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Synthesis And Applications Of Keratin Nanoparticles: Recent Advances

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Abstract
Keratin, a biodegradable and biocompatible protein derived from hooves, horns, wool, feathers, hair and other keratinous bio materials, has gained amassed attention in nanotechnology due to its inherent biological activity and structural functionality. Keratin nanoparticles (KeNps) have emerged as a promising candidate for biomedical, pharmaceutical, and environmental applications due to their innocuous nature, surface modifiability and excellent cellular interaction. This paper explores the current progressions in the synthesis techniques of keratin nanoparticles, comprising chemical, physical and green synthesis approaches. Key applications of KeNps in bio sensing, wound healing, drug delivery, tissue engineering, environmental remediation and challenges and future projections have been reviewed

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I. Introduction

Keratin belongs to a very diverse family of fibrous protein which represents a group of cysteine rich filament-forming proteins. Keratin serve as a shielding layer for the animals epidermal appendages like hair, fur, nails, scales, feathers, hooves, wool, claws, beaks, snake skins and horns [1]; [2]; [3] [4]. The cysteine residues constitutes 7-20 % of the protein [5]. Due to its high cysteine content, keratin forms strong disulfide covalent bonds, responsible for mechanical stability, chemical resistance and thermal stability [2]. The crucial role of keratins is to provide support and strength to the structural design of cells, deliver a scaffolding for the cytoskeleton of tissues and cells and mechanical functions to uphold the structure under mechanical stress, shielding animal's organs and averting loss of body fluids [6]. Globally, it is estimated that industries such as the wool, poultry and meat produce tens of millions of tons of keratin waste each year of which feather waste accounts for nearly 8.5 million tons [7]. Keratinous material is non-edible because of abundant cross linking of disulfide bonds that prevent its digestibility by humans and animal enzyme thus forming substantial quantities of solid waste that pose severe environment pollution. The vital keratin sources are depicted in Fig.1, and their varied applications are tabulated in Table 1

Table 1 Keratin sources and their different applications

S. No.	Keratin Sources	Application	References
1	Hooves	Tissue engineering, nanoparticles, synthesis and stabilizing of nanoparticles, Biomedical applications	[8]; [4]; [9]; [10]
2	Wool	Textile dyeing, hydrogels, drug carriers, facilitating dermal fibroblast cell adhesion	[10]; [11]; [12]
3	Feather	Heavy metal adsorbents, thermoplastics, wound treatment, Pharmaceuticals, cosmetics,	[5]; [9]; [13]
4	Nails	Scaffolds for bone tissue engineering	[14]
5	Fur	Bio adsorbent, stabilizing nanoparticles,	[15]; [16]
6	Fish scales	Bone tissue engineering	[17]
7	Human hair	Adsorbents for heavy metals and dyes, cell proliferation Nanoparticles synthesis, Hydrocolloids for wound dressing	[18]; [19]; [13];[20]

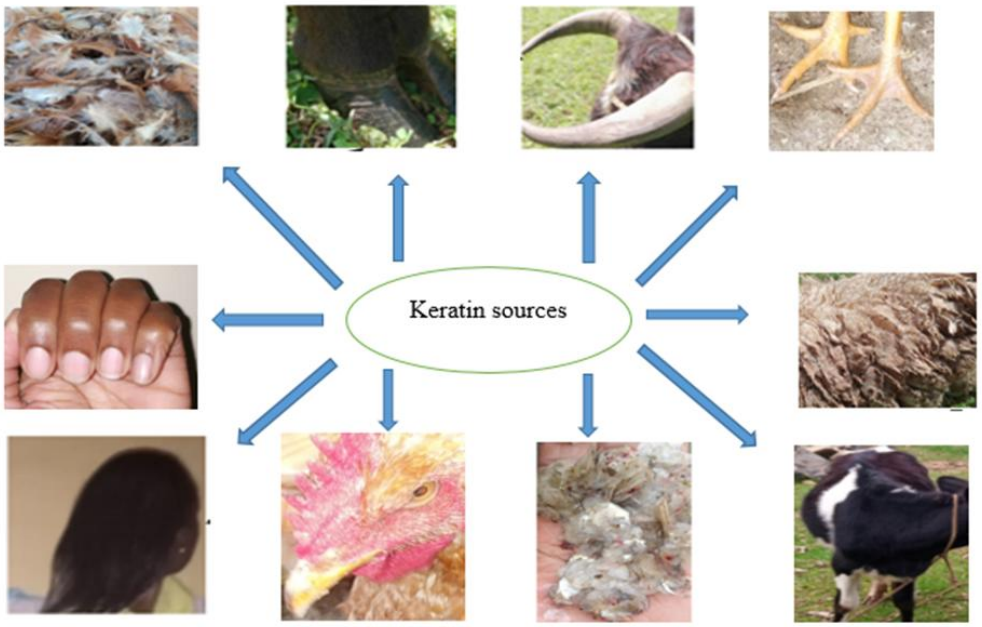

Fig 1: Sources of keratin

Keratin is a polypeptide consisting of amino acids having intermolecular bonding of cysteine and few intramolecular bonding of polar and nonpolar groups [9]. It exists in three different forms: α-, β-, and γ-keratin. α-keratin has a structure which is a left-handed alpha-helix that is coiled with other keratin molecules to form a polymerized complex. The α-keratin have intermediate filaments which are involved in the cytoskeleton. α-keratin is found in mammals' soft tissues and is the chief constituent of claws, hair, hooves, stratum corneum and wool [21]. β- keratin are located in hard tissues such as shells, scales, claws, beaks. γ-keratin is a form of keratin which is not involved in the cytoskeleton structural elements [22]. The most plentiful forms of protein are α-keratin which forms α-helix structure and β-keratin which forms β-pleated sheets [23]. Keratin-based supplies are exceptionally biocompatible, easily decomposable, has a rich tapestry of functional groups and have ability to provide support for cellular functions making it ideal for biomedical applications, biofertilizer and viable raw material for crafting biodegradable and ecologically friendly biopolymer-NP nano-composites [21];[23]; [24].

Based on unique molecular structural characteristic of keratin which includes high concentration of thiol groups and disulfide crosslinking, keratin facilitates the binding of metal ions onto the protein to obtained metal-nanoparticles. Nanotechnology encompasses designing of materials, manipulation and control of matter on nanoscale proportions [25]. Nano-sized particles are tiny constituent parts with a size range (10^{-9}-10^{-7}) m [4]. They are particles with at least one dimension less than 100 nm, [26]. Nanoparticles have distinctive properties likened to their bulk materials owing to their shape, dimensions morphology and dispersal [27]. Presently, keratin biomaterial is converted to nano-sized particles to expose new potentials, improving keratin's remarkable characteristics such as the versatility due to low cell opposition, upgraded surface area and good cellular penetration [23]. Synthesis of keratin nanoparticles involves extraction of keratin from the keratinous biomass using techniques such as sulfitolysis, hydrolysis using enzymes, superheated water, strong acids and alkalis, use of ionic liquids, oxidative and reductive approaches [28]. Nano sized keratin particles are obtained through various fabrication techniques such as electro spraying [12] self-assembly or aggregation, ionic gelation [29], electro spinning, spin coating, drop casting, freeze drying, soft lithography, nano imprinting, ultra sonication, inkjet printing [30], desolvation and controlled precipitation methods [28].

Keratin nanoparticles maintain the biological relevance, functional adaptability and mechanical firmness in addition to other benefits such as enhanced bioavailability, controlled release of therapeutic agents, targeted drug delivery and larger surface area. This makes the nanoparticles suitable for applications in biomedical, environmental remediation and cosmetics and other technological fields [31]. Keratin nanoparticles (KeNps) can encapsulate both hydrophilic and hydrophobic molecules owing to their hydrophilic and hydrophobic nature and their surface can be functionalized for precise applications [32].

II. Keratin Extraction

Keratin is one of the most plentiful existing bio-renewable biopolymers and favorite bioresource for production of bio-based materials [9], [33], [34]. With the hasty worldwide progress of the rigorous breeding industry, large-scale breeding of livestock yields millions of tons of keratin. Keratin from wool, hair (α-keratins)

and feathers (β-keratin) have been extensively scrutinized owing to their massive obtainability from fur processing, slaughterhouse, poultry and tanning industries and their subsequent prospective for large-scale usage [6], [35], [36]. Wool and feathers contain up to 95 % of keratin [21]. Chicken feathers comprises of up to 91 % of keratin, while human hair is contains 65- 95 % of keratin [37]; [38]. Chemical structure of a cattle hoof shows that it has about 90% crude protein which is predominantly α-helical conformation with an assortment of β-sheet [39]. Extraction method strongly dependent on form of keratinous biomass, nature of extract solution and the reaction approaches variables. Among the extensively used methods in keratin extraction includes chemical biological, microwave heating and Steam flash explosion.

Chemical methods

Extraction of keratin can be achieved by cleavage of hydrogen inter-chain links and covalent bonds [32]. The four main types of chemical approaches includes: oxidation, reduction, use of ionic liquids and hydrolysis [9]. There are chemical approaches which cleave disulfide links using chemicals such as mercaptoethanol, peroxides, dithiothreitol and sulfites which are detrimental, often noxious and problematic to handle. Other approaches can be physicochemical basing on diverse reaction settings that can break down the bio protein such as superheated water treatments, hydrothermal, microwave-assisted methods or steam explosion and use of ionic liquids [30].

Oxidation approaches can be employed using oxidative reagents such as peracetic acid to produce oxidized form of keratin known as keratose, Reduction methods using reducing agents such as thioglycolic acid, sodium m-bisulphite, dithiothreitol and sodium bisulphite tailed by NaOH treatment [40]; [6];[3]. Scholars are now attentive on new eco-friendly techniques of extraction using high density steam explosion and use of L-cysteine for keratin dissolution [30]. L- Cysteine with the chemical formula HOOCCH (NH_2) CH_2SH, is α- amino acid. Thiols of L- Cysteine, (SH) group take part in redox reactions, in which it gets oxidized to protein disulfides. L-cysteine can be used in place of 2- sulfanylethanol since it can give a good yield of undamaged keratin [41]; [30]. L-cysteine can be oxidized or may form disulfide bridge R-S-S-$CH_2CH(NH_2)COO^-$. The disulfide link is broken in keratin as shown below:

$$R\text{-}S\text{-}S\text{-}R + 2\text{-}SCH_2CH(NH_2)COO^- \longleftrightarrow 2RS^- + {}^-OOC(NH_2)CHCH_2S\text{-}SCH_2CH(NH_2)COO^-$$

Biological methods

Biological methods such as enzymatic hydrolysis using microbial fermentation and keratinase a proteolytic type of enzymes from bacteria, actinomycetes and keratinophilic fungi are ecofriendly [30]. Keratinase are found in the membrane, inside or outside of the cells. The *bacillus* types such as *B. subtilis* and *B. pumilus* have shown a prospective for commercial scale production of the enzyme [42]. Other microorganisms which breaks down keratin are *Chryseobacterium, Amycolatopsis, Fervidobacterium Lysobacter Kocuria, Thermoactinomyces, Stenotrophomonas, Staphylococcus, Vibrio, Streptomyces* [3]. Their activity has been reported from low to high pH and temperatures [43]. The enzymes break down hard keratin converting them to high nutrient proteins hydrolysates. The biodegradation yields are amino acids and peptides [42]. The technique involves low energy intake hence there is no damage to essential peptides such as lysine, methionine and tryptophan which are thermo sensitive [44]. The technique is safe, simple, produces a high yield and it is eco-friendly nevertheless, the technique involves high cost of production, poor reproducibility and the microbial disintegration can effortlessly damage the peptide links in the structure giving a yield to keratin with low molecular weight [45]. The use of enzymes for industrial application is slowed down by the long production cycle of enzymes methods [30].

Microwave heating

Microwave (MW) technique is an extension of superheated technique carried out in existence of an alkali or an acid in a heated container at 170°C – 180 °C for 60 min. During the irradiation process, the activation energy for extraction of keratin is lowered. The radiations penetrate deeply into the keratin structure and destabilize disulphide links [46]. It is a green extraction technique in which absorbed microwave electromagnetic radiations are altered to heat energy, which ensures uniform thermal effect with no temperature gradient [47]. The biomolecules energy uptake occurs evenly with temperature rise in the reactor. It is an efficient process due to homogeneous heating attained in a short time. [21]. Pulidori et al., used microwave radiations applied using coaxial dipole antennae in extraction of keratin from feathers in acetic acid 70 % v/v loaded in a MW supported vessel wrapped in a metallic grid to prevent loss of MW. Using extraction power of 90 W with nonstop stirring at a temperature of 104.6 °C, the effect of extraction time, solvent/ feathers ratio and heating modes (MW heating and conventional heating) were investigated [48]. By collecting the non-feathers, drying, determining it's mass and comparing with the initial mass of feathers used, the % yield of hydrolysis was reported. From the findings, Pulidori and coworkers reported a highest yield of 26 ± 2 % using MW heating, solvent to feathers ratio 150:1 and a contact tie of 5 hours [48].

Steam Flash Explosion (SFE)

SFE is a hydrothermal technique which uses saturated steam under high pressure for small intervals of 1-10 min on keratin material kept at a temperature of 180 °C -230 °C in the reactor. When the pressure is lowered rapidly towards the end of the treatment, an explosive decompression and rapture of the biomaterial fibers occur [21]. It is a physical technique which involves discharge of steam under high pressure in a taped-up container. The technique breaks disulfide cross links without altering the fibers' chemical composition, although raising steam pressure lowers thermal decomposition energy and fiber crystallinity [6]. The method is advantageous since perilous chemicals are not involved, though the process may be limited by resultant large particles sizes in most of the resultant solid matrix [46]. Wei and coworkers extracted keratin from goat hair using stem explosion by spraying the hair with water 100 % (w/w, basing on goat's weight) and positioned in a test bed chamber for explosion. The steam pressure was adjusted from 1.3 to 1.8 Mpa and temperature adjusted from 465 K to 480 K for treatment duration of from 20 to 30 seconds. The extracted keratin was frozen in liquid nitrogen and disintegrated using a disintegrator set at a rate of 25,000 rpm to get the powder used in removal of dyes in dyeing waste water [16].

Synthesis of Keratin nanoparticles

Various techniques have been employed in synthesis of keratin nanoparticles. The fabrication technique and settings affect nanoparticles' shapes, weight and sizes. The technique selected should tune the nanoparticles for precise application [22]. Some of the synthesis techniques includes:

Desolvation Technique

The most common technique, involving the adding of a desolvating agent (e.g., ethanol or acetone) to an aqueous keratin solution, resulting in particle precipitation. Crosslinking agents like genipin or glutaraldehyde are habitually used to stabilize the nanoparticles [29]. Khamees et al. synthesized keratin nanoparticles using 1µL of 8% glutaraldehyde and 8 mL of ethanol on 100 mg of chicken feathers' keratin in 2 mL of water and obtained nanoparticles with sizes 9-10 nm after 24 hours on a stirrer at 40 rpm. Nanoparticles obtained were centrifuged for 20 min at 20 000 rpm and lyophilized [49]. Nanthavanan et al. synthesized nanoparticles with a particle size of 79.6 nm using the same technique to obtain nanoparticles which were centrifuged at 10000 rpm for 20 mins before lyophilization [50]. Desolvation has also been used in synthesis of keratin nanoparticles which were infused with other substances or as drug carriers. For instance Martella et al. obtained spherical keratin nanoparticles with sizes (115.6 ± 24.0 nm) using 2.92 µL of 8 % glutaraldehyde (0.4 µL/mg) on wool keratin. Nanoparticles obtained were centrifuged at 120,000 rpm and aggregated with paclitaxel mixed with ethanol in the dark for 1 hour followed by centrifugation at 12,000 rpm. Paclitaxel loaded with keratin with a magnitudes of~150 nm hydro diameter were obtained [51].

Electro spraying (Electrodynamic spraying)

Electro spraying is a technique based on high voltage in which keratin solutions are atomized into fine droplets that form nanoparticles upon solvent evaporation. Nanoparticles size is influenced by electro spraying settings such as feed rate, polymer concentration and nozzle collector distance [28]. In electro spraying, an electric field (15-25 kV) applied on droplets generates electric charge on keratin solution drops producing a coulomb force. When the coulomb force overcomes the cohesive forces between the solution molecules, surface tension diminishes and nanoparticles are formed [29]. Electro spraying has the following advantages; First, the process can be conducted at ambient pressure and temperature situations, which is beneficial for sensing biomolecules and living cells, secondly, encapsulation efficiency can be maximized using the technique due to the probable nonexistence of external medium which permits migration or dissolution of water-soluble cargos, and thirdly the size dispersal is narrow. These makes the technique appropriate for nano medicine [52]. Keratin sponge is dissolved in formic acid (0.5 % w/v) at 70 °C for 24 hours, to prepare electro spraying solution [28]. Nanoparticles with size range 32-72 nm obtained through electrospraying of keratin extracted from wool keratin has been reported by Ebrahimgol et al. [53]. Guo and colleagues in 2019, obtained keratin-coated Keratin coated- polyvinyl alcohol (PVA) nano sized fibres by electro spraying keratose solution extracted from human hair at 15 kV with a volume rate set at 0.1 mL/hr, and a distance of 12 cm from tip to collector. KeNps with size distributions 250-350 nm were obtained [54].

Self-Assembly

Utilizes the amphiphilic nature of keratin, which self-assembles into nano-sized subdivisions under controlled pH and temperature. Self-assembly ensues when a disorderly system impulsively forms a well-organized assembly due to specific interactions amid species [29]. It arises due to attachments of chemical groups [55]. The technique is conducted under mild settings, doesn't involve high temperatures or toxic solvents [29]. In self-assembly, nanoparticles with small dimensions are attained due to the balanced composition of hydrophilic

and hydrophobic amino acid ends [18]. Local interactions between molecules due to electrostatics, disulfide linkage, van der Waals and and hydrogen bonds which are commonly weak allows for keratin protein self-assembly to be formed [29];[30]. Abbasi et al. fused keratin nanoparticles using human hair keratin using self-assembly technique through a 3 days dialysis of keratin solution using a cellulose tubing at 12 °C. The resultant solution was lyophilized to get keratin nanoparticles [18].

Micro emulsion

Micro emulsion encompasses addition of a surfactant to a polar and non-polar solvents such as water-in-oil emulsion, nanoparticles formation in the solvents is followed by crosslinking and purification [56]. Micro emulsions consist of nanometer-sized water drops disseminated in organic phase [29]. Co-surfactants and surfactants stabilize the boundary of the polar and non-polar solvents [57]. The surfactants produce aggregates due to called reverse micelles which stabilize the system [29]. The reverse micelles in the oil or organic phase is due to dispersion of water nano-drops in the oil part (w/o) micro emulsion. Water nano-parts constantly collide due to Brownian motion and combine stimulating inter-micellar exchange enabling micellar exchange. Nanoparticles' constricted sizes dispersal and shapes are regulated by adjusting composition of water or protein concentration, oil and surfactant mixture using micro emulsion technique [57]. The technique has a good size regulation however the surfactants and organic solvents need to be removed after the process [29].

Green Synthesis

Green synthesis involves use of biopolymers or plant extracts as reducing and stabilizing agents, eliminating the need for noxious reagents [58]; [27]. Microbes such as yeast, bacteria, algae and fungi acts as substrates [59]. Enzymes secreted by microbes delivers extractability for the bio synthesis of nanoparticles which is alternative step to green synthesis which omits stabilizing reagents. The *keratinases* from fungi or bacteria is more eco-friendly, but less frequently used for direct fabrication of nanoparticles [60].

Ultra-sonication

Ultra sonication is one of the top-down techniques in which keratin particles converts to smaller with sonication period [26]. It involves breaking down larger particles to nanoparticles using ultrasonic device [5]. In various researches, keratin nanoparticles synthesis through sonication has been reported for instance Cheng and associates synthesized nanoparticles with particle sizes (345.5 ± 15.3) nm through ultra-sonication by dissolving a varied ratio of kerateine (KTN) and keratose (KOS) in ultrapure water in different ratios and injecting the mixture into dilute HCl via a needle at a rate of 0.25 mL/ min. Ultra sonication was performed using a 400 W powered ultrasonic cell [40]. Mousavi and coworkers synthesized KeNps by dissolving keratin in water mixed with tris base to form a mild alkaline setting. The mixture was shaken at 400 rpm at room temperature for 16 hrs and sonicated by a sonicator rated 300 W for 10 mins [5]. In other studies, Shankar and Shiv fused KeNps by adding 1 L of chicken feather keratin solution drop wise to 200 mL of 1 M HCl in a bath type sonicator to obtain keratin nanoparticles with average size 47 ± 15 nm [24]. In other studies, Wang and coworkers synthesized keratin nanoparticles by dissolving chicken feather keratin in deionized water with different concentrations (5-12.5 mg/mL). A 20 mL of acetic acid (pH 3) was added for dispersing the solvent under sonication. KeNps with sizes 180.9 – 219.7 nm were obtained when keratin concentration was raised from 5 mg/L to 12.5 mg/L [61]. A summary of popular techniques of synthesis of keratin nanoparticles from various sources is presented in Table 2.

Table 2. Popular Techniques used for Synthesis of keratin nanoparticles from various sources

Method	Keratin Source	Mechanism	Particle Size	Strengths	Limitations	Reference
Acid Hydrolysis	Human hair	Sulfuric acid breakdown	25–75 nm	Fast, efficient, single-step	Strong acid handling, dialysis needed	[23]
Desolvation + Sonication	Feather (glycol-water)	Solvent precipitation + sonication	200–800 nm	Mild, scalable, tunable size	Requires ultrasonication, dialysis	[5].
Ionic Gelation	Wool	Electrostatic complexation	~176 nm	Solvent-free, drug-loading built-in	Limited to cationic cargo systems	[62].
Desolvation + Crosslinking	Feather	Alcohol precipitation + glutaraldehyde	~70–80 nm	Consistent size, antibacterial effects	Use of toxic glutaraldehyde	[50]
Sonication-Assisted	Feather-derived	Ultrasonic fragmentation	Variable (fine)	Simple, minimal chemicals	May need optimization for uniformity	[63].

Isoelectric Precipitation	Wool keratin	pH-triggered protein precipitation	Micron to nano	Green, crosslinker-free	Can produce heterogeneous sizes	[12]

Industrial Applications of Keratin Nanoparticles

Drug Delivery

Nanoparticles have been verified to be worthwhile in medicine since they execute protective activity against drug degradation, upsurge drug bioavailability, regulate drug release, improve solubility of hydrophobic drugs, alter pharmacokinetics, selectively increase cellular uptake and target precise sites affected by the disease [29]. The properties of keratin nanoparticles such as biodegradability, biocompatibility, low immunogenicity and its physicochemical properties makes it suitable for production of nano carriers. KeNps have been used to deliver various drugs such as doxorubicin, aspirin, curcumin, and paclitaxel. Their capability to penetrate biological obstructions and sustain drug release makes them attractive carriers [13]. Paclitaxel (PTX) is one of the highly effective anticancer drug used however, its optimal efficacy is limited by low solubility in aqueous surrounding and side effects such as neutropenia, neurotoxicity and hypersensitivity. KeNps have a high loading ratio and able to load PTX lyophilic drug without need for purification [11]. For instance Martella and associates prepared keratin loaded paclitaxel (PTX-KeNps) using aggregation and desolvation approaches. Aggregation technique was achieved without need for toxic cross linking-agents. The effect of the PTX-KeNps, unloaded PTX and KeNps on cancer cells was investigated for 24 hours. Cell internalization of the drug was confirmed by transformed cell morphology due to inhibition of cell division through binding to microtubules cytoskeleton. The Loaded PTX-Ker retained activity while being explored. PTX-KeNps demonstrated superior cytotoxic blockage and oxidative damage of osteosarcoma cells compared to PTX ox keratin alone [51]. Foglietta et al. synthesized PTX-Ker using PTX and sheep wool keratin via aggregation method and investigated the efficacy of the loaded drug on tumor cells. The synthesized PTX-KeNps guaranteed a steady and regulated release of PTX. The PTX-KeNps inhibited tumor cells' viability and induced apoptosis [11]. Aluigi and coworkers prepared KeNps using sheep wool keratin loaded the nanoparticles using doxorubicin through ionic gelation and aggregation techniques in absence of cross linkers, surfactants or organic solvents. The efficacy of drug release was examined using unloaded doxorubicin (DOX) and keratin loaded doxorubicin (DOX-KeNps) through dialysis diffusion. Aggregation routine formed smaller nanoparticles with a lower loading capacity, while ionic gelation resulted in nanoparticles with a higher loading capacity and exhibited a pH-responsive drug release. The DOX-KeNps showed superior potential against human breast carcinoma cells than unloaded DOX [62]. In other studies, Costa and coworkers encapsulated terbinafide, an antifungal with adverse side effects when taken orally with keratin nanoparticles and investigated the controlled release of terbinafide from therapeutic functionalized textile in different biomimetic solutions which included acidic sweat solutions, micellar solution and PBS-buffer. By testing the activity of the functionalized textile on tychophyton rubmum, a distinguished inhibition was detected, with the highest release in acid sweat and PBS. The blend made it possible to develop new therapeutic textiles for activity against trichophyton rubrum [64].

Wound Healing

Keratin yields mechanically tough biomaterial which enhances cell proliferation for wound healing and supports skin regeneration [6]. It is considered to be a promising preference due to its intrinsic cell growth, proliferation ability, biodegradability, biocompatibility and natural profusion [13]. The improved wound healing due to KeNps is associated with vascularization, collagen deposition, remodeling and improved epithelization [20]. KeNps based is used in wound healing in form of films, hydrogels, nanofibres and 3 D blended scaffolds [30] KeNps-based hydrogels and films promote faster wound closure and lessen inflammation. Biomaterials from keratin based mucoadhesive drugs have been formulated in form of hydrogels, films, wound dressing and scaffolds [40]. Keratin enables self-assembly of rigid hydrogels or films for wound healing. Guo et al investigated the suitability of KeNps from human hair in accelerating dermal wound healing using wound models made at the dorsal surfaces on Sprague- Dawley rats for invivo studies. The wounds were enclosed with 0.5 mg KeNps and secured with Tegaderm film. A wound enclosed with Tegaderm film only was used as control. The wound treatments and Tegaderm covering changes were done every 3 days until wound healing was observed. The effect of KeNps on HaCaT keratinocytes cell proliferation for in vitro studies. Using 3-(4, 5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) assay, the cell proliferation was determined. The cells viability was measured using a microplate reader at 490 nm, with the untreated cells activity set at 100 %. Extend of proliferation on HaCaT cells with KeNps was compared with the proliferation of cells cultured without KeNps. The findings indicated that KeNps enhanced cell proliferation and improved dermal wound healing [20].

Tissue Engineering

Keratin has the possibility to interact positively with cells, improving the differentiation of stem cells [30]. The amino acid sequence in keratin, ARG-GLY-ASP and LEU-ASP-VAL is similar to those found on

extracellular matrix proteins. This exemplifies a source for formulation of biomaterials which are readily acknowledged by human body and heighten cell attachment [1]. It has been used in the form of films, scaffolds, nanofibres and 3D scaffolds joined with other constituents to produce antibacterial activity and better mechanical properties for skin repair, when incorporated to porous membranes with fibroin, it demonstrates enhanced cell adhesion providing a substitute guide to vascular tissue regeneration [30]. Keratin scaffolds can mimic the extracellular matrix, providing structural support for cell attachment and growth. Keratin nanofibres plays a pronounced role in tissue engineering due to their capability to reinstate the natural extracellular matrix role [21]. Applications include bone, vascular, cartilage and neural tissue regeneration [65]. In 2019, Guo et al prepared KeNps coated PVA nanofibres using human hair keratin by electro spinning and electro spraying approaches and used the KeNps/PVA on neural cells. The PC12 and C6 cells were cultured, detached and seeded on KeNps/PVA nanofibres. Fluorescence images were taken after 24 hours of incubation to determine the number of cells in KeNps/PVA and in control mixture containing uncoated PVA. Cell viability was conducted using MTT assay and measuring absorbance at 490 nm. KeNps/PVA showed an improvement in cell morphology, cell morphology, cell adhesion and increased cell viability compared to pure PVA nanofibres [54].

Biosensors

Nanotechnology has made it possible for sensitive biosensors to be formed [66]. Most of the worn-out sensors are released as electronic wastes which contaminate the environs. Hydrogel sensors are safe and biocompatible however to improving the mechanical strength of ionic hydrogels involves wastage of conductivity, hindering stable signal transition and sensitivity. Keratin hydrogels are ecofriendly, biocompatible, highly conductive and skin matchable [67]. Functionalized KeNps serve as bio recognition elements for biosensors due to their reactive surface groups and biocompatibility [68]. Keratin bio sensors are safe, highly conductive, ecofriendly and comfortable [67]. Humidity sensor based on keratin films has been reported by Hamouche et al,. The biosensor displayed small hysteresis and high sensitivity [68]. Gao et al., designed a sensor for movement detection using keratin hydrogel with a low noticeable strain of 0.25%, insignificant hysteresis swift responsiveness, and operational resilience, which were promising for gaining whole-body motion signals unceasingly [67].

Environmental Applications

KeNps is used as bio adsorbent due to the various functional groups such as the amino groups, carboxylic groups and other functional groups provides the binding sites for the removal of dyes and heavy metals in wastewater, offering a sustainable approach to environmental remediation [18];[15]. For instance, Abbasi et al., investigated the suitability of human hair keratin nanoparticles in removal of crystal violet dye. At optimum conditions, a maximum efficiency of 75.97 % in dye removal was attained.[18]. In other studies, Mousavi and coworkers investigated suitability of chicken feathers' keratin nanoparticles in removal of Cu^{2+} ions, and reported highest adsorption capacity of 30 mg/ g, with optimum dose of 3g/L of KeNps [5]. In 2024, Chowdhury et al., designed graphene oxide-keratin based nanocomposite which was effectively used in removal of dyes in tannery waste water. The removal efficiency was over 98 % within 20 minutes indicating that the nanocomposite was highly effective [15].

Challenges and Future Perspectives

A number of challenges have been reported on synthesis and application of keratin nanoparticles. Some of the challenges include scalability. It is hard to produce large quantities with uniform size and properties. The particle sizes obtained varies depending on the technique used to synthesize the nanoparticles and the reaction conditions [53]; [29]. Crosslinking toxicity may limit clinical applications. Use of toxic cross linkers such as glutaraldehyde initiate immune responses, induce calcification and devitalize cells. This is due to the presence of toxic aldehyde residues from the crosslinker [69]. Variability in storage stability of KeNps affects its application. The stability of keratin is influenced by environmental factors such as temperature, water and presence of salt ions. The situations influence the structural properties of keratin at nano scale. KeNps needs optimal storage settings to prevent degradation or aggregation [70]. Purity and standardization of KeNps varies. There is variability in keratin sources, synthesis techniques and conditions. This affects reproducibility of the results influencing the suitability of the particles for various applications [13].

Green synthesis and use of biocompatible cross linkers such as genipin instead of epoxide and glutaraldehyde to address noxiousness concerns [69]. Hybrid nanocomposites keratin can be combined with other biopolymers (e.g., chitosan, collagen) to improve functional properties. The nanocomposites have enhanced efficiency and helps to overcome the problem of handling and isolation of nanoparticles [53]; (Seghir et al., 2020). There is need for incorporation of smart drug delivery systems (e.g.,enzyme-triggered or pH-responsive KNPs) for personalized medicine. This will help in lowering the drugs' side effects and control drug release at the selected site, in order to improve healing effect [71]. Due to keratins' compatibility with the cells, efficiency in drug

delivery and its tenability, there is need for further in vivo studies and clinical trials to authenticate efficacy and safety [72].

III. Conclusion

The paper has outline of recent advances in synthesis and applications of keratin nanoparticles. Keratin nanoparticles exemplify a versatile and eco-friendly platform with significant potential in biomedicine applications in tissue engineering in form of scaffolds, films and nanofibres, drug delivery and environmental science as adsorbents for heavy metals and organic impurities. The nanoparticles are synthesized through various physical and chemical techniques. The ongoing development of safer, scalable synthesis techniques and multifunctional nanocomposites will pave the way for broader industrial and clinical applications. Future efforts should focus on translational research, standardization, and regulatory approval to harness the full potential of keratin-based nanotechnology.

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