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Evaluation of wound healing potential of cuttlefish bone extract: An experimental study

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Abstract
Wound healing remains a significant clinical challenge, particularly in cases of chronic wounds and delayed healing. This experimental study investigates the therapeutic potential of cuttlefish bone extract (CBE) as a novel wound healing agent. Trace amounts matrix proteins, has been traditionally used in various medicinal applications. The study employed both *in vitro* and *in vivo* experimental models to evaluate the wound healing properties of CBE. Cell proliferation assays using human dermal fibroblasts demonstrated enhanced cellular migration and proliferation rates when treated with CBE concentrations ranging from 50-200 µg/mL. *In vivo* studies using a rat excisional wound model showed accelerated wound closure, improved re-epithelialization, and enhanced collagen deposition in CBE-treated groups compared to controls. Histological analysis revealed increased angiogenesis and reduced inflammatory infiltration in wounds treated with CBE. The extract demonstrated antimicrobial activity against common wound pathogens, including Staphylococcus aureus and Pseudomonas aeruginosa. These findings suggest that cuttlefish bone extract possesses significant wound healing potential through multiple mechanisms, including enhanced cellular proliferation, improved angiogenesis, antimicrobial activity, and modulation of inflammatory responses. Further clinical studies are warranted to validate these promising preclinical results.

Keywords: Cuttlefish bone extract, wound healing, tissue regeneration, calcium carbonate, antimicrobial activity, angiogenesis, collagen synthesis

Introduction

Wound healing is a complex, inflammation, proliferation, and remodelling. The restoration of tissue integrity requires precise coordination of cellular activities, growth factor signalling, extracellular matrix synthesis, and angiogenesis. Despite advances in wound care technologies, chronic wounds and impaired healing continue to pose significant challenges in clinical practice, affecting millions of patients worldwide and imposing substantial economic burdens on healthcare systems.

Traditional wound care approaches often fall short in addressing the multifactorial nature of wound healing, particularly in patients with comorbidities such as diabetes, vascular diseases, or immunocompromised states. This has driven researchers to explore novel therapeutic agents derived from natural sources, which may offer advantages including biocompatibility, reduced side effects, and multi-target mechanisms of action.

Bioactive compounds with therapeutic potential. The marine environment's unique conditions have led to the evolution of organisms with distinctive biochemical properties that may be harnessed for medical applications. Among these, cuttlefish (Sepia species) represents an underexplored resource with potential therapeutic applications. Cuttlefish bone, the internal shell of cuttlefish, is form of aragonite, along with organic matrix proteins, chitin, and various trace elements.

The structural composition of cuttlefish bone suggests several mechanisms by which it might promote wound healing. Calcium ions play crucial roles in cellular signalling pathways involved in wound repair, including platelet activation, coagulation cascade, and cellular migration. The aragonite form of calcium carbonate found in cuttlefish bone exhibits unique crystalline properties that may influence its biological activity. Additionally, the organic matrix components, including proteins and glycoproteins.

Biocompatibility and osteoconductive properties of cuttlefish bone in bone tissue engineering applications. However, its potential for soft tissue wound healing remains largely unexplored. The porous structure of cuttlefish bone, combined with its chemical composition, suggests it may serve as both a source of bioactive compounds and a scaffold material for tissue regeneration.

Furthermore, marine-derived calcium carbonate has shown promising antimicrobial properties, which could be particularly beneficial in wound care where infection prevention is crucial. The ability to simultaneously promote healing while preventing microbial colonisation would represent a significant advantage over conventional wound care products.

This study aims to comprehensively evaluate the wound healing potential of cuttlefish bone extract through systematic *in vitro* and *in vivo* experimental approaches. We hypothesise that cuttlefish bone extract will demonstrate significant wound healing properties through multiple mechanisms, including enhanced cellular proliferation, improved angiogenesis, antimicrobial activity, and appropriate modulation of inflammatory responses. The findings from this research may contribute to the development of novel, natural wound care therapies with improved efficacy and safety profiles.

Methods

Preparation of Cuttlefish Bone Extract

Fresh cuttlefish bones were obtained from local seafood markets and thoroughly cleaned to remove organic debris. Mechanical grinder, and passed through a 200-mesh sieve. Aqueous extraction was performed by mixing 100g of cuttlefish bone powder with 1 L of distilled water and heating at 80°C for 4 hours under continuous stirring. The

concentrated extract was lyophilised to obtain dried cuttlefish bone extract (CBE) powder, which was stored at -20°C until use.

Chemical Characterization
The chemical composition of CBE was analysed using X-ray diffraction (XRD) for crystalline structure identification, Fourier-transform infrared spectroscopy (FTIR) for functional group analysis, and inductively coupled plasma mass spectrometry (ICP-MS) for elemental composition. Protein content was determined using the Bradford assay.

Cell Culture Studies
Human dermal fibroblasts (HDFs) were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in 5% CO_2. Cell viability was assessed using the MTT assay after 24, 48, and 72 hours of treatment with CBE concentrations ranging from 25-400 μg/mL. Cell migration was evaluated using a scratch wound assay.

Antimicrobial Activity Testing
The antimicrobial properties of CBE were evaluated against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa using disc diffusion and broth microdilution methods. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined according to Clinical and Laboratory Standards Institute guidelines.

Animal Studies
Male Wistar rats (200-250g) were used following institutional animal care guidelines and ethical approval. Animals were anaesthetised using ketamine-xylazine, and full-thickness excisional wounds (1.5 cm diameter) were created on the dorsal surface. (n=8 each): control (saline), CBE 50 mg/mL, CBE 100 mg/mL, and positive control (commercial wound gel). Treatments were applied topically twice daily for 14 days.

Wound Assessment
Wound healing was monitored by photographing wounds daily and calculating wound closure percentage using ImageJ software. On days 7 and 14, tissue samples were collected for histological analysis. Immunohistochemistry was performed for CD31 (angiogenesis marker) and α-smooth muscle actin (myofibroblast marker).

Statistical Analysis
Data were analysed using SPSS 25.0 software. Results are expressed as mean ± standard deviation. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test, with $p<0.05$ considered significant.

Results
Chemical Characterisation of Cuttlefish Bone Extract
XRD analysis confirmed that the cuttlefish bone extract contained predominantly the aragonite form of calcium carbonate (94.2% ± 2.1%), with minor traces of calcite. FTIR spectroscopy revealed characteristic peaks at 1085 cm^{-1}, 874 cm^{-1}, and 712 cm^{-1} corresponding to carbonate groups, along with peaks at 1650 cm^{-1} and 1540 cm^{-1} indicating the presence of amide bonds from protein components. ICP-MS analysis showed calcium as the major element (38.2% ± 1.5%), followed by magnesium (0.8% ± 0.2%), strontium (0.3% ± 0.1%), and trace amounts of zinc, manganese, and iron. The protein content was determined to be 12.4% ± 0.8%, while total carbohydrate content was 3.2% ± 0.4%.

Cell Viability and Proliferation
MTT assay results demonstrated that CBE concentrations up to 200 μg/mL were non-cytotoxic to human dermal fibroblasts, with cell viability remaining above 95% at 72 hours. Interestingly, CBE concentrations of 50-150 μg/mL showed significant enhancement of cell proliferation compared to controls, with peak activity observed at 100 μg/mL (134.2% ± 8.7% of control, $p<0.001$). Higher concentrations (300-400 μg/mL) showed dose-dependent cytotoxic effects, with cell viability dropping to 68.3% ± 5.2% at 400 μg/mL.

Cell Migration Assay
The scratch wound assay revealed that CBE significantly enhanced fibroblast migration in a dose-dependent manner. At 48 hours post-treatment, wound closure percentages were 45.2% ± 3.8% for control, 62.7% ± 4.5% for 50 μg/mL CBE, 78.9% ± 5.2% for 100 μg/mL CBE, and 71.3% ± 4.9% for 150 μg/mL CBE (all $p<0.01$ compared to control). The optimal concentration of 100 μg/mL showed 74.5% faster wound closure compared to untreated controls.

Antimicrobial Activity
CBE demonstrated broad-spectrum antimicrobial activity against all tested pathogens. The minimum inhibitory concentrations were 125 μg/mL for S. aureus, 250 μg/mL for E. coli, and 187.5 μg/mL for P. aeruginosa. Minimum bactericidal concentrations were 250 μg/mL, 500 μg/mL, and 375 μg/mL, respectively. The antimicrobial activity was attributed to the calcium carbonate content and organic matrix components, which likely disrupted bacterial cell wall integrity and interfered with metabolic processes.

***In Vivo* Wound Healing Assessment**
Macroscopic evaluation of wound healing showed significant acceleration in CBE-treated groups. By day 7, wound closure percentages were 42.3% ± 5.1% for control, 58.7% ± 6.3% for CBE 50 mg/mL, 71.2% ± 4.8% for CBE 100 mg/mL, and 65.9% ± 5.7% for positive control. Complete wound closure was achieved by day 12 in the CBE 100 mg/mL group compared to day 16 in controls ($p<0.001$). The higher concentration of CBE (100 mg/mL) demonstrated superior healing compared to the commercial wound gel.

Histological Analysis
Histological examination revealed enhanced tissue regeneration in CBE-treated wounds. By day 7, CBE-treated groups showed increased granulation tissue formation, improved re-epithelialization, and reduced inflammatory cell infiltration compared to controls. The epithelial layer thickness was significantly greater in the CBE 100 mg/mL group (127.4 ± 12.8 μm) compared to the control (89.6 ± 8.4 μm, $p<0.01$). Masson's trichrome staining demonstrated enhanced collagen deposition and better-organised collagen fibre arrangement in CBE-treated wounds, indicating improved tissue remodelling.

Angiogenesis and Myofibroblast Assessment

Immunohistochemical analysis for CD31 revealed significantly increased micro vessel density in CBE-treated wounds. The vessel count per high-power field was 8.3 ± 1.2 for control, 14.7 ± 2.1 for CBE 50 mg/mL, and 18.9 ± 2.8 for CBE 100 mg/mL ($p<0.001$). α-smooth muscle actin staining showed increased myofibroblast differentiation in CBE-treated groups, indicating enhanced wound contraction and tissue remodelling. The myofibroblast count was 2.3-fold higher in the CBE 100 mg/mL group compared to the control ($p<0.01$).

The findings from this comprehensive experimental study demonstrate that cuttlefish bone extract possesses significant wound healing potential through multiple synergistic mechanisms. The combination of enhanced cellular activities, antimicrobial properties, and promotion of angiogenesis positions CBE as a promising natural therapeutic agent for wound care applications. With appropriate development and clinical validation, CBE could contribute to improved patient outcomes and reduced healthcare costs in wound management.

Discussion

The present study provides compelling evidence for the wound healing potential of cuttlefish bone extract through multiple complementary mechanisms. Our findings demonstrate that CBE significantly enhances wound healing through improved cellular proliferation, enhanced migration, antimicrobial activity, increased angiogenesis, and appropriate tissue remodelling. These results support our initial hypothesis and suggest that CBE could represent a valuable addition to the arsenal of natural wound healing agents.

Mechanisms of Action

The wound healing properties of CBE appear to be mediated through several interconnected pathways. The high calcium carbonate content, particularly in the aragonite form, likely plays a crucial role in cellular signalling and activation. Calcium ions are essential cofactors in numerous cellular processes, including platelet aggregation, coagulation cascade activation, and cellular migration. The sustained release of calcium from CBE may create an optimal microenvironment for wound healing by maintaining adequate calcium levels necessary for these processes.

The protein components identified in CBE, comprising 12.4% of the extract, may contain bioactive peptides that directly stimulate cellular activities. Marine-derived proteins often possess unique amino acid sequences that can act as growth factors or growth factor analogues, promoting cellular proliferation and differentiation. The enhanced fibroblast proliferation and migration observed in our study suggest that these protein components may be interacting with specific cellular receptors to activate pro-healing pathways.

The antimicrobial properties of CBE represent another significant advantage in wound care applications. Chronic wounds often suffer from bacterial colonisation and biofilm formation, which impede healing and can lead to serious complications. The broad-spectrum activity of CBE against common wound pathogens, including gram-positive S. aureus and gram-negative bacteria, suggests multiple antimicrobial mechanisms. The calcium carbonate component may disrupt bacterial cell wall integrity, while organic matrix components could interfere with bacterial metabolism or adhesion.

Angiogenesis and Tissue Remodelling

The significant increase in microvessel density observed in CBE-treated wounds indicates enhanced angiogenesis, which is crucial for supplying nutrients and oxygen to the healing tissue. The 2.3-fold increase in vessel density compared to controls suggests that CBE actively promotes endothelial cell proliferation and vessel formation. This may be mediated through the release of pro-angiogenic factors or through direct interaction with endothelial cells.

The enhanced myofibroblast differentiation observed in CBE-treated wounds is particularly important for wound contraction and collagen synthesis. Myofibroblasts are specialised cells that combine the contractile properties of smooth muscle cells with the synthetic capabilities of fibroblasts. Their increased presence in CBE-treated wounds correlates with the improved collagen organisation and enhanced tissue remodelling observed histologically.

Comparison with Existing Treatments

The superior performance of CBE compared to commercial wound gel in our study is noteworthy. While the commercial product likely contains synthetic polymers and antimicrobial agents, CBE demonstrated faster wound closure and better tissue quality through natural mechanisms. This suggests that the multi-component nature of CBE provides synergistic effects that surpass single-target approaches.

The dose-response relationship observed in our study, with optimal effects at 100 mg/mL, indicates that CBE activity is concentration-dependent. Higher concentrations may lead to excessive calcium levels or protein overload that could potentially interfere with normal cellular processes. This emphasises the importance of proper dosing in clinical applications.

Clinical Implications and Future Directions

The promising results obtained in this study suggest several potential clinical applications for CBE. The extract could be formulated into hydrogels, creams, or wound dressings for treating various types of wounds, including diabetic ulcers, pressure sores, and surgical wounds. The antimicrobial properties make it particularly suitable for infected or high-risk wounds.

However, several considerations must be addressed before clinical translation. First, standardisation of the extraction process and quality control measures needs to be established to ensure consistent potency and safety. The natural variability in cuttlefish bone composition, depending on species, location, and season, could affect extract properties. Second, comprehensive toxicological studies, including sensitisation and irritation testing, are necessary to establish safety profiles for human use.

The mechanism of action studies could be expanded to investigate specific molecular pathways involved in CBE-mediated wound healing. Proteomics and genomics approaches could identify the active protein components and their cellular targets. Additionally, the role of trace elements such as strontium and zinc in the healing process warrants further investigation.

Limitations and Considerations

Our study has several limitations that should be acknowledged. The animal model, while well-established, may not fully recapitulate human wound healing processes. The acute wound model used does not address the complexity of chronic wounds, where CBE might be most clinically relevant. Future studies should include chronic wound models and assessment of healing in compromised conditions such as diabetes or infection.

The extraction method used in this study, while effective, may not optimise the recovery of all bioactive components. Alternative extraction methods, including enzymatic digestion or supercritical fluid extraction, could potentially yield extracts with enhanced activity. Additionally, fractionation studies could identify the most active components for targeted therapy development.

Environmental and Sustainability Considerations

The use of cuttlefish bone as a source of therapeutic compounds raises important sustainability questions. Cuttlefish populations and harvesting practices must be carefully managed to ensure environmental protection. However, cuttlefish bone is often a waste product from seafood processing, making its utilisation for medical applications an attractive example of waste valorisation and circular economy principles.

The findings from this comprehensive experimental study demonstrate that cuttlefish bone extract possesses significant wound healing potential through multiple synergistic mechanisms. The combination of enhanced cellular activities, antimicrobial properties, and promotion of angiogenesis positions CBE as a promising natural therapeutic agent for wound care applications. With appropriate development and clinical validation, CBE could contribute to improved patient outcomes and reduced healthcare costs in wound management.

Conclusion

This experimental study demonstrates that cuttlefish bone extract possesses significant wound healing potential through multiple synergistic mechanisms. The extract effectively enhanced fibroblast proliferation and migration, exhibited broad-spectrum antimicrobial activity, and accelerated wound closure in animal models. The optimal concentration of 100 mg/mL CBE achieved superior healing outcomes compared to commercial wound treatments, with complete wound closure occurring 4 days earlier than controls. Histological analysis confirmed enhanced angiogenesis, improved collagen organisation, and appropriate tissue remodelling without adverse effects. The aragonite form of calcium carbonate, combined with bioactive proteins and trace elements, appears to create an optimal microenvironment for wound healing. These findings support the potential development of CBE-based wound care products as safe, effective, and sustainable alternatives to conventional treatments. Future research should focus on clinical trials, mechanistic studies to identify active components, and optimisation of extraction and formulation methods. The utilisation of cuttlefish bone, often a waste product from seafood processing, also represents an environmentally conscious approach to natural product development in wound care therapeutics.

References

1. Singer AJ, Clark RA. Cutaneous wound healing. New England Journal of Medicine,1999:341(10):738–746.
2. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature,2008:453(7193):314–321.
3. Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, *et al*. Human skin wounds a major and snowballing threat to public health and the economy. Wound Repair and Regeneration,2009:17(6):763–771.
4. Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. Advances in Wound Care,2015:4(9):560–582.
5. Maver T, Mastnak T, Mihelič M, Maver U, Kleinschek KS, Stana-Kleinschek K. Synthetic and natural biomaterials for wound healing applications. Materials,2021:14(7):1724.
6. Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR. Marine natural products. Natural Product Reports,2017:34(3):235–294.
7. Rajesh S, Koshi E, Philip K, Mohan A. Antimicrobial peptides an alternative for innovative medicines. Asian Pacific Journal of Tropical Biomedicine,2011:1(5):401–412.
8. Kim SK, Mendis E. Bioactive compounds from marine processing byproducts a review. Food Research International,2006:39(4):383–393.
9. Rocha J, Peixe L, Gomes NCM, Calado R. Cnidarians as a source of new marine bioactive compounds. Marine Drugs,2011:9(10):1860–1886.
10. Ivankovic H, Tkalcec E, Orlic S, Ferrer GG, Schauperl Z. Hydroxyapatite formation from cuttlefish bones kinetics. Journal of Biomedical Materials Research A,2010:95(4):1203–1212.
11. Rocha JHG, Lemos AF, Agathopoulos S, Kannan S, Oktar FN, Valerio P, *et al*. Scaffolds for bone restoration from cuttlefish. Bone,2005:37(6):850–857.
12. Kim SK, Ravichandran YD, Khan SB, Kim YT. Prospects of the cosmeceuticals derived from marine organisms. Biotechnology and Bioprocess Engineering,2008:13(5):511–523.
13. Paoletti MG, Buscardo E, VanderJagt DJ, Pastuszyn A, Glew RH, Cerda H, *et al*. Nutrient content of earthworms consumed by Ye'Kuana Amerindians of the Alto Orinoco of Venezuela. Proceedings of the National Academy of Sciences of the United States of America,2003:100(21):12381–12384.
14. Velasco MA, Narváez-Tovar CA, Garzón-Alvarado DA. Design, materials, and mechanobiology of biodegradable scaffolds for bone tissue engineering. Biomedical Research International,2015:2015:729076.
15. Chen S, Shi J, Zhang M, Chen Y, Wang X, Zhang J, *et al*. Mesenchymal stem cell-laden anti-inflammatory hydrogel enhances diabetic wound healing. Scientific Reports,2015:5:18104.
16. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair and Regeneration,2008:16(5):585–601.
17. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiological Reviews,2003:83(3):835–870.
18. Reinke JM, Sorg H. Wound repair and regeneration. European Surgical Research,2012:49(1):35–43.

19. Krafts KP. Tissue repair the hidden drama. Organogenesis,2010:6(4):225–233.
20. Tonnesen MG, Feng X, Clark RA. Angiogenesis in wound healing. Journal of Investigative Dermatology Symposium Proceedings,2000:5(1):40–46.
21. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. Clinics in Dermatology,2007:25(1):9–18.
22. Gonzalez ACO, Costa TF, Andrade ZA, Medrado ARAP. Wound healing a literature review. Anais Brasileiros de Dermatologia,2016:91(5):614–620.
23. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing a cellular perspective. Physiological Reviews,2019:99(1):665–706.
24. Schultz GS, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing. Wound Repair and Regeneration,2009:17(2):153–162.
25. Xue M, Jackson CJ. Extracellular matrix reorganisation during wound healing and its impact on abnormal scarring. Advances in Wound Care,2015:4(3):119–136.
26. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration mechanisms, signalling, and translation. Science Translational Medicine,2014:6(265):265sr6.
27. Guo S, DiPietro LA. Factors affecting wound healing. Journal of Dental Research,2010:89(3):219–227.
28. Flanagan M. Wound healing and skin integrity: principles and practice. John Wiley Sons,2013.
29. Boateng JS, Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: a review. Journal of Pharmaceutical Sciences,2008:97(8):2892–2929.
30. Dhivya S, Padma VV, Santhini E. Wound dressings: a review. Biomedicine,2015:5(4):22.