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The Use of Keratin in Biomedical Applications

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Abstract: Keratins are naturally derived proteins that can be fabricated into several biomaterials morphologies including films, sponges and hydrogels. As a physical matrix, keratin biomaterials have several advantages of both natural and synthetic materials that are useful in tissue engineering and controlled released applications. Like other naturally derived protein biomaterials, such as collagen, keratin possess amino acid sequences, similar to the ones found on extracellular matrix (ECM), that may interact with integrins showing their ability to support cellular attachment, proliferation and migration. The ability of developing biomaterials that mimic ECM has the potential to control several biological processes and this is the case for keratin which has been used in a variety of biomedical applications due to its biocompatibility and biodegradability. This review describes the progress to date towards the use of keratin in the field of wound healing, tissue engineering and drug delivery applications, with highlight to reports of particular relevance to the development of the underlying biomaterials science in this area.

Keywords: Biomaterials, cell proliferation, drug delivery, films, keratin, oxidative extraction, reductive extraction, scaffolds, wound healing.

1. INTRODUCTION

Biomaterial is a material that replaces either a tissue or a function within the body [1]. For this purpose many researchers have explored the use of natural polymers due to their intrinsic ability to perform very specific biochemical and structural roles. The similarity between natural polymers and the macromolecules forming the extracellular matrix (ECM) suggests an innate ability to interact with cells from the host tissues presenting better biocompatibility when compared to synthetic materials [2, 3]. Several natural polymers such as fibrin, fibronectin, collagen, elastin, gelatin, alginate, silk fibroin, chitosan and hyaluronic acid have been widely used in a broad spectrum of biomaterial development. In particular, proteins are considered a sophisticated group due to their ability to function as a synthetic ECM [4]. Within this group, keratin protein has emerged as potential biopolymers for the fabrication of new biomaterials. Advances in the extraction, purification and characterization of keratin proteins derived from wool and human hair, have led to the development of a keratin-based biomaterials platform. Keratin is characterized by highly repetitive amino acid sequences that result in the formation of relatively homogeneous secondary structures *via* self-assembly. Its ability to self-assemble into various physical states such as films, sponges and nanoparticles was exploited for the development of new biomaterials with improved properties that allow their application in controlled delivery systems and tissue engineering. This review addresses the properties of keratin and its use on biomaterial production that target different biomedical applications.

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2. KERATIN PROPERTIES

Keratin is the major structural fibrous protein providing outer covering such as hair, wool, feathers, nails and horns of mammals, reptiles and birds [5]. Keratin proteins are self-assembled into fibers in the follicle by a high proliferative process controlled by more than 30 growth factors and cytokines [6-10]. After extrusion through the skin, the fiber is formed into a highly stable structure formed by covalent, ambient oxygen catalyzed disulfide crosslinking and non-covalent interactions, which can occur between separate polypeptide chains (intermolecular) but also, between different points of the same polypeptide chain (intramolecular).

Keratin fibers, such as wool and human hair, consist of two major morphological parts: the cuticle layer which is composed of overlapping cells that surround the cortex, the inner part of the fiber. The cortex comprises spindle-shaped cortical cells that are separated from each other by a cell-membrane complex, which consists of non-keratinous proteins and lipids [11-15]. The cuticle cells comprise 10% of the total weight and are laminar with a rectangular shape forming a sheath of overlapping scales surrounding the cortex [13, 16, 17]. Keratin proteins can be roughly classified into two groups: the intermediate filament proteins (IFPs) and the matrix proteins. The most abundant are the IFPs also known as α-keratin that reside in the fiber cortex. They have α-helical secondary structure, are low in sulfur content and have an average molecular mass in the range of 40-60 kDa. The matrix proteins or γ-keratin are globular, have a low molecular weight and are noted for the high content in either cysteine, glycine and tyrosine residues. The ones with high

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sulfur content can be divided in high sulfur proteins (HSPs) or ultra-high sulfur proteins (UHSPs) depending on their cysteine content and have a molecular weight in the range of 11-26 kDa. The high glycine-tyrosine proteins (HGTPs) have a molecular weight between 6 and 9 kDa [18-20]. The matrix proteins functions surround the IFPs and interact with them through intermolecular disulfide bonds [15]. The formation of the crosslinked IF-matrix composite is crucial in conferring to the α-keratin their high mechanical strength, inertness and rigidity [21]. The packing of the IFPs combined with the matrix proteins form the macrofibrils within the cortex [21, 22]. There is also another group of keratin proteins, the β-keratin. These form the majority of the cuticle and their function is to protect keratin fibers from physical and chemical damage. The β-keratin is difficult to extract and do not form especially useful reconstituted structures [22, 23].

3. EXTRACTION OF KERATIN FROM KERATINACEOUS SUBSTRATES

Keratins are removed from the cortex first by using chemicals to break the disulfide bonds that are prevalent in keratinized tissues. The alpha and gamma-keratins are converted to their non-crosslinked forms by oxidation [22, 24-26] or reduction [22, 27-29], during which cystine is converted to either cysteic acid or cysteine, respectively. The free proteins extracted with denaturating solvents produce a solution that can be purified by filtration and dialysis. Oxidative extraction with peracetic acid or hydrogen peroxide e.g., yields keratins referred to as "keratoses". These are hygroscopic, non-disulfide crosslink, water-soluble and, susceptible to hydrolytic degradation at extremes pH values due to polarization of the backbone caused by the electron withdrawing properties of the cysteic acid. These characteristics lead to biomaterials that can degrade relatively fast *in vivo*, i.e. on the order of days to weeks. Reductive extraction [commonly uses dithiothreitol (DTT), 2-mercaptoethanol (2-Me) and thioglycolic acid (TGA) with a protein extraction of approximately 80% [30]. Extraction with sodium bisulfite forms cysteine and Bunte salts residues, temporally modified S-sulfo group [5, 31, 32]. With this method the keratin extracted is about 30% of the total protein content of the original wool [33]. Reduced keratins or "kerateines" are, less polar and as a consequence less soluble in water, more stable at extreme pH and, can be re-crosslinked through oxidative coupling of cysteine groups. This results in biomaterials that persist *in vivo* for weeks to months. Recently, it was reported the extraction of keratin using a hydrophobic ionic liquid with a maximum of keratin yield of 21% [34].

4. KERATIN-BASED BIOMATERIALS FABRICATION

The interest of using keratin as a biomaterial in medical applications is based on several key properties that contribute to the overall physical, chemical and biological behavior of these biomaterials. Extracted keratin proteins have an intrinsic ability to self-assemble and polymerize into fibrous and porous films gels and scaffolds. The spontaneous self-assembly of keratin has been studied extensively at both microscale [35-37] and macroscale levels [38]. Furthermore, the presence of cell adhesion sequences, arginine-glycine-aspartic acid (RGD) and leucine-aspartic acid-valine (LDV) on the keratin protein derived from wool and hair, makes keratin biomaterials able to support cell attachment and growth [39, 40]. These are the same sequences found in several extracellular matrix (ECM) proteins [41, 42]. The processing of keratin protein solutions into derivatives physical states such as gels, films and scaffolds start to appear in the literature in 1972 [43-45], then the first study using a keratin biomaterial appeared wherein a vascular graft coated with keratin was successfully implanted into a dog for more than 200 days without thrombosis [46]. Since then, keratin have been studied for its application in biomaterials for wound healing [47-51] bone regeneration [52], hemostasis [53] and recently in peripheral nerve repair [54, 55].

4.1. Keratin Films and Coatings

Keratin films can be prepared by solvent casting. This technology is becoming increasingly attractive for the production of films with extremely high quality requirements. The advantage of this technology includes uniform thickness distribution, maximum optical purity, and extremely low haze and is a technique easy to use. The ability of keratin solution to self-assemble into films was described by Yamauchi *et al.* [56] and, the physicochemical properties and biodegradability of the solvent-cast keratin films were evaluated. Pure keratin films presented low mechanical strength but the addition of glycerol resulted in transparent films, with increased mechanical strength, flexible and biodegradable both *in vitro* (trypsin) and *in vivo* (subcutaneous implantation in mice) [56]. Furthermore, these films proven to promote and increased cell adhesion and growth when compared to collagen and glass [57]. Like many natural-derived biomaterials, keratin-based ones present poor mechanical strength. As a consequence, several approaches for controlling the physical and biological properties have been studied such as, blend systems with natural [58-62] and synthetic [33, 63] polymers, use of crosslinking agents [64] and new preparation techniques [31, 65, 66]. Addition of chitosan to keratin forms strong and flexible films with improved swelling properties. Furthermore, the composite film demonstrated to be a good substrate for mammalian cell culture [60]. Lee *et al.* first reported keratin-silk fibroin (SF) blend films. This study aimed to understand the interactions that occur between the two proteins and how they affect the overall properties of the biomaterials. The results indicated that addition of keratin induces the transition from random coil to β-sheet of the SF chains. It was consider that keratin plays a similar role of a polar solvent due to the abundant polar groups in its amino acid composition [59]. Moreover, the antithrombogenicity and biocompatibility of keratin-SF films was improved when compared to pure keratin and SF films [67]. In a different study, the properties of keratin-SF films were further studied and, it was described that keratin and SF interactions are not simply additive but the two proteins are able to establish intermolecular interactions like hydrogen bonding. Furthermore, degradation rates are controlled by blend composition. The knowledge and strength of the interactions as well the degradation rates allows the design of matrices with controlled-release properties [62]. In addition to natural biopolymers, keratin interaction with synthetic polymers has also been studied [33, 63]. The proper-

ties of keratin films blended with poly(ethylene oxide) (PEO) were described by Tonin *et al.* [33], it was concluded that at appropriate levels, keratin is able to inhibit PEO crystallization. Moreover, PEO interferes with the keratin self-assembly, inducing a more thermally-stable β-sheet secondary structure. The intermolecular interactions between keratin and polyamide 6 (PA6) have also been studied and, the results indicated that the addition of keratin improves the miscibility and hydrophilicity [63]. Another common technique to improve the mechanical strength is the use of crosslinking agents. Keratin films prepared by casting a reduced keratin solution, were chemically crosslinked with ethylene glycol diglycidyl ether (EGDE) and glycerol diglycidyl ether (GDE) [64]. It was reported that keratin crosslinked films have similar mechanical properties and improved waterproof characteristics when compared to keratin-chitosan films previously described [60]. Furthermore, chemical crosslinking does not have detrimental effects on cell biocompatibility [64]. Improved mechanical properties can also be achieved by alternative fabrication techniques. Compression molding of S-sulfo keratin powder for the production of films was developed [31]. The mechanical properties of the films can be modulated by controlling the molding temperature and water content. The biocompatibility was also demonstrated by cell adhesion and proliferation onto the films [31]. Recently, Yang *et al.* described the use of keratin for layer-by-layer (LbL) self-assembly [66]. Herein, the film is formed by depositing alternating layers of oppositely charged materials. Keratin has an isoelectric point (pI) of 3.8. Therefore, at neutral pH keratin is negatively charged and will act as a polyanion in LbL assembly. When the pH of the solution is set below its pI, the net charge of the keratin molecule is reversed to positive. Thus, keratin can be used as a polycation for LbL assembly. With this technique, keratin films with controlled thickness will be easily produced. In addition, it will be possible to easily incorporate bioactive compounds into the layers by electrostatic interactions. Although the mechanical properties were not evaluated, it is clear that a multilayer film will possess an increased mechanical strength when compared to a unilayer film. Reichl *et al.* demonstrated two approaches for substrate coating: keratin precipitation with thricloroacetic acid (TCA) and casting a keratin nanosuspension. The latter presented improved cell growth in comparison to uncoated polystyrene or TCA coating [68].

Keratin presents high versatility as it has been used in the development of *in vitro* assays. The limited source of human nail plate for studying drug permeation, for the treatment of nail diseases, have led researchers to develop a nail plate model made of human hair keratin films. Keratin was extracted using reductive conditions and the films prepared by solvent evaporation. The produced films were suitable for permeation experiments with regard to its mechanical stability and water resistant property [69]. In another study, regenerated keratin and ceramides were combined for the development of membranes that simulate the stratum corneum [70]. The membranes can represent a simplified model to assay the *in vitro* skin permeability study for small drugs avoiding the use of animal or human skin.

4.2. Keratin 3D-Constructs

The ability of extracted keratin to self-assemble into three dimensional porous structures has led to their development as scaffolds for biomedical applications. The sponge scaffolds were fabricated by lyophilization of an aqueous keratin solution after controlled freezing. This resulted in sponges with homogeneous porous microstructures. Lyophilization or freeze-drying technique is based upon the principle of sublimation. The protein solution, of desired concentration, is frozen and solvent is removed by lyophilization under the high vacuum. Porous structures are formed from the voids left by the removal of the solvent. Thus, the frozen solvent acts as porogen to produce porous materials. The pore size can be controlled by the freezing rate and pH; a fast freezing rate produces smaller pores. Wool keratin sponges were first reported by Tachibana *et al.* [39]. The sponges exhibited cell attachment and proliferation over a long-term cultivation period of 23-43 days [39]. The diameter and interconnectivity of the scaffolds pores is important for obtaining adequate cellular infiltration and nutrient delivery. Sponges with controlled pore size and porosity were developed by Katoh *et al.* by means of S-sulfo keratin sponges prepared by a compression-molded/particulate leaching (CM/PL) technique. The sponges presented adequate mechanical properties and were water-insoluble [32]. In another study, a reduced keratin solution was mixed with dried calcium alginate beads. After lyophilization and leaching, a keratin sponge with high porosity and interconnectivity was obtained, in addition to cellular adhesion and growth [42]. Functionalization of the active free cysteine residues in the keratin sponges can be achieved by chemical treatments with iodoacetic acid, 2-bromoethylamine and iodoacetamide to produce carboxyl-, amino- and amide-sponges. These chemically-modified keratin sponges have been shown to mimic ECM proteins and, the large presence of active groups allows further hybridization with bioactive molecules. Tachibana *et al.* demonstrated the hybridization of keratin sponges with calcium phosphate. Two types of hybrids sponges were obtained by either chemically binding of calcium and phosphate or, by trapping commercially available hydroxyapatite particles within the modified keratin sponge. Both hybridized sponges supported osteoblasts cultivation and altered their differentiation pattern [52]. Keratin carboxyl-sponges have also been functionalized with bone morphogenetic protein-2 (BMP-2). BMP-2 was confined within the modified keratin sponge, without protein loading into the surrounding media, which was accompanied by preosteoblasts differentiation and growth [71]. The *in vivo* biodegradation of keratin sponges, with bars shape, was assessed by subcutaneously implantation in adult rats [72]. It was showed a gradual decrease in the mass of the bars over the 18 weeks of study. On the contrary, the elastic modulus of the bars decrease abruptly indicating an internal change in the structure and shape of the keratin bars [72].

Electrospining technology has become popular for the fabrication of scaffolds because it is a simple, rapid and efficient method for producing nanofibrous mats which have unique properties such as high surface to volume ratio, high porosity and morphologically similarity to natural ECM which promote cell adhesion, proliferation and migration [73]. In recent years much attention has been focused on the

electrospining of biopolymers such as silk fibroin [74, 75], collagen [76], fibrinogen [77], gelatin [78] and elastin [79]. Nevertheless, keratin has not been widely used due to the poor mechanical properties of regenerated keratin that hinders its processability and restricts its practical applications to blending with appropriate polymers. Yuan *et al.* [80] fabricated the poly(hydroxybutylate-co-hydroxyvalerate) (PHBV)/keratin composite nanofibrous mats concluding that the presence of keratin produced beads on the mats due to the broad molecular weight distribution and low dissolvality of keratin. In another study, wool keratin was used to improve the cell affinity of poly(L-lactic acid) (PLLA) [81]. PLLA/keratin nonwoven fibrous was produced by electrospining of the blend solutions and osteoblasts cells were used to evaluate the cellular behavior of the composite membrane. The results indicated that the presence of keratin improved the interactions between osteoblast cells and the polymeric membranes. Aluigi *et al.* fabricated composite nanofibers consisted of keratin and poly(ethyleneoxide) (PEO) using water as a solvent. As a result, regularly shaped nanofibers could be obtained at the ratio of 50/50 and polymer concentration of 7-10% [82, 83]. In an additional study, they evaluated the chemical, physical and rheological characteristics of electrospun PEO/keratin mats concluding that the presence of keratin increase the viscosity of the solutions [84]. Recently, the same author produced mats of wool keratin and polyamide 6 (PA6) blends to be evaluated as adsorbents of copper (II) ions, the adsorption tests revealed that keratin-based nanofibers highly adsorb copper ions and its highly pH-dependent, being the optimum pH above the isoelectric point of keratin [85]. In a different study, keratin was modified with iodoacetic acid to enhance its solubility in organic solvent. Modified keratin was further blended with PEO and electrospun to produce mats. The results indicated that the nanofibers become thinner and more homogeneous due to the presence of keratin with an enhanced cell adhesion and proliferation [86].

5. KERATIN-BASED DRUG DELIVERY SYSTEMS

Drug delivery which takes into consideration the carrier, the route of administration and the target, has evolved into a strategy of processes and devices designed to enhance the efficacy of therapeutic agents through controlled release. For many drug applications controlled drug delivery has even become a prerequisite to achieve therapeutic efficacy and/or avoid adverse side effects [87, 88]. Controlled drug delivery systems are not only to protect and stabilize the incorporated drug but also help to maintain significant local levels for sustained therapeutic response at low frequency of administration. Biomaterials for controlled drug delivery systems have to meet several requirements. They need to be biocompatible, biodegradable, non-toxic, cheap and easy to process. The ability to fabricate a variety of drug delivery constructs with different morphologies such as films, gels, foams, microparticles and scaffolds contributes to a broad application spectrum of the biomaterial. Mild processing technologies are preferred, and the amount of organic solvents must be minimized when sensitive agents are incorporated in order to reduce toxic effects of residual solvents. Moreover, the release profiles of a delivery system should be adjustable in order to achieve spatiotemporal control of clinically relevant therapeutic concentrations. A variety of polymers have been investigated for drug delivery purposes. Synthetic macromolecules including polyesters, polyorthoesters, polyanhydrides, polyphosphazenes, and polyphosphoesters have found broad application [89]. Natural polymers including alginates, chitosan, cellulose, collagen, gelatin, silk fibroin and elastin remain attractive due to their biocompatibility and biodegradability, their similarity to biological macromolecules and the potential for chemical or physical modification [2, 90]. However, there remains a need for biomaterials that can be highly controlled in terms of composition and sequence, structure and architecture, mechanical properties and function. To address these requirements, the exploration of keratin as a biomaterial for controlled drug delivery has widely expanded over the last few years. This article will review recent developments in this area of research.

Natural polymers have the advantage of being rich in reactive chemical groups (hydroxyl, carboxyl, amide, sulfhydryl) which make them more hydrophilic and capable of interacting with bioactive molecules. The most common and easiest way of incorporating drugs into keratin biomaterials is by dissolving or mixing them directly into the keratin solution before processing. The challenge of this method is to ensure that there is no detrimental impact of the fabrication process on the integrity and bioactivity of the drug. Such protocols were suggested for the preparation of drug loaded keratin films [91, 92]. Fujii *et al.* developed a new procedure for the extraction of keratin from human hair without a surfactant agent, with a protein extraction yield greater than 70%. The developed films were effective on the controlled-release of alkaline phosphatase (ALP) that retains its biological activity after incorporation into keratin films for at least 14 days [91]. In another study, blend films of keratin and silk fibroin (SF/K films) were developed to incorporate small peptide sequences with the ability to control the activity of human neutrophil elastase (HNE). To evaluate the release mechanism present, BSA-FITC was used as a model. The results indicated that the release is fast and is dependent on the keratin amount. Methanol treatment, used to induce insolubility of SF leads to higher crystallinity, making the film rigid and compact. Although this treatment leads to physical cross-linking of the films, incorporation of keratin, of hydrolytic nature, increased the release rates due to keratin dissolution causing more void volume for the release of the compound. The results indicated that the release rate can be modulated by film composition and that the mechanism is dominated by film degradation and diffusion [92]. In this way, keratin can be used to increase the release in highly hydrophobic and non-degradable systems. Recently, keratose was mixed with bone morphogenetic protein 2 (BMP-2) fabricated into a scaffold and implanted into a critical-size rat femoral defect [93]. Paracetic acid oxidation of keratin used to produce keratose biomaterial introduces sulfonic acids which confers a net negative charge to the protein chains [94, 95]. The recombinant human BMP-2 growth factor used is positively charged at acid and neutral pH levels [96] which allows its incorporation into the scaffold through electrostatic interactions. This permits localized and controlled growth factor delivery, with biological functionality, during the bulk degradation of the keratose scaffold which in turns promotes the regeneration of critical-size bone defect.

Drugs may alternatively be incorporated post-fabrication [97, 98]. Keratin was obtained by oxidative extraction yielding keratose powder. This was further resuspended to form hydrogels and the drugs ciprofloxacin [97] and halofuginone [98] were incorporated in this step through electrostatics interactions. Keratin-ciprofloxacin and keratin-halofuginone hydrogels support the sustained release of the drugs over 3 weeks and 7 days respectively, with biological activity. In addition, it was shown that the release is mediated by the keratin degradation as previously reported [92, 99]. Physical adsorption is a simple immobilization process and is based on relatively weak or moderate electrostatic, van der Waals, hydrogen and hydrophobic interactions, the binding stability of the drugs can vary depending on environmental conditions which can result in uncontrolled release as observed in the above studies. To overcome this, covalent immobilization has been widely used since it has the advantage of providing stable attachment of bioactive agents to biomaterials. This is an efficient strategy to control the release profile of the immobilized drugs since these are retained for longer time periods at the delivery site when compared to adsorption [100]. In a recent study, diclofenac was added to keratin solution to prepared drug-loaded keratin films. These were further crosslinked with transglutaminase (TGase). The results indicated that diclofenac release is closely related to the solubility of keratin films and, TGase crosslinking delayed the release due to the improvement of the mechanical properties of the films [99]. The presence of free cysteine residues within keratin sponges allows the immobilization of bioactive agents. Lysozyme was immobilized in a keratin sponge *via* disulfide and thioether bonds. Disulfide-linked lysozyme was released over a 21-days period unlike lysozyme linked *via* thioether bonds that remains in the sponge up to two months. This study showed that the release properties from keratin sponges can be controlled by the selection of the crosslinker [101]. Belcarz *et al.* demonstrated that gentamicin covalently bound to keratin-coated-hydroxyapatite (HAP) granules showed more controlled release profiles that analogues gelatin-coated-HAP and non-coated granules [102].

The incorporation of drugs into nanoparticles is another option. Recently, Li *et al.* reported the development of keratin-*g*-polyethylene glycol (PEG) nanoparticles loaded with doxorubicin (DOX). The results indicated that the loading efficiency increases with the increase of the keratin content on the keratin-*g*-PEG nanoparticles due to the formation of hydrogen bonds between keratin and the drug. DOX release was investigated at glutathione (GSH) concentrations corresponding to those present in cells and blood plasma. It was shown that higher release rates are obtained at intracellular level (higher GSH concentration) with efficient internalization showing the promising applications of keratin-*g*-PEG as drug carriers for cancer therapy [103].

6. KERATIN IN BIOMEDICAL APPLICATIONS

Keratin have a strong potential for development as clinically relevant biomaterials because they are abundant, bioactive and a realistic source of autologous proteins. Several studies have been made to evaluate the use of keratin in biomedical applications. Recently, keratin films were used for ocular surface reconstruction [104] that uses human amniotic membrane (AM). The results suggested that keratin films could represent the replacement of the amniotic membrane in ophthalmology because keratin films are more transparent and stiffer than AM with similar levels of corneal epithelial cells attachment and proliferation. Shen *et al.* [105] developed keratin hydrogels for the treatment of acute myocardial infarction. Human hair derived keratin hydrogels were injected in the hearts promoting angiogenesis without incremental inflammation, attenuated adverse heart remodeling and preserved cardiac function. Aboushwareb *et al.* [53] revealed the hemostatic properties of keratin. It was hypothesized that keratin hydrogel has the ability to adsorb fluid and bind cells to act as an effective hemostatic agent. The results demonstrated that keratin hydrogel derived from human hair was efficient in stopping hemorrhage and improved survival in a rabbit model of liver injury, with comparable efficacy of commercial hemostatic agents. Serpinski *et al.* [55], Hill *et al.* [106] and Lin *et al.* [107] demonstrated the effectiveness of keratin hydrogels in the promotion of peripheral nerves regeneration using animal models. The studies revealed that keratin biomaterial is neuroconductive and contain regulatory molecules capable of enhancing nerve tissue regeneration by enhancing the activity of Schwann cells.

Keratin powder used as an absorbent wound dressing showed the promotion of skin healing due to the release of keratin derivative peptides to the wound [108]. Water-soluble keratin peptides derived from an oxidative extraction from human hair were shown to be wound-healing agents enhancing the proliferation of human dermal fibroblasts [50]. More recently, keratin derivatives obtained either by oxidative and reductive methods were applied to burn wounds using animal and human models. The burn wounds treated with keratin materials showed a decrease in wound size and accelerated wound healing. Cross-linked keratin powder, films and hydrogels showed significant proliferation of wound healing cell lines like microvascular endothelial cells, keratinocytes and fibroblasts. Moreover, incubation of keratin materials with lymphocytes (T cells) and activated lymphocytes showed, respectively, no proliferation and normal growth, indicating that keratin materials are non-immunogenic and that the body's normal cell-mediated immune response is not inhibited by keratin materials. These were also applied to wounds on animals (rats) and humans, and a faster healing of the wounds treated with keratin materials was observed and, in the human model, with reduced pain [47, 49]. Recently, it was investigated the biological mechanism underlying the observed clinical benefits of keratin-based products as wound treatments [109]. The results suggested that the beneficial effects of keratin are related to its positive effects on re-epithelialization *via* stimulation of keratinocyte migration and production of collagen type IV and VII. Nunez *et al.* [110] suggested that keratose can act as a colloid in fluid resuscitation applications providing viscosity and oncotic properties that may be beneficial during acute ischemic events. After infusion in cremaster muscle of rats, a significant vasodilation was observed. Xu *et al.* [111] applied human hair keratin scaffolds for subcutaneous implantation and for treating full-thickness skin defects in rats. The data confirmed the biodegradation, biocompatibility and wound healing function of keratin biomaterials. Compared with the self-healing process of full-thickness wounds, keratin scaffolds led to earlier vascularization, less contraction

and newly formed hair follicles. Keratin was also effectively blended with other components to form new wound dressings. Keratin–collagen sponges were used in rats showing tissue compatibility and accelerated wound healing by stimulating cell proliferation and vascularization [112]. An analogue keratin–collagen sponge containing poly 2-hydroxy ethylmethacrylate was applied to burn wounds in rats. The composite showed healing promotion by allowing *in vivo* construction of tissue engineered epidermis [113]. In another recent study, keratin–gelatin used in full-thickness wounds in dogs promoted the healing due to the early presence of hair follicles, sebaceous gland and normal thickness of the epidermis [114].

7. CONCLUSIONS

The choice of biomaterials available continues to grow rapidly with new materials often claiming advantages over those already existing. Keratin is one of the most abundant proteins in mammals and it can be obtained from different sources including wool, human hair, and chicken feathers among others. Keratin biomaterials have several advantages of both natural and synthetic materials that are useful in biomedical applications. The presence of thiol groups on keratin biomaterials allows its functionalization and the presence of lysine and arginine residues enables it to be cleaved *in vivo* by trypsin. The better understanding of keratin properties, extraction and fabrication procedures influence the final characteristics of keratin biomaterials. Owing the excellent biological compatibility and the promotion of cell attachment and binding, keratin has experience increasing interest in the field of wound healing, tissue engineering and controlled release applications. Nevertheless, this review highlights the limitations of keratin regarding its mechanical properties which in turn hampers its use in broader applications. The most common approach, as described herein, to overcome this issue is to blend keratin with other synthetic and natural polymers with improved mechanical properties. A synergistic effect is often observed, at the same time that the mechanical properties of keratin are improved; the biocompatibility of the synthetic materials in the blends is also enhanced. This review also demonstrated that there are few published studies exploring the ability to achieve the controlled release of drugs from keratin biomaterials. The studies addressed herein indicated that the release of drugs from keratin systems is mediated by keratin degradation. Therefore, the main challenge in controlling the drug release from keratin systems is to control the degradation of the devices. Overcoming this issue, it will be possible to exploit and design keratin devices with modulated release for several applications. In the last year, several studies were published highlighting the valuable properties of keratin, namely its biocompatibility and the intrinsic cellular recognition. However, many research results are far from clinical applications and commercialization. The better understanding of keratin properties and how they influence the final biomaterial properties will allow a broader commercialization of keratin materials.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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