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Review

Mechanobiology of force transduction in dermal tissue

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Background/aims: The influence of mechanical forces on skin has been examined since 1861 when Langer first reported the existence of lines of tension in cadaver skin. Internal tension in the dermis is not only passively transferred to the epidermis but also gives rise to active cell-extracellular matrix and cell–cell mechanical interactions that may be an important part of the homeostatic processes that are involved in normal skin metabolism. The purpose of this review is to analyse how internal and external mechanical loads are applied at the macromolecular and cellular levels in the epidermis and dermis.

Methods: A review of the literature suggests that internal and external forces applied to dermal cells appear to be involved in mechanochemical transduction processes involving both cell–cell and cell–extra-cellular matrix (ECM) interactions. Internal forces present in dermis are the result of passive tension that is incorporated into the collagen fiber network during development. Active tension generated by fibroblasts involves specific interactions between cell membrane integrins and macromolecules found in the ECM, especially collagen fibrils. Forces appear to be transduced at the cell–ECM interface via re-arrangement of cytoskeletal elements, activation of stretch-induced changes in ion channels, cell contraction at adherens junctions, activation of cell membrane-associated secondary messenger pathways and through growth factor-like activities that influence cellular proliferation and protein synthesis.

Conclusions: Internal and external mechanical loading appears to affect skin biology through mechanochemical transduction processes. Further studies are needed to understand how mechanical forces, energy storage and conversion of mechanical energy into changes in chemical potential of small and large macromolecules may occur and influence the metabolism of dermal cells.

Key words: skin – extracellular matrix – mechanochemical transduction – collagen fibrils – gap junctions – integrins – secondary messengers

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The extracellular matrix (ECM) is the substrate for cell adhesion, growth and differentiation, and it provides mechanical support to surrounding tissue and organs (1). It is well known that connective tissue cells adapt their ECM to changes in externally applied mechanical loads during wound healing (2). For this response to occur, a feedback mechanism must exist by which, cells that sense mechanical stress via their substrate, respond by altering patterns of protein expression, thus remodeling their ECM to meet changing mechanical requirements (1). In addition to their ability to adapt to externally applied loads, cells have the ability to generate their own internal forces through the production of cytoskeletal tension (2, 3). These externally applied forces, and internal cytoskeletal forces appear to be integrated together with other environmental signals, which are then transduced into a biochemical response in the cell cytosol and nucleus (3). In order to understand how external and internal forces are transduced in skin, it is first necessary to analyse how these forces are applied to the epidermis and dermis. The purpose of this paper, then, is to review the literature on cell–ECM interactions that are important to understanding how internal and external mechanical forces are transduced into chemical changes through a process of mechanochemical transduction.

Mechanical forces acting on skin

Skin is a multilayered composite material composed of an upper cellular layer, the epidermis, which is between 0.06 and 1.00 mm thick,

connected to a lower layer, the dermis, containing cells and ECM, that is 1–4 mm thick (4). External forces are transmitted through the epidermis to the dermis and the underlying subcutaneous tissues, while internal forces are transmitted through the dermis to the epidermis (Fig. 1). Internal forces in the skin exist as passive tension in the collagen fibrils of the dermis that are approximately directed along Langer's lines (see section on Mechanical Properties of Skin) and are augmented by active cytoskeletal tension (2). The active cellular tension also acts approximately along Langer's lines and is produced by fibroblast contraction of collagen fibrils in the extracellular matrix (2). In the absence of external forces, the internal tension acting on the collagen fibrils of the dermis causes tension to occur at keratinocyte–keratinocyte cell junctions. External forces applied to the skin surface at the air–epidermis interface also increase the tension at keratinocyte–keratinocyte cell junctions (Fig. 1) as well as change the state of stress in the dermis. Transmission of external forces through the epidermis to the dermis occurs through a number of possible mechanisms including: (a) keratinocyte–keratinocyte interactions in the epidermis; (b) keratinocyte–ECM interactions that occur in basement membranes at the dermal–epidermal junction; (c) macromolecular–macromolecular interactions that occur in the dermis; (d) macromolecular–fibroblast interactions in the dermis; and (e) fibroblast–fibroblast interactions in the dermis.

Thus, both internal tension (see 1 through 5 in Fig. 1) as well as externally applied loads affect the mechanobiology of both epidermis and dermis. In addition, there are a number of possible mechanisms by which internal and external stresses are transmitted through the skin. In the following discussion, we will attempt to analyse how forces are transferred through the epidermis and dermis by considering the structure of each of these skin layers.

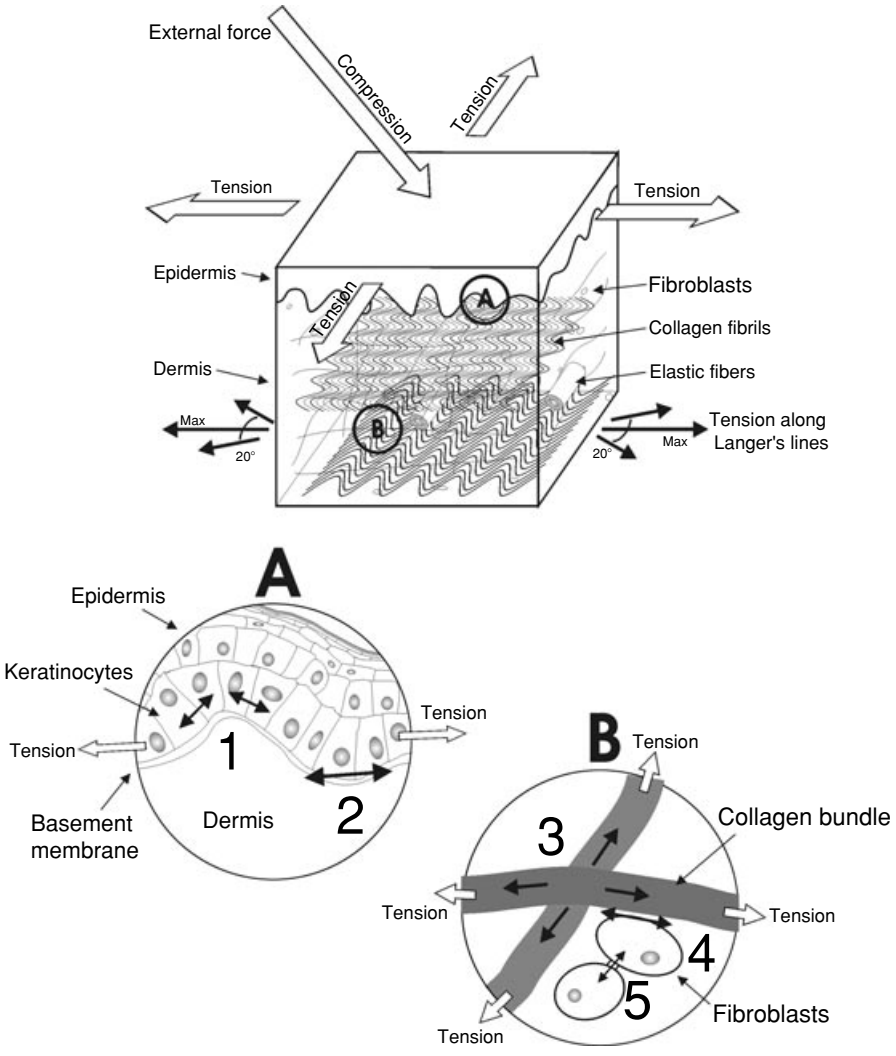

Fig. 1. External and internal force transmission in skin. Forces applied at the air–epidermis interface include shear forces due to friction and tensile and compressive forces. Both shear forces due to friction applied to the epidermis and compressive forces applied to the epidermis increase the tension in the dermis. Tension in the dermis arises from stretching of the collagen fibrils that run almost parallel to Langer's lines. Internal forces in the dermis consist of passive tension in the collagen fibrils and active cellular tension that occur as fibroblasts contract the collagen fibrils. (A) Illustration of how tension in the epidermis leads to stretching of basal epithelial cell junctions and results in tension at the basal epithelial cell–basement membrane interface. (B) Diagram showing tension in collagen fibrils in dermis that arises from tension along Langer's lines and the resulting stretching of collagen–fibroblast and fibroblast–fibroblast attachment sites.

Epidermis composition and mechanobiology

External forces applied to the epidermis include normal forces that result when skin is compressed or stretched in tension, and shear forces that result from friction (Figs 1 and 2). Internal tension along Langer's lines in the dermis is transmitted to the epidermis and results in providing tension at keratinocyte–keratinocyte cell junctions. The epidermis consists of several cell layers beginning with a layer of viable basal keratinocytes that differentiate into a cornified non-viable layer of squamous epithelium that covers the surface (see Fig. 2). The various cells of the epidermal layer differ in size, shape, and position and physical properties, which reflect the state of differentiation of the cells in their respective layers. In addition, the exact nature of the mechanical loading on individual cell layers differs depending on the distance from the basement membrane and the level of external loading acting at the air–epidermal interface. In the absence of external loading, the stress is highest at the interface between the epidermis and the dermis; in areas of skin under high levels of external loading, such as on the hands and feet, the epidermis thickens, suggesting that epidermal thickness is controlled by the balance of external and internal forces acting on/in this layer.

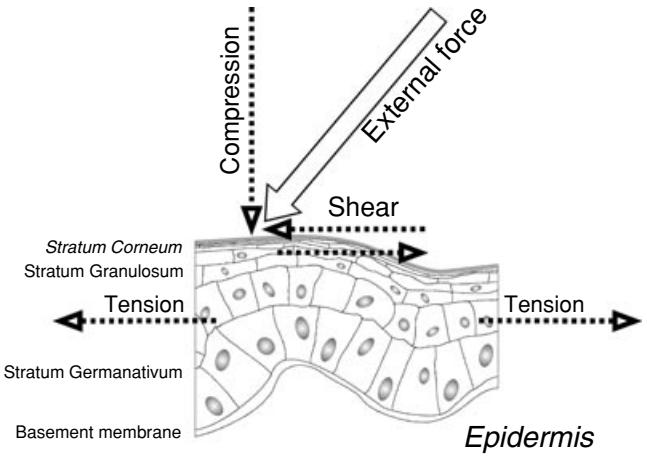

Fig. 2. Force transmission at keratinocyte–keratinocyte cell junctions. High magnification view of Fig. 1A showing tension that arises in the epidermis as a result of external forces and internal tension in the collagen fibrils in the dermis. External forces applied at the skin surface as well as the passive and active tension in the collagen fibrils of the dermis result in stretching and activation of keratinocyte–keratinocyte cell junctions. External forces applied to the epidermis stretch keratinocyte–keratinocyte cell junctions and keratinocyte cell membranes resulting in thickening of the epidermis.

Basal keratinocytes are joined together by desmosomal junctions and attached to the underlying basement membrane by hemidesmosomes and integrin receptors. Both types of junctions involve low molecular weight and keratin intermediate filaments (K14 and K15) that extend throughout the cytoplasm and insert into the attachment plaques of the junctions at the cell periphery (6, 7). Forces are transmitted between epithelial cells via specific cell adhesion molecules including cadherins (8–10); and mechanical loading leads to activation of secondary messengers that affect genetic expression and cell growth, as discussed further below.

Together with actin microfilaments and microtubules, keratin filaments make up the cytoskeleton of vertebrate epithelial cells (see 4 for a review). Keratins belong to a family of intermediate filament proteins that form α-helical coiled-coil dimers associated laterally and end-to-end to form 10 nm diameter filaments (4). Keratin and actin filaments and microtubules form an integrated cytoskeleton that preserves the shape and structural integrity of the keratinocyte as well as serves to transmit mechanical loads. Keratins account for about 30% of the total protein in basal cells (14).

Above the basement membrane and basal cell layer in the epidermis is found the spinous layer consisting of three to four cell layers of keratinocytes that are polyhedral in shape (4). Keratin accounts for up to 85% of the total protein in the cells of the spinous layer and is presumed to stiffen the cell cytoskeleton (4). Above this layer is the granular cell layer consisting of two to three cell layers where the keratin filaments are associated with a protein, profilaggrin, to form keratohyaline granules (4). Profilaggrin, is a high molecular weight, histidine-rich, phosphorylated polymer composed of monomers joined by link proteins (11–13) and is known to bind calcium (14). The protein constituents of the cornified cell envelope become crosslinked by calcium dependent, epidermal transglutaminases within granular cells. A cytosolic form of epidermal transglutaminase is present in granular cells, and a particulate membrane bound form is found in spinous and granular layer cells (15, 16) These transglutaminases are believed to form lysine-derived crosslinks between envelope protein precursors, which

make up the cell envelope that forms when the granular cells flatten, become dehydrated and cornify (17).

The boundary between the epidermis and dermis is a basement membrane; it can be described by four planes proceeding from the basal epidermal side to the dermal side: (a) the border of the basal keratinocyte; (b) the lamina lucida, an electron lucent layer that lies beneath the epidermis; (c) the lamina densa, an electron dense layer also known as the basal lamina; and (d) the reticular lamina or subepidermal zone consisting of connective tissue immediately below the epidermis (4). The mechanical continuity at the epidermal–dermal junction, as well as between the keratinocytes, is key to normal transfer of internal and external mechanical forces between the epidermis and the dermis.

The epidermis and dermis are connected through the hemidesmosome attachment plaques. The bullous pemphogoid antigen (BPA) is associated with the attachment plaque of the hemidesmosomes, where it appears to colocalize with the $\alpha 6\beta 4$ integrin receptor (18). The bullous pemphogoid antigen (BPA) may also be found in the lamina lucida adjacent to the hemidesmosome (19, 20). Keratin filaments of the basal keratinocyte insert into attachment plaques connecting the cell cytoskeleton with the cell surface and to the matrix of the upper dermal layer, the papillary dermis. Anchoring filaments composed of type VII collagen that span the lamina lucida, are usually increased in density beneath the hemidesmosomes. In most regions of the skin, both ends of the anchoring fibrils are embedded into the lamina densa (21, 22). In patients with a genetic connective tissue disease affecting type VII collagen, termed epidermolysis bullosa, the epidermis spontaneously separates from the dermis upon application of external forces (23). This observation underscores the importance of maintaining the mechanical continuity between the dermis and epidermis in order to maintain the normal mechanical force transfer.

Thus, external forces applied to the epidermis and/or internal forces present in dermis are transmitted through the cornified layer, and lead to the stretching of keratinocyte–keratinocyte cell junctions and the epidermal–dermal junction. These forces appear to be transmitted between epithelial cells via cadherins and may result in activation of secondary messengers, which alter gene expression and protein synthesis.

Dermis and mechanobiology

The mechanical behavior of the dermis dominates the mechanical behavior of skin under normal conditions (24, 25), and therefore internal forces in collagen fibrils in dermis are an important source of mechanical stimuli that affect mechanochemical transduction in the dermis and epidermis. The dermis is organized into two regions on the basis of differences in connective tissue density and arrangement (4). The upper layer, the papillary dermis, is approximately twice the thickness of the epidermis and is composed of loose, small diameter collagen fibers and immature elastic fibers. The bulk of the lower dermis, the reticular layer, contains large interwoven collagen fibers and mature elastic fibers that are coated with proteoglycans (PGs) and other fibril-associated macromolecules. Elastic fibers are composed of elastin, fibrillins and microfibrillar proteins. Fibroblasts are more abundant in the papillary dermis compared to the reticular dermis and have a higher rate of metabolic activity, an enhanced capacity for proliferation and a longer replicative life span (26, 27). Fibroblasts seen in dermis appear to be associated with the surface of collagen fibrils and in some areas, cell–cell connections are apparent (4).

The primary macromolecular components of the dermis include collagens, elastin, fibrillins, proteoglycans (PGs), fibronectin and hyaluronan. Fibril forming collagens in dermis (Fig. 3), including types I, III and V, interact with fibril associated collagens with interrupted triple helices (FACITs) including types XII and XIV. Non-fibril forming collagens in dermis include types IV, VI, and VII. The fibril-associated collagens with interrupted triple helices (FACITs) contain a short C-terminal domain that forms a triple helix, which is thought to be associated with fibril forming collagens (28). The collagen fibrils observed in the electron microscope images consist of mixtures of types I and V; these fibrils are coated with type III (29) (Fig. 3). Small diameter type I collagen fibrils containing the amino terminal propeptides are found in the papillary layer (30). Types XII and XIV collagen have been specifically localized along the surface of the banded fibrils in skin (31). The molecular structure of these fibril-associated collagens suggest that they may interact with the surface of banded fibrils through their triple helical domains and bend or protrude away from the surface in the flexible non-helical domains (29).

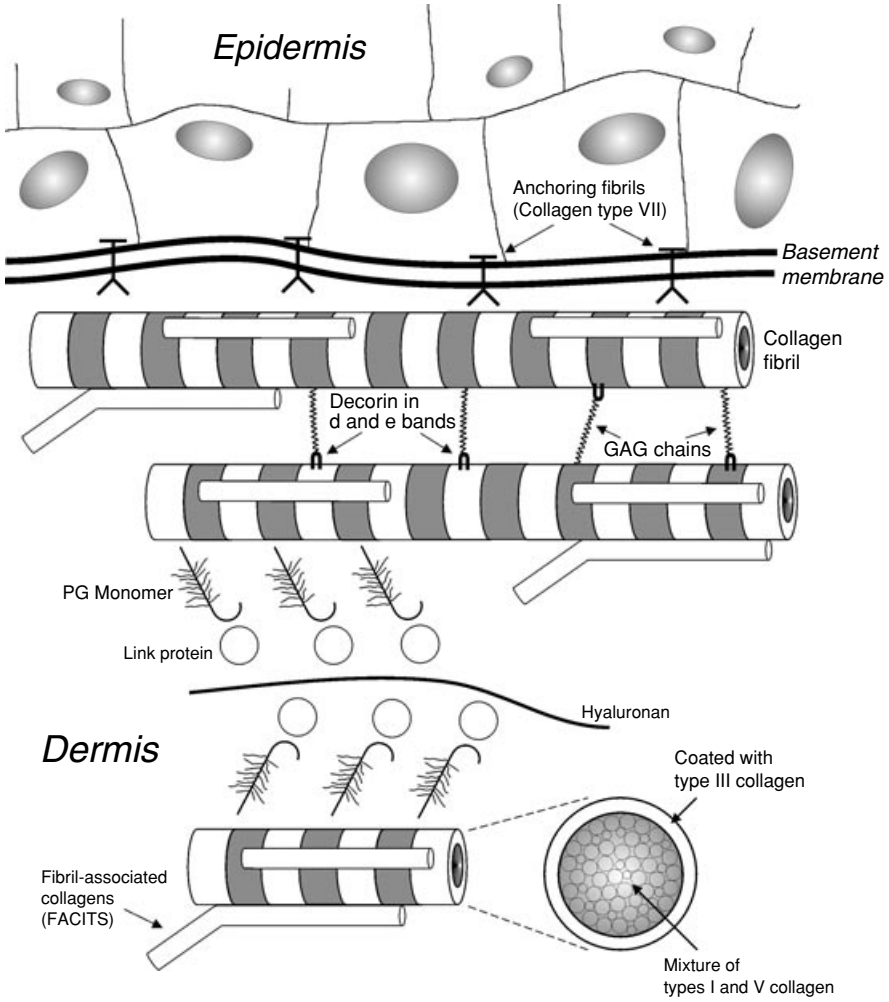

Fig. 3. Macromolecular components of skin. This diagram illustrates the primary macromolecular components of dermis including collagens, proteoglycans (PGs), and hyaluronan. Dermis is composed of collagen fibrils containing types I, III and V collagen that are separated by proteoglycan aggregates containing proteoglycan monomers, link protein and hyaluronan. Collagen fibrils are coated with the proteoglycan decorin as well as with fibril associated macromolecules including tenascin-C and type XII collagen. The dermis is anchored to the epidermis by type VII collagen.

Type VI collagen is found within the dermis where it is organized into 3 nm beaded filaments that are interwoven between the banded fibrils, and within the matrix between the fiber bundles (32, 33). Type VII collagen is found in anchoring fibrils that connect the dermis and epidermis at the dermal–epidermal junction (22). Type IV collagen is a major component of basement membranes located at the dermal–epidermal junction (34).

Tenascin-C is an extracellular matrix glycoprotein that contributes to the mechanical stability of extracellular matrix through its interaction with collagen fibrils; the interaction appears to be mediated by decorin (35). Tenascin-C is not normally expressed in adults, but appears under pathologic conditions (36) and during joint development (37). It is also expressed in the presence of mechanical loading (37). Both tenascin-C and type XII collagen are minor components found in the dermis that are associated with collagen fibrils and are affected by mechanical loading. Unfortunately, it is not clear how mechanical loading of collagen fibrils affects these components.

Fibers composed of type I and III collagens are found in both the papillary and reticular dermis; the type III to type I ratio is somewhat higher in the papillary layer as compared to the reticular layer (39) with type I collagen comprising about 80–90% of the total collagen content. The mean fractional volume of collagen fibers determined from stereological data is reported to be relatively constant for both papillary and reticular dermis and is between about 66% and 69% (38). In the young adult, the collagen in the papillary dermis appears as a feltwork of randomly orientated thin fibers; while in the reticular dermis it consists of loosely interwovn, large, wavy, randomly orientated collagen bundles (4). Reportedly, the spaces between individual collagen bundles decrease with age, which is reflected as an increase in collagen fiber density (4). The fibers comprising the bundles appear to unravel with increasing age (4). In general, collagen concentration in the skin

of rats is known to increase up to six months of age, after which it decreases (40). The type III collagen content of rat skin falls from 33% at two weeks of age to 18.6% at one year (40).

Elastic tissue in skin forms a three-dimensional network of branched fibers of variable diameter and elastin content (41), which spans from the papillary layer to the deep dermis. Mechanically, the elastic fiber network of skin is in parallel with that of collagen (24, 25), and in skin from older individuals, elastic fibers appear to fray and contain holes (4, 55). Diameters of elastic fibers increase from about 1 μm to 2 μm in proceeding from the papillary to the reticular dermis (4). They form a continuous network that can be isolated by treatment with strong alkali and autoclaving after removal of other components (41). Individual elastic fibers are composed of what was originally believed to be an amorphous core of elastin (42–45), which constitutes up to 90% of the fiber and a microfibrillar component consisting of 10–12 nm diameter fibrils (46, 47). Later studies suggest that elastin may contain regions with different levels of order (48–50). The microfibrils are composed of fibrillins and microfibrillar-associated glycoproteins (51, 52). Oxytalan fibers found in the papillary layer are composed of bundles of 12 nm microfibrils with little associated elastin (53). They extend into the papillary dermis from the dermal–epidermal junction, where they merge with elaunin fibers, with a higher elastin content, and then join elastic fibers in the deeper dermis (4, 41). Elastic fibers in the deep dermis contain about 90% elastin. The relative volume of elastic fibers increases from about 0.7% to about 2.5% in proceeding from the papillary to the recticular dermis (54).

Skin contains a number of glycosaminoglycans (GAGs) including hyaluronan, which is not connected to a protein core, and a number of proteoglycans consisting of a protein core to which GAG side chains are attached. Proteoglycans found in skin include heparin/heparan sulphate proteoglycan, chondroitin-6-sulphate proteoglycan found primarily associated with basement membranes (56), chondroitin sulphate/dermatan sulphate proteoglycan in dermal matrix (57), and low levels of keratan sulphate (4) (Fig. 3). The high molecular weight chondroitin sulphate PG in skin is versican, while the low molecular weight keratan sulphate containing members of the leucine-rich proteoglycan family found in skin include lumican, decorin and biglycan (58). Decorin and biglycan have small molecular sizes and consist of one (decorin) or two (biglycan) GAG chains attached to a core protein with a molecular weight of about 40 kDa (59). Both decorin and biglycan share homologueous core proteins containing 7–24 repeats of characteristic leucine rich amino acid motifs (60). Decorin has been shown to bind to type I collagen via leucine rich regions designated 4–5 while biglycan does not bind (61). Lumican is found to be associated with fibrillar collagens in skin and in its absence animals develop abnormally thick collagen fibrils (62).

The hyaluronan content of skin has been estimated to be between 0.03% and 0.09% (63), while dermatan sulphate represents 30–40% of dermal proteoglycans (64). The GAG content is reported to decrease with respect to the amount of protein with increased age (65), and a recent report suggests that there is a decrease in the proportion of versican and an increase in decorin with increased age, which is associated with the appearance of a small PG that may be a catabolic fragment of decorin (66).

Thus, the dermis contains a variety of macromolecular components that are involved in the transmission and distribution of forces. The collagen fibers appear to transfer internal tension and external loads to the surrounding ECM containing non-fibril forming collagens, FACITs, other minor collagen types, proteoglycans, elastic fibers and other macromolecular components as well as to fibroblasts. External forces applied to the skin not only cause stretching of keratinocyte–keratinocyte cell junctions and the epidermal–dermal junction, but also appear to lead to the stretching of elastic and collagen fibril networks as well as collagen–fibroblast and fibroblast–fibroblast interfaces. Therefore, the response of collagen fibers to mechanical loading appears to be an important aspect of mechanobiology of the skin.

Mechanical properties of skin

The mechanical behavior of skin reflects the passive behavior of the elastic and collagen fibers in the dermis, as well as an active component reflecting keratinocyte–keratinocyte, fibroblast–fibroblast and fibroblast–ECM interactions. The passive mechanical properties of skin to a first approximation reflect the behavior of collagen and elastic networks that make up the majority of the macromolecular components of the dermis

(67–69). Elastic fibers are believed to contribute to the initial low modulus portion of the behavior providing recovery to the collagen networks in skin (70), while the collagen fibers prevent premature mechanical failure of skin (24, 71). Collagen fibers have been modelled as a biaxial orientated wavy fiber network, which aligns with the load direction during elastic fiber deformation (24, 25). When skin is stretched in uniaxial tension, the collagen fibers become aligned with the load axis, and the skin on stretching takes on a tendon-like morphology (24). The mechanical behavior of skin is age dependent and is a function of (a) the degree of crosslinking of collagen fibers (b) degradation of the elastic fiber network, and (c) age dependent changes in PGs (55, 72–75). Skin appears to maintain its thickness and extensibility up to the seventh decade as opposed to the recovery capabilities, which decrease with increased age (76).

The passive uniaxial tensile stress–strain behavior of skin is composed of three phases (67) (Fig. 4). Up to strains of about 30%, the collagen network offers little resistance to deformation and the behavior is dominated by the elastic fibers (70). Between strains of about 30% and 60% the collagen fibrils begin to offer resistance to deformation. During this linear portion of the stress–strain curve, the collagen component dominates the deformation (24, 72) and appears to involve stretching of the flexible regions within crosslinked collagen molecules (77–79). The yield and failure region (strains above 60% for skin) appears to involve fibril defibrillation (69), which occurs as a result of fibril stretching and slippage (79).

The mechanical behavior is further understood by examining the elastic (energy storage) and viscous (energy loss) stress–strain curves (Fig. 5). Elastic stress–strain curves reflect energy that is stored via elastic and collagen fiber stretching, while the viscous stress–strain curves reflect energy lost through fibrillar slippage (77–79) (Fig. 5). Analysis of elastic stress–strain curves for skin and dermis suggest that the elastic and collagen fibril networks are in parallel and can be fit using two Maxwell elements (a Maxwell element is an elastic spring in series with a viscous dashpot) in parallel (78, 80). The elastic moduli of the springs in the two Maxwell elements are consistent with the elastic moduli of elastic fibers and collagen fibrils, respectively (77–80). This suggests that at low degrees of deformation, the collagen fibrils remain wavy and offer little resistance to

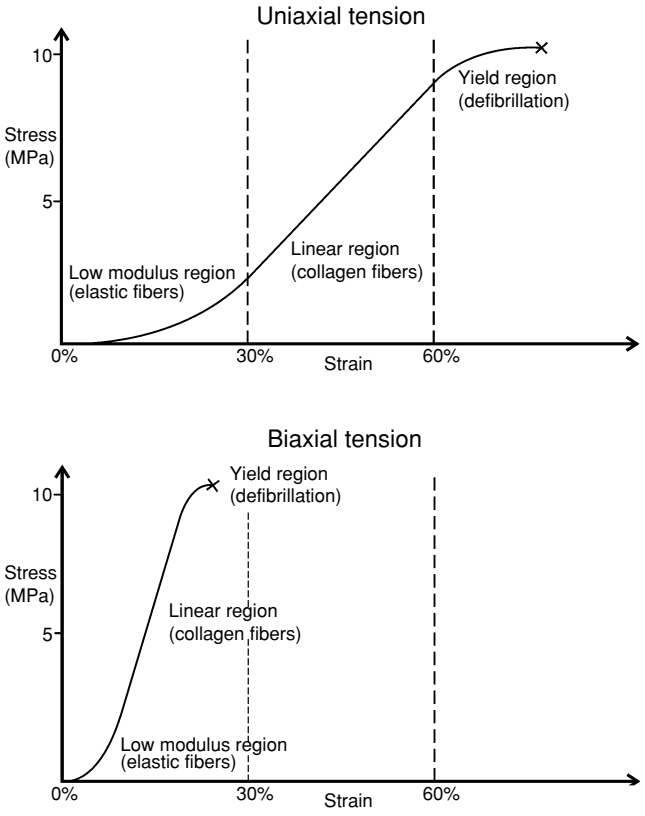

Fig. 4. Generalized stress–strain curve for skin and dermis in uniaxial and biaxial tension. (top) Diagrammatic representation of the stress–strain curve for human skin and dermis excised from the surrounding skin and tested in uniaxial tension. The low modulus (modulus is equal to the slope of the stress–strain curve) portion of the stress–strain curve is reported to represent straightening of the wavy collagen fibers and stretching of elastic fibers, while the linear region represents stretching and slippage of collagen molecules within crosslinked collagen fibers and collagen fibril slippage. The final yield (failure) region is a reflection of defibrillation of the collagen fibrils, which results in loss of fibrillar structure. (bottom) Biaxial tensile loading results in the lateral compression of the stress–strain curve and the reduction in the strain before entry into the linear region occurs.

deformation as previously hypothesized (70); and that the slope of the elastic stress–strain curve reflects stretching of first elastic and then collagen fibers (77–80). The slope of the elastic stress–strain curve of collagen does not depend on strain rate supporting the model of collagen fibers as almost 'ideal' spring-like elements (80). At high strain rates the slope of the viscous stress–strain curve decreases (80), which is consistent with reports that skin shear-thins and is thixotropic (80, 81). Additionally, both the elastic and viscous properties of skin change during ageing (73, 80).

In the 1860s Langer first recognized the presence of a biaxial passive pretension in normal skin from observations that circular holes punched out of cadavers became enlarged and elliptical due to

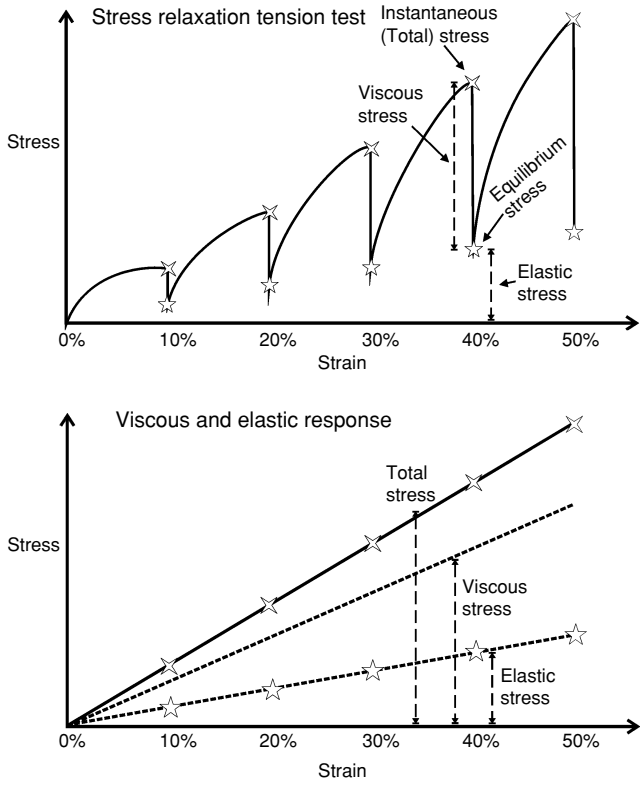

Fig. 5. Incremental, total, elastic and viscous stress–strain curves for skin and dermis. (top) Incremental stress–strain curves are obtained by stretching skin through a series of strain increments and allowing the stress to relax at each strain increment until it does not change with increasing time before another strain increment is added. The total stress can be separated into elastic and viscous stress–strain curves (bottom). The slope of the elastic stress–strain curve is a measure of collagen and elastic fiber stretching while the slope of the viscous stress–strain curve is a measure of fiber slippage (25, 77–80).

the inequality of the prestress along and across the major axes of the ellipse (82–84). By connecting the long axes of all the ellipses, he created a pattern of lines that were used to diagram the direction of maximum tension within the skin. A recent study of the biaxial mechanical properties of skin indicates that the prestress in skin is greatest for the arms, sternum, thigh, patella and tibia while it is lowest for the back; the prestress in skin is about 10% of the maximum stress or about 1 MPa (75). This level of prestress varies from region to region and may be important in regulating ECM metabolism through mechanochemical transduction. Because of the passive biaxial prestress (75), skin *in vivo* is characterized by a stress–strain curve that is condensed and shifted to the left as is diagrammed in Fig. 4 and also appears to operate in the beginning of the linear region of the stress–strain curve.

The maximum stress in skin occurs after a biaxial strain of about 20%, a value that decreases with increased age (75). Since the principal stresses occur at angles of $+/-10^{\circ}$ with respect to Langer's lines (75) this observation supports the conclusion that the direction of Langer's lines is within an angle of about 10° to the direction of maximum prestress in skin. The isotropy of skin is known to be highest in areas that have the highest prestresses (75). However, the active contribution of fibroblast tension on the collagen network (2, 85) also needs to be taken into consideration. This contribution would shift the stress–strain curve even further to the left, suggesting that the extensibility of skin *in vivo* is limited by the presence of a biaxial strain on the collagen fibrils in the dermis (Fig. 4).

Thus, the mechanical behavior of skin *in vivo* is influenced by the direction of external loading, the level of pretension and the rate at which the load is applied. Since the pretension in skin can be as high as 1 MPa, this suggests that skin appears to operate in the upper linear region of the stress–strain curve and that collagen fibers bear the load even in the absence of external loading. Since fibroblasts rest on the surface of collagen fibrils in dermis, internal and external tensile loading of skin would lead to direct stretching of fibroblast cell membrane–collagen fibril interfaces and fibroblast–fibroblast cell attachments. Conversely, active fibroblast tension applied to the surface of collagen fibrils could lead to increased tension in the dermis. These two cases will be discussed further below.

Influence of external forces on the skin

The influence of mechanical forces on skin structure and remodeling has been studied extensively in an attempt to: understand wound healing and reduce hypertrophic scarring (86); increase the skin surface area using balloon expanders (87, 88); study the reorganization and contraction of fibroblast seeded collagen matrices in the absence (89, 90) and in the presence of external mechanical loading (91) and; understand the influence of gravity on the properties of skin (92–94).

Application of external forces to skin affects both cell–cell and cell–ECM interactions. The use of compression dressings placed over areas of skin with hypertrophic scarring results in resorption of some of the underlying scar tissue (86), while the

application of tension affects reorganization of fibroblast seeded collagen matrices (2). Tissue expansion, using an expandable balloon placed in the dermis that applies tension to the epidermis, results in flattening of the basal cells (95–97) and changes in keratinocyte morphology from columnar to cuboidal (97). Epidermal hyperplasia has been observed with increased cellularity in basal and suprabasal layers with up-regulated mitotic activity of epidermal cells in balloon expanded skin (95–97). The epidermis undergoes significant thickening while the dermis and subcutaneous tissue are significantly thinner after expansion (96, 98). It has been hypothesized that even gravity has an effect in modifying cellular behavior (99). These studies underscore the need to better understand how cells in the ECM respond to external mechanical loading.

Anatomic location also affects the influence of external forces on the mechanical behavior of skin. The influence of the force of gravity on skin has been proposed to play a significant role in protecting against oedema formation in the legs when the body is in the upright posture (100). In younger individuals, changes in skin elasticity and distensibility of the lower extremities (that is associated with increased gravitational loading that occurs during standing) has been shown to be higher compared to older individuals, suggesting that a diurnal variation in skin elasticity occurs in younger individuals (92). However, in aged individuals, skin is less elastic and no diurnal variation in elasticity or distensibility is observed (92). Other reported effects of external loading on skin mechanics include gravity induced facial oedema and modification of skin mechanical properties of the leg after use of compression dressings (93, 94).

Thus, the influence of tension and compression on skin components supports the conclusion that tensile stresses applied to skin appear to stimulate cellular proliferation, while the application of compressive forces appear to lead to resorption of the underlying tissue. These observations have important implications with respect to wound healing and the design of devices used to treat wounds.

How are internal stresses developed within skin?

Internal stress in skin arises from forces exerted by cells on their ECM as well as from the tension that is incorporated into the collagen fibril network during development. Removal of the epidermis that occurs during trauma does not result in large changes in skin structure and function. However, loss of full thickness skin results in relaxation of the internal stresses that exist in the collagen fibrils of the dermis, requiring the application of external tension through the use of sutures to close skin defects. This observation leads to the conclusion that the internal forces in the dermis are larger than those in the epidermis.

Internal forces in the dermis arise from internal tension in the collagen fiber network, and active fibroblast–collagen and fibroblast–fibroblast interactions. Isometric tension has been measured in muscle and non-muscle cells attached to a substrate (101); release of these gels from the substrate results in spontaneous contraction (102). These results indicate that living cells exert internal tensile stresses on their surrounding ECM, which in part accounts for the release of stress encountered when a piece of full thickness skin is excised from the surrounding skin. Internal forces within the skin are presumeably generated by tensile forces inherent to collagen fibrils and fibers, fibroblast cytoskeletal contraction of collagen fibrils (2) and fibroblast–fibroblast contractile forces in the dermis. Further research is needed to identify if these mechanisms are involved in internal tension generation.

Such cellular tensile traction forces have been identified as arising from the cell cytoskeleton. Ingber hypothesized that forces exerted by the extracellular matrix on cells may be in equilibrium with forces exerted by cells on the extracellular matrix (103). He (103) proposed that forces are transmitted to and from cells through the extracellular matrix with changes in mechanical forces and cell shape acting as biological regulators. Ingber further hypothesized that cells use a tension-dependent form of architecture, termed tensegrity, to organize and stabilize their cytoskeleton. He noted that mechanical interactions between cells and their extracellular matrix appear to play a critical role in cell regulation by switching cells between different gene products (103). Ingber (3) suggested that cytoskeletal tension is the major force acting on living cells, and that all external mechanical loads are imposed on a preexisting force balance. Cells anchor to the underlying extracellular matrix and surrounding cells by physically coupling their tensed cytoskeletal filaments. These cytoskeletal filaments cluster in

localized adhesion sites termed focal adhesion complexes to form cell-substrate and cell–cell interactions (3).

Integrin adhesion receptors connect extracellular matrix components to cytoskeletal elements, and have been implicated in mediating signal transduction through the cell membrane in both directions (104). Integrin adhesion receptors are heterodimers of two different subunits termed α and β (104). They contain a large extracellular matrix domain responsible for binding to substrates, a single transmembrane domain and a cytoplasmic domain that in most cases consists of 20–70 amino acid residues (105). Integrin-cell membrane associated talin interactions may explain how dynamic structural changes at the cell membrane may lead to cytoskeletal changes in the cytosol (106). Integrins mediate signal transduction through the cell membrane in both directions by a process involving ligand binding. The process of ligand binding to integrins causes signals to be transmitted into the cell, and results in cytoskeletal reorganization, gene expression and cellular differentiation (outside-in signaling). On the internal cellular side of the membrane, signals within the cell can also propagate through integrins and regulate integrin-ligand binding affinity and cell adhesion (inside-out signaling) (105).

External loading of the ECM has been suggested by several authors to be related to actin filament rearrangement in the cytoskeleton. Actin filaments in the cytoskeleton play an important role in determining cell shape and delineating cell motility, as well as participating in other cellular functions (103, 107). Cell culture studies have shown that when cells are grown on a mechanically stretched substrate, the actin cytoskeleton of the cell is reorganized into bundles of actin filaments (stress fibers) orientated in a specific direction (108, 109). The ability of the cytoskeleton to reorganize into orientated actin filaments suggests that stresses in the ECM can be transduced into changes in the cell cytoskeleton. It can be speculated that conversely, changes in the cell cytoskeleton can be transduced into stresses in the ECM.

Although eukaryotic cells employ integrins to bind directly to collagen fibers; they also have receptors for fibronectin and other components of the ECM. Cells bind to extracellular matrix collagen fibers via integrin subunits α1β1 and α2β1 (110) through a six-residue (glycine-phenylalanine-hydroxyproline-glycine-glutamic acid-arginine) sequence (111) that is present in the b2 and d bands of the collagen positive staining pattern (25, 79, 80) (Fig. 6). The integrins are heterodimeric transmembrane receptors that are composed of an α regulatory subunit and a β signal transducing unit (see 112 for a review). The α subunit imparts ligand specificity, enabling the heterodimer to bind to specific extracellular matrix (ECM) or basement membrane (BM) components and the β subunit provides a regulatory function. Results of recent studies suggest that integrin-containing focal complexes behave as mechanosensors exhibiting directional assembly

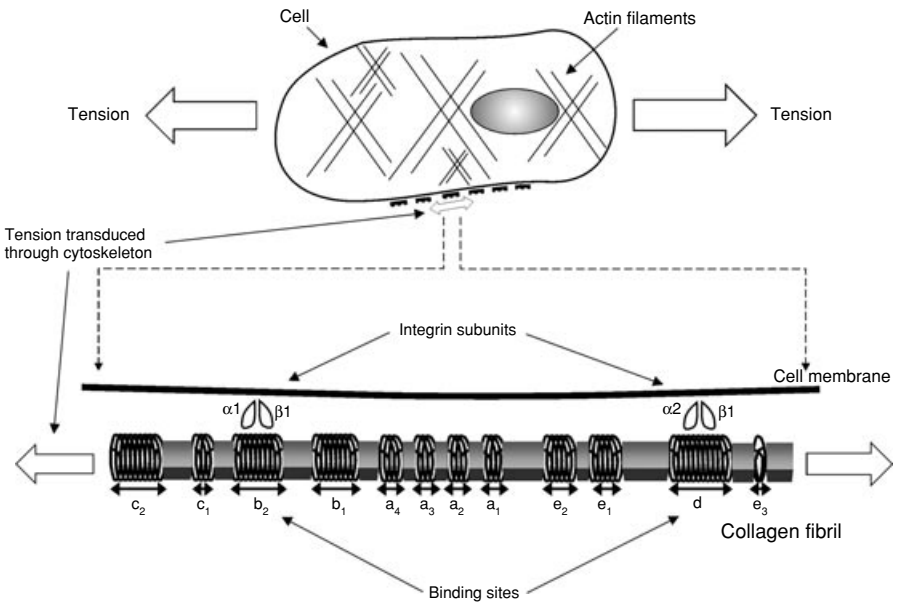

Fig. 6. Cell–collagen fibril interactions in dermis. Diagram illustrating fibroblast–collagen fibril binding in the extracellular matrix through interactions between integrin dimers α1β1 and α2β1 and regions containing charged residues on collagen. The integrin units are modeled to bind to flexible regions in bands b2 and d (represented as springs) in the positive staining pattern of the collagen fibril. Tension and changes in tension in the collagen fibrils found in ECM results in stretching of integrin subunits and components bound to the cell membrane between the two attachment sites and putatively explains some of the possible changes to the cell cytoskeleton and membrane that lead to secondary messenger activation within the cell that are diagrammed in Fig. 8.

in response to local forces (3). It has been reported in studies of fibroblasts grown in a relaxed collagen gel that collagen binding integrins are involved in down-regulating expression of genes for collagen α1(I) and up-regulating collagenase (MMP1) mRNA (113), suggesting that direct collagen-fibroblast tension may be required to maintain dermal structure and function. Strain has been shown to induce alterations in integrin expression of α5β1 and α2β1, which are ligands for fibronectin and collagen, respectively (114).

Fibroblasts can also bind to fibronectin in the ECM through integrin subunits α5β1. Wang and Stamenovic (115) have shown that upon stretching fibroblasts grown on a silicon membrane coated with fibronectin exhibit reorganization of the actin cytoskeleton to an angle of about 60°. Upon fibronectin adhesion, the short cytoplasmid domain of the β1 subunit binds to proteins, which in turn associates with and reorganizes actin filaments to form focal adhesions. Upon further activation of secondary messenger pathways in cells of the ECM through Rho GTPases, changes in actin cytoskeleton lead to integrin clustering, which facilitates the polymerization and assembly of ECM on the cell surface and enables stable substratum attachment (116, 117).

Fibroblast–fibroblast interactions in the dermis may also contribute to generation of the internal tension in skin. Ragsdale et al. (118) showed that fibroblasts were stretched in tension after spontaneous contraction of neighboring cells. They postulated that mechanical transmission of tensile forces between neighboring fibroblasts occurs through adherens junctions. Mechanical forces applied to fibroblast adherens junctions activate N-cadherin associated stretch-sensitive calcium permeable channels, increasing actin polymerization (120). In some cell types, mechanical perturbation leads to a transient increase in intracellular calcium that propagates from cell to cell (119).

Thus, internal stresses in dermis arise from internal tension in the collagen fiber network, isometric tension generated by fibroblast traction on collagen fibrils through integrin adhesion, and fibroblast–fibroblast interactions through adherens junctions that are mechano-sensitive calcium permeable channels. The consequences of perturbations in mechanical loading may include cytoskeletal reorganization, and changes in gene expression and cell differentiation.

Internal stress generation in a contracting fibroblast populated collagen matrix model

Fibroblast–collagen interactions have been studied by culturing fibroblasts in a collagen matrix, scaffold or lattice (see 3 for a recent review). Forces exerted by cells on the collagen matrix cause the matrix to contract even in the absence of external mechanical loading. If the collagen resists deformation, forces exerted by fibroblasts on the matrix cause isometric tension to develop, indicating that the state of cellular mechanical loading appears to regulate how fibroblasts respond (2). The ability of cells to contract a collagen matrix depends on the actin cytoskeleton (101); if actin polymerization is inhibited, matrix contraction is impaired (10).

The effects of mechanical forces have been studied on both isolated fibroblasts and fibroblasts cultured in a collagen matrix. The transcriptional profile of genes induced by fibroblasts grown in collagen lattices suggests that mechanical stimulation leads to a 'synthetic' fibroblast phenotype characterized by induction of connective tissue synthesis while simultaneously inhibiting matrix degradation (121). Mechanically loaded cells, grown on laminin or elastin or other substrates, expressed higher levels of procollagen mRNA and incorporated more labeled proline into protein than unstressed cells (122). Fibroblasts grown in a 3-dimensional collagen lattice have been shown to align themselves with the direction of principle strain (123) and to adopt a synthetic fibroblast phenotype characterized by induction of connective tissue synthesis and inhibition of matrix degradation (121). Under these conditions they show *de novo* transcription of the COL1A1 gene and pro-α1(I) collagen mRNA (124). Fibroblasts grown in collagen lattices can generate a force of approximately 10^{-10} N as a result of a change in cell shape and attachment (123) and maintain a tensional homeostasis of approximately $40–60 \times 10^{-5}$ N per million cells (123). Cell contraction of 3-D collagen matrices is opposite to the direction of applied loads (125) and increased external loading is followed immediately by a reduction in cell-mediated contraction (125). Stress relaxation in a collagen matrix results in activation of a cellular Ca^{2+}-dependent adenyl cyclase signaling pathway that leads to an increase in both cyclic adenosine monophosphate (cAMP) and free arachidonic acid (102).

Fibroblasts in cell culture that are not aligned with the force direction, show a several-fold increase in matrix metalloproteinase activity (MMP1, MMP2 and MMP3) suggesting that cells that are unable to align with the direction of the applied load, remodel their matrix more rapidly than orientated cells (126). The above observations suggest that fibroblast alignment with collagen fibrils found in the matrix results in matrix stability as opposed to matrix catabolism that occurs when cells are not aligned.

Cells grown in matrices that are restrained to eliminate contraction develop isometric tension (101); whereas cells grown in matrices floating freely in cell culture medium remain mechanically unloaded. Mechanically loaded fibroblasts develop prominent actin stress fibers and organize a fibronectin matrix. Treatment of such cells with transforming growth factor beta (TGF-β) results in cellular differentiation into myofibroblasts, expression of α-smooth muscle actin and formation of stress fibers (127). Fibroblasts grown in collagen matrices in the absence of mechanical loading down-regulate the extracellular-signal-regulated kinases (ERK) pathways, which leads to quiescence (128). Some of the cells undergo apoptosis as a result of loss of mechanical loading (129, 130). Integrins appear play a role in cellular apoptosis induced by a reduction or absence of mechanical loading. Primary human fibroblasts display a marked reduction of apoptosis in mechanically relaxed collagen matrices in the presence of adhesion blocking antibodies to integrins α1β1 and α2β1 (131). Also cells that lack α2 integrin or those undergoing depolymerization of F-actin display no apoptosis in mechanically relaxed matrices (131).

Tenascin-C and type XII collagen are associated with collagen fibrils in ECM as discussed above, and their synthesis appears to be up-regulated by mechanical loading. In matrices bearing high mechanical loads, mRNA expression and synthesis of tenascin-C and collagen XII are up-regulated (132–134). Stretch response promoter regions have been identified in both tenascin-C and collagen type XII genes. These regions have the same structural motif that has been implicated in the response of endothelial cells to platelet-derived growth factor (PDGF-B) under the influence of shear stress (135, 136).

Studies of fibroblast populated collagen lattices suggest that fibroblasts grown under the influence of external tensile loads exhibit a 'synthetic' phenotype characterized by induction of connective tissue synthesis and inhibition of matrix degradation. In addition, the cells appear to align with the external stress direction and undergo a reduction in cell-mediated contraction. In the absence of mechanical loading, fibroblasts grown in collagen matrices down-regulate secondary messenger pathways that may result in cellular apoptosis.

How do external forces affect epidermal cells?

There are numerous studies in the literature suggesting that both keratinocytes and fibroblasts are capable of transducing mechanical forces in skin. Cells from mechanically active environments appear to be able to couple signals from forces applied through β-integrins to up-regulate the production of cytoprotective cytoskeletal proteins including filamin A (140). Application of a cyclic compressive force has been shown to increase keratin synthesis and decrease cell division of epidermal cells leading to the conclusion that cyclic loading promotes differentiation of epidermal cells (137). Strain induced changes in keratinocyte function are modeled to be modulated through increased expression of mRNA of interleukin-1 (138). Expression of IL-1 by keratinocytes as a result of mechanical strain may activate vascular endothelium and promote local inflammation (139).

Takei et al. (141) studied the effects of cyclic strain on protein kinase C (PKC) activation and translocation in cultured keratinocytes. Isolated keratinocytes subjected to cyclic strain exhibit a significant increase in cell proliferation, DNA synthesis and protein synthesis as compared to stationary or constantly loaded cells (141) (Fig. 7). Takei et al. (141) also reported a strain-induced reduction in the levels of cyclic adenosine monophosphate, protein kinase A (PKA) and prostaglandin E2 (PGE2) as compared to stationary controls (141). In another study, mechanical stretching of skin was shown to alter cell shape and trigger biochemical signaling in keratinocytes through mitogen activated protein (MAP) kinases (142). Cell stretch, shown to activate extracellular signal regulated kinase ½ (ERK1/2), was reversed by treatment with monoclonal antibodies to β1 integrins (142) suggesting that mechanochemical transduction involves the β integrin subunit in keratinocytes.

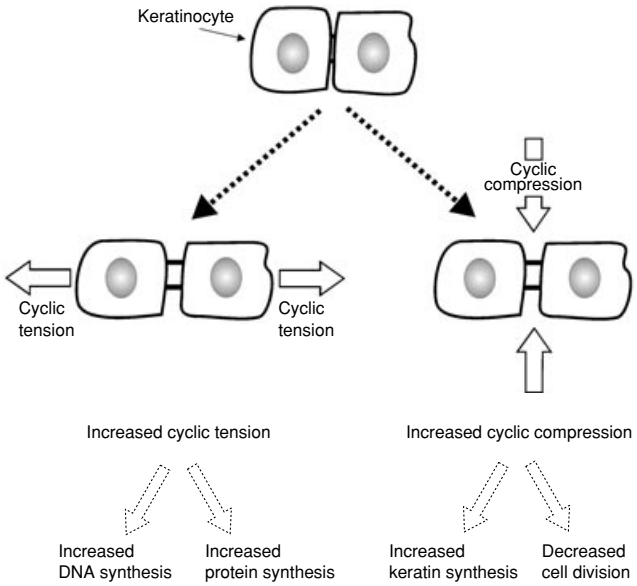

Fig. 7. Putative affects of compressive and tensile loading on cell proliferation and protein synthesis. Diagram illustrating how cyclic compressive loading down regulates cell division of keratinocytes, while cyclic tensile loading up regulates cell division. Protein synthesis, however, is up-regulated by both types of mechanical loading.

Growth factors such as platelet derived growth factor and angiotension II have been implicated in strain-induced cellular growth in vascular tissues (143, 144). Two growth factors in skin, epidermal growth factor (EGF) and TGF-β appear to act via cytoskeletal molecules or protein kinase families and may play a role in mechanical strain induced changes in skin cells (see 138 for a review). The EGF receptor has been shown to bind to actin binding proteins and cause reorganization of the actin filament system (145). The addition of EGF to its receptor leads to phosphorylation of tyrosine residues on the EGF receptor (EGFR), and to colocalization of F-actin and the EGF receptor (146). Cytoskeletal-linked EGFR has been postulated to induce cell proliferation through the microfilament system (138). Membrane ruffling occurs at sites that are rich in actin, EGFR, phospholipase C and tyrosine phosphorylated proteins (147, 148), all of which are essential for strain-induced signal transductions (141). EGF-induced effects on keratinocytes involve high affinity receptors (149) and involve the tyrosine kinase pathway (150).

Thus, the application of external forces to epidermal cells leads to a number of events including up-regulation of production of cytoprotective cytoskeletal proteins, cell proliferation, changes in cell shape, activation of MAP kinase and ERK ½ pathways, and activation of growth factor pathways (protein kinase). These changes subsequently affect gene expression and protein synthesis as described further below.

How are external forces transduced in the epidermis?

As discussed above, the epidermis is stretched due to the tension transmitted from the underlying dermis. This stretching causes tension in the contacts between cells in the epidermis and tension in the cell–BM interface. The contacts between the two adjoining cell membranes are stabilized by specific cell adhesion molecules (CAMs), which include the Ca^{2+}-dependent cadherins. These molecules appear to lead the way for cell–cell communications (8, 9). In the epithelial cells, cadherins are concentrated within adherens junctions (151) and their extracellular domains interact with cadherins on adjacent cells, while their cytoplasmic domains provide attachment to the actin cytoskeleton via catenins and other cytoskeletal proteins (10, 151). The Rho family is required for the establishment and maintenance of cadherin-based adherens junctions (152, 153). The type of cadherin expressed in a cell can affect the specificity (151) and the physiologic properties of cell interactions (10).

Epithelial cells also appear to attach to ECM components via integrin subunits and in this manner can transfer stress via cell–ECM interactions. Epithelial cells that express the integrin subunit α6β4 associate with transmembrane collagen VII and with intermediate filament linker proteins (154). This association enables the integrin subunits to cluster into macrostructures referred to as hemidesmosomes prominent in epithelium such as epidermis (155). Minor epidermal integrins include α2β1 (collagen laminin), α5β1 (fibronectin) and wound healing induced αvβ5.

In the epidermis and its appendages, basal keratinocytes utilize integrins to adhere to other components of the underlying basement membrane (BM), which is rich in extracellular matrix macromolecules. The predominant epidermal integrins are α3β1 and α6β4, both of which bind laminin 5, the major ECM component of BM (156). Interactions including anchorage and mobility between laminin 5 and keratinocytes are mediated through integrins α6β4 and α3β1 respectively (18, 157). Deposition of laminin 5 over-exposed dermal collagen in epidermal wounds allows

keratinocytes to interact via α6β4 integrin subunits and to switch from RhoGTPase-dependent adhesion on collagen to PI3K-dependent adhesion and spreading on laminin 5. The later event is mediated by integrin subunits α3β1 (158). Keratinocyte cultures implicate both integrin dimers in adhesion, proliferation, and stem cell maintenance. Results of a recent study suggest that keratinocyte migration requires α2β1 integrin–mediated interaction with laminin 5 (159). Ablation of β1 integrin results in severe defects in epidermal proliferation, BM formation and hemidesmosome stability (155). When α6 or β4 is missing, hemidesomosomes are absent and epidermal adhesion to the underlying BM is impaired (160).

In addition to their function as sites for mechanical energy transduction, matrix adhesions participate in signaling processes that go on within the cell cytoplasm. Focal contacts contain several types of signaling molecules including tyrosine phosphatases, tyrosine kinases and adaptor proteins (161, 162). Focal contacts appear as both adhesion and signal sites, conveying information from the ECM into the cell.

Thus, the internal tension transmitted from the dermis to the epidermis results in stretching of epithelial adherens junctions, which are sensitive to stretch activation. Tensile loading of epithelial basement membranes that also occurs due to internal stresses in the dermis results in stretching of integrin receptors on epidermal cells, which affects gene expression and protein synthesis as described further below.

What are the general cellular mechanisms that may be triggered by external mechanical loading?

During cell adhesion, the initial binding of integrins to their ECM ligands leads to their activation and clustering, and to assembly of focal adhesion complexes, which serve as 'assembly lines' for signaling pathways. The signaling pathways include protein kinases, adaptor proteins, guanidine exchange factors and small GTPases that are recruited to these sites and may directly trigger mitogen-activated protein kinases (MAPK) pathways or with growth factors as well as activation of the NF-κB pathway (163–165) (Fig. 8).

G proteins are another family of membrane proteins believed to modulate mechanochemical transduction pathways (166). Mechanical stimulation changes the conformation of G protein that leads to growth factor-like changes that initiate secondary messenger cascades leading to cell growth (167). It has been reported that cyclic strain of smooth muscle cells significantly decreased steady state levels of G protein and adenylate cyclase activity (168). Muscular stimulation also appears to be coupled with PLC and G protein activation in small arteries (169).

In addition to activation of signaling pathways, mechanical stress triggers activation of stretch-activated ion channels. These ion channels have been identified in a number of cells (170) and have been studied extensively in muscle cells (171, 172). Stretch activated channels are reported to permit passage of cations including Ca^{2+}, K^{+} and Na^{+}, although a few anion channels are reported to be sensitive to mechanical stimulation (170, 173). In muscle cells Ca^{2+} influx through voltage-gated channels induces a transient elevation in intracellular Ca^{2+} levels (174). Ca^{2+} influx is activated by mechanical stimulation and leads to membrane depolarization and cell contraction (172). The presence of extracellular Ca^{2+} appears to be a requirement for its influx as a result of stretch-induced cell contraction in muscle cells (176). It appears to enhance the sensitivity of intracellular calcium on subsequent signal transduction through activation of cascades such as activation of protein kinase C (176, 177). Strain induced Ca^{2+} signal transmission appears to involve the actin microfilament system since an actin polymerization inhibitor was found to abolish Ca^{2+} responses that were induced by mechanical strain (178).

Both Ca^{2+} and K^{+} channels have been implicated in mediating stretch induced changes in cells (166). Ca^{2+} ion involvement in phospholipase C (PLC) activation has been proposed, which catalyses the generation of PLC-derived inositol phosphates and diacylglycerol (DAG) necessary for PKC activation (179, 180), while K^{+} channel involvement has also been postulated (181, 182) (Fig. 8).

The presence of mechanical forces at the cell–ECM interface may not only affect stretch-activated ion channels, but may also modulate changes in cell membrane structure with membrane associated changes in the cell cytoskeleton. Actin binding proteins, including vinculin, and gelsolin, are related to the phosphoinsitide and PKC pathways and may undergo or lead to

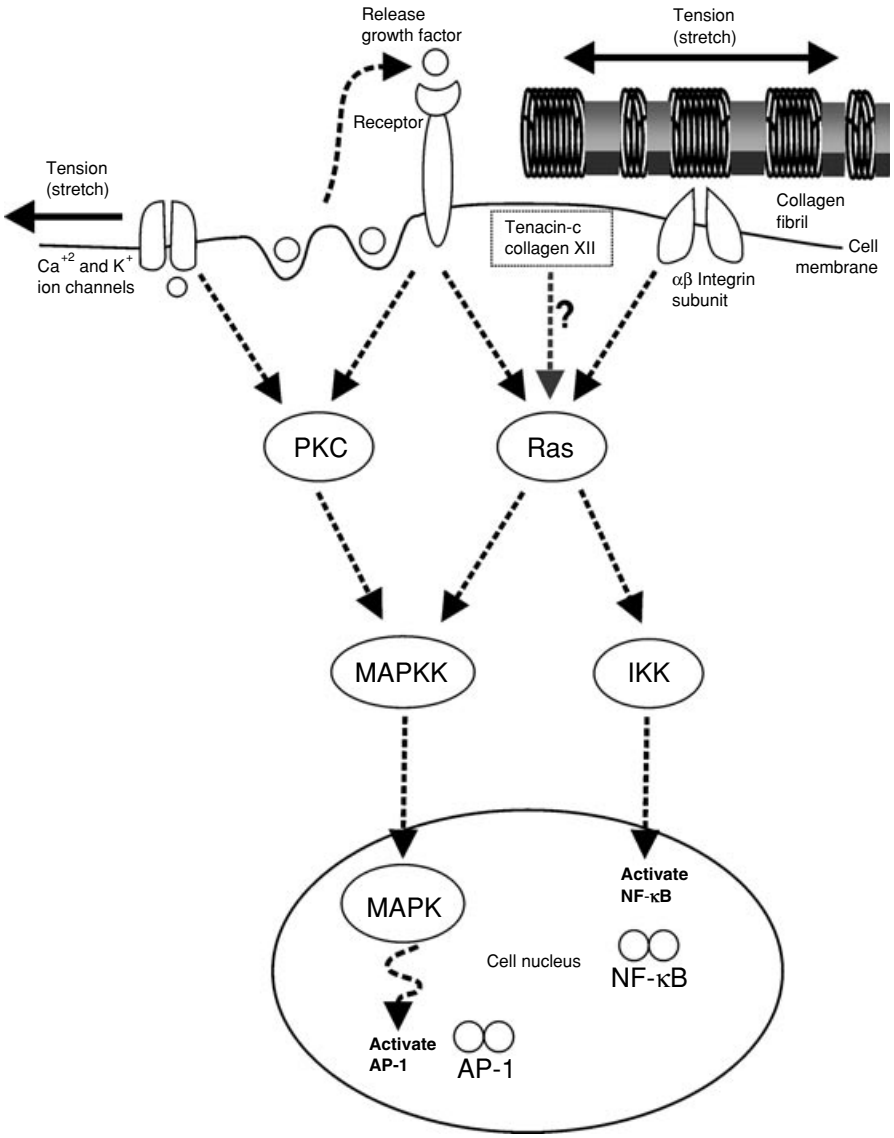

Fig. 8. Generalized, oversimplified scheme for how external mechanical stress is transduced into changes in cell genetic expression. Tensile stresses applied to the cell causes stretching of intracellular junctions, activation of membrane ion channels, or release of growth factors that leads to activation of secondary messengers. Secondary messenger activation, in turn, leads to activation of factors such as NF-κB that binds to promoter sequences in genes such as those expressed for tenascin-C and type XII collagen. Stretching of ECM-integrin contacts and cell intercellular junctions are known to trigger MAP kinases pathways (MAPKKK, MAPKK, and MAPK) via the GTPase Ras. MAPKs translocate to the nucleus and activate transcription factors such as AP-1. Alternately, members of the MAP kinase kinase kinase (MAPKKK) family have been shown to activate the I-κB kinase (IKK) complex, which phosphorylates I-κB and leads to the release of NF-κ, which in turn, translocates to the nucleus. In the nucleus, NF-κB binds to its target promoter sequence. Another putative route for MAPK activation is via autocrine release of growth factors and activation of protein kinase C (PKC). Mechanical stretch also leads to activation of stretch response promoter regions in tenascin-C and type XII collagen genes in a similar manner to platelet derived growth factor B (1, 195).

conformational changes during membrane activation (183). It is likely that intracellular tension could modulate cytoskeleton filament organization and cellular alignment with external forces and transduce signals among adjacent cells (166).

Protein kinase C (PKC) has been proposed to play a pivotal role in signal transduction by mediating a variety of biological responses (184, 185). Hydrolysis of phospholipids in cell membranes, catalysed by PLC, produces inositol phosphates and diacyglycerol, leading to PKC activation (166). Mechanical strain in muscle cells activates PLC, phospholipase A2 and phospholipase D, DAG and inositol triphosphate (177, 186, 187). Inhibition of PLC activation abolishes the strain-induced responses in endothelial cells (178). $PLC_{\gamma 1,2}$ is stimulated by the receptor tyrosine as well as the EGF receptor, suggesting that EGF exhibits its stimulatory effect through the PLC and PKC pathway (166). PLCβ is also activated in association with G protein that could activate mitogen-activated kinase (MAPK) through PKC-related pathways (187, 188). The mitogen-activated kinase (MAPK), also known as extracellular signal-regulated protein kinase (ERK), plays an important role in cell signaling (189, 190). It is interesting to note that changes in ERK and MAPK activities have been observed in aged human skin (191).

Secondary messengers implicated in strain-induced cellular responses include cyclic AMP (cAMP) and prostaglandin E_2 (PGE_2). cAMP is reported to influence protein cell growth, differentiation and protein synthesis (192, 193). Cyclic strain results in increased protein production by keratinocytes and a decrease in cAMP and

cAMP-dependent protein kinase A activity (PKA) (194). Keratinocytes exposed to strain also show decreased levels of cAMP (194) indicating that PGE_2 has a similar effect to cAMP when keratinocytes are strained (138).

A number of cellular processes are triggered by the application of external mechanical forces to epidermal and dermal cells. Cell adhesion to collagen via integrin receptors, stretch activation of ion channels, changes in cell membrane structure, and stretch activation of growth factor receptors all cause activation of a number of signaling pathways that lead to activation of MAPK kinase pathways and changes in gene expression, cell proliferation and protein synthesis.

Conclusion

Internal and external forces applied to keratinocytes and fibroblasts appear to be involved in mechanochemical processes in skin. These processes involve both cell–cell and cell–ECM interactions (1, 195). Internal forces present in dermis are the result of tension that is developmentally incorporated into the collagen fiber network. Passive tension in the collagen fiber network is responsible for the existence of lines of principal tension in skin that result in Langer's lines. The maximum tension occurs at angles of $\pm 10^{\circ}$ with respect to Langer's lines, giving rise to a biaxial state of tensile stress in the collagen network of dermis. The exact magnitudes of the tensions vary from location to location in skin and are reflected in the variation in mechanical properties, namely the stiffness and extensibility of skin.

Beyond the passive tension that exists in the collagen network of skin, there is an active tension generated by fibroblasts in dermis that is responsible for wound contraction and contraction of collagen lattices. This active tension may involve specific interactions between cell membrane integrins and macromolecules in the ECM, especially collagen fibrils. Forces appear to be transferred at the cell–ECM interface via rearrangement of cytoskeletal elements that include actin filaments. In addition, mechanical forces at the cell–ECM interface may activate stretch-induced changes in ion channels, cell contraction at adherens junctions, cell membrane associated secondary messenger pathways and lead to growth factor-like activities that influence cellular proliferation and protein synthesis.

Tension in the collagen network of the dermis is transferred to the epidermis through attachments such as anchoring fibrils and basement membrane components. Although maximum tension occurs in the dermis, there is stress transfer to the epidermis, since the two structures are physically connected. The resulting tension in the epidermis leads to stretching of keratinocytes and may play a role in maintaining cell proliferation, DNA and protein synthesis. However, it is apparent that external mechanical loading causes thickening of the epidermis possibly by increasing the tension experienced by keratinocytes in the epidermis.

Although we have some clues concerning the importance of the effects of internal and external mechanical loading on skin biology, we are only beginning to contemplate the implications of the significance of mechanochemical transduction and its relationship to molecular biology and biochemistry. In order to move ahead in understanding how mechanical forces, energy storage and conversion of mechanical energy into changes in chemical potential of small and large macromolecules may influence living systems, further progress is necessary to provide bridges between the biological and physical sciences.

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