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Possible Beneficiation of Waste Chicken Feathers Via Conversion into Biomedical Applications

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Abstract

Keratin-based waste materials such as wool and waste chicken feathers are driving investigations to beneficiate them. The poultry industry in South Africa generates about 230 million kg of waste chicken feathers per annum, which makes them the abundant keratin source. most of which is disposed of by landfilling or combustion. The current disposal techniques through landfilling or combustion are not environmentally friendly. Thus, methods for beneficiation of the waste are needed. Considering that chicken feathers are comprised of mainly keratin, it is plausible that the keratin can be exploited for application in biomedical applications. However, keratin biomaterials have not found a breakthrough in clinical applications. The keratin can be recovered in the form of fibres or dissolved from feathers in suitable solvents. Regenerated keratin biomaterials can take the form of hydrogels, membranes, films, sponges, scaffolds, and nanofibres. These materials possess excellent properties that can be applied to different fields, including the health sector. Currently, there is no review paper that puts together all possible beneficiations of waste chicken feathers keratin in biomedicine. Therefore, this work exposes the chemistry and characteristics of keratin from different sources including chicken feather keratin in relation to their potential use in the biomedical applications. This review also highlights the properties of regenerated keratin and corresponding biomaterials, including electrospun regenerated keratin fibres for biomedical applications. Keratin nanofibres, also possess advanced properties for biomedical applications due to nanofibres reception in medical applications. Keratin is one biopolymer that can function as an acceptable biopolymer. The review indicates that there is a need for biopolymers as many fields rely on petroleum-based polymers which tend to have biocompatibility limits and are unsustainably resourced.

***Keywords:** Regenerated keratin; Keratin; Waste chicken feathers; Keratin-based waste material*

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Introduction

In various fields, there is a need for biopolymers that can be used as alternative sources to petroleum-based polymers that have biocompatibility problems and/or environmentally unfriendly production. Keratin is one biopolymer that can replace some of these materials [1]. Keratin is fibrous protein that has high concentrations of sulphur in its amino acids which are responsible for mechanical, thermal and chemical stability properties [2]. Protein-based biomaterials can facilitate cell-to-cell and cell-to-material interactions which makes them more biocompatible than their counterparts [3]. Previous research work has shown that regenerated keratin can be used for different applications such as in cosmetics, animal feedstocks, fertilisers and pharmaceutical engineering applications [4,5].

Potential biomedical applications of regenerated keratin also include materials for drug delivery, tissue engineering and wound healing [2,6]. These materials are made in forms that are relevant to their applications; the regenerated keratin materials can be membranes, films, powders, sponges and fibres [2,7-9]. The fact that keratin can blend with other polymers such as polyethene oxide, polylactic acid and so forth, to supplement each other widens it fields of applications [10,11]. It also plays a vital role in dissolving drugs in the body; hence, keratin protein supports dissolution of drugs to body cells. Therefore, pure and or blended regenerated keratin membranes can be used for drug delivery because of their properties match the human stratum corneum [2]; pores in keratin film provide excellent drug delivery property for the film, even though the keratin as a biopolymer has not been characterised in terms extracellular polymeric substance (EPS) and soluble microbial product (SMP), the fact that it is a protein and has a high molecular weight may suggest has a high ratio of EPS than SMP [8]. Regenerated keratin partnered with glycerol to make clear films can be used as wound dressing materials [7]. The aim of this report is to review the possible economical way of keratin extraction and possibilities of valorisation of chicken feathers keratin into regenerated keratin nanofibres.

Sources and related classifications of keratin

There are different types of keratins depending on the classification that is used. One way to classify keratin is based on the sulphur content, which is proportional to the amount of cysteine, the main amino acid of keratin. Keratins with 1,5 to 2% and 4 to 8% sulphur content are referred to as soft and hard keratins, respectively [5,6]. This sulphur content is proportional to structural stability and some degree of resistance to forces before fracture [7]. Both soft and hard keratin contain protein mixture that is set in a filament matrix structure which is supported by sulphide bond [8]. The other method of keratin classification is based on the source of keratin concerned; this filament protein, keratin is mainly found in vertebrates and reptiles, including chickens. The keratin proteins from these two classes are distinguishable by the way in which polypeptide chains are laid, which may either form alpha helices or beta pleated sheets that result in alpha-keratin and beta keratin, respectively. Alpha and beta keratins of mammals and reptiles are found in stratum corneum, horns, nails, hooves, claws, hagfish slime threads, scales, shells such as that of tortoise and beaks, claws and feathers of birds [9-11].

The earlier mentioned soft keratin is typically found in the skin while hard keratin is found in nails, hooves, horns, hair including wool and feathers [6]. Human skin consists of stratum corneum which is 70% keratin [2] while a human hair is 90% keratin [12,13]. The hair keratin consists of 50-60% alpha keratin while nail keratin, contrariwise, has characteristics of both hair and skin keratins in varying proportions [14,15], with 21.9% of amino acids being cysteine and serine [16]. Wool and feathers are the most abundant sources of keratin worldwide due to their utilisation in the textile and food industry, respectively [17,18]. The usage of wool in textile industry results in masses of un-spinnable short wool waste [19]. Wool is

95% keratin, of which 60% wt is soft keratin, and 26% is hard keratin [20]. Comparatively, feathers consist of 90% beta keratin [21]. The poultry industry produces the bulk of chicken feathers as waste during meat production [22]. Valuable materials produced from regenerated keratin include electrospun nanofibres [20]. Regeneration of keratin via electrospinning demands its extraction from its source. Equally, keratin must be extracted from chicken feathers for the production of nanofibres [23].

Chicken feathers as the most abundant source of keratin

Compassion in World Farming stated, in 2013, that 58 billion chickens are slaughtered per year [24]. Considering a 2 kg slaughter-weight of a chicken with 5-7% of feathers per chicken, a minimum of 5,8 billion kg of chicken feathers are produced per year as a by-product [25]. USA and India produce approximately 1.044 billion kg and 140 million kg chicken feathers, as waste, per year, respectively [26,27]. Two to three tons of chicken feathers can be effortlessly produced by a slaughterhouse that processes 50 000 chickens per day [28]. According to Tesfaye South Africa produces 258 million kg of chicken feathers as a by-product while producing meat [29]. Insignificant amounts of these feathers are used as useful products, for low-value applications like animal feed which cost about 13 Rand, and fertilizer; the other significant portion is considered as a waste product [30,31]. The trend of poultry production indicates an increase in chicken production.

Consequently, chicken feathers amount will increase [32]. Currently, the poultry industry disposes of the waste chicken feathers using landfilling and incineration techniques [7,33]. These disposal procedures are restricted, generate greenhouse gases such as carbon monoxide or pose a danger to the environment; chicken feathers are solid waste which pollutes land due to their degradation resistance, increase cost of production due to levy paid by poultry meat producers, covers large area and can contaminate groundwater [34,35].

Conversely, chicken feathers contain about 90% of keratin protein [25], a highly valued protein; the price of one-milligram keratin from a human cell sells for R2840 per gram [36]. Hence, keratin can be extracted from chicken feathers and be converted into essential and valuable products which can add extra value and revenue to the poultry industry. Biomedical applications are amongst the applications of keratin [3,37-39]. However, keratin extraction for these applications has not yet optimised. The succeeding section of this paper describes some of the extraction methods that are or can be used to extract keratin from waste chicken feathers.

Keratin extraction techniques

Keratin extraction involves breaking strong disulphide bonds that crosslink keratin molecules, this allows extraction of keratin protein. Depending on extraction technique, keratin chains may also be shortened during the process [40,41]. This section describes extraction techniques that can be used to extract keratin from different sources. These extracting techniques can be categorised into chemical techniques, microwave radiation technique, microbial, enzymatic extraction supercritical water and steam explosion [42]. The subsections below give a brief description of the mentioned techniques.

Chicken feather keratin can be extracted via chemical solutions or mixtures of sodium metabisulphite, sodium bisulphite, sodium sulphide, sodium hydroxide, 2-mercaptoethanol, thioglycolic acid and ionic liquids [28,40,41,43,44]. These keratin extraction methods may produce keratin proteins with different physical properties such as viscosity, molecular weight and

others which later affect the properties of the final products that will be produced from keratin. Therefore, this section aims to review the different methodologies of keratin extraction technologies for waste chicken feathers.

Chemical extraction of keratin

Chemical extraction uses chemical substances to extract keratin from keratinous fibres. The most common classes of chemicals used to extract keratin from keratinous materials are reducing agents, oxidising agents, ionic liquids and alkalines [43]. Hydrolysis extraction method requires a significant amount of alkaline chemicals such as sodium hydroxide. When the keratinous fibre is treated with an alkaline solution, keratin chain is damaged, and keratin structure is disrupted and altered through degradation of cysteine which forms thioether linkage [17]. However, cysteine is very sensitive to alkalis, hence, it quickly decomposes in their presence, therefore, the amount of cysteine decreases rapidly when compared to the reduction method. Nevertheless, the hydrolysed keratin remains undamaged during the process [19,45].

Reducing agents: On the other hand, keratin can be extracted by reduction process, where reducing agents break cystine disulphide bonds and produce cysteine. The often used reducing agents are thiol containing chemicals such as thioglycolic acid or thioglycolate salts and 2-mercaptobisulphite, sulphitolysis agents like sodium sulphite, sodium bisulphite and sodium metabisulphite [43,46]. The reducing agents are often used with denaturing agents and surfactants to enhance the extractability of keratin [42].

Extraction of keratin using sodium metabisulphite

Ayutthaya investigated keratin extraction from chicken feathers using various concentrations of sodium metabisulphite [40]. In this method, short cleaned feathers are dissolved by immersing in solutions of sodium-metabisulphite (varying from 0 to 0.5 M) with 8 M urea, 0.6 M sodium dodecyl sulphate and 5 N sodium hydroxide and stirred at 65°C for 5 hours. The next step is to filter solution and dialyse it with distilled water for three days, changing water three times a day. To further purify the extracts, the solution is then concentrated by a rotary evaporator at 40°C and 45 mbar. The concentrated keratin is then freeze-dried before determining the percentage yield of extracted keratin and store it in a closed container. The results, according to Ayutthaya paper, showed the increased in keratin percentage yield from 36.2% to 87.6% as the concentration of sodium metabisulphite increased from 0.0 M to 0.2 M respectively. Sodium metabisulphite solution of 0.2 M concentration yielded keratin with a molecular weight range of 12-20 kDa. The sodium metabisulphite concentration above 0.2 M resulted in a decrease in keratin percentage yield because most disulphide bonds of keratin are broken, leading to keratin short chains which escape dialysis tube during dialysis. Sinkiewicz and Ayutthaya separately recorded 62.9% and 60.2% from 0.5 M sodium metabisulphite extraction [40,43]. Precaution must be considered when working with sodium metabisulphite as it is slightly toxic in both LD50 oral and LD50 dermal exposure according to Hodge and Sterner acute toxicity scale. Sodium metabisulphite is harmful if swallowed, causes severe eye blindness and liberates toxic gases if it reacts with acids, hence, eye protection must be considered when working with this substance [47-49]. According to the German Federal Water Management Act, sodium metabisulphite poses slightly danger to aquatic life, therefore it must be neutralized before discharging, like treating using sodium hypochlorite solution [47].

Extraction of keratin using sodium bisulphite

This section reviews keratin extraction from chicken feathers using sodium bisulphite. In Sinkiewiczinvestigation, one gram of pre-treated feathers was immersed in 25 ml of aqueous solution of 0.5 M sodium bisulphite, 8 M urea and 0.08 M SDS.

The mixture was then stirred at 70°C for 4 hours. For purification purpose, the mixture was centrifuged at 9000 rpm for 15 minutes before filtered through a folded filter paper. The filtrate was then dialysed using regenerated cellulose (MWCO 3500-500 Da) in distilled water for 72 hours, changing the outer water every day. The keratin sediments were then washed with distilled water and centrifuged at 9000 rpm for 15 minutes. The insoluble residue was collected, dried at 105°C and weighed. The maximum percentage yield of keratin from Sinkiewicz investigation was 84%; however, the addition of 2.5% sodium hydroxide increases the percentage yield of keratin by approximately 10%.

Sodium bisulphite is non-combustible, however, precautions must be taken when handling it because it is harmful if swallowed, it liberates toxic gases when it reacts with acids. It slightly endangers aquatic life; hence, its disposal must be controlled by stirring into sodium hypochlorite [47-50].

Extraction of keratin using sodium sulphide

Sodium sulphide can be used to extract keratin from chicken feathers; In Gupta investigation 50 g of blended clean chicken feathers was put into 2 L of 0.5 M sodium sulphide solution. The mixture was heated to the temperature of 30°C and pH was maintained between 10-13 while the mixture was continuously stirred for 6 hours. For purification purpose, the mixture was then filtered and centrifuged at 10 000 rpm for 5 minutes. The liquid was filtered again to make it particle free. 5.3 M ammonium sulphate was added dropwise to feather solution at 1:1 ratio to precipitate protein. The solution is then centrifuged at 10 000 rpm for 5 minutes, and the particles are collected. The collected solid particles are washed with 1000 ml of deionised water and centrifuged at 10 000 rpm for 5 minutes. The solid particles are collected, dissolved in 2 M sodium hydroxide, centrifuged at 10 000 for 5 minutes. The liquid is collected and stored. The percentage yield of keratin from Gupta procedure was recorded to be 53% relative to 50 g of starting chicken feathers [28]. Despite the simplicity of this method, sodium sulphide is very dangerous; it is a permeator that also causes skin irritation that may result in inflammation and eye irritation which can injure or cause blindness in case of contact. The substance is also dangerous when ingested and inhaled. Precautions must be considered when handling sodium sulphide because severe over-exposure can produce lung damage, choking and unconsciousness or death; moreover, sodium sulphide is slightly water endangering.

Extraction of keratin using Shindai method

In this method, Sinkiewicz investigated keratin extraction using 2-mercaptoethanol. To execute this method, one gram of pre-treated feathers is immersed in 25 ml of aqueous solution of 1.66M 2-mercaptoethanol with 8 M urea and 0.2 M Tris-HCl at pH 8.0. The mixture is then stirred at 70°C for 2 hours. To purify the extract, the mixture is centrifuged at 9000 rpm for 15 minutes and then filtered through a folded filter paper before dialysed using regenerated cellulose (MWCO 3500-5000 Da) in distilled water for 72 hours, changing the outer water every day. The keratin sediments are then washed with distilled water and centrifuged at 9000 rpm for 15 minutes. The insoluble keratin residues are then dried at 105°C and weighed to determine percentage yield of keratin. The percentage yield of keratin from Sinkiewicz investigation was 90% [43]. Yin used the similar method of keratin extraction from chicken feather and obtained 93% keratin yield; hence, this method can be replicated to obtain this high percentage yield of keratin in just 2 hours of dissolving feathers [51].

Nevertheless, 2-mercaptoethanol is moderately toxic in both oral and dermal exposure; it causes skin irritation, severe eye damage, and allergic skin reaction. It causes damage to organs through continued or frequent exposure if consumed. 2-

mercaptoethanol is also toxic to marine life with enduring effect. Moreover, it is a high-water endangering substance. 2-mercaptoethanol must be kept below 30°C due to its combustibility.

Extraction of keratin using thioglycolic acid

The other keratin extraction method is by use of thioglycolic acid; Gupta extracted keratin from 50 g of chicken feathers using 2 L of 0.5 M thioglycolate solution with 0.1 N sodium hydroxide. The mixture is then heated to 30°C while the solution is being stirred and pH maintained between 10-13 for 6 hours. To purify the extract, the mixture is then centrifuged at 10 000 rpm for 5 minutes, and the collected liquid is filtered to remove insoluble residues further. To refine the extracted keratin, it is precipitated by adding 5.3 M of ammonium sulphate solution dropwise, while stirring the solution. Keratin solid particles are then collected after 5 minutes centrifugation at 10 000 rpm. The percentage yield of keratin obtained from Gupta investigation was 8.8% [28]. However, Hatakeyama obtained percentage yield of 75% when they used thioglycolic acid and sodium hydroxide to extract keratin from wool, a method that can be adapted for keratin extraction from chicken feathers, while allowed to dissolving time of 16 hours for a solution pH value of 13 [52].

Thioglycolic acid is toxic if swallowed, in contact with skin or if inhaled. The liquid causes severe skin burn and eye damage, hence, protective gear must be worn to avoid skin contact, inhalation and eye irritation. This substance should be stored in a temperature range of 2°C to 8°C due to its combustibility [47-49,53]. According to the German Federal Water Management Act, thioglycolic acid slightly endangers aquatic life, thus, precautional measures must be taken [50].

Extraction using ionic liquids: Idris (2014) and Xie (2005) reported the use of imidazole ionic liquids such as 1-allyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium chloride and 1-butyl-3-methylimidazolium bromide to extract keratin. The reports show that chlorine-containing ionic liquids are more effective than other ionic liquids, this might be due to nucleophilic strength of chlorine ions as compared to other halogen ions. The extracted keratin by these liquids is mainly composed of beta-sheet structure than alpha helix structure which seems to be destroyed during extraction process [1,19]. Moreover, the extract from wool showed greater thermal stability than the original wool. Ionic liquids are said to be environmentally friendly and cost-effective because of their recyclable ability which minimises their disposal to the environment and reduces the consumption of fresh raw ionic liquids [44].

Extraction of keratin using imidazole ionic liquids

Imidazole ionic liquids can also be used to extract keratin from chicken feathers; Ji used imidazole ionic liquids 1-allyl-3-methylimidazolium chloride [Amim]Cl, 1-butyl-3-methylimidazolium chloride [Bmim]Cl and 1-butyl-3-methylimidazolium bromide [Bmim]Br. These liquids were used in proportion to different weights of Na_2SO_3 and water to dissolve chicken feathers. The mixture of liquids with chicken feathers is then heated, to advance disulphide bond cleavage, under magnetic stirring condition. The sample is filtered by suction filtration before the protein is precipitated by adding water to the mixture. Keratin solid particles are then collected using suction filtration; at this stage, the sample is ready for percentage yield determination. Ji investigation obtained reported a maximum yield of 75.1% using [Bmim]Cl ionic liquid under extraction time of 60 minutes at 90°C [44].

Precautional measured must be taken when one works with imidazole ionic liquids because they cause skin irritation, acute eye irritation and may cause respiratory irritation [47,49]. The liquids also pose a danger if swallowed due to its moderate

toxicity level on Hodge and Sterner acute toxicity scale [48]. According to the German Federal Water Management Act, [Bmim]Cl is highly dangerous to water life with chronic effect [50]. Wang and Cao extracted keratin from chicken feathers using hydrophobic ionic liquid, IL,1-hydroxyethyl-3-methylimidazolium bis(trifluoromethanesulfonic)amide ([HOEMIm][NTf_2]) and sodium hydrogen sulphate. The maximum percentage yield of keratin obtained from the investigation of Wang and Cao was recorded as low as 21.5%. The addition of the ionic liquid increased the percentage yield of keratin up to some extent depending on the mass ratio of ionic liquid, feathers, and $NaHSO_3$ [41]. The primary objective of keratin extraction from chicken feathers is to valorise chicken feathers. Hence, the percentage yield of keratin is prioritised during chicken feather dissolving; therefore, keratin extraction by the [HOEMIm][NTf_2] liquid is not efficient on keratin recovery. This substance, 1-hydroxyethyl-3-methylimidazolium bis(trifluoromethanesulfonic) amide is moderately toxic if swallowed according to Hodge and Sterner acute toxicity scale, a precaution against swallowing is vital; it is also hazardous to aquatic life [47,50].

Oxidising agents: Keratin can also be extracted by an oxidation method, where oxidising agents like peracetic acid, performic acid, potassium permanganate, sodium hypochlorite and hydrogen peroxide are used to break cystine disulphide bonds and release cysteine amino acids with sulfonic groups as side chains. The extracted keratin may contain alpha helix structures and beta sheet structure, in which oxidation by peracetic acid or performic acid allows their separation due to their solubility at different pH values [42].

Costs of the reviewed methods of chemical extraction of keratin: Cost of implementing the keratin extraction procedure is one of the vital factors for choosing a keratin extraction technique. This section is, therefore, describing them, excluding purification after cooking the mixture. The Table 1 below illustrates the unit costs of the materials and equipment, as per Sigma-Aldrich Pty. Ltd catalogue, that is used in the keratin extraction procedures, described above, at laboratory scale [36].

TABLE 1. **Material for keratin extraction, for selected methods, and cost per, in rand, per gram.**

Materials	**Unit cost (Rand per gram)**
Sodium metabisulphite	0.86
Urea	2.04
Sodium dodecyl sulphite	1.59
Sodium hydroxide	1.69
Sodium bisulphite	0.32
Sodium sulphide	0.85
2-Mercaptoethanol	55.4
Tris-HCl	3.09
Thioglycolic acid	6.4

The tabulated unit costs in Table 1, with concentrations of chemicals that are required to prepare 100 ml keratin extraction mixtures and electrical energy consumed, as per Eskom tariffs, during extraction are the bases of the cost calculations. Table 2 shows the costs of keratin extraction and keratin percentage yields of various extraction techniques [54].

TABLE 2. **Keratin extracting methods, cost of extraction and percentage yield of keratin.**

Keratin extraction method	Cost (rands)	%Yield of Keratin
Thioglycolic acid	33.24	75
Sodium sulphide	50.28	53
Sodium bisulphite	111.08	84
Sodium metabisulphite	176.02	87
2-Mercaptoethanol (Shindai)	758.80	90-93

As it is clearly seen in Table 2, an increase in extraction cost from thioglycolic acid to 2-mercaptoethanol and cost dependent percentage yield with an exception of thioglycolic acid which seems not to correlate with others. Shindai, 2-mercaptoethanol, method is the expensive extraction technique (Table 2), however, the keratin percentage yield may attract the interests of the researchers.

Enzymatic hydrolysis

Keratin extraction in a very high or low pH for extended time at high temperatures produces low molecular weight peptide fragments because chemicals break both disulphide and peptide bonds, and this limits several biomedical applications [23,55,56]. The enzymatic hydrolysis of keratin is the use of biological catalysts to catalyze chemical reaction during keratin extractions. Enzymatic hydrolysis requires mild treatment conditions hence conserves the functional properties of extracted keratin [23].

Microwave-assisted keratin extraction

Microwave keratin extraction uses microwave radiation to extract keratin from fibres. The extraction is done by using the principle of direct heating the molecules of the material using microwave energy. The transformation of electromagnetic energy to thermal energy occurs when the mechanisms, ionic induction and dipole rotation take place simultaneously [57]. Keratinous fibres can also be put into superheated water at the temperature ranging from 150-180°C in a microwave reactor [58]. Shavandi used microwave radiation varying power in a range of 150-570 Watts [42]. The power was applied for up to 7 minutes at a temperature of 180°C. Microwave-assisted keratin extraction is advantageous in a way that it may take place in low temperatures over a short period of time.

Supercritical water and steam explosive

In supercritical water and steam explosive keratin extraction, steam is forced into keratinous tissue and cells of biomass. The high-temperature steam penetrates tissues and cells, pressurising and then rapidly depressurises them. This causes an explosion in millisecond reaction which releases keratin. The process results in reduced molecular weight of keratin and loss of mechanical properties [42,59].

Applications of keratin biopolymer in biomedicine

Keratin biomaterials have been produced and experimentally used in several biomedical applications, this is due to their biocompatibility, biodegradability and the capability of keratin to act as an extracellular matrix to facilitate cell adhesion. The

following subsections highlight areas of focus in applications of feather keratin-based biomaterials to emphasize the potential biomedical applications of keratin extracted from waste chicken feathers.

Drug delivery carriers

Drug delivery systems are technologies that are engineered for the targeted delivery and or controlled release of therapeutic substances to improve health and extend lives [60]. Several synthetic and natural polymers have been used as drug carriers. Even though synthetic polymers are often used compared to natural polymers due to modifiable properties to cover a wide range of application, they pose side effects. Therefore, the focused has turned to natural polymers for safe use as drug delivery carriers. Multiple proteins, including keratin, have been investigated in the development of biomaterials, and keratin-based materials showed positive outcomes. Researches are underway to improve this system to make it more effective and precise; one way to do this is to incorporate nanotechnology and biomaterials in development of drug delivery carrier [61]. Keratin biocompatibility property, that is due to the presence of amino acids, allows keratin to be modified to meet drug delivery requirement [62] (Table 3).

TABLE 3. **Drug delivery keratin-based carriers, keratin sources and results of investigations.**

Type of Keratin biomaterials	**Keratin source**	**Drug delivery ability**	**References**
Keratin film	Chicken feathers	Drugs were loaded and released successfully. Good mechanical properties films provided a continuous release of loaded drug for up to 12 hours.	[51]
Keratin-hydrotalcite hybrid films	Merino wool	support fibroblast cells adhesion and growth suggesting their potential use as drug delivery systems	[63]
Keratin graft polyethylene glycol (Keratin-g-PEG)	Wool	The drug released from the loaded keratin-*g*-PEG nanoparticles showed that it can be internalized into the cells efficiently, and the loaded drug indicated a faster release into the nuclei of the cells	[64,65]
keratin/doxorubicin nanoparticles	Human hair	Keratin-based drug carrier is potential for cancer therapy. Keratin/doxorubicin nanoparticles were able to catalyse nitric oxide release from blood endogenous donor	[66]
Unextracted	Cancer cell plasma membrane in human	The study establishes keratin 1 as a new marker for breast cancer targeting	[67]
Keratin Hydrogel	Chicken feathers	The cumulative release of the anticancer drug (Dox·HCl) reached 93.3% within 16 h, and the cumulative release rate of macromolecular drug (BSA) got to 75.9% in 24 h. Therefore, the keratin-based biopolymer hydrogel with	[68]

		interpenetrating network structure, pH-sensitivity and temperature sensitivity are potentially applied to sustain drug carrier	
Keratin films with catalysed crosslinking	Wool	Films showed a lower drug release ratio in which drugs can be loaded and released over a longer period for prolonged healing. These films also showed an increase in tensile strength and decrease in elongation at break.	[69]
Keratin hydrogel	Chicken feathers	The hydrogels were able to release 97% drugs for 24 hours	[70]
Keratin hydrogel	Wool	Keratin hydrogel and chemically modified keratin hydrogels showed good drug delivery carrier properties, with a delivery rate that ranges from 1-3 days.	[71]

Wound dressing

Wound management is a necessity for wound healing process. To archive this wound dressing and wound treatment play vital roles. The wound dressing material may include multiple layers to maintain moist environment at the wound, be able to control excess exudates, the material must be able to protect wound from the outside environment, adhere well to the skin and be comfortable to the body part [72]. Wound dressing materials are in various forms including, but not limited to, nanofibrous mats, hydrogel, films, sponges or foams. Keratin has a potential of being incorporated in the development of wound dressing materials due to its biocompatibility property and more. Investigations are ongoing to introduce keratin wound dressing biomaterials to clinical application. Table 4 highlights some keratin wound dressing materials which have been developed from across the world.

TABLE 4. **Wound dressing keratin-based biomaterials, keratin sources and results of investigations.**

Type of Keratin biomaterials	**Keratin source**	**Observations**	**References**
Keratin/gelatin nanofibres with polyurethane as an outer layer	Human hair	*In vivo* study, keratin nanofibres mat gave reduction in wound area at 4 days, and better wound repair at 14 days with a thicker epidermis and larger number of newly formed hair follicles, thus, this material could be a good candidate for wound dressing applications.	[73]

Keratin nanofibres	Chicken feathers	Three keratin blended wound dressing materials were prepared, namely keratin nonwoven (KN), keratin-sodium alginate (KSAN) and keratin-chitosan (KCN). *In vivo* observed at 15, 17, 21 and 23 days showed better wound dressing effects of KSAN and KCN than KN.	[74]
Keratin powder	Mouse fur	Fur keratin-derived protein dressings significantly accelerated wound healing in the mouse mode, which is a good outcome.	[75]

Tissue engineering

Damaged body tissues or cells may require the assemble of functional construct that restores, maintains, improves them or the whole organ. Generally, cells are the building blocks of tissues which are the basic unit of body functioning. This is tissue engineering, it evolved from the field of biomaterials development to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues, however, this field is now extending using tissues as biosensors and chips that detect threads agents and toxicity test, respectively. The tissue engineering process begins with building a scaffold from synthetic and or natural sources. Keratin can be used in development of scaffolds for tissue engineering [76-80].

Electrospinnability of keratin

Keratin nanofibres could be used in wide range of keratin biomedical applications including drug delivery carrier, wound dressing and tissue engineering, therefore, it is important to gather the evidence of chicken feather electrosinnability in order to valorise them. To verify the electrospinnability of keratin, it is important to recall the properties of keratin, that might affect its electrospinnability, and relate them to the basic requirements of electrospinning. Examples of how the keratin properties influences the electrospinnability of this biopolymer are described below.

Keratin properties in relation to its electrospinnability

Viscosity is one of the most important parameters on electrospinning. The viscosity of the solution and its electrical properties determine the extent of elongation of the solution. These influence the diameter and other morphological properties of the resultant electrospun fibres [81]. However, this property has limits, at low viscosity; it is common to find beads along the fibres deposited on the collector. When the viscosity increases, there is a gradual change in the shape of the beads from spherical to spindle-like until a smooth fibre is obtained. High viscosity discourages the bending instability to set in for a longer distance as it emerges from the roller surface. As a result, the jet path is reduced and the bending instability spreads over a smaller area [82,83]. This reduced jet path also means that there is less stretching of the solution resulting in a larger fibre diameter. However, when the viscosity is high enough, it may discourage secondary jets from breaking off from the main jet which may contribute to the increased fibre diameter [84]. Therefore, determining the viscosity range of each polymer to be spun is required. One of the factors that affect the viscosity of the solution is its molecular weight. The molecular weight of the spannable polymers ranges from 13 kDa to about 200 kDa [85] (Table 5).

TABLE 5. **Keratin-based scaffolds for tissue engineering, keratin sources and results of investigations.**

Type of Keratin biomaterials	Keratin source	Tissue engineering ability	References
Keratin/Gelatin/Chitosan	Hooves	The scaffold exhibited good porosity and interconnectivity of pores, and cells demonstrated good cell viability of keratin scaffold.	[76]
Keratin/poly (ε-caprolactone) nanofibres mat (keratin/PLC)	Human hair	Test showed that fibroblast cells adhered more to keratin/PLC mat than PLC. And blood clothing time test indicated that the mats are blood compatible which makes them potential scaffolds for vascular tissue engineering	[77]
Powdered scaffolds	Chicken feathers	*In vitro* cell viability test indicated that the scaffolds are biocompatible and support cell growth, this were positive results for tissue engineering application	[78]
Keratin based hydrogels	Human hair	*In vitro* study showed positive results for peripheral nerve regeneration over 6 months period.	[79]
Keratin/chitosan mats	Human hair	The cells that were cultured in nanofibers showed growth, forming the layer on the scaffold, mimicking the epidermis tissue.	[80]

The Aluigi investigation of the structure and properties of keratin/polyethylene oxide nanofibres shows extracted keratin molecular weight ranges from 11kDa to 60kDa in which a large distribution of it falls within the molecular weight range of spannable polymers [46]. The molecular weight of the polymer may also influence electrical conductivity and surface tension of the solution; these properties have significant effect on electrospinnability of a polymeric material [86].

Electrical conductivity of the polymer solution is one of the requirements for electrospinning because the polymer solution must allow charge induction to form Taylor cones and then polymer jets. Aluigi investigation also reported that increasing the amount of keratin in electrospinning solution results in significant increase of solution electrical conductivity which might be due to the polarity of its amino acids [46]. Nevertheless, the polymer solution electrical conductivity can always be improved by adding an electrical conductive substance like salts or conductive solvents even though this compromises the diameter of the nanofibres [87,88].

Surface tension is also a critical factor in electrospinning. The formation of droplets, beads and fibres depends on the surface tension of solution. Generally, high surface tension of a solution inhibits the electrospinning process because of instability of the jets and the generation of sprayed droplets [89]. Low surface tension of the spinning solution helps electrospinning to occur at a lower electric field [90]. However, not necessarily a lower surface tension of a solvent will always be more suitable for electrospinning, surface tension determines the upper and lower boundaries of the electrospinning window if all other variables are held constant [91-93]. The surface tension mostly depends on the composition of the solvent, hence, extracted

keratin may insignificantly influence surface tension of the electrospinning solution. The following section reveals the work that have been done on electrospinning of keratin to keratin nanofibres.

Electrospinability of keratin

Literature shows the existence of biopolymer keratin nanofibres [58], therefore, the co-existence of keratin from the worldwide abundant chicken feathers [32], and applications of large surface area-to-volume ratio nanofibres [94] is a phenomenon that shall be embraced. Nanofibrous materials have been investigated for their extravagant applications such as gas-sensor ability [95]. Biocomposite nanofibres in urea biosensing have also been investigated and showed improvements, over existing technology, in properties such as response time and sensitivity to lower concentrations of urea [96]. Moreover, nanofibrous materials in biomedical applications have drawn much attention due to their abilities in biomedicine field [3]. Polyvinyl alcohol nanofibres can controllably release ketoprofen when it is used as a drug carrier [97]. Antimicrobial wound dressing nanofibres materials loaded with silver nanoparticles for aerobic bacteria reduction showed excellent properties and antibacterial effects [98]. Also, researchers revealed potential nanofibres applications in tissue engineering, including tissue scaffolding [99,100]. Nanofibres biomedical applications are advanceable by petrochemical polymers replacement or blending with biopolymers, this enhances nanofibres matrix-to-cell interactions. Thus, keratin is one of the biopolymers that can supplement petrochemical polymers lacking properties for biomedical applications. Therefore, electrospinnability of keratins, including chicken feathers keratin is vital to nanofibres materials biomedical applications [3]. Although pure keratin nanofibres are non-existence, researchers have managed to produce keratin blended nanofibres [40].

Wool alpha keratin can blend, with good interaction with, with polyvinyl alcohol to form nanofibrous materials which have improved thermal and mechanical properties as compared to keratin properties. The wool alpha keratin-polyethene blend nanofibres, with various ratios, can be spun which suggests that alpha keratin blends with a range of synthetic polymers [20,46]. In addition, human hair alpha keratin blends with, at least one synthetic polymer, poly(ε-caprolactone) and electro-spun to nanofibres that can act as a composite base of uniform fibre morphology and suitable mechanical properties for biomedical applications [101]. The other type of keratin, beta keratin from chicken feathers, has not yet been electrospun to 100% keratin nanofibres rather blended with other electrospinnable synthetic polymers such as polylactic acid (PLA) even though the mats reflect poor mechanical properties and instability in water [102]. This keratin/PLA composite nanofibrous material can be used as scaffolds for tissue engineering in biomedicine [103].

Keratin-based materials have not made it to any commercial industry across the spectrum despite their potential applications and a demand for such products. All the work that has been done on these keratin-based materials proves to be, somehow, inadequate to commercialise them, therefore, more investigations must be done to eliminate all the limitations that prohibit commercialisation of these materials. Table 6 summarises some of the work that has been done regarding converting keratin to keratin nanofibres. This table does not include work that has not been discussed in this section; a lot more work has been done on the electrospinnability of keratin.

For each investigation, Table 6 shows the source of keratin, extraction technique of keratin, type of polymer that was used to blend with keratin to improve its properties and reference.

TABLE 6. **Advancements of the properties of keratin nanofibres by blending keratin with synthetic polymers.**

Source of Keratin	Extraction Technique	Type of Polymer used	Aim	Reference
Chicken feathers	Sodium metabisulphite	Polylactic acid	To advance processibility of keratin	[40]
Wool	Sodium metabisulphite	Polyvinyl alcohol	To enhance wool-keratin properties	[20]
Wool	Sodium metabisulphite	Polyethylene oxide	Biomedical application	[46]
Human hair	Trizma base	Poly(ε-caprolactone)	Biomedical application	[101-103]
Human Epidermis	2-mercaptoethanol	Polylactic acid	Biomedical application	[104,105]

Discussion

Regenerated keratin can also be used in nanofibres form that is produced via electrospinning [106]. In electrospinning, a high voltage is applied to a polymer fluid to charge it; when charges within the fluid reached a critical amount, a fluid jet will erupt from the droplet resulting in the formation of a Taylor cone. The electrospinning jet travels towards the region of lower potential, which in most cases is a grounded collector [106-108]. This process results in the formation of microfibres and nanofibres. The high surface area to volume ratio, flexibility, and some mechanical properties are some of the properties that draw attention to nanofibres applications [94].

Applications of nanofibre products in various fields such as tissue engineering, drug delivery carriers, cancer diagnosis, optical sensors, oil-water separation, air filtration and lithium-air battery are driving forces for improvement in production of nanofibres for such applications. Thus, keratin nanofibres can be used for these nanofibres applications [109,110]. Like other forms of keratin materials, keratin nanofibres have thus far been produced by blending keratin with another polymer like polyethene and polyvinyl alcohol [46,20]. Keratin-based nanofibres are an alternative to nanofibres of petroleum polymers as they possess an improved cell to material interaction since they are protein based [3].

Conclusion

Persistence of a problem of keratin-based waste, including waste chicken feathers, indicates the need for further investigation of their valorisation process. Pure keratin is worth paying attention to due to its high price per kilograms. Variety of keratin-based products across different fields is sufficient to valorise keratin-based waste by-product, especially waste chicken feathers from the poultry industry. There are adequate keratin extraction techniques that can assist in the valorisation of keratin-based waste by-products. Electrospinnability of keratin blend enhances its potential for biomedical applications especially as nanofibres receive an overwhelming welcome to the same field. Nonexistence of breakthrough of keratin-based biomaterial in the clinical applications shows innovative opportunities for further investigation of keratin biomaterials, including chicken feather keratin-based biomaterials. Extensive research and development work is required to develop appropriate technologies for utilisation of waste chicken feathers as a source of some of the proposed biomedical applications mentioned in this review.

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