



National University of Science and Technology Politehnica Bucharest

Doctoral School "Chemical Engineering and Biotechnology"

Department of Science and Engineering of Oxide Materials and Nanomaterials

PhD Tehsis

PhD Supervisor:

Prof. Dr. Ecaterina Andronescu

PhD Student:

MSc. Eng. Denisa-Maria Radulescu

PhD Committee Members:

S.L.Dr.Ing. Ionela Andreea Neacșu

CSII.Dr.Ing. Bogdan Ștefan Vasile

Prof. Dr. Eng. Adrian-Vasile Surdu

Bucharest 2025





National University of Science and Technology Politehnica Bucharest

Doctoral School "Chemical Engineering and Biotechnology"

Department of Science and Engineering of Oxide Materials and Nanomaterials

Green Metal Oxide Nanoparticles with Silk Fibroin for Soft Tissue Engineering

PhD Supervisor: Prof. Dr. Ecaterina Andronescu

PhD Student: MSc. Eng. Denisa-Maria Radulescu

Bucharest 2025

Table of Contents

Part I. State-of-the-Art Review 5

1. Introduction 5

1.1. Importance of Scaffold Materials 6

1.2. Wound Healing Management 7

2. Green Synthesis Metal Oxide Nanoparticles 9

2.1. Principles of Green Nanotechnology 11

2.2. Plant-Mediated Green Synthesis 12

2.3. Bacteria-Mediated Green Synthesis 14

2.4. Algae-Mediated Green Synthesis 15

2.5. Fungi-Mediated Green Synthesis 16

3. Biomedical Applications of Polymers 17

3.1. Classification of Polymers in Tissue Engineering 18

3.2. Biodegradability and Biocompatibility of Polymers 25

3.3. Role of Polymers in Scaffold Design 26

4. Natural Polymers: Chitosan and Silk Fibroin 26

4.1. Chitosan: Structure, Properties, and Applications 27

4.2. Silk Fibroin: Structure, Properties, and Biomedical Uses 27

4.3. Synergistic Effects of Chitosan-Silk Fibroin Composite Scaffolds 30

5. Design and Fabrication of Nanoparticle-Embedded Scaffolds 31

5.1. Metal Oxide Nanoparticle Integration into Chitosan-Silk Fibroin Scaffolds 32

5.1.1 Zinc Oxide Nanoparticles 33

5.1.3 Magnesium Oxide Nanoparticles 34

5.2. Mechanisms of Wound Healing Enhancement by Nanoparticles 34

5.3. Antibacterial and Anti-inflammatory Properties of Metal Oxide NPs 35

6. Applications in Wound Healing and Tissue Regeneration 37

6.1. Preclinical and Clinical Studies in Soft Tissue Engineering 38

6.2. SF Scaffold Challenges and Solutions 43

Part II. Personal Contribution 44

1. General and Specific Objectives of the Research 44

2. List of Published Articles 45

3. Results of the Research 47

4. Conclusion 145

4.1. General conclusions 145
4.2. Original contributions and perspectives 148
4.3. Future Perspectives and Emerging Directions 150
5. References 152

1. State of the Art - Introduction

Traumatic or pathological soft tissue injuries represent a serious worldwide health concern, requiring novel therapeutic strategies in addition to conventional wound care. In this direction, a possible approach of tissue engineering is represented by scaffolds development. These are biomaterials defined as temporary matrices that imitate the extracellular matrix (ECM) to facilitate tissue regeneration[1, 2]. The body's main barrier of defense is the skin, which is made up of the epidermis, dermis, and subcutaneous layers. Moreover, the intricate wound healing process of the skin includes four interrelated stages: hemostasis, inflammation, proliferation, and remodeling [3-5]. Conventional wound dressings have drawbacks such as limited functionality, poor skin adherence, insufficient healing conditions, and the potential for infection when changed often [6, 7].

By offering crucial structural support and promoting cellular migration, adhesion, and tissue regeneration during all stages of healing, scaffolds help overcome these constraints [8, 9]. Biocompatibility, non-toxicity, antibacterial qualities, suitable mechanical strength, bioactivity, regulated biodegradability, and flexibility are all necessary for the ideal scaffold [10]. Thus, to allow for vascularization, nutrition transport, and cellular penetration while preserving mechanical integrity, the design necessitates meticulous pore size and architecture optimization [11]. Multifunctional biomaterials that resemble the human extracellular matrix (ECM) and offer regulated breakdown and release of bioactive chemicals are the main focus of contemporary scaffold development [12]. In addition, by improving the mechanical, electrical, and biological qualities of biomaterials, the incorporation of nanoparticles (NPs) into scaffolds for tissue engineering has transformed biomedical applications [13]. Compared to conventional NPs synthesis methods, green synthesis techniques have become well-known as economical and ecologically benign substitutes for conventional chemical processes, which frequently use dangerous materials [14]. By using biological resources such as bacteria, fungi, algae, and plant extracts as natural reducing and stabilizing agents, these sustainable techniques enhance biocompatibility while lessening their negative effects on the environment [15].

Compared to microbial approaches, plant-mediated synthesis offers scalable production and quick kinetics, making it the most effective green option [16]. Alkaloids, amino acids, proteins, enzymes, and polysaccharides are examples of phytoconstituents that have dual functions as

capping and reducing agents, preventing agglomeration while also regulating the size and form of nanoparticles [17, 18]. Additionally, the synthesis usually consists of three successive stages: the preparation of the extract, the addition of the metal precursor, and the activation, growth, and stabilization phases that result in the production of nanoparticles [19]. Apart from plant-mediated methods, alternate pathways with distinct benefits are offered by bacterial, algal, and fungal-mediated synthesis. Notably, bacterial synthesis uses a variety of biomolecules, such as proteins, carbohydrates, exopolysaccharides, and functional groups, and provides high yields via both extracellular and intracellular mechanisms [20-22]. Similar to this, algae-based synthesis makes use of high levels of bioactive compounds and the ability to accumulate metals, which allows for continuous production without the need for further fertilizers [23, 24]. Furthermore, fungi-mediated methods take advantage of flexibility, scalability, and adaptation in a variety of environmental settings, resulting in the production of stable, distinct nanoparticles via both extracellular and intracellular pathways [25, 26].

Moving on to biomedical applications, polymers play important roles in biomedical engineering, especially in drug delivery systems, scaffold building, and wound healing. For tissue regeneration applications, their adjustable qualities—such as porosity, biocompatibility, and biodegradability—make them perfect [64, 65]. Polymers are thus categorized according to their synthesis methods (copolymerization, homopolymerization, interpenetrating networks), crosslinking type (physical vs. chemical), and source (natural vs. synthetic) [27, 28]. Alginate, collagen, gelatin, cellulose, hyaluronic acid, and dextran are examples of natural polymers that facilitate cell attachment and differentiation and have inherent biocompatibility and biodegradability [29-43]. By creating moist conditions, absorbing exudates, and permitting regulated drug release, these materials aid in the healing of wounds. Poly(ε-caprolactone), polyvinyl alcohol, poly(lactic acid), and polyvinylpyrrolidone, on the other hand, offer improved mechanical stability and adjustable characteristics, but they may also raise issues with biocompatibility [34, 44-49].

In terms of crosslinking mechanisms, physical crosslinking provides dynamic reactivity to environmental stimuli by utilizing transient molecular interactions via hydrophobic, hydrogen bonding, and ionic crosslinking [50, 51]. In contrast, chemical crosslinking provides better mechanical strength and thermal stability by creating permanent covalent connections through

photo-polymerization, Michael-type addition, and Schiff base production [52]. When it comes to stability, responsiveness, and degrading characteristics, the choice of crosslinking techniques ultimately comes down to the particular application needs.

Among natural polymers, silk fibroin (SF) and chitosan (CS) are two of the most promising for use in wound healing applications. In particular, chitosan, which is produced by deacetylating chitin, has intrinsic antibacterial qualities that are strengthened by protonation in acidic conditions and result in electrostatic interactions with negatively charged bacterial membranes [53, 54]. Its antioxidant, hemostatic, and anti-inflammatory qualities also encourage collagen deposition and fibroblast proliferation while lowering the formation of scars [55-57]. However, composite development is required due to chitosan's mechanical constraints, which include brittleness and low solubility [55]. Silk fibroin, which is derived from Bombyx mori cocoons, complementarily offers remarkable mechanical qualities due to its hierarchical structure, which consists of P25 glycoprotein complexes, heavy chains, and light chains [58, 59]. While α-helices add flexibility, β-sheet crystallites buried in semi-amorphous matrices offer structural stability via hydrogen bonding [60]. Furthermore, SF is perfect for tissue engineering applications because of its ECM-mimetic qualities, environmental resistance, and thermal stability [61-63]. The protein also promotes cell adhesion, migration, and differentiation by means of certain signaling pathways, such as PI3K, JNK, MEK, and NF-κB [64].

Thus, by combining the mechanical strength and stability of silk fibroin with the antibacterial and hemostatic qualities of chitosan, chitosan-silk fibroin composites take advantage of synergistic effects [65]. Consequently, while resolving the limitations of individual components, the resultant scaffolds exhibit better cellular contacts, self-healing capacities, underwater adherence, and malleability [66, 67]. As a result, by offering regulated drug release, moisture retention, and structural support, these composites produce the best conditions for wound healing.

The development of multifunctional wound dressings has evolved with the addition of metal oxide nanoparticles to chitosan-silk fibroin scaffolds, building on these composite foundations. Because of their antibacterial, anti-inflammatory, and tissue-regenerative qualities, zinc oxide (ZnO), copper oxide (CuO), and magnesium oxide (MgO) nanoparticles in particular provide clear benefits [68]. Technically, there are several ways to accomplish integration, including direct blending, template-directed synthesis, and biological inclusion techniques, each of which offers

varying degrees of control over the distribution and characteristics of nanoparticles [69]. When looking at individual components, ZnO nanoparticles show excellent photostability, biocompatibility, and absorption of wide-spectrum radiation, along with low toxicity and environmental safety [70, 71]. Mechanistically, they promote angiogenesis, cell proliferation, ECM remodeling, and granulation tissue creation by zinc ion release [72-74]. Additionally, the antioxidant and anti-inflammatory qualities promote collagen synthesis and tissue regeneration while lowering oxidative stress [75, 76]

Similarly, via generating reactive oxygen species (ROS), inhibiting respiratory enzymes, and disrupting membranes, CuO nanoparticles exhibit potent antibacterial action[77]. Enhanced dermal fibroblast proliferation, higher collagen and elastin production, improved integrin expression, and boosted angiogenesis through copper ion release are among their therapeutic advantages for wound healing [78]. To reduce cytotoxicity issues, however, size, surface chemistry, and concentration must be carefully considered [79]. Furthermore, MgO nanoparticles have outstanding stability and biocompatibility along with broad-spectrum antipathogenic qualities [80]. Mechanistically, their antibacterial activities include membrane rupture and ROS creation, whereas their benefits for wound healing include favorable macrophage phenotypic regulation, VEGF production promotion, and improved angiogenesis through magnesium ion release [81].

The implementation of metal oxide nanoparticle-embedded polymer scaffolds from laboratory to clinical applications has advanced significantly in recent research. In multiple types of experimental models, investigations have demonstrated encouraging outcomes in terms of biocompatibility, wound healing acceleration, and antibacterial activity [82]. There are still a number of obstacles to overcome, though, such as the requirement for uniform characterization procedures, limited clinical scalability, cytotoxicity issues with nanoparticle aggregation, and a lack of long-term safety evidence [83]. However, preclinical research has shown that scaffolds with improved mechanical qualities, controlled disintegration, and improved cellular responses can be successfully used in antimicrobial therapies, diabetic wound healing, and bone regeneration [84]. Clinical application still needs to address manufacturing uniformity, regulatory approval processes, and thorough safety review methodologies in spite of these advancements. Currently, a

major gap that needs to be filled for therapeutic viability is the lack of extensive animal investigations and human clinical trials.

Future studies should concentrate on enhancing the incorporation of bioactive molecules, regulating degradation rates, refining nanoparticle integration techniques, and creating intelligent responsive systems. Notably, innovative polymer composite designs combined with green synthesis techniques provide sustainable avenues for next-generation wound healing therapies. Furthermore, more accurate control over scaffold performance and therapeutic efficiency will be possible with further research into the interactions between inorganic phases and polymer secondary structures. The field's advancement toward customized regenerative medicine solutions ultimately relies on addressing present issues while preserving the biocompatibility and environmental sustainability benefits that make scaffolds embedded with green-synthesized metal oxide nanoparticles attractive options for groundbreaking wound healing applications. To guarantee safe and efficient translation from bench to bedside, interdisciplinary cooperation involving materials scientists, biomedical engineers, doctors, and regulatory specialists will be necessary for success.

Objectives and Originality of the Doctoral Thesis

This PhD thesis' main goal was to combine natural polymers with green-synthesised metal oxide nanoparticles to create multifunctional, biocompatible scaffolds for enhanced wound healing and tissue regeneration. The main goal of this research was to enhance the biological activity and scaffold architecture so that the scaffolds could replicate the natural extracellular matrix (ECM) and provide structural and mechanical support while also promoting vital regenerative processes like angiogenesis, cell proliferation, and antimicrobial defense. By using this method, the thesis aimed to help develop next-generation wound dressing materials that bring together sustainable nanotechnology and regenerative medicine to provide more effective, secure, and individualized chronic wound care solutions.

The study proceeded with a thorough analysis of the scaffold materials already in use in tissue engineering and wound healing in order to accomplish these goals. Significant drawbacks of existing technologies were brought to light by this investigation, including poor mechanical

stability, subpar biodegradability, low antibacterial efficacy, and inadequate moisture balance control. Aware of these drawbacks, natural polymers—particularly chitosan and silk fibroin—were chosen as the basis for the scaffold design. These polymers were selected due to their complementary qualities: silk fibroin offers superior mechanical strength and a positive interaction with cells, while chitosan is well known for its biocompatibility, biodegradability, and antibacterial qualities. When these two polymers have been combined, composite scaffolds that could handle several problems at once were created. The mechanical integrity, swelling capacity, biodegradability, and moisture retention of chitosan–silk fibroin scaffolds—all of which are critical for effective skin regeneration—were thus assessed in the thesis.

The production and incorporation of metal oxide nanoparticles (ZnO, CuO, and MgO) into these polymeric scaffolds was another key goal. The utilization of green chemical techniques to create the nanoparticles was one of the work's unique features. In particular, the hazardous chemicals commonly utilized in the manufacture of conventional nanoparticles were eliminated by using orange peel extract as a natural reducing and capping agent. In addition to supporting waste valorization and the circular economy's tenets, this strategy was economical, environmentally friendly, and biomedically acceptable. It has been demonstrated that the phytochemicals found in orange peel, including flavonoids, phenolics, and alkaloids, efficiently control the nucleation, development, and stability of nanoparticles. Reproducibility and suitability for biomedical applications were confirmed by the synthesis, which yielded nanoparticles with a regulated shape and a size range between 13 and 19 nm.

The thesis's originality is found in both the methodical evaluation and integration of these nanomaterials into functional scaffolds, as well as in the environmentally friendly method of nanoparticle manufacturing. XRD, SEM, TEM, FTIR, and zeta potential studies were all combined in a thorough characterization methodology to evaluate the nanoparticles' physicochemical and biological characteristics. A methodological benchmark for next studies in the area of green nanomaterials for biomedical applications is established by this exacting approach.

To produce composite materials with improved biological performance, the green-synthesized nanoparticles were subsequently added to chitosan–silk fibroin scaffolds. Nanoparticle concentrations ranged from 0.05 to 0.15% w/w, and scaffold compositions were

adjusted using CS:SF ratios of 1:1 and 2:1. Scaffolds with 0.1–0.15% nanoparticles had the best balance of mechanical strength, swelling capacity, antibacterial activity, and biocompatibility, according to this methodical optimization. Crucially, the study demonstrated unambiguous relationships between the distribution of nanoparticles, scaffold microstructure, and biological results. In addition to validating the design approach used in this thesis, these structure–property–function correlations offer design guidelines that can be applied to future scaffold development in regenerative medicine.

The therapeutic potential of the nanocomposite scaffolds was validated by their biological performance. According to *in vitro* assessment, the scaffolds had broad-spectrum antibacterial action and promoted fibroblast adhesion, proliferation, and tissue regeneration. The discovery that the scaffolds worked against both common bacterial strains and strains resistant to antibiotics, such as methicillin-resistant *Staphylococcus aureus* (MRSA), was very noteworthy. The increasing incidence of antibiotic resistance is one of the most urgent issues facing wound care today, and this result directly addresses it. The scaffolds created in this thesis provide a novel treatment approach by offering antimicrobial protection via nanoparticle-mediated processes as opposed to traditional antibiotics.

The proof that adding nanoparticles improved the scaffold's porosity and swelling capacity while preserving its structural integrity represents another unique contribution. In applications involving wound healing, this mix of characteristics is essential since scaffolds need to be able to absorb exudates, retain moisture, permit oxygen permeability, and still be strong enough to offer mechanical protection. When compared to conventional passive wound dressings, the dual enhancement in regenerative and antibacterial capabilities marks a substantial advancement toward active and responsive biomaterials that actively contribute to the healing process. This research is further unique since it integrates sustainability and therapeutic performance thoroughly. The thesis developed a replicable and scalable approach for the development of sustainable biomedical materials by validating the full process chain, from the use of orange peel waste to the creation of effective wound dressings. This accomplishment shows that clinical effectiveness and environmental responsibility do not have to conflict; rather, they can work together to provide cutting-edge healthcare solutions.

Thus, the primary objective of this PhD thesis was to develop multifunctional scaffolds that actively promote wound healing while simulating the extracellular matrix. The uniqueness of this approach is found in the combination of innovative polymeric scaffolds, green nanoparticle synthesis, and methodical material and biological outcome optimization. Collectively, these contributions lay the foundation for the creation of clinically and environmentally sound next-generation wound care products. Thus, in addition to advancing the field of biomaterials, the work opens the door for intelligent responsive dressings, customized wound care solutions, and sustainable biomedical manufacturing processes that tackle the twin issues of environmental sustainability and human health.

General Conclusions

The current study conducted an extensive investigation of wound healing technologies, the focus being on the synergistic design and integration of natural biopolymers and metal oxide NPs for scaffold fabrication. Wound care has experienced significant progress in the past few decades, evolving from passive, inert materials toward improved bioactive platforms capable of actively impacting the healing process. In addition, the combination of tissue engineering, green nanotechnology, and smart biomaterials has enabled the manufacture of multifunctional scaffolds that not only mimic the ECM but also mediate biological responses required for regenerative healing. One of the most important concerns addressed in this study was represented by the inability of traditional wound dressings to meet the multifaceted needs of intricate wound environments, especially for chronic and non-healing ulcers. Thus, it is well known that traditional materials like dry gauze or basic hydrocolloids possess low antibacterial activity, lack dynamic reactivity, and frequently cause additional tissue harm when removed. These constraints underlined the need for more advanced, bioresponsive materials capable of providing structural support, preventing infection, and promoting cellular regeneration in a coordinated manner.

This thesis investigated the development of scaffolds by employing biocompatible polymers—specifically CS and SF—and the addition of metal oxide NPs produced via green methods. Due to their inherent biocompatibility, biodegradability, and specialized biological activities, CS and SF have a high potential for tissue regeneration applications. In this direction, CS provides antibacterial, anti-inflammatory, and hemostatic features, whereas SF provides mechanical strength, elasticity, and structural mimicry of the ECM. Hence, the combination of these polymers

into composite matrices offered an excellent basis for scaffold systems that improve stability, hydration capacity, and cell proliferation support. Furthermore, the introduction of green-synthesized ZnO, CuO, and MgO NPs showed a significant impact on scaffold functionality. Each type of NP provides various therapeutic benefits. For example, ZnO exhibits antioxidant and angiogenic effects, CuO provides a strong antibacterial and collagen-inducing characteristic, and MgO offers anti-inflammatory activity and endothelial cell proliferation. In addition, the green synthesis pathway reduces toxicity and environmental impact while increasing the biocompatibility of the particles. This approach kept the synthesized nanocomposite scaffolds within safe toxicity limits, preserving cellular viability and decreasing potential immunological reactions. One of the most appealing aspects of this work is its interdisciplinary approach. The study combines materials science, nanotechnology, cellular biology, and regenerative medicine to present a biomimetic solution that takes part in the wound healing cascade. The developed scaffolds interact with the wound microenvironment throughout the healing process, including hemostasis, inflammation, proliferation, and remodeling. During the early stages, their structural integrity provides a temporary matrix for clot formation. Moreover, CS should be able to regulate immune function via the inflammatory stage, whereas NPs should reduce bacterial colonization. Further, as proliferation progresses, the porous architecture that contains embedded NPs would enable fibroblast infiltration, angiogenesis, and re-epithelialization. Finally, during the remodeling phase, scaffold biodegradability is adjusted to match ECM synthesis, allowing native tissue to regain control over structural function.

Apart from the mentioned functional performance, the morphology of the scaffolds and their physicochemical features should be designed carefully to address wound-specific difficulties. Thus, the porosity, mechanical strength, degradation rate, and swelling behavior of the scaffold should be carefully tuned to maintain an appropriate balance of mechanical support and biological activity. Controlling the synthesis parameters and crosslinking techniques could improve the load-bearing capability, flexibility of the nanocomposite scaffold, and regulated therapeutic agent release. All the mentioned properties are required for long-term in vivo efficacy. Furthermore, new fabrication techniques like as electrospinning, freeze-drying, and chemical crosslinking enable precise control of scaffold design and composition. These approaches permit scaffolds and hydrogel matrices fabrication, capable of storing and gradually releasing NPs and bioactive substances in response to physiological cues from the wound. Such responsiveness is critical in

dealing with dynamic wound settings, especially in diabetic or infected wounds where the local pH, bacterial load, and inflammation fluctuate considerably over time.

Furthermore, the study also makes a significant contribution by critically evaluating the biocompatibility and cytotoxicity of nanoparticle-enhanced biomaterials. While the scientific community has long recognized the possible hazards posed by metallic and metal oxide NPs in biological systems, the present research has also been focused on green synthesis as a viable alternative to traditional approaches. The synthesized NPs exhibit enhanced dispersability, size control, and reduced aggregation tendencies after being reduced and stabilized with plant-derived phytochemicals and microbial agents. This mechanism not only improves their biological functionality but also minimizes unfavorable cellular responses. The green synthesis approach can provide not only environmental sustainability but also a higher level of integration with bio-based polymer matrices, which is critical for long-term scaffold stability.

From the biological performance point of view, *in vitro* investigations and literature-supported experimental results confirmed the functional significance of the investigated scaffolds. These included improved cell viability, efficient fibroblast and keratinocyte proliferation, and strong antibacterial activity against both Gram-positive and Gram-negative pathogens. The ability of CS-SF scaffolds to absorb exudate, preserve a moist healing environment, and allow for oxygen permeability has been demonstrated to be vital in preserving cell function and facilitating fast tissue reconstruction. Furthermore, the localized release of metal ions from the incorporated NPs resulted in a bioactive medium that could speed the production of granulation tissue, collagen deposition, and angiogenesis. All these processes are necessary for successful skin regeneration. Another essential part of the investigation is represented by the intricacy of wound physiology and how it interacts with scaffold characteristics. Chronic wounds, such as diabetic ulcers and pressure sores, are distinguished by persistent inflammation, bacterial colonization, and poor re-epithelialization. The architecture of the scaffold promptly targeted these pathophysiological barriers by combining antibacterial, antioxidant, and anti-inflammatory features into a single structurally supporting matrix. This comprehensive approach emphasizes the transition in current biomaterials design from passive fillers to active players in the wound microenvironment.

Furthermore, one of the most attractive characteristics of the scaffolds is their ability to respond to tailored therapy. With the capacity to customize NP concentration, scaffold porosity, degradation

rate, and mechanical qualities, the system offers a versatile platform for patient-specific requirements. For example, in individuals with poor vascularization, angiogenesis-promoting NPs like ZnO and MgO may be prioritized. CuO-enhanced scaffolds may provide superior antibacterial efficacy in diseased or high-risk wounds. This tailored scaffold optimization is consistent with the future trajectory of precision healthcare, highlighting the research's translational potential. One of the key recommendations for future research is the introduction of smart or stimulus-responsive parts into scaffold systems. Materials that can respond to changes in pH, temperature, oxidative stress, or enzyme activity allow for on-demand drug release and real-time wound monitoring. Such intelligent systems have the potential to transform wound treatment into an interactive process in which scaffold behavior changes dynamically based on the healing status. Furthermore, integrating wireless biosensors and telemetry systems may enable remote patient monitoring, minimizing hospital visits and boosting treatment adherence.

Furthermore, while *in vitro* data and preclinical models yield encouraging results, clinical translation necessitates extensive long-term safety and efficacy investigations. The biodegradability of scaffolds and the future usage of surplus NPs in vivo environment must be thoroughly tested to avoid systemic toxicity and ensure biosafety. Regulatory systems must also be carefully navigated, especially when it comes to the approval of nanomaterials for human use. As a result, interdisciplinary collaboration among biological researchers, doctors, material scientists, and regulatory agencies will be critical in moving this technology from the laboratory to clinical practice. Another promising area for future research is the utilization of hybrid nanocomposites, which mix inorganic NPs with organic medicinal compounds such as antibiotics, peptides, or growth factors. This dual-functional method could improve scaffold performance by providing both immediate antibacterial activity and long-term tissue regeneration. Furthermore, the development of modular or multilayered scaffolds may enable sequential therapeutic delivery, better replicating the natural wound healing time frame. For example, an initial layer might release antimicrobial agents, followed by angiogenic and regenerative compounds as the lesion heals.

Considering the wider impact of this study, it is clear that the field of regenerative medicine is experiencing a paradigm shift. Biomaterials are now created to take an active role in biological processes rather than only providing structural support. The scaffolds investigated in this thesis are a prime example of this change. By combining the synergistic potential of biopolymers and

nanotechnology, they provide a multifunctional solution that can solve the multifarious issues of wound healing. Furthermore, their development promotes a more sustainable and ecologically responsible biomedical business, which is becoming increasingly relevant in light of global environmental issues.

To summarize, our study highlighted the promise of green-synthesized nanoparticle-embedded biopolymeric scaffolds as a novel and comprehensive method to wound healing and tissue regeneration. The combination of chitosan and silk fibroin provided a strong and bioactive framework, and the addition of metal oxide NPs improved the scaffolds' therapeutic properties. This comprehensive technique successfully addressed the structural, biochemical, and microbiological elements of wound treatment, opening the way for the next generation of bioresponsive, safe, and long-lasting medical materials. The subsequent phases include further research into the interaction dynamics between scaffold components and living tissues, large-scale clinical validation, and going on refining of fabrication procedures. As the area advances, the insights gathered from this thesis will make a significant contribution to the creation of smart, tailored, and environmentally sound solutions for global health concerns in tissue repair and regenerative medicine.

Perspectives and Emerging Directions

A solid basis for the creation of multipurpose, long-term scaffolds for the healing of chronic wounds has been laid by the research described in this thesis. However, a number of encouraging avenues that can further develop the theoretical and practical contributions of this work are revealed, as is the case with any quickly developing scientific subject. The strengthening of interdisciplinary cooperation is one aspect. Advances in wound healing materials demand a comprehensive combination of chemical engineering, biology, nanotechnology, mathematics, and electronic sciences rather than relying exclusively on materials science. In order to ensure the reproducibility and scalability of green synthesis, chemical engineering will remain crucial in understanding the physicochemical interactions between polymers and nanoparticles. At the same time, the creation of scaffolds that may actively interact with cells is made possible by the insights that molecular biology and regenerative medicine provide into the intricate signaling pathways that govern skin restoration.

The transition from lab research to clinical application could be accelerated by using computational modeling and mathematical simulation to provide prediction tools for optimizing scaffold construction, nanoparticle dispersion, and mass transport phenomena in chronic wound settings. Furthermore, by combining bioelectronics and nanotechnology, it may be possible to create intelligent scaffolds with sensors that can track local parameters like pH, oxygen levels, or bacterial activity. This would turn wound dressings from passive barriers into active platforms for diagnosis and treatment.

Optimizing oxygen delivery mechanisms in chronic wound dressings is a second line of research that builds on the existing findings. Green-synthesised ZnO, CuO, and MgO nanoparticles that demonstrated antibacterial and regenerative properties were the main focus of this study. Future research could expand on this line of inquiry by examining biological alternatives like red blood cells, which are already utilized in biomedical contexts for their oxygen-transport function, or by incorporating additional oxygen-releasing agents like MnO and CeO_2 nanoparticles. Multifunctional scaffolds that combine oxygen release with structural and antibacterial properties should greatly enhance healing outcomes. Addressing the hypoxic environment of chronic wounds is still a crucial concern. The improvement of antibacterial techniques is a third perspective. This thesis used ZnO, CuO, and MgO nanoparticles to show broad-spectrum antibacterial efficacy, including against resistant pathogens like MRSA. However, the worldwide increase in antibiotic resistance necessitates ongoing research into novel remedies. By precisely controlling nanoparticle size, shape, and surface chemistry, microfluidic platforms with adjustable flow parameters, precursor compositions, and reducing agents will enhance antimicrobial efficacy while preserving cytocompatibility.

The intricate architectural design of scaffolds presents another opportunity for future advancement. Although chitosan–silk fibroin scaffolds were optimized in this work at 1:1 and 2:1 ratios and nanoparticle concentrations of 0.05–0.15% w/w, multilayered or gradient scaffolds can be developed to make even more progress. With several layers intended for mechanical protection, moisture regulation, angiogenic stimulation, and antimicrobial defense, these would more accurately mimic the hierarchical organization of human skin. The development of patient-specific wound dressings may be made possible by additive manufacturing techniques like 3D or 4D

bioprinting, which could allow for exact control over scaffold porosity, orientation, and functionalization.

Lastly, sustainability will continue to serve as a compass for future approaches. The current thesis has shown how agricultural waste, such as orange peel, can be a useful starting point for the synthesis of nanoparticles, minimizing environmental effects and promoting the ideas of the circular economy. The relationship between therapeutic performance and environmental stewardship will be further strengthened by expanding the use of this strategy to other biomass sources and increasing production levels. From waste biomass to biomedical application, a completely sustainable chain can be used as a template for healthcare technology of the future.

The development of intelligent, multipurpose, and environmentally friendly biomaterials that combine structural support, oxygen release, antimicrobial protection, and diagnostic capabilities is, in summary, the key to the future of wound dressing research. In addition to supporting this vision, the work done for this thesis offers a foundation for further interdisciplinary research. The field is well-positioned to provide wound care solutions that are both environmentally conscious and revolutionary for patient outcomes by embracing sustainability, clinical efficacy, and technology innovation.

List of Published Articles

List of Articles Published:

1. **Radulescu, D.-M**.; Neacsu, I.A.; Grumezescu, A.-M.; Andronescu, E. New Insights of Scaffolds Based on Hydrogels in Tissue Engineering. Polymers 2022, 14, 799. https://doi.org/10.3390/polym14040799.

   Q1, I.F. = 5

2. **Radulescu, D.-M.;** Surdu, V.-A.; Ficai, A.; Ficai, D.; Grumezescu, A.-M.; Andronescu, E. Green Synthesis of Metal and Metal Oxide Nanoparticles: A Review of the Principles and Biomedical Applications. Int. J. Mol. Sci. 2023, 24, 15397. https://doi.org/10.3390/ijms242015397.

   Q2, I.F. = 4.9

3. **Radulescu, D. M**., Andronescu, E., Vasile, O. R., Ficai, A., Vasile, B. S. Silk fibroin-based scaffolds for wound healing applications with metal oxide nanoparticles. Journal of Drug Delivery Science and Technology, 2024, 96, 105689. https://doi.org/10.1016/j.jddst.2024.105689

   Q1, I.F. = 4.9

4. **Radulescu, D.-M**., Neascu I.A., Vasile B.S., Surdu V.-A., Andronescu E. Green Synthesis of Copper, Zinc, and Magnesium Oxide Nanoparticles Using Orange Peel Extract. U.P.B. Sci. Bull., 2025, Series B, Vol. 87, Iss. 3.

   Q4, I.F. = 0.3

5. **Radulescu, D.-M**., Vasile B.S., Surdu V.-A;Trusca R.-D.; Ilie C.-I.; Popescu R.C., Andronescu E.; Drumea V.; Ditu L.-M. Antimicrobial chitosan-silk fibroin scaffolds with green-synthesized metal oxide nanoparticles. Journal of Drug Delivery Science and Technology, 2025, 114, 107463. https://doi.org/10.1016/j.jddst.2025.107463

   Q1, I.F. = 4.9

6. **Radulescu, D.-M.;** Neacsu, I.A.; Vasile, B.S.; Surdu, V.-A.; Oprea, O.-C.; Trusca, R.-D.; Chircov, C.; Popescu, R.C.; Ilie, C.-I.; Ditu, L.-M.; et al. Orange Peel-Mediated Green Synthesis of ZnO and CuO Nanoparticles: Evaluation for Antimicrobial Activity and Biocompatibility in Tissue Engineering. Int. J. Mol. Sci. 2025, 26, 8781. https://doi.org/10.3390/ijms26188781

   Q1, I.F. = 4.9

List of Conference Attendance:

1. **Radulescu Denisa-Maria**, Ecaterina Andronescu. Alexandru-Mihai Grumezescu. Ionela-Andreea Neacsu. Adrian-Ionut Nicoara. "Future Trends of Scaffolds Applied in Tissue Engineering" *Applications Chemistry in Nanosciences and Biomaterials Engineering (Nanobiomat 2021) Summer Edition*.
2. **Radulescu Denisa-Maria**, Ecaterina Andronescu. Alexandru-Mihai Grumezescu. Ionela-Andreea Neacsu. Adrian-Ionut Nicoara. "Advanced Active Molecules for Wound Healing Application" *Applications Chemistry in Nanosciences and Biomaterials Engineering (Nanobiomat 2021) Winter Edition*.
3. **Radulescu Denisa-Maria**, Ecaterina Andronescu. Alexandru-Mihai Grumezescu. Ionela-Andreea Neacsu. Adrian-Ionut Nicoara. "Advances in Green Synthesis of Metal Oxide Nanoparticles used in Tissue Engineering" *Applications Chemistry in Nanosciences and Biomaterials Engineering (Nanobiomat 2022) Summer Edition*.
4. **Radulescu, Denisa-Maria**, Ionela Andreea Neacsu, Alexandra Catalina Barca, Alina Maria Holban Bogdan Vasile, Ecaterina Andronescu. "Green Syntheses of Oxidic Nanoparticles and Medical Applications " Paper presented at Conferinta Nationala de Chimie, Editia XXVI, Calimanesti, Romania, October 4-7, 2022.
5. **Radulescu Denisa-Maria**, Ecaterina Andronescu. Alexandru-Mihai Grumezescu. Ionela-Andreea Neacsu. Adrian-Ionut Nicoara. "Latest Research in Green Synthesis of Metal Oxide Nanoparticles Applied in Tissue Engineering." *Applications Chemistry in Nanosciences and Biomaterials Engineering (Nanobiomat 2022) Winter Edition*.
6. **Radulescu Denisa-Maria**, Ecaterina Andronescu. Alexandru-Mihai Grumezescu. Surdu Adrian Vasile, Adrian-Ionut Nicoara. "Green Synthesis of Metal Oxide Nanoparticles Applied in Soft Tissue Engineering" *Applications Chemistry in Nanosciences and Biomaterials Engineering (Nanobiomat 2023) Summer Edition*.

7. **Radulescu Denisa-Maria**, Neacsu Ionela Andreea, Vasile Bodgan Stefan, Andronescu Ecaterina. "Green Synthesis of Zinc, Magnesium, and Copper Oxide Nanoparticles Using Orange Peel Extract". *Applications Chemistry in Nanosciences and Biomaterials Engineering (Nanobiomat 2024) Winter Edition*.
8. **Radulescu Denisa-Maria**, Vasile Bodgan Stefan, Surdu Vasile-Adrian, Trusca Roxana-Doina, Ilie Cornelia-Ioana, Andronescu Ecaterina Andronescu, Ditu Lia-Mara. *"Sustainable Wound Dressings: Chitosan-Silk Fibroin Scaffolds with Green-Synthesized MgO Nanoparticles for Enhanced Healing and Antibacterial Action" 4th International Conference on Bioengineering and Polymer Science Brasov, Romania,* June 2-5, 2025.

References

[1] Z. Mao, X. Bi, F. Ye, P. Du, X. Shu, L. Sun, J. Guan, X. Li, S. Wu, The relationship between crosslinking structure and silk fibroin scaffold performance for soft tissue engineering, International Journal of Biological Macromolecules 182 (2021) 1268-1277.

[2] A. S, N.R. Nadar, S. Shivakumar, S.S. C, S. P, S. Varalakshmi V, R. Lenka, R. S, Harnessing natural polymers and nanoparticles: Synergistic scaffold design for improved wound healing, Hybrid Advances 8 (2025) 100381.

[3] W. Weng, L. Wang, L. Fan, X. Ding, X. Wang, Ordered micro-nano structured biomaterials for wound healing, Biomedical Technology 8 (2024) 104-114.

[4] C. Ding, X. Liu, S. Zhang, S. Sun, J. Yang, G. Chai, N. Wang, S. Ma, Q. Ding, W. Liu, Multifunctional hydrogel bioscaffolds based on polysaccharide to promote wound healing: A review, International Journal of Biological Macromolecules 259 (2024) 129356.

[5] J.W. Sojobi, O.L. Bankole, D.C. Ulelu, O.S.I. Fayomi, Examination into nanomaterials, nanofibers and nanoparticles advances for wound healing and scaffolds in tissue engineering application: A review, Results in Surfaces and Interfaces 18 (2025) 100380.

[6] P. Wang, F. Cai, Y. Li, X. Yang, R. Feng, H. Lu, X. Bai, J. Han, Emerging trends in the application of hydrogel-based biomaterials for enhanced wound healing: A literature review, International Journal of Biological Macromolecules 261 (2024) 129300.

[7] V. Popescu, V. Cauni, M.S. Petrutescu, M.M. Rustin, R. Bocai, C.R. Turculet, H. Doran, T. Patrascu, A.M. Lazar, D. Cretoiu, V.N. Varlas, B. Mastalier, Chronic Wound Management: From Gauze to Homologous Cellular Matrix, Biomedicines, 2023.

[8] A.G. Tabriz, D. Douroumis, J. Boateng, 3D printed scaffolds for wound healing and tissue regeneration, Therapeutic Dressings and Wound Healing Applications (2020) 385-398.

[9] S.M. Naghib, S.N. Hosseini, A. Beigi, 3D 4D printing of chitosan-based scaffolds for wound dressing applications, Carbohydrate Polymer Technologies and Applications 8 (2024) 100594.

[10] X. Yang, W. Mo, Y. Shi, X. Fang, Y. Xu, X. He, Y. Xu, Multifunctional bilayer scaffold with controlled drug release and kinetics for wound healing applications, Journal of Drug Delivery Science and Technology (2025) 106619.

[11] D. Gao, W.D. Shipman, Y. Sun, J.Z. Glahn, L. Beraki, H.C. Hsia, Macroporous scaffolds based on biomass polymers and their applications in wound healing, Journal of Bioresources and Bioproducts 10(1) (2025) 14-31.

[12] X. Ding, Y. Yu, Y. Zu, Self-healing hydrogels based on the Knoevenagel condensation reaction for wound healing, Biomedical Technology 2 (2023) 70-76.
[13] Y. Huang, X. Guo, Y. Wu, X. Chen, L. Feng, N. Xie, G. Shen, Nanotechnology's frontier in combatting infectious and inflammatory diseases: prevention and treatment, Signal Transduction and Targeted Therapy 9(1) (2024) 34.
[14] N. Monica Ahmad, A. Husaini Mohamed, N.A. Hasan, N. Zainal- Abidin, M. Zaini Nawahwi, A. Mohamad Azzeme, Effect of optimisation variable and the role of plant extract in the synthesis of nanoparticles using plant-mediated synthesis approaches, Inorganic Chemistry Communications 161 (2024) 111839.
[15] C. Pechyen, B. Tangnorawich, S. Toommee, R. Marks, Y. Parcharoen, Green synthesis of metal nanoparticles, characterization, and biosensing applications, Sensors International 5 (2024) 100287.
[16] E. Takele Assefa, G. Shumi, K. Mohammed Gendo, G. Kenasa, N. Roba, Review on green synthesis, characterization, and antibacterial activity of CuO nanoparticles using biomolecules of plant extract, Results in Chemistry 8 (2024) 101606.
[17] N. Bahari, N. Hashim, K. Abdan, A. Md Akim, B. Maringgal, L. Al-Shdifat, Role of Honey as a Bifunctional Reducing and Capping/Stabilizing Agent: Application for Silver and Zinc Oxide Nanoparticles, Nanomaterials 13(7) (2023) 1244.
[18] D.R. Jaishi, I. Ojha, G. Bhattarai, R. Baraili, I. Pathak, D.R. Ojha, D.K. Shrestha, K.R. Sharma, Plant-mediated synthesis of zinc oxide (ZnO) nanoparticles using Alnus nepalensis D. Don for biological applications, Heliyon 10(20) (2024) e39255.
[19] C.d.M. Strieder, D.L.P. Macuvele, C. Soares, N. Padoin, H.G. Riella, Plant-mediated green synthesis of cerium oxide nanoparticles: A critical perspective of some unclear issues, Journal of Materials Research and Technology 30 (2024) 6376-6388.
[20] R. Saberi Riseh, M. Gholizadeh Vazvani, Green synthesis of metal nanoparticles using plant growth promoting rhizobacteria and application in agriculture, Plant Nano Biology 10 (2024) 100111.
[21] P. Singh Jassal, D. Kaur, R. Prasad, J. Singh, Green synthesis of titanium dioxide nanoparticles: Development and applications, Journal of Agriculture and Food Research 10 (2022) 100361.
[22] A. Singh, P.K. Gautam, A. Verma, V. Singh, P.M. Shivapriya, S. Shivalkar, A.K. Sahoo, S.K. Samanta, Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review, Biotechnology Reports 25 (2020) e00427.
[23] Annu, M. Sahu, S. Singh, S. Prajapati, D.K. Verma, D.K. Shin, From green chemistry to biomedicine: the sustainable symphony of cobalt oxide nanoparticles, RSC Advances 14(45) (2024) 32733-32758.
[24] A. Mukherjee, D. Sarkar, S. Sasmal, A Review of Green Synthesis of Metal Nanoparticles Using Algae, Frontiers in Microbiology 12 (2021).
[25] E.A. Adebayo, M.A. Azeez, M.B. Alao, A.M. Oke, D.A. Aina, Fungi as veritable tool in current advances in nanobiotechnology, Heliyon 7(11) (2021).
[26] M. Rezghi Rami, M. Meskini, B. Ebadi Sharafabad, Fungal-mediated nanoparticles for industrial applications: synthesis and mechanism of action, Journal of Infection and Public Health 17(10) (2024) 102536.

[27] E.T.J. Chong, J.W. Ng, P.-C. Lee, Classification and Medical Applications of Biomaterials–A Mini Review, BIO Integration 4(2) (2023).
[28] A. Berradi, F. Aziz, M.E. Achaby, N. Ouazzani, L. Mandi, A Comprehensive Review of Polysaccharide-Based Hydrogels as Promising Biomaterials, Polymers 15(13) (2023) 2908.
[29] A. Aldhaher, F. Shahabipour, A. Shaito, S. Al-Assaf, A.A.M. Elnour, E.B. Sallam, S. Teimourtash, A.A. Elfadil, 3D hydrogel/ bioactive glass scaffolds in bone tissue engineering: Status and future opportunities, Heliyon 9(7) (2023) e17050.
[30] A. Barbu, B. Neamtu, M. Zăhan, G.M. Iancu, C. Bacila, V. Mireșan, Current Trends in Advanced Alginate-Based Wound Dressings for Chronic Wounds, Journal of Personalized Medicine 11(9) (2021) 890.
[31] C. Oliveira, D. Sousa, J.A. Teixeira, P. Ferreira-Santos, C.M. Botelho, Polymeric biomaterials for wound healing, Frontiers in Bioengineering and Biotechnology 11 (2023).
[32] L. Suamte, A. Tirkey, P.J. Babu, Design of 3D smart scaffolds using natural, synthetic and hybrid derived polymers for skin regenerative applications, Smart Materials in Medicine 4 (2023) 243-256.
[33] R. Álvarez-Chimal, J.Á. Arenas-Alatorre, M.A. Álvarez-Pérez, Nanoparticle-polymer composite scaffolds for bone tissue engineering. A review, European Polymer Journal 213 (2024) 113093.
[34] M. Chelu, A.M. Musuc, Advanced Biomedical Applications of Multifunctional Natural and Synthetic Biomaterials, Processes 11(9) (2023) 2696.
[35] R. Andreazza, A. Morales, S. Pieniz, J. Labidi, Gelatin-Based Hydrogels: Potential Biomaterials for Remediation, Polymers 15(4) (2023) 1026.
[36] F. Zheng, X. Yang, J. Li, Z. Tian, B. Xiao, S. Yi, L. Duan, Coordination with zirconium: A facile approach to improve the mechanical properties and thermostability of gelatin hydrogel, International Journal of Biological Macromolecules 205 (2022) 595-603.
[37] S. Iravani, R.S. Varma, Cellulose-Based Composites as Scaffolds for Tissue Engineering: Recent Advances, Molecules 27(24) (2022) 8830.
[38] A.P.C. Almeida, J.N. Saraiva, G. Cavaco, R.P. Portela, C.R. Leal, R.G. Sobral, P.L. Almeida, Crosslinked bacterial cellulose hydrogels for biomedical applications, European Polymer Journal 177 (2022) 111438.
[39] H.N. Abdelhamid, A.P. Mathew, Cellulose-Based Nanomaterials Advance Biomedicine: A Review, International Journal of Molecular Sciences 23(10) (2022) 5405.
[40] M. Jiang, H. Fang, H. Tian, Latest advancements and trends in biomedical polymers for disease prevention, diagnosis, treatment, and clinical application, Journal of Controlled Release 380 (2025) 138-174.
[41] J. Arnold, J. Chapman, M. Arnold, C.Z. Dinu, Hyaluronic Acid Allows Enzyme Immobilization for Applications in Biomedicine, Biosensors 12(1) (2022) 28.
[42] S. Wei, P. Xu, Z. Yao, X. Cui, X. Lei, L. Li, Y. Dong, W. Zhu, R. Guo, B. Cheng, A composite hydrogel with co-delivery of antimicrobial peptides and platelet-rich plasma to enhance healing of infected wounds in diabetes, Acta Biomaterialia 124 (2021) 205-218.
[43] C. Liu, S. Yue, R. Li, L. Wang, K. Zhang, S. Wang, S. Yu, F. Shafiq, Y. Liu, W. Qiao, Synthesis of a series of dextran-based DA-AHA hydrogels for wound healing dressings, European Polymer Journal 221 (2024) 113521.

[44] S. Prete, M. Dattilo, F. Patitucci, G. Pezzi, O.I. Parisi, F. Puoci, Natural and Synthetic Polymeric Biomaterials for Application in Wound Management, Journal of Functional Biomaterials 14(9) (2023) 455.
[45] C. Mukherjee, D. Varghese, J.S. Krishna, T. Boominathan, R. Rakeshkumar, S. Dineshkumar, C.V.S. Brahmananda Rao, A. Sivaramakrishna, Recent advances in biodegradable polymers – Properties, applications and future prospects, European Polymer Journal 192 (2023) 112068.
[46] M.S. Singhvi, S.S. Zinjarde, D.V. Gokhale, Polylactic acid: synthesis and biomedical applications, Journal of Applied Microbiology 127(6) (2019) 1612-1626.
[47] E.E. Doğan, P. Tokcan, M.E. Diken, B. Yilmaz, B.K. Kizilduman, P. Sabaz, Synthesis, Characterization and Some Biological Properties of PVA/PVP/PN Hydrogel Nanocomposites: Antibacterial and Biocompatibility, Advances in Materials Science 19(3) (2019) 32-45.
[48] S.M.H. Gillani, A. Mughal, R.A.A. Khan, M.H. Nawaz, Z. Razzaq, M.S. Ismat, R. Hussain, A. Wadood, S. Ahmed, B. Minhas, M. Abbas, T. Vayalpurayil, M.A.U. Rehman, Development of hybrid polyvinylpyrrolidone/carboxymethyl cellulose/collagen incorporated oregano scaffolds via direct ink write printing for potential wound healing applications, International Journal of Biological Macromolecules 278 (2024) 134528.
[49] M.I. Khan, P. Paul, S.K. Behera, B. Jena, S.K. Tripathy, C. Stålsby Lundborg, A. Mishra, To decipher the antibacterial mechanism and promotion of wound healing activity by hydrogels embedded with biogenic Ag@ZnO core-shell nanocomposites, Chemical Engineering Journal 417 (2021) 128025.
[50] Y. Liu, J. Wang, H. Chen, D. Cheng, Environmentally friendly hydrogel: A review of classification, preparation and application in agriculture, Science of The Total Environment 846 (2022) 157303.
[51] X. Huang, Y. Zheng, J. Ming, X. Ning, S. Bai, Natural polymer-based bioadhesives as hemostatic platforms for wound healing, International Journal of Biological Macromolecules 256 (2024) 128275.
[52] P. Zhang, J. Qi, R. Zhang, Y. Zhao, J. Yan, Y. Gong, X. Liu, B. Zhang, X. Wu, X. Wu, C. Zhang, B. Zhao, B. Li, Recent advances in composite hydrogels: synthesis, classification, and application in the treatment of bone defects, Biomaterials Science 12(2) (2024) 308-329.
[53] R.M. Ibrahim, E.A. Kamoun, N.M. Badawi, S.H. El-Moslamy, M. kh, S.A. Salim, Cutting-edge biomaterials for advanced biomedical uses: self-gelation of l-arginine-loaded chitosan/PVA/vanillin hydrogel for accelerating topical wound healing and skin regeneration††Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4ra04430d, RSC Advances 14(42) (2024) 31126-31142.
[54] X. Zhang, Y. Liang, S. Huang, B. Guo, Chitosan-based self-healing hydrogel dressing for wound healing, Advances in Colloid and Interface Science 332 (2024) 103267.
[55] X. Lin, X. Zhang, Y. Wang, W. Chen, Z. Zhu, S. Wang, Hydrogels and hydrogel-based drug delivery systems for promoting refractory wound healing: Applications and prospects, International Journal of Biological Macromolecules 285 (2025) 138098.
[56] B. Anna, D. Solaiman, S. Alexey, D. Sali, Pharmacological and biological effects of chitosan, Research Journal of Pharmacy and Technology 13(2) (2020).

[57] P. Feng, Y. Luo, C. Ke, H. Qiu, W. Wang, Y. Zhu, R. Hou, L. Xu, S. Wu, Chitosan-Based Functional Materials for Skin Wound Repair: Mechanisms and Applications, Frontiers in Bioengineering and Biotechnology 9 (2021).
[58] Z. Yan, T. Zhang, Y. Wang, S. Xiao, J. Gao, Extracellular vesicle biopotentiated hydrogels for diabetic wound healing: The art of living nanomaterials combined with soft scaffolds, Materials Today Bio 23 (2023) 100810.
[59] S.-L. Wang, X.-W. Li, W. Xu, Q.-Y. Yu, S.-M. Fang, Advances of regenerated and functionalized silk biomaterials and application in skin wound healing, International Journal of Biological Macromolecules 254 (2024) 128024.
[60] Y. Song, C. Hu, Z. Wang, L. Wang, Silk-based wearable devices for health monitoring and medical treatment, iScience 27(5) (2024) 109604.
[61] R. Harishchandra Yadav, M. Kenchegowda, M. Angolkar, M. T S, R. Ali M. Osmani, S. Palaksha, H. Veerabhadrappa Gangadharappa, A review of silk fibroin-based drug delivery systems and their applications, European Polymer Journal 216 (2024) 113286.
[62] S. Bogadi, R. Malayandi, P. Vasanth Raj, A. Suresh Kumar, M. Parvathaneni, M. Kumar Kundu, M. Rabiul Islam, F.S. Khan, P. Tagde, T. Kumar Mondal, A. Alexiou, M. Papadakis, V. Venkata Satyanarayana Reddy Karri, Silk fibroin and sericin: Multifunctional formulations for treating diabetic wound healing, European Polymer Journal 220 (2024) 113465.
[63] A.P. Madappura, S. Madduri, A comprehensive review of silk-fibroin hydrogels for cell and drug delivery applications in tissue engineering and regenerative medicine, Computational and Structural Biotechnology Journal 21 (2023) 4868-4886.
[64] T. Zhang, R. Zhang, Y. Zhang, P.R. Kannan, Y. Li, Y. Lv, R. Zhao, X. Kong, Silk-based biomaterials for tissue engineering, Advances in Colloid and Interface Science 338 (2025) 103413.
[65] C. You, C. Wang, Z. Ma, Q. Yu, S. Liu, Review on application of silk fibroin hydrogels in the management of wound healing, International Journal of Biological Macromolecules 298 (2025) 140082.
[66] L. Zhang, W. Zhang, Y. Hu, Y. Fei, H. Liu, Z. Huang, C. Wang, D. Ruan, B.C. Heng, W. Chen, W. Shen, Systematic Review of Silk Scaffolds in Musculoskeletal Tissue Engineering Applications in the Recent Decade, ACS Biomaterials Science & Engineering 7(3) (2021) 817-840.
[67] G. Li, S. Sun, Silk Fibroin-Based Biomaterials for Tissue Engineering Applications, Molecules 27(9) (2022) 2757.
[68] X.Y. Xu, T.H.M. Tran, H. Perumalsamy, D. Sanjeevram, Y.-J. Kim, Biosynthetic gold nanoparticles of Hibiscus syriacus L. callus potentiates anti-inflammation efficacy via an autophagy-dependent mechanism, Materials Science and Engineering: C 124 (2021) 112035.
[69] Z. Xu, L. Shi, M. Yang, L. Zhu, Preparation and biomedical applications of silk fibroin-nanoparticles composites with enhanced properties - A review, Materials Science and Engineering: C 95 (2019) 302-311.
[70] R. Nayal, D. Mejjo, M.Y. Abajy, Anti inflammatory properties and safety of green synthesized metal and metal oxide nanoparticles: A review article, European Journal of Medicinal Chemistry Reports 11 (2024) 100169.

[71] A.C. Paiva-Santos, A.M. Herdade, C. Guerra, D. Peixoto, M. Pereira-Silva, M. Zeinali, F. Mascarenhas-Melo, A. Paranhos, F. Veiga, Plant-mediated green synthesis of metal-based nanoparticles for dermopharmaceutical and cosmetic applications, International Journal of Pharmaceutics 597 (2021) 120311.
[72] M. Huston, M. DeBella, M. DiBella, A. Gupta, Green Synthesis of Nanomaterials, Nanomaterials 11(8) (2021) 2130.
[73] E.R. Ghomi, M. Shakiba, A.S. Ardahaei, M.A. Kenari, M. Faraji, S. Ataei, P. Kohansal, I. Jafari, M. Abdouss, S. Ramakrishna, Innovations in Drug Delivery for Chronic Wound Healing, Curr Pharm Des 28(5) (2022) 340-351.
[74] C.T. Anuradha, R. Krishna Sharma, Nanobiotechnology driven wound care solutions: A critical review of bio-synthesized nanoparticles' applications, Results in Surfaces and Interfaces 18 (2025) 100369.
[75] S.N. Nandhini, N. Sisubalan, A. Vijayan, C. Karthikeyan, M. Gnanaraj, D.A.M. Gideon, T. Jebastin, K. Varaprasad, R. Sadiku, Recent advances in green synthesized nanoparticles for bactericidal and wound healing applications, Heliyon 9(2) (2023) e13128.
[76] O. Castaño, S. Pérez-Amodio, C. Navarro-Requena, M.Á. Mateos-Timoneda, E. Engel, Instructive microenvironments in skin wound healing: Biomaterials as signal releasing platforms, Advanced Drug Delivery Reviews 129 (2018) 95-117.
[77] K. Velsankar, A.K. R.M, P. R, M. V, S. Sudhahar, Green synthesis of CuO nanoparticles via Allium sativum extract and its characterizations on antimicrobial, antioxidant, antilarvicidal activities, Journal of Environmental Chemical Engineering 8(5) (2020) 104123.
[78] T. Munusamy, S. Rajeshkumar, Chapter 4 - Green synthesis of nanoparticles for wound healing, in: P. Kesharwani (Ed.), Recent Advances in Nanomedicines Mediated Wound Healing, Academic Press2025, pp. 47-68.
[79] L. Lyu, W. Cao, G. Yu, D. Yan, K. Deng, C. Lu, C. Hu, Enhanced polarization of electron-poor/rich micro-centers over nZVCu-Cu(II)-rGO for pollutant removal with H2O2, Journal of Hazardous Materials 383 (2020) 121182.
[80] H. Bhoi, S. Tiwari, G. Lal, K.K. Jani, S.K. Modi, P. Seal, V. Saharan, K.B. Modi, J.P. Borah, K. Punia, S. Kumar, Green synthesis and characterization of Mg0.93Na0.07O nanoparticles for antimicrobial activity, cytotoxicity and magnetic hyperthermia, Ceramics International 48(19, Part A) (2022) 28355-28373.
[81] M. Liu, W. Zhang, Z. Chen, Y. Ding, B. Sun, H. Wang, X. Mo, J. Wu, Mechanisms of magnesium oxide-incorporated electrospun membrane modulating inflammation and accelerating wound healing, Journal of Biomedical Materials Research Part A 111(1) (2023) 132-151.
[82] M.I.H. Mondal, T. Chowdhury, M.H. Islam, M. Nuruzzaman, Development of nano ZnO loaded chitosan-sericin nano-biocomposite- a rapid homeostasis and effective wound healing agent, Materials Today Chemistry 42 (2024) 102446.
[83] M. Ramezani Farani, M. Farsadrooh, I. Zare, A. Gholami, O. Akhavan, Green Synthesis of Magnesium Oxide Nanoparticles and Nanocomposites for Photocatalytic Antimicrobial, Antibiofilm and Antifungal Applications, Catalysts 13(4) (2023) 642.
[84] Z. Guo, L. Yan, B. Zhou, P. Zhao, W. Wang, S. Dong, B. Cheng, J. Yang, X. Wang, B. Li, In situ photo-crosslinking silk fibroin based hydrogel accelerates diabetic wound healing

through antibacterial and antioxidant, International Journal of Biological Macromolecules 242 (2023) 125028.