Hydrogels of levan polysaccharide: A systematic review

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Abstract:	Levan is a fructose-based homopolysaccharide renowned for its unique properties, including exceptional adhesive strength, self-assembly capability, low viscosity, and bioactivities such as prebiotic, anti-cancer, anti-inflammatory, and anti-diabetic effects. These characteristics have created increasing interest in levan-based biomaterials over the past decade, positioning levan as a highly under-explored biopolymer for a wide range of applications, from medicine to cosmetics. As a result, levan-based hydrogels have emerged as promising biomaterials in drug delivery, tissue engineering, and cosmetic formulations, owing to their extracellular matrix-mimicking structure, tunable mechanical properties, and controlled cargo release capabilities. This review is the first to comprehensively examine the advancements in levan-based hydrogel research, systematically analyzing their biomedical applications and comparing them with other biopolymer-based hydrogels. Key questions regarding levan's potential as an alternative to established hydrogel systems are explored, highlighting areas requiring further research. By assessing trends and findings in the literature, this review provides an overview of the advantages, limitations, and prospects of levan hydrogels. Our analysis establishes a foundation for the continued development of levan-derived biomaterials, fostering broader adoption in biomedical and industrial applications.
Opposed Reviewers:	
Response to Reviewers:	

Highlights

- the first comprehensive review of levan-based hydrogels
- analysis of state-of-the-art literature followed PRISMA protocol
- hydrogel reports are limited and mainly for biomedical applications
- they remain unexplored in various applications areas like food

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Wednesday, May 07, 2025

To: Prof. Ying Liang

Editor

International Journal of Biological Macromolecules

Central South University of Forestry and Technology,
Changsha, China

Dear Prof. Ying Liang,

Please find enclosed the revised version of our manuscript **IJBIOMAC-D-25-10428** entitled **" *Hydrogels of Levan Polysaccharide: a systematic review*"** which we are submitting for exclusive consideration of publication as a review article in the International Journal of Biological Macromolecules. This study represents **the first comprehensive analysis of levan-based hydrogels and their applications**.

Following the reviewers' comments, the whole manuscript underwent a comprehensive change, paragraphs were added, formats were adjusted and a new Figure was added. All the revisions made in the manuscript are marked in red. Moreover, the "Response to Reviewer's Comments" attached to this letter lists all the comments as well as our point-by-point responses. We hope to have addressed all suggestions and believe that these modifications have improved the manuscript to a great extent.

As corresponding author, on behalf of all the authors, I would like to thank you and all the reviewers for the time and effort put into careful reading and helpful commenting on our work.

Thank you again for your consideration of our work and we hope to hear from you soon.

Sincerely Yours,

Prof. Ebru Toksoy Oner
Marmara University
Industrial Biotechnology and Systems
Biology (IBSB) Research Group

Response to Reviewer's Comments

Reviewer #1:

The current manuscript reports a systematic review on hydrogels of levan polysaccharide. The obtained results could be useful information for researchers who worked in the similar field. In general, this is an interesting review.

Section 3. Since this manuscript is not about the properties of levan, it is suggested to simplify this section.

We thank Reviewer 1 for the constructive suggestions. As noted, the primary focus of this review manuscript is on levan-based hydrogels rather than levan itself. The section titled 'Why levan?' was included to provide a general overview of the key physicochemical and biological properties of levan, intended to guide researchers in developing novel hydrogel formulations. These properties may also offer additional insights depending on the application, especially when compared with hydrogel studies involving other polymers in the literature. To enhance clarity, we have revised this section as well as the rest of the mannuscript to more clearly explain the relevance of these properties to hydrogel design, and we have removed or streamlined sections as appropriate.

Reviewer #2:

This manuscript deals with levan-based hydrogels. The paper gives a systematic overview of the results in this field and compares materials based on levan with hydrogels based on other polysaccharides. This new field with wide-ranging potential applications will undoubtedly be of interest to readers. Certainly, the review could be inspiring for new research.

However, the discussion could be expanded in some sections:

1. The authors used the WoS database for their search. What about other databases such as the patent database or dissertations?

Authors thank the Reviewer for this valuable comment. Firstly, the WoS search was updated during the revision from March to May 2025. While the total number of documents and their distribution among specific polysaccharides changed, the model predicted data remained the same as well as the specific percentages reported for chitosan and alginate.

A patent search was conducted using four major databases—WIPO's PATENTSCOPE, Google Patents, Espacenet (by the European Patent Office - EPO) and The Lens by querying the terms "levan" and "hydrogel" across each platform. The Lens, Google Patents and Espacenet gave a total of 7143, 4518 and 448 patent hits, respectively. A Frontpage search on Patentscope resulted in a total of four patents. Just to compare, the

same Frontpage search for "chitosan and hydrogel" and "alginate and hydrogel" yielded 2969 and 2412 hits, respectively. Whwn these four patents were examined, " KR1020190066936 - Levan/Hap Organic-Inorganic Hybrid Filler and Method For Producing Same" and " KR1020190041272 - Medical Bio-Filler Material Using Levan Hydrogel" were granted to Korea Institute Of Ceramic Engineering And Technology in 2019 by Republic of Korea and they were related to cosmetic applications of levan, PF127 and CMC containing hydrogels. The Russian patent "RU0002815367 - Biocomposite Material Based On Natural Polysaccharides" was granted to Potapova Valentina Vladimirovna and involved a mixture of alginate and levan from Paenibacillus polymyxa. The final patent wasn also a Russian patent " RU0002819701 - Hydrogel Biocomposite Based On Bacterial Polysaccharides For Use In Tissue Engineering" issued in 2024 and involved a hydrogel composite of bacterial cellulose of *Komagataeibacter sucrofermentans* and *P. polymyxa* levan. The Cooperative Patent Classification (CPC) code A23V 2250/5068 was specific for fructans (including levan) used as food ingredients or additives in foodstuffs or non-alcoholic beverages. On the other hand, this code did not yield any hydrogel related patent hit.

The following sentences were added to the Section on state-of-the-art.

"A patent search was also conducted across four major databases, namely, World Intellectual Property Organization (WIPO)'s Patentscope, Google Patents, Espacenet (by the European Patent Office, EPO), and The Lens—using the keywords "levan" and "hydrogel." The Lens, Google Patents, and Espacenet yielded 7,143, 4,518, and 448 hits, respectively. A Frontpage search on Patentscope returned 4 results only (same search for chitosan and alginate instead of levan yielded 2969 and 2412 hits, respectively). Two Korean patents (KR1020190066936 and KR1020190041272) granted in 2019 focused on levan-based hydrogels containing PF127 and CMC for cosmetic applications [27, 28]. In 2024, two Russian patents (RU0002815367 and RU0002819701) described biocomposite hydrogels involving *Paenibacillus polymyxa*-derived levan with one combined with alginate (RU0002815367), and the other with *Komagataeibacter sucrofermentans* bacterial cellulose (RU0002819701) for tissue engineering applications. The Cooperative Patent Classification (CPC) code A23V 2250/5068, which pertains to fructans used in food applications, was identified as relevant to levan but did not yield any hydrogel-related results."

Thesis search was performed from a more general platform (Google) since each country has its own database for thesis (see https://ndltd.org/thesis-resources/find-etds/ for the specific links). Keywords "Thesis" and "levan hydrogel" yielded only 3 relevant results all of which were from our research group, namely,

- *Halomonas* levan hydrogels for skin tissue engineering applications, SS Selvi - 2019 - search.proquest.com
- Development of Injectable Levan Hydrogels for the Treatment of Uterine Fibroids, MY Kandur - 2023 - search.proquest.com

- Levan as a Biomaterial for the Conservation of Cultural Heritage, R Sağlam - 2022 - search.proquest.com

When the Proquest data base was searched through Dissertations & Theses for "hydrogel", "levan" and "levan hydrogel", 1028, 186 and 6 results were found. These 6 were listed as follows:

- 3D Microfluidics for Environmental Pathogen Detection and Single-Cell Phenotype-To-Genotype Analysis, Zhu, Yanzhe. California Institute of Technology ProQuest Dissertations & Theses, 2020. 30555368. (acknowledgement to Axl LeVan)

- Novel, Rapid and Cost-Effective Methods for Concentration, Detection and Monitoring of Waterborne Pathogens in Resource-Limited Settings, Wu, Xunyi. California Institute of Technology ProQuest Dissertations & Theses, 2021. 30555422. (acknowledgement to Axl LeVan)

- Additive Manufacturing of 3D Functional Materials: From Surface Chemistry to Combustion-Derived Materials, Yee, Daryl W. California Institute of Technology ProQuest Dissertations & Theses, 2020. 30555434. (acknowledgement to Axl LeVan)

- Remodeling Characteristics and *in vivo* Healing of Low Viscosity Polyurethane Biocomposites for Bone Regeneration, Talley, Anne Douglas. Vanderbilt University ProQuest Dissertations & Theses, 2016. 10753468. (approved by M. Douglas LeVan, Ph.D.)

- Aqueous Aerosols in Atmospheric Chemistry and Airborne Diseases, Gu, Alan Yalun. California Institute of Technology ProQuest Dissertations & Theses, 2022. 30554081. (acknowledgement to Axl LeVan)

- The Emerging Mechanochemistry of Naphthopyran, McFadden, Molly Elizabeth. California Institute of Technology ProQuest Dissertations & Theses, 2023. 30961645. (acknowledgement to Axl LeVan)

These clearly showed that there are only very few thesis studies on levan hydrogels. I pinpoint this fact, the following sentence was added to the conclusions section:

"Despite the growing interest in levan-based hydrogels, there are only a limited number of MSc and PhD theses dedicated to this topic worldwide, highlighting a significant gap in academic research training that should be addressed through increased graduate-level investigations across institutions globally."

2. What is the regulatory framework for the introduction of hydrogels in practice?

The regulatory framework for introducing hydrogels into practice depends on their intended application—medical, cosmetic, food, or industrial. In medical and healthcare contexts, hydrogels are regulated as medical devices or drug-device combinations by authorities like the FDA in the US and under the EU Medical Device Regulation (MDR). These require classification based on risk, biocompatibility testing (e.g., ISO 10993), sterility validation, clinical evaluation, and compliance with manufacturing standards such as GMP or ISO 13485. For cosmetic uses, regulations focus on safety and proper labeling, with notification procedures like the EU's Cosmetic Products Notification Portal (CPNP). In the food sector, hydrogels must be approved as safe additives, such as GRAS substances in the US or approved E numbers in the EU. Industrial applications are generally less tightly regulated but may fall under environmental or chemical safety rules like EPA or REACH, especially if human exposure is possible.

When FDA database is searched for levan, the only relevant output is about inulin "Agave Inulin Generally Recognized as Safe (GRAS) Notice for IMAG Organic, Agave Inulin Extracted from Agave tequilana Weber var. azul" where levan is only mentioned as a structural form of fructan.

As also explained in much detail in Catoira et al. (2020), under the new European Medical Device Regulation (EU 2017/745), hydrogels—particularly those used as implantable materials—are classified as Class III medical devices, the highest risk category. This classification subjects them to strict regulatory scrutiny from the early stages of development through commercialization. Manufacturers must conduct comprehensive risk analysis and management, especially when using animal-derived materials, and compile extensive technical documentation, including clinical evaluation data. A Quality Management System (QMS), typically compliant with ISO 13485, is required, and the device must pass conformity assessments by a Notified Body. Once approved, a CE mark is granted, enabling marketing within the EU. Additionally, traceability is reinforced through the Unique Device Identification (UDI) system and the EUDAMED database. When EUDAMED database is searched for levan hydrogels, there was no relevant output.

The absence of levan hydrogels in these databases was also confirmed by a recent article that reviewed the clinical landscape of both injectable and non-injectable hydrogels, highlighting hydrogel-based medical products that have received FDA or EMA approval or are currently being investigated in active clinical trials (Clegg et al., 2024).

To emphasize these, the following paragraphs were added in the conclusions section :

" The regulatory framework for introducing hydrogels into practice depends on their intended application—medical, cosmetic, food, or industrial. In medical and healthcare contexts, hydrogels are regulated as medical devices or drug-device combinations by authorities like the FDA in the US and under the EU Medical Device Regulation (MDR). These require classification based on risk, biocompatibility testing (e.g., ISO 10993), sterility validation, clinical evaluation, and compliance with manufacturing standards such

as GMP or ISO 13485 (Catoira et al., 2020). For cosmetic uses, regulations focus on safety and proper labeling, with notification procedures like the EU's Cosmetic Products Notification Portal (CPNP). In the food sector, hydrogels must be approved as safe additives, such as GRAS substances in the US or approved E numbers in the EU. Industrial applications are generally less tightly regulated but may fall under environmental or chemical safety rules like EPA or REACH, especially if human exposure is possible.

Levan hydrogels are not currently present in major regulatory or clinical databases such as the FDA or European Database on Medical Devices (EUDAMED) and no approved or clinically investigated levan-based hydrogel products have been identified (Clegg et al., 2024). This clearly shows that they have not yet reached the stage of regulatory recognition or clinical translation despite the broader clinical interest in hydrogel technologies.

To advance the clinical and commercial translation of levan-based hydrogels, future research should prioritize comprehensive in vivo studies. While preliminary in vivo evaluations have demonstrated promising biocompatibility, the current body of evidence remains limited and insufficient to fully assess their behavior under complex physiological conditions. Systematic in vivo testing is essential to evaluate not only biocompatibility but also immunogenicity, biodegradability, mechanical stability, and therapeutic efficacy across diverse biomedical applications. Such studies are critical for meeting the safety and efficacy standards set by regulatory agencies such as the FDA and European Medicines Agency (EMA) and represent a necessary milestone before progressing to human clinical trials and market adoption. "

References :

Catoira, M.C., González-Payo, J., Fusaro, L. et al. Natural hydrogels R&D process: technical and regulatory aspects for industrial implementation. J Mater Sci: Mater Med 31, 64 (2020). https://doi.org/10.1007/s10856-020-06401-w

Clegg, J.R.; Adebowale, K.; Zhao, Z.; Mitragotri, S. Hydrogels in the clinic: An update. Bioeng. Transl. Med. 2024, 9, e10680.

3. Apart from biomedicine, which areas of application for levan hydrogels have the greatest potential to be developed in the future?

We thank the Reviewer for this insightful comment. While levan hydrogels have predominantly been investigated in biomedical applications, as discussed throughout the review, they also show considerable potential in other fields. For instance, their use in the food industry is discussed in Section 5.5, and their recent application in conservation science, highlighted in Section 5.6, suggests broader relevance.

To address this further, we have added the following paragraph to the manuscript to emphasize the potential of levan hydrogels in environmental applications:

“One of these emerging areas is environmental science. Levan hydrogels possess key features—such as high water absorption capacity, adjustable porosity, biodegradability, and non-toxicity—that make them suitable for environmental uses. These include their application as adsorbents in wastewater treatment and as carriers in controlled-release systems for agricultural chemicals (Dong et al., 2024). Moreover, a study by Phengnoi et al. (2019) demonstrated that blending levan with polyvinyl alcohol (PVA), a synthetic polymer with low degradability, led to increased degradation rates with higher levan content, further supporting their potential in eco-friendly materials.”

References:

Dong, Y., Ghasemzadeh, M., Khorsandi, Z., Sheibani, R., & Nasrollahzadeh, M. (2024). Starch-based hydrogels for environmental applications: A review. *International Journal of Biological Macromolecules*, *269*, 131956. https://doi.org/10.1016/j.ijbiomac.2024.131956

Phengnoi, P., Thongmee, K., Tiptipakorn, S., Boekfa, B., & Kuttiyawong, K. (2019). Biodegradation of Levan Polymer / Poly (Lactic Acid) (PLA) blend. *IOP Conference Series Materials Science and Engineering*, *526*(1), 012025. https://doi.org/10.1088/1757-899x/526/1/012025

4. It is known that levan (as well as some other polysaccharides) has certain disadvantages in the production of hydrogels, such as weak mechanical properties, fast degradation time and susceptibility to microbial contamination.

What strategy is used to overcome these shortcomings?

Authors thank the Reviewer for highlighting this important point. It is indeed recognized that native levan, like many natural polysaccharides, faces challenges such as limited mechanical strength, rapid degradation, and vulnerability to microbial contamination when used in hydrogel formulations. Also, the uncharged nature of levan prevents it from forming a stable hydrogel on its own. This can lead to poor mechanical properties and rapid degradation. To address these issues, several strategies have been employed. One common approach is the chemical or physical crosslinking of levan with other polymers—both natural and synthetic—such as polyvinyl alcohol (PVA), chitosan, or gelatin, which improves the structural integrity and mechanical properties of the resulting hydrogels (see Table 1 and relevant sections in the manuscript. Additionally, blending levan with polymers that possess antimicrobial activity or incorporating antimicrobial agents (e.g., silver nanoparticles or essential oils) can mitigate contamination risks. The degradation rate can also be modulated by adjusting the degree of crosslinking, molecular weight of levan, or by forming composite networks that provide controlled stability. These modifications aim to tailor levan-based hydrogels for specific applications, enhancing their performance without compromising their biocompatibility.

The following paragraphs have been added to the manuscript.

“This self-assembly behavior contributes to the formation of 3D networks, which are characteristic of hydrogels, while maintaining low viscosity in solution. Although levan's low viscosity is advantageous for injectable hydrogel systems, it can also hinder the development of mechanically strong and stable hydrogels. This limitation can be addressed by optimizing reaction conditions to relax the self-assembled structures, employing chemical crosslinking, or forming composite hydrogels."

"Furthermore, the inherently fast biodegradation rate of levan can be tuned by chemical modifications or by integrating it into composite hydrogels, allowing for better control over its stability and degradation kinetics in various application environments. “

Reviewer #3:

Tekin et al. presented an interesting manuscript about hydrogels of levan polysaccharide, an emerging field because of the interesting properties of this biopolymer as authors have stated in the manuscript. Some revisions should be done before publishing at IJBM.

- Authors should include a figure that explains the differences between hydrogels crosslinking (physical or chemical), and what is the role of levan in each case.

Following this valuable suggestion, the following Figure was added to the manuscript as Figure 2 and related context was discussed.

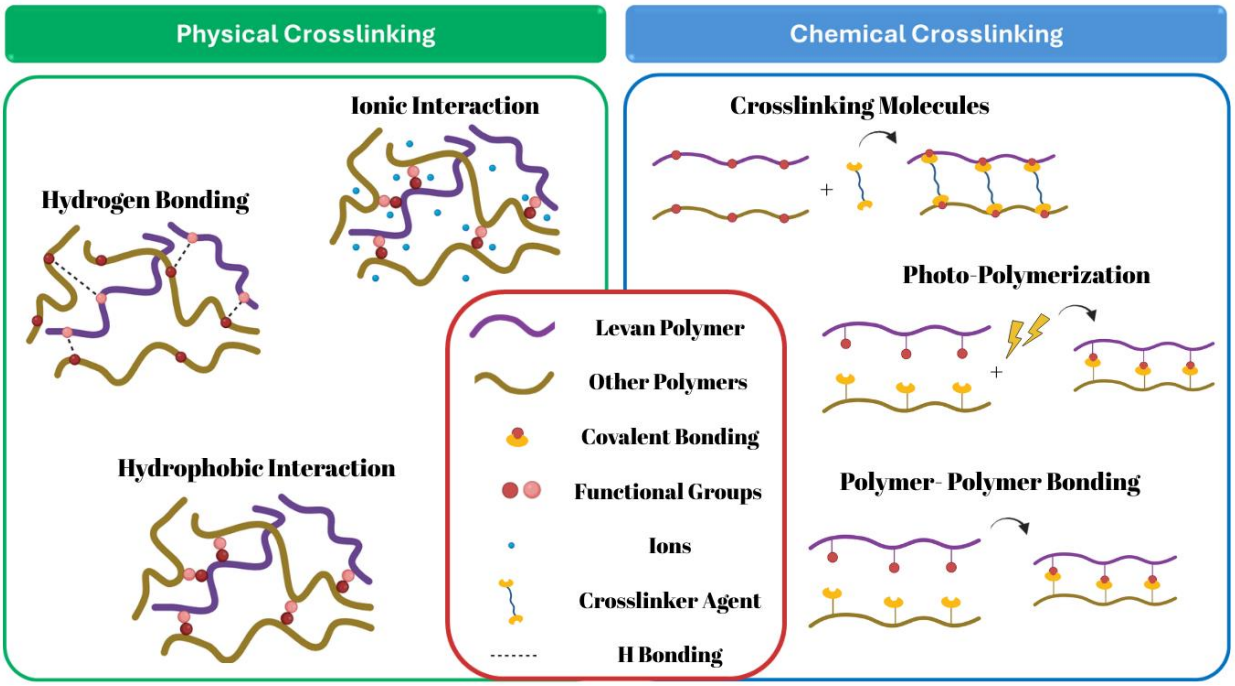

Figure 2. Physically and chemically crosslinked levan-based hydrogels (Created with Canva.com).

- More information about hydrogels should be included in the introduction. Perhaps after line 78, before presenting the objective of the work.

Following paragraph was added to the text for a better explanation and more information about hydrogels

"Compared to rigid scaffolds or dry polymeric systems, polysaccharide-based hydrogels offer flexibility, soft structure, high porosity, and water-holding capacity that promote cell viability, proliferation, matrix remodeling, and better resemblance to the native living tissue. In the formation of hydrogels, the use of polysaccharides that contain different chemical groups or whose chemical modifications are relatively easier allows both chemical and physical crosslinking. Thus, depending on the requirements and the area of use, it is possible to obtain chemically, mechanically, and dimensionally stable hydrogels formed with covalent bonds or to obtain hydrogels that do not require crosslinkers and whose preparation process is easier and faster. Due to their extracellular matrix-mimicking properties, biocompatibility, flexible synthesis methods, and suitable physical properties, they have found broad applications as artificial soft tissue biomaterials in various biomedical fields, such as biosensors, and tissue engineering and regenerative medicine [6, 7]. Furthermore, with a smart hydrogel approach, polysaccharide-based hydrogels can be tuned to respond to environmental stimuli such as pH, temperature, electrical and magnetic fields, ionic strength or light and can change some of their properties such as their wettability, degradability, swelling, and mechanical or surface properties. These attunable properties making them attractive especially for smart drug delivery systems and personalized medicine [8, 9]."

- page 3, After numerical analysis (papers published about hydrogels) Authors should explain why there are only 21 papers related with levan hydrogels if you compare with other polysaccharides. The reason is, mainly, levan viscosity. Authors should deepen on this property.

As the Reviewer rightly noted, there are currently very few publications specifically focused on levan hydrogels. Although the levan polymer has been known for over a century (Toksoy Öner et al., 2016), it remained largely overlooked until the early 2000s, when the number of publications on levan began to increase significantly. This surge in interest is primarily due to advances in addressing the high production cost of levan—a major limiting factor in its wider application. Numerous researchers have tackled this challenge, and today, several high-yield production platforms exist for both microbial and enzymatic levan. These developments have been discussed in depth in the Book of Fructans (J. Combie, E. Toksoy Öner, Fructan Production Processes, The Book of Fructans (2023), pp. 187–199. https://doi.org/10.1016/b978-0-323-85410-8.00009-0) as well as in the conclusions section of this manuscript.

The inherently low viscosity of levan, often perceived as a drawback, can actually be advantageous in microbial production systems, where it helps maintain stable culture rheology even at high product concentrations—as observed with xanthan. Moreover, this low viscosity results from levan's self-assembling behavior, which under controlled conditions can be harnessed in the design of smart delivery systems. A paragraph elaborating on the viscosity and self-assembly behavior of levan has been added to Section 3.

Reference:

E. Toksoy Öner, J. Hernández, J. Combie, Review of levan polysaccharide: from a century of past experiences to future prospects, Biotechnol. Adv. 34 (2016) 827–844.

- section 3, maybe viscosity issues can be explained here as well.

Thanks to Reviewer for this constructive addition. The required section was added to section 3.

"This self-assembly behavior contributes to the formation of 3D networks, which are characteristic of hydrogels, while maintaining low viscosity in solution. Although levan's low viscosity is advantageous for injectable hydrogel systems, it can also hinder the development of mechanically strong and stable hydrogels. This limitation can be addressed by optimizing reaction conditions to relax the self-assembled structures, employing chemical crosslinking, or forming composite hydrogels. "

- sections 3.1, 3.2, 3.3., 3.4 explains the current uses of levan, because of its properties, but they do not explain why these properties can be used to create hydrogels. Authors should reinforce this part of the review.

As also noted above, the primary focus of this review manuscript is on levan-based hydrogels rather than levan itself. The section titled 'Why levan?' was included to provide a general overview of the key physicochemical and biological properties of levan, intended to guide researchers in developing novel hydrogel formulations. These properties may also offer additional insights depending on the application, especially when compared with hydrogel studies involving other polymers in the literature. To enhance clarity, we have revised this section as well as the rest of the mannuscript to more clearly explain the relevance of these properties to hydrogel design, and we have removed or streamlined sections as appropriate.

- section 4. Authors should clarify that "there is not a paper that presents levan-hydrogel only formed by levan". All papers combined levan with other polysaccharide or they use a crosslinker. This issue has to be addressed properly.

The authors thank the Reviewer for this very important point. Indeed, similar to dextran, native levan is an uncharged and highly water-soluble polymer that cannot form stable hydrogels in its unmodified form due to the absence of charged groups and insufficient intermolecular interactions required for three-dimensional network formation. Therefore, hydrogel formation with levan typically necessitates structural modification and/or chemical or physical crosslinking. To clarify this point, the following sentence has been added to the main text: “Due to its uncharged nature and water solubility, native levan does not readily form stable hydrogels without chemical or physical crosslinking or prior structural modification.”

- section 4 must be extended. Authors should explain here why polymer (or fructans) functionalization is important. Some paragraphs about fructan functionalization to create hydrogels are needed.

Authors would like to thank the Reviewer for this important point. Following the suggestion, Section 4 has been extended and one Figure (Figure 2) was added to illustrate the discussed items.

The added paragraphs are as follows :

" Chemical crosslinking involves the formation of covalent bonds between polymer chains, either directly or via low-molecular-weight crosslinkers, resulting in hydrogels with enhanced long-term stability and mechanical strength. In contrast, physical crosslinking relies on non-covalent interactions, often leading to heterogeneous polymer networks and hydrogels that are structurally less stable and mechanically brittle. Nevertheless, physically crosslinked hydrogels formed via weak interactions, such as ionic or electrostatic interactions, hydrophobic interactions, hydrogen bonding, or van der Waals forces, can exhibit advantageous properties, including self-healing capability and responsiveness to external stimuli [99].

The choice of cross-linking method depends on the physicochemical properties of the polymer. Due to its uncharged nature and water solubility, native levan does not readily form stable hydrogels without chemical or physical crosslinking or prior structural modification. Therefore, either levan or its chemical derivatives are mixed with other polymers to promote physical crosslinking, or chemical crosslinkers are used to establish covalent bonds and enhance hydrogel stability. Furthermore, the inherently fast biodegradation rate of levan can be tuned by chemical modifications or by integrating it into composite hydrogels, allowing for better control over its stability and degradation kinetics in various application environments.

In literature, there are reports on physically linked levan hydrogels such as injectable levan/ CMC /Pluronic F127 blends [27, 28]. In both studies, it was stated that there is hydrophobic association and hydrogen bonding between levan, Pluronic F127, and CMC, and especially that the poly(ethylene oxide) group of Pluronic F127 interacts with levan, and therefore levan is necessary for physical crosslinking. In another study, ionically crosslinked levan/gellan blends were examined, and it was hypothesized that under alkaline conditions, gellan interacts with levan via ionic crosslinking between hydroxyl and carboxyl groups to form a hydrogel network [29]. On the other hand, relatively more studies used chemical crosslinking to obtain better mechanical stability, including photo-polymerized methacrylated *Bacillus* levan [34] or chitosan crosslinked oxidized *Bacillus* levan [36]. In these studies, functional groups were introduced into levan to facilitate its reaction with crosslinkers or other polymers. For instance, methacrylate-modified levan enables covalent bonding with photoinitiated crosslinkers such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) or Irgacure 2959 [34]. Similarly, the incorporation of aldehyde groups through oxidation allows levan to form Schiff base linkages with amine-containing polymers like chitosan [36]. However, there are also examples in which unmodified levan has been directly employed to form chemically crosslinked hydrogels such as glutaraldehyde crosslinked *Zymomonas* levan/PVA [35], and BDDE crosslinked *Halomonas* levan [37 - 42]. Figure 2 illustrates various types of chemically and physically crosslinked levan-based hydrogels while Figure 3 gives the classification of levan hydrogels.

"

- Table 1, Z. mobilis example, 3rd row, should be expressed as: Levan/PVA crosslinked glutaldehyde

The suggested correction was made in Table 1.

- Conclusion. Authors should include as future research, the importance of carry out in vivo experiments, due to the lack of knowledge, as an essential milestone to deliver these hydrogels to market.

The authors thank the Reviewer for this insightful comment highlighting the importance of i*n vivo* studies as a critical step toward clinical and commercial translation of levan-based hydrogels. We have revised the conclusion to better reflect this point and emphasize the necessity of further *in vivo* experimentation. The following paragraph was added to the conclusions section:

"To advance the clinical and commercial translation of levan-based hydrogels, future research should prioritize comprehensive in vivo studies. While preliminary *in vivo* evaluations have demonstrated promising biocompatibility, the current body of evidence remains limited and insufficient to fully assess their behavior under complex physiological

conditions. Systematic *in vivo* testing is essential to evaluate not only biocompatibility but also immunogenicity, biodegradability, mechanical stability, and therapeutic efficacy across diverse biomedical applications. Such studies are critical for meeting the safety and efficacy standards set by regulatory agencies such as the FDA and European Medicines Agency (EMA) and represent a necessary milestone before progressing to human clinical trials and market adoption."

Minor typos:

We are grateful to reviewers for their careful audits. All minor typos have been corrected as listed and highlighted with red in the main text.

+ line 172, "a crucial"
+ NaOH and $CaCl_2$ in Table 1
+ Uppercase for pNIPA in Table 1
+ line 487, "combined" was replaced with "incorporated"
+ All bacteria names in the text were written following the universal standards including those pointed by the Reviewer (line 524, line 560, line 568, line 608, line 615, line 645)

Hydrogels of Levan Polysaccharide: A Systematic Review

Aybüke Tekin[a], Selay Tornacı[a], Defne Boyacı[b], Suming Li[c], Sonia Calligaris[d], Hana Maalej[e], Ebru Toksoy Öner[a*]

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Abstract

Levan is a fructose-based homopolysaccharide renowned for its unique properties, including exceptional adhesive strength, self-assembly capability, low viscosity, and bioactivities such as prebiotic, anti-cancer, anti-inflammatory, and anti-diabetic effects. These characteristics have created increasing interest in levan-based biomaterials over the past decade, positioning levan as a highly under-explored biopolymer for a wide range of applications, from medicine to cosmetics. As a result, levan-based hydrogels have emerged as promising biomaterials in drug delivery, tissue engineering, and cosmetic formulations, owing to their extracellular matrix-mimicking structure, tunable mechanical properties, and controlled cargo release capabilities. This review is the first to comprehensively examine the advancements in levan-based hydrogel research, systematically analyzing their biomedical applications and comparing them with other biopolymer-based hydrogels. Key questions regarding levan's potential as an alternative to established hydrogel systems are explored, highlighting areas requiring further research. By assessing trends and findings in the literature, this review provides an overview of the advantages, limitations, and prospects of levan hydrogels. Our analysis establishes a foundation for the continued development of levan-derived biomaterials, fostering broader adoption in biomedical and industrial applications.

Keywords

Levan; Polysaccaharide; Hydrogel; Biocompatibility; Biomaterials; Tissue Engineering

1. Introduction

Throughout history, significant milestones in humanity have been marked by discoveries in human physiology, diseases and treatments. However, the search for solutions to health problems dates back even further, as people have always relied on natural or synthetic materials from their environment to facilitate wound healing, repair damaged tissues and organs, and even enhance food preservation and safety [1]. Today, with increasing clinical and industrial needs, there is a growing demand for materials, devices, and techniques not only for diagnosing and treating diseases but also for ensuring safety, improving food quality, extending shelf life, and enhancing health benefits [2,3]. Biomedical and food sciences have emerged as multidisciplinary fields in response to these needs, aiming to improve human health by addressing genetic, environmental, and dietary factors. The size of the biotechnology market, which includes both biocompatible and functional, natural or synthetic materials that meet the definition of "biocompatibility", is expected to increase from 1.75 trillion to approximately 4.61 trillion USD from 2025 to 2034 [4]. This trend indicates growing interest in the biomedical, food and cosmetic sectors, as well as in the development of biomaterials for diverse applications.

Polysaccharides, the most common biocompatible polymers in nature, have been used throughout human history for their nutritional and healing properties due to their structural and functional characteristics that make them multifunctional bioactive materials [5]. Today, they are widely utilized not only in diagnosis, regenerative medicine, gene therapy, drug targeting, and tissue engineering, but also in food and nutrition sectors for enhancing food texture, stability, and bioavailability, as well as for their prebiotic and health-promoting effects. Polysaccharide-based hydrogels are three-dimensional colloidal solids that retain water in their 3D network structure. Compared to rigid scaffolds or dry polymeric systems, polysaccharide-based hydrogels offer flexibility, soft structure, high porosity, and water-holding capacity that promote cell viability, proliferation, matrix remodeling, and better resemblance to the native living tissue. In hydrogel formation, polysaccharides with diverse functional groups enable both chemical and physical crosslinking. Depending on application, this allows for the development of covalently bonded hydrogels with enhanced chemical, mechanical, and dimensional stability, as well as crosslinker-free hydrogels that are easier and faster to prepare [6]. Due to their extracellular matrix-mimicking properties, biocompatibility, flexible synthesis methods, and suitable physical properties, they have found broad applications as artificial soft tissue biomaterials in various biomedical fields, such as controlled drug and cargo release, biosensors, and tissue engineering and regenerative medicine [6, 7]. Furthermore, with a smart hydrogel approach, polysaccharide-based hydrogels can be tuned to respond to environmental stimuli such as pH, temperature, electrical and magnetic fields, ionic strength or light and can change some of their properties such as their wettability, degradability, swelling, and mechanical or surface properties. These attunable properties make them attractive especially for smart drug delivery systems and personalized medicine [8, 9]. While chitosan [10], dextran [11], and alginate [12] are common natural polysaccharides used in hydrogel formulations, levan-based hydrogels have recently gained increasing attention in health-related applications [13]. Levan, a natural homopolysaccharide of fructose, has been part of the human diet for centuries. However, compared to inulin-type fructans, which have a well-established market market as prebiotics, our knowledge of levan remains limited [14,15]. Despite this, levan's remarkable properties, such as biocompatibility, biodegradability, antioxidant, immune boosting, prebiotic, heparin-mimicking, anti-aging, and cryoprotectant effects, have piqued scientific interest, leading to extensive research on its production, derivatives, and levan-based

biomaterials [16,17,18]. Challenges such as high production costs and limited commercial availability, have hindered its widespread industrial use, promoting research into new production systems [19]. This has led to the discovery of more affordable methods for producing levan, opening up new avenues for its used in cosmetics, medicine, pharmaceuticals, and the food sector [20]. However, further research is still needed before levan can become a widely used product in these fields.

This review systematically examines trends and research on levan-based hydrogels and their applications in various biomedical fields, such as drug delivery, tissue engineering, and cosmetics. Several key questions are addressed, including why levan is an ideal biomaterial for biomedical applications, whether levan-based hydrogels can compete with other biomaterial-based hydrogels, and which sectors have extensively researched them. The literature is compared with other biomaterial-based hydrogels, revealing the advantages and limitations of levan-based hydrogels in various applications. This review aims to provide a solid foundation for future research and development in levan-based hydrogel and biomaterial applications.

2. State-of-the art

In order to gain insight into the current literature on levan-based hydrogels, a systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. The search strategy involved quering Scopus, ScienceDirect, and other journal websites using keywords such as “Levan”, “Levan hydrogels”, and “hydrogel”. No date or language restrictions were applied. To explore the sector-specific uses, additional keywords, such as "drug delivery", "tissue engineering", "cosmetics", and “food industry” were incorporated. From an initial set of 141 records, screening identified 15 relevant documents, after removing duplicates and out-of-scope articles. Further, a search on the Web of Science (WoS) database revealed a total of 124,017 documents related to hydrogels as of May 2025. Starting from the first studies with silica gels in 1946, the number of documents increased exponentially after the 2000s, and according to the best model fit, a total of 20,236 documents will be published in 2025 alone and an additional 182,458 reports by the end of 2030. When the search was narrowed down to specific polysaccharides by adding keywords, the total number of documents was 16,639 (chitosan), 13,508 (alginate), 8,884 (cellulose), and 6,666 (hyaluronic acid), followed by starch (1,946), dextran (1,837), pectin (990), and xanthan (836). On the other hand, adding "levan" as a keyword reduced the total number of documents to 21 that were only less than 0.02% of all hydrogel documents and many orders of magnitude less than common polysaccharides like chitosan (13.4%) or alginate (10.9%). While one of these 21 documents only cited levan and hence was discarded, another one was a review article on the recent advancements in pharma and healthcare applications of exopolysaccharide composites where authors also considered levan as a promising biopolymer and briefly discussed a few levan-based biomaterials in a short paragraph [21]. The remaining 19 research articles also included those from the previous search, and they were screened for their relevance. The first mention of levan as a hydrogel component appeared in Castillo and López-Munguia’s 2004 study, which focused on the enzymatic synthesis of high molecular weight levan by *Bacillus subtilis* levansucrase, resulting in a highly viscous levan precipitate resembling a hydrogel [22]. Other early studies highlighted levan’s hydrogel-like properties in *B. subtilis* biofilms [23] and *Erwinia tasmaniensis*-produced levan, which formed sticky hydrogels at high concentrations [24]. The hydrogel’s swelling behavior

was also explored in enzymatically synthesized levan from *Erwinia amylovora* levansucrase [25]. Another interesting study on encapsulating *Gluconobacter oxydans* cells in poly(vinyl alcohol) (PVA) hydrogels reported interactions of the polymer network with bacterial extracellular polysaccharide (EPS) components including levan as well as acetan, cellulose, and dextran [26].

In 2018, Choi et al. introduced the first physically crosslinked levan hydrogels using *Zymomonas mobilis* levan mixed with Pluronic F127 and carboxymethylcellulose (CMC) [27]. The group further incorporated hydroxyapatite to improve the hydrogel's injectability, biocompatibility, and long-acting anti-wrinkle efficacy [28]. Similarly, blends of gellan and *Erwinia herbicola* levan were ionically crosslinked to create shear-thinning hydrogels for tissue engineering [29]. Other studies explored the use of *E. herbicola* levan mixed with CBD oil to form sponges (rather than hydrogels) for wound healing applications [30]. Likewise, Maria et al. (2021) reported that a *Bacillus* levan isolated from honey showed the capacity to form emulsion hydrogels with omega-3 polyunsaturated fatty acids (PUFA) from ray liver oil and chia oil [31].

In 2017, the first covalent use of levan in a hydrogel structure was reported, where carboxymethylated derivative of levan from *Halomonas smyrnensis*, *Halomonas* levan, was employed as a cross-linker for temperature-responsive N-isopropyl acrylamide (pNIPA) hydrogels [32]. Then, another group used the same procedure to obtain short carboxymethylated *Zymomonas* levan, attached catechol, and then synthesized hydrogels with high adhesivity [33].

Further advancements included the photochemical crosslinking of methacrylated *Bacillus* levan to form covalent hydrogels, which were characterized for their structural and mechanical features, biodegradability, and cytocompatibility [34]. These studies were followed by the use of glutaraldehyde crosslinked *Zymomonas* levan/PVA hydrogels as pathogen-capturing filters [35], chitosan crosslinked oxidized *Bacillus* levan hydrogels loaded with curcumin for wound dressing applications [36], as well as 1,4-butanediol diglycidyl ether (BDDE) crosslinked *Halomonas* levan hydrogels for controlled release of Amphotericin B (AmB) [37], Resveratrol [38], *Plantago lanceolata* extracts [39], and as cryoprotectants for probiotic bacteria [40]. These BDDE/*Halomonas* levan hydrogels were also integrated with polyacrylamide to obtain interpenetrating hydrogel networks for uses in the conservation of cultural heritage [41]. Most recently, these hydrogels also showed promise in guided bone regeneration [42].

A patent search was also conducted across four major databases, namely, World Intellectual Property Organization (WIPO)'s Patentscope, Google Patents, Espacenet (by the European Patent Office, EPO), and The Lens—using the keywords "levan" and "hydrogel." The Lens, Google Patents, and Espacenet yielded 7,143, 4,518 and 448 hits, respectively. A Frontpage search on Patentscope returned 4 results only (same search for chitosan and alginate instead of levan yielded 2,969 and 2,412 hits, respectively). Two Korean patents (KR1020190066936 and KR1020190041272) granted in 2019 focused on levan-based hydrogels containing PF127 and CMC for cosmetic applications [27, 28]. In 2024, two Russian patents (RU0002815367 and RU0002819701) described biocomposite hydrogels involving *Paenibacillus polymyxa*-derived levan with one combined with alginate (RU0002815367), and the other with *Komagataeibacter sucrofermentans* bacterial cellulose (RU0002819701) for tissue engineering applications. The Cooperative Patent Classification (CPC) code A23V

2250/5068, which pertains to fructans used in food applications, was identified as relevant to levan but did not yield any hydrogel-related results.

3. Why levan?

Levan is a versatile, non-structural homopolysaccharide composed primarily of fructose units linked by β-2,6 glycosidic bonds, with different degrees of branching at the β-2,1 position. This unique structure allows levan to be synthesized naturally by a range of microorganisms, including archaea, fungi, bacteria, and a few plants [43]. Due to the difficulty of extracting levan from plants in large quantities, most of the levan used in current studies is derived from microbial sources through cloning levan-related genes [44, 45].

Levan has several properties that make it extremely useful for use as a hydrogel formulation in a variety of applications. First, it is an amphiphilic polymer soluble in both water and oil, but insoluble in most organic solvents, except dimethyl sulfoxide (DMSO). Such solubility profile gives levan the ability to self-assembly in water into densely packed spheres with a diameter of 25-250 nm, resulting in a low viscosity of its aqueous solutions [46]. This self-assembly behavior contributes to the formation of 3D networks, which are characteristic of hydrogels, while maintaining low viscosity in solution. Although levan's low viscosity is advantageous for injectable hydrogel systems, it can also hinder the development of mechanically strong and stable hydrogels. This limitation can be addressed by optimizing reaction conditions to relax the self-assembled structures, employing chemical crosslinking, or forming composite hydrogels.

Another important feature of levan is its suitability for chemical derivatization methods, such as sulfation, phosphonation, and oxidation [47]. Chemically-modified levan derivatives exhibit distinct properties, such as the anticoagulant activity of sulfated *Halomonas* levan [48] or the enhanced anticarcinogenic activity of oxidized *Halomonas* levan [49], which may support the design of hydrogels with diverse properties tailored for specific applications. Besides, a distinguishing feature of levan compared to other bioactive polymers is its remarkable adhesive strength. This is due to the high number of interactions, such as hydrogen bonds, Van der Waals forces, and interactions between the abundant hydroxyl groups within the structure, which contribute to levan’s strong adhesion properties [50]. This adhesive strength is especially significant in the context of microbial biofilms, where levan serves not only as a crucial structural component to protect the microbial community but also as a nutrient reservoir, enabling survival under nutrient-deprived conditions [51].

There is growing interest in the use of bioactive polymers in hydrogel design across diverse fields, from biomedicine to the food industry. Unlike traditional hydrogels that primarily serve as passive carriers, these polymers can actively interact with biological systems, thereby enhancing the material’s functionality and biocompatibility depending on the application. Levan, with its wide range of bioactivities, holds significant potential to confer additional therapeutic or functional benefits when incorporated into hydrogel systems. Figure 1 summarizes various bioactivities of levan polysaccharide with the state of the art literature discussed in the following sections.

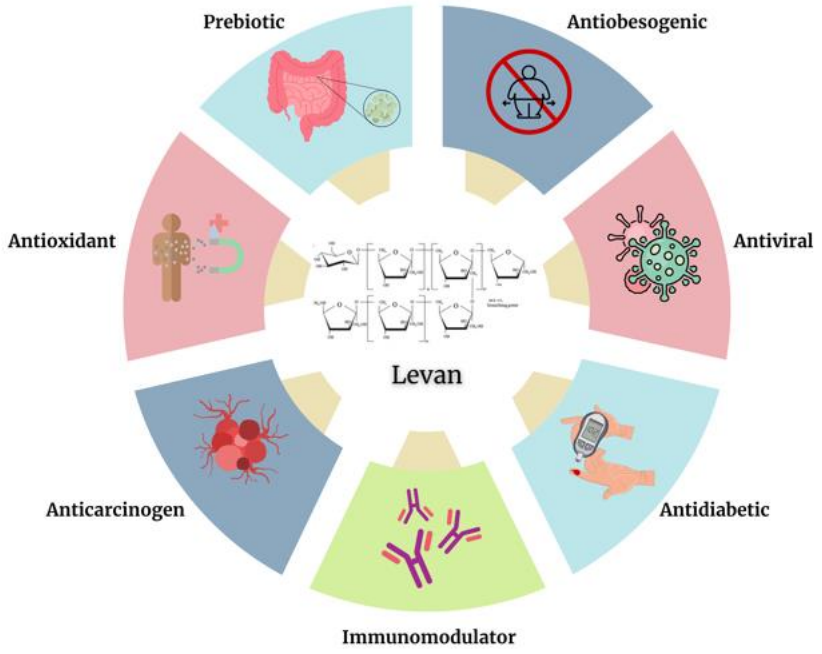

Figure 1. Bioactivities of Levan polymer (Created with Canva.com).

3.1. Levan as a Potential Prebiotic

Prebiotics are non-digestible food ingredients that promote the growth or activity of beneficial microbes in the body. Their degradation products, short-chain fatty acids (SCFAs), have gained attention for influencing the microbiota and general health, including effects beyond the gastrointestinal system [52, 53, 54]. In a study conducted with fructans of different molecular weights, it was observed that high molecular weight levan was more effective than low molecular weight fructooligosaccharides in terms of increasing the production of SCFAs and contributing to the diversity of the intestinal microbiota [55]. For prebiotics to be used by the beneficial microbiota, they must reach the colon without being digested in the upper intestine [56]. Fructans, which meet this requirement, differ from glucans in that they are only metabolized by the colon microbiota [51, 57]. Today, inulin-type fructans are the most researched group for their effects on microbiota, immunity, and intestinal barrier function [58]. Although levan is less researched, there are studies on levan as a potential prebiotic [15, 59]. It has been reported that when chicken [59], pigs [60], or rats [61] were fed with levan, breast meat and feed ratio increased, ammonia levels decreased, digestive abilities improved, and beneficial bacteria were abundant. There are even studies where levans' prebiotic activity was higher than inulin. For example, levan produced from *Erwinia* sp. significantly enhanced microbial community growth compared to inulin, likely due to its higher degree of

polymerization (DP), which allowed for prolonged persistence in the colon [62]. Moreover, levan from *Streptococcus salivarius* had higher or similar prebiotic activity scores against four probiotic bacteria compared to inulin [63] while levan from *Bacillus siamensis* and *Bacillus velezensis* supported the growth of *Streptococcus thermophilus* DKT-3 better than inulin [64].

3.2. Anti-diabetic and Anti-obesogenic Activity of Levan

Levan, unlike glucans such as cellulose and starch, is broken down into fructose rather than glucose, making it a potentially more suitable option for diabetic patients [65]. Dahech et al. (2011) demonstrated that orally administered levan reduced hyperglycemia and oxidative stress in diabetic rats, also protecting against hepatic and pancreatic toxicity [66]. Further studies reported that levan supplementation lowered blood sugar and serum cholesterol levels—critical factors in diabetes management [67, 68, 69]. Moreover, in obese rats on a high-fat diet, levan suppressed diet-induced obesity and hyperlipidemia, reducing total cholesterol while increasing HDL levels [70]. Additionally, a combination of levan and fermented ginseng was shown to reduce hyperlipidemia, fat accumulation, body weight gain, and improve glucose homeostasis and leptin resistance [71]. These findings highlight the biotherapeutic potential of levan in improving diabetes, diabetes-related metabolic syndromes, and obesity.

3.3. Anti-cancer, Antioxidant and Antiviral Activities of Levan

Cancer, characterized by uncontrolled cell growth due to DNA mutations, remains a significant health challenge due to the diversity in tumor types and treatment responses, prompting growing interest in natural agents alongside traditional therapies like chemotherapy and radiotherapy [72, 73]. Levan is one of the natural polymers that have been extensively studied for their anticancer potential across various cancer types where the structural properties of levan, such as its chain length and degree of branching, were foud to play a crucial role in its anticancer efficacy [74]. Recently, nanoparticles coated with *Zymomonas* levan (Np-Lev) have shown significant antiproliferative activity against breast cancer and melanoma cells [75], while levan combined with doxorubicin significantly reduced expression of cancer-related genes in hepatocellular carcinoma cells [74]. Moreover, the combined use of bacterial levan and benzimidazole derivative (BMPE) exhibited notable antimetastatic effects in a triple-negative breast cancer mouse model via immunomodulation and redox regulation [76].

Oxidative stress, caused by excessive accumulation of reactive oxygen species (ROS), can result in cellular damage such as DNA disruption, protein misfolding, lipid peroxidation, and membrane dysfunction, which in turn are associated with diseases like type 2 diabetes, cardiovascular and neurodegenerative disorders, and ischemia [77]. In this context, levan has been shown to effectively suppress peroxidation, donate electrons, and terminate free radical chain reactions, acting as a potent antioxidant [78]. Studies have demonstrated that levan derived from *Bacillus* strains and its sulfated forms possess strong antioxidant properties [64, 79, 80]. Similarly, levan isolated from *Acetobacter xylinum* and *Leuconostoc mesenteroides* cultures have also been reported to exhibit strong antioxidant and anti-inflammatory effects [44, 81].

Viruses with their complex structures and historical impact on pandemics, have driven

increased interest in antiviral agents, particularly following the global COVID-19 pandemic. Levan has been studied for its antiviral properties, with *Bacillus* levan shown to affect respiratory HPA1, H5N1, and enteric adenovirus type 40 [82]. More recently, same researchers found that crude, dialyzed, and sulfated derivatives of levan produced from *Enterococcus faecalis* cultures showed antivirus activity against Newcastle disease virus (NDV) [83].

3.4. Immunomodulatory Activity of Levan

The immune system plays a crucial role in defending against infections and cancer and understanding its mechanisms is essential for recognizing its role in diseases, interactions with other systems, and the development of new therapeutic strategies [84]. While traditional treatments like corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to suppress inflammation [85], their long-term use is limited by significant side effects [86], driving research into more selective and natural treatment approaches for immune modulation with fewer adverse effects [87, 88].

There are numerous reports in the literature on the immunomodulatory activity of levan polysaccharides from various sources, but only a few of them have investigated the related molecular mechanism [89]. *In vivo* oral administration of *B. subtilis* natto levan was found to reduce the serum level of ovalbumin-specific Immunoglobulin E (IgE), modulate the T helper 2 (Th2) cell response, and pattern recognition was found to be mediated by Toll-like receptor (TLR) 4 [90]. TLR4 are receptors that recognize conserved pathogen-associated molecular patterns (PAMPs) and thus constitute the first line of defense [91]. In addition, they recognize lipopolysaccharide (LPS) glycolipids found in the outer membranes of both commensal and pathogenic Gram-negative bacteria [92, 93]. A recent study showed that LPS is the actual molecular determinant of the immunomodulatory property of levan toward TLR4 receptor-expressing innate immune cells [94].

Some studies provide a better understanding of the changes in the immunomodulatory properties of structurally different levan produced from different sources. For example, while levan produced from *L. mesenteroides* increased the levels of interleukin-4 (IL-4), an important anti-inflammatory cytokine [81], levan obtained from *Paenibacillus bovis* sp. did not cause any modulation in IL-4 levels [95]. In addition, levan of different molecular weights from the same species has been observed to have different effects on inflammation-related pathways. For example, levan from *B. subtilis* altered iNOS, COX-2 gene expression [96] and nitric oxide (NO) production [97] in RAW264.7 macrophages depending on molecular weight. Moreover, a relatively recent study used methacrylated levan as a bio-ink for 3D bioprinting of bone tissue scaffolds. In addition to good bioprintability, the samples supported the expression of the anti-inflammatory marker CD206 [98]. These results suggest that the inherent immunomodulatory effect may make levan an ideal polymer for hydrogels in tissue repair and regeneration applications, as it will reduce the possibility of rejection by the body.

4. Synthesis of Levan-based hydrogels

Properties of hydrogels such as mechanical strength, self-healing ability, swelling capacity, and biodegradability depend on cross-linking processes. Hydrogel synthesis is generally achieved through chemical or physical crosslinking methods. Chemical crosslinking

involves the formation of covalent bonds between polymer chains, either directly or via low-molecular-weight crosslinkers, resulting in hydrogels with enhanced long-term stability and mechanical strength. In contrast, physical crosslinking relies on non-covalent interactions, often leading to heterogeneous polymer networks and hydrogels that are structurally less stable and mechanically brittle. Nevertheless, physically crosslinked hydrogels formed via weak interactions, such as ionic or electrostatic interactions, hydrophobic interactions, hydrogen bonding, or van der Waals forces, can exhibit advantageous properties, including self-healing capability and responsiveness to external stimuli [99].

The choice of cross-linking method depends on the physicochemical properties of the polymer. Due to its uncharged nature and water solubility, native levan does not readily form stable hydrogels without chemical or physical crosslinking or prior structural modification. Therefore, either levan or its chemical derivatives are mixed with other polymers to promote physical crosslinking, or chemical crosslinkers are used to establish covalent bonds and enhance hydrogel stability. Furthermore, the inherently fast biodegradation rate of levan can be tuned by chemical modifications or by integrating it into composite hydrogels, allowing for better control over its stability and degradation kinetics in various application environments.

In literature, there are reports on physically linked levan hydrogels such as injectable levan/ CMC /Pluronic F127 blends [27, 28]. In both studies, it was stated that there is hydrophobic association and hydrogen bonding between levan, Pluronic F127, and CMC, and especially that the poly(ethylene oxide) group of Pluronic F127 interacts with levan, and therefore levan is necessary for physical crosslinking. In another study, ionically crosslinked levan/gellan blends were examined, and it was hypothesized that under alkaline conditions, gellan interacts with levan via ionic crosslinking between hydroxyl and carboxyl groups to form a hydrogel network [29]. On the other hand, relatively more studies used chemical crosslinking to obtain better mechanical stability, including photo-polymerized methacrylated *Bacillus* levan [34] or chitosan crosslinked oxidized *Bacillus* levan [36]. In these studies, functional groups were introduced into levan to facilitate its reaction with crosslinkers or other polymers. For instance, methacrylate-modified levan enables covalent bonding with photoinitiated crosslinkers such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) or Irgacure 2959 [34]. Similarly, the incorporation of aldehyde groups through oxidation allows levan to form Schiff base linkages with amine-containing polymers like chitosan [36]. However, there are also examples in which unmodified levan has been directly employed to form chemically crosslinked hydrogels such as glutaraldehyde crosslinked *Zymomonas* levan/PVA [35], and BDDE crosslinked *Halomonas* levan [37 - 42]. Figure 2 illustrates various types of chemically and physically crosslinked levan-based hydrogels while Figure 3 gives the classification of levan hydrogels.

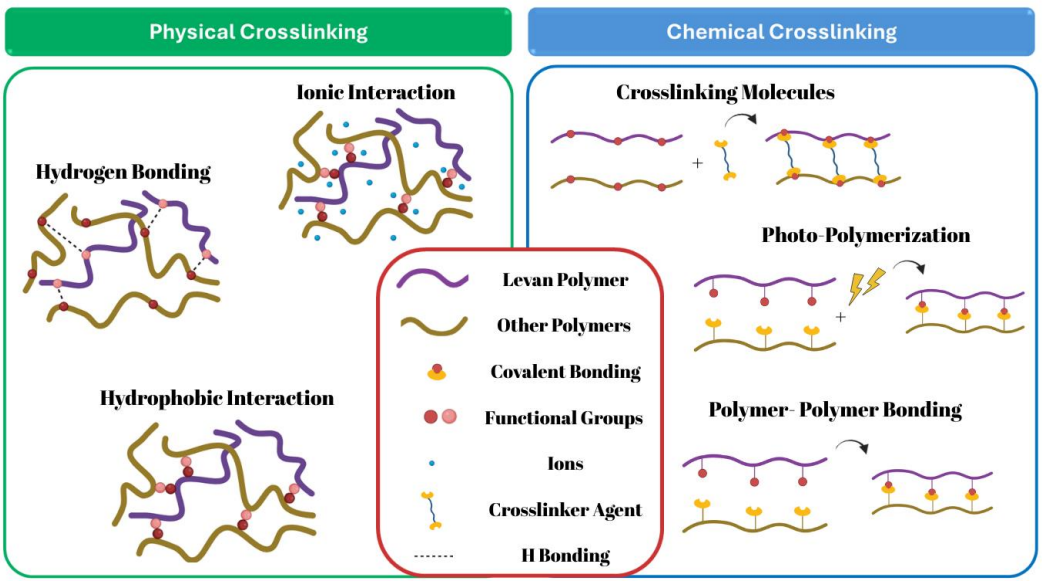

Figure 2. Physically and chemically crosslinked levan-based hydrogels (Created with Canva.com).

The good biocompatibility of hydrogels under physiological conditions, their degradability, and the low toxicity of the formed degradation products are important issues in the selection of the crosslinking agent [99]. Although hydrogels with good mechanical stability are formed by chemical crosslinking, crosslinking agents are generally synthetic and can cause toxic effects [100]. In a related study, levan was used to crosslink pNIPA hydrogels as an alternative to bis-acrylamide (BAAm), aiming to eliminate its potential toxic effects [32].

So far, mostly BDDE crosslinked levan hydrogels are reported where the formation of covalent bonds occurs as a result of the reaction between the epoxide groups of BDDE and the primary hydroxyl groups on the levan backbone. However, in a recent review by Wojtkiewicz et al. (2024) [101], it was noted that long-term use of BDDE-crosslinked hydrogels can lead to tissue hardening and redness in the application area after several years, potentially due to BDDE itself or its byproducts released during hydrogel disintegration, as indicated by clinical findings.On the other hand, physical crosslinking offers a valuable alternative to avoid the potential risks associated with chemical methods.

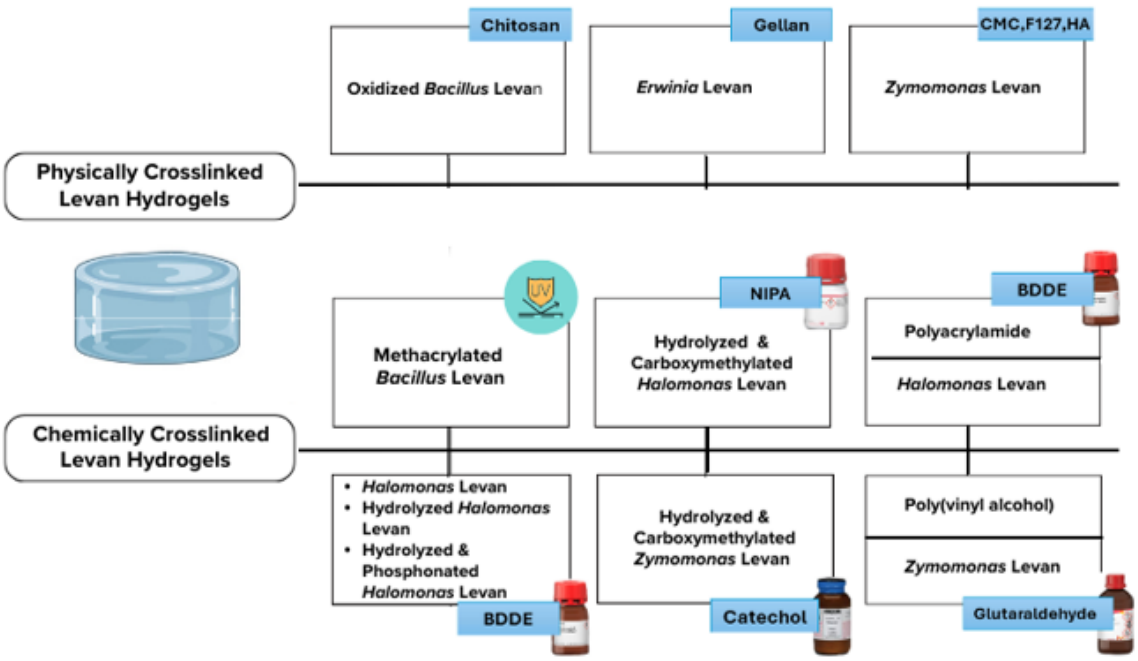

Figure 3. Classification of levan-based hydrogels (Created with Canva.com)

5. Applications of Levan Hydrogels

Levan-based hydrogels have emerged as promising biomaterials in various biomedical applications, particularly in drug delivery systems, tissue engineering, and regenerative medicine, as well as in cosmetics. Their unique physicochemical properties, including biocompatibility, biodegradability, and stimuli-responsiveness, make them suitable candidates for controlled and targeted drug release, offering improved stability, bioavailability, and efficacy. In Table 1 are summarized the key findings on levan-based hydrogels in various sectors to provide a general overview of their diverse applications.

Table 1. Hydrogels of Levan

Source of Levan	Hydrogel structure	Application	Reference
Bacillus subtilis	Levan was methacrylated and then photo-polymerized	Mechanically stable, slightly degradable and cytocompatible gels suitable as starting materials in common additive manufacturing processes like bioprinting or stereolithography	[34]
	Levan was oxidized with sodium metaperiodate and then mixed with chitosan for the gelation	Bio- and hemo-compatible drug delivery system and wound dressing material with sustained curcumin release	[36]
Erwinia herbicola	Levan/gellan blends crosslinked ionically with NaOH and $CaCl_2$	Shear-thinning properties showed high potential of levan-gellan hydrogel for its use as an injectable material in medical and cosmetic industries	[29]
Halomonas elongata	BDDE crosslinked levan hydrogels loaded with liposomes containing *Plantago lanceolata* L. Extracts	Biocompatible and biodegradable levan-based herbal liposome-loaded hydrogels for acute wound healing	[39]
Halomonas smyrnensis	Hydrolyzed, carboxylated and then methacrylated levan crosslinked with *N*-isopropyl acrylamide (NIPA)	Thermoresponsive levan/pNIPA hydrogels for the controlled release of 5-ASA for the treatment of inflammatory bowel diseases	[32]
	BDDE crosslinked levan hydrogels	Amphotericin B release for exerting antifungal activity against *Candida albicans*	[37]
	Hydrolyzed and	Controlled release of resveratrol	[38]

	phosphonated levan crosslinked with BDDE	for skin tissue engineering	
	Levan, acrylamide and methylene-bis-acrylamide solution crosslinked with BDDE and Ammonium persulfate	IPN-based Levan/PA hydrogels for conservation of cultural heritage	[41]
	Hydrolyzed levan cross-linked with BDDE	Levan hydrogels for guided bone regeneration and bone tissue engineering	[42]
Zymomonas mobilis	Pluronic F127/CMC/levan mixture	Injectable hydrogels to be used as an alternative to hyaluronic acid (HA) based dermal fillers in soft tissue augmentation	[27]
	Hydroxyapatite containing Pluronic F127/CMC/levan hydrogels	*In vivo* stability of the composite hydrogel was enhanced with high anti-wrinkle efficacy maintained for 8 weeks and showing high collagen production	[28]
	Levan/PVA crosslinked with glutaldehyde	Virus capture efficiency of levan−PVA hydrogels was higher than that of commercial cotton swabs	[35]
	Catechol conjugated carboxymethylated levan hydrogels via oxidation-induced catechol/quinone (covalent) and Fe^{3+}-(non-covalent) mediated coordinative crosslinking	Levan-catechol conjugate hydrogel for wound healing applications	[33]

5.1. Pharmaceutical Applications

Direct clinical use of active pharmaceutical ingredients (APIs) “as is” is limited since drugs are sensitive chemicals and are adversely affected by body conditions (pH, temperature, enzymes, etc.), low bioavailability, and low absorption [102]. Therefore, delivery systems that transport therapeutics to relevant sites in the body in a controlled manner are necessary for effective drug delivery. Traditional drug delivery systems require high and continuous doses due to untargeted distribution and uncontrolled release of the drug. This is contrary to keeping the plasma drug concentration above the minimum effective concentration (MEC) and below the toxic concentration and may cause side effects [103]. There is increasing interest in controlled and targeted drug delivery systems (DDS), designed to release the correct dose of a therapeutic agent directly to the desired site and within the required period. These systems maximize the effectiveness of the therapeutic agent and minimize possible side effects [104].

The conventional DDS methods, like oral tablets and injections, often suffer from limitations like poor bioavailability, systemic side effects, and the need for frequent administration [105]. Advanced drug delivery systems have been developed to overcome these challenges, and so many new options, such as liposomes, nanoparticles, microneedles, polymeric carriers, and hydrogels offering unique advantages, improve health care day by day [106]. Among them, hydrogels have gained considerable attention due to their cross-linked hydrophilic polymer network that can retain large amounts of water. When loaded with drugs, they can hold the drug inside and gradually release it in a controlled manner due to their porous structure and swelling properties [107]. Nowadays, more specific versions of hydrogels, such as stimuli (pH, temperature, enzymes, etc.) responsive, injectable, or nanogels, are being designed, thus rapidly progressing towards more efficient and effective drug delivery systems [108,109].

The use of levan-based drug delivery systems may overcome, to a great extent, the challenges of the conventional modes of drug administration. So far, various drugs have been loaded into levan-based-drug delivery systems for targeted and controlled delivery. After levan polysaccharide was first reported to be suitable for delivering peptides, proteins, and macromolecular drugs [110], *Halomonas* levan was used for controlled release of vancomycin [111], curcumin [112], paclitaxel [113], and resveratrol [114]. Additionally, levan derived from *Bacillus licheniformis* has demonstrated high potential as a pharmaceutical excipient in topical drug formulations [115]. Recent studies include *B. subtilis* levan-based nanoparticles loaded with the antiretroviral drug - dolutegravir for potential use in HIV treatment [116] and levan-shelled hydrophobic silica nanoclusters encapsulating doxorubicin as an ultrasound-responsive drug delivery system for cancer treatment [117]. These studies reveal that levan is a promising polymer for drug delivery strategies, not only as a carrier but also with its bioactivity.

When designing drug delivery systems, some important issues should be considered. The first one is the ability to provide targeted delivery. Hydrogels have been demonstrated as the ideal pharmaceutical carrier in biomedicine due to their high porosity and water content through their unique three-dimensional (3D) crosslinked network structure [118]. Stimuli-responsive hydrogels, also known as smart or intelligent hydrogels, have become the most studied systems for drug delivery [119]. They are able to respond to environmental changes like temperature, pH, or magnetic fields, controlling not only drug administration but also the timing and release profiles, with their sol-gel phase transition behavior influenced by their sensor characteristics and surrounding conditions [120].

Examples of levan-based-stimuli-responsive hydrogels include thermoreactive levan/N-isopropyl acrylamide (levan/pNIPA) hydrogels for controlled release of 5-aminosalicylic acid (5-ASA) used in the treatment of inflammatory bowel diseases. Osman et al. (2017) used methacrylated, hydrolyzed, and carboxymethylated forms of *Halomonas* levan as crosslinkers to prepare levan-pNIPA hydrogel for the first time [32]. The biocompatibility of the obtained hydrogels with L929 cells was evaluated, and data showed that the biocompatibility was improved with higher levan percentage in hydrogels. Also, when different concentrations and temperatures were tested, addition of levan brought the volume phase transition temperature closer to the human body temperature.

Every drug delivery system, including hydrogels, has a limited residence time in the targeted release region. This is between 2-5 hours for the stomach and 2-6 hours for the small intestine, and at the end of this period, the content is transferred to the next step of the system, such as the colon. Therefore, it is important to test how much and how quickly a hydrogel will

release the encapsulated drug. Since it is known that the type and content of crosslinker used in hydrogel production change the drug release characteristics, the levan/pNIPA hydrogel system [32] was compared with the same system where chitosan was used as a crosslinker instead of levan [121]. It is observed that both hydrogels released approximately 90% of the drug in 2 hours under the same conditions (37 °C, pH 7.4). Both hydrogels can release an effective amount of the drug in a sufficient time. However, while 100% of the drug was released in 4 hours in the chitosan hydrogel, this period was 6 hours in the levan hydrogel. This difference can be attributed to the pH-sensitive structure of chitosan, suggesting that levan may be a good alternative for medications that need to be released over a longer period.

The second important point to consider when designing drug-delivery hydrogels is the swelling capacity. The reason is the high influence of swelling on drug loading efficiency (DLE) and drug encapsulation efficiency (DEE), which are mandatory for an adequate drug delivery system. It is worth noting that the swelling capacity of a hydrogel depends on the polymer used and the degree of cross-linking. While high crosslinking causes lower water absorption capacity, lower crosslinking degree leads to lower mechanical stability [38,122]. So, the optimum cross-linking degree should be selected for enhanced drug delivery. Another essential factor influencing swelling is the pH of the environment. Therefore, in a study by Demici et al. (2020) to estimate swelling capacity, tests were performed with selected hydrogels that have an optimum polymer/crosslinker ratio (*Halomonas* levan/BDDE) in different solutions [37]. The results showed that the swelling capacity of the hydrogels was pH dependent, higher pH leading to higher swelling, and the maximum equilibrium degree was reached in 1 hour. Antifungal and cytotoxicity tests also revealed the high potential of levan hydrogels in the transport of pH-sensitive drugs such as amphotericin B (AmB) [37].

The third and possibly most crucial factor is the biocompatibility of the drug delivery system. Hydrogels, especially those produced from natural polymers, are known as biocompatible since they are not toxic or injurious and do not cause immunological rejection in living tissues [107]. Considering the need for more systematic studies on levan-based hydrogels to widen their use in drug delivery systems and biomedical applications, chemically modified forms were used to synthesize biocompatible hydrogels. Recently, levan produced by *H. smyrnensis* and its hydrolyzed and phosphonated derivatives crosslinked with BDDE were produced for the first time for resveratrol delivery [38]. Results revealed the superior *in vitro* biocompatibility of levan hydrogels with a human keratinocyte cell line (HaCaT). Additionally, cell attachment to phosphonated hydrogels was higher since phosphonate groups affect cellular attachment via mimicking protein-protein and protein-natural polymer interactions [123]. This finding proved that hydrogels produced from chemically modified forms of levan polymer have superior potential in the pharmaceutical and cosmetic industries.

5.2. Levan Hydrogels in Tissue Engineering

Tissue engineering (TE), a rapidly growing field, is used to substitute diseased or damaged tissues with functional ones, aiming to restore or enhance the function of the affected tissues. Nowadays, hydrogels, due to their unique properties, have become an integral part of tissue engineering, especially in enhancing tissue function and promoting cell growth, differentiation, and regeneration [124,125]. When selecting biomaterials to form hydrogels, several factors need to be considered, including mechanical properties, biocompatibility, gelation time and swelling capacity [126]. The understanding that natural polymers, especially

polysaccharides, have the potential to meet these properties positions levan as an attractive candidate for tissue engineering applications.

The first study on the synthesis of photo-crosslinkable levan derivatives and their use for hydrogel production was reported by Berg et al. (2018) [34]. Levan from *B. subtilis* was chemically modified via methacrylation with glycidyl methacrylate (GMA), methacrylic anhydride (MAA), or 2-isocyanatoethyl methacrylate (IEM) and then photo-polymerized in the presence of different photoinitiator systems and irradiation units. The resulting hydrogels were mechanically stable and cytocompatible. Furthermore, it was shown that changing the type of crosslinker and the crosslinking density altered the degradation rate of the hydrogels, thus making these gels suitable candidates for both drug delivery and tissue engineering applications. Considering the complexity and high cost of materials used in tissue engineering in terms of preparation and application processes, there is a high need for new-generation biomaterials that can be produced at low cost, have high bioactivity, and are sustainable [127]. The increasing use of natural polymers such as gelatin, hyaluronic acid, etc., in tissue engineering applications has encouraged researchers to explore the potential of levan in this area [128]. In a recent study, levan hydrogels were incorporated with Bio-Oss®, a traditional xenograft used in guided bone regeneration applications (GPR), at different ratios. The results showed that, in addition to higher biocompatibility, levan containing hydrogels exhibited higher new bone formation, osteoblast density, and new vessel formation compared to the group containing only Bio-Oss® [42].

5.3. Applications in Wound Healing

Wounds, which are pathological cases caused by various diseases and physical-chemical damages, are classified as acute or chronic depending on the type of damage and the duration of the healing process [129]. Wound healing is a complex biological process that involves the coordination of various tissues and cell types, such as fibroblasts and keratinocytes, to repair damaged skin or other tissues. Wound dressing materials play a critical role in accelerating the healing process by providing an optimal environment for tissue regeneration [130, 131]. A suitable and effective wound dressing material should possess key properties, including biocompatibility, non-toxicity or non-allergenicity, mechanical strength, air permeability, moisture retention, antimicrobial activity, and ease of removal [132, 133]. Conventional wound dressings often fail to meet these requirements, making biopolymer-based hydrogels a promising alternative due to their morphological similarity to the extracellular matrix and ability to mimic natural tissue structures [134, 135, 136, 137, 138].

Levan has gained attention for its wound healing potential. Its anti-inflammatory, antibacterial and high adhesive properties, contribute significantly to tissue repair [139]. Levan can support cell proliferation, particularly of fibroblasts and keratinocytes, which are essential for wound closure and immune response regulation [140, 141]. Additionally, levan can enhance the mechanical stability of wound healing systems, promoting faster recovery. As proof of that, *Bacillus mojavensis* Levan-PVA nanofibers have been shown to accelerate wound healing due to the water-holding properties of levan combined with the film-forming ability of PVA [142]. Increasing levan-PVA concentration further enhances tensile strength and cell viability, leading to better healing outcomes [143] showed increasing levan-PVA concentration causes higher tensile strength and improved cell viability. In addition, porous microbial levan-based sponges loaded with cannabis oil (Lev@CBDs) exhibited a suitable swelling ratio and enough thermal stability to use even after being exposed to harsh conditions for a long period. Also, produced

sponges exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa,* the two most known bacteria that cause infection in wound areas [30]. In addition to its bioactivity on the wound area, levan can also be used as a natural reducing and coating material for microelement nanoparticles (NPs) [144] due to the presence of -OH groups on the polymer chains [145].

In some cases, using more than one polymer for the synthesis of hydrogels called hydrogel composites is preferred to obtain an effective drug delivery system, as they offer excellent properties of contained biomaterials, which other drug delivery systems cannot quickly achieve for the controlled release of drugs to specific sites. Basically, by cross-linking through covalent bonding/ionic interactions/hydrogen bonding of levan with other polymers, hydrogels with increased structural integrity can be formed. To improve wound healing, the oxidized form of levan from *B. subtilis* was crosslinked with chitosan by Veerepandian et al. (2023) [36]. Schiff's base reaction between oxidized levan and chitosan leads to formation of hydrogels without need for an initiator such as UV light or chemical cross-linkers, making hydrogels more biocompatible and suitable for wound healing. All the hydrogels had highly interconnected porous networks due to the large number of functional groups in the structure of levan and chitosan that help cell attachment, proliferation, and nutrient transfer. The hydrogels showed better thermal stability than neat levan, and the presence of chitosan made the swelling behavior of hydrogels pH-dependent (pH 2.6). The results from the hemocompatibility tests via direct and indirect methods showed non-significant differences compared to the control group. In another study, levan-catechol conjugate (LC) was synthesized by combining the carboxyl group of CM-levan with the amino group of dopamine using 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) chemistry. This conjugation improves the produced hydrogels' water-proof ability as well as fast swelling over the first 30 min of incubation, which makes these hydrogels good candidates for wound healing applications. Furthermore, the increased blood clotting rate, low endotoxin levels, and faster wound closure in the SD rat model compared to the control suggested that it can be used as a biomedical adhesive in wound healing process [33]. Moreover, the wound healing efficacy of the composite material developed by loading herbal liposomes into levan-based hydrogel structures was investigated by Altıntaş and Çelik (2023) [39]. Hydrogel structures were developed for the first time with levan produced by *Halomonas elongata* 153B halophilic bacteria for wound healing. Extracts from *P. lanceolata* L., commonly known as a wound herb, which has cell regeneration ability and anti-bacterial activity in the wound area, were obtained. Then, extract-loaded liposomes were prepared, and were loaded into the levan-based hydrogels for controlled release into the wound area. The wound healing efficacy of the herbal liposome-loaded levan-based hydrogel was evaluated in an *in vitro* wound model. Results indicated that the developed hydrogels are a promising therapeutic approach for the healing of acute wounds [39].

5.4. Cosmetics

The skin, which is the largest organ of the body, has the basic functions of protecting the body from pathogens and external environmental elements (radiation, impact, heat, etc.) and maintaining homeostasis by maintaining body temperature and humidity [146]. Cosmetic substances such as lotions, creams, masks, etc. are generally products used to maintain or improve the current condition and can be produced from various raw materials [147]. However, some cosmetic products contain several ingredients like parabens, which can harm the skin

and hair [148]. Recently, natural products are rapidly gaining attention due to their biocompatibility, sustainability, and high contribution to skin health. Cosmetic product users are more interested in skin and environment-friendly, biodegradable ingredients instead of synthetic additives. For this reason, bioactive formulations are widely available in skincare, haircare, and personal care items [149]. Levan, on the other hand, has remarkable properties such as skin whitening, moisturizing, and reduction of skin irritants [150, 151].

While the selection of raw materials used in cosmetic formulas varies depending on which area they will be applied in, today the cosmetic industry is showing much more interest in hydrogel-based cosmetic products due to their properties such as biocompatibility, elasticity, and the ability to hold excess water [152, 153, 154]. Hydrogels, which can be used to help restore skin elasticity, softness, and moisture, can also facilitate drug penetration by loosening the skin barrier [155]. Synthetic polymers and biopolymers are widely used in commercially available cosmetic products. Biodegradable polymers, especially polysaccharides, have been attracting much attention of scientists looking for new compounds with cosmetic properties because they can be eliminated by normal metabolic pathways and do not cause toxicity and accumulation in the body [156, 157].

The variety of properties of levan mentioned above makes it a useful and safe biomaterial for cosmetics. In the literature, lots of research showed the suitability of using levan in cosmetics. For example, the stimulation of human fibroblasts and keratinocytes by *Z. mobilis* levan [158], as well as a whitening agent by inhibition of melanin production [159]. Lewińska et al. (2022) [160] developed tonic, gel, and cream formulations containing surfactin-stabilized *B. subtilis* levan nanoparticles (NP) and nanoemulsion (NE) nanosystems. They reported that these formulations improved skin hydration, elasticity, wrinkle depth, and also skin discoloration. In addition, levan, which also met the requirement of non-toxicity, did not show cytotoxicity on human dermal fibroblasts (NHDF) [161]. In *in vitro* experiments with *Halomonas* levan and its derivatives, increased cell proliferation, skin barrier function, and rapid wound healing ability were observed [139]. In addition, the cosmetic product needs to maintain its stability for a long time when the approximate period of use is evaluated. Da Silva et al. (2022) [162] reported the maintenance of moisturizing, antioxidant activity, and stability of the product consisting of *B. subtilis* natto levan and almond oil for 3 months. In a study, carboxymethyl levan (CML)-hEGF nanoparticles designed to overcome the low stability of human epidermal growth factor (hEGF) maintained over 100% cell proliferation activity for 6 weeks [163]. This is one of the important studies supporting the potential use of levan as a cosmeceutical because hEGF is a signaling molecule that stimulates the growth and motility of keratinocytes and fibroblasts in epithelial tissues [164]. Moreover, in a study aimed at enhancing the effects of levan with biocompatible actives such as natural compounds and vitamins, levan was combined with natural compounds such as aloe vera extract, avocado oil, and vitamin E [165]. The cosmeceutical formulations were non-toxic and completely vegan, as well as showing good stability. Another study also showed that levan-containing digestive extract reduces the irritating effect of ionic surfactants and is therefore a suitable ingredient for the formulation of harmless body wash cosmetics [166].

Soft tissue fillers are applications developed as an alternative to plastic surgery due to reasons such as low life risk, fast recovery time, and instant effect. The material used for an effective application should be biocompatible, non-allergenic, and stable [167, 168]. Fillers are essentially divided into two categories: non-biodegradable and biodegradable, according to the properties of the raw material used [169]. Non-biodegradable fillers are less tolerated in the body than biodegradable ones and therefore can cause serious side effects. Hyaluronic

acid (HA) fillers are approved by Food and Drug Administration (FDA) and known for providing the ideal features mentioned today and dominate the sector [167]. Choi [27] developed an injectable levan-based hydrogel for use as a dermal filler for soft tissue augmentation and reported that this system could be an alternative to hyaluronic acid (HA)-based dermal fillers. The injectable hydrogels were formed by combining Pluronic F127, CMC, and levan derived from *Z. mobilis* with hydrophobic and non-covalent interactions. The levan hydrogel and HA-based hydrogels were tested in an animal disease model both *in vitro* and *in vivo*. Rheological results showed that the elastic modulus of levan-based hydrogel (~6 kPa) was higher than that of the HA-based hydrogel (~2.8 kPa), and the interconnected porous structures were similar to each other. In addition, no cytotoxicity was observed in human adult dermal fibroblasts (hADF) in the levan-treated group, and enhanced cell proliferation was observed. Since collagen synthesis is another important factor in enhancing the anti-wrinkle effect [170], hydrogels were also evaluated for their ability to improve collagen synthesis. hADF cells treated with levan showed higher expression of the type I collagen gene and improved anti-wrinkle efficacy in the wrinkle mouse model compared to HA. Later, by adding hydroxyapatite to the prepared hydrogels, their *in vivo* stability was extended to maintain the anti-wrinkle effect [28]. Levan hydrogel was biocompatible and stable longer *in vivo* than Pluronic F127 or HA, indicating that levan has high potential as a new material for effective dermal filler compared to HA *in vivo*. Therefore, levan-based dermal fillers are an up-and-coming, low-cost alternative to overpriced HA-based hydrogels. Additionally, due to its surface morphology, thermal behavior, rheological properties, and gel-forming features, enzymatically produced *E. amylovora* levan was also proposed as a promising candidate for cosmeceutical applications [25]. In this study, levan and other commercial hydrogels, such as carrageenan and guar gum, showed similar rheological behavior, similar porous network microstructure, and endothermic temperature.

Normally, shear-thinning hydrogels are mostly ideal for use in the cosmetic industry; however, chemical modification, which is usually applied for synthesis, causes high gelation time and chemical toxicity. Nair and Choudhury [29] produced levan/gellan composite hydrogels using levan from the *E. herbicola* solution and low acyl gellan polysaccharide, aiming to eliminate the problems caused by chemical modification by using natural polysaccharides. The results showed that the composite hydrogels exhibited remarkable mechanical properties and easy injectability. In addition, it was observed that when the ratio of levan or gellan polysaccharide in the composite hydrogels was increased, the swelling percentage also increased due to free hydroxyl groups compared to hydrogels consisting of equal amounts of each polysaccharide [171]. In addition, it was shown that the hydrogels had 97% water retention capacity and sufficient cross-linking with strong structures, revealing the significant potential of levan-gellan hydrogel for use as an injectable material in the medical and cosmetic industries.

5.5. Potential Applications in Food Science

Levan-based hydrogels hold significant promise in the food industry due to their natural origin, biocompatibility, and functional properties such as antioxidant, antidiabetic, and anti-obesogenic activity. Different authors proposed the use of levan in association with other biopolymers to form films feasible as edible coatings. For instance, levan-gellan gum [172], levan-chitosan [173], levan-vanillin [174], and oxidized levan-gelatin [175] films have been proposed as a coating to be applied on the surface of food products to improve their shelf life.

Moreover, the gelling properties of levan could be exploited for the design of functional foods with improved overall acceptability while delivering prebiotic levan. Being levan, able to form hydrogels, its usage in all gel-based foods could be tested. However, to this aim, it would be fundamental to have a deep understanding of structure-function relationships at different lengths of scales (from nano to micro and macro scale). Today, the possible interactions of levan with other biopolymers, such as proteins or other carbohydrates, in complex food systems are still underexplored but highly demanded for the design of novel sustainable food with designed health-improving capacity. In this context, Hundschell et al. (2022) [176] studied the influence of levan on the thermally induced gel formation of β-lactoglobulin. The presence of levan increased the water-binding capacity of the gel network. Besides, some studies elucidated the structuring ability of levan produced by microbial fermentation in bread. The exopolysaccharide levan is reported to be able to impact the bread quality [177]. *B. subtilis* levan produced from coconut inflorescence extract was studied not only for its technological properties but also as a sweetener [178]. The possibility of exploiting levan also as an alternative and innovative sweetener is particularly interesting in the attempt to reduce the use of artificial non-nutritive sweeteners, such as saccharin, cyclamate, and aspartame. Considering both the health and technological functionalities of levan, it seems to be an ideal polymer that can be used in many areas, from packaging to the food industry, as an ingredient in many food formulations.

Furthermore, thinking about the possibility of converting hydrogels into oleogels, i.e., oil-based gels with macroscopic properties mimicking those of solid fats [179, 180], or into aerogels, i.e., highly porous materials designed as delivery systems [181], it cannot be underestimated the possibility to use levan hydrogels in the field of oleogelation or aerogelation.

5.6. Other Applications

To minimize the health, economic, and social damage that epidemics can cause, it is important to detect and capture the pathogens that cause them as soon as possible and many strategies are applied in today's literature. Some of these are magnetic nanoparticles (MNPs) for the detection of various infectious diseases [182], superparamagnetic iron oxide nanoparticles (SPIONs) functionalized with peptides obtained from salivary protein, a mesoporous PDMS sponge for foodborne pathogens [183], magnetic beads (MB) coated with specific molecules for pathogen detection from blood samples [184], and a silk protein-based microbial trap [185]. It is known that carbohydrates on the mucosal tissue surface act as receptors for pathogens during infection and prevent invasion by preventing pathogens from passing through the cell membrane [186]. With this approach, carbohydrate materials that will be designed by taking inspiration from a natural process may be useful as pathogen trappers. Li et al. (2011) [187] used carbohydrate-functional chitosan nanofibers to capture the influenza virus. Moreover, carbohydrate-enriched graphene sheets [188] and glycosylated stimuli-responsive polyacrylamide microspheres with AgNPs [189] not only selectively encapsulated pathogens but also enabled the killing of captured bacteria.

Kim et al. (2020) [35] proposed hydrogels made of levan produced from *Z. mobilis* and glutaraldehyde poly(vinyl alcohol) (PVA) as influenza virus capture and recovery materials. The approach that levan would promote virus capture by effectively interacting with the lectin, hemagglutinin and nucleoprotein (NP) of the virus was confirmed by RT-PCR and ELISA tests. According to the results, the virus capture efficiency of levan-PVA hydrogels was higher than

commercial cotton swabs due to the lack of the lectin binding function of PVA alone. To test the capture capacity of levan hydrogels in different transmission routes, the authors also produced bioaerosols containing influenza viruses and tested their capture with a filter material containing the hydrogels. RT-PCR analyses showed that levan hydrogels increased the capture of virus droplets compared to the control group. This study showed that levan-based hydrogels could be simple and cost-effective materials for pathogen capture and recovery.

Another interesting application is the use of levan hydrogels in conservation science. Cultural heritages play a major role in ensuring the historical, social, and economic integrity of the society. Even traditional restoration methods applied to protect them from destructive environmental factors such as temperature, light, microorganisms, and erosion can damage the heritage [190]. On the other hand, in recent years, new methods have been developed to overcome the limitations of traditional restoration techniques and materials, and the interest in sustainable, environmentally friendly, and non-toxic systems has increased. Recently, the application of materials science and nanoscience to the preservation of works of art has led to the development of advanced cleaning systems and approaches that are transforming the preservation of cultural heritage [191]. The first suggestion of gels with their ability to provide appropriate protection and cleaning due to the osmotic balance and flexibility they provide [192], followed by the production of more advanced and responsive gels [193], inorganic nanomaterials [194], microemulsions [195], biocomposite films [196], hybrids such as fibroin-nanocellulose composite [197] and chitosan-based coatings [198] are among the materials used in studies carried out to protect cultural heritage today, and it is noteworthy that carbohydrates are frequently used in this sector due to their outstanding properties.

Based on the many important properties of levan-based hydrogels, a study was conducted to investigate the potential use of levan polysaccharides in areas other than health services, such as conservation. Saglam et al. (2023) [41] investigated the potential use of IPN-based enzymatic levan-polyacrylamide hydrogels (EL-PA) in paper protection applications. According to the results of hydrogel characterization in terms of structural, morphological, rheological, and swelling kinetics, EL-PA hydrogels have larger specific surface areas compared to PA-gel, and this is one of the properties sought in surface coating applications [199]. In addition, in the FTIR analysis performed on the paper sample previously coated with hydrogel, as a result of the separation of the hydrogel, only typical bands associated with cellulose were observed, suggesting that the hydrogels successfully removed the contaminants on the paper surface. The results showed that levan-based hydrogels have high potential in conservation science thanks to their biocompatibility and easy applicability [41].

6. Conclusion and Future Perspectives

As the demand for natural, economical, biocompatible, and biodegradable materials continues to rise, levan has emerged as a promising polysaccharide with a wide range of applications in various fields, from medicine to cosmetics. Its properties, such as adhesive strength, self-assembly, low viscosity, and its bioactivity, ranging from prebiotic and anti-cancer to anti-inflammatory and anti-diabetic, distinguished it from other polysaccharides, making it a polymer of significant interest for further research and development [15, 17]. Levan-based hydrogels, with their 3D network structures, have proven to be ideal materials for biomedical applications due to their superior biocompatibility, biodegradability, and nontoxicity. Furthermore, Levan derivatives, such as methacrylated, phosphonated, and oxidized levan,

have shown potential as parent materials for hydrogel production. These hydrogels have already been explored for applications in drug delivery, tissue engineering, cosmetics, and beyond. However, challenges remain, including high production costs of levan and variability in its properties, such as molecular weight, chain length, and branching patterns which depend on the producing organisms and production conditions. These issues must be addressed to optimize levan-based hydrogels for broader industrial applications [45].

In this systematic review, the physicochemical and functional properties of levan polymers as ideal hydrogel raw materials are examined, together with the wide range of usage areas of existing hydrogels from drug delivery to conservation science. In addition, in some sections, an objective perspective is provided by comparing the hydrogel structures that are currently accepted as gold standards with levan-based hydrogels. In light of the studies conducted on levan hydrogels to date and in drug delivery, tissue engineering, cosmetics, and other different fields, it can be expected that the potential usage areas of these hydrogels will increase even more in the future. One of these emerging areas is environmental science. Levan hydrogels possess key features—such as high water absorption capacity, adjustable porosity, biodegradability, and non-toxicity—that make them suitable for environmental uses. These include their application as adsorbents in wastewater treatment and as carriers in controlled-release systems for agricultural chemicals [200]. Moreover, a study by Phengnoi et al. (2019) [201] demonstrated that blending levan with polyvinyl alcohol (PVA), a synthetic polymer with low degradability, led to increased degradation rates with higher levan content, further supporting their potential in eco-friendly materials.

Despite the competitive properties of levan-based hydrogels with other natural polymer hydrogels currently used, unfortunately, this is a relatively new field and there are still not enough studies in the literature, only a handful of patents have been issued. Although most studies have been in the fields focused on drug delivery and tissue engineering, hydrogel studies in these areas are still insufficient.

A recently published article presenting a comprehensive bibliometric analysis of hydrogel research suggested that future applications of hydrogels will predominantly focus on wound healing, drug delivery, cell encapsulation, bioprinting, tissue engineering, electronic devices, and environmental applications. Notably, in the field of drug delivery, the development of responsive sustained-release nanocarriers, intelligent drug delivery systems, and targeted delivery approaches are expected to be significant trends [202]. Also, in their analysis, “wound healing”, “3D printing”, “antibacterial”, “strain”, “adhesive”, “tough”, “strain sensor”, and “inflammation” were identified as hot spots.

Moving forward, levan-based hydrogels need to be developed further to enhance their functionality and applicability. One important avenue of research is the investigation of levan’s interaction with both synthetic and natural materials used in hydrogel production. The properties of composite hydrogels, formed by combining levan with other materials, also warrant further study.

The regulatory framework for introducing hydrogels into practice depends on their intended application—medical, cosmetic, food, or industrial. In medical and healthcare contexts, hydrogels are regulated as medical devices or drug-device combinations by authorities like the FDA in the US and under the EU Medical Device Regulation (MDR). These require classification based on risk, biocompatibility testing (e.g., ISO 10993), sterility validation, clinical evaluation, and compliance with manufacturing standards such as GMP or

ISO 13485 [203]. For cosmetic uses, regulations focus on safety and proper labeling, with notification procedures like the EU's Cosmetic Products Notification Portal (CPNP). In the food sector, hydrogels must be approved as safe additives, such as GRAS substances in the US or approved E numbers in the EU. Industrial applications are generally less tightly regulated but may fall under environmental or chemical safety rules like EPA or REACH, especially if human exposure is possible.

Levan hydrogels are not currently present in major regulatory or clinical databases such as the FDA or European Database on Medical Devices (EUDAMED) and no approved or clinically investigated levan-based hydrogel products have been identified [204]. This clearly shows that they have not yet reached the stage of regulatory recognition or clinical translation despite the broader clinical interest in hydrogel technologies. To advance the clinical and commercial translation of levan-based hydrogels, future research should prioritize comprehensive *in vivo* studies. While preliminary *in vivo* evaluations have demonstrated promising biocompatibility, the current body of evidence remains limited and insufficient to fully assess their behavior under complex physiological conditions. Systematic *in vivo* testing is essential to evaluate not only biocompatibility but also immunogenicity, biodegradability, mechanical stability, and therapeutic efficacy across diverse biomedical applications. Such studies are critical for meeting the safety and efficacy standards set by regulatory agencies such as the FDA and European Medicines Agency (EMA) and represent a necessary milestone before progressing to human clinical trials and market adoption.

Despite the growing interest in levan-based hydrogels, there are only a limited number of MSc and PhD theses dedicated to this topic worldwide, highlighting a significant gap in academic research training that should be addressed through increased graduate-level investigations across institutions globally. In conclusion, while levan hydrogels currently show significant promise in several biomedical applications, their full potential has yet to be realized. Future research will likely expand their use, not just as bioactive delivery systems, but also in more diverse and functional areas, benefiting from their natural, biocompatible, and versatile properties.

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Hydrogels of Levan Polysaccharide: A Systematic Review

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Abstract

Levan is a fructose-based homopolysaccharide renowned for its unique properties, including exceptional adhesive strength, self-assembly capability, low viscosity, and bioactivities such as prebiotic, anti-cancer, anti-inflammatory, and anti-diabetic effects. These characteristics have created increasing interest in levan-based biomaterials over the past decade, positioning levan as a highly under-explored biopolymer for a wide range of applications, from medicine to cosmetics. As a result, levan-based hydrogels have emerged as promising biomaterials in drug delivery, tissue engineering, and cosmetic formulations, owing to their extracellular matrix-mimicking structure, tunable mechanical properties, and controlled cargo release capabilities. This review is the first to comprehensively examine the advancements in levan-based hydrogel research, systematically analyzing their biomedical applications and comparing them with other biopolymer-based hydrogels. Key questions regarding levan's potential as an alternative to established hydrogel systems are explored, highlighting areas requiring further research. By assessing trends and findings in the literature, this review provides an overview of the advantages, limitations, and prospects of levan hydrogels. Our analysis establishes a foundation for the continued development of levan-derived biomaterials, fostering broader adoption in biomedical and industrial applications.

Keywords

Levan; Polysaccaharide; Hydrogel; Biocompatibility; Biomaterials; Tissue Engineering

1. Introduction

Throughout history, significant milestones in humanity have been marked by discoveries in human physiology, diseases and treatments. However, the search for solutions to health problems dates back even further, as people have always relied on natural or synthetic materials from their environment to facilitate wound healing, repair damaged tissues and organs, and even enhance food preservation and safety [1]. Today, with increasing clinical and industrial needs, there is a growing demand for materials, devices, and techniques not only for diagnosing and treating diseases but also for ensuring safety, improving food quality, extending shelf life, and enhancing health benefits [2,3]. Biomedical and food sciences have emerged as multidisciplinary fields in response to these needs, aiming to improve human health by addressing genetic, environmental, and dietary factors. The size of the biotechnology market, which includes both biocompatible and functional, natural or synthetic materials that meet the definition of "biocompatibility", is expected to increase from 1.75 trillion to approximately 4.61 trillion USD from 2025 to 2034 [4]. This trend indicates growing interest in the biomedical, food and cosmetic sectors, as well as in the development of biomaterials for diverse applications.

Polysaccharides, the most common biocompatible polymers in nature, have been used throughout human history for their nutritional and healing properties due to their structural and functional characteristics that make them multifunctional bioactive materials [5]. Today, they are widely utilized not only in diagnosis, regenerative medicine, gene therapy, drug targeting, and tissue engineering, but also in food and nutrition sectors for enhancing food texture, stability, and bioavailability, as well as for their prebiotic and health-promoting effects. Polysaccharide-based hydrogels are three-dimensional colloidal solids that retain water in their 3D network structure. Compared to rigid scaffolds or dry polymeric systems, polysaccharide-based hydrogels offer flexibility, soft structure, high porosity, and water-holding capacity that promote cell viability, proliferation, matrix remodeling, and better resemblance to the native living tissue. In hydrogel formation, polysaccharides with diverse functional groups enable both chemical and physical crosslinking. Depending on application, this allows for the development of covalently bonded hydrogels with enhanced chemical, mechanical, and dimensional stability, as well as crosslinker-free hydrogels that are easier and faster to prepare [6]. Due to their extracellular matrix-mimicking properties, biocompatibility, flexible synthesis methods, and suitable physical properties, they have found broad applications as artificial soft tissue biomaterials in various biomedical fields, such as controlled drug and cargo release, biosensors, and tissue engineering and regenerative medicine [6, 7]. Furthermore, with a smart hydrogel approach, polysaccharide-based hydrogels can be tuned to respond to environmental stimuli such as pH, temperature, electrical and magnetic fields, ionic strength or light and can change some of their properties such as their wettability, degradability, swelling, and mechanical or surface properties. These attunable properties make them attractive especially for smart drug delivery systems and personalized medicine [8, 9]. While chitosan [10], dextran [11], and alginate [12] are common natural polysaccharides used in hydrogel formulations, levan-based hydrogels have recently gained increasing attention in health-related applications [13]. Levan, a natural homopolysaccharide of fructose, has been part of the human diet for centuries. However, compared to inulin-type fructans, which have a well-established market market as prebiotics, our knowledge of levan remains limited [14,15]. Despite this, levan's remarkable properties, such as biocompatibility, biodegradability, antioxidant, immune boosting, prebiotic, heparin-mimicking, anti-aging, and cryoprotectant effects, have piqued scientific interest, leading to extensive research on its production, derivatives, and levan-based

biomaterials [16,17,18]. Challenges such as high production costs and limited commercial availability, have hindered its widespread industrial use, promoting research into new production systems [19]. This has led to the discovery of more affordable methods for producing levan, opening up new avenues for its used in cosmetics, medicine, pharmaceuticals, and the food sector [20]. However, further research is still needed before levan can become a widely used product in these fields.

This review systematically examines trends and research on levan-based hydrogels and their applications in various biomedical fields, such as drug delivery, tissue engineering, and cosmetics. Several key questions are addressed, including why levan is an ideal biomaterial for biomedical applications, whether levan-based hydrogels can compete with other biomaterial-based hydrogels, and which sectors have extensively researched them. The literature is compared with other biomaterial-based hydrogels, revealing the advantages and limitations of levan-based hydrogels in various applications. This review aims to provide a solid foundation for future research and development in levan-based hydrogel and biomaterial applications.

2. State-of-the art

In order to gain insight into the current literature on levan-based hydrogels, a systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. The search strategy involved quering Scopus, ScienceDirect, and other journal websites using keywords such as “Levan”, “Levan hydrogels”, and “hydrogel”. No date or language restrictions were applied. To explore the sector-specific uses, additional keywords, such as "drug delivery", "tissue engineering", "cosmetics", and “food industry” were incorporated. From an initial set of 141 records, screening identified 15 relevant documents, after removing duplicates and out-of-scope articles. Further, a search on the Web of Science (WoS) database revealed a total of 124,017 documents related to hydrogels as of May 2025. Starting from the first studies with silica gels in 1946, the number of documents increased exponentially after the 2000s, and according to the best model fit, a total of 20,236 documents will be published in 2025 alone and an additional 182,458 reports by the end of 2030. When the search was narrowed down to specific polysaccharides by adding keywords, the total number of documents was 16,639 (chitosan), 13,508 (alginate), 8,884 (cellulose), and 6,666 (hyaluronic acid), followed by starch (1,946), dextran (1,837), pectin (990), and xanthan (836). On the other hand, adding "levan" as a keyword reduced the total number of documents to 21 that were only less than 0.02% of all hydrogel documents and many orders of magnitude less than common polysaccharides like chitosan (13.4%) or alginate (10.9%). While one of these 21 documents only cited levan and hence was discarded, another one was a review article on the recent advancements in pharma and healthcare applications of exopolysaccharide composites where authors also considered levan as a promising biopolymer and briefly discussed a few levan-based biomaterials in a short paragraph [21]. The remaining 19 research articles also included those from the previous search, and they were screened for their relevance. The first mention of levan as a hydrogel component appeared in Castillo and López-Munguia’s 2004 study, which focused on the enzymatic synthesis of high molecular weight levan by *Bacillus subtilis* levansucrase, resulting in a highly viscous levan precipitate resembling a hydrogel [22]. Other early studies highlighted levan’s hydrogel-like properties in *B. subtilis* biofilms [23] and *Erwinia tasmaniensis*-produced levan, which formed sticky hydrogels at high concentrations [24]. The hydrogel’s swelling behavior

was also explored in enzymatically synthesized levan from *Erwinia amylovora* levansucrase [25]. Another interesting study on encapsulating *Gluconobacter oxydans* cells in poly(vinyl alcohol) (PVA) hydrogels reported interactions of the polymer network with bacterial extracellular polysaccharide (EPS) components including levan as well as acetan, cellulose, and dextran [26].

In 2018, Choi et al. introduced the first physically crosslinked levan hydrogels using *Zymomonas mobilis* levan mixed with Pluronic F127 and carboxymethylcellulose (CMC) [27]. The group further incorporated hydroxyapatite to improve the hydrogel's injectability, biocompatibility, and long-acting anti-wrinkle efficacy [28]. Similarly, blends of gellan and *Erwinia herbicola* levan were ionically crosslinked to create shear-thinning hydrogels for tissue engineering [29]. Other studies explored the use of *E. herbicola* levan mixed with CBD oil to form sponges (rather than hydrogels) for wound healing applications [30]. Likewise, Maria et al. (2021) reported that a *Bacillus* levan isolated from honey showed the capacity to form emulsion hydrogels with omega-3 polyunsaturated fatty acids (PUFA) from ray liver oil and chia oil [31].

In 2017, the first covalent use of levan in a hydrogel structure was reported, where carboxymethylated derivative of levan from *Halomonas smyrnensis*, *Halomonas* levan, was employed as a cross-linker for temperature-responsive N-isopropyl acrylamide (pNIPA) hydrogels [32]. Then, another group used the same procedure to obtain short carboxymethylated *Zymomonas* levan, attached catechol, and then synthesized hydrogels with high adhesivity [33].

Further advancements included the photochemical crosslinking of methacrylated *Bacillus* levan to form covalent hydrogels, which were characterized for their structural and mechanical features, biodegradability, and cytocompatibility [34]. These studies were followed by the use of glutaraldehyde crosslinked *Zymomonas* levan/PVA hydrogels as pathogen-capturing filters [35], chitosan crosslinked oxidized *Bacillus* levan hydrogels loaded with curcumin for wound dressing applications [36], as well as 1,4-butanediol diglycidyl ether (BDDE) crosslinked *Halomonas* levan hydrogels for controlled release of Amphotericin B (AmB) [37], Resveratrol [38], *Plantago lanceolata* extracts [39], and as cryoprotectants for probiotic bacteria [40]. These BDDE/*Halomonas* levan hydrogels were also integrated with polyacrylamide to obtain interpenetrating hydrogel networks for uses in the conservation of cultural heritage [41]. Most recently, these hydrogels also showed promise in guided bone regeneration [42].

A patent search was also conducted across four major databases, namely, World Intellectual Property Organization (WIPO)'s Patentscope, Google Patents, Espacenet (by the European Patent Office, EPO), and The Lens—using the keywords "levan" and "hydrogel." The Lens, Google Patents, and Espacenet yielded 7,143, 4,518 and 448 hits, respectively. A Frontpage search on Patentscope returned 4 results only (same search for chitosan and alginate instead of levan yielded 2,969 and 2,412 hits, respectively). Two Korean patents (KR1020190066936 and KR1020190041272) granted in 2019 focused on levan-based hydrogels containing PF127 and CMC for cosmetic applications [27, 28]. In 2024, two Russian patents (RU0002815367 and RU0002819701) described biocomposite hydrogels involving *Paenibacillus polymyxa*-derived levan with one combined with alginate (RU0002815367), and the other with *Komagataeibacter sucrofermentans* bacterial cellulose (RU0002819701) for tissue engineering applications. The Cooperative Patent Classification (CPC) code A23V

2250/5068, which pertains to fructans used in food applications, was identified as relevant to levan but did not yield any hydrogel-related results.

3. Why levan?

Levan is a versatile, non-structural homopolysaccharide composed primarily of fructose units linked by β-2,6 glycosidic bonds, with different degrees of branching at the β-2,1 position. This unique structure allows levan to be synthesized naturally by a range of microorganisms, including archaea, fungi, bacteria, and a few plants [43]. Due to the difficulty of extracting levan from plants in large quantities, most of the levan used in current studies is derived from microbial sources through cloning levan-related genes [44, 45].

Levan has several properties that make it extremely useful for use as a hydrogel formulation in a variety of applications. First, it is an amphiphilic polymer soluble in both water and oil, but insoluble in most organic solvents, except dimethyl sulfoxide (DMSO). Such solubility profile gives levan the ability to self-assembly in water into densely packed spheres with a diameter of 25-250 nm, resulting in a low viscosity of its aqueous solutions [46]. This self-assembly behavior contributes to the formation of 3D networks, which are characteristic of hydrogels, while maintaining low viscosity in solution. Although levan's low viscosity is advantageous for injectable hydrogel systems, it can also hinder the development of mechanically strong and stable hydrogels. This limitation can be addressed by optimizing reaction conditions to relax the self-assembled structures, employing chemical crosslinking, or forming composite hydrogels.

Another important feature of levan is its suitability for chemical derivatization methods, such as sulfation, phosphonation, and oxidation [47]. Chemically-modified levan derivatives exhibit distinct properties, such as the anticoagulant activity of sulfated *Halomonas* levan [48] or the enhanced anticarcinogenic activity of oxidized *Halomonas* levan [49], which may support the design of hydrogels with diverse properties tailored for specific applications. Besides, a distinguishing feature of levan compared to other bioactive polymers is its remarkable adhesive strength. This is due to the high number of interactions, such as hydrogen bonds, Van der Waals forces, and interactions between the abundant hydroxyl groups within the structure, which contribute to levan's strong adhesion properties [50]. This adhesive strength is especially significant in the context of microbial biofilms, where levan serves not only as a crucial structural component to protect the microbial community but also as a nutrient reservoir, enabling survival under nutrient-deprived conditions [51].

There is growing interest in the use of bioactive polymers in hydrogel design across diverse fields, from biomedicine to the food industry. Unlike traditional hydrogels that primarily serve as passive carriers, these polymers can actively interact with biological systems, thereby enhancing the material's functionality and biocompatibility depending on the application. Levan, with its wide range of bioactivities, holds significant potential to confer additional therapeutic or functional benefits when incorporated into hydrogel systems. Figure 1 summarizes various bioactivities of levan polysaccharide with the state of the art literature discussed in the following sections.

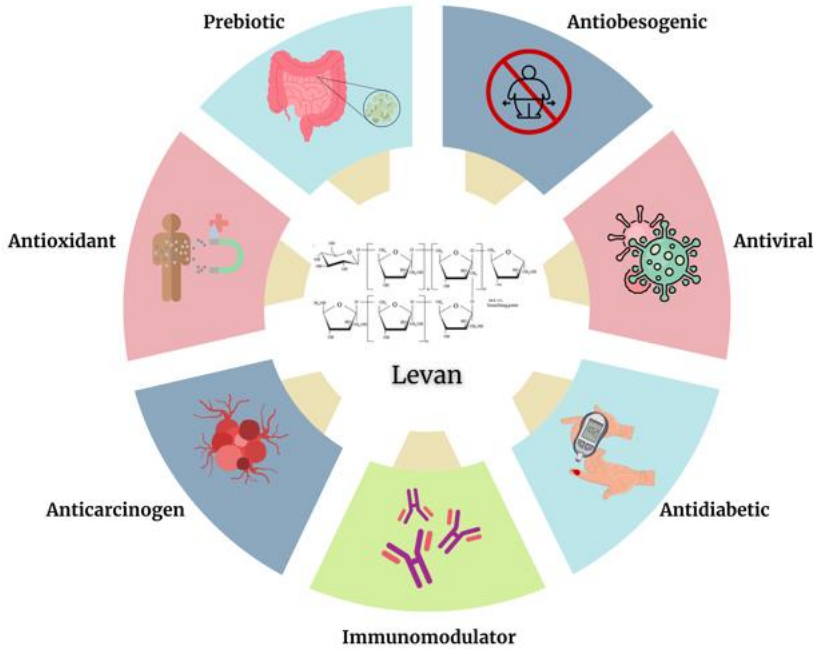

Figure 1. Bioactivities of Levan polymer (Created with Canva.com).

3.1. Levan as a Potential Prebiotic

Prebiotics are non-digestible food ingredients that promote the growth or activity of beneficial microbes in the body. Their degradation products, short-chain fatty acids (SCFAs), have gained attention for influencing the microbiota and general health, including effects beyond the gastrointestinal system [52, 53, 54]. In a study conducted with fructans of different molecular weights, it was observed that high molecular weight levan was more effective than low molecular weight fructooligosaccharides in terms of increasing the production of SCFAs and contributing to the diversity of the intestinal microbiota [55]. For prebiotics to be used by the beneficial microbiota, they must reach the colon without being digested in the upper intestine [56]. Fructans, which meet this requirement, differ from glucans in that they are only metabolized by the colon microbiota [51, 57]. Today, inulin-type fructans are the most researched group for their effects on microbiota, immunity, and intestinal barrier function [58]. Although levan is less researched, there are studies on levan as a potential prebiotic [15, 59]. It has been reported that when chicken [59], pigs [60], or rats [61] were fed with levan, breast meat and feed ratio increased, ammonia levels decreased, digestive abilities improved, and beneficial bacteria were abundant. There are even studies where levans' prebiotic activity was higher than inulin. For example, levan produced from *Erwinia* sp. significantly enhanced microbial community growth compared to inulin, likely due to its higher degree of

polymerization (DP), which allowed for prolonged persistence in the colon [62]. Moreover, levan from *Streptococcus salivarius* had higher or similar prebiotic activity scores against four probiotic bacteria compared to inulin [63] while levan from *Bacillus siamensis* and *Bacillus velezensis* supported the growth of *Streptococcus thermophilus* DKT-3 better than inulin [64].

3.2. Anti-diabetic and Anti-obesogenic Activity of Levan

Levan, unlike glucans such as cellulose and starch, is broken down into fructose rather than glucose, making it a potentially more suitable option for diabetic patients [65]. Dahech et al. (2011) demonstrated that orally administered levan reduced hyperglycemia and oxidative stress in diabetic rats, also protecting against hepatic and pancreatic toxicity [66]. Further studies reported that levan supplementation lowered blood sugar and serum cholesterol levels—critical factors in diabetes management [67, 68, 69]. Moreover, in obese rats on a high-fat diet, levan suppressed diet-induced obesity and hyperlipidemia, reducing total cholesterol while increasing HDL levels [70]. Additionally, a combination of levan and fermented ginseng was shown to reduce hyperlipidemia, fat accumulation, body weight gain, and improve glucose homeostasis and leptin resistance [71]. These findings highlight the biotherapeutic potential of levan in improving diabetes, diabetes-related metabolic syndromes, and obesity.

3.3. Anti-cancer, Antioxidant and Antiviral Activities of Levan

Cancer, characterized by uncontrolled cell growth due to DNA mutations, remains a significant health challenge due to the diversity in tumor types and treatment responses, prompting growing interest in natural agents alongside traditional therapies like chemotherapy and radiotherapy [72, 73]. Levan is one of the natural polymers that have been extensively studied for their anticancer potential across various cancer types where the structural properties of levan, such as its chain length and degree of branching, were foud to play a crucial role in its anticancer efficacy [74]. Recently, nanoparticles coated with *Zymomonas* levan (Np-Lev) have shown significant antiproliferative activity against breast cancer and melanoma cells [75], while levan combined with doxorubicin significantly reduced expression of cancer-related genes in hepatocellular carcinoma cells [74]. Moreover, the combined use of bacterial levan and benzimidazole derivative (BMPE) exhibited notable antimetastatic effects in a triple-negative breast cancer mouse model via immunomodulation and redox regulation [76].

Oxidative stress, caused by excessive accumulation of reactive oxygen species (ROS), can result in cellular damage such as DNA disruption, protein misfolding, lipid peroxidation, and membrane dysfunction, which in turn are associated with diseases like type 2 diabetes, cardiovascular and neurodegenerative disorders, and ischemia [77]. In this context, levan has been shown to effectively suppress peroxidation, donate electrons, and terminate free radical chain reactions, acting as a potent antioxidant [78]. Studies have demonstrated that levan derived from *Bacillus* strains and its sulfated forms possess strong antioxidant properties [64, 79, 80]. Similarly, levan isolated from *Acetobacter xylinum* and *Leuconostoc mesenteroides* cultures have also been reported to exhibit strong antioxidant and anti-inflammatory effects [44, 81].

Viruses with their complex structures and historical impact on pandemics, have driven

increased interest in antiviral agents, particularly following the global COVID-19 pandemic. Levan has been studied for its antiviral properties, with *Bacillus* levan shown to affect respiratory HPA1, H5N1, and enteric adenovirus type 40 [82]. More recently, same researchers found that crude, dialyzed, and sulfated derivatives of levan produced from *Enterococcus faecalis* cultures showed antivirus activity against Newcastle disease virus (NDV) [83].

3.4. Immunomodulatory Activity of Levan

The immune system plays a crucial role in defending against infections and cancer and understanding its mechanisms is essential for recognizing its role in diseases, interactions with other systems, and the development of new therapeutic strategies [84]. While traditional treatments like corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to suppress inflammation [85], their long-term use is limited by significant side effects [86], driving research into more selective and natural treatment approaches for immune modulation with fewer adverse effects [87, 88].

There are numerous reports in the literature on the immunomodulatory activity of levan polysaccharides from various sources, but only a few of them have investigated the related molecular mechanism [89]. *In vivo* oral administration of *B. subtilis* natto levan was found to reduce the serum level of ovalbumin-specific Immunoglobulin E (IgE), modulate the T helper 2 (Th2) cell response, and pattern recognition was found to be mediated by Toll-like receptor (TLR) 4 [90]. TLR4 are receptors that recognize conserved pathogen-associated molecular patterns (PAMPs) and thus constitute the first line of defense [91]. In addition, they recognize lipopolysaccharide (LPS) glycolipids found in the outer membranes of both commensal and pathogenic Gram-negative bacteria [92, 93]. A recent study showed that LPS is the actual molecular determinant of the immunomodulatory property of levan toward TLR4 receptor-expressing innate immune cells [94].

Some studies provide a better understanding of the changes in the immunomodulatory properties of structurally different levan produced from different sources. For example, while levan produced from *L. mesenteroides* increased the levels of interleukin-4 (IL-4), an important anti-inflammatory cytokine [81], levan obtained from *Paenibacillus bovis* sp. did not cause any modulation in IL-4 levels [95]. In addition, levan of different molecular weights from the same species has been observed to have different effects on inflammation-related pathways. For example, levan from *B. subtilis* altered iNOS, COX-2 gene expression [96] and nitric oxide (NO) production [97] in RAW264.7 macrophages depending on molecular weight. Moreover, a relatively recent study used methacrylated levan as a bio-ink for 3D bioprinting of bone tissue scaffolds. In addition to good bioprintability, the samples supported the expression of the anti-inflammatory marker CD206 [98]. These results suggest that the inherent immunomodulatory effect may make levan an ideal polymer for hydrogels in tissue repair and regeneration applications, as it will reduce the possibility of rejection by the body.

4. Synthesis of Levan-based hydrogels

Properties of hydrogels such as mechanical strength, self-healing ability, swelling capacity, and biodegradability depend on cross-linking processes. Hydrogel synthesis is generally achieved through chemical or physical crosslinking methods. Chemical crosslinking

involves the formation of covalent bonds between polymer chains, either directly or via low-molecular-weight crosslinkers, resulting in hydrogels with enhanced long-term stability and mechanical strength. In contrast, physical crosslinking relies on non-covalent interactions, often leading to heterogeneous polymer networks and hydrogels that are structurally less stable and mechanically brittle. Nevertheless, physically crosslinked hydrogels formed via weak interactions, such as ionic or electrostatic interactions, hydrophobic interactions, hydrogen bonding, or van der Waals forces, can exhibit advantageous properties, including self-healing capability and responsiveness to external stimuli [99].

The choice of cross-linking method depends on the physicochemical properties of the polymer. Due to its uncharged nature and water solubility, native levan does not readily form stable hydrogels without chemical or physical crosslinking or prior structural modification. Therefore, either levan or its chemical derivatives are mixed with other polymers to promote physical crosslinking, or chemical crosslinkers are used to establish covalent bonds and enhance hydrogel stability. Furthermore, the inherently fast biodegradation rate of levan can be tuned by chemical modifications or by integrating it into composite hydrogels, allowing for better control over its stability and degradation kinetics in various application environments.

In literature, there are reports on physically linked levan hydrogels such as injectable levan/ CMC /Pluronic F127 blends [27, 28]. In both studies, it was stated that there is hydrophobic association and hydrogen bonding between levan, Pluronic F127, and CMC, and especially that the poly(ethylene oxide) group of Pluronic F127 interacts with levan, and therefore levan is necessary for physical crosslinking. In another study, ionically crosslinked levan/gellan blends were examined, and it was hypothesized that under alkaline conditions, gellan interacts with levan via ionic crosslinking between hydroxyl and carboxyl groups to form a hydrogel network [29]. On the other hand, relatively more studies used chemical crosslinking to obtain better mechanical stability, including photo-polymerized methacrylated *Bacillus* levan [34] or chitosan crosslinked oxidized *Bacillus* levan [36]. In these studies, functional groups were introduced into levan to facilitate its reaction with crosslinkers or other polymers. For instance, methacrylate-modified levan enables covalent bonding with photoinitiated crosslinkers such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) or Irgacure 2959 [34]. Similarly, the incorporation of aldehyde groups through oxidation allows levan to form Schiff base linkages with amine-containing polymers like chitosan [36]. However, there are also examples in which unmodified levan has been directly employed to form chemically crosslinked hydrogels such as glutaraldehyde crosslinked *Zymomonas* levan/PVA [35], and BDDE crosslinked *Halomonas* levan [37 - 42]. Figure 2 illustrates various types of chemically and physically crosslinked levan-based hydrogels while Figure 3 gives the classification of levan hydrogels.

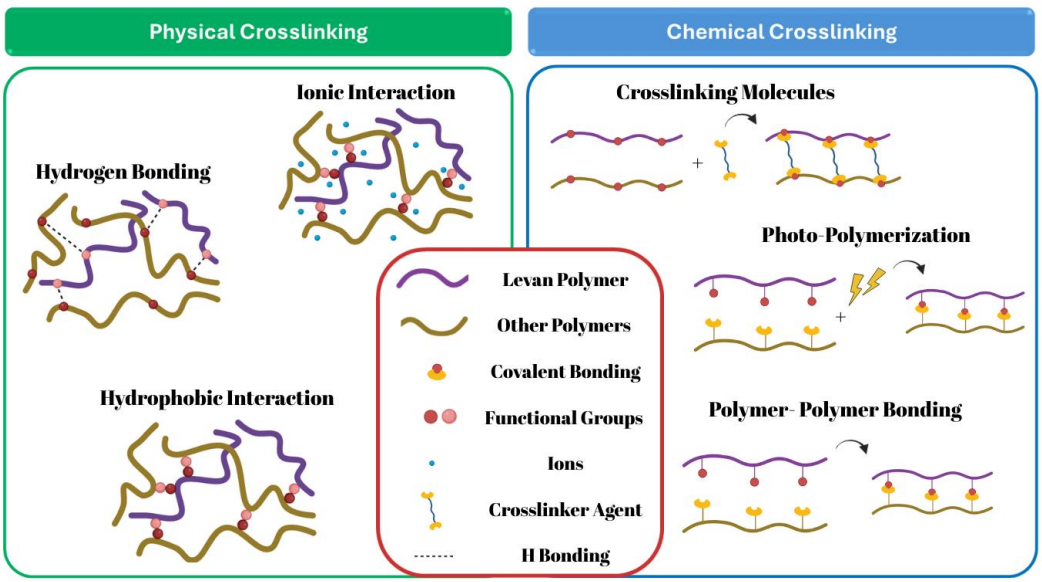

Figure 2. Physically and chemically crosslinked levan-based hydrogels (Created with Canva.com).

The good biocompatibility of hydrogels under physiological conditions, their degradability, and the low toxicity of the formed degradation products are important issues in the selection of the crosslinking agent [99]. Although hydrogels with good mechanical stability are formed by chemical crosslinking, crosslinking agents are generally synthetic and can cause toxic effects [100]. In a related study, levan was used to crosslink pNIPA hydrogels as an alternative to bis-acrylamide (BAAm), aiming to eliminate its potential toxic effects [32].

So far, mostly BDDE crosslinked levan hydrogels are reported where the formation of covalent bonds occurs as a result of the reaction between the epoxide groups of BDDE and the primary hydroxyl groups on the levan backbone. However, in a recent review by Wojtkiewicz et al. (2024) [101], it was noted that long-term use of BDDE-crosslinked hydrogels can lead to tissue hardening and redness in the application area after several years, potentially due to BDDE itself or its byproducts released during hydrogel disintegration, as indicated by clinical findings.On the other hand, physical crosslinking offers a valuable alternative to avoid the potential risks associated with chemical methods.

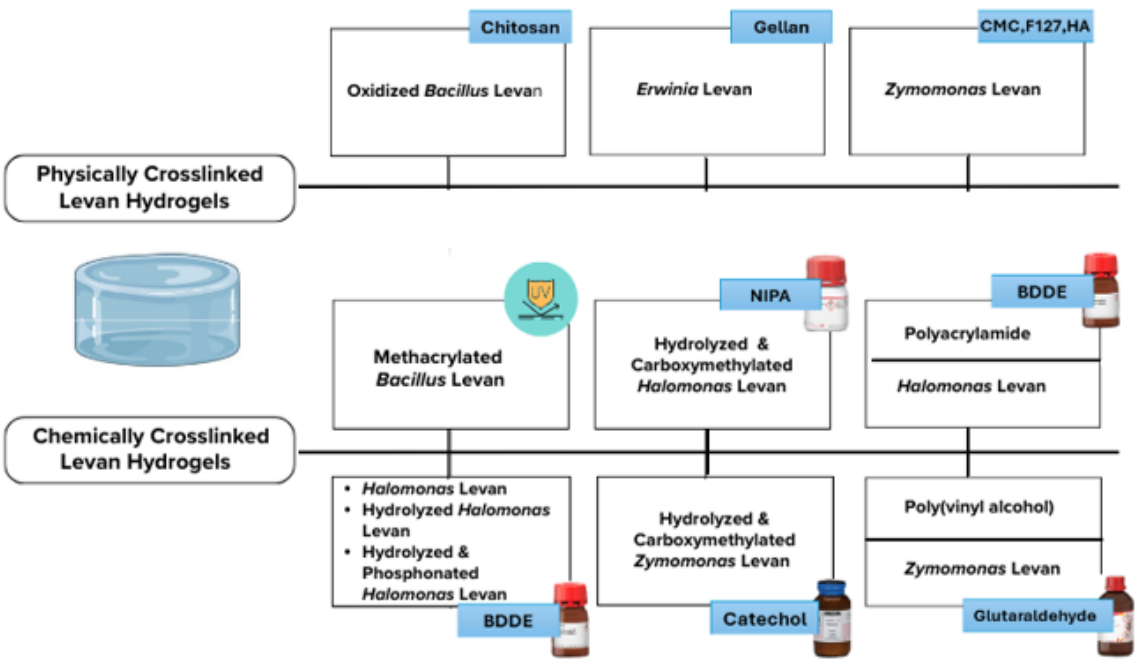

Figure 3. Classification of levan-based hydrogels (Created with Canva.com)

5. Applications of Levan Hydrogels

Levan-based hydrogels have emerged as promising biomaterials in various biomedical applications, particularly in drug delivery systems, tissue engineering, and regenerative medicine, as well as in cosmetics. Their unique physicochemical properties, including biocompatibility, biodegradability, and stimuli-responsiveness, make them suitable candidates for controlled and targeted drug release, offering improved stability, bioavailability, and efficacy. In Table 1 are summarized the key findings on levan-based hydrogels in various sectors to provide a general overview of their diverse applications.

Table 1. Hydrogels of Levan

Source of Levan	Hydrogel structure	Application	Reference
Bacillus subtilis	Levan was methacrylated and then photo-polymerized	Mechanically stable, slightly degradable and cytocompatible gels suitable as starting materials in common additive manufacturing processes like bioprinting or stereolithography	[34]
	Levan was oxidized with sodium metaperiodate and then mixed with chitosan for the gelation	Bio- and hemo-compatible drug delivery system and wound dressing material with sustained curcumin release	[36]
Erwinia herbicola	Levan/gellan blends crosslinked ionically with NaOH and $CaCl_2$	Shear-thinning properties showed high potential of levan-gellan hydrogel for its use as an injectable material in medical and cosmetic industries	[29]
Halomonas elongata	BDDE crosslinked levan hydrogels loaded with liposomes containing *Plantago lanceolata* L. Extracts	Biocompatible and biodegradable levan-based herbal liposome-loaded hydrogels for acute wound healing	[39]
Halomonas smyrnensis	Hydrolyzed, carboxylated and then methacrylated levan crosslinked with *N*-isopropyl acrylamide (NIPA)	Thermoresponsive levan/pNIPA hydrogels for the controlled release of 5-ASA for the treatment of inflammatory bowel diseases	[32]
	BDDE crosslinked levan hydrogels	Amphotericin B release for exerting antifungal activity against *Candida albicans*	[37]
	Hydrolyzed and	Controlled release of resveratrol	[38]

	phosphonated levan crosslinked with BDDE	for skin tissue engineering	
	Levan, acrylamide and methylene-bis-acrylamide solution crosslinked with BDDE and Ammonium persulfate	IPN-based Levan/PA hydrogels for conservation of cultural heritage	[41]
	Hydrolyzed levan cross-linked with BDDE	Levan hydrogels for guided bone regeneration and bone tissue engineering	[42]
Zymomon as mobilis	Pluronic F127/CMC/levan mixture	Injectable hydrogels to be used as an alternative to hyaluronic acid (HA) based dermal fillers in soft tissue augmentation	[27]
	Hydroxyapatite containing Pluronic F127/CMC/levan hydrogels	*In vivo* stability of the composite hydrogel was enhanced with high anti-wrinkle efficacy maintained for 8 weeks and showing high collagen production	[28]
	Levan/PVA crosslinked with glutaldehyde	Virus capture efficiency of levan−PVA hydrogels was higher than that of commercial cotton swabs	[35]
	Catechol conjugated carboxymethylated levan hydrogels via oxidation-induced catechol/quinone (covalent) and Fe^{3+}-(non-covalent) mediated coordinative crosslinking	Levan-catechol conjugate hydrogel for wound healing applications	[33]

5.1. Pharmaceutical Applications

Direct clinical use of active pharmaceutical ingredients (APIs) “as is” is limited since drugs are sensitive chemicals and are adversely affected by body conditions (pH, temperature, enzymes, etc.), low bioavailability, and low absorption [102]. Therefore, delivery systems that transport therapeutics to relevant sites in the body in a controlled manner are necessary for effective drug delivery. Traditional drug delivery systems require high and continuous doses due to untargeted distribution and uncontrolled release of the drug. This is contrary to keeping the plasma drug concentration above the minimum effective concentration (MEC) and below the toxic concentration and may cause side effects [103]. There is increasing interest in controlled and targeted drug delivery systems (DDS), designed to release the correct dose of a therapeutic agent directly to the desired site and within the required period. These systems maximize the effectiveness of the therapeutic agent and minimize possible side effects [104].

The conventional DDS methods, like oral tablets and injections, often suffer from limitations like poor bioavailability, systemic side effects, and the need for frequent administration [105]. Advanced drug delivery systems have been developed to overcome these challenges, and so many new options, such as liposomes, nanoparticles, microneedles, polymeric carriers, and hydrogels offering unique advantages, improve health care day by day [106]. Among them, hydrogels have gained considerable attention due to their cross-linked hydrophilic polymer network that can retain large amounts of water. When loaded with drugs, they can hold the drug inside and gradually release it in a controlled manner due to their porous structure and swelling properties [107]. Nowadays, more specific versions of hydrogels, such as stimuli (pH, temperature, enzymes, etc.) responsive, injectable, or nanogels, are being designed, thus rapidly progressing towards more efficient and effective drug delivery systems [108,109].

The use of levan-based drug delivery systems may overcome, to a great extent, the challenges of the conventional modes of drug administration. So far, various drugs have been loaded into levan-based-drug delivery systems for targeted and controlled delivery. After levan polysaccharide was first reported to be suitable for delivering peptides, proteins, and macromolecular drugs [110], *Halomonas* levan was used for controlled release of vancomycin [111], curcumin [112], paclitaxel [113], and resveratrol [114]. Additionally, levan derived from *Bacillus licheniformis* has demonstrated high potential as a pharmaceutical excipient in topical drug formulations [115]. Recent studies include *B. subtilis* levan-based nanoparticles loaded with the antiretroviral drug - dolutegravir for potential use in HIV treatment [116] and levan-shelled hydrophobic silica nanoclusters encapsulating doxorubicin as an ultrasound-responsive drug delivery system for cancer treatment [117]. These studies reveal that levan is a promising polymer for drug delivery strategies, not only as a carrier but also with its bioactivity.

When designing drug delivery systems, some important issues should be considered. The first one is the ability to provide targeted delivery. Hydrogels have been demonstrated as the ideal pharmaceutical carrier in biomedicine due to their high porosity and water content through their unique three-dimensional (3D) crosslinked network structure [118]. Stimuli-responsive hydrogels, also known as smart or intelligent hydrogels, have become the most studied systems for drug delivery [119]. They are able to respond to environmental changes like temperature, pH, or magnetic fields, controlling not only drug administration but also the timing and release profiles, with their sol-gel phase transition behavior influenced by their sensor characteristics and surrounding conditions [120].

Examples of levan-based-stimuli-responsive hydrogels include thermoreactive levan/N-isopropyl acrylamide (levan/pNIPA) hydrogels for controlled release of 5-aminosalicylic acid (5-ASA) used in the treatment of inflammatory bowel diseases. Osman et al. (2017) used methacrylated, hydrolyzed, and carboxymethylated forms of *Halomonas* levan as crosslinkers to prepare levan-pNIPA hydrogel for the first time [32]. The biocompatibility of the obtained hydrogels with L929 cells was evaluated, and data showed that the biocompatibility was improved with higher levan percentage in hydrogels. Also, when different concentrations and temperatures were tested, addition of levan brought the volume phase transition temperature closer to the human body temperature.

Every drug delivery system, including hydrogels, has a limited residence time in the targeted release region. This is between 2-5 hours for the stomach and 2-6 hours for the small intestine, and at the end of this period, the content is transferred to the next step of the system, such as the colon. Therefore, it is important to test how much and how quickly a hydrogel will

release the encapsulated drug. Since it is known that the type and content of crosslinker used in hydrogel production change the drug release characteristics, the levan/pNIPA hydrogel system [32] was compared with the same system where chitosan was used as a crosslinker instead of levan [121]. It is observed that both hydrogels released approximately 90% of the drug in 2 hours under the same conditions (37 °C, pH 7.4). Both hydrogels can release an effective amount of the drug in a sufficient time. However, while 100% of the drug was released in 4 hours in the chitosan hydrogel, this period was 6 hours in the levan hydrogel. This difference can be attributed to the pH-sensitive structure of chitosan, suggesting that levan may be a good alternative for medications that need to be released over a longer period.

The second important point to consider when designing drug-delivery hydrogels is the swelling capacity. The reason is the high influence of swelling on drug loading efficiency (DLE) and drug encapsulation efficiency (DEE), which are mandatory for an adequate drug delivery system. It is worth noting that the swelling capacity of a hydrogel depends on the polymer used and the degree of cross-linking. While high crosslinking causes lower water absorption capacity, lower crosslinking degree leads to lower mechanical stability [38,122]. So, the optimum cross-linking degree should be selected for enhanced drug delivery. Another essential factor influencing swelling is the pH of the environment. Therefore, in a study by Demici et al. (2020) to estimate swelling capacity, tests were performed with selected hydrogels that have an optimum polymer/crosslinker ratio (*Halomonas* levan/BDDE) in different solutions [37]. The results showed that the swelling capacity of the hydrogels was pH dependent, higher pH leading to higher swelling, and the maximum equilibrium degree was reached in 1 hour. Antifungal and cytotoxicity tests also revealed the high potential of levan hydrogels in the transport of pH-sensitive drugs such as amphotericin B (AmB) [37].

The third and possibly most crucial factor is the biocompatibility of the drug delivery system. Hydrogels, especially those produced from natural polymers, are known as biocompatible since they are not toxic or injurious and do not cause immunological rejection in living tissues [107]. Considering the need for more systematic studies on levan-based hydrogels to widen their use in drug delivery systems and biomedical applications, chemically modified forms were used to synthesize biocompatible hydrogels. Recently, levan produced by *H. smyrnensis* and its hydrolyzed and phosphonated derivatives crosslinked with BDDE were produced for the first time for resveratrol delivery [38]. Results revealed the superior *in vitro* biocompatibility of levan hydrogels with a human keratinocyte cell line (HaCaT). Additionally, cell attachment to phosphonated hydrogels was higher since phosphonate groups affect cellular attachment via mimicking protein-protein and protein-natural polymer interactions [123]. This finding proved that hydrogels produced from chemically modified forms of levan polymer have superior potential in the pharmaceutical and cosmetic industries.

5.2. Levan Hydrogels in Tissue Engineering

Tissue engineering (TE), a rapidly growing field, is used to substitute diseased or damaged tissues with functional ones, aiming to restore or enhance the function of the affected tissues. Nowadays, hydrogels, due to their unique properties, have become an integral part of tissue engineering, especially in enhancing tissue function and promoting cell growth, differentiation, and regeneration [124,125]. When selecting biomaterials to form hydrogels, several factors need to be considered, including mechanical properties, biocompatibility, gelation time and swelling capacity [126]. The understanding that natural polymers, especially

polysaccharides, have the potential to meet these properties positions levan as an attractive candidate for tissue engineering applications.

The first study on the synthesis of photo-crosslinkable levan derivatives and their use for hydrogel production was reported by Berg et al. (2018) [34]. Levan from *B. subtilis* was chemically modified via methacrylation with glycidyl methacrylate (GMA), methacrylic anhydride (MAA), or 2-isocyanatoethyl methacrylate (IEM) and then photo-polymerized in the presence of different photoinitiator systems and irradiation units. The resulting hydrogels were mechanically stable and cytocompatible. Furthermore, it was shown that changing the type of crosslinker and the crosslinking density altered the degradation rate of the hydrogels, thus making these gels suitable candidates for both drug delivery and tissue engineering applications. Considering the complexity and high cost of materials used in tissue engineering in terms of preparation and application processes, there is a high need for new-generation biomaterials that can be produced at low cost, have high bioactivity, and are sustainable [127]. The increasing use of natural polymers such as gelatin, hyaluronic acid, etc., in tissue engineering applications has encouraged researchers to explore the potential of levan in this area [128]. In a recent study, levan hydrogels were incorporated with Bio-Oss®, a traditional xenograft used in guided bone regeneration applications (GPR), at different ratios. The results showed that, in addition to higher biocompatibility, levan containing hydrogels exhibited higher new bone formation, osteoblast density, and new vessel formation compared to the group containing only Bio-Oss® [42].

5.3. Applications in Wound Healing

Wounds, which are pathological cases caused by various diseases and physical-chemical damages, are classified as acute or chronic depending on the type of damage and the duration of the healing process [129]. Wound healing is a complex biological process that involves the coordination of various tissues and cell types, such as fibroblasts and keratinocytes, to repair damaged skin or other tissues. Wound dressing materials play a critical role in accelerating the healing process by providing an optimal environment for tissue regeneration [130, 131]. A suitable and effective wound dressing material should possess key properties, including biocompatibility, non-toxicity or non-allergenicity, mechanical strength, air permeability, moisture retention, antimicrobial activity, and ease of removal [132, 133]. Conventional wound dressings often fail to meet these requirements, making biopolymer-based hydrogels a promising alternative due to their morphological similarity to the extracellular matrix and ability to mimic natural tissue structures [134, 135, 136, 137, 138].

Levan has gained attention for its wound healing potential. Its anti-inflammatory, antibacterial and high adhesive properties, contribute significantly to tissue repair [139]. Levan can support cell proliferation, particularly of fibroblasts and keratinocytes, which are essential for wound closure and immune response regulation [140, 141]. Additionally, levan can enhance the mechanical stability of wound healing systems, promoting faster recovery. As proof of that, *Bacillus mojavensis* Levan-PVA nanofibers have been shown to accelerate wound healing due to the water-holding properties of levan combined with the film-forming ability of PVA [142]. Increasing levan-PVA concentration further enhances tensile strength and cell viability, leading to better healing outcomes [143] showed increasing levan-PVA concentration causes higher tensile strength and improved cell viability. In addition, porous microbial levan-based sponges loaded with cannabis oil (Lev@CBDs) exhibited a suitable swelling ratio and enough thermal stability to use even after being exposed to harsh conditions for a long period. Also, produced

sponges exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa,* the two most known bacteria that cause infection in wound areas [30]. In addition to its bioactivity on the wound area, levan can also be used as a natural reducing and coating material for microelement nanoparticles (NPs) [144] due to the presence of -OH groups on the polymer chains [145].

In some cases, using more than one polymer for the synthesis of hydrogels called hydrogel composites is preferred to obtain an effective drug delivery system, as they offer excellent properties of contained biomaterials, which other drug delivery systems cannot quickly achieve for the controlled release of drugs to specific sites. Basically, by cross-linking through covalent bonding/ionic interactions/hydrogen bonding of levan with other polymers, hydrogels with increased structural integrity can be formed. To improve wound healing, the oxidized form of levan from *B. subtilis* was crosslinked with chitosan by Veerepandian et al. (2023) [36]. Schiff's base reaction between oxidized levan and chitosan leads to formation of hydrogels without need for an initiator such as UV light or chemical cross-linkers, making hydrogels more biocompatible and suitable for wound healing. All the hydrogels had highly interconnected porous networks due to the large number of functional groups in the structure of levan and chitosan that help cell attachment, proliferation, and nutrient transfer. The hydrogels showed better thermal stability than neat levan, and the presence of chitosan made the swelling behavior of hydrogels pH-dependent (pH 2.6). The results from the hemocompatibility tests via direct and indirect methods showed non-significant differences compared to the control group. In another study, levan-catechol conjugate (LC) was synthesized by combining the carboxyl group of CM-levan with the amino group of dopamine using 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) chemistry. This conjugation improves the produced hydrogels' water-proof ability as well as fast swelling over the first 30 min of incubation, which makes these hydrogels good candidates for wound healing applications. Furthermore, the increased blood clotting rate, low endotoxin levels, and faster wound closure in the SD rat model compared to the control suggested that it can be used as a biomedical adhesive in wound healing process [33]. Moreover, the wound healing efficacy of the composite material developed by loading herbal liposomes into levan-based hydrogel structures was investigated by Altıntaş and Çelik (2023) [39]. Hydrogel structures were developed for the first time with levan produced by *Halomonas elongata* 153B halophilic bacteria for wound healing. Extracts from *P. lanceolata* L., commonly known as a wound herb, which has cell regeneration ability and anti-bacterial activity in the wound area, were obtained. Then, extract-loaded liposomes were prepared, and were loaded into the levan-based hydrogels for controlled release into the wound area. The wound healing efficacy of the herbal liposome-loaded levan-based hydrogel was evaluated in an *in vitro* wound model. Results indicated that the developed hydrogels are a promising therapeutic approach for the healing of acute wounds [39].

5.4. Cosmetics

The skin, which is the largest organ of the body, has the basic functions of protecting the body from pathogens and external environmental elements (radiation, impact, heat, etc.) and maintaining homeostasis by maintaining body temperature and humidity [146]. Cosmetic substances such as lotions, creams, masks, etc. are generally products used to maintain or improve the current condition and can be produced from various raw materials [147]. However, some cosmetic products contain several ingredients like parabens, which can harm the skin

and hair [148]. Recently, natural products are rapidly gaining attention due to their biocompatibility, sustainability, and high contribution to skin health. Cosmetic product users are more interested in skin and environment-friendly, biodegradable ingredients instead of synthetic additives. For this reason, bioactive formulations are widely available in skincare, haircare, and personal care items [149]. Levan, on the other hand, has remarkable properties such as skin whitening, moisturizing, and reduction of skin irritants [150, 151].

While the selection of raw materials used in cosmetic formulas varies depending on which area they will be applied in, today the cosmetic industry is showing much more interest in hydrogel-based cosmetic products due to their properties such as biocompatibility, elasticity, and the ability to hold excess water [152, 153, 154]. Hydrogels, which can be used to help restore skin elasticity, softness, and moisture, can also facilitate drug penetration by loosening the skin barrier [155]. Synthetic polymers and biopolymers are widely used in commercially available cosmetic products. Biodegradable polymers, especially polysaccharides, have been attracting much attention of scientists looking for new compounds with cosmetic properties because they can be eliminated by normal metabolic pathways and do not cause toxicity and accumulation in the body [156, 157].

The variety of properties of levan mentioned above makes it a useful and safe biomaterial for cosmetics. In the literature, lots of research showed the suitability of using levan in cosmetics. For example, the stimulation of human fibroblasts and keratinocytes by *Z. mobilis* levan [158], as well as a whitening agent by inhibition of melanin production [159]. Lewińska et al. (2022) [160] developed tonic, gel, and cream formulations containing surfactin-stabilized *B. subtilis* levan nanoparticles (NP) and nanoemulsion (NE) nanosystems. They reported that these formulations improved skin hydration, elasticity, wrinkle depth, and also skin discoloration. In addition, levan, which also met the requirement of non-toxicity, did not show cytotoxicity on human dermal fibroblasts (NHDF) [161]. In *in vitro* experiments with *Halomonas* levan and its derivatives, increased cell proliferation, skin barrier function, and rapid wound healing ability were observed [139]. In addition, the cosmetic product needs to maintain its stability for a long time when the approximate period of use is evaluated. Da Silva et al. (2022) [162] reported the maintenance of moisturizing, antioxidant activity, and stability of the product consisting of *B. subtilis* natto levan and almond oil for 3 months. In a study, carboxymethyl levan (CML)-hEGF nanoparticles designed to overcome the low stability of human epidermal growth factor (hEGF) maintained over 100% cell proliferation activity for 6 weeks [163]. This is one of the important studies supporting the potential use of levan as a cosmeceutical because hEGF is a signaling molecule that stimulates the growth and motility of keratinocytes and fibroblasts in epithelial tissues [164]. Moreover, in a study aimed at enhancing the effects of levan with biocompatible actives such as natural compounds and vitamins, levan was combined with natural compounds such as aloe vera extract, avocado oil, and vitamin E [165]. The cosmeceutical formulations were non-toxic and completely vegan, as well as showing good stability. Another study also showed that levan-containing digestive extract reduces the irritating effect of ionic surfactants and is therefore a suitable ingredient for the formulation of harmless body wash cosmetics [166].

Soft tissue fillers are applications developed as an alternative to plastic surgery due to reasons such as low life risk, fast recovery time, and instant effect. The material used for an effective application should be biocompatible, non-allergenic, and stable [167, 168]. Fillers are essentially divided into two categories: non-biodegradable and biodegradable, according to the properties of the raw material used [169]. Non-biodegradable fillers are less tolerated in the body than biodegradable ones and therefore can cause serious side effects. Hyaluronic

acid (HA) fillers are approved by Food and Drug Administration (FDA) and known for providing the ideal features mentioned today and dominate the sector [167]. Choi [27] developed an injectable levan-based hydrogel for use as a dermal filler for soft tissue augmentation and reported that this system could be an alternative to hyaluronic acid (HA)-based dermal fillers. The injectable hydrogels were formed by combining Pluronic F127, CMC, and levan derived from *Z. mobilis* with hydrophobic and non-covalent interactions. The levan hydrogel and HA-based hydrogels were tested in an animal disease model both *in vitro* and *in vivo*. Rheological results showed that the elastic modulus of levan-based hydrogel (~6 kPa) was higher than that of the HA-based hydrogel (~2.8 kPa), and the interconnected porous structures were similar to each other. In addition, no cytotoxicity was observed in human adult dermal fibroblasts (hADF) in the levan-treated group, and enhanced cell proliferation was observed. Since collagen synthesis is another important factor in enhancing the anti-wrinkle effect [170], hydrogels were also evaluated for their ability to improve collagen synthesis. hADF cells treated with levan showed higher expression of the type I collagen gene and improved anti-wrinkle efficacy in the wrinkle mouse model compared to HA. Later, by adding hydroxyapatite to the prepared hydrogels, their *in vivo* stability was extended to maintain the anti-wrinkle effect [28]. Levan hydrogel was biocompatible and stable longer *in vivo* than Pluronic F127 or HA, indicating that levan has high potential as a new material for effective dermal filler compared to HA *in vivo*. Therefore, levan-based dermal fillers are an up-and-coming, low-cost alternative to overpriced HA-based hydrogels. Additionally, due to its surface morphology, thermal behavior, rheological properties, and gel-forming features, enzymatically produced *E. amylovora* levan was also proposed as a promising candidate for cosmeceutical applications [25]. In this study, levan and other commercial hydrogels, such as carrageenan and guar gum, showed similar rheological behavior, similar porous network microstructure, and endothermic temperature.

Normally, shear-thinning hydrogels are mostly ideal for use in the cosmetic industry; however, chemical modification, which is usually applied for synthesis, causes high gelation time and chemical toxicity. Nair and Choudhury [29] produced levan/gellan composite hydrogels using levan from the *E. herbicola* solution and low acyl gellan polysaccharide, aiming to eliminate the problems caused by chemical modification by using natural polysaccharides. The results showed that the composite hydrogels exhibited remarkable mechanical properties and easy injectability. In addition, it was observed that when the ratio of levan or gellan polysaccharide in the composite hydrogels was increased, the swelling percentage also increased due to free hydroxyl groups compared to hydrogels consisting of equal amounts of each polysaccharide [171]. In addition, it was shown that the hydrogels had 97% water retention capacity and sufficient cross-linking with strong structures, revealing the significant potential of levan-gellan hydrogel for use as an injectable material in the medical and cosmetic industries.

5.5. Potential Applications in Food Science

Levan-based hydrogels hold significant promise in the food industry due to their natural origin, biocompatibility, and functional properties such as antioxidant, antidiabetic, and anti-obesogenic activity. Different authors proposed the use of levan in association with other biopolymers to form films feasible as edible coatings. For instance, levan-gellan gum [172], levan-chitosan [173], levan-vanillin [174], and oxidized levan-gelatin [175] films have been proposed as a coating to be applied on the surface of food products to improve their shelf life.

Moreover, the gelling properties of levan could be exploited for the design of functional foods with improved overall acceptability while delivering prebiotic levan. Being levan, able to form hydrogels, its usage in all gel-based foods could be tested. However, to this aim, it would be fundamental to have a deep understanding of structure-function relationships at different lengths of scales (from nano to micro and macro scale). Today, the possible interactions of levan with other biopolymers, such as proteins or other carbohydrates, in complex food systems are still underexplored but highly demanded for the design of novel sustainable food with designed health-improving capacity. In this context, Hundschell et al. (2022) [176] studied the influence of levan on the thermally induced gel formation of β-lactoglobulin. The presence of levan increased the water-binding capacity of the gel network. Besides, some studies elucidated the structuring ability of levan produced by microbial fermentation in bread. The exopolysaccharide levan is reported to be able to impact the bread quality [177]. *B. subtilis* levan produced from coconut inflorescence extract was studied not only for its technological properties but also as a sweetener [178]. The possibility of exploiting levan also as an alternative and innovative sweetener is particularly interesting in the attempt to reduce the use of artificial non-nutritive sweeteners, such as saccharin, cyclamate, and aspartame. Considering both the health and technological functionalities of levan, it seems to be an ideal polymer that can be used in many areas, from packaging to the food industry, as an ingredient in many food formulations.

Furthermore, thinking about the possibility of converting hydrogels into oleogels, i.e., oil-based gels with macroscopic properties mimicking those of solid fats [179, 180], or into aerogels, i.e., highly porous materials designed as delivery systems [181], it cannot be underestimated the possibility to use levan hydrogels in the field of oleogelation or aerogelation.

5.6. Other Applications

To minimize the health, economic, and social damage that epidemics can cause, it is important to detect and capture the pathogens that cause them as soon as possible and many strategies are applied in today's literature. Some of these are magnetic nanoparticles (MNPs) for the detection of various infectious diseases [182], superparamagnetic iron oxide nanoparticles (SPIONs) functionalized with peptides obtained from salivary protein, a mesoporous PDMS sponge for foodborne pathogens [183], magnetic beads (MB) coated with specific molecules for pathogen detection from blood samples [184], and a silk protein-based microbial trap [185]. It is known that carbohydrates on the mucosal tissue surface act as receptors for pathogens during infection and prevent invasion by preventing pathogens from passing through the cell membrane [186]. With this approach, carbohydrate materials that will be designed by taking inspiration from a natural process may be useful as pathogen trappers. Li et al. (2011) [187] used carbohydrate-functional chitosan nanofibers to capture the influenza virus. Moreover, carbohydrate-enriched graphene sheets [188] and glycosylated stimuli-responsive polyacrylamide microspheres with AgNPs [189] not only selectively encapsulated pathogens but also enabled the killing of captured bacteria.

Kim et al. (2020) [35] proposed hydrogels made of levan produced from *Z. mobilis* and glutaraldehyde poly(vinyl alcohol) (PVA) as influenza virus capture and recovery materials. The approach that levan would promote virus capture by effectively interacting with the lectin, hemagglutinin and nucleoprotein (NP) of the virus was confirmed by RT-PCR and ELISA tests. According to the results, the virus capture efficiency of levan-PVA hydrogels was higher than

commercial cotton swabs due to the lack of the lectin binding function of PVA alone. To test the capture capacity of levan hydrogels in different transmission routes, the authors also produced bioaerosols containing influenza viruses and tested their capture with a filter material containing the hydrogels. RT-PCR analyses showed that levan hydrogels increased the capture of virus droplets compared to the control group. This study showed that levan-based hydrogels could be simple and cost-effective materials for pathogen capture and recovery.

Another interesting application is the use of levan hydrogels in conservation science. Cultural heritages play a major role in ensuring the historical, social, and economic integrity of the society. Even traditional restoration methods applied to protect them from destructive environmental factors such as temperature, light, microorganisms, and erosion can damage the heritage [190]. On the other hand, in recent years, new methods have been developed to overcome the limitations of traditional restoration techniques and materials, and the interest in sustainable, environmentally friendly, and non-toxic systems has increased. Recently, the application of materials science and nanoscience to the preservation of works of art has led to the development of advanced cleaning systems and approaches that are transforming the preservation of cultural heritage [191]. The first suggestion of gels with their ability to provide appropriate protection and cleaning due to the osmotic balance and flexibility they provide [192], followed by the production of more advanced and responsive gels [193], inorganic nanomaterials [194], microemulsions [195], biocomposite films [196], hybrids such as fibroin-nanocellulose composite [197] and chitosan-based coatings [198] are among the materials used in studies carried out to protect cultural heritage today, and it is noteworthy that carbohydrates are frequently used in this sector due to their outstanding properties.

Based on the many important properties of levan-based hydrogels, a study was conducted to investigate the potential use of levan polysaccharides in areas other than health services, such as conservation. Saglam et al. (2023) [41] investigated the potential use of IPN-based enzymatic levan-polyacrylamide hydrogels (EL-PA) in paper protection applications. According to the results of hydrogel characterization in terms of structural, morphological, rheological, and swelling kinetics, EL-PA hydrogels have larger specific surface areas compared to PA-gel, and this is one of the properties sought in surface coating applications [199]. In addition, in the FTIR analysis performed on the paper sample previously coated with hydrogel, as a result of the separation of the hydrogel, only typical bands associated with cellulose were observed, suggesting that the hydrogels successfully removed the contaminants on the paper surface. The results showed that levan-based hydrogels have high potential in conservation science thanks to their biocompatibility and easy applicability [41].

6. Conclusion and Future Perspectives

As the demand for natural, economical, biocompatible, and biodegradable materials continues to rise, levan has emerged as a promising polysaccharide with a wide range of applications in various fields, from medicine to cosmetics. Its properties, such as adhesive strength, self-assembly, low viscosity, and its bioactivity, ranging from prebiotic and anti-cancer to anti-inflammatory and anti-diabetic, distinguished it from other polysaccharides, making it a polymer of significant interest for further research and development [15, 17]. Levan-based hydrogels, with their 3D network structures, have proven to be ideal materials for biomedical applications due to their superior biocompatibility, biodegradability, and nontoxicity. Furthermore, Levan derivatives, such as methacrylated, phosphonated, and oxidized levan,

have shown potential as parent materials for hydrogel production. These hydrogels have already been explored for applications in drug delivery, tissue engineering, cosmetics, and beyond. However, challenges remain, including high production costs of levan and variability in its properties, such as molecular weight, chain length, and branching patterns which depend on the producing organisms and production conditions. These issues must be addressed to optimize levan-based hydrogels for broader industrial applications [45].

In this systematic review, the physicochemical and functional properties of levan polymers as ideal hydrogel raw materials are examined, together with the wide range of usage areas of existing hydrogels from drug delivery to conservation science. In addition, in some sections, an objective perspective is provided by comparing the hydrogel structures that are currently accepted as gold standards with levan-based hydrogels. In light of the studies conducted on levan hydrogels to date and in drug delivery, tissue engineering, cosmetics, and other different fields, it can be expected that the potential usage areas of these hydrogels will increase even more in the future. One of these emerging areas is environmental science. Levan hydrogels possess key features—such as high water absorption capacity, adjustable porosity, biodegradability, and non-toxicity—that make them suitable for environmental uses. These include their application as adsorbents in wastewater treatment and as carriers in controlled-release systems for agricultural chemicals [200]. Moreover, a study by Phengnoi et al. (2019) [201] demonstrated that blending levan with polyvinyl alcohol (PVA), a synthetic polymer with low degradability, led to increased degradation rates with higher levan content, further supporting their potential in eco-friendly materials.

Despite the competitive properties of levan-based hydrogels with other natural polymer hydrogels currently used, unfortunately, this is a relatively new field and there are still not enough studies in the literature, only a handful of patents have been issued. Although most studies have been in the fields focused on drug delivery and tissue engineering, hydrogel studies in these areas are still insufficient.

A recently published article presenting a comprehensive bibliometric analysis of hydrogel research suggested that future applications of hydrogels will predominantly focus on wound healing, drug delivery, cell encapsulation, bioprinting, tissue engineering, electronic devices, and environmental applications. Notably, in the field of drug delivery, the development of responsive sustained-release nanocarriers, intelligent drug delivery systems, and targeted delivery approaches are expected to be significant trends [202]. Also, in their analysis, “wound healing”, “3D printing”, “antibacterial”, “strain”, “adhesive”, “tough”, “strain sensor”, and “inflammation” were identified as hot spots.

Moving forward, levan-based hydrogels need to be developed further to enhance their functionality and applicability. One important avenue of research is the investigation of levan’s interaction with both synthetic and natural materials used in hydrogel production. The properties of composite hydrogels, formed by combining levan with other materials, also warrant further study.

The regulatory framework for introducing hydrogels into practice depends on their intended application—medical, cosmetic, food, or industrial. In medical and healthcare contexts, hydrogels are regulated as medical devices or drug-device combinations by authorities like the FDA in the US and under the EU Medical Device Regulation (MDR). These require classification based on risk, biocompatibility testing (e.g., ISO 10993), sterility validation, clinical evaluation, and compliance with manufacturing standards such as GMP or

ISO 13485 [203]. For cosmetic uses, regulations focus on safety and proper labeling, with notification procedures like the EU's Cosmetic Products Notification Portal (CPNP). In the food sector, hydrogels must be approved as safe additives, such as GRAS substances in the US or approved E numbers in the EU. Industrial applications are generally less tightly regulated but may fall under environmental or chemical safety rules like EPA or REACH, especially if human exposure is possible.

Levan hydrogels are not currently present in major regulatory or clinical databases such as the FDA or European Database on Medical Devices (EUDAMED) and no approved or clinically investigated levan-based hydrogel products have been identified [204]. This clearly shows that they have not yet reached the stage of regulatory recognition or clinical translation despite the broader clinical interest in hydrogel technologies. To advance the clinical and commercial translation of levan-based hydrogels, future research should prioritize comprehensive *in vivo* studies. While preliminary *in vivo* evaluations have demonstrated promising biocompatibility, the current body of evidence remains limited and insufficient to fully assess their behavior under complex physiological conditions. Systematic *in vivo* testing is essential to evaluate not only biocompatibility but also immunogenicity, biodegradability, mechanical stability, and therapeutic efficacy across diverse biomedical applications. Such studies are critical for meeting the safety and efficacy standards set by regulatory agencies such as the FDA and European Medicines Agency (EMA) and represent a necessary milestone before progressing to human clinical trials and market adoption.

Despite the growing interest in levan-based hydrogels, there are only a limited number of MSc and PhD theses dedicated to this topic worldwide, highlighting a significant gap in academic research training that should be addressed through increased graduate-level investigations across institutions globally. In conclusion, while levan hydrogels currently show significant promise in several biomedical applications, their full potential has yet to be realized. Future research will likely expand their use, not just as bioactive delivery systems, but also in more diverse and functional areas, benefiting from their natural, biocompatible, and versatile properties.

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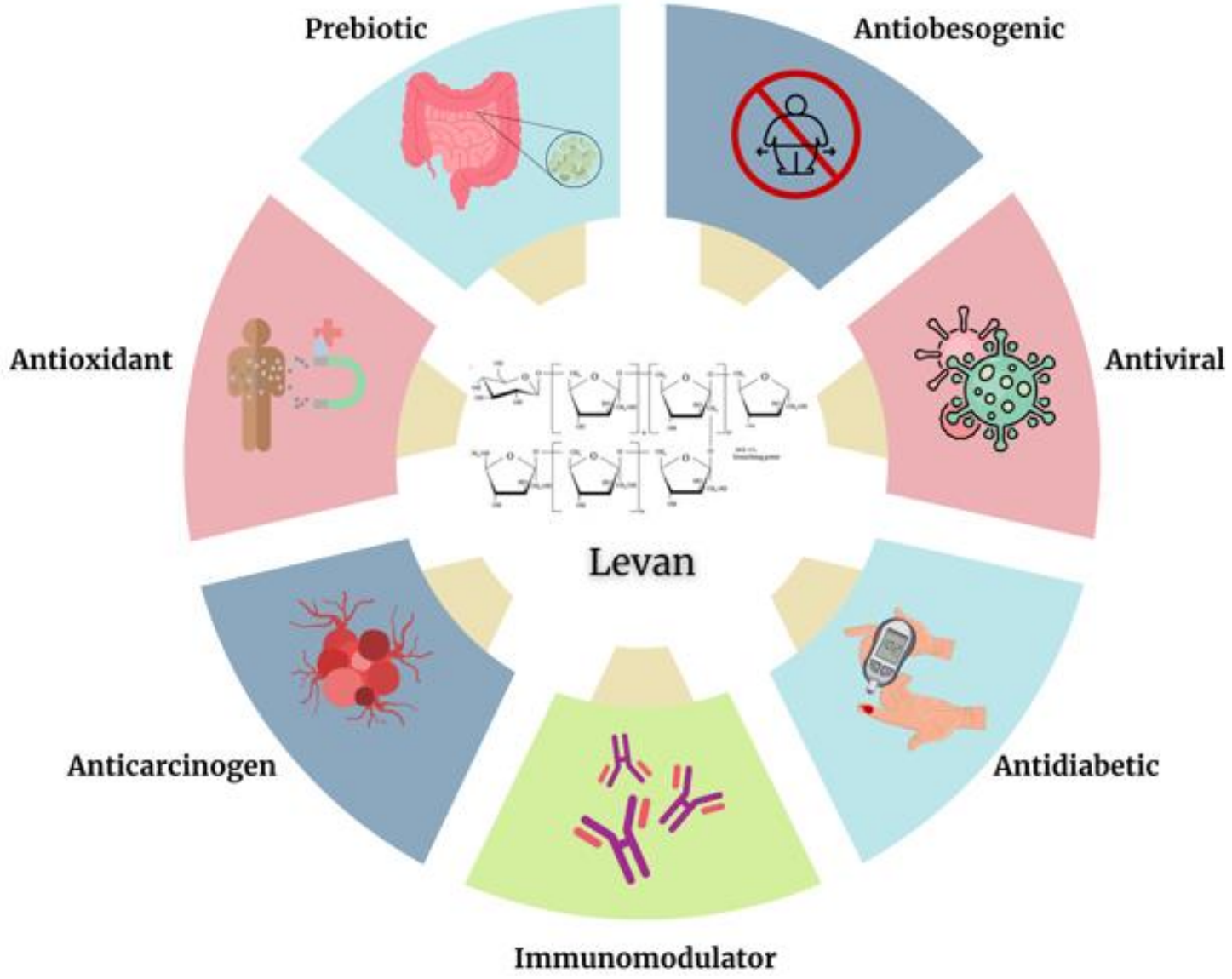

Figure 1. Bioactivities of Levan polymer (Created with Canva.com)

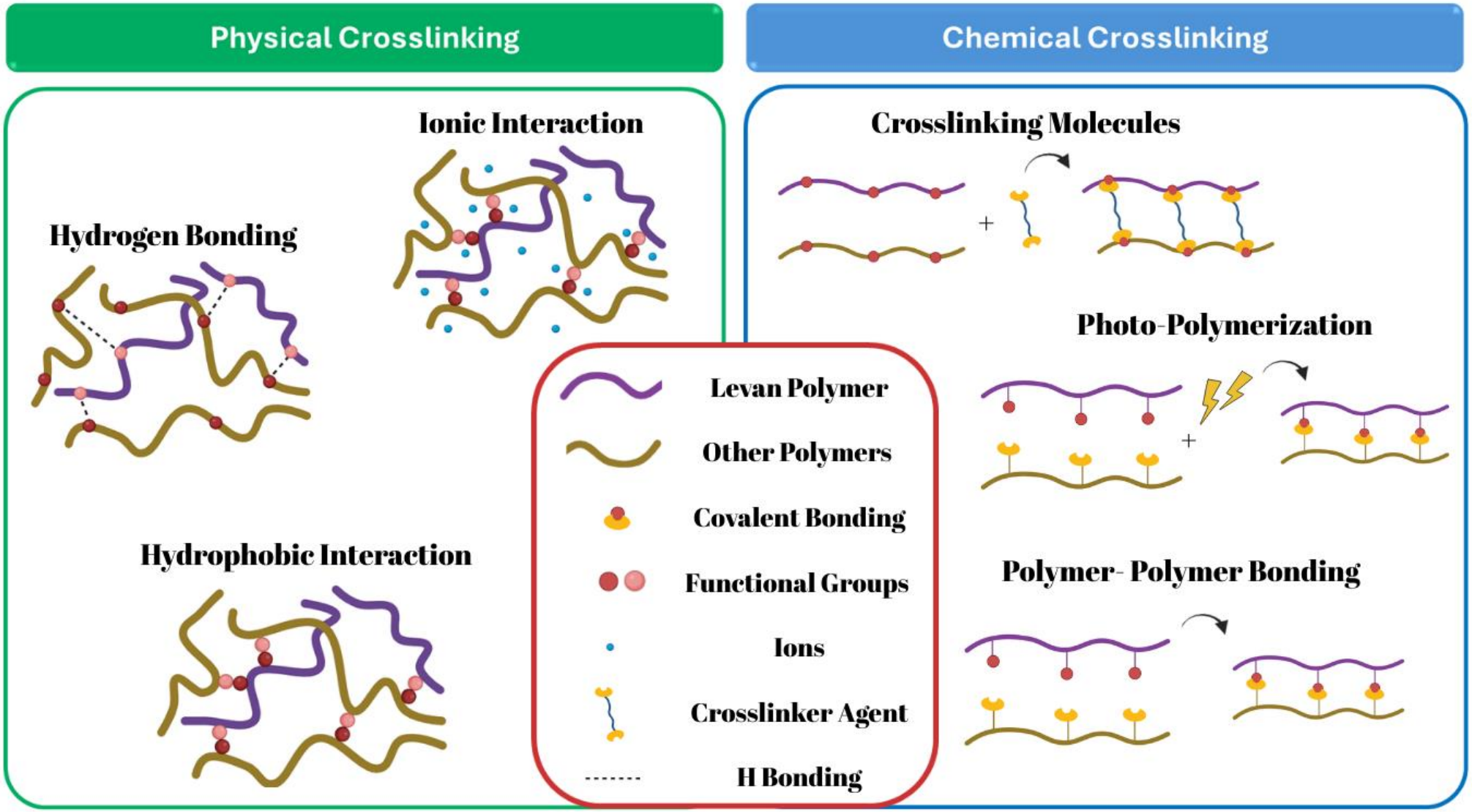

Figure 2. Physically and chemically crosslinked levan-based hydrogels (Created with Canva.com)

Figure 3

Click here to access/download;Figure;Figure 3 revised.pptx

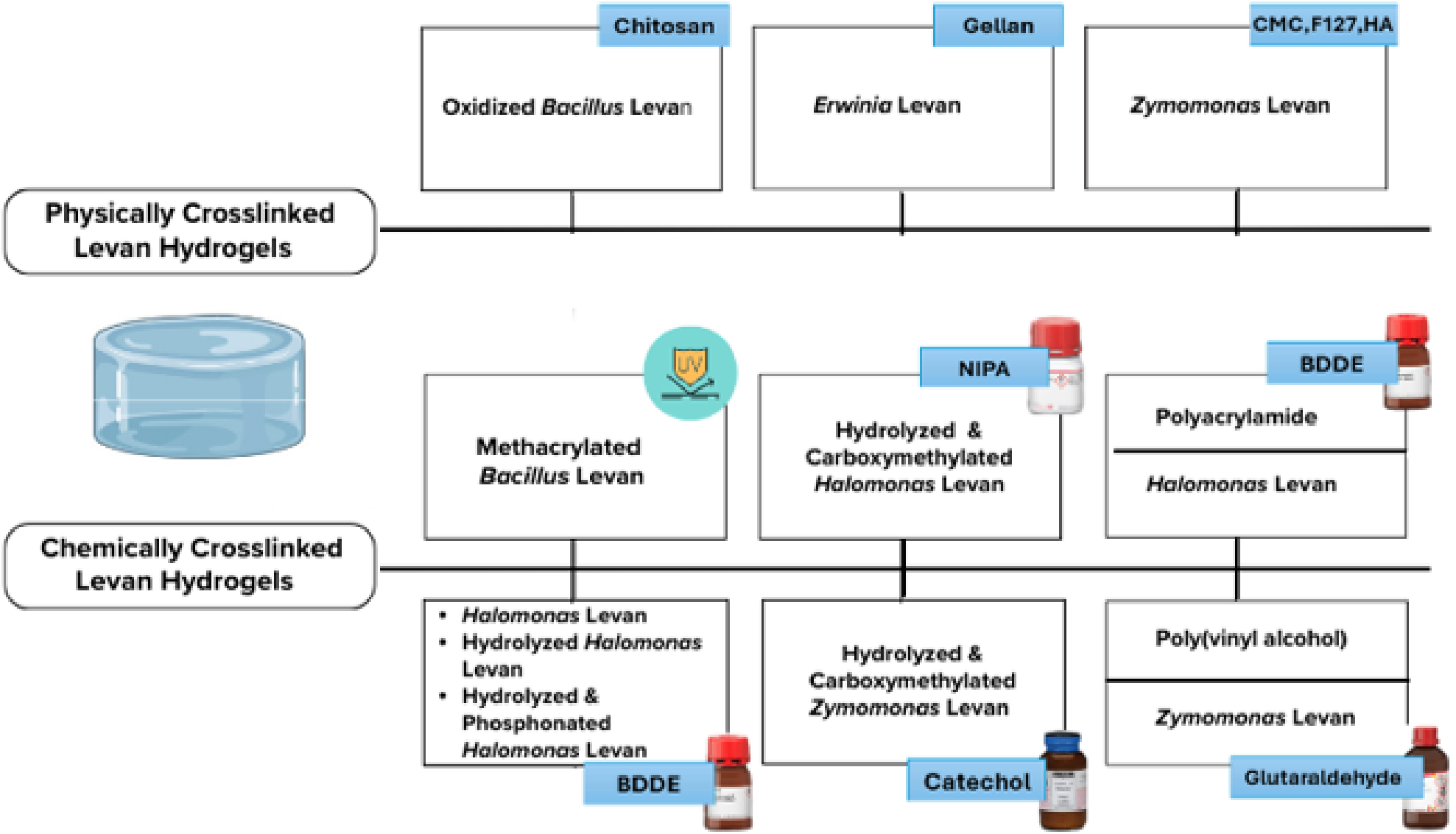

Figure 3. Classification of Levan-based hydrogels (Created with Canva.com)

Table 1. Hydrogels of Levan

Source of Levan	Hydrogel structure	Application	Reference
Bacillus subtilis	Levan was methacrylated and then photo-polymerized	Mechanically stable, slightly degradable and cytocompatible gels suitable as starting materials in common additive manufacturing processes like bioprinting or stereolithography	[34]
	Levan was oxidized with sodium metaperiodate and then mixed with chitosan for the gelation	Bio- and hemo-compatible drug delivery system and wound dressing material with sustained curcumin release	[36]
Erwinia herbicola	Levan/gellan blends crosslinked ionically with NaOH and $CaCl_2$	Shear-thinning properties showed high potential of levan-gellan hydrogel for its use as an injectable material in medical and cosmetic industries	[29]
Halomonas elongata	BDDE crosslinked levan hydrogels loaded with liposomes containing *Plantago lanceolata* L. Extracts	Biocompatible and biodegradable levan-based herbal liposome-loaded hydrogels for acute wound healing	[39]
Halomonas smyrnensis	Hydrolyzed, carboxylated and then methacrylated levan crosslinked with *N*-isopropyl acrylamide (NIPA)	Thermoresponsive levan/pNIPA hydrogels for the controlled release of 5-ASA for the treatment of inflammatory bowel diseases	[32]
	BDDE crosslinked levan hydrogels	Amphotericin B release for exerting antifungal activity against *Candida albicans*	[37]
	Hydrolyzed and phosphonated levan crosslinked with BDDE	Controlled release of resveratrol for skin tissue engineering	[38]
	Levan, acrylamide and methylene-bis-acrylamide solution crosslinked with	IPN-based Levan/PA hydrogels for conservation of cultural heritage	[41]

	BDDE and Ammonium persulfate		
	Hydrolyzed levan cross-linked with BDDE	Levan hydrogels for guided bone regeneration and bone tissue engineering	[42]
Zymomon as mobilis	Pluronic F127/CMC/levan mixture	Injectable hydrogels to be used as an alternative to hyaluronic acid (HA) based dermal fillers in soft tissue augmentation	[27]
	Hydroxyapatite containing Pluronic F127/CMC/levan hydrogels	*In vivo* stability of the composite hydrogel was enhanced with high anti-wrinkle efficacy maintained for 8 weeks and showing high collagen production	[28]
	Levan/PVA crosslinked with glutaldehyde	Virus capture efficiency of levan−PVA hydrogels was higher than that of commercial cotton swabs	[35]
	Catechol conjugated carboxymethylated levan hydrogels via oxidation-induced catechol/quinone (covalent) and Fe^{3+}-(non-covalent) mediated coordinative crosslinking	Levan-catechol conjugate hydrogel for wound healing applications	[33]