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Structure–property relationships of meta-kerateine biomaterials derived from human hair

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ABSTRACT

The structure–property relationships of kerateine materials were studied by separating crude hair extracts into two protein sub-fractions, referred to as α- and γ-kerateines, followed by their de novo recombination into meta-kerateine hydrogels, sponges and films. The kerateine fractions were characterized using electrophoresis and mass spectrometry, which revealed that the α-fraction contained complexes of type I and type II keratins and that the γ-fraction was primarily protein fragments of the α-fraction along with three proteins of the KAP-1 family. Meta-kerateine materials with increased amounts of γ-kerateines showed diminished physical, mechanical and biological characteristics. Most notably, materials with higher γ-content formed less elastic and less solid-like hydrogels and sponges that were less hydrolytically stable. In addition, a model biological assay showed that meta-kerateine films with greater amounts of γ-kerateines were less supportive of hepatocyte attachment. Investigation into the mechanism of attachment revealed that hepatocyte adhesion to meta-kerateines is not mediated by the β1 integrin subunit, despite the presence of LDV binding motifs within the type I α-keratins. This work to define the role of protein composition on biomaterial function is essential for the optimization of keratin biomaterials for biomedical applications.

1. Introduction

For more than a century keratins have been extracted from hair and wool and used for medical, textile and cosmetic applications [1]. The excellent bioactivity and physiochemical properties of these protein extracts have recently led to the development of a keratin-based biomaterial platform for tissue engineering [2–4]. 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, all of which are important for biomaterial scaffolds. In addition, the natural abundance, intrinsic biocompatibility, and mechanical durability of keratins have shown promise in the field of biomaterials.

α-Keratins are structural proteins that assemble as intermediate filaments and form the bulk of cytoplasmic epithelia and epidermal appendages (e.g., hair, wool, horns, hooves, and nails). Mammalian keratins are classified as either epithelial or trichocytic keratins based on their structure, function, and regulation [5]. Although these proteins have closely related secondary structures, distinct differences in the amino acid sequences contribute to measurable differences between the filamentous structures. Most notably, trichocytic keratins contain a much higher content of cysteine residues in their non-helical domains and thus form tougher and more durable structures than do epithelial keratins [6–8].

Within the cortex of the hair fiber there are two main groups of keratins: (1) alpha-keratins (molecular weight 40–60 kDa) [9] and (2) high sulfur matrix proteins (molecular weight 5–40 kDa). Collectively, the hair fiber consists of 50–60% α-keratins and 20–30% matrix proteins [10]. The α-keratins assemble to form microfibrous structures known as keratin intermediate filaments (KIF) that impart toughness to the hair fiber. The matrix keratins function primarily as a disulfide crosslinker or "glue" that holds the cortical superstructure together and are termed keratin associated proteins or KAP [11], and are themselves relatively smaller, globular keratins. In total there are 17 functional human hair keratin genes and more than 85 KAP genes that contribute to the hair structure [5,12]. The interactions between KIF and KAP within the hair fiber are not entirely defined, although it is postulated that there is intermolecular disulfide bonding between individual KAP as well as between KAP and the cysteine-containing terminal domains of KIF [6–8,10,11].

Numerous methods have been established and published for the extraction and purification of keratins from wool and hair [10,13–16]. These methods rely on chemical processes to break down the extensive disulfide crosslinked network of the fiber, combined with or followed by an extraction step in which the

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