Journal of Renewable Materials

Tech Science Press

DOI: 10.32604/jrm.2024.056251

REVIEW

Synthesis and Properties of Biomimetic Self-Assembling Structures from Poultry Feather Keratin

Sara Mattiello and Carlo Santulli*

School of Science and Technology, Università di Camerino, via Gentile III da Varano 7, Camerino, 62032, Italy

*Corresponding Author: Carlo Santulli. Email: carlo.santulli@unicam.it

Received: 17 July 2024 Accepted: 19 September 2024 Published: 20 January 2025

ABSTRACT

Taking a widely contaminated yet abundant waste, such as poultry feathers, and extracting keratin from this structure appears to be a real challenge whenever the preservation of the secondary structure of the protein is desired. This process would allow exploiting it in ways (e.g., in the biomedical field) that are inspired by a structure that is primarily designed for flight, therefore capable specifically of withstanding flexure and lateral buckling, also with very low thicknesses. The preservation of the structure is based on disulfide crosslinks, and it is offered with preference by some chemical treatments, mainly those based on ionic liquid and on a reduction process. However, the degree of preservation cannot always be precisely assessed; however, beyond chemical characterization, the formation of homogeneous gels can also suggest that the process was successful in this sense. An extraction respectful of nature's intentions, considering that the secondary structure builds up according to the very function of the feathers in the animal, can be deemed to be biomimetic. In particular, biomimetic extractions comply with the very characteristics the protein was designed for to serve in the specific environmental and mechanical situation in which it is inserted. This review tries to elucidate in which cases this aim is achieved and for which specific applications a chicken feather keratin that has preserved its secondary structure can be suited.

KEYWORDS

Keratin extraction; secondary structure; self-assembly; chicken feathers

Nomenclature

[Amim]Cl	1-Allyl-3-methyl-1H-imidazol-3-ium chloride
[Bmim]Br	1-Butyl-3-methylimidazolium bromide
[Bmim]Cl	1-Butyl-3-methylimidazolium chloride
[HOEMIm]	1-hydroxyethyl-3-methylimidazolium bis(trifluoromethanesulfonyl)amide
FTIR	Fourier transform infrared spectroscopy
ILs	Ionic Liquids
[NTf_2]	1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
PBAT	Poly(butylene adipate-co-terephtalate)
PCL	Polycaprolactone
PVA	Poly(vinylalcohol)
SAXS	Small angle X-ray scattering

1 Introduction

A biomimetic approach to materials would regard the possibility of promoting their self-assembly in a way that allows the growth of autonomous structures to be used for functional purposes [1,2]: this procedure is particularly sought in the case of peptides and proteins [3]. More specifically, the typical structures of proteins, such as collagen, elastin, and keratin, all include some degree of α-helices arrangements [4]. The most recent novelty, however, is constituted by the possibility of obtaining these structures out of the material extracted from animal waste, which enables the establishment of correlations between their aspect and the route by which self-assembly has been realized [5]. As far as protein extraction is concerned, extracted material takes the form of hydrolysates and small peptides [6]. These nanomaterials can have a role in acquiring a biomimetic function towards appropriate training exerted on non-natural protein backbones [7]. In other words, some extraction methods confer to the material a higher capability to resist proteolysis than others and can be regarded as adapted to its biomimicking [8].

In practice, keratin-based waste from different origins/biological structures can be used for the purpose: these include, among others, sheep wool in the form of hydrolyzed peptides [9], or in more structured form for direct blending with biopolymers [10], where a biomimetic approach might reduce the final risk of generating other hazardous material difficult to dispose of [11]. Other keratin residues in search of sustainable routes for disposal are human hair [12], epidermal waste [13], nails [14] or claws [15], and poultry feathers [16], on which this review specifically focuses.

Chicken feathers can be considered dense shells with a porous core, which provides lightness and is able to be compressed or even locally torqued without being buckled [17]. Their application as fibers (chicken feather fibers, CFF) in composites, purposely separated from the rachis [18], though proposed in many cases, does reduce this structural complexity to the bare tensile support of a polymer resin [19]. On the other hand, keratin extraction may result in the destruction of the composite structure, which is the scope of the biomimetic process to avoid. The main issue is unlocking the protein by extracting it without damaging its secondary structure and, therefore, promoting the self-assembly of the keratin structure in another geometry from the original one [20]. The secondary structure is mainly influenced by the position of the side chains and hence the degree of close packing in the protein, which for keratin is controlled by the presence of a number of different motifs [21], namely α-helical, β sheets, β-turn, and random coil structures [22].

In practice, the organization of chicken feathers includes, as the effect of the judicious combination of α and β forms, the combined presence of crossed-lamellar structure (300–600 nm thick) in lateral walls of rachis and barbs, arranged into alternate layers of crossed-fibers [23]. A significant grade of preservation of this structure out of keratin extraction is particularly beneficial in terms of toughness, which also allows its blending with other proteins isolated, e.g., from soy, for the formation of composite films [24].

The isolation of keratin from poultry feathers has been demonstrated of interest in the last few decades, especially, but not exclusively, in the food-related and the cosmetics sector [25]. Here, the preservation of secondary structure is not always considered essential [26], while rather preventive purification processes, based, e.g., on ethanol, ozone, and sodium chlorite, are given larger significance [27]. Another possibility, which is specific to poultry-originated keratin is the capability to absorb some metals, e.g., in soil treatment, such as cadmium, nickel, chromium, and zinc [28]. In some cases, nonetheless, the use of keratin from poultry feathers can be considered biomimetic, more explicitly whenever the architecture of the folding structure is considered and used to achieve specific functionalities. This has been, e.g., recently performed in the case of carbonized feathers therefore used as biochar [29], where the preservation of keratin structure did result in the possible application to the removal of the residues of drugs, such as amoxicillin, from aqueous solutions [30]. Keratin biochar is also particularly adapted to be possibly blended with other similarly abundant biomass waste, such as is the case for sugarcane bagasse,

which suggests synergistic effects to be obtained from adapted doping strategies between the two feedstocks [31]. However, the energy-intensive character of these processes based on carbonization has also suggested that to use chicken feathers with a biorefinery approach, biodegradation/metabolism by the action of bacteria would represent a more suitable route towards the extraction of free amino-acids and soluble proteins [32].

To allow the potential use of keratin extracted, hence solubilized, from chicken feathers, a possible approach is based on the fabrication of autogenous cross-linked gels, e.g., serving as plant growth media [33]. On the one hand, this route can provide some mechanical performance that might ease application and compete, at least as blends, with other categories of gel structures, such as those obtained from polysaccharides, e.g., starch [34], alginates [35], or guar gum [36]. On the other hand, restoring secondary structure, hence protein cross-linking, starting from disulfide links, but not limiting to them, would represent a more natural application of keratin, therefore providing also other characteristics, such as controlled water retention, elongation, and strength [37]. These properties were exploited for some uses, such as wound healing, using a blended film with polysaccharides [38], or in a more general sense, in the biomedical engineering sector, including, e.g., also applications for drug delivery [39].

This review concentrates on chicken feathers since they constitute a very large waste of the food-related production sector, and therefore, their functional use would possibly result in a circular economy approach, offering a larger value to waste. Using poultry feathers in a biomimetic way for the production of engineered structures does involve preserving as much as possible their features during extraction, in the understanding that keratin-based structures do present a number of fundamental biomimetic properties in a thermal insulation and low-density context, which include reversible adhesion, structural coloration and the possibility to offer super-hydrophobic surfaces [40]. To achieve this potential, it is important, though, to preserve as much as possible the secondary structure of feather keratin, which will be described in Section 2. A number of methods exist for the extraction of keratin from feathers, which are reported in Section 3.

Following this, the discussion does particularly concentrate on those works where keratin is extracted with the preservation of its secondary structure, which can be suggested to represent a biomimetic application of poultry feathers' keratin. With this aim, bio-inspired materials based on poultry feather keratin are discussed, after general considerations of the keratin role in these materials (Section 4.1), being either exclusively based on keratin or as a significant component of a blend with another biopolymer (Section 4.2). Finally, applications with particular reference to the bio-inspired potential, therefore with evidence of self-assembly, are discussed (Section 4.3). Conclusions and potential for future research are offered in Section 5.

2 Structure of Keratin in Feathers

The design of a chicken feather is constituted on hierarchical levels, depicted in Fig. 1 [41], namely the basal calamus, the main structure of the rachis, from which barbs, and then barbules irradiate. Recent studies also emphasized the variable characteristics of feather design between broiler chicken, reared for meat production, and layer chicken, which are intended at egg production instead [42]. This occurs since the feathers have specialized functions, in particular, contour feathers are dedicated to flight, down feathers serve for insulation, and small ornamental ones are aimed at signaling for social activity. The different dimensional levels represented are deemed to constitute the base for the engineering of feathers' functionality, comprising flight ability and thermal insulation properties [43]. In other words, they are able to transform through a controlled and tailored spiraliform arrangement, a unidimensional appendage into a volumetric body capable of actively sustaining the flight action [44]. In particular, the section of the rachis comprises epicortex, with crossed-fiber architecture, made of β keratin, which offers a trabeculae-like support [45], then cortex and the medullary pith with its foam-like structure [46]. The latter has recently been proposed as to offer some bio-inspired action of thermal insulation due to its

cellular geometry [47]. Keratin fibers are arranged in a cross-like architecture being coated with amorphous protein: this disposition is connected to the need to withstand specific forces during flight, mainly by flexural and shear loads, maintaining a sufficient flexibility notwithstanding the required stiffness offered by the protein structure [48]. In particular, an uninterrupted structural connection appears to be formed between the cortex of the rachis and the barbs [49]. The idea is that the rooting of barbs within the rachis is helpful in withstanding the aerodynamic forces during flight [50]. As a matter of fact, the whole structure of the feather optimizes bending stiffness to sustain loading in flight: in that respect, the heavily deformed structure is then recovered by immersion in water, aimed at simulating air moisture effect [51].

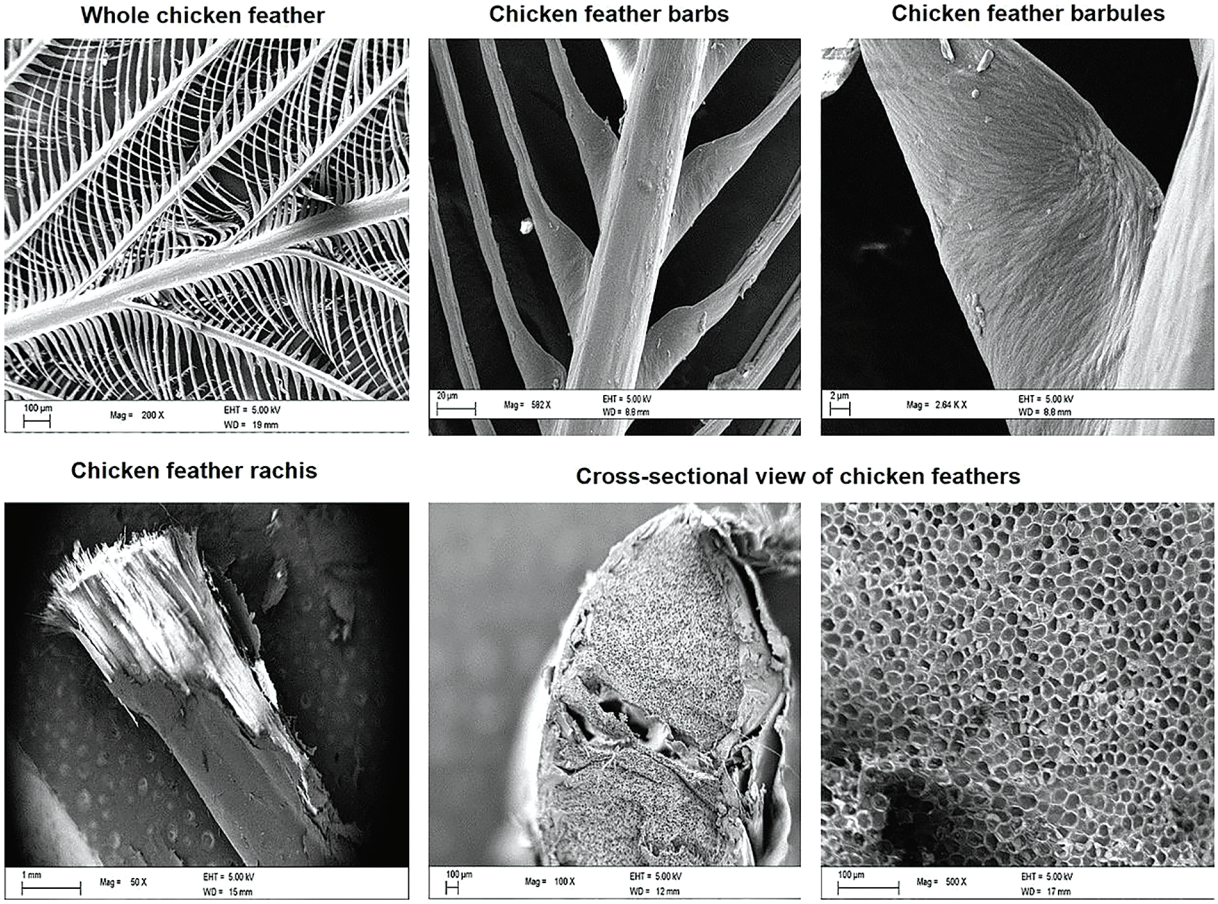

Figure 1: Different parts of keratin feathers, showing hierarchical structure (Reproduced with permission from Reference [43], © Elsevier 2017)

At a microscopical level, though, the structure of feather keratin takes the form of microfibrils, less ordered with respect to alpha-keratin, yet still following a composite arrangement such as fiber + matrix [52]. It has been also suggested that the natural model followed during feather development, and that has therefore to be accounted for in the self-assembly process, is that of a simple twisted β-sheet [53], as indicated in Fig. 2, which details the different dimensional levels. Investigations carried out by small angle X-ray scattering (SAXS) have demonstrated the more compact short-range organization of feather keratins with respect to wool keratin [54].

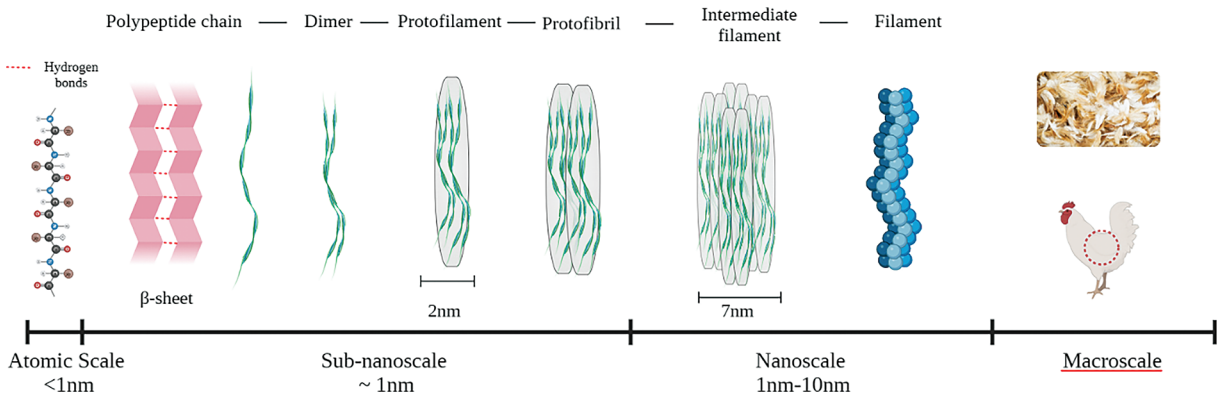

Figure 2: Schematic representation of keratin structure ranging from the atomic scale to the macroscale (Adapted from Reference [20] © Elsevier 2022)

Going more into detail, into the molecular level, keratin is generally rich in cysteine, which creates a strong covalent disulfide bond, which results in crosslinking, promoting therefore an increased hardness of the structures [55]. This ultimately contributes to cornification, hence conversion into a horn structure, of epithelial cells of feathers [56].

3 Methods for the Extraction of Keratin from Feathers and Preservation of the Secondary Structure

Different methods are available to extract keratin from feathers (a summary of the various techniques adopted is offered in Fig. 3), among which this review gives preference to those that are able to preserve its secondary structure, which results also in maintaining its antioxidant potency [57]. A number of chemical methods like reduction [58], ionic liquid [59] and alkaline hydrolysis [60], are used for keratin extraction, but not all of them preserve the secondary structure. The completeness of the secondary structure preservation is difficult to assess, even for recent studies that concentrate on the morphological characteristics of the keratin extracted from feathers the amount of β sheets crosslinked by disulfide bonds was not easily measurable from microscopical observation [61]. More reliable measurements can be offered by quantitative Raman spectroscopy, especially by comparing the intensity of the peaks representing S-S disulfide bridges and β sheets, typically around 521 and 1662 cm^{-1}, respectively [62]. Apart from the extraction process, it is also worth noting that the application of tensile stress on the keratin structure might lead to a more reduced preservation of disulfide bonds, due to their stretching, which might also be ascribed to the agitation process during chemical action [63].

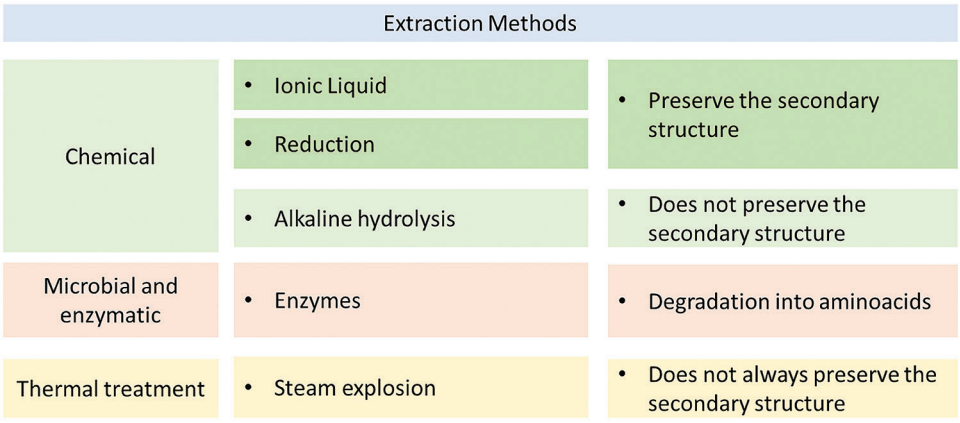

Figure 3: Summary of extraction methods used for feathers keratin

In particular, alkaline hydrolysis, also facilitated by cetrimonium bromide, resulted in a significant disruption of the hydrogen links and in the virtual absence of peptides at 200°C [64]. Often alkaline methods are assisted by microbial and enzymatic ones [65] using keratinases [66]. These can operate autonomously in a biorefinery concept, involving diversification of products and full use of waste resources [67]. This contributes to the complete use of the whole of feather residues, in a circular economy approach, as reported in Fig. 4. Further assistance can be provided by ultrasound irradiation [68], or by microwave treatment [69], where the texture and morphology of extracted material can be controlled by the time and energy of irradiation [70]. In all these cases, the final product yielded are functional protein + hydrolysates of interest for the food industry [71]. Another important factor, which in particular influenced the preservation of the secondary structure, while not directly related with the adopted method, is the retention time in the alkaline solution, since keratin is typically soluble in variable amounts depending on the pH of the relevant environment [72].

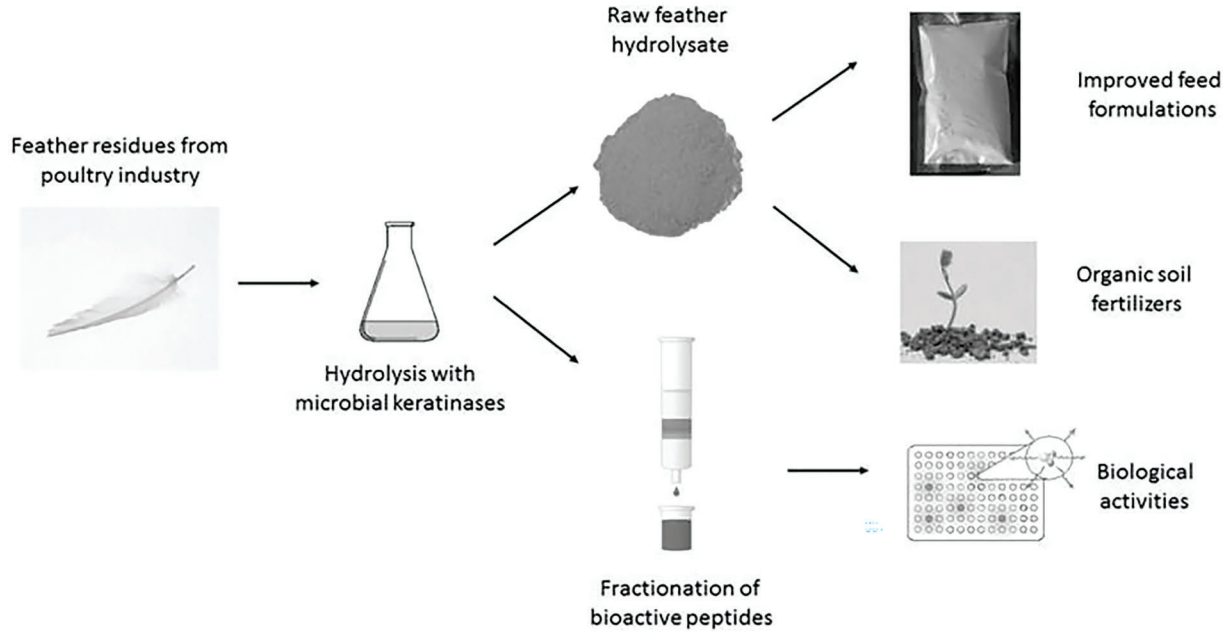

Figure 4: Different destinations of poultry feathers by a microbial enzymatic extraction (Reproduced with permission from Reference [67], © Elsevier 2015)

Ionic liquids (ILs) method for the extraction preserves the structure of the keratin, at least partially, with yields as high as 45% in turkey feathers [73]. In particular, on chicken feathers, the use of ILs [Amim]Cl, [Bmim]Cl and [Bmim]Br have been studied by Ji et al. [74], demonstrating that the imidazole liquid [Bmim]Cl can extract keratin from feathers, the solvent of IL is non-volatile and easy recyclable. Other hydrophobic IL have been used, such as [HOEMIm] [NTf_2], which offers molecular weights for extracted keratin in the order of 10,000 [75]. Moreover, with extraction using ionic liquids, there is no discharge of pollutants, which goes in agreement with circular economy and eco-friendliness, since the ionic liquid is easily separated from keratin, and it benefits the recycling of the salt, being not detrimental to the quality of extracted keratin [76].

The reduction method that uses mercaptoethanol has a high yield, and preserves the secondary structure of the keratin, yet being harmful for the possible non-preservation of mercaptan in the final product, hence its possible discharge [77]. The reduction with sulfitolysis method, using sodium sulfate or sodium

metabisulfite, is the most extensively used for the extraction of keratin due the good yield, low toxicity, and the likely preservation of the secondary structure of the protein [78]. Treatment with thiourea leads also to sufficient self-assembly properties, which are deemed to depend on the preservation of cysteine residues, with possible re-oxidation of free cysteine thiols, specifically responsible for the possible recoiling of the protein: this method has been widely applied on wool keratin [79]. A symptom of the degree of potential self-assembly of extracted keratin is described by the formation of homogeneous gels, normally at rather neutral pH. As a consequence, methods based on sulfitolysis treatment offer larger availability due to the larger presence of sulfato-kerateines, for biomedical applications [80].

Thermal treatments for the extraction of keratin appear to be quicker and are therefore prevalently used in the industrial sector. The steam flash explosion is a process based on a hydrolysis in high temperature and pressure, the exposure of keratin to these conditions allows obtaining the disintegration of the proteins [81], and a significant modification of the secondary structure with an increase of the disorder domains [82]. Furthermore, it can even assist alkaline hydrolysis into increasing the extraction rate of keratin from feathers up to over 65% [83]. However, although β sheets are comparatively more diffuse than α-helical, β-turn, and random coil structures, steam explosion does maintain on the other side a tertiary structure of partially coiled proteins in a generally more polar environment [84]. In this context, the use of supercritical water does also induce an extensive depolymerization of keratin into aliphatic chains [85]: in other contexts, this process appears particularly suitable for the production of bio-hydrogen [86].

4 Bio-Inspired Materials Obtained from Poultry Feathers with Preservation of Secondary Protein Structure

4.1 General Considerations

In this section, the structures that have been obtained using keratin extracted from chicken feathers with preservation of protein secondary structure, further down defined as “conservative methods”, therefore with the possibility of self-assembly, are presented and discussed. As from the above considerations, the preservation of secondary structure is allowed by extracting keratin through chemical processes (ionic liquids, and reduction) and all the works that will be mentioned in this section will involve the use of the aforementioned methods. Possibly the most suitable demonstration that the secondary structure has been preserved is linked to Fourier transform infrared spectroscopy (FTIR), finding out that cysteine-S-sulfonated residues are still present in the sample, which are able to promote self-assembly [87]. This is usually correlated with a 1024 cm^{-1} sharp peak observed in the FTIR results [88].

To recognize the function of keratin and its self-assembly, it is sometimes complicated. This was noticed in early studies on avian feathers, in particular, due to the fact that the fit-for-purpose alternance of α and β structures with specific monomers, defined as φ keratins, leads to a non-obvious relation between the subunits and the tissue morphology [89]. In other words, the macroscopic geometry does not immediately recall the function, such as it occurs instead in other cases, for example ligno-cellulosic fibers, which are clearly designed for structures intended for tension and possibly torque [90]. Provided the preservation of the secondary structure is adequately achieved, feather keratin arranged in the β form suggests the prevalence of the resistance to defect propagation, described as toughness, rather than the bare mechanical strength [91]. This exceptional toughness is achieved by incorporating matrix and filaments into an only protein, whose structural preservation is therefore of paramount importance [92].

It has been elsewhere recognized that the characteristics of crosslinked gels formed from keratin obtained from chicken feathers are different from those from other sources, such as hair and wool, namely as for viscoelastic properties and in particular a higher cell proliferation [93]. In terms of blending, poultry keratin can be associated with a large variety of biodegradable and conventional polymers, as reported in [94,95]. The already mentioned autogenous cross-linking of poultry feather keratin gels has also potential to offer bio-based cross-linking agents in other contexts, such as rubber,

avoiding on one hand the use of carbon black as hardener, while on the other side the high nitrogen content of feathers would delay the thermal degradation of rubber up to 400°C [96]. However, this is not the kind of operation that would provide any bio-inspired sense to the keratin structure, which is used as just the replacement of the synthetic counterpart, trying to match as much as possible its performance. The same concept applies when using keratin as a bare filler for lignin and bio-epoxy to increase possibly to 100% the bio-based content of a composite [97].

4.2 Development and Function of Keratin Materials and Blends Extracted with Conservative Methods

The preservation of the secondary structure in chicken keratin can serve to various purposes, which enhance the characteristics of the material beyond its bare properties of hardness and toughness, enabling applications that span from the biomedical field to the production of biodegradable materials to other technical applications, such as water treatment. This potential has particularly been demonstrated in connection with the large availability of keratin waste products to be reprocessed, which offer large amounts of material for study [98]. Typically, to grow beyond the nanometric level, the material needs to be added with some plasticizers/biopolymers, a large variety of which was demonstrated effective to the purpose. The use of keratin feather fibers by bare alkali treatment, therefore at a micrometric dimensional level, taking the example of lignocellulosic fillers, while it allows introducing large amounts of fibers (up to 60 wt.% in [99] using PLA and PBAT), on the other hand it penalizes tensile strength in polymers, suggesting rather their use for acoustic panels, or similar applications, generally as bio-insulation [100]. It is also noteworthy that preservation of secondary structure is not particularly sought for when extracted feather fibers are only exploited in terms of their compressive densification in a sponge-like geometry, such as in [101], where enzymatic extraction was preferred [102]. In this sense, other applications are not in need of any particular efficiency for keratin extraction, such as it is the case for oil spill absorption [103], use as natural flocculants for the treatment of potato starch wastewater [104], or heavy metal ions (e.g., hexavalent chromium) removal from wastewater [105], as an alternative to the use of polysaccharide absorbers, such as chitosan [106]. However, also for this application, an extraction of keratin with reduction by sodium sulfite and sodium hydroxide showed effectiveness over a larger spectrum of metals [107].

The same applies when keratin fibers are supposed to be employed as fillers possibly with considerable tensile elongation and in small tenors in biopolymer blends, such as for PLA/PHB in [108], where strain at break was brought as high as to 140%. Here, it can be suggested that protein hydrolysates would do their job better in terms of low-quantity fillers for tensile elongation, with no need for preservation of the secondary structure. Conversely, the extraction of keratin capable of potential self-assembly opens the field to further sectors, which require smaller film thicknesses and more controllable mechanical properties, especially in shear, as desirable in applications such as biomedical and bioplastics. The quality of self-assembly through preservation of the secondary structure offers keratin with higher shear properties, which enable molding of structure for wound healing and tissue regeneration [109].

To avoid including chicken keratin in other polymers, which is likely to be a suitable approach for keratin with self-assembling properties, a possible solution is its plasticization through polyols, such as glycerol. An amount of glycerol between 2% and 10% was used in [110], processing bioplastics at 60°C, from feathers extracted using sodium sulfide. Another work on sodium sulfide extraction from chicken feathers used glycerol in an amount of 3.5%, adding then a smaller amount of microcrystalline cellulose (0.2%) in a sodium hydroxide solution for 48 h again at 60°C, to offer improved mechanical properties [111]. The idea was to offer a bioplastic film, which could be aimed at various applications, including biomedical, pharmaceutical and generally biopolymer development. Other films with chicken keratin extracted by sulfitolysis, yet with plasticization enhanced by citric acid, did include 25% glycerol [112]. Adding more glycerol gradually affects tensile strength and solubility, whilst increasing elongation at

break up to 35% glycerol content, where still swelling is below 17% for a urea-sodium sulfide high yield (73%) extraction [113]. A possible alternative polyol plasticizer for chicken keratin is sorbitol, which offered good performance in a 2-mercaptoethanol extraction with concentrated urea solution using sodium dodecyl sulfate (SDS) [114].

Passing to the blends of conservatively extracted keratin with chicken feathers with biopolymers, the variety of solutions attempted appear considerable and would especially depend on the application that was proposed for the keratin-based structure. It is also noteworthy that some polymers, such as poly (ethylene oxide) (PEO), are able to hinder the self-assembly of cysteine residues, and therefore keratin blending with them might not always be desirable for the production of biomaterials, though it eases electrospinning of fibers [115].

In particular, in the biomedical field, keratin nanoparticles are particularly effective in producing drug delivery systems, where their distinct advantages are their generous surface area, and encapsulation efficiency, which results in a controlled drug release [116]. Successful examples have been provided using poly(vinyl alcohol) (PVA) in crosslinked films with dialdehyde starch [117,118]. In other uses, the objective might also be orienting the specific polymer towards more focused properties through its blending with potentially self-assembling keratin [119]. This occurred for example with polycaprolactone (PCL)-human hair keratin blend coated with hydroxyapatite particles, when the objective is to fabricate scaffolds for human bone regeneration [120].

The capability of keratin to contribute to faster regeneration and to promote hydration in tissue engineering is well recognized [121]. More recently, also the combination of an adapted reduction process and the capability to regenerate natural tissue has received a considerable deal of attention [122–124]. In the case of the use of keratin from chicken feathers, a limited number of studies, summarized in Table 1, do possibly represent combinations with polymers that do not exclude in principle a self-assembly process, because of keratin extraction performed through reduction processes.

Table 1: Studies on biopolymer composites with keratin from chicken feathers obtained with reduction processes

Biopolymer	Keratin extraction method	Amount of keratin	Application	Reference
Chitosan	Dialysis precipitated by HCl	0.5% w/v nanoparticles	Bone tissue regeneration	[125]
Chitosan/PLA	Feather fibers	Up to 4%	Bone tissue regeneration	[126]
PVA/PVP/ starch	NaOH	56% w/v	Biomedical hydrogel	[127]
PHB	Sodium sulfide-L-cysteine	20% w/v	Scaffolds	[128]
PVA	0.1 M Na_2S and 5% NaOH	20% w/v	Neural repair scaffold	[129]

A conclusion can be that most studies on keratin from poultry feathers do not effectively preserve the secondary structure of the protein, though in general this would appear to be necessary to improve the application profile of the material, especially in terms of upcycling, whenever this is obtained from industrial waste. As a consequence, the following section does concentrate on those studies where explicitly this characteristic leading to self-assembly is declared or evident and possibly the extraction method for keratin is tailored to obtain this result.

4.3 Self-Assembled Structures: Nanoparticles and Nanofibers

To summarize what has been exposed previously, when maintained in a natural system and at adapted conditions of pH, temperature, etc. Keratin has the tendency to self-assemble into functional structures (e.g., hair, nails, feathers…) due to their specific amino acid sequences and interactions.

A natural example of self-assembly of beta keratin fibers is the photonic system that is created inside the feathers of some birds which generates the structural color of the feather itself [130,131].

In general, self-assembly is an intrinsic property of keratin proteins that is favored by environmental conditions during the experimental process. In particular, the self-assembly potential does depend on the chemical action performed, including nature of the chemical involved pH, temperature, and retention time, yet also may be affected by the mechanical action, such as in the case of vapor pressure for steam explosion, which hinders the preservation of cysteine residues [132]. Cysteine residues present in keratin proteins form the S-S bonds, which are responsible for the structural stability and self-assembly. At the same time the hydrophobic and hydrophilic regions of the proteins allow it to interact with itself and other molecules in aqueous environments, promoting self-assembly [133]. This suggested the potential use of keratin waste-based materials also in the field of biomedical scaffolding [134].

Self-assembling properties are found in the formation of keratin nanoparticles. Keratin nanoparticles have a high tendency to create interparticle bonds, leading to the creation of larger structures such as nanofibrils or nanolayers. The reconstruction of disulfide bonds during dialysis, used during the extraction method, is crucial for the structural integrity and stability of the keratin nanoparticles. These bonds help maintain the folded structure of the proteins and promote aggregation. Hydrogen bonds between the backbone and side chains of keratin molecules stabilize the secondary and tertiary structures, facilitating the formation of beta-sheets and subsequent self-assembly into nanoparticles [135]. Hydrophobic regions of keratin molecules tend to aggregate to minimize exposure to the aqueous environment, driving the self-assembly process. By modifying the incubation time, the temperature, together with pH and concentration of keratin in solution, the dimensions and characteristics of the nanostructures can be controlled, offering various building blocks that allow a tailored and effective design of the nanostructure passing from 1-D to 3-D geometries at the nano-level, as detailed in Fig. 5. This suggests to better orient the envisaged application in the biomedical field offering further advantages are low immunogenicity, colloidal stability and biodegradability [136].

Dialysis process can also influence the mechanism of self-assembly creating a gel. The mechanism called “gelation” is due to the intermolecular interactions and the reformation of disulfide bonds. Viscoelastic properties of this kind of gels can be controlled by manipulating disulfide bond reoxidation and cross-link density during the dialysis process. Keratin gels are primarily stabilized by disulfide bonds, though complete dissolution is only possible by disrupting hydrophobic interactions and hydrogen bonds as well. As suggested above, the appearance and viscoelastic properties of the gels are also influenced by pH and temperature.

The self-assembly of keratin fibers plays a crucial role in determining their mechanical properties and performance; this characteristic was also observed in the wet spinning process. To start from an extraction by chemical process, using mercaptoethanol, helps manipulating disulfide bonds, essential for building the keratin fibers. In particular, the self-assembly of keratin fibers via controlled disulfide bond formation involves the gradual recovery of secondary structures and the creation of ordered protein configurations. This process ultimately improves the mechanical properties of the fibers, including their toughness and durability. For instance, the regenerated keratin fibers exhibit breaking strain and toughness that were much higher than those of cotton and linen. Their toughness was nearly equivalent to that of viscose fibers. These results indicate that keratin fibers, which retain their secondary structures through continuous production, are well-suited for practical applications.

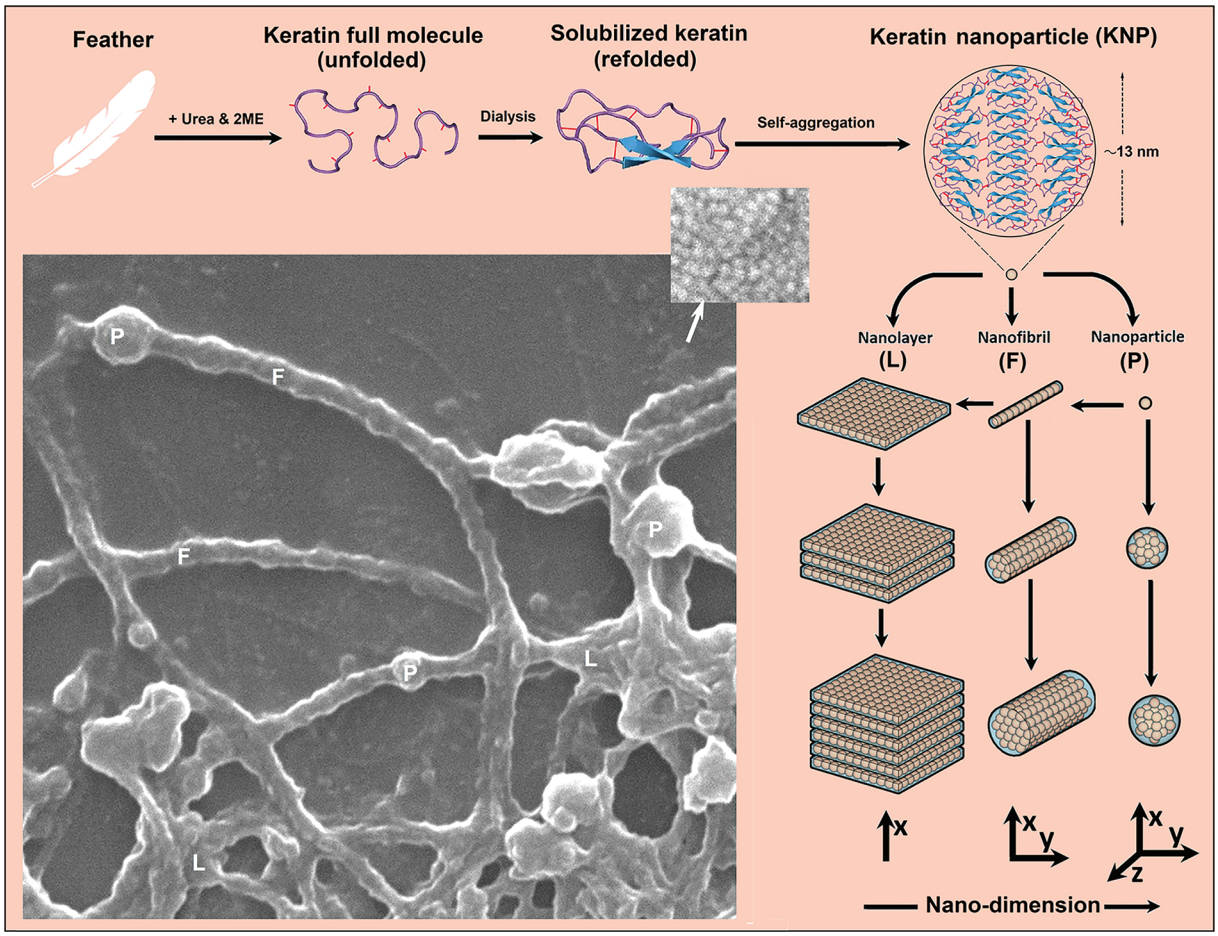

Figure 5: A schematic diagram demonstrating how keratin nanoparticles can be formed and served as the building blocks for higher nanostructures. Letters P, F and L indicate nanoparticles, nanofibrils and nanolayers, respectively (Reproduced with permission from Reference [133], © Elsevier 2020)

The ability to control and utilize this natural self-assembly process opens possibilities for creating advanced materials for biomedical applications, such as wound dressings, drug delivery systems, and tissue engineering scaffolds. In Wang et al. [137], for the production of hydrogel, it was used as an extraction method, a reduction and then an oxidation, that preserve the secondary structure of keratin. The hydrogel formation was favored by the use of H_2O_2 which promotes the formation of disulfide bonds, finally the hydrogel was used as an effective base for the formation of scaffolds useful for cell proliferation in wound healing. Similar applications have been tested by Polesca et al. [76], starting from the extraction process based on ionic liquids, they create a film of pure keratin. The formation of the film is favored by using temperatures between 50°C and 60°C. They also demonstrated non-toxicity to cells and the *in vitro* wound healing study demonstrates that this type of film improves the proliferation of keratinocytes and fibroblasts, accelerating wound healing up to 16 h.

5 Conclusions

Self-assembly of chicken feather extracted keratin, therefore intended as use in biomedical devices, such as for scaffolds and wound dressing, received some degree of attention in recent studies. On the other hand, not many works that resulted in regenerating the disulfide bonds explicitly declared the potential for

prospective self-assembly of obtained biomaterials, despite the fact that the attention towards the synthesis of nanoparticles does appear to be gradually increasing. The outcome of this review indicated the reduction extraction processes as the most suitable for the purpose, including those with use of sodium sulfite or mercaptoethanol, or also those with ionic liquids, and even more surprisingly, the extraction of keratin through steam explosion.

A final comment would concern the fact that future developments in this field, also given the very large availability of poultry feather would also be likely to invest in larger value applications for extracted keratin, which will necessarily involve the preservation of secondary structure to enable its self-assembly process in the form of nanoparticles, gels, and blends with biopolymers.

Acknowledgement: None.

Funding Statement: The authors received no specific funding for this study.

Author Contributions: Study conception and design: Carlo Santulli; data collection: Sara Mattiello, and Carlo Santulli; draft manuscript preparation: Sara Mattiello, and Carlo Santulli. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: No new data were generated.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

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