Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Antibacterial and wound healing applications of curcumin in micro and nano-scaffolds based on chitosan, cellulose, and collagen

Mehran Alavi[1,2*], Musa Moetasam Zorab[3], Morahem Ashengroph[1*], Qassim Hassan Aubais Aljelehawy[4], Danial Kahrizi[2]

[1]*Department of Biological Science, Faculty of Science, University of Kurdistan, Sanandaj, Kurdistan, Iran*
[2]*Nanobiotechnology Department, Faculty of Innovative Science and Technology, Razi University, Kermanshah, Iran*
[3]*Department of Physics, University of Halabja, Kurdistan Region, Iraq*
[4] *Department of Chemistry, College of Education, University of Alqadisiya, Iraq*

ARTICLE INFO

Original paper

Article history:
Received: December 09, 2021
Accepted: March 06, 2022
Published: March 31, 2022

Keywords:
Antibacterial activity; wound healing; curcumin; micro/nano-scaffolds; biocompatible polymers

ABSTRACT

About 80% higher risk of amputation resulting from microbial infection was indicated for patients with diabetic foot ulcers (DFUs). Micro and nano-scaffolds made of natural polymers specifically cellulose, chitosan, and collagen can donate the biocompatibility, biodegradability, and bioavailability properties appropriate to accelerate wound closure before microbial biofilm formation. The antimicrobial activity of these wound dressings can be improved by the incorporation of bioactive compounds extracted from medicinal plant species such as curcumin. Low water solubility and poor bioavailability are recognized as two main disadvantages of curcumin, lipophilic phytopolyphenol, which could be controlled by targeted polymeric micro and nano-scaffolds. Consequently, this review has discussed the capacity and challenges of these types of formulations according to recent investigations.

DOI: http://dx.doi.org/10.14715/cmb/2022.68.3.2

Introduction

Various infectious diseases have been complicated by the increasing of multidrug-resistant microorganisms particularly pathogenic bacteria (1-4). For example, an infected wound such as DFU is a serious health disease resulting from multidrug-resistant microorganisms including bacteria and fungi that may lead to death in patients (5-8). As a medical aspect, biocompatibility, bioavailability, and biodegradability properties of new micro and nano-formulations to heal infected wounds should be considered as critical factors. In this regard, using of biomaterials is increasing due to these properties and also the reduction of hazardous compounds in the ecosystem (9, 10). Biomaterials specifically primary and secondary metabolites of medicinal plants can also increase the therapeutic effect of other drugs (11, 12). For instance, the curcumin compound is extracted naturally from the *Curcuma longa* plant species, a member of *Zingiberaceae* family (13-15). Different isomers of this natural phenol are related to its keto-enol tautomerism property with keto and enol isomers in water and organic solvent, respectively. electron density of curcumin indicates the active sites of these metabolites with the ability to participate in redox reaction by the molecular electrostatic potential (**Figure 1**) (16). In addition to usage in cosmetic and food industries, various therapeutic applications were indicated for this material including antitumor, antimicrobial, and wound healing activities by anti-inflammatory, antiseptic, and antioxidant functions (17,18). However, hydrophobicity, low stability properties in physiological fluids as well as side effects including diarrhea, nausea, and reduction in cell proliferation, increased apoptosis, focal necrosis in the liver, as well as decreased body weight in a high dose of curcumin, can be major complications to achieve an efficient drug formulation (19, 20). In addition to secondary

*Corresponding author. E-mail: m.ashengroph@uok.ac.ir and mehranbio83@gmail.com

metabolites of medicinal plants (21-23), natural polymers such as chitosan, cellulose, and collagen can increase the bioavailability and biodegradability of curcumin in micro and nanoformulations (24-26). Additionally, these polymers or their derivatives can contribute positively to the wound healing phase involving hemostasis, inflammation (inflammatory response, immune recruitment, and pathogen removal), proliferation, and maturation (**Figure 2**) (27, 28). Synergistic activity has resulted from antimicrobial activity, anti-inflammatory, and cell growth promotion of formulated curcumin by these polymers (15, 29, 30). Challenges for these formulations are related to curcumin release and inhibition of multi-drug-resistant bacteria specifically in chronically infected wounds such as DFUs (31). It is worth noting that 150-fold augmented risk of amputation and an enhanced risk of mortality within 18 months were indicated for patients with DFUs caused by biofilm formation in wound tissues (32). Delayed wound healing commonly results from symbiotic colonization of bacteria and fungi in biofilm due to hindering antibiotic penetration (33). In recent years, a plethora of studies illustrated the significant progress in micro and nanoformulations, and the present review has endeavored to assess these advancements and related challenges in detail.

Cellulose

Controlled and sustained release of curcumin is critical to achieving wound healing and antimicrobial effects at an appropriate time, wherein NMs of cellulose present an efficient option (34). Cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs) may be prepared via treatments of ball milling, acidic hydrolysis, chemical, and ultrasound from bacterial herbal, marine animals, and algal cellulose fibers (35). Modification of these NM is carried out by two main strategies including hydroxyl substitution (acetylation and TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) oxidation) and polymer grafting through coupling agents and ring-opening (36). Prepared CNCs with a mean length of 159 nm and thickness of 0.4 mm from cotton fiber demonstrated the sustained release of curcumin as a value of 98.9% after 36 h of treatment as well as 99% of growth reduction in Gram-negative and Gram-positive bacteria. Consequently, the closure of the wound was significant and complete after 7 and 12 days, respectively relative to placebo control. Interestingly, 99% growth inhibition against methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli*, *Proteus mirabilis*, *Bacillus coagulans*, *Streptococcus* sp. bacteria and *Candida albicans* fungus was observed after 15 washing wound dressing (32).

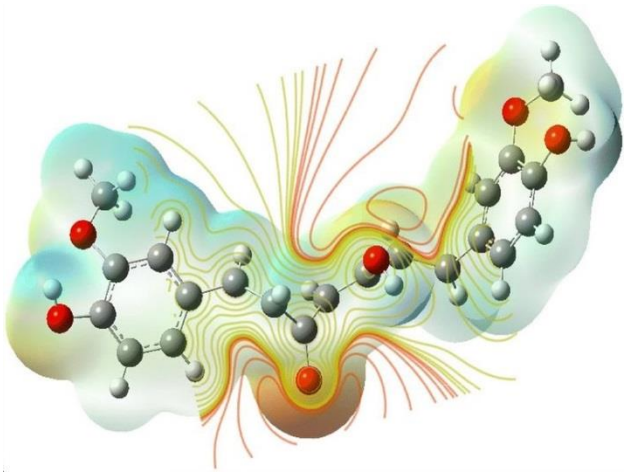

Figure 1. Active sites of curcumin according to map of electron density (under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/)) (16).

Chitosan

Encapsulation of AgNPs via polymeric micro and nanoformulations can be performed as a smart strategy to decrease the cytotoxicity of AgNPs in physiological conditions. For this purpose, chitosan-g-polyacrylamide was exploited to load AgNPs through a grafting copolymerization reaction (grafting one or more homopolymers as branches onto a main polymer chain) using potassium N,N′-methylenebisacrylamide and persulfate as crosslinker and initiator agents, respectively. In this formulation, chitosan by 20 and 40 weight % resulted in a spherical shape with particle sizes of 18.48 and 23.67 nm, respectively (37). In a comparative investigation, three types of sponge composites encompassing chitosan-alginate-curcumin, chitosan-gelatin-curcumin, and chitosan-collagen-curcumin were prepared by a simple safe a freeze-drying technique.

Chitosan-gelatin-curcumin displayed more antibacterial activity compared to other composites against *E. coli* and *S. aureus* by inhibition zone diameter of 24 and 18 mm, sequentially with good cell reproduction after 10 days of scar treatment rat sample (38). Mechanical properties, wettability, swelling, and metabolic stability of polymeric micro and nanoformulations can be improved by using pluronic

block copolymers (39). In this way, membranes based on chitosan-curcumin incorporated in pluronic copolymers exhibited curcumin release (in a medium containing Tween 80 after the period of ~300 h) and high swelling degree with values of ~60% and >800%, respectively (40). Combination of curcumin with metal or metal oxide NPs can cause synergistic antibacterial activity; as an example, curcumin can form a complex with TiO_2 and then loaded on chitosan to increase bacterial inactivation of antibiotic-resistant Gram-positive and Gram-negative bacteria by synergistic effect, water absorption capacity, wound healing after 14 days as well as improve drug release (41).

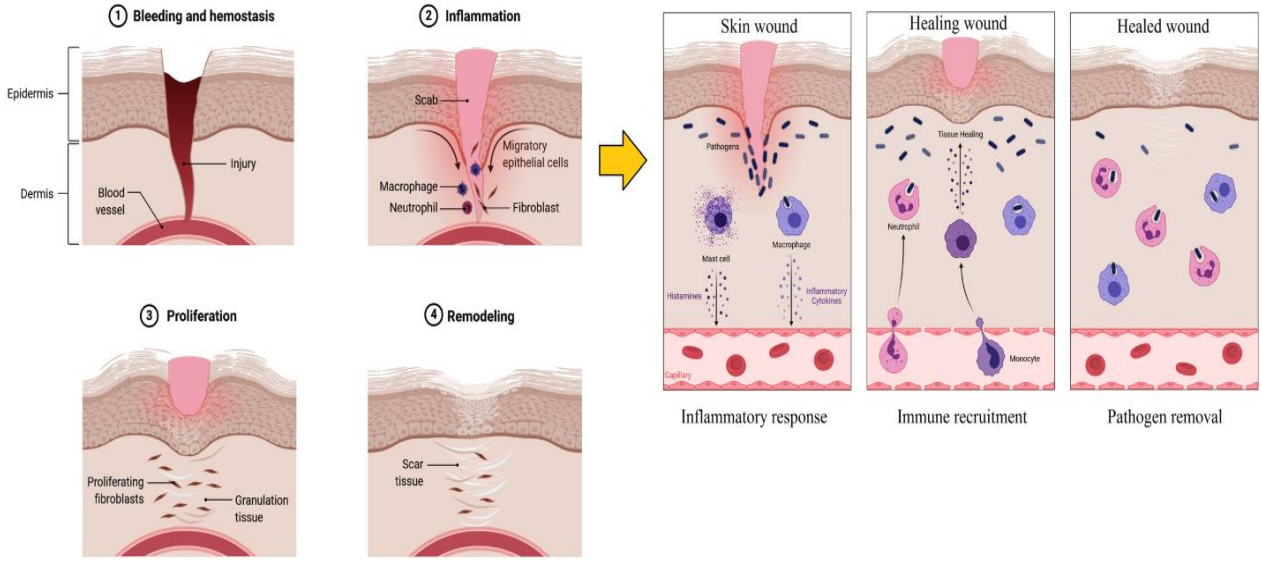

Figure 2. Wound healing phases with three sub-phases related to an inflammatory response (created with BioRender.com) (42).

Collagen

The wound healing property of collagen is based on cell attachment via abundant arginine–glycine–aspartic acid (RGD) integrin-binding motifs, which play critical roles in cellular migration, growth and differentiation resulting in increased angiogenesis and granulation tissues in wound site (43, 44). Collagen in combination with other antimicrobial materials such as MNPs/MONPs, antibiotics and biopolymers can accelerate the healing of infected chronic wounds (45). Lipid nanoparticles (LNP) are suitable carriers for encapsulation of therapeutic agents such as curcumin owing to the abilities of bioavailability and sustained drug release in physiological conditions. However, for wound dressing application, collagen can enhance formulation stability and promote differentiation, migration and cell adhesion. In this regard, collagen hydrogels composed of curcumin-loaded LNP showed 100% release after 25 days in saline buffer without cytotoxicity (18). Acting as drug carriers, polymeric nanospheres and microspheres composed of natural or synthetic polymers can encapsulate antimicrobial drugs, to release them in a controlled and sustained way (46). Scaffold based on porous collagen-CNCs-curcumin/gelatin microspheres showed high porosity, antibacterial activity, anti-inflammatory and accelerated dermis regeneration of infected burn wounds in rat models after 21 postoperative days. Sustained curcumin release for this scaffold was ~27.8% within the first 24h and 100% by 240h. However, this formulation showed less antibacterial activity against Gram-positive and Gram-negative bacteria in comparison with curcumin-collagen-CNC. It is important to note that gelatin in this scaffold can lead to a slower release of curcumin, but reduce antibacterial capacity. In addition, the role of collagen-curcumin combination can be suppression of NF-κB followed by reduced expression of pro-inflammatory cytokines of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (47). Nanoformulation of synthetic polymers such as polyvinyl alcohol (PVA), poly ε-caprolactone (PCL), and polyethylene glycol (PEG) with collagen can enhance the mechanical properties of wound dressings. As shown in Figure 3a, firstly, curcumin was loaded on a copolymer of PCL-PEG-PCL (PCEC) to produce nanofibers and then incorporated into collagen type I and PVA to prepare composite film (CPCF). In addition to antibacterial activity against *E. coli* and *S.*

aureus, this composite film showed regular arranged and dense distributed collagen fibers after 15 days of treatment of surgical wound site in rat samples (48).

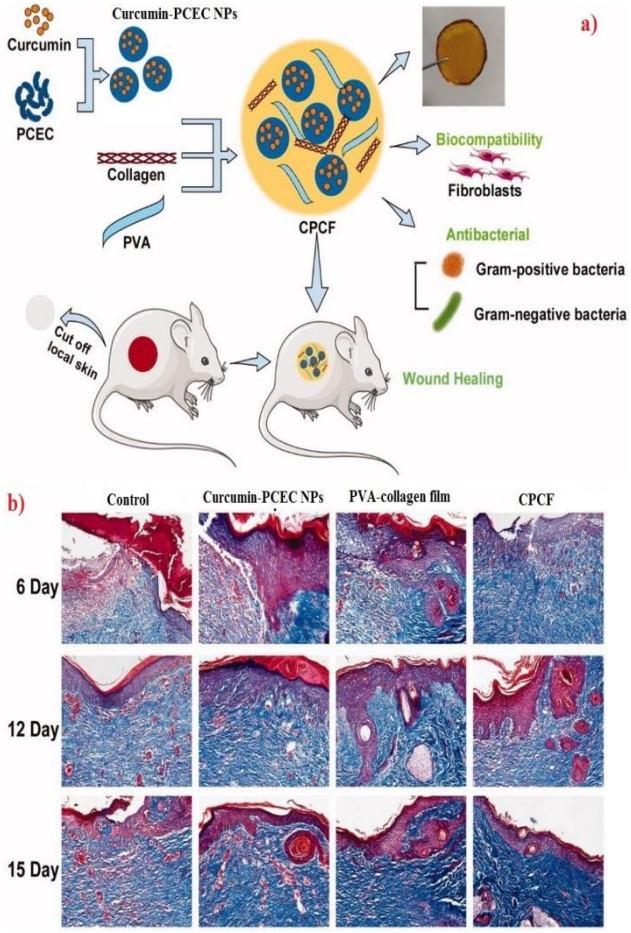

Figure 4. a) Synthesis steps of a composite film of CPCF based on curcumin, PCEC, PVA, and collagen b) formation of collagen fibers in surgical wound site; control is an untreated group (open access article distributed under the terms of the Creative Commons CC BY license) (48).

Conclusion

An infected wound such as DFU is a serious health problem resulting from multidrug-resistant microorganisms that may lead to death in patients. Curcumin as a phenolic compound has been illustrated as appropriate antimicrobial, anti-inflammatory, antioxidant, anticarcinogenic, and wound healing, antiseptic, and antioxidant activity. However, its low stability, extremely high hydrophobicity and bioavailability in physiological conditions can make it difficult to formulate in concentrations above the MIC for the treatment of infected wounds. To overcome this hindrance, the application of natural polymers particularly cellulose, chitosan, and collagen may be an effective strategy. Among these polymers, chitosan has intrinsic antibacterial activity because of its high density of positive charge, which can facilitate the interaction of formulation with the negative charge of the bacterial envelope as the first step of bacterial inactivation. However, the stability of cellulose is more than chitosan appropriate for wound dressing application. Cellular migration, growth and differentiation and granulation tissues in wound sites also should be considered to prepare an efficient wound dressing scaffold by adding collagen polymer. Curcumin in combination with collagen can suppress NF-κB followed by reduced expression of pro-inflammatory cytokines of IL-1β, IL-6 and TNF-α in rat models. Therefore, it can be concluded that each of these polymers has a unique property, which can be formulated with curcumin in a combination way at a safe concentration and weight ratio.

Acknowledgments

None.

Conflict interest

The authors declare no conflict of interest.

References

1. Abbas-Al-Khafaji ZK, Aubais-aljelehawy Qh. Evaluation of antibiotic resistance and prevalence of multi-antibiotic resistant genes among Acinetobacter baumannii strains isolated from patients admitted to al-yarmouk hospital. Cell Mol Biomed Rep. 2021;1(2):60-8.
2. Almasian-Tehrani N, Alebouyeh M, Armin S, Soleimani N, Azimi L, Shaker-Darabad R. Overview of typing techniques as molecular epidemiology tools for bacterial characterization. Cell Mol Biomed Rep. 2021;1(2):69-77.
3. Aubais aljelehawy Qh, Hadi Alshaibah LH, Abbas Al- Khafaji ZK. Evaluation of virulence factors among Staphylococcus aureus strains isolated from patients with urinary tract infection in Al-Najaf Al-Ashraf teaching hospital. Cell Mol Biomed Rep. 2021;1(2):78-87.
4. Alavi M, Hamblin MR, Martinez F, Kennedy JF, Khan H. Synergistic combinations of metal, metal oxide, or metalloid nanoparticles plus antibiotics against resistant and non-resistant bacteria. Micro Nano Bio Aspects. 2022;1(1):1-9.
5. Zubair M. Prevalence and interrelationships of foot ulcer, risk-factors and antibiotic resistance in foot ulcers in diabetic populations: A systematic review and meta-analysis. World J Diabetes. 2020;11(3):78-89.
6. Alavi M, Rai M. Antisense RNA, the modified

CRISPR-Cas9, and metal/metal oxide nanoparticles to inactivate pathogenic bacteria. Cell Mol Biomed Rep. 2021;1(2):52-9.
7. Bariz K, De Mendonça R, Denis O, Nonhoff C, Azzam A, Houali K. Multidrug resistance of the extended-spectrum beta-lactamase-producing Klebsiella pneumoniae isolated in Tizi-Ouzou (Algeria). Cell Mol Biol. 2019;65(8):11-7.
8. Leyva-Gómez G, González-Torres M, Alcalá-Alcalá S, Bernal-Chávez SA, Morales-Morfin JC, González-Del Carmen M, et al. Development of films from natural sources for infections during wound healing. Cell Mol Biol. 2021;67(1):96-100.
9. Alavi M, Kennedy JF. Recent advances of fabricated and modified Ag, Cu, CuO and ZnO nanoparticles by herbal secondary metabolites, cellulose and pectin polymers for antimicrobial applications. Cellulose. 2021;28(6):3297-310.
10. Amirthalingam S, Rajendran AK, Mani P, Rangasamy J. Perspectives and Challenges of Using Chitosan in Various Biological Applications. In: Jayakumar R, Prabaharan M, editors. Chitosan for Biomaterials III: Structure-Property Relationships. Cham: Springer International Publishing; 2021. p. 1-22.
11. Ahani H, Attaran S. Therapeutic potential of Seabuckthorn (Hippophae rhamnoides L.) in medical sciences. Cell Mol Biomed Rep. 2022;2(1):22-32.
12. Prasathkumar M, Sadhasivam S. Chitosan/Hyaluronic acid/Alginate and an assorted polymers loaded with honey, plant, and marine compounds for progressive wound healing—Know-how. Int J Biol Macromol. 2021;186:656-85.
13. Bilal I, Xie S, Elburki MS, Aziziaram Z, Ahmed SM, Jalal Balaky ST. Cytotoxic effect of diferuloylmethane, a derivative of turmeric on different human glioblastoma cell lines. Cell Mol Biomed Rep. 2021;1(1):14-22.
14. Aziziaram Z, Bilal I, Zhong Y, Mahmod AK, Roshandel MR. Protective effects of curcumin against naproxen-induced mitochondrial dysfunction in rat kidney tissue. Cell Mol Biomed Rep. 2021;1(1):23-32.
15. Alavi M, Adulrahman NA, Haleem AA, Al-Râwanduzi ADH, Khusro A, Abdelgawad MA, et al. Nanoformulations of curcumin and quercetin with silver nanoparticles for inactivation of bacteria. Cell Mol Biol. 2022;67(5):151-6.
16. Manimaran S, SambathKumar K, Gayathri R, Raja K, Rajkamal N, Venkatachalapathy M, et al. Medicinal Plant Using Ground State Stabilization of Natural Antioxidant Curcumin by Keto-Enol Tautomerisation. Nat prod bioprospect. 2018;8(5):369-90.
17. Abd El-Hack ME, El-Saadony MT, Swelum AA, Arif M, Abo Ghanima MM, Shukry M, et al. Curcumin, the active substance of turmeric: its effects on health and ways to improve its bioavailability. J Sci Food Agric. 2021;101(14):5747-62.
18. Laghezza Masci V, Taddei A-R, Courant T, Tezgel O, Navarro F, Giorgi F, et al. Characterization of Collagen/Lipid Nanoparticle–Curcumin Cryostructurates for Wound Healing Applications. Macromol Biosci. 2019;19(5):1800446.
19. Soleimani V, Sahebkar A, Hosseinzadeh H. Turmeric (Curcuma longa) and its major constituent (curcumin) as nontoxic and safe substances: Review. Phytother Res. 2018;32(6):985-95.
20. Ibrahim Fouad G, Ahmed KA. Curcumin Ameliorates Doxorubicin-Induced Cardiotoxicity and Hepatotoxicity Via Suppressing Oxidative Stress and Modulating iNOS, NF-κB, and TNF-α in Rats. Cardiovasc Toxicol. 2022;22(2):152-66.
21. Alavi M, Karimi N. Hemoglobin self-assembly and antibacterial activities of bio-modified Ag-MgO nanocomposites by different concentrations of Artemisia haussknechtii and Protoparmeliopsis muralis extracts. Int J Biol Macromol. 2020;152:1174-85.
22. Karim MB, Kanaya S, Altaf-Ul-Amin M. Antibacterial Activity Prediction of Plant Secondary Metabolites Based on a Combined Approach of Graph Clustering and Deep Neural Network. Mol Inform. 2022;n/a(n/a):2100247.
23. Ahmadi S, Ahmadi G, Ahmadi H. A review on antifungal and antibacterial activities of some medicinal plants. Micro Nano Bio Aspects. 2022;1(1):10-7.
24. Mobaraki M, Bizari D, Soltani M, Khshmohabat H, Raahemifar K, Akbarzade Amirdehi M. The Effects of Curcumin Nanoparticles Incorporated into Collagen-Alginate Scaffold on Wound Healing of Skin Tissue in Trauma Patients. Polymers. 2021;13(24):4291.
25. Shoueir KR, El-Desouky N, Rashad MM, Ahmed MK, Janowska I, El-Kemary M. Chitosan based-nanoparticles and nanocapsules: Overview, physicochemical features, applications of a nanofibrous scaffold, and bioprinting. Int J Biol Macromol. 2021;167:1176-97.
26. Alavi M, Varma RS. Antibacterial and wound healing activities of silver nanoparticles embedded in cellulose compared to other polysaccharides and protein polymers. Cellulose. 2021;28(13):8295-311.
27. Alavi M, Rai M. Chapter 11 - Antibacterial and wound healing activities of micro/nanocarriers based on carboxymethyl and quaternized chitosan derivatives. In: Rai M, dos Santos CA, editors. Biopolymer-Based Nano Films: Elsevier; 2021. p. 191-201.
28. Stern D, Cui H. Crafting Polymeric and Peptidic Hydrogels for Improved Wound Healing. Adv

Healthc Mater. 2019;8(9):1900104.
29. Alven S, Buyana B, Feketshane Z, Aderibigbe BA. Electrospun Nanofibers/Nanofibrous Scaffolds Loaded with Silver Nanoparticles as Effective Antibacterial Wound Dressing Materials. Pharmaceutics. 2021;13(7):964.
30. Basit HM, Mohd Amin MCI, Ng S-F, Katas H, Shah SU, Khan NR. Formulation and Evaluation of Microwave-Modified Chitosan-Curcumin Nanoparticles—A Promising Nanomaterials Platform for Skin Tissue Regeneration Applications Following Burn Wounds. Polymers. 2020;12(11):2608.
31. Ribeiro DML, Carvalho Júnior AR, Vale de Macedo GHR, Chagas VL, Silva LdS, Cutrim BdS, et al. Polysaccharide-Based Formulations for Healing of Skin-Related Wound Infections: Lessons from Animal Models and Clinical Trials. Biomolecules. 2020;10(1):63.
32. Tong WY, bin Abdullah AYK, binti Rozman NAS, bin Wahid MIA, Hossain MS, Ring LC, et al. Antimicrobial wound dressing film utilizing cellulose nanocrystal as drug delivery system for curcumin. Cellulose. 2018;25(1):631-8.
33. Pouget C, Dunyach-Remy C, Pantel A, Schuldiner S, Sotto A, Lavigne J-P. Biofilms in Diabetic Foot Ulcers: Significance and Clinical Relevance. Microorganisms. 2020;8(10):1580.
34. Vachanont T, Torgbo S, Sukyai P. Release Kinetic Model and Antimicrobial Activity of Freeze-Dried Curcumin-loaded Bacterial Nanocellulose Composite. Polym Sci - A. 2020;62(3):218-27.
35. Patil TV, Patel DK, Dutta SD, Ganguly K, Santra TS, Lim K-T. Nanocellulose, a versatile platform: From the delivery of active molecules to tissue engineering applications. Bioact Mater. 2022;9:566-89.
36. Alavi M. Modifications of microcrystalline cellulose (MCC), nanofibrillated cellulose (NFC), and nanocrystalline cellulose (NCC) for antimicrobial and wound healing applications. e-Polymers. 2019;19(1):103-19.
37. Ferfera-Harrar H, Berdous D, Benhalima T. Hydrogel nanocomposites based on chitosan-g-polyacrylamide and silver nanoparticles synthesized using Curcuma longa for antibacterial applications. Polym Bull. 2018;75(7):2819-46.
38. Naghshineh N, Tahvildari K, Nozari M. Preparation of Chitosan, Sodium Alginate, Gelatin and Collagen Biodegradable Sponge Composites and their Application in Wound Healing and Curcumin Delivery. J Polym Environ. 2019;27(12):2819-30.
39. Alavi M, Rai M, Varma RS, Hamidi M, Mozafari MR. Conventional and novel methods for the preparation of micro and nanoliposomes. Micro Nano Bio Aspects. 2022;1(1):18-29.
40. Enumo A, Argenta DF, Bazzo GC, Caon T, Stulzer HK, Parize AL. Development of curcumin-loaded chitosan/pluronic membranes for wound healing applications. Int J Biol Macromol. 2020;163:167-79.
41. Marulasiddeshwara R, Jyothi MS, Soontarapa K, Keri RS, Velmurugan R. Nonwoven fabric supported, chitosan membrane anchored with curcumin/TiO2 complex: Scaffolds for MRSA infected wound skin reconstruction. Int J Biol Macromol. 2020;144:85-93.
42. Alavi M, Nokhodchi A. Antimicrobial and Wound Treatment Aspects of Micro- and Nanoformulations of Carboxymethyl, Dialdehyde, and TEMPO-Oxidized Derivatives of Cellulose: Recent Advances. Macromol Biosci. 2020;20(4):1900362.
43. Acosta S, Quintanilla-Sierra L, Mbundi L, Reboto V, Rodríguez-Cabello JC. Elastin-Like Recombinamers: Deconstructing and Recapitulating the Functionality of Extracellular Matrix Proteins Using Recombinant Protein Polymers. Adv Funct Mater. 2020;30(44):1909050.
44. Abdel-Mohsen AM, Frankova J, Abdel-Rahman RM, Salem AA, Sahffie NM, Kubena I, et al. Chitosan-glucan complex hollow fibers reinforced collagen wound dressing embedded with aloe vera. II. Multifunctional properties to promote cutaneous wound healing. Int J Pharm. 2020;582:119349.
45. Alavi M, Rai M. Topical delivery of growth factors and metal/metal oxide nanoparticles to infected wounds by polymeric nanoparticles: an overview. Expert Rev Anti-Infect Ther. 2020;18(10):1021-32.
46. Alavi M, Webster TJ. Recent progress and challenges for polymeric microsphere compared to nanosphere drug release systems: Is there a real difference? Bioorg Med Chem. 2021;33:116028.
47. Guo R, Lan Y, Xue W, Cheng B, Zhang Y, Wang C, et al. Collagen-cellulose nanocrystal scaffolds containing curcumin-loaded microspheres on infected full-thickness burns repair. J Tissue Eng Regen Med. 2017;11(12):3544-55.
48. Leng Q, Li Y, Pang X, Wang B, Wu Z, Lu Y, et al. Curcumin nanoparticles incorporated in PVA/collagen composite films promote wound healing. Drug Deliv. 2020;27(1):1676-85.