SYNTHESIS AND CHARACTERIZATION OF KERATIN BASED HYDROGEL LOADED WITH SILVER NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

Project report submitted in fulfillment of the

requirement for the degree of

Master of Science

in

Biotechnology

By

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Under the supervision of

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CANDIDATE'S DECLARATION

I hereby declare that the work presented in this report entitled "**SYNTHESIS AND CHARACTERIZATION OF KERATIN BASED HYDROGEL LOADED WITH SILVER NANOPARTICLES FOR BIOMEDICAL APPLICATIONS**" in fulfilment of the requirements for the award of the degree of Master of Science in Biotechnology submitted in the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over a period from July 2022 to May 2023 under the supervision of **Dr. Ashok Kumar Nadda,** Assistant Professor, Department of Biotechnology and Bioinformatics.

I also authenticate that I have carried out the above-mentioned project work under the proficiency stream biotechnology.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

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This is to certify that the above statement made by the candidate is true to the best of my knowledge.

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SUPERVISOR's CERTIFICATE

This is to certify that the work presented in the project report titled "**SYNTHESIS AND CHARACTERIZATION OF KERATIN BASED HYDROGEL LOADED WITH SILVER NANOPARTICLES FOR BIOMEDICAL APPLICATIONS**" in partial fulfilment of the requirement for the award of degree of **Masters of Science** in **Biotechnology** submitted to the department of Biotechnology and Bioinformatics, Jaypee University of Information Technology Waknaghat. Is an authentic record of work carried out during the period of August 2023 to May 2024 under the supervision of **Dr. Ashok Kumar Nadda**, Assistant Professor, Department of Biotechnology and Bioinformatics.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
ECM	Extra Cellular Matrix
XRD	X-ray Diffraction
FTIR	Fourier Transform Infra-Red Spectroscopy
Ag	Silver
AgNP	Silver nanoparticles
NP	Nanoparticles
Ch-g-PVA	Chitosan-
PVA	Poly Vinyl Alcohol
Ag@Ker	Silver Keratin Scaffold
G’	elastic modulus
FT	Freeze Thaw cycle
g	Gram
G”	Viscous modulus
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ABTS	2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
Ag-K	Silver- Keratin Hydrogel

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Abstract

This study is focused on synthesis of a high value product from poultry biomass used in biomedical applications. The product formed is a 3-D polymerised, hydrophilic, biocompatible, biodegradable, antibacterial hydrogel loaded with green synthesized silver nanoparticles from *Tridax procumbens*. Use of chicken feathers lowers the environmental pollution. Keratin, an in-soluble, fibrous, hydrophobic, biodegradable protein found abundantly in the chicken feathers that is around 90%. The Hydrogel were mixed with various solutions like PVP, PVA which provide a strong and robust structure to the hydrogel by acting as an aqua sponge and an absorptive material for discharge from wounds. Further these hydrogels were then loaded with AgNP which were synthesised from *Tridax procumbens,* The AgNP provide a better wound heling effect as, they act as antimicrobial and prevent the growth of microbes in the area where the hydrogel have been placed, enhancing the wound healing effects of the Hydrogel.

Keywords: *Biomaterial, Hydrogel, Tridax procumbens, Silver, nanoparticles, wound healing, antimicrobial.*

CHAPTER-1

INTRODUCTION

Keratin biomaterials, which are found in large quantities in hair, fleece, hooves, nails, quills, and horns, have recently emerged as a significant force in tissue engineering and regenerative medicine. Referring to a collection of proteins rich in cysteines that form filaments, keratin has a wide range of roots in the human genome. It makes up the majority of intermediate fiber properties and forms the two largest homology bunches: keratin types I and II[1]. As research delves deeper into their potential, keratin-based biomaterials—including hydrogel dressings—are being examined for their critical role in wound healing, emphasizing their inherent functionality, fundamental support, amazing biocompatibility, and advantageous biodegradability features[1].

Beyond keratin, further developments in fabric sciences have led to the invention of hydrogel wound dressings enhanced by silver nanoparticles. In addition to meeting the essential criteria—such as maintaining a moist environment and being biocompatible and biodegradable—this advancement seeks to provide an ideal wound dressing arrangement that also combines restorative operators to promote tissue remodeling and reduce resistant responses[1]. A new age in wound care has begun with the combination of silver nanoparticles and keratin-based hydrogel, which not only makes dressings more affordable but also ready to deliver improved healing results[1].

• Hydrogel Foundation for Biomedicine

Hydrogels have been a cornerstone in the biomedical sector for more than 50 years; they were initially introduced with the development of crosslinked hydroxyethyl methacrylate (HEMA) hydrogels [2]. These materials are known for their unique three-dimensional viscoelastic systems that swell with water and sustain particles and cells. This feature makes them quite reasonable for a variety of biological applications [2].

1.1 Hydrogels: Properties and Categorization

The distinctive feature of hydrogels is their polymeric matrixes, which expand in water but do not disintegrate, because to their strong thermodynamic affinity for the

soluble [2]. Hydrogels may be further catagorized into two main groups based on their source:

1.1.1 Natural hydrogels: they contain hyaluronic acid, collagen, fibrin, and derivatives of common substances such alginate and chitosan [2].

1.1.2. Synthetic Hydrogels: Cases include poly(acryl amide) and poly(ethylene glycol) diacrylate. Despite the fact that their final structure may change depending on the circumstances of polymerization, they are usually preferred because of their repeatability [2].

By modifying the concentration, structure, and utility of the used monomer or cross-linker, hydrogels' fundamental and mechanical properties may be tailored to the needs of the user [2].

1.2 Headways and Applications in Biomedicine

Hydrogels' biocompatibility and tissue-like properties have led to their widespread use in a variety of biomedical applications [2]. These a Cell-based treatments and therapies, Burn dressings that are created and wound healing Bone and cartilage healing.

1.2.1 Frameworks for drug delivery

Hydrogels are also essentially different in other fields including controlled medication discharge, bio-separations, and electrophoresis, demonstrating their versatility beyond coordinated biological applications biomedical applications [3].

1.2.2 Developments and Prospects

The exponential growth of logical distributions on the topic, which highlights the growing curiosity among the logical community, is indicative of the ongoing research and innovation in hydrogels [2]. Their use in sophisticated restorative applications has expanded due to advancements; these include immunotherapy, plastic surgery, and even water treatment, where they are used to regulate or decontaminate pollutants [3].

Additionally, the potential of hydrogels to detect natural hazards like heavy metal particles and to maintain and retain tall liquids in applications like surge control devices has been studied [3].

Due to their ability to mimic the extracellular lattice, their permeability, and their high-water content—all of which are essential for tissue design—hydrogel research in medicine is continuing to advance [4] [5]. Hydrogels' incorporation with other biomaterials and technologies is expected to help improve their suitability and viability in biomedicine and other fields as research advances.

1.3 The Properties of Hydrogel and Wound Healing

Due in large part to their ability to maintain the moist wound environment—which is essential for successful healing—hydrogels have become essential materials in the field of wound care [6]. Their ability to absorb wound exudates and conform to the wound bed enhances their practicality in restorative settings [6]. Hydrogels may also be engineered to include bioactive substances like antibacterial experts and development components, which are essential for accelerating the healing process [6].

1.3.1 Phases of the Healing Process

The process of wound healing may consist of four distinct yet related stages, arranged in a complicated grouping:

enlargement, remodeling, aggravation, and hemostasis [6]. To ensure successful recovery, each step is essential and must to occur in the precise order listed. By providing the essential elements and the ideal setting for each organization, hydrogels regress these steps [6].

1.3.2 Difficulties with Persistent Injuries

Severe wounds affect millions of people worldwide and slow down during the fiery stage before healing [6]. Factors include prolonged hyper-inflammation and macrophage disruption are fundamental in the development of these wounds [6]. Furthermore, chronic wounds often get contaminated with microorganisms, forming biofilms that promote healing by disrupting cellular migration and increasing inflammation [6].

1.3.3 Hydrogels that Replicate Extracellular Network Structure

Because hydrogels may mimic the ECM, a fundamental element of tissue healing, they are particularly effective [6]. The three fundamental processes for tissue integration and recovery that they promote are cellular attachment, tissue jetty, and

cellular signaling [6]. Specific hydrogels, such as those derived from alginate, collagen, and hyaluronic acid, are especially useful for replicating extracellular matrix functions [6].

1.3.4 Utilization in Burn Injury Management

Hydrogels improve the healing process in the context of burn injuries by providing cooling effects, pain relief, and infection prevention [6]. By balancing the skin's natural flexible flexibility and suppleness, their ability to mimic the physical characteristics of an intact dermis also contributes to the reduction of scarring [6].

1.3.5 Wide Selection of Hydrogel Adhesives

From ordinary polymers like chitosan and gelatin to customized choices like polyethylene glycol and polyvinyl liquor, the variations in hydrogel features are enormous [6]. This variety enables the production of bandages specifically tailored to specific types of wounds and healing phases [6]. Prominent commercial products include DermaSyn®, Neoheal® Hydrogel, and Aquacel® Ag, which have demonstrated widespread effectiveness in managing chronic wounds [6] [7].

1.3.6 Innovation in Hydrogel Technology Advances

Subsequent advancements in hydrogel innovation have focused on enhancing their characteristics to better satisfy the needs of continuous wound care [7]. Improvements include the development of hydrogels with enhanced re-epithelialization capabilities, antibacterial effects, and pH-responsive sedate discharge [7]. Hydrogels are a viable option because of their multifunctional qualities as well as their innate biocompatibility and biodegradability. for future biomedical applications [7].

1.4 Keratin's Role in Biomedical Hydrogels

It is well acknowledged that keratin, a naturally occurring biomaterial, moves naturally and is compatible with human tissues. It is an ideal part of biological applications like tissue construction, wound healing, and sedate conveyance frameworks since it creates self-assembled structures important for cellular recognition and activity [8]. Due to its cysteine-rich composition, which allows for physical crosslinking when exposed to oxygen and confers quality and flexibility that

is advantageous for hydrogel systems, keratin possesses the inherent potential to form these complex structures [8].

1.4.1 Keratin based hydrogel for Tissue Design

Keratin based hydrogel have shown significant promise in tissue construction, particularly in the healing of nerves. Researchers have demonstrated the neuroinductive properties of these hydrogels, which promote healing in mouse models of peripheral nerve injury. In order to elude traditional tactile nerve autografts, the hydrogels promote this by acting as a transient network that obstructs axon healing and advances beneficial recuperation [9].

1.4.2 Interaction of Keratin Hydrogels with Cells

There is a substantial interaction between keratin hydrogels and cellular devices. The keratins in human hair facilitate the migration of Schwann cells, which are essential for nerve function. By initiating cellular multiplication and movement and upregulating the production of characteristics essential for neuronal workouts, these keratins help the repair and healing of damaged nerves. [9].

1.5 Keratin Sources' Financial and Environmental Benefits

Keratin is derived from abundant resources like human hair and chicken feathers, which are often seen as waste in the poultry industry. This not only provides an environmentally beneficial way to dispose of waste, but it also makes using keratin to manufacture hydrogels more affordable. Particularly feather keratins have shown promise as a source of therapeutically important biomaterials, capable of polymerizing in aqueous environments to create robust hydrogel structures [10].

1.6 Keratin Hydrogels: Fundamental and Practical Properties

Keratin based hydrogel are distinguished by their excellent mechanical qualities, hydrophobic characteristics, and high molecular weight. These characteristics make them suitable for various biological applications where stability and hardness are needed. Additionally, these hydrogels' crystallinity files range from 30 to 50%, showing a strong structure that can bolster various biomedical capacities [10].

Particularly useful is keratin's unique ability to self-assemble into porous, sinewy structures. This characteristic enables the production of hydrogels that, rather than

essentially restoring cellular connections, promote cell growth, which is essential for tissue healing and repair [9]. Additionally, keratin materials derived from specific protein divisions have high concentrations of sulfide bunches, which improves their capacity to absorb moisture. This is crucial in applications like wound healing, where controlling exudates is crucial [10].

In summary, keratin-based hydrogels provide a versatile platform for biomedical applications by utilizing their shared source, inherent natural activities, and capacity for self-assembly to provide solutions that are successful and long-lasting.

1.7 Keratin based hydrogel: A Renewable Structure for Lattice Sustainability Elements

1.7.1 Source and Fabricating: The keratin source and the manufacturing techniques used to create keratin-based hydrogels have a significant impact on the hydrogels' sustainability. These constituents determine not only the inherent appearance of the hydrogels but also their suitability for medicinal purposes [4].

1.7.2. Recycling Potential: It is essential to look at the possible results for recycling or reusing keratin-based hydrogels after their first therapeutic use. This strategy might reduce waste and improve the biomaterials' overall acceptability for use in healthcare settings [4].

1.7.3. Environmental and Moral Considerations: It is essential for analysts to evaluate the natural impact and the ethical recommendations of using Keratin based hydrogel. To ensure maintainable honing, factors such the keratin's origin, especially if it comes from animals, and the generating forms' environmental friendliness need to be properly taken into account [4].

1.8 Properties and Procedures for Expository

X-ray diffraction (XRD) and Fourier change infrared spectroscopy (FTIR) examination:

These theoretical techniques are applied to consider the auxiliary characteristics of Keratin based hydrogel. The functional groups and holding patterns may be distinguished using FTIR analysis, and the crystallinity record, which varies from 30 to 50%, can be somewhat understood by XRD examination [10].

Tests for swelling and dissolvability are essential for determining how much solid material is present in the hydrogel and how effectively it can absorb water. These qualities are essential for restorative applications where controlling moisture, such as bandages for wounds, is crucial [10].

Table1: Properties of Keratin-Based Hydrogels Analyzed

Property	**Analytical Technique**	**Importance in Medical Application**
Functional Groups and Bonds	Fourier Transform Infra-Red Spectroscopy (FTIR)	Determines chemical stability and reactivity
Crystallinity Index	X-ray Diffraction (XRD)	Affects mechanical strength and degradation rate
Water Absorbance Capacity	Swelling and Solubility Tests	Essential for moisture management in wound care

This detailed analysis and consideration of supportability factors highlights the promise of keratin-based hydrogels as a workable and environmentally responsible substitute in the field of biomedicine, Keratin based hydrogel:

1.9 Healing of Wounds

Because they can hold on to exudates and provide a moist healing environment, keratin-based hydrogels are extremely feasible for use in wound care. For successful wound-mending forms to advance, this attribute is essential [10]. These hydrogels provide improved wound care by regulating the surrounding environment and acting as a conduit for biomolecules that support the healing process [10]. Additionally, the presence of keratinocytes in these hydrogels influences the preparation for healing by causing them to migrate away from the wound edge, so contributing significantly to tissue regeneration and reducing scarring [10].

1.9.1 Updated Definitions for the Adequacy of Made Progress

In order to enhance the wound-healing capabilities of keratin-based hydrogels, ingredients such as honey, chitosan, and aloe vera are added. Aloe vera gel is well-

known for its genuine application in the treatment of burns and skin wounds, promoting beneficial effects [10]. In addition to being biocompatible, chitosan also adds its non-toxic and non-allergic qualities. Additionally, biodegradability, which attracts users as a component for medicinal uses [10]. Because of its antimicrobial, anti-inflammatory, and antioxidant properties, nectar is used extensively in wound care [10].

1.9.2 Tissue design and drug delivery

Keratin-based hydrogels provide a controlled release framework in the drug delivery domain that enhances medication bioavailability. Ensuring that medications are correctly delivered to the intended spot requires the use of this application [8]. These hydrogels provide a foundation for tissue construction that supports the growth of underutilized tissues, like bone or cartilage, which is essential for the advancement of beneficial tissue development [8].

1.9.3 Properties Neuroinductive

Keratin based hydrogel have demonstrated neuroinductive characteristics, which makes them useful in models of damage to peripheral nerves. These hydrogels provide an advantage over traditional palpable nerve autografts by acting as a transient lattice that interrupts axon repair and improves effective recuperation [8]. The relationship with Schwann cells is particularly noteworthy since keratin hydrogels cause these cells to proliferate and migrate while upregulating essential characteristics for neural functions [8].

1.9.4 Applications for Hemostasis

Keratin-based hydrogels synthesised from keratin of human hair have demonstrated adequate hemostatic performance in crisis therapy circumstances. As demonstrated in a rabbit exhibition, this treatment is fundamental to limiting death in situations of severe liver damage [8]

Table 2: Key Applications of Keratin-Based Hydrogels in Biomedicine

Application Area	Functionality	Key Components	Benefits
Wound Healing	Moisture management, delivery of biomolecules	Keratinocytes, Aloe vera, Chitosan, Honey	Promotes tissue regeneration, reduces scarring
Drug Delivery	Controlled release systems	Keratin-based matrix	Enhances drug bioavailability
Tissue Engineering	Scaffold for tissue growth	Keratin-based scaffold	Supports development of functional tissues
Neuro regeneration	Provisional matrix for nerve regeneration	Neuroinductive properties	Facilitates nerve repair and functional recovery
Hemostatic Agent	Control bleeding in emergency medical conditions	Hemostatic properties	Effective in severe trauma scenarios

This comprehensive diagram underscores the flexibility and viability of keratin-based hydrogels over different biomedical applications, highlighting their potential as an economical and compelling arrangement in advanced medication.

1.9.5 Biomedical Applications of Silver Nanoparticle-loaded Hydrogels

Silver nanoparticles (AgNPs) are eminent for their powerful antibacterial properties, which have been saddled for centuries [5]. These nanoparticles are especially esteemed within the biomedical field for their wide range of applications, counting antimicrobial gel details and different restorative gadgets such as catheters and inserts [5]. Their integration into hydrogel dressings speaks to a noteworthy headway in wound care, advertising upgraded restorative results through their one of a kind physical, chemical, and natural characteristic [5].

1.10 Antibacterial and Antimicrobial Components

AgNPs apply their antimicrobial impacts by entering microbial cells, disturbing vital cellular components such as mitochondria and ribosomes, and interferometer with imperative biomolecules like proteins and DNA [5]. This infiltration comes about in cellular poisonous quality and oxidative stretch, fundamentally through the era of receptive oxygen species (ROS) and free radicals [5]. The adequacy of AgNPs against a wide extend of pathogens, counting microscopic organisms, infections, and organisms, is especially famous for its potential to combat drug-resistant strains [5] [11].

1.10.1 Bio composite Materials for Wound Mending

The improvement of bio composite materials that consolidate AgNPs is driven by the require for productive antibacterial movement in wound mending applications [5]. These materials, counting hydrogel frameworks, are planned to bolster the recuperating handle in both burn and wound care by leveraging the tall surface zone to volume proportion of AgNPs, which improves their microbial interaction [5] [11]. Strikingly, AgNP-loaded hydrogels can discharge nanoparticles that are retained by nearby cells, possibly affecting recuperating forms at the cellular level [5].

1.10.2 Characterization and Union Strategies

The characterization of AgNPs is basic to guaranteeing their adequacy and security in restorative applications. Methods such as UV-Vis spectroscopy, FT-IR, SEM, TEM, and XRD are utilized to examine the properties of nanoparticles, giving basic information on their measure, shape, and chemical composition [11]. Also, green amalgamation strategies utilizing common sources like plant extricates offer an eco-friendly approach to creating AgNPs, minimizing natural affect whereas keeping up restorative adequacy [11].

1.10.3 Progressed Hydrogel Definitions

Later considers have investigated the joining of AgNPs into particular hydrogel definitions, such as Polyacrylamide and Ch-g-PVA hydrogels. These ponders address the optimization of nanoparticle measure and discharge energy to progress the helpful results in wound recuperating [11] [12]. For occurrence, AgNPs synthesized utilizing green tea leaf extricate and stacked onto Ch-g-PVA hydrogels have illustrated great antibacterial action against common pathogens like E. coli and S. aureus [12].

1.10.4 In Vivo and In Vitro Adequacy

The adequacy of AgNP-loaded hydrogels has been tried in different models to affirm their potential in clinical settings. While a few thinks about have appeared promising comes about in quickening wound mending and improving the expression of recuperating markers in rodent models, point by point comes about of these in vivo thinks about give a more profound understanding of the components at play [13]. So also, in vitro tests utilizing fibroblast cells have been conducted to assess the wound mending capabilities of these hydrogels, in spite of the fact that particular results from these thinks about were not point by point [12].

Table 3: Key Properties and Applications of AgNP-Loaded Hydrogels

Property	Application	Impact on Healing
Antibacterial Activity	Wound Dressings	Reduces infection risk
High Surface Area to Volume Ratio	Enhanced Cellular Interaction	Improves efficacy in wound management
ROS Generation	Antimicrobial Mechanism	Aids in rapid microbial clearance
Eco-friendly Synthesis	Sustainable Production Methods	Reduces environmental impact
Characterization Techniques	Ensuring Safety and Efficacy	Validates application in medical use

These experiences into the biomedical applications of silver nanoparticle-loaded hydrogels highlight their critical potential in upgrading wound recuperating, advertising a promising road for progressed helpful techniques in present day pharmaceutical.

1.10.5 Biomedical Applications of Keratin-Based Hydrogel with AgNP

AgNPs have a long history of utilize in wound mending due to their antimicrobial, anti-inflammatory, and antioxidative properties, making them exceedingly successful against a wide range of pathogens, counting gram-negative and gram-positive microscopic organisms, parasites, and infections [14]. In modern restorative hone,

silver-based dressings are broadly utilized, and progressing inquire about proceeds to investigate inventive combinations of these nanoparticles with other materials [14].

1.11 Antibacterial Viability and Recuperating Improvement

Keratin-based wound dressings that consolidate silver nanoparticles (Ker/Ag) illustrate predominant pliable quality and a slower debasement rate compared to choices where silver nanoparticles are simply joined to the surface of keratin platforms (Ag@Ker) [16]. This auxiliary judgment is vital for supported bolster in wound care. Both sorts of frameworks display surprising antibacterial properties with an ideal concentration of 10 μg mL−1 of AgNPs, successfully lessening the estimate of contaminations and quickening the mending handle [16].

1.11.1 Controlled Silver Discharge

One of the key preferences of Ker/Ag platforms is their capacity to preserve a straight silver discharging proportion amid the beginning 5-7 days post-application. This controlled discharge is advantageous for maintaining antibacterial properties over time whereas maintaining a strategic distance from the biotoxicity that can happen with concentrated bursts of silver [16]. Moreover, the moderate discharge of silver from these frameworks has been appeared to quicken cell multiplication, as demonstrated by cytotoxicity tests [16]

1.11.2 Provocative Reaction and Epithelialization

Utilizing Ker/Ag platforms in irresistible creature models and through histological thinks about has illustrated their viability in hindering incendiary reactions and quickening epithelialization. This contributes essentially to their potential as prevalent wound repair materials [16].

Table 4: Comparative Properties of Keratin-Based Hydrogels with and without Silver

Property	Without Ag Scaffolds	With Ag Scaffolds
Tensile Strength	Higher	Lower
Degradation Rate	Slower	Faster
Antibacterial Property	Excellent with 10 μg mL−1 AgNPs addition	Excellent with 10 μg mL−1 AgNPs addition
Silver Releasing Ratio	Linear during the initial 5-7 days, beneficial for wound care	Less controlled, potential for biotoxicity
Cell Proliferation	Accelerated by slow silver release	Standard
Inflammatory Response	Reduced inflammation, faster healing	Standard inflammatory response
Epithelialization	Enhanced epithelialization	Standard epithelialization

The integration of silver nanoparticles inside keratin-based hydrogels not as it were upgrading their inalienable properties but too presents unused functionalities that are basic for progressed wound care. This combination leverages the antimicrobial adequacy of silver with the biocompatibility and auxiliary benefits of keratin, displaying a compelling arrangement for present day therapeutic applications in wound administration.

Chapter- 2

REVIEW OF LITERATURE

Keratin, a wide category of insoluble proteins, plays an essential part in shaping the structure of cytoplasmic epithelia and epidermal members. It is divided in to two primary sorts:

difficult and delicate keratins, each serving unmistakable capacities inside organic frameworks [17]. This stringy basic protein is fundamentally to different natural structures such as hair, nails, horns, feet, fleece, plumes, and the epithelial cells within the furthest layers of the skin [18].

2.1 Sorts of Keratins

Keratin proteins are subdivided based on their auxiliary structure into alpha-keratins and beta-keratins. Alpha-keratins, which are fundamentally stringy and helical, are found in well evolved creatures. In differentiate, beta-keratins, comprising of parallel sheets of polypeptide chains, are show in fowls and reptiles [18]. This qualification is pivotal for understanding the different mechanical properties and functionalities of keratin over diverse species.

2.2 Chemical Composition and Properties

The amino corrosive composition of keratin changes depending on its area and work inside a life form. A noteworthy component of keratin is cysteine, an amino corrosive that contributes to its Vigor through disulfide bonds connecting the protein chains. These covalent bonds give keratin with momentous solidness and resistance, basic for the defensive parts it plays in natural substances [18][21]. Keratin's insolubility in water and resistance to proteolytic proteins assist underline its solidness, with the length of keratin filaments affected by their water substance [18].

2.2.1 Keratinocytes:

The Makers of Keratin

Keratin is synthesized by specialized cells known as keratinocytes, overwhelmingly found within the skin, hair, and nails [19]. These cells are vital for the nonstop

recovery and repair of harmed epithelial tissues, guaranteeing basic astuteness and security against natural challenges.

2.3 Keratin as a Biopolymer

Recognized for its biocompatibility and biodegradability, keratin is utilized in different biomedical applications. As a common biopolymer, it is sourced from creature parts like hair, nails, plumes, horns, and claws, making it an economical choice for creating biomaterials [20]. The extraction of keratin has advanced altogether, especially from sources like hair and fleece, encouraging the creation of progressed keratin-based biomaterials stages [17].

Keratin's complex structure, comprising different proteins counting keratins and keratin-associated proteins, makes it a captivating subject for logical investigate. Its tall cysteine substance and the resultant cross-linked disulfide bonds are especially outstanding for their commitment to its mechanical quality and strength [21]. This interesting combination of properties makes keratin an important component within the field of biomaterials science, especially in applications requiring solidness and biocompatibility.

Amalgamation of Keratin-Based Hydrogel

Beginning Materials and Arrangement

2.4 Keratin Extraction:

The union handle starts with the extraction of keratin protein, essentially sourced from chicken plumes [22].

2.4.2 Arrangement of Hair:

In a few strategies, hair is utilized, preferably washed and unbleached, to preserve the keenness of the keratin [24].

• Union Strategies

Cyclic Freezing/Thawing Procedure:

This strategy was utilized to form two sorts of hydrogels, KS-50 and K-50. The method includes cyclic changes in temperature to help within the arrangement of the hydrogel structure [22].

Photo crosslinking Strategy:

A more progressed approach includes the utilize of photo-cross linkable keratin-PEG hydrogels. These are arranged by crosslinking decreased human hair keratin with a 4 arm polyethylene glycol linker, utilizing Eosin Y as a photo initiator [23]. The crosslinking response utilizes the free thiol bunches on keratins and the norbornene utilitarian bunches on the PEG linker [23].

Straightforward Freeze-Thaw Cycle:

Connected within the blend of keratin-based hydrogels from rabbit hair, this strategy is famous for its effortlessness and effectiveness [25].

Nitty gritty Union Handle

1. Oxidation:

Hair is oxidized utilizing peracetic corrosive or another appropriate reagent to break down the structure and uncover keratin [24].

2. Recuperation and Drying:

Post oxidation, the hair is recouped, flushed, and dried [24].

3. Pulverization:

The dried hair is at that point pulverized and ground into a fine powder to increase the surface zone for way better chemical responses [24].

4. Suspension and Solubilization:

The keratin powder is suspended in ammonium thioglycollate and warmed to solubilize the solvent division of the hair [24].

5. Centrifugation:

The mixture is at that point centrifuged to evacuate the insoluble division, guaranteeing a purer keratin extricate [24].

6. Dialysis and Concentration:

The supernatant gotten is dialyzed to filter it encourage and after that concentrated [24].

7. Crosslinking:

At long last, an oxidizing specialist is included to crosslink the keratin proteins, shaping a hydrogel [24].

2.5 Characterization Strategies

SEM and Fourier Transfer Infrared Spectroscopy (FTIR):

These strategies were utilized to see at the characteristics of the synthesized hydrogels, giving encounters into their fundamental and chemical properties [22].

• Improvements in Photo crosslinking

Microfabrication Strategies:

The photo crosslinking instrument licenses for advanced strategies such as micropatterning and moist turning. These techniques engage the creation of cell-laden tissue creates with moved models, making strides the congruity of keratin-based hydrogels in biomedical zones [23].

This nitty coarse graph of the amalgamation techniques underscores the adaptability and flexibility of keratin-based hydrogels, highlighting their potential totally different biomedical applications.

2.5.1 Swelling Conduct and Porosity

• Swelling Conduct of Keratin-Based Hydrogels

Swelling conduct in hydrogels may be a critical characteristic that impacts their convenience and application. The swelling extent, a quantifiable list, is utilized to depict this conduct interior hydrogels, illustrating how much water a hydrogel can hold relative to its dry weight [27]. For keratin-based hydrogels, a number of factors influence this swelling conduct:

2.6 Prepolymer Definitions and Crosslinking Conditions:

Changes inside the prepolymer mixes and the conditions in the midst of the crosslinking handle can through and through alter the swelling characteristics of keratin-based hydrogels. Frequently, another thickness of crosslinks interior the hydrogel structure comes around in diminished swelling capabilities [23].

2.Environmental Impacts:

Components such as pH levels, ionic quality, dissolvable composition, temperature, and presentation to light play crucial parts in choosing the swelling conduct. These common conditions can either update or curb the hydrogel's capacity to swell [28].

3.Material Composition:

The specific beauty care products of the hydrogel as well oversees its swelling capacity. For event, hydrogels with lower crosslink thickness or those with hydrophobic components tend to appear different swelling practices [29].

2.7 Porosity and Its effect on Hydrogel Properties

Porosity interior hydrogels is another essential calculate that impacts not because it was swelling conduct but too their mechanical quality and dispersal characteristics. Exceedingly penetrable hydrogels are by and huge related with higher swelling extents and progressed mass transport capabilities, which are valuable for distinctive biomedical applications [27]. The porosity of keratin- based hydrogels can be influenced by:

1.Crosslinking Thickness:

Comparative to its effect on swelling, the thickness of crosslinks inside the hydrogel impacts its porosity. The next crosslinking thickness commonly comes around in a less porous structure, which can affect the hydrogel's by and huge value [23].

2.Photocurrent Impacts:

In events where photocurrent is included, especially with negative values due to electrolyte buildups, the porosity can be adjusted. These buildups, when revealed to light, release electrons that travel to the cathode, altering the assistant porosity of the hydrogels [28].

2.8 Manufacturing Shapes and Their Impacts

The methodologies utilized inside the manufacturing of keratin-based hydrogels additionally play a critical portion in characterizing their swelling and porosity characteristics:

1.Lyophilization and Rehydration:

These shapes are particularly essential in shaping the estimations and assistant insight of 3D printed keratin hydrogels. They can inside and out impact both the porosity and swelling conduct of the extreme hydrogel item [30].

2.Sol Division Examination:

After manufacturing, the sol division (%) is calculated to choose the mass incident, which gives encounters into the steadiness and judgment of the hydrogel structure [30].

• Fundamental Examination

To energize get it the properties of keratin-based hydrogels, distinctive characterization techniques are utilized:

• Crystallinity Record:

This investigation makes a distinction in choosing the essential composition of the hydrogels, revealing whether they are semi-crystalline or indistinguishable. Such encounters are crucial for anticipating how the hydrogels will carry on beneath differing conditions [26].

These nitty abrasive examinations and changes inside the union handle allow for the optimization of keratin-based hydrogels, fitting them for specific applications where swelling conduct and porosity are fundamental factors.

2.9 Mechanical Properties

2.9.1 Graph of Mechanical Quality in Keratin-Based Hydrogels

Keratin-based hydrogels appear modestly down and out mechanical properties due to their complex composition and the challenges related with effective self-assembly [31]. To address these limitations, a bioinspired method counting recombinant proteins was utilized, basically overhauling the Vigor of these hydrogels [31].

2.10 Self-Assembly and Mechanical Overhaul

1.Homotypic and Heterotypic Self-Assembly:

Examiners conducted tests with various different self-assembly utilizing chosen sort I and sort II keratins. Different combinations were attempted to recognize key space structures and their vitality [31].

2.Impact on Mechanical Quality:

The think approximately revealed critical associations between space structures and the mechanical quality of the hydrogels. This understanding makes a difference in moving forward the mechanical properties through crucial nuclear arrange [31].

• Headways in Biomedical Applications

The as of late laid out keratin hydrogels outlined predominant execution in mechanical properties, which has critical recommendations for biomedical applications. These hydrogels are particularly compelling in controlled cure release and progressing skin recuperation [31].

2.11 Rheological Properties

2.11.1 Viscoelastic Conduct:

The rheological considers around appeared that the adaptable modulus (G') of the keratin hydrogels was basically higher than the thick modulus (G"), certifying their gel-like viscoelastic nature [25].

2.11.2 Effect of Freeze-Thaw (FT) Cycles:

The flexible modulus made strides with a growing number of FT cycles, showing reversible thixotropic conduct, which is profitable for distinctive lively biomedical applications [25].

2.12 Compressive Quality and Assistant Examination

Overhaul with FT Cycles:

The compressive quality of the RHK hydrogel extended generally with the number of FT cycles, appearing a arrange relationship between planning strategies and mechanical Vigor [9]. Keratin Strands from Chicken Plumes:

These fibres are popular for their awesome mechanical properties, counting regard to the hydrogels deduced from them. Their hydrophobic conduct and moo thickness make them fiscally sensible options for hydrogel era [29].

2.12.1 Compressive Moduli Extend

The keratin-PEG hydrogels shown a wide run of compressive moduli, from 1.9 kPa to 45 kPa. This changeability is subordinate on the concentrations of the prepolymer and Eosin Y utilized amid the amalgamation prepare, permitting for customization concurring to particular application needs [23].

• Cytocompatibility and Cellular Interaction

Keratin-based hydrogels have been tried for cytocompatibility and have appeared to bolster the connection, spreading, and multiplication of fibroblast cells. This property is pivotal for applications including tissue building and wound recuperating [23].

2.13 The Part of Silver Nanoparticles

• Antibacterial Properties and Applications

AgNP have long been recognized for their strong antibacterial properties, making them a profitable component in different restorative and nonmedical applications. These applications run from antimicrobial gel details to more complex employments such as dressings for wound recuperating, orthopaedic applications, restorative catheters and rebellious, inserts, and coatings for contact focal points [32].

• Investigate and Characteristics

The logical community has appeared impressive intrigued in AgNPs, driving to broad investigate pointed at understanding their physical, chemical, and organic characteristics. This inquiries about too investigates the components by which AgNPs apply their impacts and their potential employments in both restorative and nonmedical areas [16]. The viability of AgNPs as antimicrobial specialists is impacted by their measure, shape, and concentration, which direct their interaction with microbial cells [32].

• Eco-Friendly Union Strategies

Later headways within the union of AgNPs have emphasized the significance of green chemistry approaches. These strategies utilize characteristic sources like plant extricates and parasites, which not as it were diminish destructive chemical utilize but to improve the biocompatibility of the nanoparticles [33]. Such green-synthesized AgNPs are progressively connected in different biomedical areas, counting

diagnostics, wound recuperating, tissue treatment, immunotherapy, regenerative pharmaceutical, dentistry, and biosensing [33].

• Integration into Hydrogels

Joining AgNPs into hydrogels has appeared promising comes about, especially in wound treatment applications. For occasion, a gel composed of poly(N-isopropylacrylamide-co-acrylamido-2-methylpropane sulfonic corrosive) (NIPAMSA) utilized AgNPs to encourage the decrease of para-nitrophenol to 4-aminophenol [17]. Additionally, chitosan-grafted polyvinyl liquor hydrogels containing AgNPs shown amazing antibacterial action against common pathogens like E. coli and S. aureus [33].

2.14 Characterization and Optimization

The characterization of AgNPs is vital for guaranteeing their adequacy and security in biomedical applications. Methods such as UV-Vis spectroscopy, Fourier change infrared spectroscopy (FT-IR), checking electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD) are commonly utilized to examine the properties of nanoparticles [33]. Continuous investigate proceeds to address the challenges of optimizing the measure and discharge energy of AgNPs in hydrogel formulations, aiming to upgrade their viability in progressed wound mending treatments [33].

2.14.1 Upgraded Wound Mending with Keratin-Based Hydrogels

Inserting AgNPs in keratin-based hydrogels essentially boosts their antibacterial properties, which is significant for advancing quicker and more secure wound mending. Particular hydrogel frameworks, such as those containing AgNP by Mimosa tenuiflora (AgMt NPs-G), have appeared promising comes about in treating burn wounds due to their bactericidal and anti-inflammatory impacts [34]. This integration of AgNPs into keratin-based hydrogels speaks to a forward-thinking approach to creating progressed biomaterials for viable wound administration and other helpful applications.

2.14.2 Biomedical Applications of Keratin-based Hydrogels

Keratin-based hydrogels, determined from sources such as chicken plumes and human hair, show a run of properties that make them exceedingly reasonable for different biomedical applications. These hydrogels are especially esteemed for their biocompatibility, biodegradability, and capacity to encourage cellular intuitive, which are vital for restorative gadgets and helpful techniques.

2.15 Wound Mending and Tissue Building

1.Effective Wound Care:

Keratin-based hydrogels inferred from chicken plumes have appeared potential as viable wound care items due to their capacity to back cellular exercises fundamental for recuperating [29].

2.Skin Recovery:

These hydrogels encourage skin recovery by promoting cell movement and multiplication, fundamental for mending burns and other skin wounds [37].

3.Scaffolds for Tissue Engineering: Leveraging the self-assembled structures of keratin, these hydrogels serve as scaffolds that enhance cell growth and differentiation, crucial for tissue engineering applications [37].

2.16 Drug Delivery Systems

Controlled Drug Release: Keratin-based hydrogels can be used to release therapeutic agents in a controlled manner, utilizing the biodegradable nature of keratin to target drug delivery effectively [37].

Antimicrobial and Antioxidant Applications: Innovations such as chlorhexidine-loaded keratin hydrogels exhibit antioxidant and antibacterial activities, making them suitable for treating infected wounds [38].

2.17 Neuroregenerative Applications

Peripheral Nerve Regeneration: Studies have demonstrated that keratin-based hydrogels are neuroinductive, supporting regeneration in peripheral nerve injury models in mice, thus offering a potential alternative to traditional nerve grafts [17].

2.18 Haemostatic Uses

Emergency Haemostasis: In models of severe bleeding, such as lethal liver injuries in rabbits, keratin hydrogels have been effectively used as haemostatic agents, showcasing their potential in trauma care [17].

Biomedical Devices and Coatings

Medical Device Coatings: The application of keratin-based hydrogels as coatings on medical devices can improve biocompatibility and reduce bacterial adhesion, which is vital for preventing infections in medical settings [37].

3 Advancements in Biomedical Research

Supporting Stem Cell Technologies: The integration of stem cells with keratin-rich materials in tissue engineering offers a promising avenue for treating a range of musculoskeletal diseases due to the advantageous properties of keratin such as non-toxicity and tissue repair capabilities [38].

Hair Growth Studies: Intradermal injections of keratin are known to promote hair growth in animal models, providing insights into potential applications for dermatological conditions [38].

This detailed exploration of the applications of keratin-based hydrogels underlines their versatility and potential in improving patient outcomes across various medical fields.

2.19 Unique Properties of Keratin-Based Hydrogels

Keratin-based hydrogels stand out due to their distinct characteristics when compared to other hydrogels. These properties make them particularly suitable for a range of biomedical applications:

1.Biocompatibility: Keratin-based hydrogels are highly biocompatible, making them ideal for applications in tissue engineering and drug delivery systems [39].

2.Mechanical Strength: These hydrogels show good mechanical strength relative to many synthetic hydrogels. This attribute is particularly beneficial in load-bearing applications where durability and resilience are required [39].

3.Biodegradability: Unlike some synthetic hydrogels that may require surgical removal, keratin-based hydrogels naturally degrade within the body. This feature minimizes the need for invasive procedures post-treatment [39].

4.Self-healing Ability: Keratin-based hydrogels have the capacity to self-repair, restoring their structure and functionality after physical damage. This self-healing property is advantageous in dynamic biomedical environments where long-term material integrity is crucial [39].

5.Non-toxicity: The non-toxic nature of keratin-based hydrogels makes them safe for a wide range of applications, including cosmetics and skincare products, where consumer safety is paramount [39].

Table 5: Highlighting the comparative aspects of keratin-based hydrogels against other common types of hydrogels

Property	Keratin-Based Hydrogels	Other Hydrogels
Biocompatibility	High	Variable
Mechanical Strength	Higher	Lower
Biodegradability	Naturally degradable	Often non-degradable
Self-healing Capability	Present	Rarely present
Non-toxicity	Non-toxic	May contain toxic residues

This comparative analysis underscores the suitability of keratin-based hydrogels for advanced biomedical applications, leveraging their unique properties for enhanced performance and safety. The ongoing research and development in this area continue to expand the potential uses of these hydrogels, particularly in areas where traditional materials may fall short [25][39].

2.20 Loading Silver Nanoparticles into Keratin-based Hydrogels

AgNP integrated into keratin-based hydrogels through a meticulous process that enhances both their mechanical and antibacterial properties. This process involves several key steps, each critical to achieving the desired functionality of the hydrogel.

- Method of Loading AgNPs

1.Silver Ion Adsorption: Initially, PVA/Keratin films are immersed in a silver nitrate solution, which facilitates the adsorption of silver ions onto the film [40].

2.Reduction Process: Following ion adsorption, the films are then immersed in an ascorbic acid solution. This step is important because it reduces the adsorbed silver ions to metallic silver nanoparticles (AgNPs) [40].

2.21 Resulting Enhancements in Hydrogel Properties

- Mechanical Strength: The crosslinking of the PVA/Keratin/AgNPs films post-AgNP loading significantly enhances their mechanical strength. This improvement is vital for applications requiring robustness and durability [40].
- Antibacterial Capability: These films exhibit superior antibacterial abilities, effectively inhibiting the growth of harmful bacteria such as *Staphylococcus aureus and Escherichia coli [40]*.
- Thermal Stability: Thermogravimetric analysis shows that these films have reduced weight loss at elevated temperatures, indicating enhanced thermal stability due to the presence of AgNPs acting as interchain cross-linkers [41].

➢ Biomedical Applications and Performance

- Biocompatibility and Cell Proliferation: The non-toxic nature of the PVA/Keratin/AgNPs films supports cell proliferation, making them well utilizable for various biomedical applications where tissue interaction is crucial [41]. Antibacterial Functionality: The films achieve their antibacterial function through the direct contact and gradual release of AgNPs, ensuring the elimination of surrounding bacteria [41].
- Inflammatory Response and Epithelialization: In infectious animal models and histological studies, these films have been shown to effectively inhibit the inflammatory response and accelerate epithelialization, demonstrating their potential in medical treatments [42].

2.22 Comparative Performance with Other Scaffolds

- Tensile Strength and Degradation: Keratin/AgNPs blend scaffolds (Ker/Ag) exhibit larger tensile strength and a slower degradation rate compared to other types, making them more attractive for wound repair materials [42].

- Ag Release Kinetics: These scaffolds display a linear Ag releasing ratio in the initial 5-7 days, which aids in providing continuous antibacterial properties while avoiding biotoxicity that can occur with a focused release [42].
- Cell Proliferation: The slow and continuous release of Ag from these scaffolds has been found to accelerate the proliferation of cells, which is beneficial for tissue regeneration and healing processes [42].

This detailed process of loading silver nanoparticles into keratin-based hydrogels not only enhances the material's inherent properties but also broadens the scope of their application in the field of biomedicine, particularly in areas requiring robust antimicrobial and mechanical performance.

2.23 Keratin Sources and Extraction Methods

Keratin, a fundamental component in numerous biological structures, is sourced from various keratin-rich materials. These materials, often by-products of the ago-industry, include chicken feathers, beaks, claws, nails, horns, hooves, human hair, and toenails [4][5]. Among these, chicken feathers are notably abundant and sustainable, making them a primary source for keratin extraction [5].

2.23.1 Extraction Methods

1.Chemical Hydrolysis: This method involves treating keratinous materials with chemicals to break down the proteins. While efficient, it often leads to a reduction in nutritional value due to amino acid loss [4].

2.Alkaline Hydrolysis: Performed at 80°C using 2% NaOH for three hours, this method typically yields about 25% of the keratin [4].

3.Enzymatic and Microbial Treatment: Utilizing keratinolytic and proteolytic enzymes, this method breaks down keratin more gently, preserving more of its properties [4].

4.Microwave Irradiation: This modern technique reduces the activation energy required for extraction and provides uniform heating, enhancing the efficiency of the process [4].

5.Steam Explosion: A hydrothermal process that uses high-pressure saturated steam for short intervals to effectively extract keratin [4].

6.Thermal Hydrolysis: Also known as the superheated process, this involves treating the biomass with water under various pressure and temperature conditions until the protein is converted into oligopeptides [4].

2.23.2 Optimization and Reuse of By-Products

Extraction methods are continually optimized at the lab scale to maximize yield and efficiency. By-products from these processes, such as feather meal, are often reused as fertilizers or animal feed supplements, closing the loop and preventing environmental pollution [5]

Table 6: Keratin Sources and Their Extraction Methods

Source	Method of Extraction	Key Benefits
Chicken Feathers	Chemical, Enzymatic, Microwave	High yield, sustainable source
Wool	Alkaline Hydrolysis, Thermal	Up to 95% keratin content, effective extraction
Human Hair	Chemical Hydrolysis, Steam Explosion	Abundant, diverse applications
Hooves and Horns	Enzymatic, Thermal Hydrolysis	Efficient breakdown, minimal environmental impact

This comprehensive approach to sourcing and extracting keratin not only supports the production of advanced biomaterials but also contributes to sustainable practices in the industry.

2.24 Safety and Biocompatibility Assessments

Recent studies have underscored the importance of thoroughly assessing the safety and biocompatibility of keratin-based hydrogels integrated with silver nanoparticles. Various in vitro and in vivo studies have been conducted, although more extensive research is necessary to fully ascertain their safety profiles [43]. For instance, a study involving poly (vinyl alcohol)/keratin films with AgNPs aimed at preventing SSIs in delayed sternal closure procedures demonstrated promising results in terms of safety and biocompatibility, suggesting their potential for broader clinical adoption [44].

2.24.1 Enhancements in Mechanical Strength and Antibacterial Properties

The integration of AgNPs into keratin-based hydrogels significantly enhances their mechanical strength and antibacterial efficacy. For example, suturable poly (vinyl alcohol)/keratin films loaded with AgNPs have shown improved mechanical properties and antibacterial activity, making them suitable for applications such as delayed sternal closure in congenital heart disease surgeries [45]. Additionally, the development of keratin hydrogels with embedded AgNPs has demonstrated superior antibacterial properties and enhanced wound healing capabilities compared to control groups [43].

2.24.2 Novel Applications in Wound Healing

Keratin-based hydrogels with AgNPs have been specifically noted for their efficacy in wound healing applications. A study utilizing hydrogels with silver nanoparticles synthesized from Mimosa tenuiflora extracts for treating second-degree burns highlighted their bactericidal and anti-inflammatory effects, which are crucial for effective recovery [45]. Similarly, the use of nanofibers made from human hair keratein combined with PEO/PVA and AgNPs has shown excellent antibacterial activity against a range of bacteria, further supporting their use in medical materials [44].

2.24.3 Comparative Studies on Keratin-Based Wound Dressings

Research comparing different types of keratin-based wound dressings has provided insights into their performance and efficacy. One study examined keratin/AgNPs blend scaffolds and keratin scaffolds with AgNPs attached to the scaffold's surface, revealing that the former displays a larger tensile strength and a slower degradation rate due to the uniform dispersion of AgNPs [51].

Both types of scaffolds exhibited excellent antibacterial properties, with the Ker/Ag scaffolds showing a beneficial linear silver releasing ratio in the initial days post-application, which helps maintain continuous antibacterial effects and reduces biotoxicity risks [51].

2.25 Advanced Synthesis Techniques and Their Implications

The methods used to incorporate AgNPs into hydrogels are crucial for optimizing their biomedical applications. Silver nanoparticles can be integrated into hydrogels either through in situ synthesis or by mixing pre-synthesized nanoparticles with the hydrogel matrix. These techniques enhance the hydrogels' antimicrobial activity and are applicable in photothermal therapy and localized drug release, broadening the scope of their use in fields such as tissue engineering and biosensing [49].

This exploration of recent studies and findings not only illustrates the advancements in the field of keratin-based hydrogels with silver nanoparticles but also highlights the ongoing need for research to address existing challenges and fully harness their potential in biomedical applications.

3 Silver Nanoparticles: An Antimicrobial Agent

AgNP renowned for their broad-spectrum antimicrobial properties, which include antibacterial, antifungal, and antiviral activities. These nanoparticles are capable of penetrating bacterial cell walls, altering cell membrane structures, and increasing membrane permeability. This penetration leads to the production of reactive oxygen species and interruption of DNA replication through the release of silver ions, culminating in bacterial death [54].

2.26 Mechanism of Action

1.Cell Membrane Disruption: AgNPs can disrupt cell membranes and release silver ions, which generate reactive oxygen species and interact with cellular components like proteins, DNA, and respiratory enzymes [52].

2.Interaction with Cellular Components: Inside the cell, AgNPs show an affinity for interacting with sulphur or phosphorus groups, altering their structure and functions, which may lead to cell wall rupture and increased cytotoxic effects [53].

3.Respiratory Chain Alteration: By interacting with thiol groups in enzymes, AgNPs may damage the respiratory chain in the inner membrane, activating pathways that lead to apoptosis[53].

- Antimicrobial Efficacy

AgNPs have demonstrated significant antibacterial activity against a variety of pathogens, including methicillin-resistant Staphylococcus aureus, penicillin-resistant Enterococcus faecalis, and Mult resistant Mycobacterium tuberculosis [52].

These nanoparticles have also been effective in inhibiting biofilm formation, which is crucial for treating chronic infections and preventing the spread of bacteria [52].

2.27 Applications in Biomedical Materials

Wound Dressings: AgNPs are incorporated into various materials such as membranes, topical applications, and nanofibers, enhancing their antibacterial efficacy for use in wound dressings [52].

Surgical Applications: For instance, suturable poly (vinyl alcohol)/keratin films loaded with AgNPs have been used for delayed sternal closure in surgeries, providing temporary protection against bacterial infections [45].

Dental Treatments: In dentistry, AgNPs have been used in products ranging from acrylic resins to titanium coatings for implants, exploiting their antibacterial properties to prevent infections [54].

2.28 Challenges and Adaptations

The development of resistance by microorganisms to AgNPs necessitates ongoing research into new combinations of nanomaterials and antibiotics to maintain their efficacy [52].

The size, shape, and coating of AgNPs are critical factors that influence their antibacterial and cytotoxic effects, with smaller nanoparticles showing a higher dissolution rate and enhanced antibacterial activity [55].

By leveraging the unique properties of AgNPs, researchers and medical professionals continue to explore and expand their applications across various fields of medicine and healthcare, aiming to harness their full potential while mitigating associated risks.

2.29 Release Kinetics of Silver Nanoparticles

The release kinetics of silver nanoparticles (AgNPs) from keratin-based hydrogels are crucial in determining their effectiveness and safety in biomedical applications. Studies have shown that the release patterns can vary significantly depending on the

composition and structure of the hydrogel. For instance, a 3-dimensional electro spun nanofibrous scaffold (3DENS) made of Poly(vinyl alcohol) (PVA), keratin, and chitosan incorporated with AgNPs exhibited a rapid release of about half of the total AgNPs within the first 6 hours [57]. This initial burst was followed by a more gradual increase, reaching a plateau with a maximum release of 25% to 30% after 24 hours [57].

2.29.1 Controlled Release for Reduced Toxicity

To mitigate potential toxicity issues, controlling the release rate of AgNPs is essential. The suturable poly (vinyl alcohol)/keratin film loaded with AgNPs for delayed sternal closure (DSC) techniques demonstrated significantly enhanced mechanical strength and a controlled release mechanism, which is critical for reducing the risk of silver-induced toxicity [48][56]. The controlled release ensures that the AgNPs are delivered at a therapeutic rate that is effective yet safe for the patient over the required duration of treatment.

2.29.2 Non-Fickian Diffusion Mechanism

Further analysis of the release kinetics from the 3DENS scaffolds indicates that the diffusion mechanism of AgNPs does not follow the simple Fickian diffusion. Instead, it is better described by the Korsmeyer-Peppas model with adjusted regression coefficients ranging from 0.981 to 0.989, suggesting a non-Fickian diffusion mechanism [57]. This implies that the release of AgNPs is influenced by more complex factors than just the concentration gradient, including the interactions between the nanoparticles and the hydrogel matrix.

2.29.3 Linear Release for Continuous Antibacterial Effect

Another aspect of the release kinetics is the linear releasing ratio observed in Ker/Ag scaffolds. These scaffolds maintain a linear release of AgNPs for the first 5-7 days, which is beneficial for providing continuous antibacterial properties without the risk of sudden spikes in silver concentration that could lead to biotoxicity [9]. This steady release pattern is crucial for applications where prolonged antimicrobial activity is necessary, such as in wound dressings and other medical implants.

By understanding and optimizing the release kinetics of AgNPs from keratin-based hydrogels, researchers can enhance both the efficacy and safety of these innovative biomedical materials.

2.30 Clinical Application in Delayed Sternal Closure

The application of Polyvinyl Alcohol (PVA)/Keratin/Silver Nanoparticles (AgNPs) films in clinical settings, particularly for delayed sternal closure surgeries, showcases a significant advancement in medical treatments. These films have been utilized effectively to protect the chest cavity from bacterial infections, which are a common complication in such surgeries. The integration of AgNPs into the keratin-based hydrogel films provides an antimicrobial property that is critical in preventing post-operative infections, thereby enhancing patient recovery and reducing hospital stay durations [45][48].

2.31 Biomedical Applications

Keratin-based nanoparticles (KNPs) have emerged as a significant tool in the realm of targeted drug delivery. Their high surface area, tunable biodegradability, and excellent biocompatibility make them ideal for controlled release applications. These properties, coupled with the ability to easily functionalize KNPs, enhance their potential in delivering therapeutic agents directly to the target site, thereby minimizing side effects and improving treatment efficacy [43].

2.31.1 Tissue Engineering and Regenerative Medicine

Keratin-based hydrogels have shown considerable promise in tissue engineering, particularly in applications such as dental pulp regeneration and bone regeneration. These hydrogels support odontogenic differentiation and reparative dentine formation, which are crucial for dental health. Moreover, their application in bone regeneration has demonstrated significant improvements in bone-to-implant contact over various time periods, highlighting their potential as a scaffold material in regenerative therapies [59].

2.31.2 Wound Healing Applications

Keratin-based hydrogels, especially those enhanced with silver nanoparticles, have demonstrated excellent capabilities in promoting wound healing. These hydrogels not

only expedite the healing process but also reduce the risk of infection, which is crucial in treating both acute and chronic wounds. The presence of silver nanoparticles adds an antimicrobial property that is essential for preventing infection in wound sites [45][60].

2.31.3 Haemostatic Applications

The rapid haemostatic properties of chicken feathers keratin-based nanoparticles make them invaluable in emergency and surgical settings. These materials can effectively stop bleeding in less than 10 seconds, which is critical in saving lives and improving outcomes in traumatic injuries [43].

2.31.4 Antimicrobial Applications

Various keratin-based hydrogel composites, such as Keratin-Chitosan-Tricalcium Phosphate (KCTPs), have shown both cytocompatibility and antimicrobial efficacy. These properties make them suitable for use in regenerative endodontic therapy, where preventing microbial infections is crucial for successful treatment outcomes [59].

2.31.5 Enhanced Biocompatibility and Safety

Studies have consistently shown that keratin-based materials are biocompatible and exhibit minimal toxicity, which is essential for any biomedical application. For instance, the PVA/Keratin/AgNPs films used in delayed sternal closure surgeries have proven to be non-toxic to human smooth muscle cells (HSMCs), underscoring their safety for clinical use [45].

2.31.6 Innovative Biomedical Products

The versatility of keratin-based nanoparticles extends beyond traditional medical applications. They have been used in creating innovative products such as fertilizers, musical instruments, and even hair extensions, showcasing the broad potential of these materials in various industries [43].

These applications of keratin-based hydrogels and nanoparticles highlight their versatility and potential in a wide range of biomedical fields, from drug delivery systems to regenerative medicine, offering promising solutions for many medical challenges.

2.32 Advantages of Keratin-Based Hydrogels

Keratin-based materials are highly regarded for their intrinsic biocompatibility and biodegradability, which are critical for medical applications. These properties ensure that keratin-based hydrogels are non-toxic to human tissues and can be broken down naturally by the body without causing harm or adverse reactions [62].

2.32.1 Enhanced Mechanical Properties

The structural integrity of keratin-based hydrogels is a significant advantage, especially in applications requiring robustness and durability. For instance, suturable poly (vinyl alcohol)/keratin films loaded with silver nanoparticles have shown enhanced tensile strength, making them suitable for demanding medical procedures such as delayed sternal closure in congenital heart disease treatments [45][48].

2.32.2 High Water Absorbency

One of the standout features of keratin-based hydrogels is their high water absorbency. This characteristic is particularly beneficial in wound healing, as it helps maintain a moist environment around the wound, which is conducive to faster and more effective healing [62].

2.32.3 Controlled Delivery of Biomolecules

Keratin-based hydrogels are excellent mediums for the controlled delivery of biomolecules. They can be engineered to release therapeutic agents at a sustained rate, which is essential for treatments requiring long-term medication administration at the target site, thereby improving the efficacy of the treatment and reducing side effects [62].

2.32.4 Customization with Bioactive Compounds

The versatility of keratin-based hydrogels allows for the inclusion of various bioactive compounds to enhance their healing properties. The addition of substances like aloe vera, chitosan, and honey has been shown to significantly improve the healing capabilities of these hydrogels, making them even more effective in clinical applications [62].

2.32.5 Superior Wound Healing Capabilities

Studies have demonstrated that keratin-based hydrogels, particularly those enhanced with silver nanoparticles, provide superior wound healing capabilities. They not only accelerate the healing process but also effectively reduce the risk of infection, a critical factor in treating both acute and chronic wounds [43][62].

2.33 Evaluating Toxicity and Immune Responses

1.Potential Toxicity Concerns: The integration of silver nanoparticles into keratin-based hydrogels necessitates careful consideration of potential toxicity. Studies have highlighted concerns regarding the focused release of silver, which can lead to biotoxicity, particularly in wound dressing applications [64][51].

2.Immune Response Evaluation: It is critical to assess how these hydrogels interact with the immune system, as inappropriate immune responses can compromise the safety and effectiveness of the hydrogels in clinical settings [64].

2.33.1 Biocompatibility Studies

In Vitro and In Vivo Assessments: The biocompatibility of keratin-based hydrogels with AgNPs has been extensively studied. For instance, PVA/Keratin/AgNPs films have shown non- toxicity and superior antibacterial ability, significantly reducing the growth rate of pathogens like Staphylococcus aureus and Escherichia coli [48].

Long-Term Stability: Ongoing research into the long-term stability and biocompatibility of these hydrogels is crucial. These studies ensure that the hydrogels can be safely used in tissue engineering and other medical applications over extended periods [53].

2.33.2 Specific Case Studies

Suturable Films for Surgical Use: A notable study involving suturable poly (vinyl alcohol)/keratin films loaded with silver nanoparticles used in delayed sternal closure surgeries demonstrated not only enhanced mechanical strength but also confirmed non-toxicity and effective antibacterial properties, making them suitable for high-risk surgical applications [3]. Low Toxicity at Specific Concentrations: Research indicates that at a concentration of 17 µg/ml, silver nanoparticles in various forms (AgNP-S, AgNP-F, and AgNP-W) exhibit very low toxicity on the HepG2

cell line while maintaining high antibacterial activity, suggesting a balance between safety and efficacy [50].

By continuously monitoring and assessing these factors, the development of keratin-based hydrogels with silver nanoparticles can be optimized for safer and more effective biomedical applications.

2.34 Overview of Synthesis Techniques for Silver Nanoparticles

Silver nanoparticles (AgNPs) can be synthesized through various methods, each affecting the size, geometry, and stability of the nanoparticles, which in turn influences their antibacterial properties. The primary methods include chemical, green, irradiation, and thermal synthesis [52].

1.Chemical Synthesis: This method allows for precise control over the size and shape of AgNPs, which is crucial for their effective integration into hydrogels for biomedical applications [52].

2.Green Synthesis: Utilizing plant extracts, such as from the medicinal plant Carduus crispus, this method not only supports the eco-friendly production of AgNPs but also results in nanoparticles with a high yield and smaller size, as evidenced by a blue shift in absorption spectra [50].

3.Irradiation Synthesis: A process that typically involves the use of gamma rays or electron beams to induce the formation of AgNPs, offering a clean and controlled environment for nanoparticle generation [52].

4.Thermal Synthesis: This method involves heating the precursor materials to form AgNPs, allowing for the manipulation of nanoparticle properties through temperature adjustments [52].

2.34.1 Key Considerations in Hydrogel Integration

Integrating silver nanoparticles into keratin-based hydrogels involves several critical considerations to ensure their effectiveness and stability within the hydrogel matrix:

Uniform Distribution: It is essential to achieve a uniform distribution of AgNPs throughout the hydrogel network to ensure consistent antimicrobial activity and mechanical properties[56].

Stability Over Time: Maintaining the stability of AgNPs in the hydrogel is crucial for prolonging the efficacy of the antimicrobial and mechanical properties of the hydrogel [14]. Controlled Size and Geometry: The synthesis method chosen impacts the size and geometry of AgNPs, which in turn affects their stability and distribution within the hydrogel. Smaller nanoparticles, such as those synthesized using Carduus crispus extract, tend to have higher stability and a more uniform distribution [8][10].

2.34.2 Nanoparticle Characteristics

The characteristics of synthesized AgNPs can vary significantly depending on the synthesis method used:

Crystallite Size and Structure: AgNPs synthesized through different methods exhibit distinct crystalline sizes and structures, such as face-centred cubic structures seen in AgNP-S, AgNP-F, and AgNP-W, with sizes of 36 nm, 13 nm, and 14 nm respectively [8].

By understanding and optimizing these synthesis methods and considerations, researchers can enhance the functionality and application of keratin-based hydrogels with embedded silver nanoparticles for various biomedical uses.

2.35 Challenges in Development

1.Efficient and Eco-friendly Keratin Extraction: Developing methods to efficiently and eco-friendly extract keratin from diverse sources such as hair and feathers is a significant challenge [43].

2.Overcoming Poor Water Solubility: Addressing the inherently poor water solubility of keratin is crucial for its broader application in hydrogel formulations [43].

2.36 Nanoparticle Design and Environmental Concerns

3.Optimizing Nanoparticles for Drug Delivery: Designing keratin-based nanoparticles that are optimized for specific drug delivery applications, including targeted and controlled release, is essential for advancing medical treatments [43].

4.Environmental Impact of Keratin Waste: The environmental impact associated with the disposal of keratin waste requires careful management to prevent ecological damage [43].

- Balancing Properties and Toxicity in Hydrogels

5.Biocompatibility vs. Mechanical Stability: Achieving an optimal balance between biocompatibility, biodegradability, and mechanical stability presents a complex challenge in the development of keratin-based hydrogels [64].

6.Engineering Anatomically Accurate Scaffolds: Creating scaffolds that accurately replicate anatomical shapes and structures is vital for effective tissue regeneration but remains technically challenging [64].

7.Managing Silver Nanoparticle Toxicity: Ensuring the biocompatibility and minimizing the potential toxicity of silver nanoparticles when integrated into hydrogels is a critical safety concern [64].

8.Addressing Biotoxicity of Silver: The focused release of silver from hydrogels can cause biotoxicity, particularly in wound dressing applications, necessitating the development of safer release mechanisms [51].

2.37 Production and Stability Challenges

9.High-Quality Keratin Extraction: Obtaining high-quality keratin from waste sources poses challenges in ensuring consistent performance of the hydrogels [56].

10.Uniform Distribution of Nanoparticles: Ensuring a uniform distribution of silver nanoparticles within the hydrogel network is essential for consistent antimicrobial activity and mechanical properties [56].

11.Long-term Stability of Nanoparticles: Maintaining the stability of silver nanoparticles within the hydrogel over time is crucial for sustaining their effectiveness [56].

12.Controlled Silver Release: Developing mechanisms to control the release of silver nanoparticles from hydrogels is necessary to prevent toxicity while maintaining therapeutic effectiveness [56].

13.Scaling Production for Industrial Use: Scaling up the production process to meet industrial application demands remains a significant hurdle [56].

2.38 Future Perspectives

1.Optimization of Keratin and AgNPs Concentrations: Future research should prioritize finding the optimal concentrations of keratin and silver nanoparticles (AgNPs) that maximize wound healing and antibacterial effectiveness without compromising safety [47][65].

2.Incorporation of Natural Extracts: Exploring the synergistic effects of combining other natural extracts with keratin and AgNPs could potentially enhance the hydrogels' wound healing and antimicrobial properties, offering more comprehensive treatment options [47][65].

- Expanding Applications in Localized Therapy and Tissue Engineering

1.Localized Antibacterial Therapy: There is significant potential for keratin-based hydrogels with AgNPs to be used in localized antibacterial therapy, particularly for treating infectious diseases. This could lead to more targeted and effective treatment modalities [53].

2.Bone Regeneration and Tissue Engineering: The application of these hydrogels in bone regeneration and other areas of tissue engineering deserves further investigation. Their ability to support tissue integration and healing could revolutionize treatments in regenerative medicine [53].

CHAPTER-3

MATERIAL AND METHODS

Material and Method

Materials:- DPPH, ABTS from (Himedia Lab.), Muller Hinton Agar, Muller Hinton Broth, Ampicillin (Himedia Lab.), *E.coli*.(ATCC 25922), *S. aureus*(ATCC 23235), Deionized water (Millipore Q.) All solvents used in this study are of analytical grade.

Method :-

3.1. Preparation of keratin lysate-

- Keratin was extracted from the chicken feather and to accomplish that, chicken feathers were taken, washed and dried.
- They were dissolved in 0.5M NaOH solution overnight.
- They were blended in a Philips mixer and grinder.
- It was filtered through a sieve.
- Then the liquid which contains keratin was kept in a dialysis membrane for 3 days, changing its water frequently.
- After three days the lysate was dialysed and was pure, and was then stored at 4°C for further use.

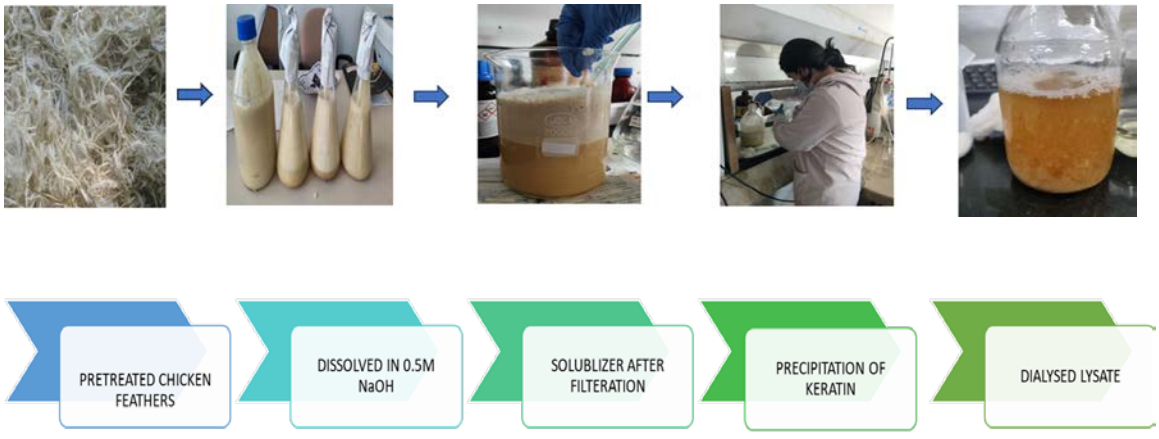

FIG 1- Steps of keratin lysate preparation

3.2. Preparation of Hydrogel-

- 10% Polyvinyl Alcohol(PVA)solution was prepared by mixing 10gram PVA in 100ml of distilled water. It was heated for half an hour at 50°C until PVA was dissolved completely.
- 10% Polyvinyl Pyrrolidone(PVP) was prepared by mixing 10 grams of PVP in 100ML of distilled water. It was heated for 10 minutes at 50°C until PVP was completely dissolved.
- Hydrogel was prepared by mixing 50ml of Keratin lysate, 30ml of PVA, 10 ml of PVA, 10 gram of starch in beaker.
- Continuously stirred at 50°C for 40 mins on a magnetic stirrer.
- Poured the solution into Petri plate/ dish.
- Froze for 18 hours at -20°C.
- Thawed the solution at room temperature for 2hours.
- Repeated the freeze-thaw cycle 3 times to form hydrogel.

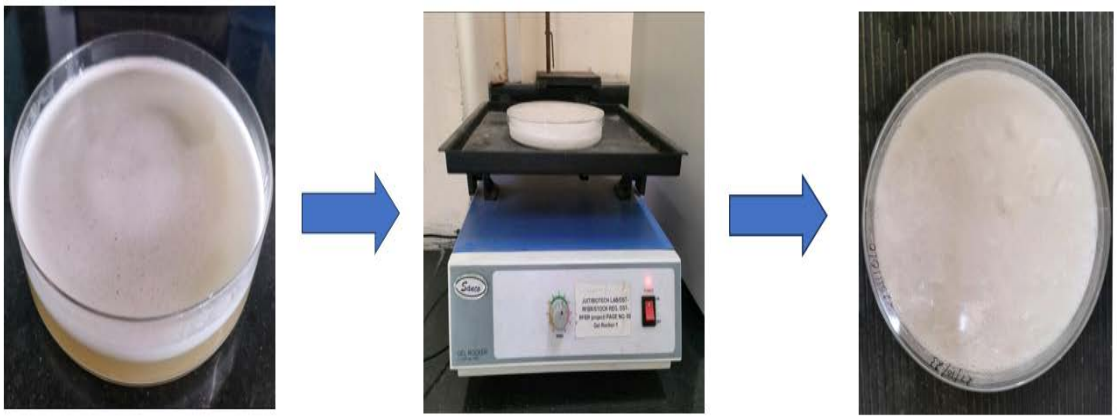

Fig 2- Synthesis of Hydrogel

3.3. Optimization of Hydrogel-

3.3.1 Optimization of PVA.

The optimization of PVA was done by changing the concentration of PVA in different samples as follows, 10, 15,20,25,30 ml respectively, while the volume of other solutions was kept constant.

3.3.2 Optimization of Keratin

The volume of keratin was changes in various sample in order 10,20,25,30,40 while keeping the volume of other solutions constant.

3.3.3 Optimization of PVP

The volume of PVP was changed (5,7.5,10,12.5,15) while keeping the volume of all the other solutions constant.

3.3.4 Optimization of Starch

The volume of starch was changed in various samples (6,8,10,12,14), while keeping all the volume of all the other solutions constant.

3.4. Preparation of Plant Extract-

- Fresh young and healthy leaves of Tridex procumbens, were collected, washed with tap water to remove any dust, and then washed with distilled water.
- Cut in to small pieces, and then weighed.
- The cut leaves were kept at 60°C for 30 mins under continuous stirring of ?
- After that the solution was allowed to cool at room temperature, and was then filtered through Wattman filter paper, and stored in cold store for further use.
- Thus 10% of Plant extract was prepared

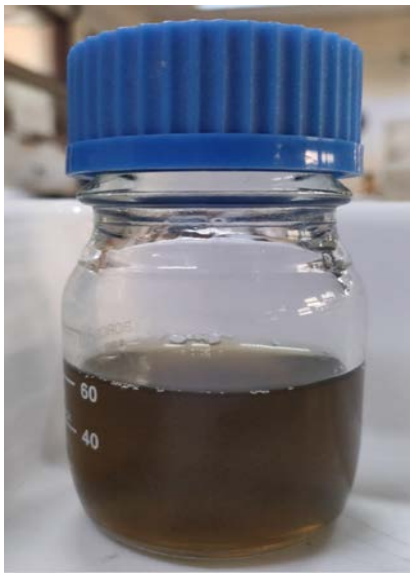

Fig 3- Synthesised Plant Extract.

3.5. Synthesis and Optimization of AgNP- 10mM of Silver nitrate was used and various different parameters were optimized like temperature, pH, concentration of AgNO3 , and time of reaction.

- Optimization of concentration

	Sample 1	Sample 2	Sample 3
Plant Extract 10%	2ml	2ml	4ml
$AgNO_3$ 10mM	2ml	2ml(5mM)	2ml

- Optimization of pH

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Plant Extract 10% (ml)	2	2	2	2	2
$AgNO_3$ 10mM (ml)	2	2	2	2	2
pH	**4.1**	**5.1**	**6.1**	**7.1**	**8.1**

- Optimization of Time of reaction

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Plant Extract (10%) (ml)	2 ml	2 ml	2 ml	2 ml	2 ml
$AgNO_3$ (10 mM) (ml)	2 ml	2 ml	2 ml	2 ml	2 ml
pH	8.1	8.1	8.1	8.1	8.1
Time of Reaction (mins)	**20**	**25**	**30**	**35**	**40**

- Optimization of temperature

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Plant Extract (10%) (ml)	2 ml	2 ml	2 ml	2 ml	2 ml
$AgNO_3$ (10 mM) (ml)	2 ml	2 ml	2 ml	2 ml	2 ml
pH	8.1	8.1	8.1	8.1	8.1
Time of Reaction (mins)	25	25	25	25	25
Temperature ^oC	**30**	**35**	**40**	**45**	**50**

Table 7- Final Optimization of AgNP

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Plant Extract (10%)	2 ml	2 ml	2 ml	2 ml	2 ml
$AgNO_3$ (10 mM)	2 ml	2 ml	2 ml	2 ml	2 ml
pH	8.1	8.1	8.1	8.1	8.1
Time of Reaction	25 mins	25 mins	25 mins	25 mins	25 mins
Temperature	30^oC	35^oC	40^oC	45^oC	50^oC

3.6 Lyophilization of Hydrogel

The hydrogel was prepared for lyophilization by following steps-

- It was kept at -80 for 2 hours,
- Then it was lyophilized overnight,
- Then the lyophilized sample was kept at room temperature for further testing's.

3.6.1 Loading Efficiency-

The loading efficiency of hydrogel was tested, in which the hydrogel was loaded with AgNP. The Hydrogel was cut in beads and weighed after lyophilization, one bead was then dissolved in 1ml of AGNP solution for 1 hour, and then weighed, to check the loading efficiency of the hydrogel.

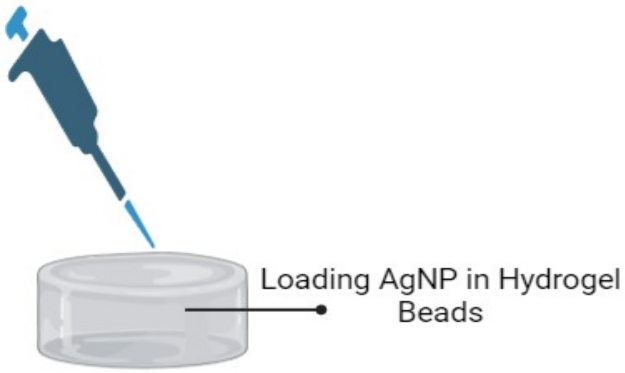

Fig 4- Visual representation of loading of AgNP on hydrogel

3.7 Physical and Chemical Characterization:

3.7.1 UV-VISIBLE SPECTROSCOPY

Using a Thermo Fisher Scientific Spectrophotometer, the UV-visible absorption spectrum of the samples was produced in order to investigate the optical absorption characteristics of the photocatalyst. Understanding the spectral characteristics of chitosan nanoparticles was made possible by spectroscopy, that were recorded at constant room temperature within the wavelength of 200-700 nm.

3.7.2 Swelling Study

The swelling study of hydrogel was studied by gravimetric method at 37oC in Phosphate buffer at pH of 7.4, it was carried out as follows.

- 1cm piece of hydrogel was dried,
- The dried sample was weighed,
- Further immersed in 20 ml of PBS,
- At different time intervals, it was removed from buffer, put on a filter paper to absorb excess moisture, and then weighed.

3.8 Bioactive properties determined by:

3.8.1 Antioxidant Assay

The free radical scavenging test to check the capacity of chitosan-based nano formulations was evaluated using the standard 2,2-diphenyl-1-Picrylhydrazyl DPPH assay. It is a free radical method based on electron transfer). The antioxidant's IC50 was determined. To determine the radical scavenging activity (RSA) formula RSA (%) = [(AcontrolAsample)/Acontrol] /100 is used.

The antioxidants are oxidized by the free radical ABTS. It is a cyan-coloured (bluish-green) reagent that becomes colourless when an antioxidant is applied. Antioxidant activity is assessed as a function of colour change intensity. The 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical scavenging test was used to test the antioxidant activity of curcumin and quercetin that had been encapsulated in chitosan. UV-Vis spectrometer was used to measure the absorbance at 734 nm (Shimadzu, Japan). The antioxidant's IC50 was determined. [43]. To determine the radical scavenging activity (RSA) formula RSA (%) = [(AcontrolAsample)/Acontrol] /100 is used

3.8.2 ANTIBACTERIAL ACTIVITY

The antibacterial action of NP and NPC will be tested using different gram-positive or negative bacteria by Antibiotic Sensitivity Test (AST) was performed using Kirby Bauer Method at different concentrations and the zone of inhibition was calculated using diameter.

Performing AST-

- Loaded the hydrogel beads with silver nanoparticles synthesized from *Tridex procumbens* plant extract.
- Performed anti- microbial susceptibility test of the loaded and unloaded beads with (*E.coli, S. aureus, B. subtilis, P. aeruginosa, and baker's yeast or S. cerevisiae*)
- A zone of inhibition was observed in the hydrogel that were loaded with nanoparticles.

CHAPTER- 4

RESULTS AND DISCUSSION

4.1. Optimization of Hydrogel

- The hydrogel was optimized and the concentration and volume of keratin and PVA were optimized.

- The optimized hydrogel was freeze dried and lyophilized and stored for further use.

Fig 5- Lyophilized hydrogel samples- A Showing whole hydrogel, B. Showing the beads cut from the hydrogel, C. Showing lyophilized hydrogel beads.

4.2. Test for Hydrophilicity of the hydrogel-

- This was done using a tensiometer.

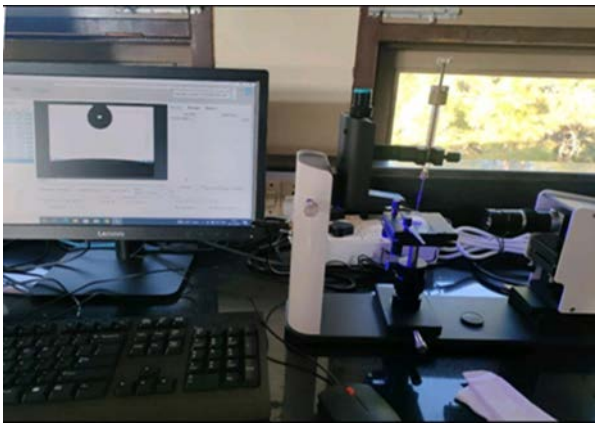

Fig 6- Testing hydrophilicity using a tensiometer

4.3. Test for Elasticity of Hydrogel-

- The synthesised hydrogel was stretched and that was noted to see the elasticity of the hydrogel.

Fig 7- Stretching the synthesised hydrogel

4.4. Optimization of AgNP

- The AgNP were optimized and was noted that the best reaction and peak was visible at 10mM AgNO3 concentration, 8.1 pH, 40oC and for 25 mins, in an orbital shaker that was set at 110 RPM.

Fig 8- Initial colour of the reaction mix after mixing

Fig 9- Final colour of the reaction after incubation

4.5. Characterization of Ag NP

• UV Vis Spectroscopy – the Ag Np synthesized were initially characterized by UV Vis Spectroscopy and the characteristic peak around 450 nm was seen which is typical of Ag nanoparticles.

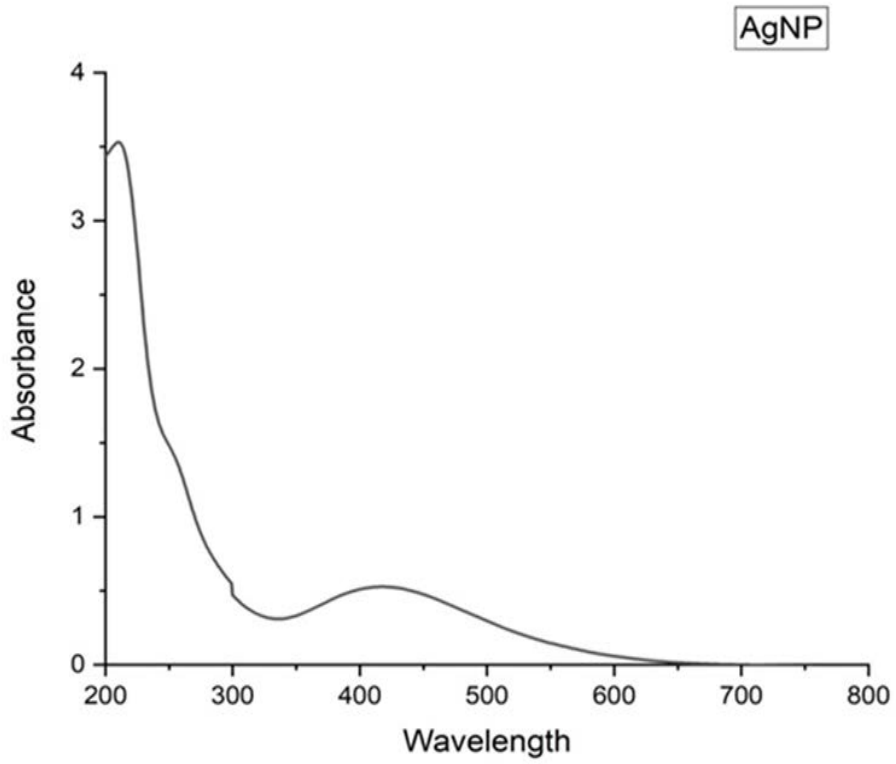

Fig 10- Spectrometric peak of synthesised AgNP

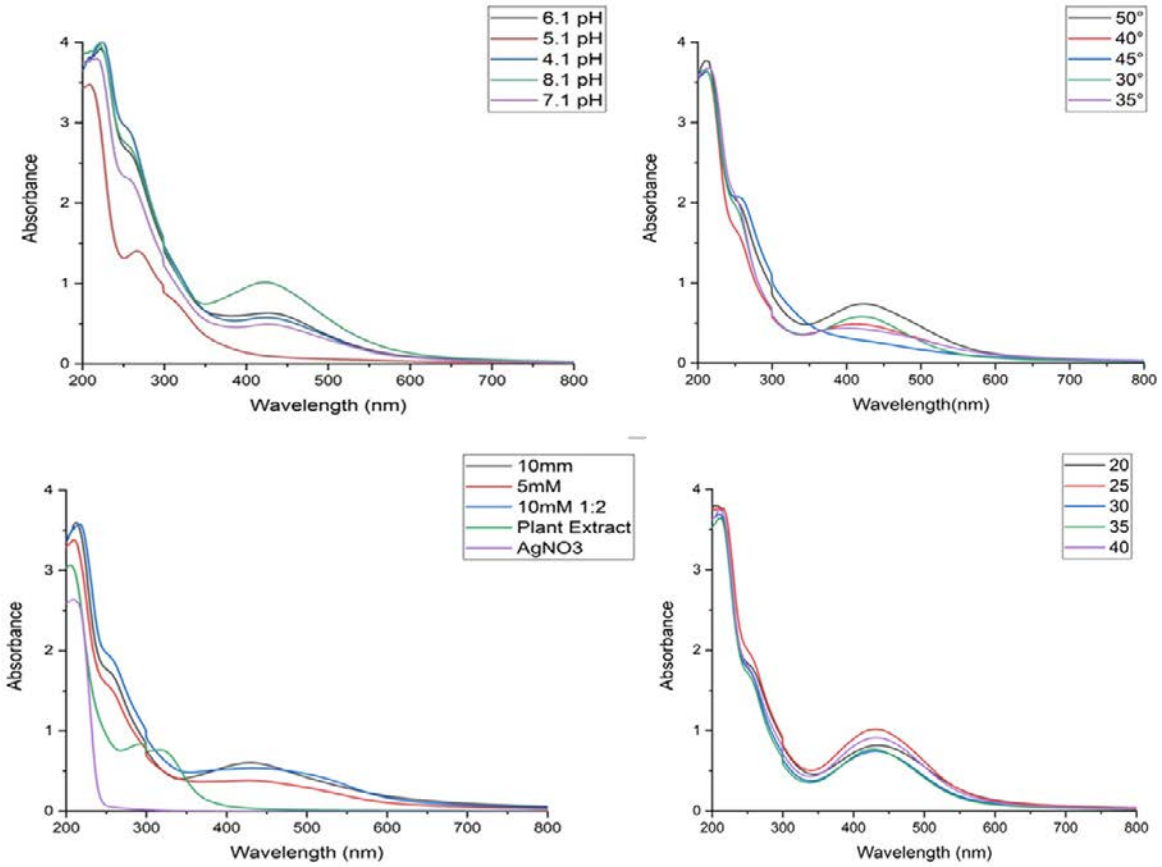

Fig 11- Optimizing of AgNP, Graph A show UV image that depicts the most stable pH for AgNP is around 8 ,B. The nanoparticles were synthesized at various different temperature, and the best peak was observed at 50ºC., C. Various different concentration of AgNO3 were used, along with different ratio of salt and Plant Extract, and it was found that the best reaction was seen at 10mM Agno3 and 1:1 salt: Plant Extract, D. The reactions were kept at various time to get the best result, and the best peak was observed at 25 mins.

4.6. ANTIMICROBIAL ASSAYS

The Antimicrobial activity of hydrogel loaded with silver nanoparticles was investigated against *E. coli* (ATCC25922), *E. coli DH5α* (ATCC68322), and *Staphylococcus aureus* using disk well diffusion assay

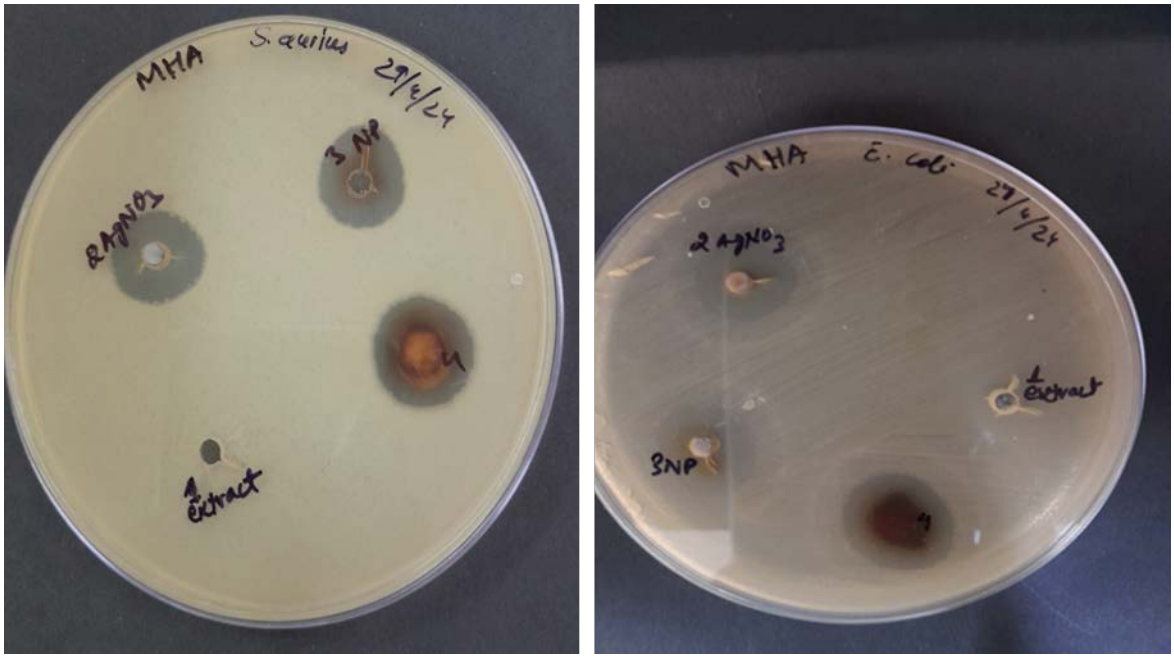

Fig 12- Antimicrobial Susceptibility test of NP and Hydrogel loaded with NP

- Swelling behaviour of Hydrogel

Time	1 hour	2 hours	3 hours	4 hours	24 hours
Control	14.56	9.417	14.66	14.07	16.50
Sample 1	11.64	12.95	11.90	15.19	9.99
Sample 2	13.10	8.06	10.35	12.19	8.79
Sample 3	40.20	13.91	13.94	18.04	8.3
Sample 4	2.40	-	2.89	2.69	0.28

4.7 Preparing band-aid from Hydrogel.

The hydrogel was made and lyophilized, and then they were cut in small disc, and applied on a band-aid, and then were loaded with green synthesised AgNP. Which can be used as an alternative band-aid for enhanced wound healing.

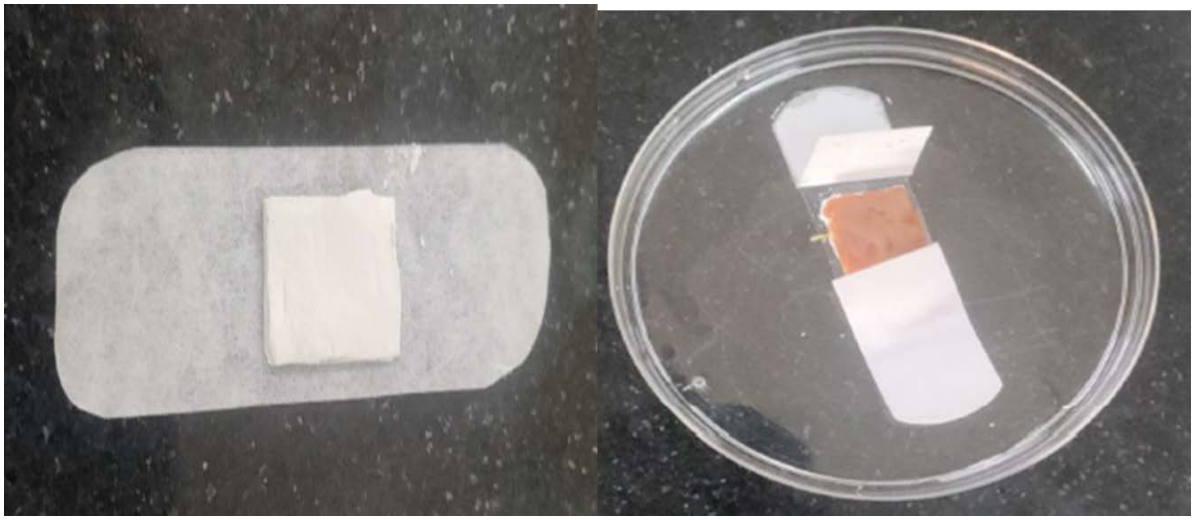

Fig 13- Hydrogel loaded with AgNP in a band aid.

CHAPTER- 5

CONCLUSION

The synthesis and characterization of keratin-based hydrogels infused with silver nanoparticles have unveiled a promising avenue for biomedical breakthroughs. By harnessing the power of green synthesis, researchers have developed a sustainable and eco-friendly approach to creating these advanced biomaterials.

Through the strategic combination of keratin, polyvinyl alcohol, and silver nanoparticles, these hydrogels exhibit a unique set of properties, including antimicrobial activity, biocompatibility, and tunable mechanical and swelling behaviour. The incorporation of silver nanoparticles not only imparts antimicrobial properties but also enhances the overall performance and efficacy of the hydrogel system.

Comprehensive characterization techniques have validated the successful synthesis and incorporation of silver nanoparticles, unveiling their structural, optical, and chemical properties within the hydrogel matrix. Furthermore, in-depth evaluations of biocompatibility, cytotoxicity, and in vivo efficacy have demonstrated the potential of these hydrogels for various biomedical applications, such as wound healing, drug delivery, and tissue engineering.

While the research in this field has yielded promising results, there is still room for further optimization and exploration. Future directions include refining the synthesis process, conducting in-depth biocompatibility studies, exploring controlled release kinetics, creating multi-functional hydrogels, and developing scalable manufacturing processes for commercialization.

As we continue to unravel the potential of these innovative biomaterials, we move closer to transformative solutions that could revolutionize the biomedical field, offering new hope for improved patient outcomes and enhanced quality of life.

The development of keratin-based hydrogels loaded with silver nanoparticles represents a significant stride towards the realization of innovative and effective biomedical solutions. By harnessing the synergistic properties of biocompatible keratin and antimicrobial silver nanoparticles, these nanocomposites hold immense potential for applications in wound healing, drug delivery, tissue engineering, and

beyond. As research in this field continues to advance, the future promises to unveil a new era of biomedical innovation, where Ag-K hydrogels play a pivotal role in improving patient outcomes and enhancing overall healthcare.

Future Perspectives

While the developed Ag-K hydrogels exhibit promising characteristics for biomedical applications, further research is necessary to fully unlock their potential.

- Optimization of Synthesis Parameters: Exploring different synthesis conditions and parameters could lead to improved control over the hydrogel's properties, such as swelling behaviour, mechanical strength, and degradation rate, tailoring them for specific applications.
- In Vitro and In Vivo Biocompatibility Studies: Comprehensive in vitro and in vivo biocompatibility assessments are crucial to ensure the safe and effective use of these hydrogels in biological systems. These studies will provide valuable insights into the hydrogels' interactions with cells, tissues, and physiological environments.
- Controlled Release Studies: Investigating the controlled release behaviour of various therapeutic agents, such as drugs, growth factors, or proteins, from the Ag-K hydrogels will enable the development of efficient and targeted drug delivery systems.
- Exploration of Synergistic Effects: Combining the Ag-K hydrogels with other bioactive compounds or materials could potentially lead to synergistic effects, further enhancing their therapeutic efficacy and expanding their applications in biomedicine.

By addressing these future directions, researchers can unlock the full potential of keratin-silver nanocomposite hydrogels and contribute to the development of innovative and effective biomaterials for various biomedical applications, including wound healing, tissue engineering, and drug delivery.

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