Mini Review

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The Use of Keratin as Potential Biomaterial for Bio-Dental Applications

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ABSTRACT

For a century, keratins extracted from different sources are being used for medical, cosmetic and textile applications. The excellent bioactivity and physiochemical properties of these protein extracts have recently led to the popularity of a keratin as biomaterial. Like other naturally derived biomaterials, keratins have the potential to form a defined, three-dimensional microstructure that supports cell infiltration, proliferation, and cell-guided tissue formation. In addition, the natural abundance, intrinsic biocompatibility, and mechanical durability of keratins have shown promise in the field of biomaterials in diverse biomedical applications. This mini review summarizes the biological properties, explores in brief the extraction methods and advances of keratin as a biomaterial in various biomedical and dental applications.

KEYWORDS: Keratin, Scaffold, Biomaterial, Pulp regeneration, Tissue engineering

INTRODUCTION

Dental caries is one of the most common prevalent chronic diseases in the world. Current restorative procedures remove carious tissue and replace it with a dental restorative material. However, these materials have little or no ability to remineralize teeth and maintain a good seal, leading to high failure rates [1]. Data for 166 million dental restorations in the United States suggest that more than half were replacements for failed restorations [2]. Hence, current research in dental material science is focused on developing bioactive materials regrow lost tissues based on the principles of tissue engineering. In addition, one of the main focuses in biomaterial research has been to develop scaffolds that mimic native tissue in structure and function. For this purpose, many investigators have explored the use of natural polymers due to their ability to perform very specific biochemical, mechanical and structural roles [3]. Natural polymers mimicking the extracellular matrix (ECM) offer advantages of good bioactivity, structural support and biodegradability over to synthetic polymers [4]. Several proteins have been investigated in relation to the development of naturally derived biomaterials, including collagen, albumin, gelatin, fibroin, chitosan and keratin. Of these, keratin have been promising polymers for developing scaffolds for tissue engineering purpose. Advances in the extraction, purification, and characterization of the protein have led to fabrication of several physical forms of coatings, films, foams, sponges and hydrogels [5-7]. Accordingly, a thorough literature search was carried out using different on-line databases (Ovid, Embase, PubMed, and Web of Science). Articles were selected based on keywords such as "keratin," "scaffold," "biomaterial," "regeneration," and "tissue engineering" in different combinations. Original research articles and selected review reports, published in the English language, were included. This was supplemented by a manual search and by examination of the bibliographies of the identified articles. Research letters to editor, abstract only articles, posters, unpublished articles and short communications were excluded but read to identify potential studies. This review aims primarily to present an overview of the biological properties of keratin extracts relevant to their role as a biomaterials and further, to discuss briefly different processing methods followed by a description of both the above-mentioned biopolymer advance as a potential biomaterial for dental applications.

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KERATIN AS A BIOMATERIAL

Keratins are a class of intermediate filament proteins (IFPs) originally comprising the broad category of insoluble proteins that form the bulk of epidermal structures (i.e., hair, wool, horns, hooves and nails) [8]. Keratins can be also classified as 'hard-keratin' or 'soft-keratin' according the sulphur content [9]. ''Hard' keratins have > 3% wt sulphur content and are primarily present in hair, wool, feather, nails and horns [10]. Hard keratins form ordered arrays of intermediate filaments (IFs) embedded in a matrix of cystine rich proteins and contribute to the tough structure of epidermal appendages. On the other hand, soft keratins (with a sulphur content <3%) consist of loosely packed bundles of IFs that helps in providing mechanical resilience [11]. Both these types of keratins have similar secondary structures but the differences in amino acid sequences are responsible for the distinct structural difference. Most notably, hard keratins contain higher content of cysteine residues in their non-helical domains that make them tougher and more durable than the epithelial soft keratins [12]. For this reason, the hard keratins have been widely investigated for their use as biomaterials for more several years now. Their structural components include outer cuticle, middle cortex and inner medulla (Figure 1). The bulk of keratins are in the cortex which can be divided into: (1) low-sulphur, "alpha" keratins which are about 50-60 % (MW 40-60 kDa), and (2) 20-30 % high-sulphur, matrix proteins (MW 10-25 kDa). The abundant alpha keratins are the intermediate filament proteins (IFPs). Keratin IFPs are the major structural component that imparts mechanical strength, inertness and rigidity [13].

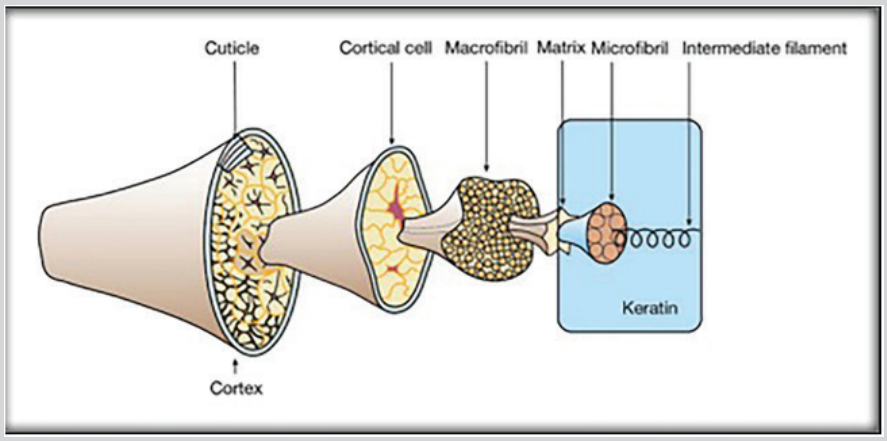

Figure 1.

Early in 1970s the research on extracted keratin proteins was focussed on developing different physical forms like films, coatings, fibres and foams. The potential use of keratin as a biomaterial in medical applications began as early as 1982 when a Japanese scientist published the first study describing the use of keratin coated, vascular grafts to control bleeding and their proven biocompatibility [14]. The main reasons for the use of keratins as a biomaterial could be listed as:

a) They are a family of structural proteins with more than 70 homologs, giving rise to an unprecedented biocompatibility

b) They have an intrinsic ability to self-assemble and polymerize into porous, fibrous scaffolds [15]

c) They have demonstrated chemotactic and cell instructive capabilities due to the presence of cell adhesion sequences, such as glutamic acid-aspartic acid-serine (EDS), arginine–glycine–aspartic acid (RGD) and leucine-aspartic acid-valine (LDV) that mimic the extracellular matrix (ECM) [16,17].

d) Implanted keratin scaffolds display minimal inflammation, support host tissue vascularization and degrade slowly

Such desirable biologic properties of keratin, plus its availability from readily renewable natural sources, have fuelled research into keratin as a biomaterial. Much has been done to establish various suitable methods for the fabrication and characterization of keratin-based new products, such as films, coatings or gels, powder, sponges, three dimensional scaffolds and fibres (Table 1-3).

Table 1: Keratin-based products based on films/coatings.

Ref.	Source	Type of Study	Experimental Approach	Application
[18]	Sheep wool	*	Keratin + RGDS (blends with natural polymers)	Excellent substrates for mammalian cell growth
[19,20]	Sheep wool	*	Keratin films + silk fibronin (blends with natural polymers)	↑antithrobogenic properties and biocompatibility
[21]	Human hair and nails	*	Keratin films	Useful materials for anti-allergic actions.

[22]	Sheep wool	*	Keratin + glycerol + chitosan films (blends with natural polymers); Keratin films chemically cross-linked with EGDE and GDE	Improved mechanical properties with antibacterial activity Good mechanical properties and biocompatibility
[23]	Human hair	*	Casting keratin precipitation with TCA and nanosuspension	Increased cell growth on nanosuspension
[24]	Human hair	*	Keratin films	Alternative for human amniotic membrane for ocular surface reconstruction
[25]	Human hair	*	Films by solvent evaporation	Nail plate model for drug permeation
[26]	Sheep wool	*	Keratin + ceramides membranes to simulate stratum corneum	Alternative model to assay the in vitro skin permeability study of small molecules.
[27]	Human hair	*	Keratin + hydroxyapatite + gentamycin coating	Cytocompatible with antibacterial activity
[28]	Human hair	**	Keratin films	Good corneal biocompatibility and transparency
[29]	Sheep wool	*	Keratin + PLLA biocomposite films	Potential scaffolds for wound dressing and tissue engineering
[30]	Human hair	*	Keratin + minocycline films	Potential application in periodontal tissue regeneration

Abbreviations: * *in vitro*, ** *in vivo*, RGDS-Cell adhesion peptide Arg-Gly-Asp-Ser; EGDE - Ethylene glycol diglycidyl ether; GDE-Glycerol diglycidyl ether; TCA-Trichloroacetic acid; bFGF: Basic fibroblast growth factor.

Table 2: Based on powders/ sponges / fibres.

Ref.	Source	Type of Study	Experimental Approach	Application
[31]	Sheep wool	*	Keratin sponge	Support long-term and high-density cell cultivation
[32]	Sheep wool	*	Hybridized keratin sponge + calcium phosphate	Support osteoblast adhesion and proliferation
[16]	Sheep wool	*	Hybridized keratin carboxy sponge + BMP 2	Localized differentiation of osteoblasts
[6,33]	Sheep wool	*	Keratin sponges by CM/PL method	Scaffold with desired pore size and porosity, improves cell adhesion and proliferation
[34]	Human hair	*, **	Keratin sponges	Dermal substitutes for skin regeneration
[35]	Sheep wool	**	Reconstituted keratin bars	Resorbable implant material
[36]	Sheep wool	*	Keratin + PEO nanofibres	Filter for air cleaning from VOCs.
[37]	Sheep wool	*	Keratin + silk fibronin blends for membranes and nanofibres	Potential application in biomedical field due to biocompatibility and antithrombogenicity
[38]	Sheep wool	*	Keratin-g-PEG nanoparticles + doxorubicin	Drug carriers for intracellular drug delivery for cancer therapy.
[39]	Sheep wool	*	Oxidized keratin powders	Improved wound healing
[40]	Sheep wool	**	Keratin-based wound dressings (keragel vs keramatrix with polyurethane dressings)	Accelerated wound healing and closure.

[41]	Human hair	*	Lyophilized keratin proteins in Hepes-Tyrode buffer	Keratin biomaterials as haemostatic agents
[42,43]	Human hair	**	KRF	Significant vasodilatory effects; can be used as a colloid in fluid resuscitation
[44]	Sheep wool	*, **	Reconstituted keratin bars	Resorbable implant material
[45,46]	Human hair	**	Keratin + collagen sponge	Dermal substitutes for skin regeneration

Abbreviations: * *in vitro*; ** *in vivo*; BMP 2-Bone morphogenetic protein 2; CM/PL-compression-moulding/particulate-leaching; PEO-Poly(ethylene oxide); VOCs-Volatile organic compounds; keratin-g-PEG-Keratin + poly(ethylene glycol); KRF-keratose resuscitation fluid.

Table 3: Based on gels.

Ref.	Source	Type of study	Experimental Approach	Application
[7]	Human hair	*,**	Keratin hydrogel	Neuroinductive and capable of nerve regeneration in mice
[47]	Human hair	*, **	Keratin hydrogel	Peripheral nerve regeneration in mice
[48]	Human hair	*, **	Keratin hydrogel	Haemostatic agent in rabbit model
[13]	Human hair	*, **	Keratin hydrogel	Excellent biocompatibility, peripheral nerve regeneration in rabbits
[49]	Human hair	**	Keratin hydrogel	Peripheral nerve repair in rat model
[50]	Human hair	*, **	Keratose hydrogel with BMP-2	Bone regeneration in rat model
[51]	Human hair	*, **	Keratin hydrogels	Sustained release of antibiotic (drug delivery)
[52]	Human hair	*, **	Keratin hydrogels vs chitosan gels	Promote wound healing
[53]	Human hair	**	Keratin hydrogel	Reduced ectopic bone growth compared to ACS
[54]	Human hair	*, **	Keratin hydrogel	Potential application in cardiac regeneration
[55]	Human hair	**	Halofuginone infused keratin hydrogel	Reduced abdominal adhesions in animals
[56,57]	Human hair	*,**	Keratin hydrogels	Haemostatic property in pigs
[58]	Human hair	*	Keratin hydrogel	Fibroblast attachment and proliferation
[59]	Sheep wool	*	Keratin gel with glycerol	mechanically stable gel that supports cell adhesion
[60]	Human hair	*, **	Keratin hydrogel	Delivery of mouse muscle progenitor cells and growth factors
[61]	Human hair	*, **	Ciprofloxacin-loaded keratose hydrogels	Local drug therapy that prevents infection and supports healing following cutaneous injury
[62]	Sheep wool	Clinical study	Keratin matrix and keratin gel	Enhanced wound healing
[63]	Sheep wool	Clinical study	Keratin gel	Reduced scarring following surgery
[64]	Human hair	*, **	Keratin hydrogel	Potential application in skin regeneration

[65]	Sheep wool	*	Chemically modified keratin hydrogel	Potential cell substrate and a sustained drug release carrier.
[66]	Sheep wool and human hair	*, **	Keratin hydrogels prepared by electron beam irradiation	Enhanced wound healing

Abbreviations: * *in vitro*; ** *in vivo*; BMP 2-Bone morphogenetic protein 2; ACS-Absorbable collagen sponges.

From the tables shown previously, the widespread use of keratin as a biomaterial in various biomedical applications is quite apparent. The majority of the films, castings and sponges were from wool-derived keratin IFPs whereas reconstituted hydrogels were predominantly from human hair. Van Dyke and his research group have widely investigated the role of keratin in various fields of regenerative medicine. [48,50,67].

Based on the literature search, the number of patents outweighs the number of published scientific research papers which reflects the immense popularity of keratin biomaterial. The use of keratin IFPs, in the form of putty, emulsion and gel for bone tissue engineering application, biocomposites, porous keratin constructs for wound healing applications was also studied [68-70]. Wool-derived, reconstituted keratin bars have shown osseoconductive properties on implantation in the long bones of sheep [71]. Since dentine resembles bone physically and chemically [72], these characteristics could be harnessed for dental tissue regeneration, especially in restorative and root canal therapy.

PROCESSING METHODS OF KERATIN

Keratins were extracted after the reduction and the oxidation of the disulphide bonds contained in their structure. The disulphide bonds of cystine form both inter and intra chains cross-links and are responsible for the greater stability and lower solubility of keratin compared with most proteins. Methods for oxidation and reduction can be found in the published literature [73,74]. If the extraction is carried out using an oxidant, the cysteic acid derivatives are referred to as ''keratoses''; if a reductant is used, the cysteine-containing proteins are call ''kerateines''. However, one of the most serious shortcomings concerning these methods for extracting keratin is that a large quantity of reagents, such as acids or reductants, is consumed and cannot be recycled.

Recently, ionic liquids (ILs) have received recognition as green and promising materials for potential applications in various fields because they are typically non-volatile, non-flammable, have chemical and thermal stability, and remarkable solubility [58,75]. In addition, chemical-free processes such as steam explosion, superheated water and enzymatic hydrolysis has been explored [76-78].

POTENTIAL OF KERATIN FOR DENTAL APPLICATIONS

Keratin has been widely used in various biomedical applications and for regenerative medicine. However, there use in dental application has been a few. Recently, Ajay Sharma et al. [79, 80], studied keratin hydrogels for their potential use in dental pulp regeneration. Keratin extracted from sheep wool was fabricated as keratin hydrogels and characterized based on structural, rheological and cell viability evaluations. Furthermore, the characterized KHs were investigated for their biocompatibility by implanting of the gel into exposed rat dental pulp. The subsequent reparative/regenerative pulpal response was assessed by histological and immunohistochemical analysis. The finding of this study demonstrated that the isolated wool derived keratin was cytocompatible, enhanced odontogenic differentiation behaviour, biocompatible with reparative dentine formation and therefore, may provide an alternative biomaterial source for pulp-tissue engineering. Another study developed a biocomposite comprising Keratin-Chitosan-Tricalcium Phosphate (KCTPs) demonstrated the cytocompatibility and antimicrobial efficacy of the biocomposite making it a promising scaffold material in regenerative Endodontic therapy [81]. A study by Duncan et al. [82,83] examined the effect of Keratec Hydrogel™ on the osseointegration of titanium dental implants in an ovine femoral cancellous bone model after 4 weeks healing. Implant osseointegration was qualitatively and quantitatively assessed using histological, histomorphometric and resonance frequency analyses. Keratin hydrogel (Keratec Hydrogel™, Keratec, Lincoln, New Zealand) improved the bone-to-implant contact (% BIC) of titanium implants after 2, 4, 8, 12 and 16 weeks following surgery, with the greatest improvement seen at 4 weeks Bone appeared denser and more mature around the keratin-treated test implants.

CONCLUSION

Keratins are promising biomaterials due to their unique chemistry afforded by their high sulphur content, excellent biocompatibility, ability to self-assemble and intrinsic cell adhesion motifs. Such advantages have led to further exploration of keratin biomaterial in the field of wound healing, tissue engineering, drug delivery systems and regenerative medicine. This narrative review clearly demonstrates the advantage of using keratins as potential biomaterial for dental applications. The use of synthetic polymers to enhance the mechanical properties may further broaden their applications. However, most of the published research involves laboratory experimentation, with some animal studies. With better understanding of keratin materials, their properties can be controlled and exploited, and hence more clinical trials will be possible.

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