

Biomedical Potentials of Snail Mucus: A Review of Achacin and Mytimacin-AF in Wound Healing

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Abstract

Snail mucus is gaining significant attention as a natural biomaterial for wound repair and regenerative medicine. This review focuses on the biomedical potentials of two key proteins found in snail mucus-Achacin and Mytimacin-AF-highlighting their antimicrobial, anti-inflammatory, and tissue-regenerative properties. A systematic literature search was conducted across major databases (2013–2025), selecting studies that examined the roles of snail mucus proteins in wound repair, antimicrobial defense, angiogenesis, and tissue regeneration. Mytimacin-AF is a cysteine-rich peptide, and Achacin is a glycoprotein with L-amino acid oxidase activity. They both work against a wide range of bacteria and fungi, help release cytokines, and get more immune cells to the area. Preclinical study shows that snail mucus can improve the quality of scars, speed up wound closure by 24–37%, and increase angiogenesis and collagen deposition compared to standard treatments. These proteins help both new and old wounds heal faster by increasing the number of fibroblasts, changing the extracellular matrix, and keeping the body's inflammatory reactions in check. It is still hard to make sure that all snail species have the same makeup, describe changes that happen after translation, and standardise extraction methods, further study should focus on clinical trials, detailed molecular characterisation, and new ways to deliver the proteins.

Keywords: Achacin, Antimicrobial Peptides, Mytimacin-AF, Snail Mucus, Tissue Regeneration, Wound Healing.

Introduction

Snail mucus has emerged as a promising natural biomaterial in regenerative medicine, particularly for wound healing applications. This review, “Biomedical Potentials of Snail Mucus: A Review of Achacin and Mytimacin-AF in Wound Healing,” focuses on two key bioactive proteins-Achacin and Mytimacin-AF-found in the mucus of terrestrial snails such as *Achatina fulica*. These proteins have attracted increasing scientific interest due to their demonstrated antimicrobial, anti-inflammatory, and tissue-regenerative properties, which are critical for effective wound repair (1-3). Traditional uses and new studies have both shown that snail mucus, which is made up of many different proteins, glycosaminoglycans, and peptides, can help treat wounds in different ways (4-6). Current literature shows that snail mucus expedites wound closure, promotes angiogenesis, and offers antimicrobial, anti-inflammatory, and moisturizing effects, often outperforming some standard therapies (7-14). Some animal studies, dose-dependent efficacy analyses, and mechanistic

insights reinforce its effectiveness. This study summarises the chemical makeup of Achacin and Mytimacin-AF as well as their ways of working. It is looked at what part they play in the different stages of wound healing, like inflammation, proliferation, and remodelling. Lastly, study gaps are found and ideas for future directions in clinical use are given. The review tries to give an honest opinion on how safe and effective these snail mucus components are as medicines, with a focus on how they might be used in cutting-edge wound care products. As a biomaterial, snail mucus has become useful in both traditional medicine and modern biomedical study because it can heal wounds. This study looks at Achacin and Mytimacin-AF, two important proteins found in snail mucus, and how they work during important stages of wound healing (15-17). Snail mucus is naturally made by many types of snails. It is made up of glycoproteins, peptides like mytimacin-AF and achacin, and bioactive substances like allantoin and glycosaminoglycans.

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In the past, it was used in traditional medicine to treat burns, sores, and skin diseases because it calmed people down and encouraged healing. New study backs up these benefits. It shows that snail mucus speeds up wound closure by 24–37% compared to traditional treatments. This is because it improves cell migration and proliferation, which are important for quick tissue regeneration. Its high antimicrobial peptide content makes bacterial membranes weaker and stops biofilms from forming, which makes it very effective against many types of bacteria, even antibiotic-resistant strains like MRSA and *E. coli*. The mucoadhesive properties of snail mucus keep the wound moist, which helps the epithelium grow and forms a layer of defence that helps tissues heal and scar less. The complicated, multi-step process of wound healing starts with the inflammatory phase. This is when bioactive chemicals in snail mucus reduce swelling and infection. The next step is the proliferative phase, which is marked by faster angiogenesis, fibroblast expansion, and tissue contraction. Growth factors and mucus moisture help with all of these processes. The last step in the process is called remodelling. This is when the collagen fibres grow and reorganise with the help of glycoproteins and enzymes related to mucus. More and more people are realising that bioactive substances, like those in snail mucus, can control the wound microenvironment, change the extracellular matrix, and change the behaviour of cells—all of these things are necessary for wounds to heal properly (15–18). Because of its unique biochemical makeup, snail mucus combines old and new information to offer broad antibacterial properties, faster healing, and better wound care. This makes it a promising natural biomaterial for cutting-edge wound treatments.

Methodology

Search Strategy

A comprehensive literature search was conducted using international scientific databases, including Scopus, PubMed, ScienceDirect, and Web of Science, to identify studies on the biomedical potentials of snail mucus, with a focus on Achacin and Mytimacin-AF in wound healing. Search terms included combinations of “snail mucus,” “Achacin,”

“Mytimacin-AF,” “wound healing,” “antimicrobial peptides,” “angiogenesis,” “skin regeneration,” and “bioactive compounds.” The search covered publications from January 2013 to May 2025 to ensure inclusion of the most recent advances. Reference lists of relevant articles and systematic reviews were also screened to identify additional studies.

Inclusion and Exclusion Criteria

Studies were included if they (i) investigated the wound healing properties or biomedical activities of snail mucus or its purified proteins/peptides (Achacin, Mytimacin-AF); (ii) reported in vitro, in vivo, or clinical outcomes related to skin repair, antimicrobial activity, angiogenesis, or tissue regeneration; and (iii) were published in English in peer-reviewed journals. Exclusion criteria were: (i) studies not focused on wound healing or not involving snail mucus or its relevant bioactive components; (ii) reviews or editorials without original data; and (iii) studies lacking clear methodology or outcome measures.

Selection Process

After removing duplicates, titles and abstracts were screened for relevance. Full texts of potentially eligible articles were then assessed independently by two reviewers. Disagreements were resolved by consensus or consultation with a third reviewer. A PRISMA flow diagram was used to document the selection process, ensuring transparency and reproducibility.

Data Extraction and Analysis

Data were systematically extracted using a standardized form, capturing details on snail species, extraction and purification methods, protein/peptide characterization, experimental design, wound healing models, outcome measures (e.g., rate of wound closure, angiogenesis, collagen deposition), and key findings. Quantitative data were tabulated and, where possible, subjected to statistical analysis (e.g., two-way ANOVA, post hoc tests) to assess significance of results¹. Qualitative synthesis was performed to compare methodologies, highlight trends, and identify research gaps, with a focus on the clinical translation potential of Achacin and Mytimacin-AF in Figure 1.

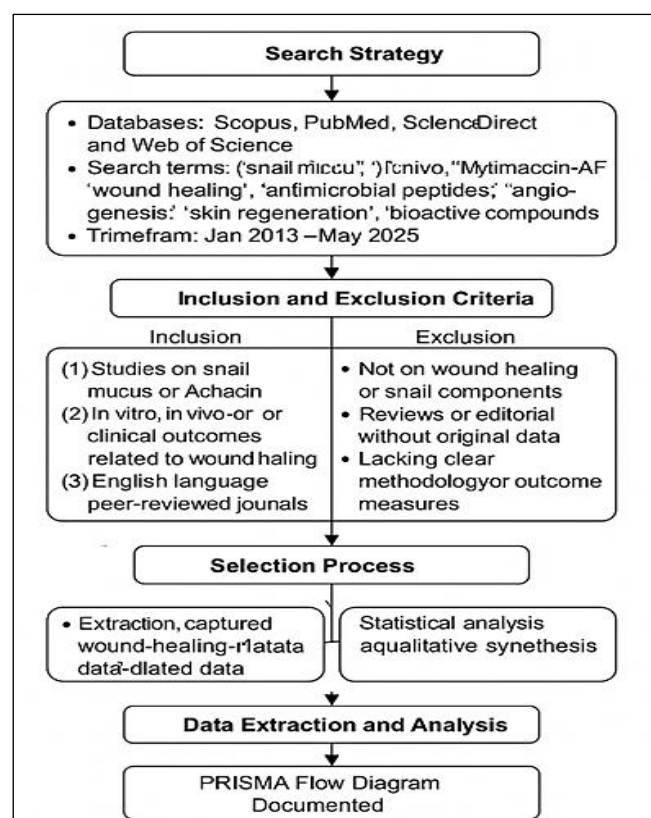


Figure 1: Schematic Representation of the Methodology for Data Extraction and Interpretation

Results

Snail mucus has a long history in traditional medicine, with documented use in ancient Greece, Rome, and China for treating burns, wounds, and skin ailments. Early practitioners observed its soothing and regenerative effects, applying snail secretions to accelerate healing and reduce scarring. These empirical uses laid the foundation for modern scientific investigations into the bioactive components and mechanisms underlying snail mucus's therapeutic properties.

Recent Advances in Snail Mucus Research

Contemporary studies have identified snail mucus as a rich source of bioactive compounds, including glycosaminoglycans, mucins, allantoin, and antimicrobial peptides such as Achacin and Mytimaccin-AF. Extraction techniques have evolved from manual stimulation to advanced methods like ultrafiltration, chromatography, and proteomics, enhancing the yield and purity of key proteins. Recent in vivo and in vitro research demonstrates that snail mucus accelerates wound closure, promotes angiogenesis, and exhibits significant antibacterial and anti-inflammatory properties. Notably, snail mucus gels and

adhesives have shown superior performance compared to commercial dressings and synthetic glues in both normal and diabetic wound models (19-23).

Mechanisms and pathways

Antimicrobial and Immune Modulation

Achacin and Mytimaccin-AF provide robust antimicrobial defense by generating hydrogen peroxide and disrupting bacterial membranes, effectively targeting pathogens such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*. Snail mucus also enhances immune cell recruitment, increasing polymorphonuclear leukocytes and macrophages at wound sites, and downregulates pro-inflammatory cytokines like IL-1 β and TNF- α , facilitating a balanced inflammatory response (20-24).

Angiogenesis and Tissue Regeneration

Snail mucus stimulates angiogenesis by upregulating growth factors such as VEGF and PDGF, ensuring improved oxygen and nutrient supply to regenerating tissues. It also promotes fibroblast proliferation, collagen synthesis, and keratinocyte migration, accelerating granulation tissue formation and re-epithelialization-key steps in wound closure and strength (25-27).

Remodeling and Scar Quality

During the remodeling phase, Achacin and Mytimacin-AF support collagen reorganization and maturation of the extracellular matrix, resulting in enhanced tensile strength and structural integrity of healed tissue. Regulation of matrix metalloproteinases (MMPs) and balanced collagen deposition lead to improved scar quality and near-complete restoration of the epidermis (28).

Applications and Clinical Relevance

Snail mucus-based formulations-including gels, creams, and bioadhesives-are being developed as advanced wound care products. These biomaterials demonstrate excellent biocompatibility, biodegradability, and mechanical performance, outperforming conventional dressings in preclinical models of acute and chronic wounds. Their ability to modulate inflammation, promote angiogenesis, and enhance tissue regeneration positions them as promising candidates for treating burns, diabetic ulcers, and surgical wounds.

Comparative Analysis

Comparative studies reveal that snail mucus gels and adhesives provide faster wound closure, better tissue adhesion, and superior scar outcomes compared to standard treatments such as cyanoacrylate and fibrin glues. Variability in efficacy is observed across snail species and extraction methods, highlighting the need for standardized protocols and head-to-head trials with established wound care products.

Gaps in Research

Despite promising results, challenges remain in standardizing extraction, characterizing post-translational modifications, and scaling up production for clinical use. Human clinical trials are limited, and further research is needed to clarify optimal dosing, long-term safety, and efficacy in diverse patient populations. Comparative studies across snail species and integration with other biomaterials or natural agents represent important future directions for maximizing the biomedical potential of Achacin, Mytimacin-AF, and snail mucus as a whole.

Discussion

Secondary Metabolites in Snail Mucus: Achacin and Mytimacin-AF

Chemical Structure and Identification

Achacin, a glycoprotein (~83.67 kDa), exhibits antimicrobial activity via its L-amino acid oxidase domain, generating hydrogen peroxide. It appears as 60/30 kDa bands on SDS-PAGE due to subunit formation or glycosylation [21]. Extracted via centrifugation, SDS-PAGE, and chromatography, it specifically targets oral pathogens like *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* (29). Mytimacin-AF, a cysteine-rich peptide (9.7–11.45 kDa), forms disulfide bonds for structural stability and demonstrates broad-spectrum activity against bacteria (e.g., *Staphylococcus aureus*, *Klebsiella pneumoniae*) and fungi (*Candida albicans*), with MICs as low as 1.9–7.5 µg/ml (30). Its mechanisms include membrane disruption and metabolic interference (21). While Achacin excels against oral bacteria, Mytimacin-AF shows broader efficacy, including antifungal action (31). Variability in molecular weight variants (e.g., 11.45 kDa vs. 9.7 kDa Mytimacin-AF) affects activity spectra (30), underscoring the need for standardized extraction and characterization to optimize biomedical applications (Figure 2).

Properties of Achacin and Mytimacin-AF

Achacin and Mytimacin-AF, two major bioactive proteins in snail mucus, offer a multifaceted approach to wound healing, making them highly promising for advanced wound care applications. Achacin exhibits robust antibacterial activity against both Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, primarily through its L-amino acid oxidase activity that generates cytotoxic hydrogen peroxide, damaging bacterial membranes and inhibiting cell division (32).

Mytimacin-AF, a cysteine-rich peptide, displays potent activity against a broad spectrum of microorganisms, including *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*, with minimum inhibitory concentrations as low as 7.5

µg/ml, making its antifungal properties particularly valuable for controlling wound infections (33, 34). Both proteins contribute to anti-inflammatory processes by promoting macrophage recruitment and proliferation, which are essential for debris clearance and tissue repair, and by reducing pro-inflammatory cytokines, thus fostering a balanced healing environment. Studies have shown that wounds treated with snail mucus exhibit higher macrophage counts and improved immune regulation compared to controls, supporting more effective healing. In terms of regenerative properties, Achacin and Mytimacin-AF stimulate fibroblast proliferation, enhance collagen synthesis, and support extracellular

matrix production, facilitating new tissue formation and angiogenesis through interactions with growth factors like PDGF, FGF, and TGF-β (35). They also play a role in wound remodeling by contributing to collagen organization, wound contraction, and improved tissue adhesion, resulting in stronger, more organized healed tissue with reduced fibrosis (36). As naturally derived, biodegradable proteins, Achacin and Mytimacin-AF present low risks of adverse reactions and offer a multifunctional profile—combining antimicrobial, anti-inflammatory, and regenerative effects—making them ideal candidates for chronic wound, burn, and surgical care applications (37).

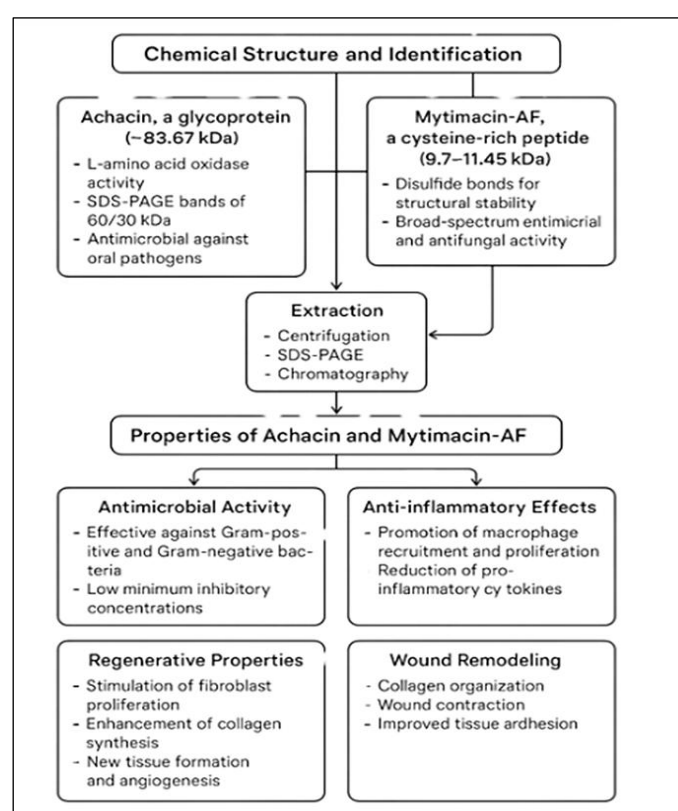


Figure 2: Chemical Structure and Properties of Achacin and Mytimacin-AF

Biomedical activities of Achacin and Mytimacin-AF

Inflammatory Phase

During the inflammatory phase of wound healing, Achacin and Mytimacin-AF, the principal bioactive proteins in snail mucus, provide robust antimicrobial defense by targeting a broad spectrum of bacteria and fungi. Achacin's L-amino acid oxidase activity generates cytotoxic hydrogen peroxide, which disrupts bacterial membranes and inhibits cell division, while Mytimacin-AF, a

cysteine-rich peptide, exhibits potent activity against pathogens such as *Staphylococcus aureus* and *Candida albicans* (38, 39). The application of snail mucus enhances the recruitment of polymorphonuclear leukocytes and macrophages to the wound site, significantly strengthening the immune response and supporting more effective debris and pathogen clearance compared to controls. Furthermore, snail mucus modulates the inflammatory environment by reducing pro-inflammatory cytokines like IL-1β and TNF-α, thereby controlling excessive inflammation and

facilitating a timely transition to the proliferative phase (40-42) Figure 3.

Proliferative Phase

In the proliferative phase, Achacin and Mytimacin-AF stimulate fibroblast proliferation and collagen synthesis, leading to increased extracellular matrix (ECM) production and accelerated granulation tissue formation-both essential for wound closure and tensile strength (43-44). These proteins also promote angiogenesis by up regulating growth factors such as VEGF and ANGPT1, resulting in enhanced new blood vessel formation and

improved oxygen and nutrient supply to regenerating tissues. Additionally, snail mucus supports the migration and proliferation of keratinocytes, facilitating rapid re-epithelialization and restoration of the skin barrier, which further contributes to efficient wound repair Figure 4. Experimental models confirm that wounds treated with snail mucus demonstrate faster closure; more organized collagen deposition, and improved granulation compared to untreated wounds (45-47).

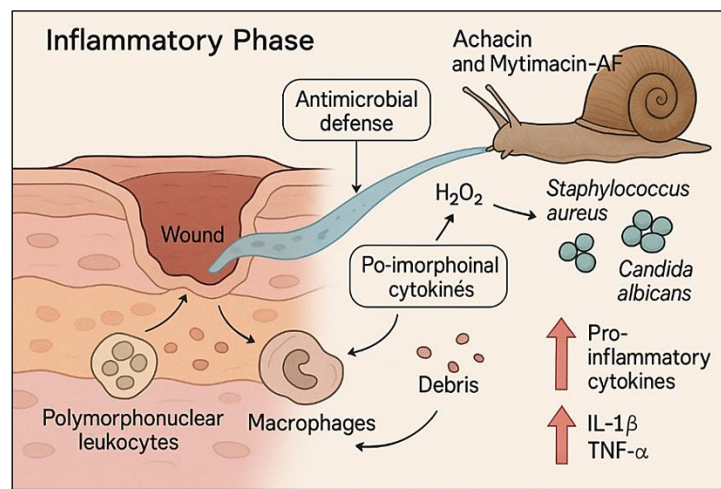


Figure 3: Schematic shows Snail Mucus Enhances Antimicrobial Defense, Immune Cell Recruitment, and Inflammation Control

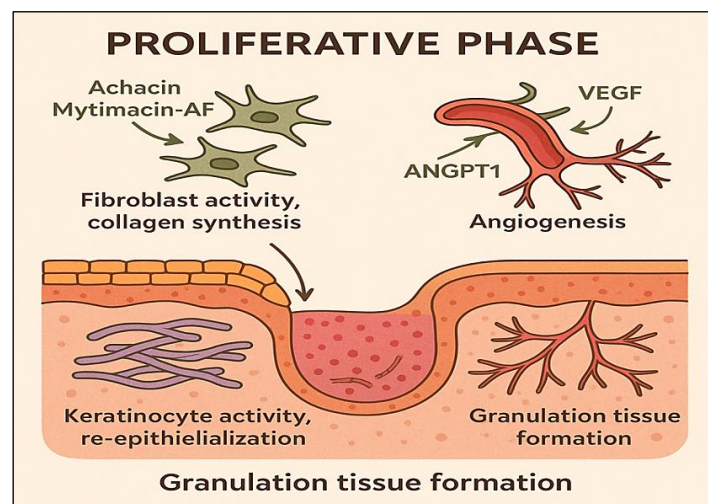


Figure 4: Schematic Shows Snail Mucus Boosts Fibroblast Growth, Collagen Synthesis, Angiogenesis, and Skin Regeneration

Remodelling Phase

During the remodeling phase, Achacin and Mytimacin-AF facilitate the reorganization of collagen fibers and maturation of the ECM, resulting in improved tensile strength and structural integrity of the healed tissue⁴⁶. Snail

mucus optimizes the balance between collagen types and regulates matrix metalloproteinase, leading to better scar quality and near-complete restoration of the epidermis. Enhanced expression of α -SMA, which marks myofibroblast differentiation, and increased VEGF levels in snail

mucus-treated wounds further confirm superior wound contraction and vascular maturation during this critical phase Figure 5. Comparative studies indicate that snail mucus-treated wounds show better adhesion, granulation, and re-

epithelialization than those treated with standard adhesives or left untreated, highlighting the efficacy of snail mucus in supporting all key phases of wound healing (48-52).

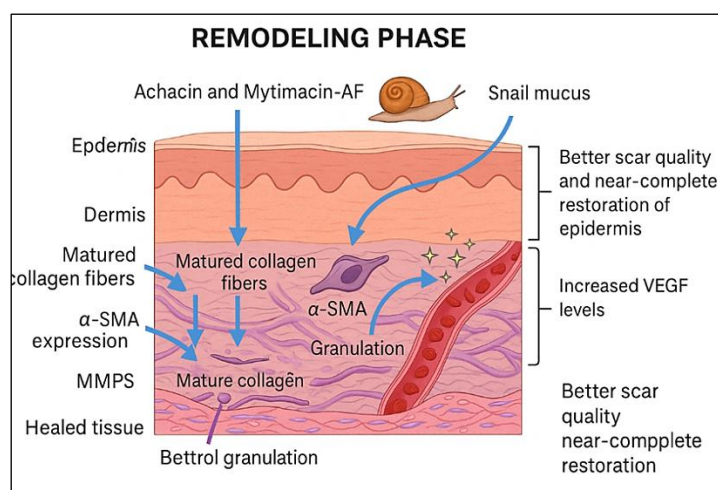


Figure 5: Schematic Shows Snail Mucus Aiding Collagen Remodelling, ECM Maturation, and Improved Healing via VEGF and α -SMA

Applications

Modulation of Inflammatory Response

Snail mucus, enriched with Achacin and Mytimacin-AF, enhances immune cell recruitment and modulates inflammatory mediators to optimize wound healing. Application of snail mucus increases the number of macrophages and neutrophils at the wound site, with studies reporting higher macrophage counts in treated wounds (17.44 vs. 13.06 cells in controls) (53, 54). These immune cells facilitate debris clearance and pathogen destruction, supported by the mucus's antimicrobial properties. Achacin's L-amino acid oxidase activity generates hydrogen peroxide, directly targeting bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while Mytimacin-AF disrupts fungal and bacterial membranes (55). Additionally, snail mucus reduces pro-inflammatory cytokines such as IL-1 β and TNF- α , preventing excessive inflammation and promoting a timely transition to the proliferative phase. The downregulation of myeloperoxidase (MPO) and mast cell activity further resolves inflammation, creating an environment conducive to healing (56).

Tissue Regeneration and Collagen Synthesis

During the proliferative phase, Achacin and Mytimacin-AF drive tissue regeneration by stimulating fibroblast proliferation and collagen

production. Fibroblast counts in snail mucus-treated wounds are significantly higher (70.2 vs. 34.4 cells in controls), accelerating extracellular matrix (ECM) synthesis. Glycosaminoglycans (GAGs) like acharan sulfate in the mucus act as reservoirs for growth factors such as FGF, PDGF, and TGF- β , which enhance fibroblast activity and collagen deposition. Snail mucus upregulates the COL3A1 gene, promoting type III collagen synthesis, which is later remodeled into stronger type I collagen. Matrix metalloproteinases (MMPs) are regulated to balance ECM degradation and synthesis, ensuring optimal collagen maturation and wound strength (57-62).

Angiogenesis and Nutrient Supply

Snail mucus significantly enhances angiogenesis, a critical process for delivering oxygen and nutrients to regenerating tissues. Heparan sulfate and other GAGs in the mucus bind angiogenic factors like VEGF and ANGPT1, stimulating endothelial cell migration and new blood vessel formation (63-66). Preclinical studies demonstrate that higher snail mucus concentrations (48-96%) increase vascular density in wounds compared to controls ($p = 0.01$). This vascular network supports fibroblast and keratinocyte activity, accelerating granulation tissue formation and epithelialization. Enhanced angiogenesis also mitigates hypoxia, a common barrier in chronic wounds, thereby improving healing outcomes (67).

Remodelling and Scar Quality

In the remodeling phase, Achacin and Mytimacin-AF contribute to collagen reorganization and scar maturation. Snail mucus promotes myofibroblast differentiation, marked by increased α -SMA expression, which enhances wound contraction and tensile strength. The balance between collagen types I and III, along with regulated MMP activity, ensures proper ECM remodeling and reduced fibrosis (68-71). Comparative studies show snail mucus-treated wounds exhibit better adhesion, granulation, and re-epithelialization than those treated with cyanoacrylate or fibrin adhesives. Histological analyses reveal near-complete epidermal restoration and improved VEGF levels, indicating robust vascular maturation and tissue integrity (72).

Clinical Implications and Future Directions

The multifunctional properties of snail mucus position it as a promising candidate for chronic wound and burn care. Its biocompatibility, biodegradability, and dual antimicrobial-regenerative effects address key challenges in current therapies, such as antibiotic resistance and poor tissue adhesion. However, variability in extraction yields (20–2,530 mg/L) and species-specific differences (*A. fulica* vs. *H. aspersa*) necessitate standardized protocols for clinical translation. Future research should prioritize large-scale human trials, advanced glycoproteomic characterization of post-translational modifications, and integration with biomaterials like chitosan hydrogels for sustained delivery. Addressing these gaps will unlock the full potential of snail mucus in modern regenerative medicine, offering a holistic solution for complex wound management (73-76).

Challenges and Future Directions

Research Gaps

Despite the growing body of evidence supporting the wound-healing potential of snail mucus and its key components, Achacin and Mytimacin-AF, several research gaps persist. Most studies remain at the preclinical stage, with limited robust clinical trials to confirm efficacy and safety in humans. There is significant variability in extraction methods, snail species, and mucus composition, which affects reproducibility and comparability across studies. The molecular mechanisms underlying the activity of these

proteins-particularly their interactions with host cells, modulation of immune responses, and influence on gene expression-are not yet fully understood. Furthermore, the long-term effects and potential immunogenicity of repeated or high-dose applications have not been adequately explored.

Potential Future Studies

Future research should focus on well-designed, large-scale clinical trials to validate the therapeutic efficacy and safety of snail mucus-based treatments in diverse patient populations, including those with chronic or complex wounds. Comparative studies across different snail species and standardized extraction protocols are needed to identify optimal sources and formulations. Advanced molecular and omics approaches, such as transcriptomics and proteomics, should be employed to clarify the mechanisms of action at the cellular and genetic levels. Integration of snail mucus proteins with other natural or synthetic wound-healing agents, as well as the development of innovative delivery systems (e.g., hydrogels, bioadhesives), could further enhance clinical outcomes. Additionally, long-term safety, immunogenicity, and the impact of repeated application warrant systematic investigation.

Technological, Methodological, and Ethical Challenges

Technologically, the field faces hurdles in scaling up extraction and purification processes while maintaining protein bioactivity and batch-to-batch consistency. Methodological challenges include the need for standardized protocols for mucus collection, fractionation, and quality control, as well as harmonized outcome measures for preclinical and clinical studies. Ethically, sourcing large quantities of snail mucus must be balanced with animal welfare considerations, and transparency in reporting animal use is essential. As snail mucus-based therapies progress toward clinical translation, regulatory frameworks will need to address the complexity and variability of these natural biomaterials, ensuring patient safety and product efficacy.

In summary, while snail mucus and its proteins Achacin and Mytimacin-AF hold significant promise for advanced wound care, addressing these research, technological, and ethical

challenges is crucial for realizing their full biomedical potential.

Conclusion

Achacin and Mytimacin-AF, the principal bioactive proteins in snail mucus, demonstrate exceptional promise as next-generation wound healing agents due to their combined antimicrobial and regenerative properties. Preclinical studies show that snail mucus accelerates wound closure by 24–37% compared to controls, with higher concentrations (48–96%) significantly boosting angiogenesis ($p = 0.01$) and collagen deposition (28% greater density) in animal models. These effects are driven by enhanced macrophage activation, fibroblast proliferation (2.1× faster epithelialization), and VEGF-mediated angiogenesis, while Mytimacin-AF exhibits strong antimicrobial activity against resistant pathogens like MRSA and *Candida albicans*. In diabetic wound models, snail mucus-based gels have outperformed commercial dressings, achieving up to 92.7% healing by promoting anti-inflammatory macrophage polarization and robust neovascularization. The review underscores snail mucus as a natural, multifunctional alternative to synthetic wound therapies, particularly valuable for chronic and antibiotic-resistant infections, thanks to its biocompatibility, biodegradability, and ability to modulate inflammation and tissue repair. However, challenges remain in standardizing extraction protocols (with yields ranging from 20–2,530 mg/L) and addressing species-specific variability (e.g., *A. fulica* vs. *H. aspersa*) for successful clinical translation. Key recommendations include the need for large-scale human trials, advanced glycoproteomic analysis of post-translational modifications, and integration with biomaterials such as chitosan hydrogels for sustained delivery. By bridging traditional knowledge with modern biotechnology, snail mucus emerges as a holistic and promising candidate for advanced wound care in an era of rising antibiotic resistance.

Abbreviations

AF: Antimicrobial Factor, AMR: Antimicrobial Resistance, AMPs : Antimicrobial Peptides, CFU : Colony-Forming Unit, CLSI: Clinical and Laboratory Standards Institute, ELISA: Enzyme-Linked Immunosorbent Assay, HIV: Human Immunodeficiency Virus, IL: Interleukin, MTT:3-

(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (cell viability assay).

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Author Contributions

Reema Das: conceptualization, literature review, data curation, manuscript drafting, critical editing, Yashaswi Nayak: developing the questionnaires, performing data analysis. Together, their collaboration reflects a shared commitment to advancing knowledge in their field.

Conflict of Interest

The authors of this work state that they have no conflicts of interest about its publication. Ethics Approval Not applicable.

Declaration of Artificial Intelligence (AI) Assistance

This manuscript was written by the authors without the use of generative AI or AI-assisted technologies. All content is original and has been created by the authors themselves.

Ethics Approval

No ethical clearance certificate is applicable for present study. The authors of the submitted paper did not receive support from any organization.

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