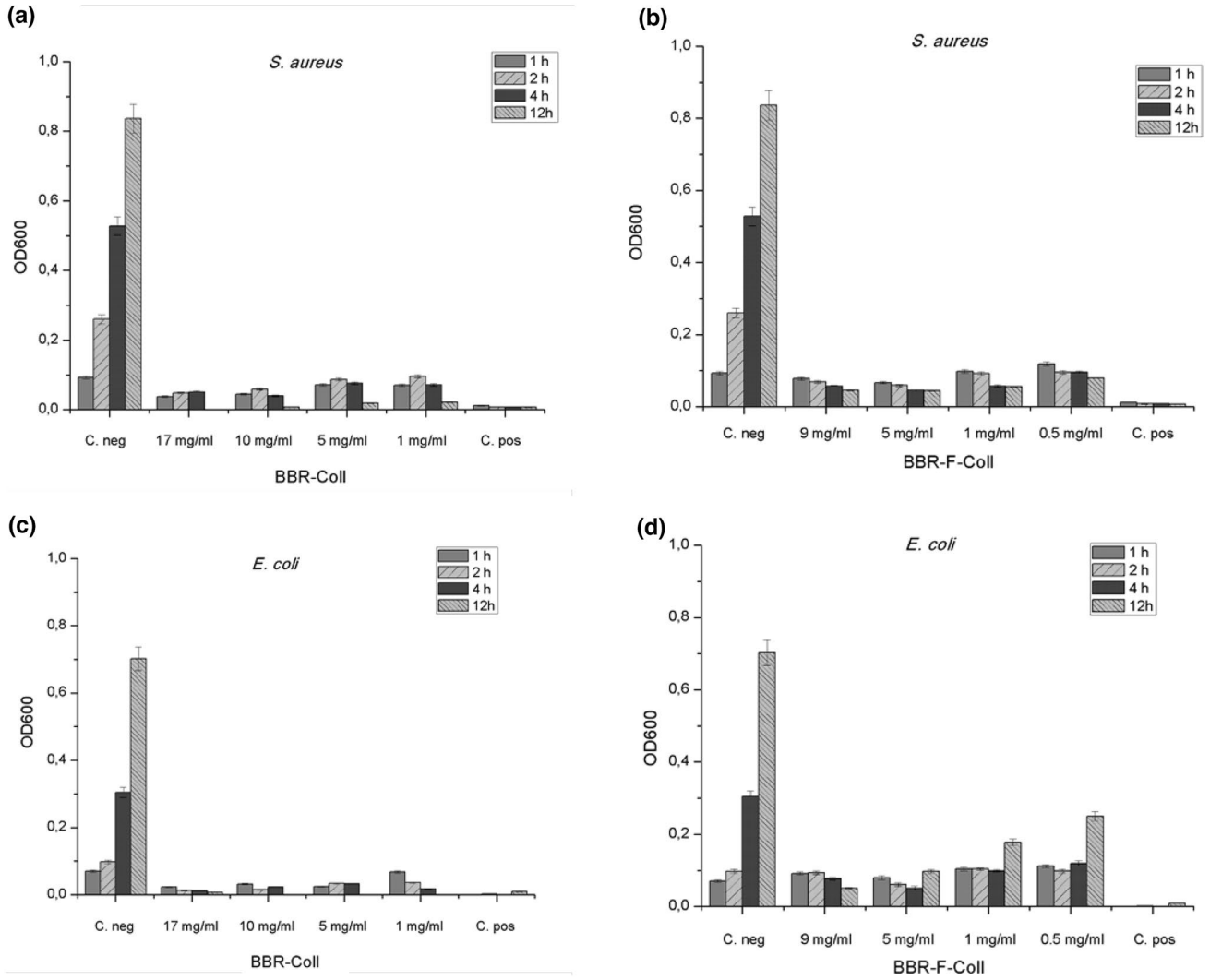


Figure 3. Results of antibacterial activity of collagen blended with berberine and natural protoberberine fraction against *S. aureus* and *E. coli*; spectrophotometrically tested with optical density 600 nm: (a) *S. aureus* treated with BBR-Coll, (b) *S. aureus* treated with BBR-F-Coll, (c) *E. coli* treated with BBR-Coll, (d) *E. coli* treated with BBR-F-Coll. *C. neg* negative control, *C. pos* positive control. Bars represent standard deviation. Time (h).

ions, and zinc titanate.[22] Collagen can be combined with other polymers such as chitosan or elastin, which influences its chemical and mechanical properties.[23] To enhance its biological properties, collagen matrices can be incorporated with plant-based antimicrobial agents.[9] In the present study, we describe a simple method to fabricate an antibacterial collagen-based composite by using natural plant compounds—BBR and BBR-F from *C. majus*. BBR is a natural alkaloid with broad-spectrum properties such as antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory effects.[24] For the biofunctionalization of collagen, we blended the biopolymer with a solution containing BBR and BBR-F. The Raman spectra confirmed that BBR was incorporated into collagen matrix and formed a composite with the polymer which showed antimicrobial activity against *S. aureus*, *E. coli*, and *C. albicans*. BBR probably linked to collagen through van der Waal forces, electrostatic forces, hydrogen bonds, or hydrophobic interactions. The method used in our present study is one of the simplest methods to biofunctionalize collagen.

Lin et al. also reported that the composite of collagen and BBR as electrospun nanofiber matrices showed antimicrobial properties against *S. aureus* and *E. coli*. The authors concluded that electrospun collagen/zein films doped with BBR could be used for treating skin bacterial infections and for wound healing.[25] Jin et al. showed that BBR incorporated into nanofibrous collagen membranes or alginate-based microspheres can prevent bacterial infection.[17] Ramanathan G. et al. reported biocompatibility and antibacterial properties of fish collagen scaffolds decorated with plant extracts of *Coccinia grandis*.

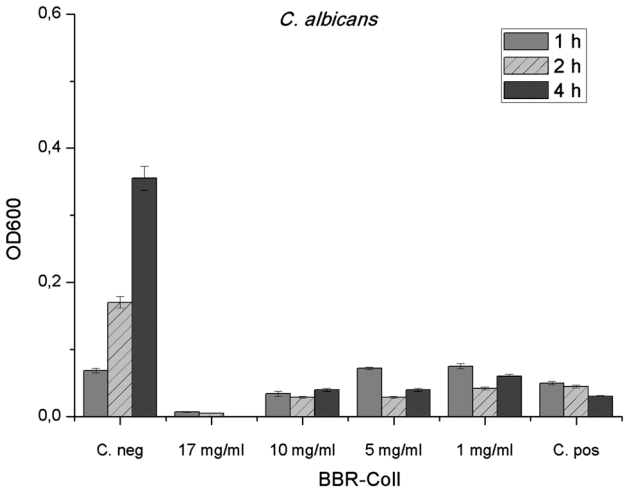


Figure 4. Antifungal activity of collagen blended with berberine against *C. albicans*; spectrophotometrically tested with optical density 600 nm. *C. neg* negative control, *C. pos* positive control. Bars represent standard deviation. Time (h).

The obtained plant-based collagen scaffolds exhibited antimicrobial properties against *S. aureus* and *E. coli*.[26] Studies on animals have reported that curcumin, a phytochemical compound obtained from *Curcuma longa* L., increases the rate of collagen synthesis.[27] Other studies have also reported that loading the natural polymer with plant bioactive compounds such as alkaloids, flavonoids, saponins, and essential oils accelerates the wound-healing process and offers protection against infections.[28] Some studies have confirmed that the incorporation of natural plant compounds into collagen can inhibit enzymes such as collagenase or myeloperoxidase,[29] which degrade collagen.[24] A previous study reported that collagen scaffolds combined with plant bioactive constituents enhanced fibroblast attachment and promoted cell proliferation. Studies conducted by Krishnan showed that herbal bioactive compounds combined with collagen play an important role in wound contraction and reepithelization.[30] Collagen mixed with natural plant compounds from *Terminalia chebula* or *Phylanthus emblica* exhibited wound-healing properties in infected wounds.[31] Thus, collagen matrices supplemented with plant-derived bioactive compounds can help to accelerate the process of wound healing because of their regenerative and antimicrobial properties.[32,33]

Research on functional biomaterials that can prevent infection is, therefore, crucial in the field of material and tissue engineering.[34] In the present study, a safe, antibacterial collagen scaffold was prepared by blending BBR and naturally obtained BBR-F from *C. majus* with fish collagen.

Conclusion

Natural plant compounds that possess antimicrobial properties can be used as natural antimicrobial agents. BBR and their derivatives belong to the group of natural plant phytochemicals with strong antibacterial activities. The present study showed that collagen composites with BBR and natural BBR-F exhibited an inhibitory effect on microorganisms. Our results demonstrated that bioactive compounds derived from plants can be successfully incorporated into natural polymers. Moreover, the natural plant compounds incorporated into collagen matrices were found to retain their antimicrobial properties. The present study suggests that such (bio)composites can be used as a potential natural dressing to support the wound-healing process and to control skin infections. Furthermore, decorating the crude collagen material with natural constituents possessing antimicrobial properties can help to prevent microbial contamination of biomaterials.[34]

Materials and methods

Materials

For this study, fish collagen was obtained from the biotechnology company Perfect Coll (Poland). The obtained collagen was isolated from fish skin of Tilapia (*Oreochromis aureus*). Berberine chloride (BBR; B3251) was purchased from Sigma-Aldrich. Protoberberine fraction (BBR-F) was obtained from *C. majus* as described previously.[35]

Preparation of collagen and berberine composite

A homogenous aqueous solution of collagen was blended with different concentrations of BBR and BBR-F. First, the plant compounds were dissolved in dimethyl sulfoxide (DMSO) and sterilized by passing through a 0.22-μm filter (Millipore). The concentrations of BBR in collagen matrices were as follows: 17 mg/ml, 10 mg/ml, 5 mg/ml, and 1 mg/ml. The concentrations of BBR-F in collagen matrices were as follows: 9.33 mg/ml, 5 mg/ml, 1 mg/ml, and 0.5 mg/ml. Next, the samples were gently centrifuged (1500 rpm, 5 min, room temperature) and left at room temperature for 3 h in order to allow plant alkaloids to disperse into the collagen matrix.

Physicochemical characterization of collagen and its composites with alkaloids

Raman spectroscopy was used for physical characterization of fish collagen and its composite with BBR and BBR-F. The Raman spectra of fish collagen were obtained in air by using the Renishaw inVia confocal Raman microscope (Renishaw, Old Town, Wotton-under-Edge, UK). Measurements were acquired at room temperature (21°C) by using an 1800 g/mm grating and a 633 nm He/Ne laser delivering 5 mW of power. The laser light was focused on the sample with a 50×/0.75 microscope objective (LEICA). Whole measurements were obtained from drops deposited on a well slide for dispersive Raman measurements under the microscope. All Raman spectra were recorded in the range of 60 to 4200 cm^{-1}. To improve the signal-to-noise ratio, the acquisition time was 20 s. Raman spectra were collected by WiRE™3.3 software linked to the instrument. Peak positions were measured using Lorentz profile with OriginPro 2020b software (Northampton, MA, USA), and all experimental data were normalized to 1.

Antimicrobial activity of collagen-BBR and collagen-BBR-F composites

Bacterial and fungal strains

To evaluate the antibacterial and antifungal activities of collagen-BBR and collagen-BBR-F composites, two bacterial species, namely *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 35218, and one fungal species, namely *Candida albicans* ATCC 10231, were used as experimental species. Both bacterial strains and *C. albicans* were obtained from the Polish Collection of Microorganisms, Wrocław, Poland.

Preparation of bacterial and fungal inoculums

E. coli and *S. aureus* strains were subcultured overnight at 37°C in a TSB liquid medium with gentle shaking. Similarly, *C. albicans* was subcultured in YPD Broth (Y1375, Millipore, Merck) liquid medium at 30°C for 10 h. The absorbance was adjusted at 600 nm by using a spectrophotometer. *E. coli*, *S. aureus*, and *C. albicans* cell suspensions were adjusted spectrophotometrically to 0.5 McFarland standard. The subcultured *E. coli* and *S. aureus* bacterial suspensions (200 µl) were added to a TSA agar medium. The subcultured *C. albicans* suspension was added to a YPD Broth agar medium.

Determination of antibacterial and antifungal activities

The collagen materials doped with different concentrations of plant phytochemicals (see "Antimicrobial effect of collagen materials doped with BBR and BBR-F" section) were used to study antimicrobial and antifungal activities. To determine the bacteriostatic and antifungal properties of the doped collagen materials, the disk diffusion method and OD measurement were used. First, the samples were blended with collagen to obtain a final concentration of 17 mg/ml, 10 mg/ml, 5 mg/ml, and 1 mg/ml (for BBR) and 9.33 mg/ml, 5 mg/ml, 1 mg/ml, and 0.5 mg/ml (for BBR-F). Next, sterile paper disks were impregnated with 20 µl of the sample, and the loaded filter paper disks were placed at precise locations on the prepared agar plates. The plates containing filter paper disks loaded with different concentrations of BBR and BBR-F were first incubated at 5°C for 2 h and then at 37°C for 24 h. After incubation, the inhibition zones were measured in millimeters and recorded (mean ± SD, $n = 2$). The diameters of the zones were compared with those of standard antibiotics.

Author contributions

AW conceptualization, investigation, formal analysis, visualization, writing original draft; MK investigation (Raman spectroscopy), visualization; MW resources; AGJ conceptualization, writing & editing, supervision.

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Data availability

The dataset are available from the corresponding author on request.

Declarations

Conflict of interest

The authors declare no conflict of interest.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1557/s43579-022-00181-w.

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References

1. S. Kadam, S. Nadkarni, J. Lele, S. Sakhalkar, P. Mokashi, K.S. Kaushik, Bioengineered platforms for chronic wound infection studies: how can we make them more human-relevant? Front. Bioeng. Biotechnol. (2019). https://doi.org/10.3389/fbioe.2019.00418
2. L. Kalan, E.A. Grice, Fungi in the wound microbiome. Adv. Wound Care **7**, 247–255 (2018). https://doi.org/10.1089/wound.2017.0756
3. T. Suchý, L. Vištejnová, M. Šupová, P. Klein, M. Bartoš, Y. Kolinko, T. Blassová, Z. Tonar, M. Pokorný, Z. Sucharda, M. Žaloudková, F. Denk, R. Ballay, Š Juhás, J. Juhásová, E. Klapková, L. Horný, R. Sedláček, T. Grus, Z. Čejka, Z. Čejka, K. Chudějová, J. Hrabák, Vancomycin-loaded collagen/hydroxyapatite layers electrospun on 3D printed titanium implants prevent bone destruction associated with S. epidermidis infection and enhance osseointegration. Biomedicines (2021). https://doi.org/10.3390/biomedicines9050531
4. A. Sorushanova, L.M. Delgado, Z. Wu, N. Shologu, A. Kshirsagar, R. Raghunath, A.M. Mullen, Y. Bayon, A. Pandit, M. Raghunath, D.I. Zeugolis, The collagen suprafamily: from biosynthesis to advanced biomaterial development. Adv. Mater. **1801651**, 1–39 (2018). https://doi.org/10.1002/adma.201801651

5. I.J. Hoppe, H. Brandstetter, E. Schönauer, Biochemical characterisation of a collagenase from *Bacillus cereus* strain Q1. Sci. Rep. **11**, 1–15 (2021). https://doi.org/10.1038/s41598-021-83744-6
6. C. Zongming, L. Yongsheng, D. Yao, Z. Zheng, H. Yibing, L. Hairong, Fabrication and characterization of a novel berberine-oleanolic acid delivery collagen I Scaffold fabrication and characterization of a novel berberine-oleanolic acid delivery collagen I Scaffold. J. Phys. (2020). https://doi.org/10.1088/1742-6596/1637/1/012105
7. M. Tallawi, E. Rosellini, N. Barbani, M.G. Cascone, R. Rai, G. Saint-pierre, A.R. Boccaccini, Strategies for the chemical and biological functionalization of scaffolds for cardiac tissue engineering : a review. J. R. Soc. Interfaces **12**, 20150254 (2015)
8. V. Dwibedi, S.K. Rath, New insight into and updates on antimicrobial agents, in *Emerging Modalities in Mitigation of Antimicrobial Resistance*. ed. by N. Akhtar, K.S. Singh (Springer, Berlin, 2022), pp. 179–217
9. F. Zhang, G. Ramachandran, R.A. Mothana, O.M. Noman, W.A. Alobaid, G. Rajivgandhi, N. Manoharan, Anti-bacterial activity of chitosan loaded plant essential oil against multi drug resistant K. pneumoniae. Saudi J. Biol. Sci. **27**, 3449–3455 (2020). https://doi.org/10.1016/j.sjbs.2020.09.025
10. J. Tan, J. Wang, C. Yang, C. Zhu, G. Guo, J. Tang, H. Shen, Antimicrobial characteristics of Berberine against prosthetic joint infection-related *Staphylococcus aureus* of different multi-locus sequence types. BMC Complement. Altern. Med. **19**, 1–10 (2019). https://doi.org/10.1186/s12906-019-2558-9
11. D. Simões, S.P. Miguel, M.P. Ribeiro, P. Coutinho, A.G. Mendonça, I.J. Correia, Recent advances on antimicrobial wound dressing: a review. Eur. J. Pharm. Biopharm. **127**, 130–141 (2018). https://doi.org/10.1016/j.ejpb.2018.02.022
12. Z. Khodabande, V. Jafarian, R. Sariri, Antioxidant activity of *Chelidonium majus* extract at phenological stages. Appl. Biol. Chem. **60**, 497–503 (2017). https://doi.org/10.1007/s13765-017-0304-x
13. S. Zielinska, M. Wójciak-Kosior, M. Dziagwa-Becker, M. Glensk, I. Sowa, K. Fijalkowski, D. Ruranska-Smutnicka, A. Matkowski, A. Junka, The activity of isoquinoline alkaloids and extracts from *Chelidonium majus* against pathogenic bacteria and Candida sp. Toxins (Basel). (2019). https://doi.org/10.3390/toxins11070406
14. A. Och, D. Zalewski, Ł Komsta, P. Kołodziej, J. Kocki, A. Bogucka-Kocka, Cytotoxic and proapoptotic activity of sanguinarine, berberine, and extracts of *Chelidonium majus* L. and berberis thunbergii DC. Toward hematopoietic cancer cell lines. Toxins (Basel) **11**, 1–19 (2019). https://doi.org/10.3390/toxins11090485
15. S. Zielinska, A. Jezierska-Domaradzka, M. Wójciak-Kosior, I. Sowa, A. Junka, A.M. Matkowski, Greater celandine's ups and downs-21 centuries of medicinal uses of *Chelidonium majus* from the viewpoint of today's pharmacology. Front. Pharmacol. (2018). https://doi.org/10.3389/fphar.2018.00299
16. W. Guo, X. Lu, B. Liu, H. Yan, J. Feng, Anti-TMV activity and mode of action of three alkaloids isolated from *Chelidonium majus*. Pest Manage. Sci. **77**, 510–517 (2021). https://doi.org/10.1002/ps.6049
17. J. Jin, M. Xu, Y. Liu, Z. Ji, K. Dai, L. Zhang, L. Wang, F. Ye, G. Chen, Z. Lv, Alginate-based composite microspheres coated by berberine simultaneously improve hemostatic and antibacterial e ffi cacy. Colloids Surf. B Biointerfaces **194**, 111168 (2020). https://doi.org/10.1016/j.colsurfb.2020.111168
18. C. Dong, Y. Lv, Application of collagen scaffold in tissue engineering: recent advances and new perspectives. Polymers (Basel) **8**, 1–20 (2016). https://doi.org/10.3390/polym8020042
19. K. Shanmugam, V. Subha, S. Renganathan, Type 1 collagen scaffold functionalized with ciprofloxacin loaded gelatin microspheres—fabrication. In vitro In vivo Eval. Histol. Biochem. Anal. **3**, 1–10 (2019). https://doi.org/10.15406/mojddt.2019.03.00072
20. S.S. Mathew-steiner, S. Roy, C.K. Sen, Collagen in wound healing. Bioengineering **8**, 63 (2021)
21. M. Raman, K. Gopakumar, Fish collagen and its applications in food and pharmaceutical industry: a review. EC Nutr. **12**, 752–767 (2018)
22. R.J.F.C. do Amaral, N.M.A. Zayed, E.I. Pascu, B. Cavanagh, C. Hobbs, F. Santarella, C.R. Simpson, C.M. Murphy, R. Sridharan, A. González-Vázquez, B. Oullivan, F.J. Orien, C.J. Kearney, Functionalising collagen-based scaffolds with platelet-rich plasma for enhanced skin wound healing potential. Front. Bioeng. Biotechnol. **7**, 1–22 (2019). https://doi.org/10.3389/fbioe.2019.00371
23. M.H. Uriarte-Montoya, J.L. Arias-Moscoso, M. Plascencia-Jatomea, H. Santacruz-Ortega, O. Rouzaud-Sández, J.L. Cardenas-Lopez, E. Marquez-Rios, J.M. Ezquerra-Brauer, Jumbo squid (Dosidicus gigas) mantle collagen: extraction, characterization, and potential application in the preparation of chitosan-collagen biofilms. Bioresour. Technol. **101**, 4212–4219 (2010). https://doi.org/10.1016/j.biortech.2010.01.008
24. D. Song, J. Hao, D. Fan, Biological properties and clinical applications of berberine. Front. Med. **14**, 564–582 (2020). https://doi.org/10.1007/s11684-019-0724-6
25. J. Lin, C. Li, Y. Zhao, J. Hu, L. Zhang, Co-electrospun nanofibrous membranes of collagen and zein for wound healing. ACS Appl. Mater. Interfaces **4**, 1050 (2012)
26. G. Ramanathan, T. Muthukumar, U. Tirichurapalli Sivagnanam, In vivo efficiency of the collagen coated nanofibrous scaffold and their effect on growth factors and pro-inflammatory cytokines in wound healing. Eur. J. Pharmacol. **814**, 45–55 (2017). https://doi.org/10.1016/j.ejphar.2017.08.003
27. R. Prasad, D. Kumar, V. Kant, Curcumin enhanced cutaneous wound healing by modulating cytokines and transforming growth factor in excision wound model in rats. Int. J. Curr. Microbiol. Appl. Sci. **6**, 2263–2273 (2017)
28. A. Sharma, S. Khanna, G. Kaur, I. Singh, Medicinal plants and their components for wound healing applications. Future J. Pharm. Sci. **7**, 1–3 (2021)
29. F. Antonio, R. Guillem, T. Sonia, C. Mattu, P. Gentile, V. Chiono, G. Ciardelli, T. Tzanov, Cross-linked collagen sponges loaded with plant polyphenols with inhibitory activity towards chronic wound enzymes. Biotechnol. J. **6**, 1208–1218 (2011). https://doi.org/10.1002/biot.201100194
30. A.K. Krishnan, S. Thomas, Recent advances on herb—derived constituents—incorporated wound—dressing materials : a review. Polym. Adv. Technol. (2019). https://doi.org/10.1002/pat.4540
31. M.S. Kumar, D. Ph, S. Kirubanandan, M. Tech, R. Sripriya, D. Ph, P.K. Sehgal, D. Ph, Triphala incorporated collagen sponge—a smart biomaterial for infected dermal wound healing. J. Surg. Res. **158**, 162–170 (2010). https://doi.org/10.1016/j.jss.2008.07.006
32. Y. Liang, Y. Liang, H. Zhang, B. Guo, Antibacterial biomaterials for skin wound dressing. Asian J. Pharm. Sci. (2022). https://doi.org/10.1016/j.ajps.2022.01.001
33. K. Joyce, G.T. Fabra, Y. Bozkurt, A. Pandit, Bioactive potential of natural biomaterials: identification, retention and assessment of biological properties. Signal Transduct. Target. Ther. **6**, 1–28 (2021). https://doi.org/10.1038/s41392-021-00512-8
34. M. Shahruzzaman, S. Hossain, T. Ahmed, S.F. Kabir, M. Minhajul Islam, A. Rahman, M. Sazedul Islam, S. Sultana, M.M. Rahman, *Biological Macromolecules as Antimicrobial Agents* (Elsevier, Amsterdam, 2022)
35. A. Warowicka, Ł Popenda, G. Bartkowiak, O. Musidlak, L. Jagoda, D. Kuźma, R. Nawrot, S. Jurga, A. Goździcka-józefiak, Phytomedicine Protoberberine compounds extracted from *Chelidonium majus* L. as novel natural photosensitizers for cancer therapy. Phytomedicine **64**, 152919 (2019). https://doi.org/10.1016/j.phymed.2019.152919