Plant Science Review

PLANT SCIENCE REVIEW

Review Article

Journal homepage: https://psr.crcjournals.org/

ISSN No: 3041-7279

Check for updates

Macromolecular Architecture of Self-Healing Bio-Films Incorporating Cryoprotective Additives: Biopolymer Design Strategies for Plant Wound and Frost Management — A Comprehensive Review

Ridhima, and Jatinder Singh*

Department of Horticulture, School of Agriculture, Lovely Professional University Phagwara, Punjab, 144401, India

04 June 2026: Received | 06 July 2026: Revised | 03 August 2026: Accepted | 05 September 2026: Available Online

*Corresponding Author: **Jatinder Singh** | Email Address: **jatinder.19305@lpu.co.in**

Citation: Ridhima, and Jatinder Singh (2026). Macromolecular Architecture of Self-Healing Bio-Films Incorporating Cryoprotective Additives: Biopolymer Design Strategies for Plant Wound and Frost Management — A Comprehensive Review. ***Plant Science Review***. **DOI: https://doi.org/10.51470/PSR.2026.07.02.01**

Abstract

Plant wounds created by pruning, grafting, mechanical damage, or pest activity remain persistently vulnerable to microbial invasion and environmental stress, particularly during cold and transitional seasons. Most traditional wound sealants are inert, petroleum based and unable to adapt to the changing conditions that plants encounter following wounding. This review outlines another approach: the design of bio-based macromolecular systems, which are able to physically protect plant wounds, and also serve as integrated cryoprotection against frost damage. Combining current knowledge on the structural biology of antifreeze proteins (AFPs) and their ice-binding mechanisms, on the self-healing chemistry of biopolymer networks (chitosan, alginate, bacterial cellulose and curli-fiber composite), and on the macromolecular composition of microbial extracellular polymeric substances (EPS), we arrive at a present knowledge about how these macromolecular building blocks could be combined into multifunctional systems for wound protection. PubMed, Web of Science and Scopus (2000–2025) databases were searched systematically: The search terms were 'antifreeze proteins', 'self-healing hydrogels', 'biopolymer wound dressings', 'extracellular polymeric substances' and 'plant cryoprotection'. We critically examine AFP structural features particularly the β-roll ice-binding domains of cereal AFPs and the leucine-rich repeat (LRR) motifs of carrot AFP and compare their thermal hysteresis (TH) and ice recrystallisation inhibition (IRI) activities. We then review the dynamic covalent and supramolecular mechanisms underlying self-healing in biopolymer networks (Schiff base, disulfide, hydrogen bonding, catechol metal coordination). The integration of AFPs into hydrogel matrices is discussed as a proposed research direction, with honest acknowledgement that combined AFP hydrogel systems have not yet been validated in plant wound contexts. Key challenges including AFP production cost, in planta stability, phytotoxicity risk, and regulatory considerations are addressed directly. This review positions the International Journal of Biological Macromolecules readership to identify the specific macromolecular design questions that must be answered before these integrated systems can advance from concept to application.

Keywords: *Antifreeze proteins, self-healing hydrogels, biopolymers, chitosan, alginate, extracellular polymeric substances, ice recrystallisation inhibition, plant cryoprotection, wound management.*

1. Introduction

Plants are continuously exposed to physical damage from pruning, grafting, storm injury, pest attack, and mechanical harvesting. Unlike animals, which can mount rapid vascular and cellular repair responses, woody plants are limited to compartmentalisation the formation of chemical and physical barriers that contain decay and prevent its spread [1]. Pruning wounds in orchards and vineyards are among the most economically significant wound types because they are large, deliberate, and often created during the periods of highest pathogen spore dispersal, typically in wet autumn and winter conditions [2]. Wood-decay fungi including Eutypa lata, Phomopsis viticola, and Neonectria species exploit these entry points, causing canker diseases responsible for significant annual losses in perennial crops [3].

Wound protection products currently available to growers fall into two broad categories: petroleum-based wound paints that provide a physical barrier and chemical fungicide treatments.

© 2026 by the authors. This is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Both have proven shortcomings the former are persistent in the environment and generally are prone to cracking during thermal cycling the latter have been associated with build-up of residues, regulatory withdrawal and resistance development [4]. Thus, there is a real need for the development of biologically active and biodegradable wound protection materials for a practical benefit.

Over the past decade, there has been significant interest on the use of bio-derived macromolecular materials such as polysaccharides and proteins as functional wound dressings in human medicine [5][6]. The biocompatibility, tunable mechanical properties and natural antimicrobial activity of chitosan, alginate, bacterial cellulose and protein-based hydrogels have all been studied [7] [8]. AFPs are also known to inhibit ice recrystallisation under sub-freezing temperatures, and the structural details of the AFPs have also progressed significantly, revealing unique ice-binding β-roll structures in cereal AFPs and leucine-rich repeats (LRR) in carrot AFP which allow them to carry out this function with remarkable efficiency [9][10].

The combination of these two lines of research self-healing biopolymer networks and cryoprotective macromolecules provides a conceptually promising design space: wound protection systems capable of physically sealing wounds, self-healing to prevent secondary damage, releasing antimicrobial agents and protecting the sensitive wound tissues from the effects of freezing. But this convergence has not yet been realized in any plant wound experiment. In this review, the available knowledge of the macromolecular science is also reviewed, and the structural and functional properties that make each class of component promising are identified, with the specific questions that must be answered to make such integrated systems a reality.

The review is specifically directed at the readership of the International Journal of Biological Macromolecules because the scientific questions are fundamentally macromolecular in nature: which AFP structural motifs retain activity when embedded in a polymer matrix? What crosslinking chemistry is compatible with both self-healing efficiency and AFP stability? How does polysaccharide chain length and degree of substitution affect AFP diffusion through a hydrogel? These are questions of macromolecular structure-function relationship, and answering them requires the analytical toolkit spectroscopy, calorimetry, molecular dynamics simulation that this journal's community routinely applies.

1.1 Search Strategy

A structured literature search was conducted in PubMed, Web of Science, and Scopus, covering publications from January 2000 to April 2025. The following search terms were used, individually and in Boolean combination: 'antifreeze protein', 'ice recrystallisation inhibition', 'self-healing hydrogel', 'biopolymer wound dressing', 'extracellular polymeric substance', 'chitosan wound', 'alginate wound', 'plant cryoprotection', 'curli fibre', and 'pruning wound protection'. Inclusion criteria required peer-reviewed primary research or systematic reviews published in English. Sources were excluded if they were grey literature, conference abstracts without corresponding publications, or commercial product documentation. The resulting reference pool was supplemented by manual cross-referencing of highly cited reviews in each sub-field.

2. Extracellular Polymeric Substances: The Macromolecular Scaffold of Biofilms

2.1 Composition and Structural Organisation

Biofilms are structured communities of microorganisms embedded within a self-produced matrix of extracellular polymeric substances (EPS). The EPS matrix is biochemically complex it contains polysaccharides, proteins, extracellular DNA (eDNA), and lipids, typically in ratios that vary by organism and growth conditions [11][12]. From a macromolecular perspective, the structural integrity of biofilms depends on physical entanglement and electrostatic interactions between these components rather than covalent crosslinking, which is why biofilms display viscoelastic rather than purely elastic mechanical behaviors [13].

Polysaccharides form the primary volumetric component of most biofilm matrices. In Pseudomonas aeruginosa, three distinct exopolysaccharides alginate, Psl, and Pel play non-redundant structural roles: alginate provides the gel-like consistency, Psl drives surface attachment and maintains the three dimensional architecture, and Pel cross-links with eDNA to enhance structural rigidity [14]. In Bacillus subtilis, the TasA fibres and BslA hydrophobin layer together create a water-repellent surface coat over an exopolysaccharide-rich interior [15]. Understanding this organisational logic is important for designing engineered biofilm-derived materials because the mechanical and functional properties of the EPS matrix are a direct product of macromolecular composition.

2.2 Curli Fibres : A Bacterial Amyloid with Engineerable Properties

Among the proteinaceous components of EPS, curli fibres produced by Enterobacteriaceae deserve particular attention. Curli are functional amyloid fibres assembled from the CsgA and CsgB subunit proteins; they form β-sheet-rich structures with Young's moduli reported in the range of 3–20 GPa, comparable to many synthetic high-performance polymers [16]. Because curli fibres are produced by non-pathogenic laboratory strains of Escherichia coli, they have been studied as building blocks for engineered biomaterials [16][17].

Research has shown that biofilm-textile composites incorporating E. coli curli fibres display self-healing behaviour upon rehydration: water mediates hydrogen bond reformation between β-sheet stacks, restoring tensile strength [16][17]. The use of curli for agricultural food crops, however, is fraught with regulatory issues as the release of GEOs into food crops is extremely complex, and would need to undergo extensive food safety and ecotoxicological testing before being released.

It is not properly recognized in much of the literature on emerging materials, nor does it limit the rapid deployment of these systems to the agricultural environment [18].

Engineered Biofilms as Bio Material Platforms

The potential of synthetic biology has led to the idea of genetically engineering organisms that can express functional proteins that can be secreted into the EPS matrix, such as AFPs, antimicrobial peptides or adhesive proteins, in addition to the production of curli fibres [19]. This 'living material' approach is appealing as it would enable the in-situ production of cryoprotective proteins without the need for separate purification and formulation. Ecologically relevant non-pathogenic soil bacteria, including species of the genera Bacillus, Pseudomonas, and Azospirillum, are already known to be associated with plant surfaces, and have established biosafety profiles when applied in an agricultural setting [21]. However, this approach is still in its infancy and is not yet fully developed and supported with measured performance data that could be used to evaluate the feasibility of its application.

3. Self-Healing Macromolecular Networks: Mechanisms and Bio-Based Systems

3.1 Principles of Self-Healing in Polymer Systems

The ability of self-healing materials to regain their physical integrity after damage is achieved by an extrinsic or intrinsic mechanism. Extrinsic systems are based on the use of microencapsulated healing agents that are ejected when damaged as pioneered [22] who used micro encapsulations filled with dicyclopentadiene in epoxy matrices. Although extrinsic systems are clearly effective they can be used only a finite number of times, as the reservoirs are depleted. Intrinsic self-healing, on the other hand, takes advantage of the reversibility of some chemical interactions in the polymer backbone, making it possible to repeatedly heal and reform the thermodynamics of the bonds [23]. Intrinsic mechanisms are thus more relevant for systems with bio-based components intended for long-term outdoor applications.

3.2 Dynamic Covalent Interactions in Biopolymers

Several classes of reversible covalent bonds have been exploited in bio-based self-healing polymers. Schiff base bonds, formed between aldehyde and amine functional groups, are among the most common in polysaccharide-based hydrogels because both aldehydes (from oxidised dextran or periodate-oxidised alginate) and amines (from chitosan) are readily accessible in biopolymer chemistry [24][25]. These imine bonds hydrolyse and reform rapidly, giving pH-responsive self-healing behaviour. Disulfide bonds provide redox-responsive reversibility and are particularly relevant in cysteine-rich protein networks [23]. Diels-Alder cycloadditions, which are thermally reversible, have been used in lignin- and furan-modified polymer networks to create robust bio-based self-healing thermosets [26].

3.3 Supramolecular Interactions: Hydrogen Bonding and Host-Guest Chemistry

Non-covalent supramolecular interactions offer an equally important route to intrinsic self-healing. Hydrogen bonding is particularly strong in polysaccharide networks because of the high density of hydroxyl groups cellulose nanocrystals in a polymer matrix for instance act as physical crosslink points that break under stress and reform on contact [27]. Catechol-functionalised polymers, inspired by mussel adhesive proteins (specifically the DOPA-rich regions of Mytilus edulis foot proteins), display remarkable self-healing under aqueous conditions because catechol groups engage in both hydrogen bonding and metal–ligand coordination, facilitating rapid bond reformation after damage [28]. The PBPA hydrogel system incorporating polydopamine-coated silver nanoparticles [29] achieved greater than 95% tensile strength recovery within 30 seconds under physiological conditions, though it should be noted that this represents an idealised single-experiment result rather than a multi-replicate characterised value.

3.4 Bio-Based Matrices Relevant to Plant Wound Applications

In terms of biopolymers most relevant for plant protection in wounds, chitosan and alginate are of particular interest, having proven safe agronomic profiles and the ability to be dynamically crosslinked. Chitosan, which is mainly derived from chitin present in the exoskeleton of crustaceans, is positively charged under mild acidic conditions and can bind to negatively-charged plant cell walls through electrostatic complexation, and has inherent antifungal activity against common post-wound pathogens [30][31]. Alginate, which is obtained from brown seaweeds, is able to create hydrogels that are stable with calcium ions and absorb exudate without sticking to the wound surface [32]. The structural properties of bacterial cellulose grown by Komagataei bacter xylinus are attractive, with high tensile strength and water retention, and could therefore be useful for wound protection films (Table 1) [33]. These materials have independently demonstrated wound-protective efficacy in controlled laboratory and small-scale field studies; the challenge is combining their strengths while incorporating AFP activity.

Bacterial cellulose in particular is noteworthy as a structural scaffold for wound films. Its nanofibrillar network creates a three-dimensional architecture analogous to collagen scaffolds in animal wound dressings, and it can be produced in a variety of geometries pellicles, microspheres, or aerogels depending on cultivation conditions [34]. The mechanical properties of bacterial cellulose are largely determined by the degree of polymerisation and crystallinity of the cellulose I polymorph, both of which can be tuned by varying the bacterial growth medium and fermentation temperature [27][33].

Table 1: Practical Applications of Wound Films

Application	Challenges	Proposed Solutions	References
Pruning Wounds	Limited adhesion and protection	Formulating stronger adhesive properties	(Bourguignon *et al.,* 2022)
Grafting	Compatibility with various plant tissues	Development of adaptable film materials	(Choi *et al.,* 2021)
Post-Harvest Protection	Resistance to environmental factors	Utilising biopolymers with antifreeze properties	(Yin *et al.,* 2018)
Disease Resistance	Preventing pathogen infiltration	Incorporation of antimicrobial agents	(Wu *et al.,* 2020)

4. Antifreeze Proteins: Structural Biology and Cryoprotective Mechanisms

4.1 Discovery, Classification, and Distribution

Antifreeze proteins are a structurally diverse group of macromolecules defined by their ability to bind to ice crystal surfaces and modify ice growth dynamics. They were first identified in Antarctic fish (notably Trematomus borchgrevinki) in the late 1960s, where they prevent lethal plasma freezing at seawater temperatures below -1°C [35]. Subsequent discovery in winter rye (Secale cereale), carrot (Daucus carota), Antarctic hairgrass (Deschampsia antarctica), and numerous cold-adapted insects and microorganisms revealed that ice-binding proteins have evolved independently multiple times, producing structurally distinct solutions to the same thermodynamic problem (Table 2) [9][36][37].

Table 2: Classification of Antifreeze Proteins (AFPs)

Type of AFP	Structural Features	Examples	Source Organisms	References
Type I	β-sheet structure, 3-fold symmetry	fish AFP from *Notothenia*	Antarctic icefish	(Daniels *et al.*, 2013)
Type II	α-helices, two distinct domains	AFP from *Pseudomonas syringae*	Bacteria	(Graham *et al.,* 2009)
Type III	Complex structures with multiple motifs	AFP from *Hirudo medicinalis*	Medicinal leech	(Fowler & Los, 2014)
Type IV	3D domain/spherical structure	AFP from *Winter flounder*	Marine fish	(Qin *et al.,* 2004)
Plant AFPs	Variable structures; often smaller	AFP from *Brassica*	Brassica species	(Duman, 2015)

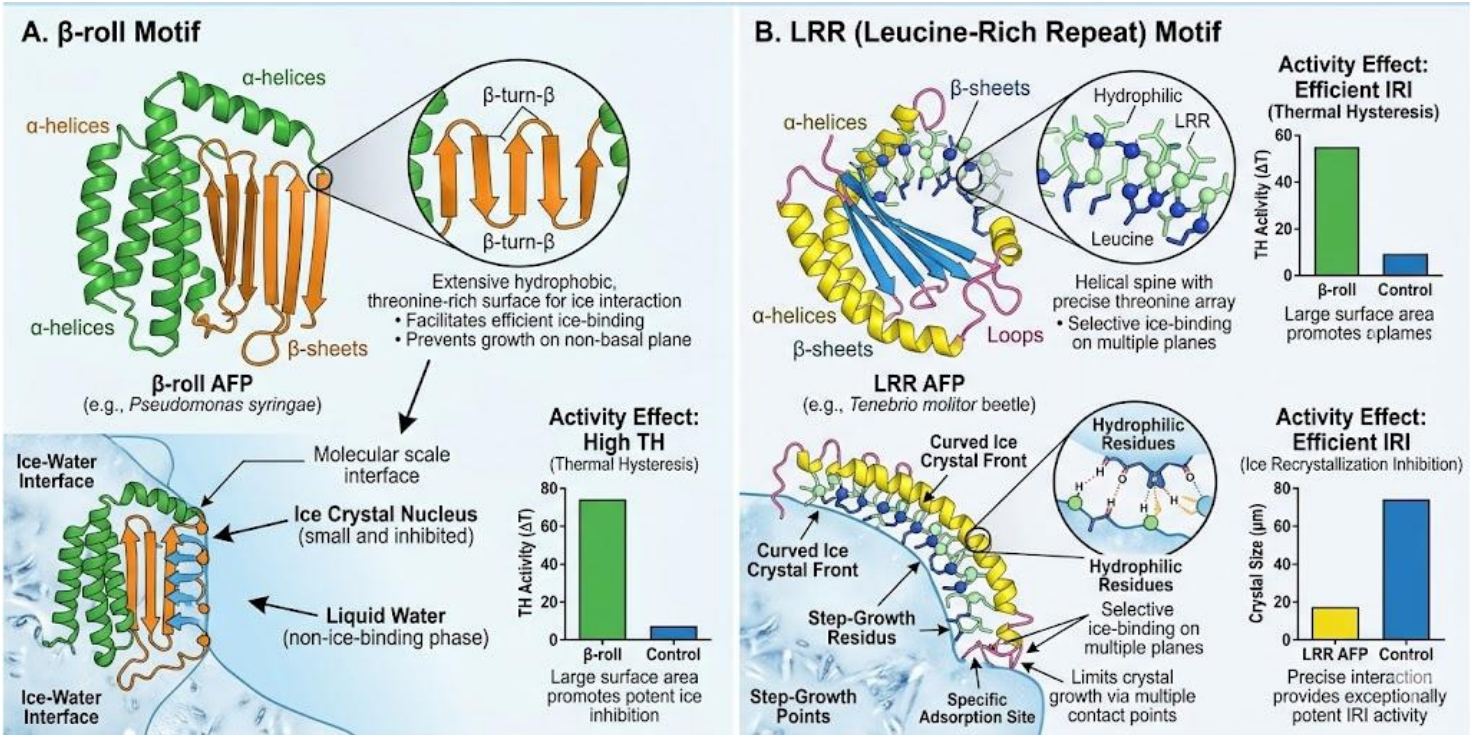

Figure 1: Structural features of Antifreeze Proteins

Table 3: Summary of Research Findings on Self-Healing Mechanisms

Mechanism	Key Studies	Applications in Wound Management	References
Dynamic Covalent Interactions	Wang *et al.,* 2020	Enhancing healing in plant tissues	(Wang *et al.,* 2020)
Supramolecular Interactions	Zhang *et al.,* 2018	Use in grafting and pruning wound protection	(Zhang *et al.,* 2018)
Hydrogen Bonding	Chen *et al.,* 2019	Improving film flexibility and recovery	(Chen *et al.,* 2019)
Ionic Interactions	Li *et al.,* 2021	Increased resistance to mechanical stress	(Li *et al.,* 2021)

Classification of AFPs into Types I–IV in fish, and separately into insect and plant types, is based on structural and sequence features rather than evolutionary homology. Plant AFPs generally fall outside the fish typology entirely; they are typically classified by their structural architecture β-roll/β-helix proteins in cereals, LRR proteins in carrot and daisy, and thaumatin-like proteins in certain trees shown in figure 1 [9]. This structural diversity is important for materials applications because different AFP architectures differ in stability, solubility, production ease, and compatibility with polymer matrices.

4.2 Structural Features of Plant AFPs

The best-characterised plant AFP is LpAFP from winter rye (Secale cereale) previously sometimes incorrectly attributed to Lolium perenne [9]. LpAFP adopts a right-handed β-helix (also described as a β-solenoid) conformation, in which parallel β-strands stack to present a flat, repetitive array of threonine residues on the ice-binding face [38]. The regularity and spacing of these threonine hydroxyls allow them to register with the hydrogen-bonding network of specific ice crystal planes, enabling tight adsorption [38][39]. The carrot AFP (DcAFP) is structurally distinct, containing leucine-rich repeat (LRR) motifs [40] its ice-binding face presents a different hydrogen bond geometry from LpAFP, and it inhibits a different subset of ice crystal faces, suggesting that AFP mixtures may provide broader protection than single-protein formulations.

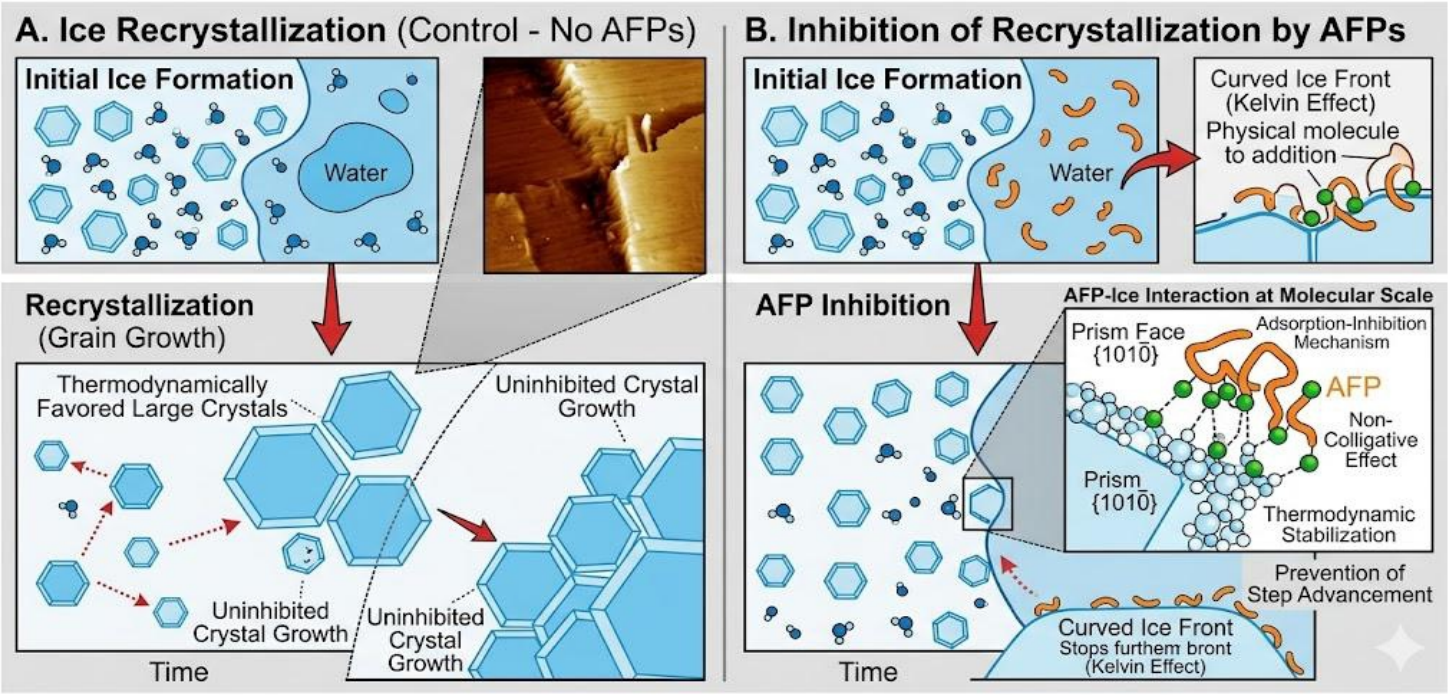

Figure 2: Mechanism of ice recrystallization and the role of AFPs

Antarctic hairgrass (Deschampsia antarctica) AFPs represent another structurally characterised group of relevance, particularly for cold-climate applications [41]. Their high IRI activity and demonstrated effectiveness in plant tissue cryopreservation contexts make them promising candidates for wound protection films intended for use in regions with harsh winters [37][41]. Importantly, many plant AFPs share structural homology with pathogenesis-related (PR) proteins particularly PR-5 (thaumatin-like) proteins which means they may carry dual functionality ice-binding and antifungal activity directed at post-wound pathogens [9][42].

4.3 Thermal Hysteresis vs. Ice Recrystallisation Inhibition

AFPs express two distinct anti-ice activities that should not be conflated in materials design. Thermal hysteresis (TH) refers to the gap created between the melting and freezing points of a solution containing AFPs it reflects the ability of adsorbed AFPs to prevent ice growth by raising the energetic cost of further crystallisation (the Kelvin/curvature effect) [39]. Fish AFPs exhibit TH values of 2–13°C, sufficient to prevent plasma freezing in polar seawater. Plant AFPs by contrast exhibit modest TH values of 0.1–0.5°C [43]. This limited TH would be insufficient to prevent tissue freezing during a hard frost, and should not be the primary design target for agricultural cold protection systems.

Ice recrystallisation inhibition (IRI) is the property that makes plant AFPs genuinely useful for cryoprotection. During freeze-thaw cycling, small ice crystals tend to coalesce into larger ones through Ostwald ripening a process driven by the thermodynamic preference for minimising surface area. Large ice crystals cause far more cellular damage than small ones. Plant AFPs adsorb to crystal surfaces and prevent this coalescence, maintaining a population of small, less injurious crystals [43][44]. Crucially, plant AFPs demonstrate IRI activity at concentrations as low as nanomolar far below those needed for TH activity meaning that even small amounts released from a wound film could have meaningful effects [44]. This distinction is important for formulation design: the required AFP loading in a wound film is determined by IRI activity requirements, not TH requirements.

4.4 Cold Signalling and Molecular Responses in Plants

The intrinsic cold response of plants must also be understood to understand why plants require external cryoprotective support. The ICE1-CBF-COR transcription cascade is still at the heart of cold acclimation where ICE1 (Inducer of CBF Expression 1) transcription factors induce CBF (C-repeat Binding Factor) genes, which then induce the expression of COR (Cold-Regulated) genes [45].

However, this classical pathway is now understood to represent only one branch of a broader regulatory network. Recent work has identified CBF-independent cold response pathways involving CAMTA transcription factors, ZAT12, and LOS4 (a DEAD-box RNA helicase) that operate in parallel [46]. Post-translational regulation of ICE1 by the E3 ubiquitin ligase HOS1 and by sumoylation adds further complexity [46]. Epigenetic mechanisms including histone H3K4 methylation at cold-responsive loci and cold-induced changes in chromatin accessibility modulate the magnitude and duration of the cold response [47].

From this signaling research there is the knowledge that the ability to cold acclimate is lower in actively growing or wounded tissue compared with dormant tissue when it comes to wound protection applications. Wounds destroy the continuity of the bark and cambium, and interrupt the gradients of solutes and signaling molecules that healthy tissue maintains.

This disruption in physiology will render the wound site more susceptible to frost injury than healthy tissue surrounding the wound, thus suggesting a physiological basis for selective, wound-specific frost protection with wound films [3][45].

4.5 Cryoprotectant Macromolecules Beyond AFPs

AFPs function most effectively in combination with other cryoprotective molecules. Osmolytes such as proline, glycine betaine, and trehalose accumulate naturally in cold-acclimated plant cells, lowering the osmotic potential of the cytoplasm and reducing the driving force for freeze-induced dehydration [48]. Trehalose ('the resurrection sugar') stabilises protein conformation during desiccation through a preferential exclusion mechanism and direct hydrogen bonding to protein surface residues [49]. This double function osmotic protection and protein stabilisation makes trehalose particularly relevant in AFP-loaded wound films because it could simultaneously protect wound tissue and preserve AFP activity during storage and application [50].

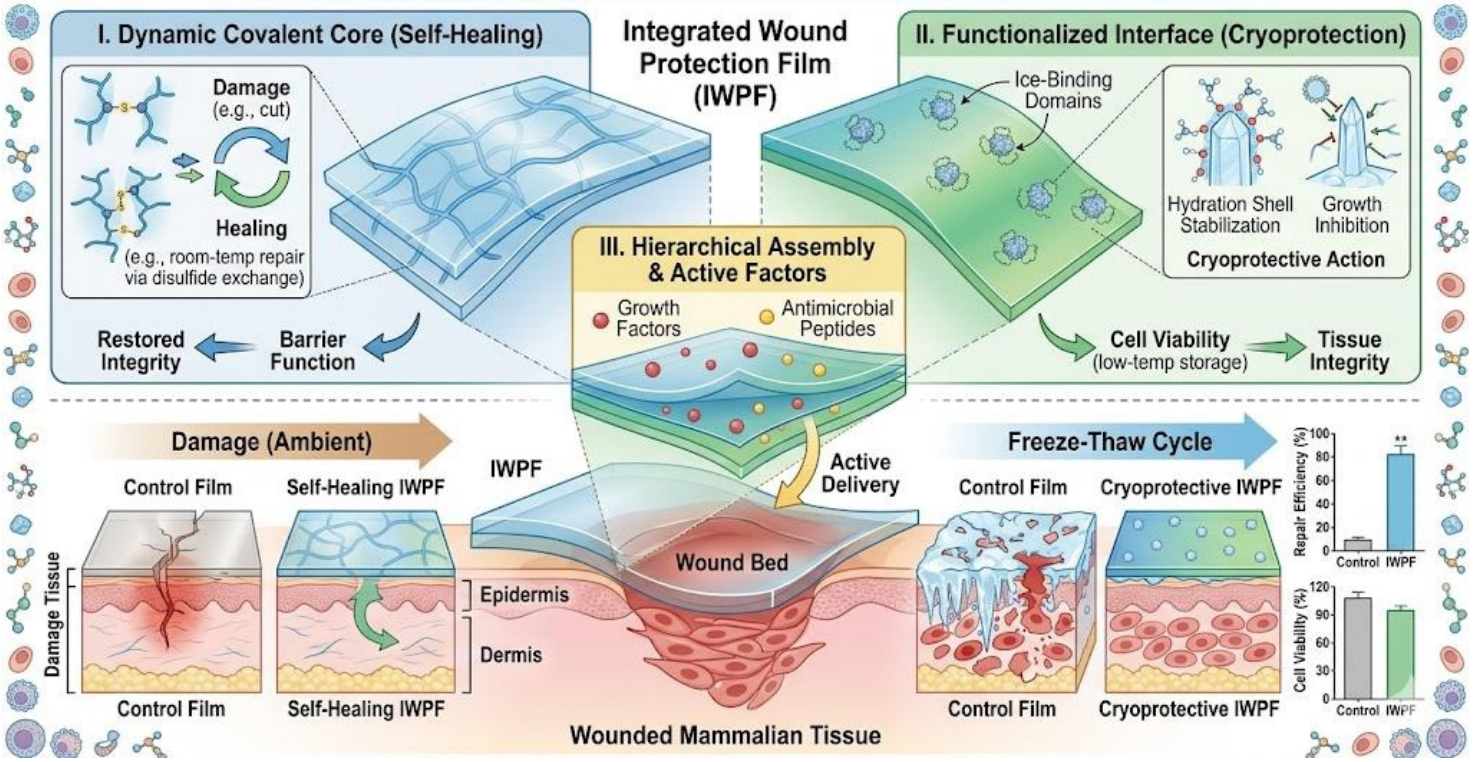

Figure 3: Proposed integrated Macromolecular framework

Glycerol, a simple colligative cryoprotectant widely used in laboratory cryopreservation, lowers the freezing point of aqueous systems by displacing water from the hydration shell of macromolecules. However, glycerol at concentrations sufficient to provide colligative protection (typically >30% v/v) would be phytotoxic to wound tissue therefore, it cannot serve as a primary cryoprotectant in open-wound applications [51]. Lower concentrations as adjuvants to AFP-based systems are more realistic, and glycerol's role in maintaining AFP hydration during film storage may be valuable at sub-toxic concentrations [49] [51].

5. Proposed Integrated Macromolecular Frameworks: Combining Self-Healing and Cryoprotection

5.1 Conceptual Basis and Scientific Rationale

A word of warning about the systems described below and the other enabling technology samples, it is important to state at the beginning of this section that these are not proven technologies, but potential avenues for research. To the best of our knowledge, there is no published study that verified a combined self-healing, AFP-releasing plant protection film that can be applied as a wound protection film.

Here follows a synthesis of evidence from neighbouring areas (biopolymer hydrogel chemistry, AFP structural biology, plant wound physiology) which indicates combinations that are scientifically feasible and systematic; deserve investigation. The rationale for combining self-healing and cryoprotective functions in a single wound film rests on three observations. First, pruning wounds must remain protected for weeks to months while callus tissue forms; any wound film that cracks or peels during this period creates unprotected entry points for pathogens [3]. Second, the period of maximum wound vulnerability overlaps with the period of maximum frost risk in temperate orchards (late autumn through early spring) [2]. Third, the biopolymer matrices most suitable for self-healing hydrogels particularly chitosan and alginate are the same matrices that have been studied as carriers for bioactive proteins, suggesting compatibility at the formulation level [32][52].

5.2 Strategy 1: AFP-Loaded Crosslinked Hydrogel Films

The most straightforward integration strategy is to load AFPs into a dynamically crosslinked hydrogel matrix that provides both a physical barrier and a reservoir for sustained AFP release. Alginate-chitosan polyelectrolyte complexes are an attractive platform because they form stable films at physiological pH, the electrostatic crosslinks are reversible (enabling self-healing when the film is rehydrated by rain or dew), and both components have established phytosafety profiles [53]. Schiff base crosslinked chitosan-aldehyde dextran networks offer an alternative dynamic bond that responds to pH, potentially providing triggered release of AFPs and antimicrobials under the mildly acidic conditions that develop when wound exudate accumulates [24].

The central macromolecular design challenge in this strategy is AFP stability within the matrix. AFP ice-binding activity depends on the correct folding of the ice-binding domain; if the β-helix or LRR structure is disrupted by crosslinking chemistry or by interactions with the matrix polysaccharides, activity will be lost. Molecular dynamics simulations of LpAFP in aqueous solution suggest that the β-helix is kinetically stable over simulation timescales [38][44], but behaviour in a high-viscosity, crosslinked gel environment has not been characterised. This is a specific, tractable experimental question that the IJBMacromolecules community is well-positioned to address through a combination of CD spectroscopy, FTIR, and IRI activity assays on AFP-loaded hydrogel films [39].

5.3 Strategy 2: Layer-by-Layer Macromolecular Assembly

Layer-by-layer (LbL) assembly of oppositely charged polyelectrolytes produces thin films with nanoscale control over composition and thickness. For a wound protection application, this technique could be used to create a multilayered film with distinct functional zones: an inner AFP-rich layer in direct contact with wound tissue for cryoprotection, an intermediate self-healing polysaccharide network for structural resilience, and an outer antimicrobial layer (chitosan, plant extract nanoparticles) as a pathogen barrier [54]. The driving force for LbL deposition would be electrostatic: alternating layers of anionic (alginate, oxidised cellulose nanofibrils) and cationic (chitosan, aminocellulose) polymers, with AFPs incorporated into designated anionic layers based on their net negative charge at physiological pH [55].

Applying Layer-by-layer films on rough irregular plant wound surfaces (as in most thin-film Layer-by-layer studies with flat substrates) would demand the adaptation of the deposition process, which could be accomplished, for example, by spraying sequential polyelectrolyte solutions instead of dip-coating. The question of maintaining adequate layer integrity on axillary surfaces of curved waxy bark remains to be answered. Bioinspired adhesives with a chitosan backbone functionalized with catechol groups have shown increased adhesiveness in moist environments, such as wound surfaces in the outdoors [28][56] and could be used as the inner adhesion layer to overcome the problem of substrate adhesion.

5.4 Strategy 3: AFP-Producing Beneficial Biofilms

A more biologically integrated approach would use AFP-secreting beneficial bacteria to form a living protective biofilm on wound surfaces. Synthetic biology tools now allow insertion of AFP expression cassettes into non-pathogenic soil bacteria, enabling constitutive or cold-inducible secretion of AFPs into the EPS matrix [19][20]. A Bacillus subtilis strain engineered to secrete LpAFP alongside its natural surfactin production would, in principle, provide both antimicrobial activity (from surfactin) and cryoprotection (from LpAFP) from a single biological agent. The ability of Bacillus biofilms to colonise plant surfaces and persist for extended periods is well documented [57].

This strategy is conceptually appealing because it exploits the self-regenerative capacity of living systems the biofilm can reconstitute itself if partially removed by rain or physical disturbance without requiring repeated applications of formulated material. However, it carries the highest regulatory complexity of the three strategies discussed here. Release of genetically modified organisms into open agricultural environments requires regulatory approval under contained-use and deliberate-release frameworks in most jurisdictions. Non-GMO approaches selecting cold-adapted bacteria that naturally produce AFP-like proteins are a lower-regulatory-burden alternative, though few agricultural bacterial strains produce AFP activity comparable to the well-characterised plant AFPs [58].

5.5 Anticipated Functional Timeline of an Integrated Film

If a combined AFP-hydrogel wound film could be developed successfully a protective role could logically be expected to follow different phases in the course of the wound healing process.

During the short-term post-application stage (0–72 hours) the main role is to create a physical barrier to retain moisture to assist in the initiation of callus and to release antimicrobial compounds from the outermost layer. The important thing is that self-healing becomes imperative during the active healing phase (3–21 days) as thermal cycling will cause the film to expand and contract, leading to the formation of microcracks which, if not filled, would expose the wound tissue. Continued AFP release during this period would ensure IRI protection during frost situations. The film is to be degraded over time (long term > 21 days) with the suberized callus tissue itself acting as a barrier function so that no residues will be left in the soil or in plants [3][59].

6. Biopolymer Matrices: Properties and Suitability for Plant Wound Films

6.1 Chitosan

Chitosan is a linear polysaccharide produced by deacetylation of chitin, consisting of β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units. The degree of deacetylation (DD) and molecular weight are the two primary parameters determining its physical and biological behaviour [30]. High-DD chitosan (>85% deacetylated) dissolves readily in dilute acids, facilitating film formation; low-DD chitosan forms more mechanically rigid films [60]. The antifungal activity of chitosan against Botrytis cinerea, Alternaria alternata, and Fusarium oxysporum all common post-wound plant pathogens has been demonstrated in multiple independent studies, though minimum inhibitory concentrations vary considerably with MW and DD [31][61].

In self-healing applications, the free amine groups ($-NH_2$) of chitosan offer reactive groups that can also undergo Schiff base reactions with the aldehyde containing crosslinkers, which will develop imine bonds that will provide healing behavior at pH above ~5 [24]. However, these bonds are pH sensitive, and the release of wound exudate will cause the whole network to become acidified and release the cargo. This is potentially a desirable trigger or an undesirable instability, depending on the application context, and experiments must be performed to characterize the plant wound microenvironment [52].

6.2 Alginate

Alginate is a block copolymer of α-L-guluronate (G blocks) and β-D-mannuronate (M blocks) derived primarily from brown seaweed. The G-block content determines the stiffness of calcium-crosslinked alginate gels high-G alginates form brittle, stiff gels while high-M alginates form softer, more elastic networks [32]. For wound film applications, high-M alginates are generally preferred because their flexibility accommodates wound surface movement. The calcium crosslinks in alginate gels are reversible in the presence of chelating agents or in calcium-limited environments, contributing to some degree of self-healing behaviour, though the rate and extent of healing after mechanical damage are lower than those achievable with covalent dynamic bond systems [32][62].

6.3 Bacterial Cellulose

Bacterial cellulose (BC), produced extracellularly by Komagataeibacter xylinus and related species, forms a nanofibrillar network of pure cellulose I with crystallinity indices of 80–90% substantially higher than plant-derived cellulose [33][34]. This high crystallinity gives BC films exceptional tensile strength (up to 300 MPa in dry state) and excellent dimensional stability. The high water-holding capacity of BC (up to 99% water by mass) makes it relevant for maintaining wound moisture balance [33]. However, BC's high crystallinity and the absence of reactive functional groups on the fibre surface limit its intrinsic self-healing capacity; chemical functionalisation (carboxylation, amination, or periodate oxidation) is required before dynamic crosslinks can be introduced [27][34].

6.4 Pectin, Starch, and Other Plant-Derived Polysaccharides

Pectin and starch, both derived from plant cell walls and seeds respectively, are relevant for plant wound films because of their natural origin, biodegradability, and compatibility with plant tissue. Pectin, a heteropolysaccharide rich in galacturonic acid, forms hydrogels through calcium crosslinking analogous to alginate, and its methyl-esterification degree determines gel behaviour [63]. The butler effect of the molecular crosslinking resulting in gelation is dependent on the methoxyl content of the pectin: high methoxyl pectins gel more readily in acidic environments, whereas low methoxyl pectins gel in mild conditions of calcium induced crosslinking, which would be suitable for use in the context of wound application. Starch-based films, less mechanically resistant are, on the contrary, totally food safe and agronomically inert [64]. Starch and chitosan or alginate are excellent additives for improving the mechanical properties, which do not reduce biodegradability [64].

7. Agricultural Applications and Scalability Considerations

7.1 Pruning Wound Management in Perennial Crops

Pruning is an annual or biennial practice in most orchard and vineyard management systems, creating millions of wound events per year across a single farm. In almond orchards of California, research has documented that the timing of pruning relative to rainfall events is a significant determinant of Botryosphaeriaceae infection rates, with wounds made during dry periods showing substantially lower infection incidence than those created immediately before winter rains [65]. A large proportion of the effect of wound sealants on long term canker incidence may be due to the fact that the first 48–72 hours after wounding may represent the most critical time, in terms of pathogen colonization rates, which a sealant may partially protect [66].

Self-healing biopolymer films are especially suitable for pruning wound applications because the wound surfaces are generally curved often waxy and also change in shape during the growing season in woody plants.

There are situations where conventional rigid sealants crack; a film that would be capable of repairing hairline cracking due to rehydration would maintain the barrier function continuously during this high-risk period without the need to reapply the film. The biodegradation process of the polysaccharide matrix should be matched with the callus formation process, duration of which depends on species from about 3-6 weeks for fast healing stone fruits to about 3-6 months for grape vine [66].

7.2 Grafting and Transplant Establishment

Grafting creates a physically vulnerable union between scion and rootstock that must be maintained under specific moisture and temperature conditions while the vascular cambium bridges across [67]. Chitosan-alginate composite coatings applied over graft unions have been shown in controlled greenhouse conditions to improve union success rates, likely through moisture maintenance and antifungal activity at the graft interface [53][67]. The integration of cryoprotectants into such coatings would be particularly valuable for grafts performed in late winter or early spring when residual frost risk is high but active growth has begun the period when grafted plants are most thermally vulnerable.

7.3 Post-Harvest Wound Protection

Mechanical harvesting and handling damage inevitably creates surface wounds on fruits and vegetables, providing entry points for Botrytis cinerea, Penicillium expansum, and Alternaria species during storage and transport [68]. Chitosan-based edible coatings with essential oil components have shown 40–60% reduction in post-harvest decay rates for strawberries, tomatoes, and citrus in multiple peer-reviewed studies [68][69]. Integration of self-healing capability into these edible coatings would extend their protective duration during the extended handling and cold-chain storage periods that are now standard for export produce [59].

7.4 Economic Considerations and AFP Cost Viability

Any realistic discussion of AFP-containing wound films must address the production cost of AFPs directly. Current estimates for recombinant AFP production in E. coli expression systems range from $50 to $200 per gram at laboratory scale, with yields of approximately 50–100 mg/L of culture under optimized conditions [42][70]. At these costs, loading AFP at even low concentrations (0.1 mg/cm^2 of film) across a hectare of pruned orchard would be prohibitively expensive.

However, several cost-reduction pathways are under investigation: plant molecular farming in tobacco or moss bioreactors, winter extraction of AFP-rich leaf apoplastic fluid from cold-acclimatised cereal crops, and the development of synthetic peptide mimetics that replicate IRI activity using shorter and cheaper-to-produce sequences [42][71].

The IRI-active minimal sequences of some AFPs have been identified, and short synthetic peptides as small as 12 amino acids have been shown to retain measurable IRI activity in vitro [71]. If effective IRI activity can be achieved at peptide concentrations one or two orders of magnitude lower than those needed for TH activity which the literature on IRI at nanomolar concentrations suggests is feasible [43][44] then the economic viability of AFP-loaded wound films improves substantially. Establishing the minimum effective AFP loading in a hydrogel wound film under field-relevant conditions is therefore one of the highest-priority experimental questions for this research area.

8. Characterisation of Macromolecular Wound Protection Systems

8.1 Structural Characterisation of Biopolymer Networks

Fourier-transform infrared spectroscopy (FTIR) is the standard first characterisation step for biopolymer films, providing information on functional group presence, crosslinking chemistry, and polymer-polymer interactions. The appearance of imine C=N stretching at 1630–1650 cm^{-1} in chitosan-aldehyde Schiff base networks confirms covalent crosslink formation [24]. For AFP-loaded films, the amide I band at 1650 cm^{-1} and amide II at 1540 cm^{-1} report on protein secondary structure specifically, the β-sheet component expected from AFP β-helix or LRR domains [38]. Changes in these bands after processing or extended storage would signal loss of AFP native conformation and likely loss of IRI activity.

Gel permeation chromatography (GPC) or size exclusion chromatography (SEC) should be used to characterise the molecular weight distribution of the polysaccharide matrix components and to monitor any degradation during processing or field exposure. Solid-state ^{13}C NMR provides information on crystallinity in cellulose-containing systems and can confirm the presence and distribution of dynamic crosslink bonds [27]. Atomic force microscopy (AFM) images the surface topography of dried wound film samples, allowing visualisation of any phase separation between AFP protein domains and the polysaccharide matrix a phenomenon that would reduce effectiveness by concentrating AFP away from the film surface [72].

Table 4: Comparison of Biopolymer Characteristics

Biopolymer	Mechanical Strength	Biodegradability	Self-Healing Potential	References
Chitosan	High; can form strong films	Biodegradable by microbial action	Moderate; capable of minor healing	(Maran *et al.,* 2017)
Alginate	Moderate; forms gel-like structures	Biodegradable in natural environments	Limited; does not self-heal	(Lee *et al.,* 2016)
Bacterial Cellulose	Very high; superior to synthetic polymers	Biodegradable; produced by microorganisms	High; effective self-healing	(Rural *et al.,* 2015)
Pectin	Low to moderate	Biodegradable; commonly found in plants	Limited; minor structural recovery	(O'Sullivan, 1999)
Starch	Moderate; varies by source	Biodegradable; widely used	Low; limited to swelling	(Zhang *et al.,* 2019)

8.2 Assessing AFP Activity in Polymer Matrices

Standard AFP activity assays include the nanoliter osmometer protocol for TH measurement and the sucrose sandwich assay for IRI quantification [43][44]. Both assays were developed for aqueous AFP solutions and require adaptation for studying AFPs embedded in or released from polymer matrices. A practical approach is to measure AFP activity in the aqueous phase after controlled incubation of a wound film sample in buffer at a defined temperature to assess how much active AFP is available for tissue penetration. Comparing IRI activity before and after AFP incorporation into the matrix and after different durations of simulated field exposure would directly answer whether the matrix environment compromises AFP function [43].

8.3 Self-Healing Efficiency Metrics

Self-healing efficiency is typically quantified as the ratio of a mechanical property (tensile strength, Young's modulus, elongation at break) measured after a prescribed healing period to the same property measured on the intact sample [23]. For wound film applications, healing should be evaluated under conditions relevant to the deployment environment: healing in a humid chamber at 4°C (simulating cold night conditions) and at 20°C (simulating daytime conditions). Equally important is assessing healing after repeated damage-heal cycles, since a pruning wound film will experience multiple thermal cycles over its functional lifetime figure 3 [22][23].

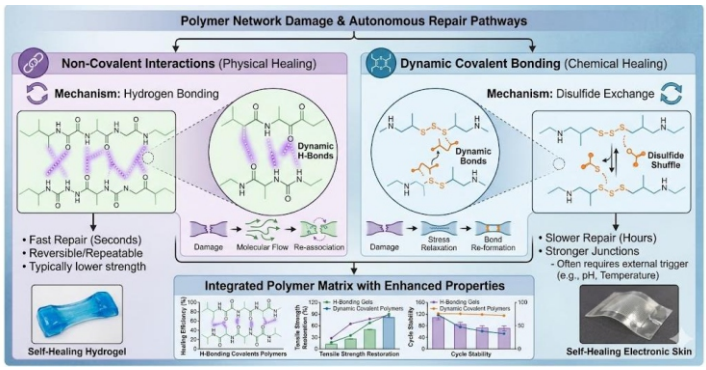

Figure 4: Self-healing mechanisms in Bio-polymers

Optical microscopy and scanning electron microscopy (SEM) allow direct visualisation of crack formation and closure, providing mechanistic insight into the healing process. In case of films that include living microorganisms (biofilm-based strategies), this shall be done in combination with cell viability after mechanical damage and healing, either by counting the cells or by measuring microbial viability with a fluorescent stain for cell viability if living cells are lost, then the ability to heal the film by a living mechanism will be compromised [20][57].

9. Challenges, Limitations, and Future Research Priorities

9.1 AFP Stability in Processed Matrices

Preserving AFP activity through the stages of material processing dissolution, film casting, drying, and storage is a non-trivial challenge. Lyophilisation is effective for preserving AFP activity in bulk solutions when appropriate excipients (trehalose, mannitol) are included [50] but the behaviour of AFPs embedded within a crosslinked polymer matrix during drying and rehydration is not well characterised. Differential scanning calorimetry (DSC) of AFP-loaded films could reveal whether dehydration induces protein unfolding, and whether rehydration restores the native folded state. This type of characterisation is straightforward and represents a logical first experiment for any group beginning work in this area.

9.2 Phytotoxicity and Crop Safety

Bio-based materials applied to open plant wounds have direct access to vascular tissue. Any component that is phytotoxic or that introduces reactive oxygen species, heavy metal contaminants, or inhibitory secondary metabolites will delay rather than promote healing. Nanoparticles (ZnO, AgNPs) proposed for enhanced antimicrobial activity in some wound dressing formulations are known to be phytotoxic at concentrations above threshold values that vary by species and tissue type [73]. Systematic phytotoxicity screening initially on callus cultures then on wounded woody plant explants is essential before any formulation is advanced to field testing.

9.3 In Planta and Field Validation

The most significant gap in the current knowledge base is the complete absence of in planta or field studies testing any AFP-containing wound film on a real crop species under realistic conditions. Laboratory results on model polymer systems provide useful mechanistic insight, but they cannot predict behaviour on a rough, waxy bark surface in variable humidity, UV exposure, and precipitation.

Proof-of-concept trials with excised woody shoots in controlled cold rooms could be a prudent intermediate step before the deployment in the field and would yield the infection rate and frost protection data required for a business case for additional investment [65][66].

9.4 Regulatory and Certification Pathways

Self-healing biopolymer films (based on chitosan and alginate) are likely to be compatible to most markets of organic certification as both materials are currently approved in several national and international organic standards. Rather, any formulation containing recombinant AFPs or genetically modified bacterial strains would involve rerouting the development process through a pesticide registration or GMO release process, which would greatly prolong the development period. Early mapping of the regulatory pathway is essential for resource allocation in any translation programme not only by determining the relevant regulatory pathway (pesticide, fertilizer, bio stimulant, novel food) [74].

9.5 Interdisciplinary Research Needs

This will involve real multidisciplinary collaboration between the disciplines which do not yet communicate well the structural biologists (who will study the AFP when it binds to ice), the polymer chemists (who will develop dynamic crosslinked networks), the plant pathologists (who will examine wound infection dynamics) and the agronomists (who will design field trial protocols). The macromolecular characterization methods that are key to the IJB Macromolecules research community (spectroscopy, calorimetry, scattering, simulation) would provide a backbone of structure-function data needed for rational formulations by the teams translating research into the clinic. Specifically, molecular dynamics simulations of the conformation of AFP in various gel environments with different cross linkage density and viscosity could complement the experimental CD and FTIR results and expedite the search for matrix compositions that do not alter the activity of the AFP [44][75].

10. Conclusions

Individually, the macromolecular science that supports the design of self-healing biopolymer wound films and antifreeze protein cryoprotection is well developed. Self-healing properties of the networks are provided by the dynamic covalent and supramolecular crosslinks in chitosan, alginate and bacterial cellulose networks. IRI activity is based on the structure of AFP β-helix and LRR ice-binding domains with nanomolar efficiency. What has not yet been achieved and what is the productive next step identified by this review is to systematically characterize their interaction when the two functional macromolecular systems are brought together in a single formulation.

This synthesis brings out several research priorities. Retention of AFP stability and activity in crosslinked biopolymer matrices need to be characterized over the entire spectrum of crosslinked density and chemistry that is relevant to wound films. Secondly, the required minimum loading for an effective IRI protection that is meaningful in wound tissue contexts needs to be established to make sure that the use of AFP containing films is both economically viable and effective in protecting the IRI. Third, before any in planta work is conducted a phytotoxicity screening of candidate formulations must be done. Fourth, regulation should be decided at an early stage of development as this will dictate which AFP production platforms are acceptable for the intended market.

While this approach is relevant to wound management, there is also a larger potential. These same principles of AFP/biopolymer integration can be applied in the design of edible post-harvest coatings for cold-chain stored produce, in the design of cryoprotective seed coatings for sowing early in frost risk areas and in the design of components of the tissue culture media for plant germplasm cryopreservation. The macromolecular structural biology tools that are available to members of this community of researchers in this journal are just what is needed to translate these applications from speculative ideas into well tested tools. What has been done so far is laid in literature of adjacent wound dressing and AFP cryopreservation, the next step is deliberate structurally-informed integration.

References

1. Flemming, H. C., & Wuertz, S. (2019). Bacteria and archaea on Earth and their abundance in biofilms. *Nature Reviews Microbiology*, *17*(4), 247-260.
2. Osorio-Marín, J., Fernandez, E., Vieli, L., Ribera, A., Luedeling, E., & Cobo, N. (2024). Climate change impacts on temperate fruit and nut production: a systematic review. *Frontiers in Plant Science*, *15*, 1352169.
3. Metcalf, D. G., & Bowler, P. G. (2013). Biofilm delays wound healing: a review of the evidence. *Burns & trauma*, *1*(1), 2321-3868.
4. Dipta, S. D., Rahman, M. M., Ansari, M. J., & Uddin, M. N. (2025). A comprehensive review of sustainable and green additive manufacturing: technologies, practices, and future directions. *Journal of Manufacturing and Materials Processing*, *9*(8), 269.
5. Percival, S. L., Swann, M. J., Daly, K., Mayer, D. O., Moodley, P., & Chen, R. (2026). An update on biofilms in acute and chronic wounds: Incidence, clinical evidence, diagnosis, prevention, and treatment. *Journal of Internal Medicine*.
6. Sahoo, K., Meshram, S., & Sahoo Jr, K. (2024). Biofilm formation in chronic infections: a comprehensive review of pathogenesis, clinical implications, and novel therapeutic approaches. *Cureus*, *16*(10).
7. Shahrajabian, M. H., & Khoshkharam, M. (2026). Recent findings and future prospects on the applications of chitosan in various fields. *Annales Universitatis Paedagogicae Cracoviensis Studia Naturae*.
8. Ugwu, C. N., Ezeibe, E. N., Emencheta, S. C., Nwagwu, C. S., Ogbonna, K. O., Ejiofor, C. V., ... & Attama, A. A. (2025). Biofilms: structure, resistance mechanism, emerging control strategies, and applications. *RSC Pharmaceutics*, *2*(6), 1376-1407.

9. Griffith, M., & Yaish, M. W. (2004). Antifreeze proteins in overwintering plants: a tale of two activities. *Trends in plant science*, *9*(8), 399-405.
10. Worrall, D., Elias, L., Ashford, D., Smallwood, M., Sidebottom, C., Lillford, P., ... & Bowles, D. (1998). A carrot leucine-rich-repeat protein that inhibits ice recrystallization. *Science*, *282*(5386), 115-117.
11. Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology*, *14*(9), 563-575.
12. Santos, J. F., del Rocío Silva-Calpa, L., de Souza, F. G., & Pal, K. (2024). Central Countries' and Brazil's Contributions to Nanotechnology. *Current Nanomaterials*, *9*(2), 109-147.
13. Donlan, R. M., & Costerton, J. W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *Clinical microbiology reviews*, *15*(2), 167-193.
14. Kostakioti, M., Hadjifrangiskou, M., & Hultgren, S. J. (2013). Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harbor perspectives in medicine*, *3*(4), a010306.
15. Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nature reviews microbiology*, *2*(2), 95-108.
16. Tanzil, A. H., Sultana, S. T., Saunders, S. R., Shi, L., Marsili, E., & Beyenal, H. (2016). Biological synthesis of nanoparticles in biofilms. *Enzyme and Microbial Technology*, *95*, 4-12.
17. Gulati, R., Sharma, S., & Sharma, R. K. (2022). Antimicrobial textile: recent developments and functional perspective. *Polymer Bulletin*, *79*(8), 5747-5771.
18. Tanzil, A. H., Sultana, S. T., Saunders, S. R., Shi, L., Marsili, E., & Beyenal, H. (2016). Biological synthesis of nanoparticles in biofilms. *Enzyme and Microbial Technology*, *95*, 4-12.
19. Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., ... & Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome*, *8*(1), 103.
20. Cheng, S., Wang, K. H., Zhou, L., Sun, Z. J., & Zhang, L. (2024). Tailoring biomaterials ameliorate inflammatory bone loss. *Advanced Healthcare Materials*, *13*(12), 2304021.
21. Guerrero-Rodriguez, I. D., Nguyen, C. M., Nguyen, K. T., & Soto-Garcia, L. (2026). Targeting Biofilms in Chronic Wounds: Emerging Strategies with Antimicrobial Nanocomposites. *Journal of Functional Biomaterials*, *17*(6), 282.
22. Bashan, Y., de-Bashan, L. E., Prabhu, S. R., & Hernandez, J. P. (2014). Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant and soil*, *378*(1), 1-33.
23. White, S. R., Sottos, N. R., Geubelle, P. H., Moore, J. S., Kessler, M. R., Sriram, S. R., ... & Viswanathan, S. (2001). Autonomic healing of polymer composites. *Nature*, *409*(6822), 794-797.
24. Antony Jose, S., Arvisu, E., & Menezes, P. L. (2026). Self-Healing Materials: Mechanisms, Properties, and Applications. *Processes*, *14*(9), 1436.
25. Estroff, L. A., & Hamilton, A. D. (2004). Water gelation by small organic molecules. *Chemical reviews*, *104*(3), 1201-1218.
26. Sharma, A., Thakur, M., Bhattacharya, M., Mandal, T., & Goswami, S. (2019). Commercial application of cellulose nano-composites–A review. *Biotechnology Reports*, *21*, e00316.
27. Hurtado-Fernández, E., Trujillo-Cayado, L. A., Álvarez-Mateos, P., & Santos, J. (2026). Extraction, Characterization and Applications of Biopolymers from Sustainable Sources. *Polymers*, *18*(5), 581.
28. Klemm, D., Heublein, B., Fink, H. P., & Bohn, A. (2005). Cellulose: fascinating biopolymer and sustainable raw material. *Angewandte chemie international edition*, *44*(22), 3358-3393.
29. Ahn, B. K., Lee, D. W., Israelachvili, J. N., & Waite, J. H. (2014). Surface-initiated self-healing of polymers in aqueous media. *Nature materials*, *13*(9), 867-872.\
30. Xiang, T., Guo, Q., Jia, L., Yin, T., Huang, W., Zhang, X., & Zhou, S. (2024). Multifunctional hydrogels for the healing of diabetic wounds. *Advanced healthcare materials*, *13*(1), 2301885.
31. Kong, M., Chen, X. G., Xing, K., & Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review. *International journal of food microbiology*, *144*(1), 51-63.
32. Dutta, P. K., Dutta, J., & Tripathi, V. S. (2004). Chitin and chitosan: Chemistry, properties and applications. *Journal of scientific and industrial research*, *63*(1), 20-31.
33. Lee, K. Y., & Mooney, D. J. (2012). Alginate: properties and biomedical applications. *Progress in polymer science*, *37*(1), 106-126.
34. Ghosh, J., Rupanty, N. S., Khan, F., Noor, T., Jahangir, R., Mirmohammad sadeghi, S., & Islam, T. (2025). Grafting modification for textile functionalization: innovations and applications. *Discover Applied Sciences*, *7*(1), 49.
35. Langer, R., & Peppas, N. A. (2003). Advances in biomaterials, drug delivery, and bionanotechnology. *AIChE Journal*, *49*(12), 2990-3006.
36. Fuller, B. J. (2004). Cryoprotectants: the essential antifreezes to protect life in the frozen state. *CryoLetters*, *25*(6), 375-388.
37. Ding, Y., Shi, Y., & Yang, S. (2024). Regulatory networks underlying plant responses and adaptation to cold stress. *Annual Review of Genetics*, *58*(1), 43-65.
38. Godoy, J. L., Pereyra, E. V., Cressa Frascot, F. N., Peralta, M. P., Cabrera, G. M., Martorell, M. M., ... & Ruberto, L. A. (2026). Cold Biotechnology: Cosmetic, Pharmaceutical, and Therapeutic Applications of Antarctic Psychrophilic Fungi. *Journal of Pharmaceutical and BioTech Industry*, *3*(3), 19.
39. Bang, J. K., Lee, J. H., Murugan, R. N., Lee, S. G., Do, H., Koh, H. Y., ... & Kim, H. J. (2013). Antifreeze peptides and glycopeptides, and their derivatives: potential uses in biotechnology. *Marine drugs*, *11*(6), 2013-2041.
40. William, N., Mangan, S., Ben, R. N., & Acker, J. P. (2023). Engineered compounds to control ice nucleation and recrystallization. *Annual Review of Biomedical Engineering*, *25*(1), 333-362.
41. Ustun, N. S., & Turhan, S. (2015). Antifreeze proteins: Characteristics, function, mechanism of action, sources and application to foods. *Journal of food processing and preservation*, *39*(6), 3189-3197.
42. Worrall, D., Elias, L., Ashford, D., Smallwood, M., Sidebottom, C., Lillford, P., ... & Bowles, D. (1998). A carrot leucine-rich-repeat protein that inhibits ice recrystallization. *Science*, *282*(5386), 115-117.
43. Salami, T. M., Sun, D. W., & Tian, Y. (2025). Advancing future food preservation with green cryoprotective agents (GCAs) to mitigate ice damage in freezing. *Food Engineering Reviews*, *17*(4), 927-945.
44. Kachhawaha, K., Singh, S., Joshi, K., Nain, P., & Singh, S. K. (2023). Bioprocessing of recombinant proteins from Escherichia coli inclusion bodies: insights from structure-function relationship for novel applications. *Preparative biochemistry & biotechnology*, *53*(7), 728-752.
45. Liu, S., Wang, W., Von Moos, E., Jackman, J., Mealing, G., Monette, R., & Ben, R. N. (2007). In vitro studies of antifreeze glycoprotein (AFGP) and a C-linked AFGP analogue. *Biomacromolecules*, *8*(5), 1456-1462.

46. Chen, Y., Sun, J., Chen, H., Yang, Y., Zhang, J., Wang, S., ... & Dai, L. (2025). Plant-derived bioactive peptides: extraction, isolation, purification, pharmacological activities, structure–activity relationship, and applications. *Journal of agricultural and food chemistry*, *73*(32), 19867-19906.
47. Thomashow, M. F. (2010). Molecular basis of plant cold acclimation: insights gained from studying the CBF cold response pathway. *Plant physiology*, *154*(2), 571-577.
48. Ullah, F., Abbas, A., Gul, H., Güncan, A., Hafeez, M., Gadratagi, B. G., ... & Li, Z. (2024). Insect resilience: unraveling responses and adaptations to cold temperatures. *Journal of Pest Science*, *97*(3), 1153-1169.
49. Elliott, G. D., Wang, S., & Fuller, B. J. (2017). Cryoprotectants: A review of the actions and applications of cryoprotective solutes that modulate cell recovery from ultra-low temperatures. *Cryobiology*, *76*, 74-91.
50. Carpenter, J. F., & Crowe, J. H. (1988). The mechanism of cryoprotection of proteins by solutes. *Cryobiology*, *25*(3), 244-255.
51. Xu, J., Lei, C., & Zhu, W. (2025). Nanomaterial-Enhanced Red Blood Cell Biopreservation: From Refrigeration to Cryopreservation. *Chembiochem*, *26*(8), e202400827.
52. Gurtovenko, A. A., & Anwar, J. (2007). Modulating the structure and properties of cell membranes: the molecular mechanism of action of dimethyl sulfoxide. *The journal of physical chemistry B*, *111*(35), 10453-10460.
53. González-Arancibia, F., Mamani, M., Valdés, C., Contreras-Matté, C., Pérez, E., Aguilera, J., ... & Andler, R. (2024). Biopolymers as sustainable and active packaging materials: fundamentals and mechanisms of antifungal activities. *Biomolecules*, *14*(10), 1224.
54. Mudge, K. (2009). A history of grafting. Horticulture Reviews, vol. 35, eds Jules Janick.
55. Bowler, P. G. (2018). Antibiotic resistance and biofilm tolerance: a combined threat in the treatment of chronic infections. *Journal of Wound Care*, *27*(5), 273-277.
56. Del Pozo, J. Á., & Patel, R. (2007). The challenge of treating biofilm-associated bacterial infections. *Clinical Pharmacology & Therapeutics*, *82*(2), 204-209.
57. Kumar, A., Vemula, P. K., Ajayan, P. M., & John, G. (2008). Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. *Nature materials*, *7*(3), 236-241.
58. Behera, A. D., & Das, S. (2023). Ecological insights and potential application of marine filamentous fungi in environmental restoration. *Reviews in Environmental Science and Bio/Technology*, *22*(2), 281-318.
59. Huston, A. L., Methe, B., & Deming, J. W. (2004). Purification, characterization, and sequencing of an extracellular cold-active aminopeptidase produced by marine psychrophile Colwellia psychrerythraea strain 34H. *Applied and environmental microbiology*, *70*(6), 3321-3328.
60. Romanazzi, G., & Feliziani, E. (2014). Botrytis cinerea (gray mold). In *Postharvest decay* (pp. 131-146). Academic Press.
61. Moradali, M. F., & Rehm, B. H. (2020). Bacterial biopolymers: from pathogenesis to advanced materials. *Nature Reviews Microbiology*, *18*(4), 195-210.
62. Versey, Z., da Cruz Nizer, W. S., Russell, E., Zigic, S., DeZeeuw, K. G., Marek, J. E., ... & Cassol, E. (2021). Biofilm-innate immune interface: contribution to chronic wound formation. *Frontiers in immunology*, *12*, 648554.
63. Abisado, R. G., Benomar, S., Klaus, J. R., Dandekar, A. A., & Chandler, J. R. (2018). Bacterial quorum sensing and microbial community interactions. *MBio*, *9*(3), 10-1128.
64. Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *science*, *284*(5418), 1318-1322.
65. Gompelman, M., van Asten, S. A., & Peters, E. J. (2016). Update on the role of infection and biofilms in wound healing: pathophysiology and treatment. *Plastic and reconstructive surgery*, *138*(3S), 61S-70S.
66. Adaskaveg, J. E., Gubler, W. D., Michailides, T. J., & Holtz, B. A. (2011). Efficacy and timing of fungicides, bactericides, and biologicals for deciduous tree fruit, nut, strawberry, and vine crops 2011.
67. Mori, Y., Nakagami, G., Kitamura, A., Minematsu, T., Kinoshita, M., Suga, H., ... & Sanada, H. (2019). Effectiveness of biofilm-based wound care system on wound healing in chronic wounds. *Wound Repair and Regeneration*, *27*(5), 540-547.
68. Ferreira, G., De-La-Cruz-Chacón, I., Boaro, C. S. F., Baron, D., & Lemos, E. E. P. D. (2019). Propagation of Annonaceous plants. *Revista Brasileira de Fruticultura*, *41*(1), e-500.
69. Romanazzi, G., Lichter, A., Gabler, F. M., & Smilanick, J. L. (2012). Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes. *Postharvest Biology and Technology*, *63*(1), 141-147.
70. Clark, M., & Adcock, L. (2019). Honey for wound management: a review of clinical effectiveness and guidelines.
71. Wisniewski, M., Bassett, C., & Gusta, L. V. (2003). An overview of cold hardiness in woody plants: seeing the forest through the trees. *HortScience*, *38*(5), 952-959.
72. Robles, V., G. Valcarce, D., & F. Riesco, M. (2019). The use of antifreeze proteins in the cryopreservation of gametes and embryos. *Biomolecules*, *9*(5), 181.
73. Harrison, J. J., Turner, R. J., & Ceri, H. (2005). Persister cells, the biofilm matrix and tolerance to metal cations in biofilm and planktonic Pseudomonas aeruginosa. *Environmental microbiology*, *7*(7), 981-994.
74. Broeckx, G., Vandenheuvel, D., Claes, I. J., Lebeer, S., & Kiekens, F. (2016). Drying techniques of probiotic bacteria as an important step towards the development of novel pharmabiotics. *International journal of pharmaceutics*, *505*(1-2), 303-318.
75. Griffith, M., Lumb, C., Wiseman, S. B., Wisniewski, M., Johnson, R. W., & Marangoni, A. G. (2005). Antifreeze proteins modify the freezing process in planta. *Plant Physiology*, *138*(1), 330-340.