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Characterisation of Microbial Property of Polyvinyl Alcohol Hydrogel Synthesised via Crosslinking Method with Incorporation of Freeze-Dried Snail Mucin

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Abstract

Wound healing has been extensively studied, where nowadays dressings accumulate bacteria growth and not permeable to the skin, causing reducing wound healing process. Hence, a wound healing material with bioactive compound containing antimicrobial property and high water absorption is highly sought. This study investigates a hydrogel from polyvinyl alcohol (PVA) and then was incorporated with freeze-dried snail mucin for enhanced properties in retaining moisture contain of skin, hydrating, and possessing antimicrobial property. The hydrogels were synthesized using a freeze-thaw crosslinking method with varying concentrations of snail mucin (1–5%), freezing durations (18–21 hours) at -75°C and thawing at room temperature for 24 hours. The prepared hydrogels were characterised for water absorption, adhesion force, and antimicrobial activity. Water absorption was measured gravimetrically by immersing the hydrogel samples in distilled water for 1 hour, adhesion was tested using ASTM F2255 standards, and antimicrobial activity was tested on *Staphylococcus epidermidis* using disk diffusion assays. The optimal formulation, containing 3% snail mucin and subjected to an 18-hour freeze-thaw cycle, demonstrated the highest water absorption capacity (80.48%) while maintaining strong adhesion (5.01 N). This result highlights a well-balanced interplay between enhanced hydrophilicity and preserved structural integrity. However, limited antimicrobial activity was observed, attributed to hydrogel changing structure during incubation and low mucin concentration. This study highlights the potential of snail mucin-loaded PVA hydrogels as an advanced wound care material, with significant improvements in water absorption and adhesion properties. Future research should focus on enhancing antimicrobial activity and hydrogel stability to ensure no bacteria growth on the hydrogel itself since it has high water content.

1. Introduction

Wound healing is a critical aspect of healthcare, addressing challenges posed by burns, surgical wounds, and chronic injuries. Regenerative hydrogels, primarily consisting of water-retentive polymers, have gained significant interest in the field of wound healing, especially for treating skin injuries [1]. Hydrogels, with their tunable physical and chemical properties, can replicate the extracellular matrix, making them highly promising for tissue regeneration and disease treatment by supporting cell growth, migration, and differentiation [2].

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However, conventional wound dressing often lack the necessary bioactivity and mechanical properties required for optimal wound healing, underscoring the need for innovative solutions to improve patient outcomes.

Existing research highlights the versatility of hydrogels in biomedical applications, particularly those based on polyvinyl alcohol (PVA). Studies have demonstrated PVA's excellent biocompatibility, high hydrophilicity, and biodegradability, making it an ideal candidate for hydrogel synthesis [3,4]. Physical cross-linking techniques for hydrogel synthesis provide a safer alternative that eliminates the toxicity concerns associated with chemical cross-linking agents [5]. The PVA polymer networks form intermolecular and intramolecular hydrogen bonds, as well as crystallites in which the PVA chains aggregate with ice crystals during the freezing process [6]. Additionally, snail mucin, a natural secretion rich in bioactive compounds, has shown promise in promoting skin regeneration, providing antimicrobial effects, and enhancing hydration [7]. While PVA hydrogels have been extensively studied, limited research exists on their combination with snail mucin to address challenges such as improving water absorption and adhesion while incorporating bioactive functionality. Furthermore, methodologies like freeze-thaw crosslinking and the incorporation of freeze-dried snail mucin require optimization for practical applications.

Although there are encouraging results, there are still gaps in our comprehension of the synergistic benefits of merging PVA hydrogels with snail mucin. Recent research has aimed to improve the performance of PVA hydrogels through various approaches, such as developing composite materials, incorporating nanostructures, and designing multi-network systems [8]. However, most studies focus on individual components rather than exploring their combined potential. Moreover, the lack of standardized methods for optimizing such hydrogels limits their applicability in clinical settings. Hydrogels can be synthesized using physical crosslinking, chemical crosslinking, or hybrid methods. Among these, physical crosslinking via freeze-thaw cycles is an eco-friendly approach that eliminates the need for toxic crosslinking agents. However, its effectiveness in incorporating bioactive compounds, such as snail mucin, remains insufficiently explored. This method relies on the formation of physical interactions, including electrostatic forces, hydrogen bonding, and pH- or temperature-dependent responses. Due to its mild processing conditions and reversible gelation, physical crosslinking is particularly advantageous for bio-extrusion applications [9].

The goal of the present study is to develop and optimize PVA hydrogels incorporating snail *Achatina fulica* mucin (SAFuM) using a freeze-thaw crosslinking method. Specifically, we aim to enhance the hydrogels' adhesion and water absorption properties, making them suitable for advanced wound care applications. We hypothesize that the integration of snail mucin will improve the bioactivity, water-absorbing capacity, and adhesion strength of the PVA hydrogels. This research predicts that the optimized hydrogel formulation will outperform conventional hydrogels in wound healing efficacy, paving the way for innovative solutions in biomedical materials.

2. Methodology

2.1 Material

This study utilized Polyvinyl Alcohol (PVA) (average molecular weight 85,000–124,000, 87–89% hydrolyzed) purchased from Sigma-Aldrich and commercial freeze-dried snail mucin powder sourced from Xi'an Lanshan Biotechnology Co., Ltd. Distilled water was used as the solvent. Equipment included a weighing machine (METTLER TOLEDO) for precise material measurements, a Haier Biomedical Ultra-Low Temperature Freezer for conducting freeze-thaw cycles.

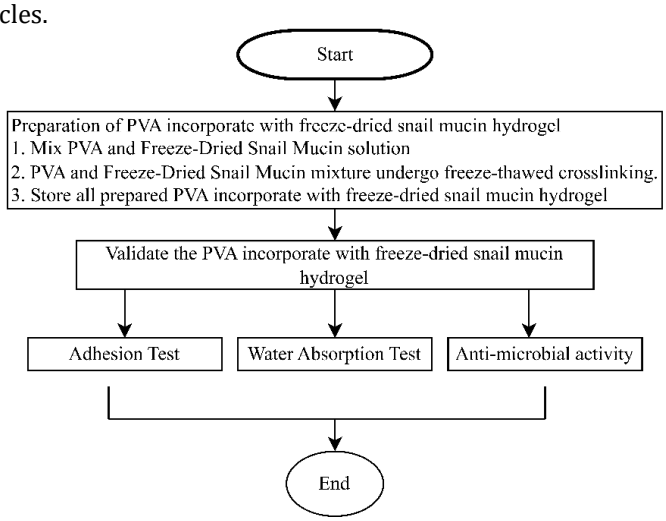

***Fig. 1*:** A flowchart containing the process of producing hydrogel with addition of SAFuM

2.2 Preparation of PVA Hydrogels with Freeze-dried Snail Mucin

The preparation process involved three main steps. First, PVA was dissolved in distilled water at approximately 95°C while being stirred vigorously until a homogeneous solution was obtained. Once the solution cooled to room temperature, freeze-dried snail mucin loadings (1-5wt%) was added and thoroughly mixed to ensure uniform incorporation. The resulting mixture was poured into molds and subjected to 1 freeze-thaw cycles, which involved freezing at -75°C followed by thawing at room temperature for 24 hours. This process facilitated physical crosslinking, producing hydrogels with desired structural properties.

2.2.1 Water absorption

Hydrogel samples (4 cm x 2 cm) were immersed in 15 ml of distilled water in labeled 100 ml cups. After weighing the samples, they were soaked for 1 hour. Post-immersion, the samples were gently dried with a lint-free cloth and re-weighed to determine the water uptake. The materials used included distilled water, hydrogel samples, and equipment such as a weighing machine, measuring cylinder, and plastic cups.

2.2.2 Adhesion Forces

The peel adhesion strength of PVA hydrogel incorporated with freeze-dried snail mucin was evaluated using ASTM F2255 standards. Hydrogel samples (2.5 cm x 1 cm) were tested with a Hot Tack Tester, where the samples were placed between two plastic surfaces. The test, conducted at a pull speed of 100 mm/s, measured the force required to separate the bonded materials. Results were shown as a graph of load (N) versus time (s).

2.3 Characterization of Optimised PVA Hydrogels with Freeze-dried Snail Mucin

2.3.1 Antimicrobial Testing

Antimicrobial activity was evaluated using disk diffusion assays with Staphylococcus epidermidis as the test microorganism. Hydrogel samples and freeze-dried snail mucin were placed on agar plates inoculated with bacteria, incubated at 37°C, and zones of inhibition were measured after 24 and 96 hours.

3. Results and Discussion

3.1 Water Absorption

Three hydrogel samples were prepared with a consistent PVA concentration of 35% (wt%), while the loading of freeze-dried snail mucin and freezing times were varied. Sample 1 contained 1% (wt%) mucin and underwent freezing for 21 hours, Sample 2 had 3% (wt%) mucin with an 18-hour freezing time, and Sample 3 included 5% (wt%) mucin with a 21-hour freezing time. The water absorption response for the samples was 46.15%, 80.48%, and 34.2%, respectively. The water absorption of hydrogels is facilitated by the hydroxyl (OH) groups in PVA, which interact with water molecules through hydrogen bonding, as well as by the porous network structure within the hydrogel [10]. The ability to form hydrogen bonds plays a key role in determining the gelation process, as well as the stability and swelling properties of the resulting gels [11]. After reaching maximum water absorption, higher concentrations of PVA hydrogel exhibit slower water loss rates [12]. The incorporation of mucin into PVA hydrogel also show more pore compare to Pure PVA hydrogel in SEM results [13]. These pores allow water to penetrate and fill the spaces, thereby increasing the overall water absorption capacity.

The results indicate that mucin loading and freezing time significantly influence the hydrogel's water absorption capacity. Sample 2, with 3% mucin and a shorter freezing time of 18 hours, exhibited the highest water absorption (80.48%), suggesting an optimal balance between hydrophilicity and structural integrity. In contrast, Sample 1 demonstrated a moderate water absorption of 46.15%, which may be attributed to the lower mucin loading and longer freezing time, resulting in a less hydrophilic network. Sample 3, despite the highest mucin loading (5%), showed the lowest water absorption (34.2%), likely due to excessive mucin disrupting the PVA network and reducing porosity.

These findings highlight the importance of optimizing mucin loading and freezing time to enhance hydrogel performance. A moderate mucin concentration at 3% and controlled freezing duration appear crucial for achieving desirable hydrophilic properties while maintaining the structural stability of the hydrogel. Further investigations may explore intermediate freezing times and mucin loadings around the 3% range to refine the hydrogel's water absorption capabilities.

Table 1: water absorption results

Sample	PVA Concentration (wt. %)	Loading of Freeze-Dried Snail Mucin (wt. %)	Freezing Times (hours)	Water Absorption (%)
1	35	1	21	46.15
2	35	3	18	80.48
3	35	5	21	34.2

3.2 Adhesion Performance

The skin adhesion strength of the PVA film is low relative to commercial adhesives as it is highly hydrophilic and water soluble [14]. PVA is inherently brittle, necessitating its combination with other polymers or modification through copolymerization to enhance its properties for use as a wound dressing [15]. The adhesion force of the polyvinyl alcohol (PVA) hydrogels incorporating freeze-dried snail mucin was measured for all three samples, revealing significant variations based on mucin loading and freezing time. Sample 1, with 1% mucin and a freezing time of 21 hours, exhibited the lowest adhesion force at 0.01 N. This result suggests limited surface interactions and bonding potential, likely due to the low mucin content.

Sample 2, with 3% mucin and an 18-hour freezing time, demonstrated the highest adhesion force at 5.01 N. This significant increase in adhesion force indicates enhanced surface interactions, possibly attributed to the optimal mucin concentration improving both hydrophilicity and interfacial bonding capabilities. In contrast, Sample 3, with 5% mucin and a freezing time of 21 hours, displayed a reduced adhesion force of 0.24 N. The decline in adhesion force at higher mucin loading could result from structural disruption or phase separation, which diminishes effective bonding surfaces.

These findings suggest that adhesion force is highly dependent on mucin loading, with an optimal concentration around 3% providing maximum adhesion. Excessive mucin incorporation, as seen in Sample 3, may compromise structural integrity and reduce adhesion performance. Further studies can investigate the relationship between adhesion force, structural morphology, and mucin distribution within the hydrogel matrix to optimize its adhesive properties.

Table 2: adhesion force results

Sample	PVA Concentration (wt. %)	Loading of Freeze-Dried Snail Mucin (wt. %)	Freezing Times (hours)	Adhesion Force (N)
1	35	1	21	0.01
2	35	3	18	5.01
3	35	5	21	0.24

3.3 Antimicrobial Testing

3.3.1 Polyvinyl Alcohol (PVA) hydrogel with freeze-dried snail mucin

The antimicrobial activity of the mucin-loaded hydrogel was tested against Staphylococcus epidermidis, but the results revealed no significant antimicrobial effects. Observations from agar plates over a 96-hour period showed continued bacterial growth, as indicated by a cloudy yellow zone around the hydrogel. This lack of antimicrobial efficacy may be attributed to the changing structure of hydrogel samples during incubation at 37°C, which compromised their structural integrity. Elevated temperatures can disrupt hydrogen bonds and microcrystalline regions, causing the physical cross-linked PVA hydrogel to melt and revert to a solution state [16]. At 37 °C, the dissociation of intramolecular and intermolecular hydrogen bonds in PVA, along with the melting of PVA microcrystals, disrupts the physical cross-linking points within the hydrogel [17]. The low concentration of freeze-dried snail mucin may have been insufficient to inhibit bacterial growth effectively.

To address these limitations, future studies should increasing the concentration of snail mucin or combining it with other antimicrobial agents could also improve efficacy. Testing against a broader range of microorganisms and refining incubation conditions could provide further insights into the antimicrobial potential of the hydrogels. Despite the observed limitations, the study highlights the significant potential of mucin-loaded PVA hydrogels for various biomedical applications, including wound care and tissue engineering.

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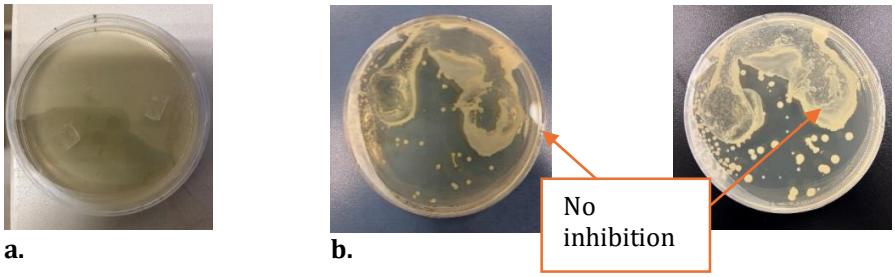

a. b.

Fig. 2: a) agar with PVA hydrogel incorporate with 3.81% freeze-dried snail mucin before incubating. b) nutrient agar with PVA hydrogel incorporates with 3.81% freeze-dried snail mucin after incubating 24 hours c) nutrient agar with PVA hydrogel incorporate with 3.81% freeze-dried snail mucin after incubating 96 hours.

3.3.2 Polyvinyl Alcohol (PVA) hydrogel

In the first plate (a), the agar contains the PVA hydrogel before any incubation. This serves as a baseline, providing the initial state of the sample. The second plate (b) depicts the nutrient agar with the PVA hydrogel after 24 hours of incubation. The agar around the hydrogel exhibits a cloudy, yellow appearance, suggesting the presence and growth of microorganisms. Moving to the third plate (c), the nutrient agar with the PVA hydrogel is shown after 96 hours of incubation. The cloudy, yellow area around the hydrogel has become more pronounced, indicating an increase in microbial growth over the extended incubation period.

The presence and proliferation of Staphylococcus epidermidis around the hydrogel locations imply that the PVA formulation was unable to effectively inhibit the growth of this common skin pathogen, even after prolonged incubation. PVA hydrogels inherently lack antibacterial properties [18].

These findings suggest that the PVA hydrogel did not exhibit effective antimicrobial activity under the tested conditions, as S. epidermidis continued to grow and proliferate around the hydrogel. A notable limitation of the experiment was the changing structure of the hydrogel samples during incubation. Elevated temperatures can disrupt hydrogen bonds and microcrystalline regions, causing the physical cross-linked PVA hydrogel to melt and revert to a solution state [16]. At 37 °C, the dissociation of intramolecular and intermolecular hydrogen bonds in PVA, along with the melting of PVA microcrystals, disrupts the physical cross-linking points within the hydrogel [17]. This likely altered their physical and chemical properties, compromising the ability to provide a stable antimicrobial environment.

To further explore the antimicrobial potential of the PVA hydrogel, additional studies would be necessary. Evaluating the hydrogel against a broader range of clinically relevant microorganisms, testing different hydrogel formulations, and optimizing the incubation parameters could provide valuable insights into the antimicrobial spectrum and the underlying mechanisms of this system.

a. b. c.

Fig. 3: a) agar with PVA hydrogel before incubating. b) nutrient agar with PVA hydrogel after incubating 24 hours c) nutrient agar with PVA hydrogel after incubating 96 hours.

3.3.3 Freeze-dried Snail Mucin

The figure presents the results of an experiment investigating the antimicrobial activity of freeze-dried snail mucin powder against the common skin bacterium, Staphylococcus epidermidis. Three agar plates are shown, each at a different stage of the experiment. The first plate (a) shows the agar with the freeze-dried snail mucin powder before any incubation. The red circles indicate the specific locations where the powder was placed on the agar surface, serving as a baseline for the experiment. The second plate (b) shows the nutrient agar with the freeze-dried snail mucin powder after a 24-hour incubation period. The red circle marks the location of the powder, and the cloudy, yellow appearance surrounding it suggests the growth and proliferation of S. epidermidis bacteria. Progressing to the third plate (c), the nutrient agar with the freeze-dried snail mucin powder is shown

after a 96-hour incubation. The red circles again indicate the powder's location, and the cloudy, yellow area has become more pronounced, implying increased bacterial growth over the extended incubation time.

These results indicate that the freeze-dried snail mucin powder did not exhibit effective antimicrobial activity against the Staphylococcus epidermidis bacteria under the experimental conditions. The presence and proliferation of the bacteria around the powder locations suggest that the snail mucin powder was unable to inhibit the growth of this common skin pathogen, even over an extended incubation period.

Further investigation would be necessary to fully understand the antimicrobial potential of snail mucin and to explore any specific formulations, concentrations, or other factors that may enhance its ability to inhibit the growth of Staphylococcus epidermidis and other clinically relevant microorganisms. Studying the biochemical composition of snail mucin and its interactions with bacterial cell structures or metabolic processes could provide valuable insights into its antimicrobial mechanisms and potential applications.

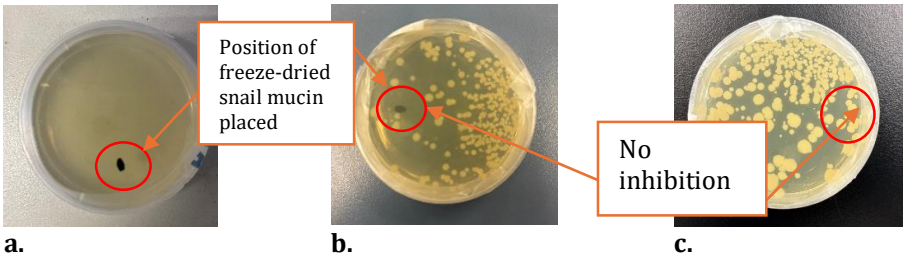

***Fig. 4*:** a) agar with freeze-dried snail mucin before incubating. b) nutrient agar with freeze-dried snail mucin after incubating 24 hours c) nutrient agar with freeze-dried snail mucin after incubating 96 hours.

4. Conclusion

This study explored the development and optimization of polyvinyl alcohol (PVA) hydrogels incorporating freeze-dried snail mucin for advanced wound care applications. The findings demonstrate that the integration of snail mucin significantly influences the hydrogels' water absorption, adhesion properties, and antimicrobial activity.

The optimized hydrogel formulation, consisting of 3% snail mucin with an 18-hour freeze-thaw cycle, exhibited the highest water absorption (80.48%) and adhesion force (5.01 N). These results highlight the importance of balancing mucin loading and freezing time to achieve desirable hydrophilic and adhesive properties. However, the antimicrobial testing revealed limitations, with both the hydrogels and freeze-dried snail mucin showing minimal efficacy against *Staphylococcus epidermidis*. This lack of antimicrobial activity was likely due to hydrogel changing structure during incubation and insufficient mucin concentration.

The study shows that PVA hydrogels have great potential for wound healing, especially when enhanced with bioactive compounds like snail mucin, even with some challenges. Future work should focus on improving thermal stability, optimizing mucin concentration, and incorporating additional antimicrobial agents to enhance the hydrogel's performance. By addressing these aspects, the formulation could bridge the gap between innovative material design and clinical application, contributing to the advancement of wound care technologies.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Kuan Tyng Yih, Aliff Hisyam; data collection: Kuan Tyng Yih; analysis and interpretation of results: Kuan Tyng Yih, Aliff Hisyam, Rama Yusvana; draft manuscript preparation: Kuan Tyng Yih, Aliff Hisyam.

Appendix A: An Example

Authors including an appendix section should do so before the References section. Multiple appendices should all have headings in the style used above. They will automatically be ordered A, B, C etc.

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