Keratin - based materials in Biomedical engineering

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Abstract. A biomaterial is used to replace tissue or its function within the living body. Many natural occurring polymers like collagen, fibrin, elastin, gelatin, silk fibroin, hyaluronic acid and chitosan, that have been broadly utilized as in biomaterial applications. In addition to this, proteins are known to be used as one of the popular biomaterials because of their capability to work as synthetic ECM. Among this, keratin is a protein used as effective biopolymers in the fabrication of many new biomaterial(s). Various new techniques have been made for their extraction and structural characterization. Keratin is being characterized as repetitive sequences of amino acid that led in the production of self-assembly. The self-assemble character of keratin has attained to develop into many physical appearances such as sponges, nano-particles and films, found helpful in many drug deliveries and biomedical tissue engineering. This manuscript detailed the advanced utilisation of keratin biomaterials in the area of tissue engineering; wound healing, drug delivery, and so on.

Keywords: tissue engineering, wound, fibre, biomaterial, self-assembly

1. Introduction
Keratin-based materials are the biodegradable natural polymers, which have brought a great revolution in the field of biomaterial field because of their definite properties such as biocompatibility, durability and biodegradability. Although, such materials would be utilized in the form of hydrogels, films, and also even as sponge forms in the applications of biomedical engineering [1]. Keratin plays a critical role in the constitution of major parts of wool, nails, hairs, feathers and hairs [2]. This current manuscript will give more insights on different sources, structural and functional properties, the history involved in the development of keratin-based biomaterial(s), their extraction method and application(s) in the directions of biomedical field, respectively.

2. Historical background of keratin research
As per the literature survey, the keratin was reported for their therapeutical applications in the 16th century by Li Shi–Zhen, chinese herbalism practitioner [3]. The term “keratin” was first introduced in literature to outline the substances obtained from animal horns, hooves and even also hard tissues in 1850. Although in the early days of the 20th century, many researchers have focused on various methods of keratin extraction. In 1920s, the main objective of research was to understand the structural, functional and extraction method of keratin protein [4]. During the periodical year of World
War, many research projects have utilized keratin for the production of textile. In 1940, the CSIR called as Council for Scientific and Industrial Research, has bought department of protein chemistry called as Division of Protein Chemistry, situated in Australia to expand the applications of wool and keratin. Moreover, the main objective of such a department was to understand the structural properties of keratin fibre [5]. Another research has been conducted through the University of Leeds and the Wool Industries Research Association in the UK, which has suggested the structure of keratin based fibre; as it has consist of a cortex followed by a layer of cuticle. Additionally, in the Netherlands, the investigators have patented a method of fabricating the keratin films and the textile fibre obtained from hooves [4]. In other words, many experimental works have been conducted between 1940 and 1970 on Keratin, which had established a new platform for the fundamentals of biomaterial(s) [6-8].

In 1970s, it has been found an exponential achievement in the techniques, explored to extract and characterize keratin(s) and their products. Several researchers have worked on the proper utilization of extracted keratin in different forms like films, gels, foams, coatings and fibres [9-11]. Additionally in past decades, the application of keratin and their derivatives for various biomedical applications such as drug delivery wound healing, tissue engineering continued to become the topic of interest. During the year of 1980s, chitosan, alginate, hyaluronic acid and collagen has become the most widely utilized biomaterials in different medical applications [12,13].

3. Keratin: structure
Keratin comprises of cysteine, as a fibrous protein associated mainly with the intermediate filaments, which are important in the formation of cytoskeleton and skin appendageal structures including wool, nails, horns, hairs and feathers [1]. Such biopolymers are widely present as depending on their variations in structure and properties; classified as hard and soft keratins on the presence of sulfur content. The hard keratin contains a higher percentage of crosslinks attached with sulfur. It also contributes to epidermal structure and mainly consists of filaments that are arranged in ordered arrays embedded within a crosslinked matrix [14,15]. Soft keratins, with low sulfur content, prepared from the bundles of cytoplasmic filaments that are loosely packed. It also provides resilience towards epithelial tissues [14].

In literature, the structure of keratins has been studied into alpha-keratins (α-keratins) and beta-keratins (β-keratins), respectively [16]. Among these distributions, the α-keratin is used as a primary constituent in the formation of nails, wool, horns, hairs and even of outermost layer of epidermis. However, on the other hand, the beta-keratin is employed as a primary component of avian and reptilian claws, beaks, scales and feathers [14,15].

In α-keratin, the polypeptide chains are mainly comprised in the form of α-helices; whereas, in case of β-keratin, they have been arranged as pleated β-sheets [17]. In a few decades, the wool has been investigated as a representative material of α-keratin [18-20]. The α-keratin filament has a diameter of 6–10 nm i-e in nanoscale and 2–4 nm for β-keratin [21,22]. Wool keratin has a 3D-structure; consists of approximate proteins (94.9%), minerals (0.5%) and some traces amount of lipid content [23,24]. Keratin consists of a polypeptide chain containing different amino acids, served as a backbone having inter-molecular as well as intra-molecular binding (Figure 1). Despite this, the disulfide, hydrogen, and ionic bonds enhanced the stability and the strength of keratin [25,26]. Such proteins are insoluble in a polar solvent, alkali, weak acids organic solvents, and protein-digesting enzymes including pepsin or trypsin. In higher concentration, these biopolymers contain amino acids such as proline, serine, cysteine and glycine; and methionine, histidine and lysine are present in a lower concentration. [27]. Three-dimensional mesh structures, resistance towards dissolution in different solvents and even high stability of keratin is mainly because of disulphide bonds. The solubilization of wool structure mostly takes place through the disarranging of keratin-complex structure. [28].
Figure 1: Structure of Keratin [50].

4. Methods used in Keratin based extraction
Initially, keratins are detached from cortex using some chemicals which are used to break down the disulphide bonds that extensively present in the keratinized tissue(s). The α-keratin and even gamma-keratin are then converted into non-crosslinked forms via the oxidation or reduction process. Moreover, during these processes, cystine gets converted into cysteic acid or cysteine. Free proteins are extracted out in the presence of denaturing solvents using filtration as well as the dialysis process. In oxidative extraction technique, keratins generally called as “keratoses” are produced by the application of hydrogen peroxide or peracetic acid. Keratoses are non-disulphide, hygroscopic and crosslinks. These are polar soluble, and highly susceptible to hydrolysis at extremely high pH, because of polarization generated by electron-withdrawing properties of cysteic acid. Although such characteristics properties can lead the biomaterials to undergo degradation relatively very fast as in vivo [29].

Reductive process of extraction can be carried out using dithiothreitol, thiolglycolic acid and 2-mercaptoethanol. Such extraction technique may form reduced keratin called as “kerateines” which are less water-soluble, stable at pH values and re-crosslinked. And these methods would remain in vivo for about weeks to months, respectively. Most employed techniques for the keratin extraction from keratin-rich biomaterials including oxidation, reduction, alkali treatment, steam explosion, microwave irradiation techniques, or by the applications of ionic liquids [30-35]. Figure 2 detailed various methods employed in the extraction and solubilization of keratin.
5. Keratin biomaterials - Biomedical applications

Various properties of keratins, concerning physical, chemical and biological aspects led to the evolution of several keratin biomaterials utilized in the biomedical application(s). The extracted keratin has a great intrinsic ability to get polymerize into porous and fibrous scaffolds which have been studied exclusively. The occurrence of self-assembly is greatly found in highly conserved structure of hair fibre; even when it has been processed correctly it is responsible for architecture, porosity and dimensionality of keratin-derived materials. Additionally, keratin based biomaterials obtained from human hair as well as wool revealed to contain cell-binding motifs like glutamic acid-aspartic acid-serine binding residues, responsible for supporting cellular binding. Though, all these properties together would create three-dimensional matrixes responsible for cellular infiltration attachment and proliferation. Keratin biomaterials play an important role in the regulatory functions found helpful in mediating the cellular behaviour of the body. Many investigators have searched various methods that modulate the physical as well as mechanical properties of keratins and their application of interest [29]. Some of the applications of keratin-based biomaterials (Table 1) are mentioned below:

- Keratin based films: used as a bone morphogenic protein carrier and even in ocular surface reconstruction
- Keratin based scaffolds employed in the urinary tract tissue engineering
- Keratin hydrogels utilized in drug delivery system and as dynamic matrices in the treatment of wound healing.
- Keratin powder employed as an absorbent in wound dressing showed the promotion of skin healing. Such application is because of the release of keratin derivative peptides to the wound [29].
Table 1: Keratin based biomaterial composition and their applications

| Sl.no. | Biomaterial composition                                                                 | Applications                                                                 | References |
|--------|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|------------|
| 1      | Aqueous Keratin dialysate incorporated with alkaline based keratin dialysate               | In-vitro as in wound healing of corneal epithelial.                          | [36]       |
| 2      | Photo active keratin derived films                                                        | Wound healing, tissue engineering                                            | [37]       |
| 3      | Keratin film cross-linked with trans-glutaminase                                          | Improve stability in artificial gastric juice environment.                   | [38]       |
| 4      | Photo cross-linkable keratin-polyethene glycol hydrogels via the thiol-norbornene "click" reaction | 2-D & 3-D cell culture substrates, microfabrication techniques.              | [39]       |
| 5      | Keratin film                                                                            | Reconstruction of ocular surface                                             | [40]       |
| 6      | Keratin, chitosan/gelatin                                                                | Soft tissue engineering                                                      | [41]       |
| 7      | Keratin –chitosan                                                                        | Wound dressing material                                                      | [42]       |
| 8      | PLA/chitosan/keratin composites                                                          | Facilitates osteoblast attachment and proliferation.                         | [43]       |
| 9      | Keratin wound dressing                                                                   | Used as hemostatic material                                                  | [44]       |
| 10     | Keratin gel                                                                              | Used as a substrate for cellular attachment and proliferation, delivery of therapeutic agents. | [45]       |
| 11     | Hydrogels in injectable forms                                                            | For repairing cardiac tissue after myocardial infarction                    | [46]       |
| 12     | Recombinant keratin proteins                                                             | Dermal wound healing.                                                       | [47]       |
| 13     | Keratin based therapeutic dermal patches                                                 | Wound healing.                                                              | [48]       |
| 14     | Keratin/poly (vinyl alcohol) nanofiber                                                    | Tissue engineering.                                                         | [49]       |

Keratin has emerged as a strong and effective biomaterial. They are bioactive and abundantly formed as a bio-sourced of autologous protein(s). Many experimental works have conducted to examine the application of keratin-based materials in the field of biomedical (Fig 2). In recent studies, keratin films were utilized in the reconstruction of the ocular surface which uses amniotic membrane (AM) of humans. Such studies revealed the fact that keratin films can be used as a replacement of Am in the ophthalmology; such films are transparent and much stiffer when compared with AM in relevant levels of attachment with the corneal epithelial cells. Keratin hydrogels derived from human hair were introduced into hearts resulting angiogenesis without producing inflammation and elevated adverse effects on the heart. Researchers revealed the potential effect of keratin hydrogels on animal models by showing the development of peripheral nerves regeneration. Such studies suggested that the keratin-based biomaterial is considered as a neuroconductive nature that helps in regulating the molecules to enhance the regeneration of nerve tissue followed by elevated Schwann cells activities [29]. Keratin powder used in wound healing dressing as an absorbent; thereby it releases its derivative i-e peptides to the wounded area which may promote the wound healing. Additionally, some polar soluble keratin peptides obtained from an oxidative method demonstrated to enhance the proliferation of fibroblasts in human skin [51-52].
Keratin derivatives were experimented on animals to treat burn wounds where it showed a significant reduction in the size of wounded area and eventually enhanced the rate of wound healing. Similarly, the other forms of keratin-based biomaterials such as cross-linked powder, hydrogels and films possessed potential proliferation of wound healing cell line areas. Another research suggested that keratin material such as keratose showed beneficial effects in acute ischemic, due to its improved viscosity as well as colloid oncotic properties [53-54]. Human hair derived keratin scaffolds were tested for skin disorders in rodents via subcutaneously. Results demonstrated the potential bio-compatibility and wound healing property of keratin-based materials [29], [54].

**Conclusions**

Several keratin biomaterials have been counterfeited and also investigated extensively their application in biomedical sciences as fibres, films, scaffolds, sponges and hydrogels. Keratin based biomaterials showed biocompatibility, specific chemical property, and also biodegradability. Despite all these properties, only a few of these biomaterials progressed to clinical trials and possessed a small share in the commercial market when compared to other biomaterials. Therefore, accompanied with a novel development of reproducible methods for keratin extraction, along with adequate mechanical and biological properties for biomedical application (s) would ultimately produce vital steps toward the tissue regenerations.

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