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Current Opinion in Cell Biology

A keratin code defines the textile nature of epithelial tissue architecture

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Abstract

We suggest that the human body can be viewed as of textile nature whose fabric consists of interconnected fiber systems. These fiber systems form highly dynamic scaffolds, which respond to environmental changes at different temporal and spatial scales. This is especially relevant at sites where epithelia border on connective tissue regions that are exposed to dynamic microenvironments. We propose that the enormous heterogeneity and adaptability of epithelia are based on a "keratin code", which results from the cell-specific expression and posttranslational modification of keratin isotypes. It thereby defines unique cytoskeletal intermediate filament networks that are coupled across cells and to the correspondingly heterogeneous fibers of the underlying extracellular matrix. The resulting fabric confers unique local properties.

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Current Opinion in Cell Biology 2023, **85**:102236

This review comes from a themed issue on **Cell Architecture (2023)**

Edited by **John Eriksson** and **Patrick Lusk**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.ceb.2023.102236

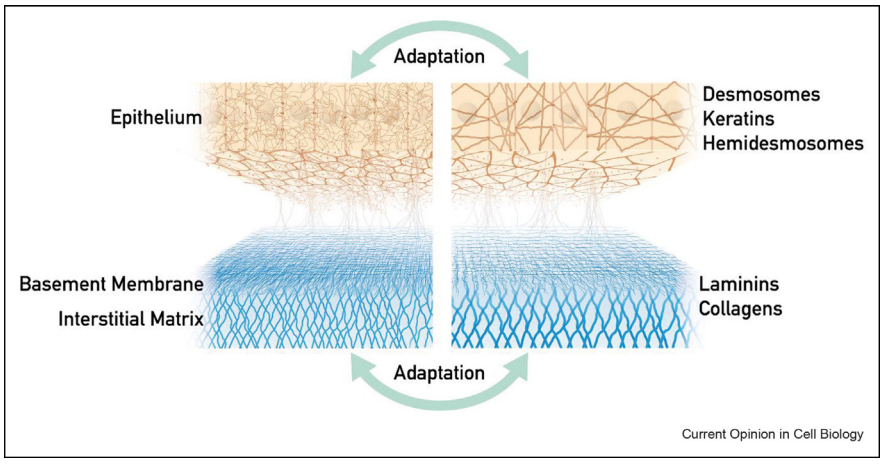
Introduction

Textiles are fiber-based materials created by weaving or knitting to produce porous, web-like structures. Products are not limited to clothing but are also found in wound dressings, different container types, houses, and even airplanes. Fiber texture,(i.e., mesh size, connectivity, and enclosed non-fibrous substances, confers unique properties on the respective materials. This manufacturing principle enhances stability while allowing lightweight design and can be coupled to specific functionalities such as defined viscoelasticity, breathability, thermoprotection, and degradability. Fiber-based (i.e., textile-like) materials are ubiquitous in the natural world. Biological fibers share many properties with fiber systems used in the technical world. They are long polymers with different degrees of deformability (bending, extension) and different dimensions (diameter) forming fabrics with defined mesh size and providing large surfaces that are functionalized (e.g., by specific binding sites). In contrast to their technical world counterparts, however, textile-like materials in the natural world can self-assemble and actively re-organize. Most importantly, they are responsive to mechanical and chemical cues guiding development and regeneration, serving as overall stress-protective systems, and causing pathology when defective. The concept of textile nature has been the basis of histology, which classifies tissues based on the unique arrangement of different cell types. While this overall concept has become somewhat outdated due to its focus on mere cellular composition, we want to revitalize it by reformulating its basic tenet. Using epithelia and their underlying extracellular matrix as an example, we will argue that tissue morphogenesis, cohesion, and function are facilitated by their unique and interconnected cytoskeletal intermediate filament (IF)-based fiber system in conjunction with the associated extracellular collagen-based fiber system (Figure 1). The resulting scaffolding exhibits astounding plasticity with tunability covering multiple length and time scales (Figure 2). It is the basis for the enormous structural and functional adaptability of epithelial tissues.

The keratin code defines epithelial fabric across multiple temporal and spatial scales

IFs are certainly the most complex of the three major cytoplasmic fiber systems that make up the cytoskeleton. In contrast to the actin-based microfilaments and the tubulin-based microtubules, IFs lack intrinsic polarity, are highly extensible and flexible, and form contiguous, interconnected transcellular networks in epithelia via desmosomal adhesions [1,2]. The epithelial keratin IF (KF) cytoskeleton is further diversified by

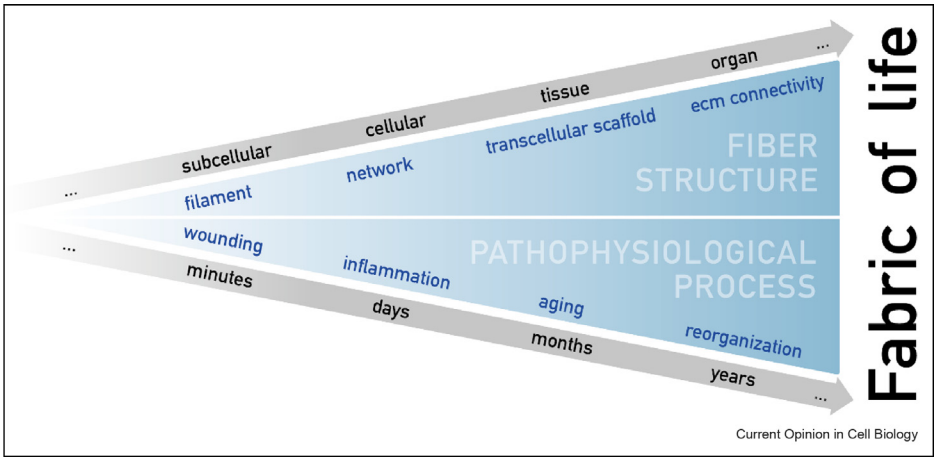
Figure 1



Schematic representation of the fiber systems forming transcellular scaffolds in interconnected epithelial cells that are connected to other types of fiber-based systems in the underlying extracellular matrix. The scheme provides an artificial view at the split interface between the epithelium on top and the extracellular matrix at the bottom. To emphasize their tight connectivity in situ, linking filamentous structures are indicated between both. The transcellular epithelial fiber scaffold encompasses the abundant keratin intermediate filaments that form 3D networks in the cytoplasm. They envelop and contact the nucleus and are connected to each other via desmosomal cell–cell adhesions. Anchorage to the extracellular matrix is mediated through hemidesmosomes, which bind via laminins to collagens. The extracellular matrix can be subdivided into the sheet-like basement membrane, which is in close apposition to the epithelium, and the more reticular interstitial matrix below, both of which can be distinguished by different collagens. The scheme further highlights that network morphology is subject to microenvironment-induced changes (compare left with right), which occur in a coordinated fashion in the physically linked epithelial and matrix fiber systems to adapt cell and tissue function.

the pairwise co-expression of 28 type I and 26 type II keratin polypeptides in humans and the mouse. They are expressed in cell type-, context- and function-dependent combinations [3,4]. It is generally accepted that each keratin polypeptide confers isotype-specific properties affecting assembly/disassembly kinetics, mechanical properties, and molecular interactions which are caused in part by isotype-specific posttranslational modifications [5,6]. We propose that the resulting diversity of IF cytoskeletons is fundamental for epithelial plasticity. The tunability of epithelial properties is thereby based on a "keratin code". It is defined by the

Figure 2



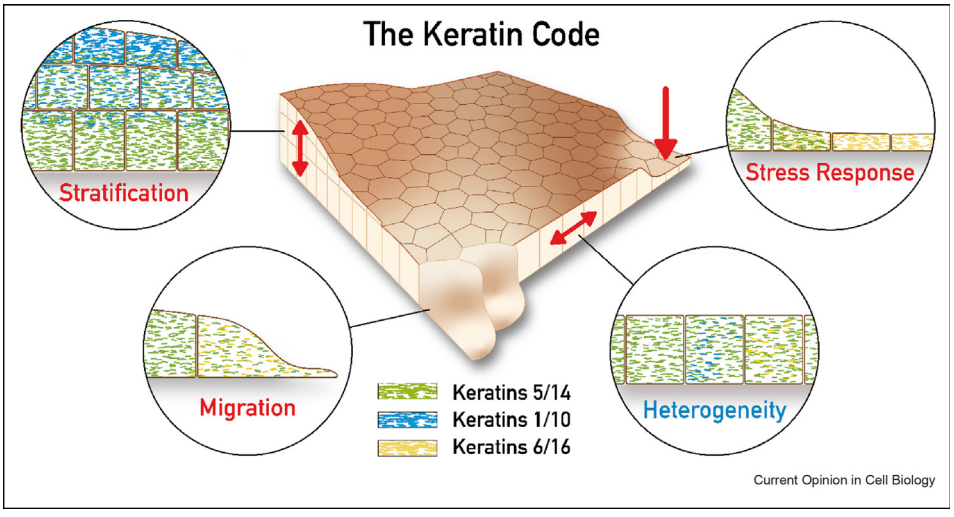
Length and time scales of fiber organization and dynamics. To understand the organization and functionality of the fiber-based fabric of life, different length and time scales must be considered. The scheme highlights the ranges most relevant for this review covering length scales from the subcellular to the organ level with the corresponding fiber-based structures and covering time scales from minutes to years with corresponding pathophysiological processes.

specific combination of keratin polypeptides and their unique posttranslational modification patterns at any point in time and any position within the cytoskeleton of a given epithelial cell. Figure 3 schematically exemplifies this type of keratin coding for properties and processes that are relevant for the epidermis. The fine-tuning of the local KF composition and modification produces an incredibly intricate and meticulously tailored fabric with spatially restricted unique functionalities. The selected examples taken from the recent literature serve to highlight aspects of the keratin code and its unique functionality.

An intriguing example of keratin isotype-specificity was recently described for the closely related type I keratins K14 and K15, which pair with the same type II keratins (typically K5) [7]. Airway basal cells respond to acute and chronic injury by switching from K15 to K14. A K14 knockout in airway basal cells inhibited their differentiation into ciliated adluminal cells but enhanced clonogenicity, whereas K15 knockout resulted in the opposite phenotype (i.e., impairment of clonogenicity but no interference with differentiation). They explained these opposing phenotypes by the isotype-specific association of K14 with the tumor suppressor stratifin/14-3-3σ. K14 knockout decreased stratifin leading to increased dNp63a oncogene expression. Büchau and colleagues [8] unveiled keratin isotype-specific regulation of hyperadhesion and desmosome composition. Hyperadhesion is facilitated by desmosomal cell—cell contacts, whereby they lose sensitivity to calcium depletion. Desmosomal hyperadhesion is not achieved in the absence of KFs. Expressing K14 in keratin type I-deficient murine keratinocytes rescued hyperadhesion, expression of K17 did not. Upregulation of desmosomal proteins in the K17 background rescued the hyperadhesion capability, suggesting an involvement of keratin isotypes in the regulation of desmosomal constituents.

The large number of isotype-dependent posttranslationally modified sites has made it difficult to elucidate their distinct functions. This is especially true for phosphorylation affecting multiple and possibly interdependent sites in cell type- and subcellular region-specific patterns. Using murine keratinocytes lacking type II keratins has helped to unravel the importance of a major phosphorylation site in the head domain of K5 [9]. Phosphomimetic mutants prevented the formation of a KF network by interference with early assembly stages. Conversely, phosphorylation-deficient mutants formed KF networks with reduced turnover. Probably the best case made for the relevance of a single posttranslational modification was reported by Guo et al. [10]. By gene replacement, they introduced a mutation in the K14 gene substituting cysteine-encoding codon 373 with an alanine-encoding codon. As expected, the mutant polypeptide presented reduced disulfide bonding. Careful analysis linked this to enhanced proliferation, a faster epidermal transit time, and altered epidermal differentiation. These effects were mediated via a 14-3-3 signaling pathway resulting in aberrant subcellular partitioning of the mechanosensitive transcriptional regulator YAP1. Findings further indicated that cell mechanics were altered in the mutant background. The derived model predicts that disulfide bridge-dependent

Figure 3



The keratin code. The scheme depicts different aspects of epithelial plasticity that are characterized by different combinations of keratin isotype expression (color code). The resulting cell- and differentiation-dependent expression of keratin isotypes, their abundance and posttranslational modifications (not shown) define the keratin code providing a unique identifier for a given epithelial cell in time and space. We hypothesize that the diverse range of epithelial functions such as stratification, migration and response to stress relies to a large extent on this keratin code, which is reflected by intra-epithelial heterogeneity.

KF network re-organization, which can occur during the transition from the basal to the suprabasal compartment, leads to perinuclear recruitment of 14-3-3σ and consecutive cytoplasmic YAP retention.

A major challenge in deciphering the keratin code and its functional relevance is the correlation of local heterogeneities in keratin composition and, more importantly, keratin modification with filament morphology (e.g., bundling, branching), protein−protein interaction (e.g., desmosomal/hemidesmosomal proteins, cytoskeletal cross-linkers, heat shock proteins, signaling proteins), and mechanical properties (e.g., viscoelasticity, stiffness). Evidence for such heterogeneities at the subcellular level was presented by Fois et al. [11], who detected increased keratin phosphorylation next to desmosomal adhesion sites. Similarly, keratin aggregates that are either induced by pathogenic keratin expression or by different types of stress are hyperphosphorylated [9,12]. It will be interesting to find out, to which degree and how posttranslational modification are involved in the association of keratins with the cytolinker plectin, which is known to affect keratin bundling and mesh size [13,14] with consequences for epithelial cell mechanics [15].

An important feature of the keratin code is its responsiveness to stimuli at very different time scales. An impressive example of a very rapid response was reported by Ratajczyk [16] who showed that association of KFs with the cytolinker epiplakin occurs within seconds (i.e., during fixation and in response to different stressors). The association is mediated by Ca^{++}-signaling and alters the dynamic properties of the keratin system. The more delayed responses occurring after wounding and during inflammation involve changes in keratin polypeptide synthesis. In the epidermis, these processes are coupled to the induction of K6, K16, and K17 ([3,5]; Figure 3). Permanent epithelial restructuring, which is characteristic for metaplasia, aging, and developmental processes is also associated with de novo expression of keratins (cf. [4]).

Desmosomal adhesion and actomyosin-dependent tension cooperate with the keratin code in defining local textile properties

The stratum-specific keratin polypeptide expression in the epidermis [4,17] suggests that the associated changes in filament type (single -> bundled -> aggregated) and filament distribution (cortical/radial -> pancytoplasmic -> cornified envelope) are major determinants of the changing basal to apical tissue texture. Another major factor is the changing force balance [18], which is believed to be driven by actomyosin-linked intercellular adherens junctions whereas the adaptation and resistance to mechanical forces are enabled by the KF-desmosome system. Thus, it has been shown that the transition from cell−matrix to cell−cell adhesion is accompanied by the reorganization of the actin system from stress fibers to cortical actomyosin [19]. This process requires the polarity protein aPKC, since aPKC depletion results in the persistence of actin stress fibers in suprabasal cells. At the same time, the physiologically occurring KF re-distribution from the cortex to the cytoplasm is abrogated and KF bundling is increased highlighting the importance of actin-dependent prestress for suprabasal keratin network organization [19]. The work by Broussard et al. [20] further emphasized the contribution of the desmosome-keratin system in this process. They observed that uncoupling of desmosomes and KFs delayed stratification, while, unexpectedly, the expression of differentiation markers accelerated through precocious activation of the mechanosensitive transcriptional regulator serum response factor (SRF). They proposed that the keratin-desmosome connection acts as a clutch to support delamination of basal cells. In support, Thomas et al. [21] examined keratin-desmosome crosstalk during apoptotic cell extrusion in simple epithelial cell monolayers. They showed that desmosomes between remaining cells are transiently associated with actomyosin cables producing junctional tension to extrude the apoptotic cells.

The differences between the basal and suprabasal cytoskeleton are also reflected by differences in desmosome composition. The best-studied switch is that from the desmosomal cadherin desmoglein 3 in basal cells to desmoglein 1 in suprabasal cells. Even more, ectopic expression of desmoglein 1 in basal keratinocytes or in simple epithelial cells promotes stratification (cf. [22]). Mechanistically, desmoglein 1 redistributes molecular tension through rearrangement of cortical F-actin via Arp1/3-dependent actin polymerization [23]. Collectively, one can conclude that the keratin cytoskeleton provides a multifunctional matrix that is tightly integrated across cell borders through desmosomes forming a contiguous transcellular network with stratum-specific properties in the epidermis, whose architecture adapts to the local force balance via mechanosensing. The resulting fabric is highly adaptable and integrates metabolic functions [24].

The epithelial and extracellular fiber systems are mechanically and functionally connected

The fabric of the epithelial fiber systems differs profoundly from that of the underlying extracellular matrix (ECM). But both are physically linked via hemidesmosomes, which have been classified as compositionally distinct type 1 in stratified and pseudostratified epithelia and type 2 in simple epithelia. Both types are attached to the basement membrane by binding to

trimeric laminins, which form reticular sheets and are, in turn, attached to the collagen type IV network. Integrin α6β4 and the bullous pemphigoid antigen BP180 of type 1 hemidesmosomes interact with laminin 332 [25]. Type 2 hemidesmosomes can also interact with α5-containing laminins (i.e., laminins 511 and 521) [26].

Conditional removal of laminin 332 provided compelling evidence for the fundamental role of type 1 hemidesmosomes in the maintenance of epidermal homeostasis in the adult skin [27]. It induced epidermal thickening and increased desquamation as a consequence of a modified differentiation program of basal keratinocytes. This was reflected by major changes in keratin expression (i.e., increased synthesis of K6a, K6b, and K16) and by increased cornified envelope proteins and cellular stress markers. Furthermore, disorganization of the actin cytoskeleton and cell shape were readily apparent.

In a similar vein, the laminin α5 chain has been shown to regulate keratin expression and the differentiation state in mammary epithelium [28]. Binding of K5/K14-positive basal cells to laminin α5 induces their differentiation into K8/K18-positive luminal cells, requiring β4 integrin and further downstream signaling. These findings are in line with a study on mammary cancer-derived epithelial acini [29] showing that their malignant transformation occurs only in the absence of a tight hemidesmosomal connection between the ECM and the keratin network. It further demonstrated that the lack of hemidesmosomal integrin clustering is regulated by a complex interplay between matrix stiffness and laminin density. While low matrix stiffness allows clustering, stiff matrix prevents it and can be rescued by increasing laminin density.

It is becoming increasingly evident that cell—cell and cell-ECM adhesion together with mechanical feedback cycles regulate epithelial function and mechanical homeostasis (cf. [30]). Mechanical homeostasis relies on the dynamic equilibrium of tensile forces transmitted between neighboring cells and the ECM. This equilibrium is based on the active regulation of the cytoskeleton and its associated junctions, which impact gene expression through their connection to the nucleus [31]. Fujiwara et al. reported that keratin networks regulate mechanotransduction through a Rho signaling pathway upstream of cell-ECM adhesion formation and organized cell migration, a pathway relevant to the pathogenesis of Epidermolysis bullosa simplex [32]. Recent data further indicate that the 3D organization of the keratin network is guided by cues from the ECM [33,34]. Sensing ECM rigidity is facilitated by crosstalk between the keratin and the actin cytoskeleton. Laly et al. [35] demonstrated that the K14 network adapts to ECM stiffening in an F-actin-dependent fashion by becoming more bundled and rigid. Further evidence for the pivotal keratin-actin crosstalk in the mechanotransduction of ECM rigidity was provided by Wang et al. [36], who reported that keratinocytes lacking integrin α6β4 exert reduced traction force and implicated the cytoskeletal linker plectin in this process. It has been proposed that plectin is involved in the F-actin-dependent organization of the keratin network into a circumferential subcortical rim and the radial keratin spokes both of which are attached to desmosomal adhesion sites [15,35,37]. Genetic removal of plectin resulted in the loss of the circumferential rim and increased mechanical fragility of cell sheets [15].

The keratin-actin crosstalk also plays an important role in epithelial cell migration on different matrix types. While actin together with focal adhesions has an exploratory probing function testing ECM stiffness and composition, keratins follow the actin system and fulfill a stabilizing function. The observed inward-directed keratin flow is always slower than the retrograde actin flow [38]. Coordination of both allows efficient directed migration. Keratins therefore provide a templating function: They reduce the speed of migration but increase its persistence [38,39]. In the presence of laminin 332 hemidesmosomes are preferentially formed at the leading front of migrating primary keratinocytes generating characteristic chevron-like structures [40]. These chevrons are laterally flanked by focal adhesions. Together, they are subject to treadmilling: Focal adhesions appear first at the leading edge. Hemidesmosomal chevrons subsequently form between paired focal adhesions. They extend ribbons of focal adhesion-flanked chevrons which serve to translocate the cell body forward. At the cell rear, focal adhesions and hemidesmosomal chevrons disassemble in reverse order. This arrangement was detected most prominently in leading cells of collectively migrating cell sheets [40].

In addition to the hemidesmosome-mediated direct association of epithelial cells to the basement membrane, the interstitial matrix underneath is also integrated into the pervasive and interactive scaffold that we refer to as the fabric of life. In some epithelia, such as the cornea and skin, collagen type VII hooks onto the fibrillar collagens type I and III of the interstitial matrix and binds collagen type IV and laminin 332 in the basement membrane forming structures referred to as anchor fibrils [41,42]. Interestingly, interstitial matrix changes impact basement membrane thickness and stiffness in the skin [43]. These changes lead to a reduction of chromatin accessibility in the stem cells of hair follicles resulting in progressively reduced regeneration potential [44].

We are currently far away from a complete understanding of subepithelial ECM heterogeneity, although

several efforts are underway to systematically explore its biochemical diversity in relation to topology and function at different interfaces [45,46].

Future perspectives and challenges

A comprehensive view of the interconnected fiber systems of the human body is needed to understand tissue morphogenesis, remodeling, and malformation as fundamental determinants of functional tissue differentiation, homeostasis, and malfunction, respectively. It requires multimodal measurements of morphological features, their biochemical composition, and mechanical properties. These analyses are needed at different lengths and time scales to understand emergent features of the keratin code and its impact on the functionality of the epithelial fabric (Figure 2). To detect the underlying mechanisms, tools have to be refined and adjusted for the examination of filaments at the subcellular level, networks at the cellular level, transcellular scaffolds at the epithelial tissue level, and their connection to the ECM fiber systems at the organ level. The analyses will help to understand acute responses occurring after wounding and in various acute stress situations, chronic responses that are induced, for example, during inflammation, and long-lasting permanent responses that take place during development, chronic stress, and aging.

Author contribution

All authors contributed to the conceptualization and writing of the manuscript.

Funding

The work of REL was funded by the Deutsche Forschungsgemeinschaft (LE566/18-2; LE566/22-2/SPP 1782; 363055819/GRK2415), JDR is supported by the Deutsche Forschungsgemeinschaft (RU2366/3-1) and a grant from the Interdisciplinary Centre for Clinical Research within the faculty of Medicine at the RWTH Aachen University. TMM was supported by the Deutsche Forschungsgemeinschaft (MA131621-2) and by DEBRA Austria Magin 5.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

We thank Adam Breitscheidel for excellent art work and Nicole Schwarz for stimulating discussions.

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