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REVIEW

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Keratin nanofibers in tissue engineering: bridging nature and innovation

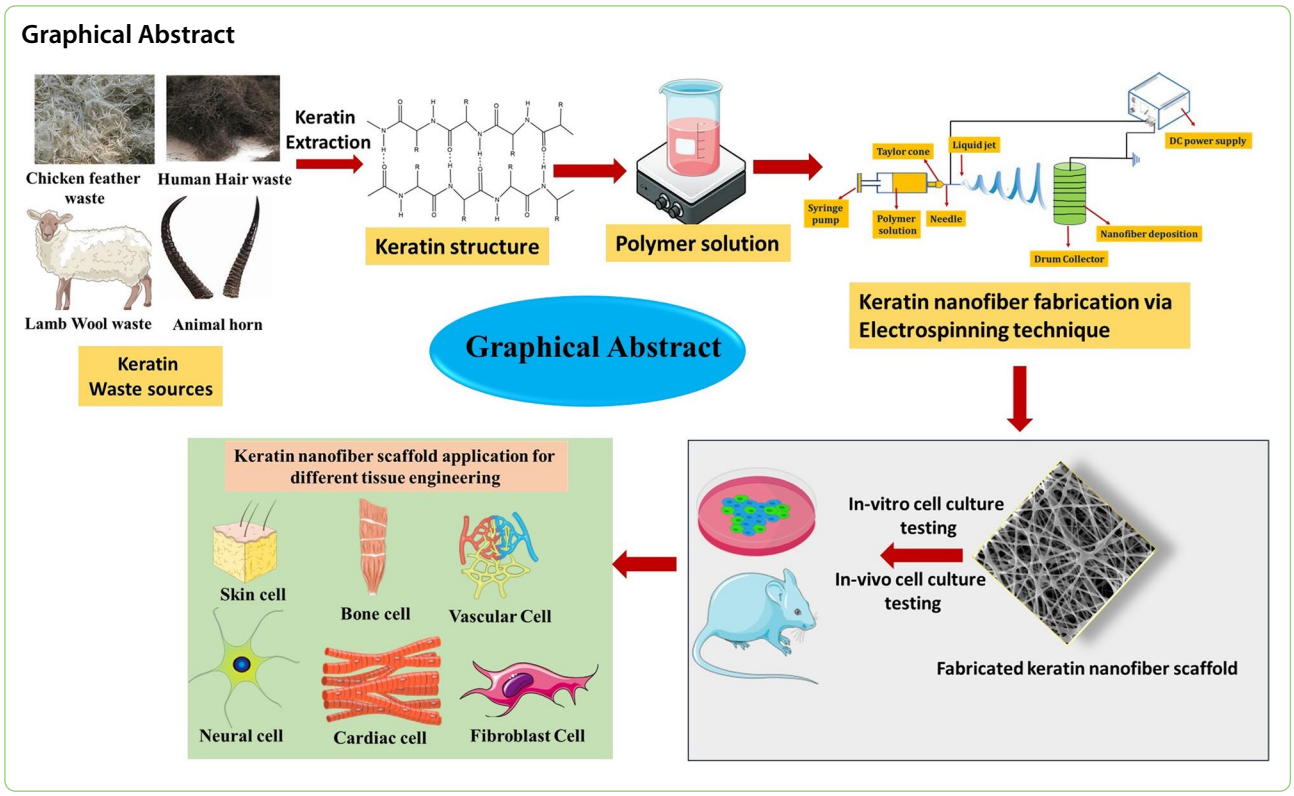
Keshaw Ram Aadil[1*], Khushboo Bhange[2*], Nitesh Kumar[1] and Gita Mishra[3]

Abstract

Tissue engineering and regenerative medicine are multidisciplinary disciplines that use technical and biological principles to create workable replacements for human tissues and organ function. Keratin, a protein found in materials like wool, feathers, and hooves, holds great promise for biomedical applications due to its unique properties. It is biocompatible, providing a suitable matrix for cell growth and tissue repair. Keratin's cysteine-rich composition facilitates cell attachment and growth, supporting the regeneration of damaged tissue. The method of electrospinning is a flexible and effective way for producing nanofibers. To generate fibers with a high surface area to volume ratio, this technique applies an electric field to draw charged threads of polymer melts or solutions. Further, the extraction, purification, and characterization of keratin proteins from hair and wool fibers have yielded significant advances over the past century, resulting in the development of keratin-based biomaterials platforms. Researchers have successfully fabricated keratin-based nanofiber scaffolds using electrospinning techniques, mimicking the natural extracellular matrix (ECM) and promoting cell infiltration and adhesion. These scaffolds have been investigated for different tissue engineering, with in vitro studies showing successful growth of skin cells on them, making them promising for wound healing and tissue repair. Keratin is a suitable biomaterial for scaffolds utilized in tissue engineering because of its biocompatibility, biodegradability, latent biological activity, and cellular binding designs. Understanding keratin nanofiber scaffolds' biocompatibility, biodegradation, stability in vitro and in vivo, and mechanism of action is essential for using them in more sophisticated applications, including clinical research. Additional research and development, in addition to advancements in related technologies, ought to create even more prospects for this versatile and fascinating biomaterial.

Keywords Keratin, Nanofiber, Tissue Engineering, Electrospinning

*Correspondence:
Keshaw Ram Aadil
kaadil7@gmail.com
Khushboo Bhange
khushboo27jan@gmail.com
Full list of author information is available at the end of the article



Introduction

An organ or tissue failing or being lost is one of the most common, catastrophic, and expensive issues in medical care. However, as the population ages and the number of new cases of organ failure rises, there is a critical scarcity of donor organs that becomes worse every year [6, 7]. An expanding multidisciplinary discipline called tissue engineering and regenerative medicine uses technical and biological principles to create workable replacements that preserve, enhance, or restore human tissues and organ function [8]. Researchers are currently using the concepts of bioengineering, material science, and cell transplantation to create biological replacements that can repair and preserve damaged or sick reproductive tissues [6, 7]. This technology is sometimes regarded as the most ideal medical treatment due of its many benefits as regrowing the patient's own organs and tissues free from severe immune rejection, inadequate biofunctionality, and poor biocompatibility is a unique characteristic of tissue engineering [45]. In recent years, a variety of methods and biomaterials have been developed to more closely resemble the microenvironment of native tissues [17]. Further, advancement in stem cell research creating new avenues for tissue engineering and cellular therapy. Adult stem cells show promise for tissue engineering applications [26]. Bioactive materials are now often used in tissue engineering and regenerative medicine applications. Tissue engineering combines cell biology, materials science, reactor engineering, and clinical research to develop new organs and tissues. The ideal conditions for cell proliferation, migration, differentiation, and framework function can be provided by a nanofiber and three-dimensional scaffold. Enhancing the scaffolds' surface and structural integrity could improve cell adhesion and proliferation [86].

The creation of cell scaffolds to encourage cell proliferation and differentiation for in vivo tissue regeneration is the first important technological advancement. In addition to giving cells a natural environment for growth and differentiation, or morphogenesis, extracellular matrix (ECM) also facilitates cell-based tissue regeneration and organogenesis [1, 88].

Nanofibers for tissue engineering

Nanoscale materials frequently exhibit unique physicochemical characteristics that offer exciting and promising prospective applications in tissue engineering. Nanomaterials are becoming more and more interesting due to their special qualities and the possible applications they could have in a variety of technical fields [58].

One of the main obstacles in the field of tissue engineering is creating scaffolds that, at the nanoscale,

Table 1 Summary of methods used for fabrication of keratin based nanofiber for biomedical applications

Methodology	Composition / Material	Application	Reference
Electrospining technique	Polycaprolactone/sodium sulphate alginate-polyvinyl alcohol (PCL/SSA)-PVA	Diabetic wound dressing /healing, drug delivery	[2]
Wool keratin, after reduction /oxidation of disulphide bond	Thioglycolic acid, dithiothreiotol and 2-mercaptoethanol	Fabrication of scaffold for tissue engineering and filtration devices	[91]
Electrospinning method	N,N-dimethilformamide (DMF) polymer solution	Drug delivery system, energy sector, textile, cosmatics	[84]
Feather keratin (FK)/PVA composite nanofiber electrospinning	Chitosan, poly e-caprolacton (PCl), formic acid and acetone	Antimicrobial active packaging, heavy metal ion absorption tissue engineering, wound dressing	[41]
PVA-Keratin nanofiber (PK-NF) using electrospinning technique	PVA, beta-mercaptoethanol, tris–HCl and SDS	Tissue engineering and drug delivery	[1]
Hydrolyzed keratin-based biomaterial and electrospinning process	γ-glycidyloxy-propyl-trimethoxy-silane (GPTMS) Gelatine – keratin hydrolizates solution	Development of *in-vitro* tissue model, skin tissue engineering	[36]
Sulphitolysis, a green method	PVA, polybutylene succinate, (PBS), polycaprolactone (PCL), poly lactic acid (PLA), gelatin	Dental implants, wound dressing, Producing nanofiber with antibacterial properties	[81]
Alkaline method for keratin extraction	Keratin dialysate (aq) and alkaline keratin dialysate	Wound healing of corneal epithelial was observed in vitro	[32]
K/PVA and K/PVA-PLA nanofiber using electrospinning method	Dichloromethane, Polylactic acid, Zinc acetate and sodium hydroxide	Pathogen killing (antimicrobial & antifungal property)	[74]
Electrospinning nanofiber	Mupirocin loaded core–shell pluronic-pectin-keratin nanofibers Pluronic, pectin, dimethyl thiazole diphenyltetrazolium bromide (MTT) powder	Skin tissue repair, Wound healing	[64]

resemble tissue architecture. The possibility of creating scaffolds that could solve this issue has been substantially expanded by the production of nanofibers. The development of nanofibers has raised the possibility of producing scaffolds that could be able to nanoscalely mimic the structure of natural human tissue. The high area surface-to-volume ratio and microporous structure of the nanofibers promote cell adhesion, proliferation, migration, and differentiation. All of these characteristics are crucial for tissue engineering applications [58].

At present, there are three methods used to produce nanofibers: phase separation, self-assembly, and electrospinning. When it comes to tissue engineering applications, electrospinning is the approach that has been investigated the most and has demonstrated the most promise [10, 12, 93].

In order to encourage cellular organization, contact guiding has been used to induce cellular elongation and orientation, and substrates with regulated micro- and nanopatterns have been created as supports for cell growth. For implantation in the host tissue, ultra-thin, micropatterned biodegradable substrates are preferred. However, these substrates must be easily retrievable, mechanically strong enough to offer sufficient support for the formation of new tissues, and maintain appropriate handling qualities [40]. Some materials are modified before their transformation into Nanofibers. For instance the degree of acetylation in chitosan affects a number of characteristics, such as its capacity to promote cell adhesion, differentiation and proliferation, inflammatory response, and rate of degradation [9]. A three-dimensional (3D) porous scaffold can offer the ideal environment for cells to proliferate, migrate, differentiate, and act as a framework. Enhancing the scaffolds' surface and structural integrity may boost cell adhesion and proliferation [86].

The extraction, purification, and characterization of keratin proteins from hair and wool fibers have yielded significant advances over the past century, resulting in the development of keratin-based biomaterials platforms. Keratin is a suitable biomaterial for scaffolds utilized in tissue engineering because of its biocompatibility, biodegradability, latent biological activity, and cellular binding designs. Extracted keratin proteins have been used to produce scaffolds for tissue engineering due to their capacity to self-assemble and polymerize into complex three-dimensional structures [86].

Recently, there has been an increase in interest in creating composite nanofibers by combining natural and synthetic polymers [89]. There are few examples of biomaterial based nanofibers have been described in Table 1.

Keratin structure sources and properties

Originally, the word "keratin" referred to a broad class of insoluble proteins that constitute the majority of cytoplasmic epithelia and epidermal appendage structures (such as hair, wool, horns, hooves, and nails) by aggregating as intermediate filaments (IFs) [32, 79]. Following further investigation into these structural proteins, mammalian keratins were divided into two groups according to their structure, function, and regulation. "Hard" keratins help give epidermal appendages their strong structure by forming ordered arrays of IFs encased in a matrix of cystine-rich proteins. "Soft" keratins give epithelial cells mechanical robustness by preferentially forming loosely packed bundles of cytoplasmic IFs. Despite the fact that the secondary structures of soft and hard keratins are closely similar, there are detectable variations in the filamentous structures due to unique amino acid sequence variances [79].

Further, keratin is a polypeptide chain consisting of two different formations: β-sheets and α-helices. Nevertheless, β-sheets are mostly present in the keratin that makes up a bird or reptile's feathers and claws. α-helices are typically present in the skin, nails, and hair of all vertebrates [80]. Keratin is a member of the scleroproteins, a family of fibrous structural proteins. This is the most prevalent structural protein present in animal hair, feathers, claws, nails, and horns. It has reduced amounts of tryptophan, methionine, and lysine but increased quantities of glycine, alanine, serine, cysteine, and valine. Additionally this biopolymer has high concentration of hydrogen bonds, hydrophobic contacts, and disulfide cross-links which give it a robust mechanical structure [62]. Unlike other structural proteins such as collagen and elastin, keratin contains a significant amount of cystine (7–13%) [4]. This distinct composition makes it an intriguing candidate for various biomedical uses. Keratin's cysteine-rich composition facilitates cell attachment and growth. It supports the regeneration of damaged tissue. It provides a suitable matrix for cell growth and tissue repair. Keratin's cysteine-rich composition facilitates cell attachment and growth. It supports the regeneration of damaged tissue. The presence of cysteine-rich proteins gives keratin its high sulfur group content. These proteins provide the fibrous structure's distinctive intra- and intermolecular disulfide connections, which give keratin-based products their good mechanical qualities and stability [81].

Keratin as a biomaterial in tissue engineering

Polysaccharide and protein are most favorable biomaterial widely used in biomedical mainly is tissue engineering and drug delivery applications. Among the various biomaterial keratin hold great promise for biomedical applications due to their unique properties. Researchers have successfully fabricated keratin-based nanofiber scaffolds using electrospinning techniques. These nanofibers mimic the natural ECM and promote cell infiltration and adhesion. Additionally, these nanofibrous meshes can also promote angiogenesis, cell adhesion, migration, growth, and differentiation, all of which are essential for the successful healing of wounds [63]. Keratin nanofiber scaffolds have been mostly investigated for skin tissue engineering. In vitro studies show successful growth of skin cells (keratinocytes, fibroblasts) on these scaffolds, making them promising for wound healing and skin repair [1, 57, 85]. Keratin's unique properties make it an

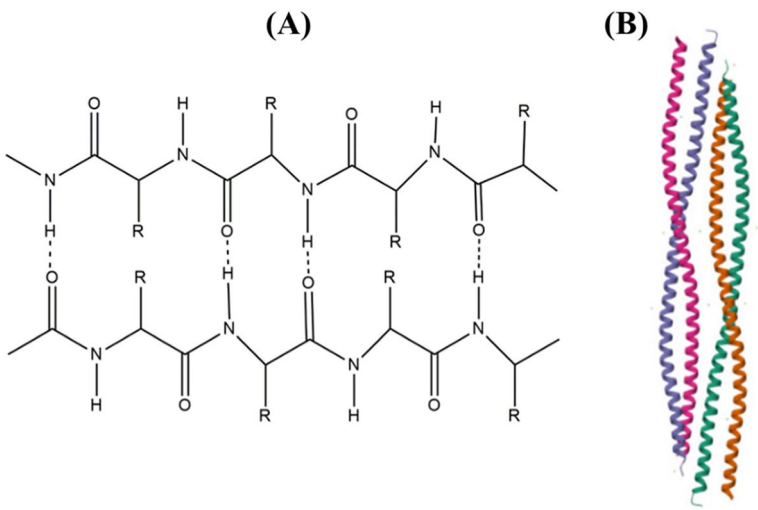


Fig. 1 Keratin molecular intra- and intermolecular interactions, (A), 3D helix structure of keratin (B) Adapted from https://www.rcsb.org/structure/6ec0. PDB DOI: https://doi.org/10.2210/pdb6EC0/pdb. Reproduced with permission of reference from (2023a). MDPI, Open Access

Fig. 2 Design parameters to be required for the synthesis of keratin nanofiber scaffold

attractive candidate for tissue engineering, especially in skin regeneration application.

The purpose of this article is to explore nanofiber scaffolds based on keratin for use in biomedical applications, specifically tissue engineering. Additionally, the production of keratin nanofibers for tissue engineering application will be covered.

Keratin extracted from has been shown in recent studies to promote the adhesion and proliferation of several cell morphologies, including as fibroblasts, osteoblasts, neuroblasts, and keratinocytes. This is solely related to the existence of cellular binding motifs that facilitate cell attachment, such as leucine-aspartic acid-valine (LVD), glutamic acid-aspartic acid-serine (EDS), and arginine-glycine-aspartic acid (RGD). These motifs repeat the interaction patterns that are supported by native Extra Cellular Matrix-like proteins (Fig. 1) [81].

In contrast to other naturally occurring polymers like starch, collagen, and chitosan, the intricate three-dimensional structure of keratin necessitates the use of severe chemical conditions in order to dissolve and extract it. The oxidation, reduction, steam explosion, microbiological approach, microwave irradiation, and use of ionic liquids are the most widely used techniques for keratin extraction [32]. The basic parameters required for the synthesis of keratin-based nanofiber scaffold is presented in Fig. 2.

Recent years have seen reports on keratin-based biomaterials for the regeneration of bone tissue. For instance, human adipose mesenchymal stem cells were able to self-differentiate into the osteogenic lineage without the need of induction agents thanks to scaffolds made of human hair keratin, jellyfish collagen, and eggshell-derived hydroxyapatite that were produced using a freeze-drying technique [5].

In another investigation, adult sheep's long bones were implanted with porous keratin-hydroxyapatite composite scaffolds for up to 12 weeks to compare the osteoconduction response to that of a collagen matrix. The rate of bone ingrowth into keratin-hydroxyapatite scaffolds was similar with the rate of keratin-hydroxyapatite resorption, and the scaffolds demonstrated a slower rate of degradation in vivo than collagen matrix [21].

This work aims to create a biocompatible and sustainable scaffold made of polycaprolactone (PCL) for bone tissue engineering by electrospinning, using keratin from human hair and calcium carbonate ($CaCO_3$) from *Pomacea canaliculata* shells, which are known to promote bone regeneration. The fibers are cytocompatible to Saos-2 osteosarcoma cells and instead, promote their

development and multiplication. The findings imply that, in comparison to pure PCL matrices, the novel addition of keratin and $CaCO_3$ into PCL nanofibers could serve as a bioactive matrix, presenting more potential for bone tissue engineering applications [29].

The potential of donkey gelatin and keratin to be combined with organic bioactive extracts like sumac, curcumin, and oak acorn to create antioxidant and antibacterial nanofibers that accelerate wound healing processes was investigated by Rapa et al. in [76]. The donkey nanofibers used in this study were based on sumac, and in an in vitro investigation, their cell viability significantly dropped to 56.25%. They also showed good biocompatibility. The nanofibers' diameters showed structural similarities to extracellular matrix components in humans, providing the ideal environment for tissue regeneration. During the first 10 min, there was a greater solubility of the sumac and curcumin extracts based on donkey nanofibers (74% and 72%). Further, study revealed that Acorn and sumac extracts displayed similar values to each other, while curcumin extract displayed similar antibacterial and antifungal performances to rivanol. Acorn and curcumin nanofibers showed significant migration rates of 89% and 85% after 24 h in vitro testing on murine fibroblast cells, respectively, highlighting these nanofibers' potential as flexible platforms for enhanced wound care applications [76].

Nanofiber fabrication techniques

Extraction of keratin for nanofiber

In contrast to other naturally occurring polymers like starch, collagen, and chitosan, the intricate three-dimensional structure of keratin necessitates the use of severe chemical conditions in order to dissolve and extract it. The oxidation, reduction, steam explosion, microbiological approach, microwave irradiation, and use of ionic liquids are the most widely used techniques for keratin extraction [32].

As keratin naturally is insoluble in water therefore it's essential to convert this protein into soluble form in order to produce nanofiber. Mostly keratin is extracted using hair, chicken feathers [83], wool [81]. They are usually purified by filtration and dialysis. Breaking the cystine (S–S) crosslinks is necessary to produce a soluble keratin. By breaking the disulphide covalent bonds into cysteine (single bond SH) and cysteine-S-sulphonated residues ($SSO3-$), keratin from wool is extracted using sulphite salts. This process, often referred to as wool sulphitolysis, yields an extraction yield of roughly 33 weight percent. Pure formic acid readily dissolves the S-sulphonated keratin [3].

Although acidic hydrolysis is quite effective, some amino acids, such as tryptophan, are destroyed throughout the process, leaving behind residue with little nutritional value. Amino acid loss is less in alkaline hydrolysis. According to a study, alkali hydrolysis using 2% NaOH at 80 °C for three hours produced a 25% yield. The pH, temperature, reaction time, and kind and concentration of acid or base used all affect the hydrolytic process' yield. Heating is sometimes used in conjunction with chemical hydrolysis to guarantee a high yield; however, excessive temperatures can cause the amino acids to be destroyed [89].

Keratin extraction is also facilitated by microorganisms like mesophilic fungi, actinomycetes, and some *Bacillus* species. For instance, savinase (a keratinolytic enzyme) extracts keratin from wool and feathers. Enzymatic hydrolysis, involving proteolysis, sulfitolysis, and deamination, is environmentally safe and cost-effective. This hydrolytic technique has gained popularity in industrial and biotechnological processes (Table 1).

Strategies to form keratin Nanofiber using electrospinning technique

In order to manufacture protein nanofibers, many researchers have chosen a bottom-up strategy, motivated by the self-assembly nature of cysteine-containing keratin proteins. These investigations have shown that keratin wool or feathers hydrolyzed in alkaline, enzymatic, or acidic solutions, or dissolved in ionic liquids, can decrosslink and detangle protein molecules, leading to the eventual regeneration of nanofibers [103].

Another technique that has been investigated for the effective and environmentally friendly isolation of keratin from wool waste is microwave induction. Sulfitolysis is a non-toxic, green method of extracting keratin, and electrospinning is used to spin the solutions into nanofibers [81]. Generating nanofibers can be done with ease and versatility using electrospinning technology. Furthermore, several groups of researchers have documented the creation of keratin nanofiber composites, in which the keratin protein-containing solution was collected from a natural source and electrospun or wet spun to assemble the nanofiber architecture in the composite [25, 89, 90]. The electrospinning process is a promising new method for producing biomaterials, as it allows solutions of polymeric material in a high electric field to be spun into uniformly thin nanoscale fibers [73]. Because of the distinctive material features and the increasing interest in nanotechnologies, electrospinning has attracted a lot of attention lately [51]. Briefly in electrospinning technique, a very thin jet of fluid is projected against the collector when a sufficiently high voltage is applied between a metallic collector and a needle, which overcomes the surface tension that holds a drop of liquid at the needle's tip. During the journey between the needle and collector, the

solvent evaporates, creating a nonwoven structure [9]. This technique is based on electrostatics principle, which uses the electrostatic repulsion forces in a high electrical field to synthesize nanofibers, is how the electrospinning process operates [52]. This approach has proven to be highly beneficial in the production of nanofibers for tissue engineering since it allows for the treatment of a wide variety of polymers to produce nanofibers with diameters ranging from 20 to 400 nm [9]. Unlike other approaches now in use, this process has shown to be dependable and economical, and it may find application in the large-scale manufacture of nanofibers [89].

In recent decades, it has been regarded as a comparatively excellent material system for the creation and design of surgical wound dressings [18]. Cui et al. showed that the electrospun crosslinked chitosan nanofiber had better mechanical properties, better wet stability, and good biocompatibility [18]. Behrens et al. used merely a commercial airbrush and pressurized CO_2 to create conformal nanofiber mats or meshes on any surface in situ using solution blow spinning [11].

Excellent mechanical characteristics including Young's modulus and failure point were demonstrated by PCL/keratin fibers [89]. SEM pictures of poly (ε-caprolactone) (PCL) keratin nanofibers fibers with varying mixes revealed homogeneous surface morphology. This superior surface shape, which is perfect for the growth and multiplication of cells [89]. The feather keratin and poly vinyl alcohol composite keratin Nanofiber had a fibrous and porous structure, among other common characteristics. For both PVA and FK/PVA fibers, the fibers were randomly aligned, smooth, and free of beads (Table 1) [41].

Keratin nanofiber functionalization

Several keratin materials have been used in attempts to create keratin nanofibers. In order to generate nanofibers, for instance, Sanchez Ramirez et al. dissolved wool keratin (WK) in formic acid at a concentration of 15–20 weight percent. These nanofibers had a distinctive structure and a sizable bead content. These WK nanofibers' circumferential orientation encourages contact with fibroblast cells, which speeds up the development of new tissue on titanium implant surfaces. In order to support tissue regeneration and repair processes, this connection is essential [81]. Further, in order to produce porous composite Thompson et al. mixed Poly (ε-caprolactone)

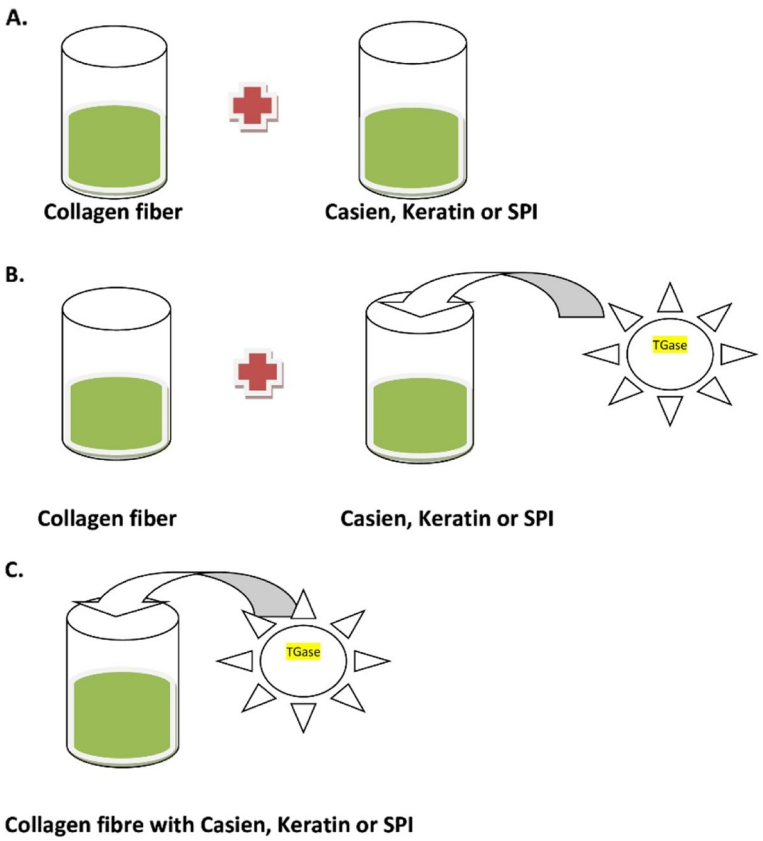


Fig. 3 Overview of the collagen fiber manufacturing process using casein, keratin, and SPI [100]

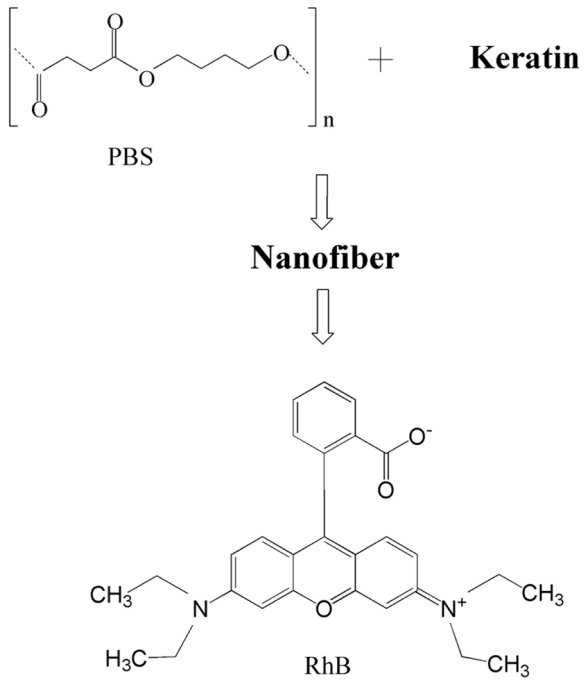


Fig. 4 Representation of chemical process for the development of rhodamine B laoding keratin nanofiber

(PCL) with 10 wt% of keratin solution [89]. To increase their potential as a scaffold for tissue engineering, the keratin components are often combined with other protein materials. For example, S. Mowafi et al. have devised a method for producing nanofibers by combining sericin and keratin. Silk's globular protein sericin is mixed with keratin and trifluoroacetic acid solvent to create fibers that are both biocompatible and encourage cell growth. Tissera et al. also extracted keratin protein using a wool/keratin-based nanofiber. The fiber was produced by treating Marino wool yarn with sulphitolysis and using electrospinning nanofiber for fabrication. The resulting transparent thin film can be used for wound dressing, drug delivery, and filtration processes [90].

Kadirvelu and colleagues developed a process to create keratin/PVA nanofibers by utilizing keratin hydrolysis. These nanofibers form a fibrous mat with exceptional thermal stability and a porous structure, making it suitable for various applications. One of the key applications of this mat is its ability to exhibit antimicrobial properties, which is beneficial for addressing infections. Additionally, the fibrous mat has shown promise in promoting wound healing, making it a versatile material with potential medical applications [50].

Keratin fiber with different polymers

M. Rapa et al. [76] conducted an experiment to increase the prevalence of chronic diseases such as accidental cuts and burns. They developed a donkey gelatin and keratin-based nanofiber with great potential for fast wound healing and resistance to pathogens. The nanofibers have high electrical conductivity in the Donkey-Keratin-Gelatin solution due to the presence of vitamins and minerals. During the process of blending Gelatin-Keratin with corn, sumac, and curcumin, the nanofibers exhibited good in-vitro biocompatibility, except for the sumac-based fibers [76]. Keratin/PBS nanofiber, PBS (Poly-butylene-succinate) is a resin of the polymer family, consisting of butylene succinate with repeating $C_8H_{12}O_4$ units. This research focuses on an interesting biomolecule for drug delivery purposes. The increased rheological properties of keratin protein are effectively used with natural or synthetic polymers.

Collagen fibers were produced and mixed with three separate treatment groups casein, keratin, or SPI (Soy Protein Isolate in a study by [100]. Upon TGase cross-linking, these fibers displayed improved mechanical characteristics and thermal stability, providing them appropriate for application in packaging materials. Proteins are cross-linked by TGase, which enhances their stability and texture. Overview of the collagen fiber manufacturing process using casein, keratin, and SPI is presented in Fig. 3.

The blending of keratin with PBS/PVA/PCL significantly improves the process of fabricating biomaterial. The Keratin/PBS solution nanofiber is loaded with rhodamine B (RhB). The presence of keratin and PBS induces a positive effect on fibroblast growth. Preparing Keratin/PBS with RhB nanofiber may significantly enhance the drug release mechanism (Fig. 4) [37].

In a study conducted by J. Choi et al. in [16], a keratin/PVA composite nanofiber was created. The keratin used in the composite was extracted from human hair using the sulphitolysis method. When combined with PVA, the resulting nanofiber displayed a remarkable characteristic – transparency. Prior to immersion in water, the keratin/PVA nanofibers exhibited a random orientation and uniform distribution with a diameter of 151 nm. However, after immersion in water, the nanofibers underwent a transformation, becoming densely packed with an increased diameter of 223 nm. This unique nanofiber structure offers the potential to enhance both mechanical strength and flexibility [16]. Different keratin based composite nanofibers are summarized in Table 2.

Table 2 Overview of keratin based nanofiber scaffold, advantages and its limitation

Biomaterials	Advantages	Limitations	Reference
Wool keratin with different polymers NFs (PBS, PCL, PLA, FIB)	Increases the electrical conductivity of non-aqueous solutions such as HFIP & FA	The fabrication involves the chemical or enzymatic attack of the disulfide group during the extraction process	[81]
PCL/keratin-based nanofiber	These materials hold tremendous potential for biomedical applications and can effectively mimic the natural extracellular matrix for tissue engineering purposes	This technique, unlike other methods, has proven reliable and cost-effective.	[89]
Sericin/trifluoroacetic acid	They play a role in the field of filtration and biomedical	For nanofiber, sericin has weak mechanical properties	[66]
Pullulan membrane	Fibroblast proliferation /migration and prevent bacterial transmission	Poor contact surface bonding	[19]
Chitin nanofiber	Development of flexible and micro patterned tissue engineering substrate	It was important to maintain the stability of the structure in water for the development of biomedical applications	[78]
Cellulose and derivatives	CNFs leading to size reduction of fibre to nanoscale	Cellulose is a crystalline water insoluble polymer	[87]
Nano cellulose derived from cellulose	Enhance crystally, high surface area, biodegradability	Cellulose nanofiber is a valuable investment despite the higher production costs, as it requires significant energy for mechanical disintegration	[68]
Wool keratin nanofiber	Nanofibers are transparent films used for drug delivery, filtration, and capacitors	Internal α-keratin bundles dissolve and denature in highly concentrated acid when higher mechanical force is applied	[90]
Keratin peptides/PVA nanofibers	Fabricated nanofibers have unique features compared to protein-peptide solution polymers, and they offer distinct advantages, very large surface area to volume ratio and superior mechanical performance compared to other biomaterials	In this method, keratin must be converted into a soluble form to function properly	[50]
Keratin/Nylon6 nanofiber	Nanofiber (NF) membranes can be used as sorbents for adsorbing heavy metals	Solubility	[98]

Nanofibers for drug delivery

The skin serves as the body's protective barrier, regulating body temperature, preventing dehydration, and shielding against harmful UV rays, bacteria, and viruses. The incidence of skin diseases is on the rise, impacting millions of people annually. Inflammatory cell like neutrophils secretes chemokins and growth factors. [109] was formed fiber and modified for process of wound healing [109]. A novel approach for treating injured cells involved the development of keratin and gelatin-based nanofibers derived from donkeys. These nanofibers have demonstrated the ability to enhance cell proliferation and migration, thereby facilitating the repair of damaged cells and promoting wound healing. Notably, the keratin/gelatin nanofibers exhibit a remarkable migration rate of 89% after 24 h when in a monolayered form within ruptured tissue [76]. In the context of drug loading into Nanofiber scaffolds, researchers have explored a range of methods to achieve this, including physical adsorption, chemical conjugation, and coating. Physical adsorption involves the initial dissolution of the drug in a suitable solvent, followed by its bonding to the scaffolds through physical means, thereby enabling effective delivery. One notable advantage of this approach is the absence of chemical bonding between the drug and the nanofiber scaffolds, preserving the integrity of both components. Natural polymers such as Gelatin and Chitosan, renowned for their hemostatic properties in wound dressing, are strategically positioned within hemostatic sponges to optimize their therapeutic efficacy. Moreover, Polyvinyl-alcohol/gelatin nanofiber scaffolds are enriched with Thrombin and Vancomycin, both of which play crucial roles in facilitating blood clotting and wound healing through their direct interaction with fibrinogen to convert it into fibrin. This intricate interplay between the nanofiber scaffolds and the loaded drugs underscores the potential for innovative advancements in the field of drug delivery and tissue engineering [48]. In the realm of ocular drug delivery systems, polymer-based nanofibers have emerged as a promising approach for targeting drugs to the eyes. Egemen and their team have been at the forefront of developing a wide array of innovative ocular drug delivery systems, including solid

lipid nanoparticles, liposomes, and biosomes. These nanofibers are particularly notable for their biocompatibility, excellent porosity, and large surface area, which allows for the efficient loading of multiple drugs. Moreover, they have also engineered a nanofiber hydrogel designed to protect the eyes while minimizing irritation. Egemen et al. detailed their successful creation of Azithromycin-loaded nanoparticles within nanofibers by blending Azithromycin with the polymers PLGA/PL/PVP, marking a significant advancement in ocular drug delivery [92].

In recent times, cancer has become a widely recognized disease, and the treatment of cancer often involves the use of anticancer medicines. However, the impact of these medicines on the body can be significant. To address this issue, researchers have made strides in developing an advanced nanofiber drug delivery system that aims to minimize adverse effects on the body. This system involves the development of various anticancer composite nanofibers, such as Chitosan/Polyurethane, poly (acrylic acid), silk fibrain/cellulose acetate, and gold-silver nanoparticles. These composite nanofibers play a crucial role in delivering drugs like Temozolomide (TMZ) and paclitaxel (PTX) specifically to target glioblastoma cancer cells. The success of drug delivery is heavily influenced by factors such as drug release temperature and pH. It has been found that maintaining a pH of 5.5 and a temperature of 43 degrees Celsius is optimal for ensuring the maximum release of the drugs. Therefore, these anticancer composite nanofibers hold substantial importance in the field of cancer treatment [49].

Applications of keratin based nanofiber scaffold in tissue engineering

Neural tissue engineering

In tissue engineering, electrospinning is an effective technique to produce nanofibrous scaffolds. Because keratin is a naturally occurring protein, it can enhance the cell affinity of scaffolds and has been utilized as a biomaterial for electrospinning in a number of biomedical applications. There is little research on the keratin-based nanofiber scaffold for neural tissue engineering. Keratin has the ability to enhance the biocompatibility and bioactivity of polymeric nanofibers. However, adding keratin to the mix nanofiber would reduce the mechanical properties of nanofibers due to keratin's poor spinnability, resulting in an inhomogeneous distribution of keratin throughout the nanofibers. As a result, adding keratin nanoparticles to the surface of polymeric nanofibers would improve their hydrophilicity and mechanical properties [38].

In this regard, Guo et al. [38] studied the keratose (oxidative keratin) nanoparticles-coating PVA nanofibers (KNPs/PVA) synthesized by electrospray deposition after electrospinning. The scaffold was tested for neural cell growth and proliferation. Their findings revealed that KNPs/PVA nanofibers had higher cyto-biocompatibility in terms of cell shape, adhesion, and proliferation than PVA nanofibers and KOS/PVA mix nanofibers. These findings showed that polymeric nanofibers containing oxidative keratin nanoparticles on their surfaces have excellent biocompatibility and mechanical qualities, making them ideal for brain tissue engineering applications [107].

Zhang et al. used a coaxial electrospinning approach to create well aligned conductive poly(L-lactic acid-co-3-caprolactone)/silk fibroin (PS)/polyaniline (PANi) nanofibers as well as NGF-loaded PS/PANi nanofibers. Their analysis of the survivability and morphology of mouse Schwann cells on nanofibrous meshes revealed that PS-PANi-1 loaded with NGF had the largest cell number after 5 days of culture, and aligned nanofibers could control cell direction. Scaffolds injected with NGF under electrical stimulation could successfully support PC12 neurite outgrowth, increasing both the percentage of neurite-bearing cells and the median neurite length [107].

Additionally, *Lycium barbarum* polysaccharide (LBP) was integrated into core–shell structured nanofibrous scaffolds. The results showed that the released LBP significantly increased the proliferation and neuronal differentiation of PC12 cells stimulated by NGF. Furthermore, Laser scanning confocal microscopy (LSCM) with immunostaining revealed that LBP-loaded scaffolds promote Schwann cell myelination and neurite outgrowth of Dorsal root ganglion (DRG) neurons. *Lycium barbarum* polysaccharide (LBP), a neuroprotective medication encapsulated in electrospun nanofibers, could be a viable candidate for tissue engineered scaffolds for peripheral nerve regeneration [95].

Skin tissue engineering

Protein-based nanomaterials are gaining popularity in the biomedical field. The researchers used a low-pressure filtration-assisted approach to create a new poly(L-lactate-caprolactone) copolymer (PLCL) nanofibrous/keratin hydrogel bilayer wound dressing loaded with fibroblast growth factor (FGF-2). The keratin hydrogel in the bilayer dressing was suggested to mimic the dermis, whereas the nanofibrous PLCL may mimic the epidermis. Furthermore, the keratin hydrogel had good porosity and a maximum water absorption of 874.09%, and its elastic modulus was approximately 44 kPa, which was comparable to the dermis' elastic modulus (2–80 kPa) [108].

Furthermore, they claimed that in vitro tests demonstrated that the bilayer dressing was both biocompatible and biodegradable. In vivo studies revealed that a PLCL/keratin-FGF-2 bilayer dressing might promote re-epithelialization, collagen deposition, skin appendage (hair follicle) regeneration, microangiogenesis formation, and the use of adipose-derived stem cells. Furthermore, the inclusion of FGF-2 is also improved cell repair. The bilayer coating also increased the interface adhesion of hydrogel/electrospinning nanofibers. They hypothesized that the PLCL/keratin-FGF-2 scaffold could be used as a biomedical wound dressing in skin tissue engineering applications [108].

The keratin/chitosan-blended nanofibrous mats were immersed in an 8% GelMA prepolymer solution containing human skin fibroblasts. The scaffold composed of keratin/chitosan-blended nanofibers and GelMA hydrogel has excellent mechanical properties and can affect re-epithelialization during skin wound healing. Ma and colleagues recently reported on the production of a bi-layered scaffold from poly(lactic-co-glycolic acid) (Res-PLGA) nanofiber mat and alginate di-aldehyde (ADA)-gelatin (GEL) crosslinking hydrogel (ADA-GEL) by immersing the Res-PLGA electrospun nanofiber mat in ADA and gelatin solution. The Res-PLGA nanofiber mat and ADA-GEL hydrogel scaffold were discovered to improve wound healing.

Using electrospinning and photopolymerization procedures, a bi-layer scaffold made of gelatin methacrylate (GelMA) hydrogel and human hair keratin/chitosan nanofiber mat was created. They carried out the manufactured nanofiber scaffold's physico-chemical, mechanical, and in-vitro cell culture analyses. They discovered that the human hair keratin/chitosan nanofiber mat's tensile strength was significantly greater than that of the pure human hair keratin nanofiber mat. In contrast to the pure chitosan nanofiber mat, the blend nanofiber mat was comparatively more conducive to HaCaT cell proliferation and keratinocyte differentiation. Additionally, human fibroblasts were encapsulated in the hydrogel layer and HaCaT cells were grown on the nanofiber layer for ten days in order to assess the viability as a skin transplant. The outcome showed that HaCaT cells created a cell layer on top of the scaffold, imitating the dermis and epidermis of skin tissue, while the encapsulated fibroblasts multiplied in the hydrogel matrix [56].

In addition, a core–shell nanofibrous wound dressing based on Pluronic-F127 (F127) with a 2 weight percent mupirocin (Mup) core and pectin (Pec)-keratin (Kr) shell was created using the coaxial electrospinning technique in the current study. The same materials were also used to create the blended nanofibers. The blended nanofibers' fiber diameter and specific surface area were around 101.56 nm and 20.16 m2/g, respectively, whereas the core–shell nanofibers' were approximately 97.32 nm and 25.26 m2/g. According to the drug release study, the core–shell nanofibers' drug release profile demonstrated a sustained release of Mup over 7 days (87.66%), whereas the blended F127-Pec-Kr-Mup nanofibers had a burst release within the first few hours (89.38% up to 48 h) and a cumulative release of 91.36% after 7 days. They proposed that the controlled release of Mup in the core–shell structure significantly improved the behavior of human keratinocytes, their angiogenic potential, and the healing of wounds in a rat model as compared to the blended structure [64].

The current study assesses the physicochemical properties of electrospun nanofibers made by combining gelatin with keratin (from wool) and sericin (from silk) to confirm their use in in vitro interaction investigations. Furthermore, they demonstrated that sericin, when combined with keratin macromolecules, can enhance the biochemical signal of gelatin, hence enhancing the in-vitro stability of gelatin-based nanofibers. In vitro data confirm a synergistic effect of sericin and keratin on human Mesenchymal Stem Cells (hMSC) proliferation—an increase of over 50% compared to other types—linked with an increase in in vitro stability directly attributed to the proteins' unique physical interaction. These findings support the use of sericin/keratin/gelatin-enriched electrospun fibers as nanostructured platforms for interface tissue engineering [94].

In another study, bacterial cellulose (BC), a nontoxic and natural biopolymer, was combined with keratin to create a nanocomposite scaffold. In this study, a new natural nanocomposite was generated for the first time by combining keratin (derived from human hair) with BC (produced by Acetobacter xylinum) to increase cutaneous fibroblast cell adhesion. In vitro cell culture investigations with human skin keratinocytes and fibroblast cells show that the unique BC/keratin nanocomposites can be used to construct skin tissues [53].

Bone tissue engineering

The significance of scaffolds in bone regeneration is very important. In this context, [61] developed a polypyrrole@keratin conductive nanofiber membrane (PPy@KNM) with high conductivity using electrospinning method and chemical in situ polymerization of pyrrole monomers. The PPy@KNM with the highest conductivity and tensile strength was synthesized using an oxidant concentration of 0.6 M and a dopant concentration of 0.5 M of p-toluenesulfonic acid (p-TSA). Furthermore, in vitro cell culture tests revealed that the conductive PPy@KNM had good biocompatibility and no cell cytotoxicity. The PPy@KNM can promote cell growth and proliferation with an

ideal current intensity of 80 μA and stimulation time of 5 min/d. The research findings demonstrated that PPy@KNM can be used as a functional biomedical material to aid wound healing [61].

Additionally, another study developed and investigated electrospun scaffolds of poly (3-hydroxybutyrate)-keratin (PHB-K)/nanohydroxyapatite (nHA) with different percentages of weight (up to 10 w/w%) of nHA and morphologies (long nanorods (HAR) and very short nanorods (HAP). The study revealed that incorporation of nHA resulted in an increase in pore size up to 16 μm, fiber integrity, and porosity of over 80%. Ca concentrations dropped by 55% for HAR-containing scaffolds and 73% for HAP-containing scaffolds after the scaffolds were submerged in simulated bodily fluid (SBF), demonstrating the bioactivity of nHA-containing scaffolds. The MG-63 cells grown on the nanocomposites demonstrated the beneficial benefits of nHA based on their cell attachment, proliferation, and viability outcomes. The findings suggest that the scaffolds made of nanocomposite materials, particularly those that contain HAP, may be appropriate for use in bone tissue engineering [82].

Additionally, in order to generate a nanofibrous scaffold, keratin (H-keratin) was isolated from hagfish slime thread and blended with a polylactic acid (PLA) polymer solution. PLA, PLA/W-keratin, and PLA/H-keratin all had comparable average diameters (~800 nm). In contrast to pure PLA or PLA/W-keratin, MG-63 cells attached and propagated more readily atop PLA/H-keratin, according to cell attachment studies. In comparison to pure PLA or PLA/W-keratin nanofibrous scaffolds, PLA/H-keratin scaffolds were able to speed up the viability, proliferation, and osteogenesis of MG-63 cells, according to assays for cell proliferation, DNA content, live/dead, and alkaline phosphatase activity. According to these results, H-keratin has a lot of potential for application as a biomaterial in bone tissue engineering and may enhance cellular attraction [108].

Cardiac tissue engineering

The limited ability of injured cardiac tissue to regenerate makes cardiovascular diseases—myocardial infarction in particular a major healthcare concern. A promising method for fixing myocardial injury is cardiac tissue engineering (CTE), that employs biomaterials that mimic the extracellular matrix of the heart. There are some reports on keratin based nano scaffold for cardiac tissue engineering.

Hydrogels, electrospun polymers, and 3D-printed cardiac patches are biomaterials that have been evaluated for cardiac tissue engineering [35, 69]. Additionally, injectable hydrogels have been developed to facilitate clinical application by enabling simple administration to damaged myocardium without the requirement for invasive procedures [72]. Bioactive compounds like peptides, microRNA, or growth factors may then be added to these materials. Nanoparticles can stabilize these biomolecules even if physiological circumstances may cause them to deteriorate. By activating nearby cells and acting as platforms for modification with bioactive chemicals, nanoparticles have been proven to be helpful in such endeavors.

Other researchers have investigated electrospinning further, utilizing the anisotropy of electrospun materials. CNTs were successfully added to an aligned poly(glycerol sebacate):gelatin gel by Kharaziha et al. They observed that this scaffold was more durable and electrically conductive than scaffolds devoid of carbon nanotubes. On this scaffold, NRVM cells maintained cell viability and displayed a more coordinated beating activity [54]. Networks of "nanoyarns" made of PCL, silk fibroin, and CNTs were created by Wu et al. via electrospinning, and they were subsequently covered in a gelatin methacrylate gel [101].

These hydrogels with nanoyarn networks promoted the development of endothelialized myocardium when NRVMs and endothelial cells were implanted in vitro. CNTs were incorporated in an electrospun chitosan/polyvinyl alcohol mesh by Mombini et al. [65]. They found that the most effective CNT concentration for encouraging mesenchymal stem cell differentiation into cardiomyocytes was 1%. They reported a significant rise in the differentiation of mesenchymal stem cells into cardiac cells using this gel and an applied current [65].

Additionally, this study explores the possibility of improving the characteristics required for successful cardiac repair using graphene nanopowder (Gnp)-enhanced polycaprolactone (PCL) scaffolds made by electrospinning. The objective of this study was to examine scaffolds with different concentrations of graphene in order to find out their mechanical, chemical, morphological, and biocompatibility properties. According to the findings, adding graphene enhances the mechanical characteristics and cellular interactions of PCL scaffolds. The optimum concentration of 1% graphene greatly improved biocompatibility and mechanical characteristics, encouraging cell attachment and growth. According to these results, Gnp-enhanced PCL scaffolds at this concentration may act as a powerful CTE substrate, providing guidance to develop biomaterials for cardiac repair that are more effective [67].

Biological interactions of keratin nanofibers

Advancement in keratin based nano-fibres brought a significant development in biomedical field because of its cellular interactions. High sulphur content, and tendency

for self-assembly and inherent cellular recognition property makes it important therapeutic agent in wound healing. Keratin based biomaterials are mostly non-immunogenic, biocompatible, biodegradable, antibacterial, antioxidant and multi-responsive in nature. Keratin biomaterials extensively used in drug delivery and tissue engineering [32, 96]. High content of cysteine (approx. 7–13%) in natural keratin extracted from hairs, nail, hooves, wool and horns benefits in cellular attachment. Keratin possesses ample cell-binding motifs i.e. arginine-glycine-aspartate, glutamate-aspartate-serine and leucine-aspartate-valine which is advantageous in cell adhesion and proliferation. Various studies reported that it is used in wound dressings due to its healing property, additionally it reduces inflammation, improves remodelling and haemostasis. Mechanical property of keratin based wound dressings are area of present research which focusses to improve strength of keratin films, hydrogels and nanofibers [106].

The major cellular component keratinocytes are basically epithelial cells of ectodermal origin. Keratinocytes are responsible for production of extracellular keratin protein, which plays a major role in re-epithelialization. Use of stem cells to improve the rate of wound healing and tissue regeneration has shown satisfying results in many studies [20]. Wound healing is a multi-step process which needs coordinated interactions between cells and growth factors. Unique properties of keratin plays a vital role in cutaneous healing [71].

A potent graft must possess properties like mechanical and physical strength, It should be non-toxic upon insertion or during degradation. Availability of keratin in large quantities and lack of its immunogenic reactions which leads to rejection makes it a successful material in wound healing [13]. Gelatin polymer combined with tofu and arginine contains strong antioxidant abilities and effective in wound healing process in rats [43, 44]. Keratin-hydroxyapatite increased rate of bone regeneration due to its bone cell integration and osteoconductive behavior [22, 23].

In a study done by Ferraro et al. keratin used as a substrate for the growth and differentiation of haematopoietic stem cells [34]. Besides, keratin scaffolds assisted in bone remodelling, bone integration and was substituted with laminar bone in twelve weeks. Keratin is easy to handle and allow manual manipulation of samples without damage [23].

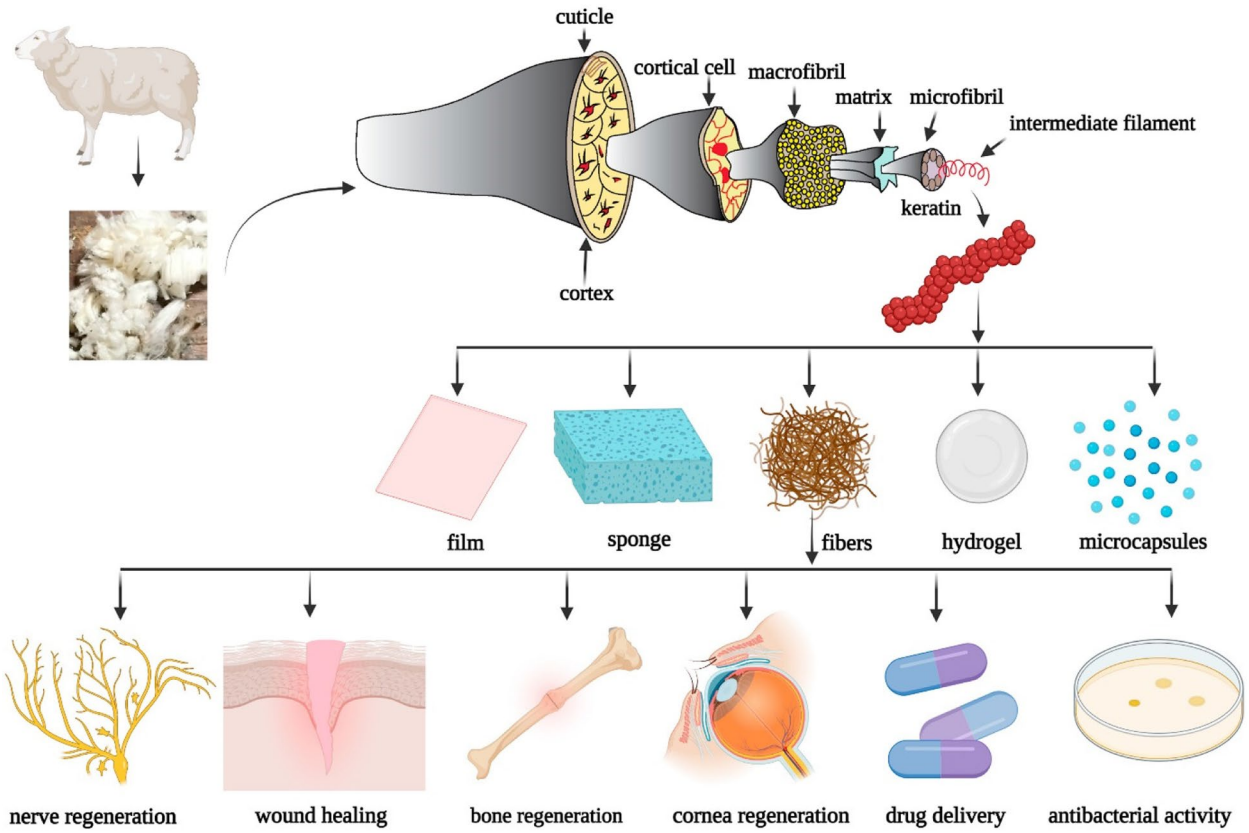


Fig. 5 Wool keratin-based compounds for tissue engineering applications are shown schematically. Adapted from [75]

Immunogenicity of keratin remain unclear, despite its potential as a graft substitute. Some studies reported that keratin based implants showed no inflammatory reactions, necrosis, pus formation and activated innate immune response [14]. Keratin based biomaterials showed anti-inflammatory effect as they augmented the level of M2 phenotype macrophages following tissue damage [97]. Many studies on keratin based treatment showed no significant immune response [15, 105]. Activation of innate immune response can trigger activation of humoral immune system. However, in some cases humoral immune reaction can sidestep the innate immune response, thus keratin scaffold may lead to antibody mediated immune reaction in the absence of acute inflammatory response [15].

Cellular keratins are mandatory inside the cell, and their extracellular existence indicates cell lysis may be after necrosis or infections secondary to viral lytic infection or necrosis. Hard alpha-keratins are present inside complete cells like follicular trichocytes or basal keratinocytes or stratum corneum or hair shaft. Some auto antikeratin antibodies have been observed in immunological assay in pathological as well as normal states [46, 59]. However, the importance of the presence of autoreactive keratin antibody remains unclear. The reconstituted keratin bone grafts in ovine model after 12 weeks not shown humoral immune response in measurement of anti-keratin antibodies or mRNA for SOCS3 proteins responsible for inflammatory cytokine response. Moreover, the innate immune response are also not stimulated by keratin based biomaterial [24]. However, further in vivo studies are necessary to fully clarify the immunogenic response of keratin Nano fibres.

Cell adhesion, influence to stemness and the ability to facilitate differentiation (adipogenesis and osteogenesis) are significant aspects in assessing the developing biomaterial used as an extracellular matrix protein. Studies on cell lines revealed that keratin nano fibers maintained normal cell structure and influenced proliferation in 3T3 fibroblasts, MG63 cells and MDCK osteoblasts [77]. Keratin coating showed better results as compared to fibronectin coating as keratin efficiently increased surface area of 3T3. In addition, keratin coating effectively increased the surface area of 3T3, and even higher than that of fibronectin coating (1.6-fold). Similar results have been found by other researchers in which it promoted cell adhesion in NIH-3T3 fibroblast cell adhesion over uncoated dish culture [42]. It also helps in higher saturation densities and number of cell yields. Keratin showed increased seeding efficiency in many cell lines from different origins, cell types and position. Thus, keratin may effectively improve cell adhesion and proliferation in pACS cells [30, 70]. Keratin coating plates improved hMSC proliferation and enhanced STRO-1 expression

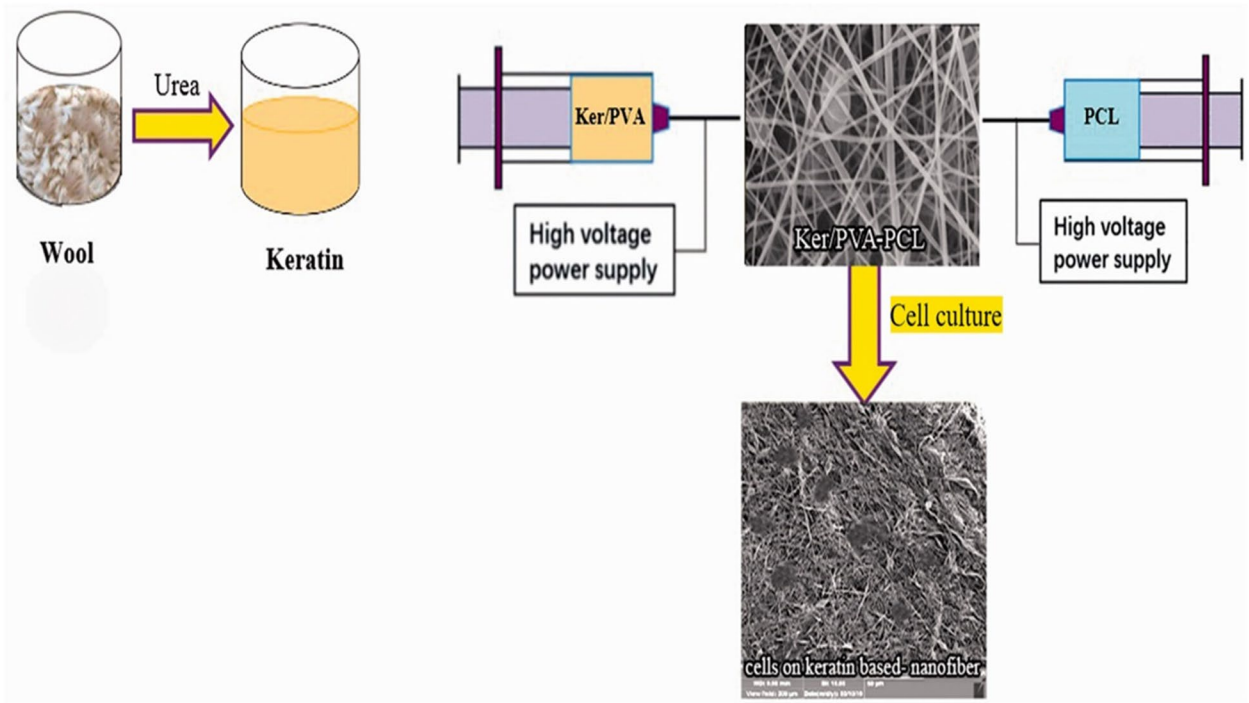


Fig. 6 The process of making keratin-PCL Nanofiber using electrospinning technique, Reproduced with permission from reference from Ranjbar-Mohmmadi et al., 2021 [74] SAGE Journal. Open Access

may be due to presence natural binding motifs [39]. The ASCs documented for bone formation both in in vivo and in vitro studies on keratin. Human hair derived keratin promoted cell adhesion and proliferation in many cells i.e. 3T3 fibroblasts, MDCK cells and MG63 osteoblasts cells [110]. Keratin improved adipogenic differentiations and upregulated LPS and PPAR-g. Some osteogenic markers COL1A1, RUNX2 and VDR also up regulated on keratin substrates. Thus, it effectively performs surface modification and scaffold fabrication in tissue engineering [102]. Keratin and its scaffolds were reported to be cyto-compatible in terms of cell proliferation. Similarly in vitro studies on keratin scaffolds seeded with osteoblasts like Saos showed adhesion, proliferation affinity and cell viability showing its potential role in tissue engineering [31]. For instance, wool keratin-based compounds for tissue engineering applications are shown schematically in Fig. 5.

In-vitro cell culture study

Blended nanofibers made from protein and synthetic polymers are a significant advancement in composite materials with structural and material properties suitable for biomedical applications [28]. For instance, Thompson et al. [89] demonstrated biomedical engineering using keratin Nanofiber. They discussed the creation of keratin-containing poly (ε-caprolactone) (PCL) nanofibers, which have unique physiochemical properties. The nanofibers were created by extracting water-soluble keratin from human hair and mixing it with PCL in different ratios. The resulting nanofibers showed uniform surface morphology, excellent mechanical properties, and good fibroblast cell viability making them potential composite materials for biomedical applications [89].

Furthermore, Fortunato et al. showed in vitro tissue models by analyzing and characterizing a hydrolyzed keratin-based biomaterial and processing it with electrospinning technology. After being extracted from poultry feathers, the biomaterial was combined with type A porcine gelatin and cross-linked with γ-glycidyloxypropyl-trimethoxy-silane (GPTMS). Following chemical-physical characterizations, electrospun nanofiber structures were produced. The effectiveness of keratin-based structures in maintaining the vitality and proliferation of human epithelial, rat neuronal, and human primary skin fibroblast cells over a 4-day period was tested in order to examine the cell response. The outcome of the trial demonstrated encouraging outcomes for the use of epithelial tissue regeneration applications [36].

Ranjbar-Mohammadi et al. have studied the synthesis of hybrid scaffolds comprising poly ε-caproactone) (PCL) and keratin/polyvinyl alcohol (Ker/PVA) for use in skin regeneration. To produce hybrid nanofibers, they first extracted keratin from wool fibers and then used the electrospinning technique. Hybrid nanofibers were collected onto a rotary drum collector after one syringe was used to extrude the Ker/PVA blend solution and the other to extrude the poly(ε-caprolactone) solution (Fig. 6). The impact of varying Ker/PVA ratios (30:70), (50:50), and (70:30) in conjunction with PCL was examined with regards to the scaffolds' shape, hydrophilicity, and mechanical attributes. The mechanical strength and suitable modulus of the Ker/PVA (50:50)-PCL nanofibers were comparable to those of normal skin [74].

Mirhaj and team [64] studied a Pluronic-F127 (F127)-based core–shell nanofibrous wound dressing with a 2 wt % mupirocin (Mup) core and pectin (Pec)-keratin (Kr) the shell was manufactured using the coaxial electrospinning method. Study revealed that during seven days, the resulting blended and core–shell nanofibers degraded by 26.65% and 32.28%, respectively. While the blended F127-Pec-Kr-Mup nanofibers had a burst release within the first few hours (89.38% up to 48 h) and an average release of 91.36% after 7 days, the core–shell nanofibers' drug release profile displayed a sustained release of Mup over 7 days (87.66%). In contrast to the blended structure, the core–shell structure significantly improved the behavior, angiogenic capacity, and wound healing of human keratinocytes in a rat model by means of the controlled release of Mup [64].

In another study, [61] investigated the electrical stimulation using conductive biomaterials exhibits significant promise for promoting skin wound healing. They studied, pyrrole monomers were chemically polymerized in situ and electrospun utilizing electrospinning technology to prepare a polypyrrole@keratin nanofiber membrane (PPy@KNM) with high conductivity. The result revealed that PPy@KNM exhibiting the maximum tensile strength and conductivity was synthesized using 0.5 M dopant and 0.6 M oxidant concentrations of p-toluenesulfonic acid (p-TSA). In vitro cell culture study demonstrated that the conductive PPy@KNM exhibited no cytotoxicity and good biocompatibility. They observed that using the PPy@KNM for electrical stimulation, it is possible to significantly speed up cell development and proliferation. An ideal current intensity of 80 μA and stimulation duration of 5 min each day are recommended. The study's findings proved that PPy@KNM can be used as a functional biomedical material to aid in the healing of wounds [61].

Further, Islam and team [47] work on fabrication of bead-free electrospun nanofibrous scaffolds made of PVOH, keratin, and chitosan after the process parameters were optimized through the use of a Box–Behnken experimental design. They found that the models, which had an R2 of 98.58 and 99.67%, were very good at

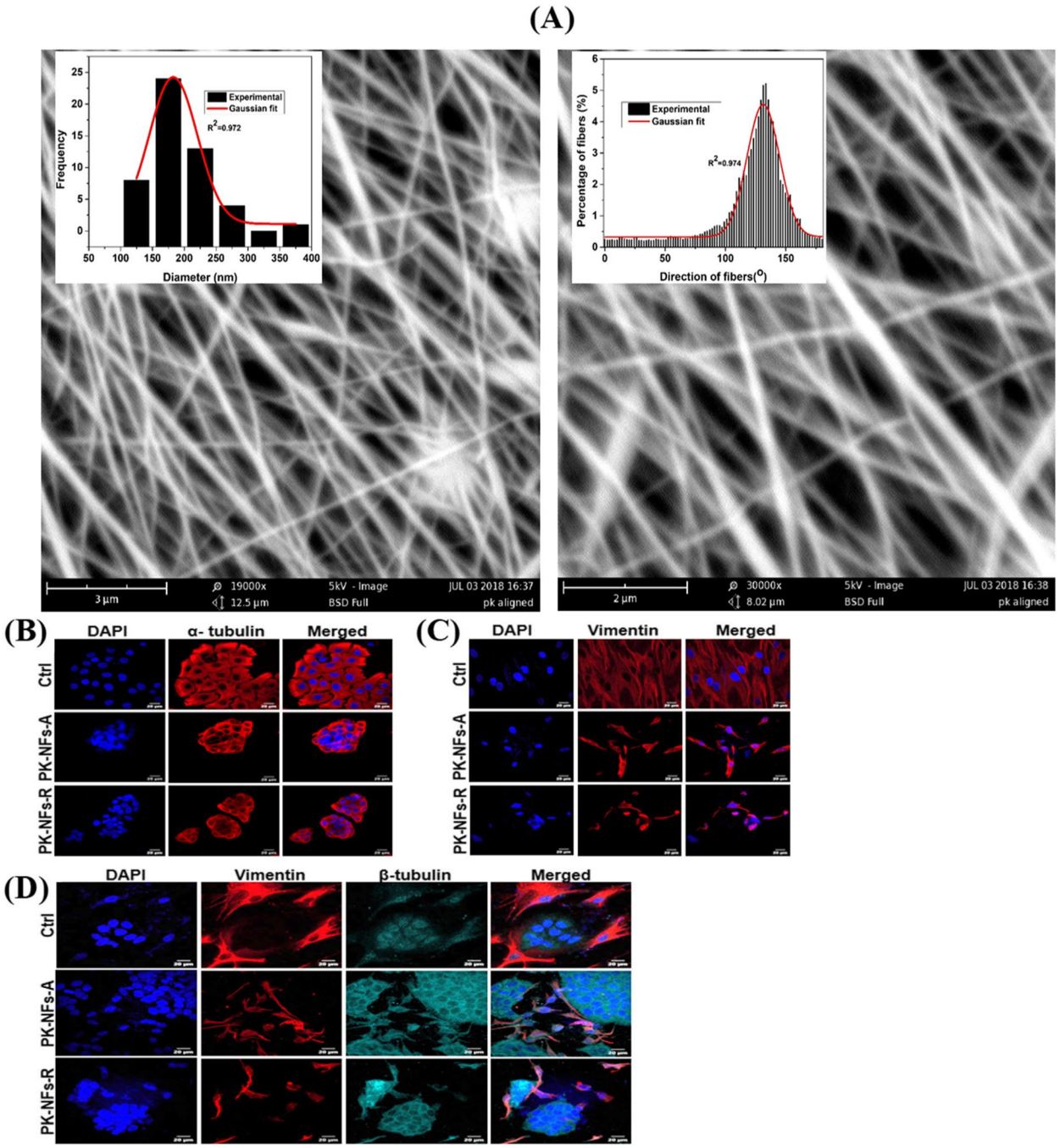


Fig. 7 **A** SEM images of keratin nanofiber, **B** The effective adhesion, infiltration, and proliferation of HaCaT and NHDF cells after their seeding on the corresponding scaffolds are revealed by immunocytochemical analysis of the cells employing the indicated cytoskeletal markers in conjunction with DAPI (nuclear). Under confocal laser scanning microscope, **B** HaCaT cells with a characteristic cuboidal morphology exhibit α-tubulin positivity at different focal planes. **C** Vimentin positivity is seen in NFDF cells with a fibroblastic shape. **D** When co-cultured HaCaT cells are stained with both vimentin and β-tubulin, NHDF cells are able to distinguish between the two populations and show how they are growing next to one another. [1]

describing responses. 15.82 kV of voltage, 0.25 mL/h of flow rate, 105 mm of spinning distance, and 30% biopolymers were found to be the ideal parameters. After verification, it was discovered that the models' accuracy fell within a reasonable range. Moreover in-vitro study on Human Aneuploid Immortal Keratinocyte (HaCaT) and Normal Human Dermal Fibroblast (NHDF) cell lines showed no cytotoxicity to the bead-free nanofibrous scaffold, allowing for cell attachment and proliferation. When co-cultured on the scaffold for 30 days, both cell lines stayed attached with flawless cell morphology, suggesting the scaffold's potential for use in biomedical applications [47].

Cell attachment and compatibility

Cell attachment is crucial for tissue engineering and regenerative medicine. Keratin Nanofibers facilitate this through surface topography, chemical functionalities, and biochemical signals. Their high surface area mimics the extracellular matrix, promoting cell adhesion and growth. The presence of carboxyl, amine, and hydroxyl groups on the keratin surface also enhances cell attachment. They support the attachment and proliferation of fibroblasts, osteoblasts, and endothelial cells, promoting wound healing, tissue engineering, and drug delivery. These Nanofibers are used in wound healing mats, tissue engineering scaffolds, and drug delivery carriers. Their excellent cell attachment and compatibility properties make them ideal for wound healing, tissue engineering, and drug delivery systems. As research progresses, keratin Nanofibers are expected to play a significant role in regenerative medicine and patient outcomes. Fibroblast cells demonstrated good cell vitality by adhering to one another and multiplying in PCL/keratin nanofibers [89]. Yamauchi et al. demonstrated that wool keratins were more adherent to the mouse L929 fibroblast cells and supportive of cell proliferation than collagen and glass [104]. Wool keratin, a type of natural protein, was utilized to increase poly (l-lactic acid)'s cell affinity [60].

Recently, Aadil and team [1] have been working on creating an electrospun nanofiber scaffold based on PVA-hair keratin (PK-NFs) for use in skin tissue engineering. They confirmed that keratin and PVA interacted by hydrophilic interaction and hydrogen bonding, and that the generated nanofiber had a mean diameter of 100–250 nm. The biodegradation analysis and contact angle revealed that PK-NFs were hydrophilic by nature and stable in an aqueous medium. The developed PK-NFs scaffolds worked well with ESCs, HaCaT, and NHDF. It's interesting to note that, as one might expect in skin tissue, both NHDF and HaCaT cell types formed adjacent to one another in the co-culture. The potential of PK-NFs scaffolds for skin tissue engineering applications is indicated by all of their findings (Fig. 7) [1].

Challenges and limitations

Despite tremendous advancements in in vitro and small animal research, present methods might not be able to achieve sustainable tissue engineering. A key consideration in this case is the selection of the biomaterial that will be used as a "scaffold" to direct the regeneration process. Effective standards for these biomaterials have been lacking for a long time, and the requirements for biodegradability and prior FDA approval for use in medical devices have dominated material selection procedures [99]. Another challenge is the characteristics and performance of the scaffold may be impacted by cell culture, tissue growth, and implantation because of material deterioration and constantly shifting interfaces [27].

Further, electrospinning has certain practical limitations in addition to its obvious benefits and wide range of applications. These include slow production rates, poor cellular infiltration and ingrowth, potential toxicity of chemical residues in electrospun fibers or postprocessing, insufficient mechanical strength for load-bearing applications, etc. Electrospun materials' porous nature allows nutrient and growth passage, but their small pore diameters and high packing density limit cellular penetration, limiting vascularization, tissue ingrowth, and uneven distribution [55].

The delicate nature of keratin-based biomaterials is a significant obstacle for tissue engineers, even with all these advantageous characteristics [33]. Regenerated keratin has poor mechanical qualities and a low molecular weight of 65–11 kDa, making it extremely fragile and challenging to work with [4].

The low stability and structural integrity of protein-based nanofibrous membranes in an aqueous environment is a challenge to overcome, despite the promising results in this regard. However, numerous approaches have been put forth, including the use of crosslinking agents like genipin, formaldehyde vapour treatments, aqueous alcohol solutions, and water vapour treatments [3].

Further, several tissue engineering initiatives have focused on the need for scaffolds that can remove waste and cell products while delivering oxygen and nutrients. The production of a hypoxic core, which typically results in cell malfunction and death, can occur when the cellular density in a scaffold is increased to resemble the density of the native tissue [27]. The application of biomaterials based on hair keratin has been restricted due to its poor mechanical qualities and brittleness in aquatic environments. Moreover, the typical animal-derived proteins including collagen, fibrin, elastin, and gelatin can

present intrinsic concerns of pathogen transmission and immunological response when combined with keratin material for scaffold production [56].

Future directions and innovations

Keratin nanofibre-based tissue engineering holds great potential for advanced wound healing, regenerative medicine, drug delivery systems, cosmetic applications, personalized medicine, bioprinting and 3D printing, environmental sustainability, mechanical properties enhancement, integration with nanotechnology, and clinical translation and commercialization. Keratin nanofibres have excellent biocompatibility and promote cell adhesion, proliferation, and differentiation, making them ideal for wound dressings. Additionally, keratin based biomaterials for tissue generation should have customizable properties, prevent inflammation, provide structural support, have controllable microstructure, and be biodegradable without toxic residues.

They can also be used in regenerative medicine to create scaffolds for tissue regeneration, and in drug delivery systems to encapsulate and release therapeutic agents at the site of injury or disease. In the cosmetic industry, keratin nanofibre-based products could rejuvenate skin, strengthen hair, and improve nail health. Personalized medicine could use keratin nanofibres to create personalized implants and tissue constructs. Additionally, keratin nanofibres could be integrated with other nanomaterials, leading to new applications in biosensing, diagnostics, and therapeutic delivery.

Conclusion

This review covered keratin nanofibers, their benefits and drawbacks, and the latest developments in their use in tissue engineering. Keratin nanofibers hold great promise for tissue engineering applications due to their unique properties, biocompatibility, and potential for cell regeneration and repair. Despite progress in in vitro and small animal studies, clinical and commercial endpoints for sustainable tissue engineering have been slow. The choice of biomaterial as a scaffold for regeneration may hinder the achievement of ultimate goals. In the end, more investigation is necessary to fully comprehend the behaviors and interactions of keratin nanofiber with cells, both in vitro and in vivo. To employ keratin nanofiber scaffolds for more advanced applications, including clinical investigations, it is crucial to understand their biocompatibility, biodegradation, in vitro and in vivo stability, and mechanism of performance. Further exploration and development, in conjunction with developments in associated technologies, should open up even more opportunities for this adaptable and exciting biomaterial.

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Authors' contributions
Keshaw Ram Aadil (KRA): conceptualization, writing the original draft review, and editing. Khushboo Bhange (KB): manuscript writing, draft review, and editing. Gita Mishra (GM): Drafting in-vitro and in-vivo section of the review drafting, and editing Nitesh Kumar (NK): Contribution to the methodology section, prepared tables and Figures. All authors reviewed the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Botany, Govt. Digvijay Autonomous Post Graduate College, Rajnandgaon 491441, Chhattisgarh, India. [2]Department of Biochemistry, Pt. J.N.M Medical College, Raipur 492010, Chhattisgarh, India. [3]Department of Zoology, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur 495009, Chhattisgarh, India.

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