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Physical determinants of cell organization in soft media

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Abstract

Cell adhesion is an integral part of many physiological processes in tissues, including development, tissue maintenance, angiogenesis, and wound healing. Recent advances in materials science (including microcontact printing, soft lithography, microfluidics, and nanotechnology) have led to strongly improved control of extracellular ligand distribution and of the properties of the micromechanical environment. As a result, the investigation of cellular response to the physical properties of adhesive surfaces has become a very active area of research. Sophisticated use of elastic substrates has revealed that cell organization in soft media is determined by active mechanosensing at cell-matrix adhesions. In order to determine the underlying mechanisms, quantification and biophysical modelling are essential. In tissue engineering, theory might help to design new environments for cells.

Keywords: Cell-matrix adhesion; Focal adhesions; Cytoskeleton; Mechanotransduction

1. Introduction

The human body consists of around 10^{13} cells, which can be classified into more than 200 different cell types. Like all complex organisms, our body is organized in a hierarchical way: cells together with extracellular matrix (ECM) form tissues, and tissues form organs. What distinguishes a clot of cells and matrix from a tissue is the well-defined organization of cells and ECM, which is closely associated with tissue function. Within a tissue, cells adopt well-defined gene expression patterns, morphologies, positions and orientations. Loss of cell organisation leads to tissue malfunction and disease. Tissue organisation affects many different physiological processes, including development, tissue maintenance, angiogenesis, and wound healing. The key question in understanding tissue organization is how cells communicate with each other and their environment to build-up organized structures. The research in cell and tissue organization principles has a long history and today we know that cells integrate information received through many different channels. Fig. 1 shows an overview of known factors influencing cell and tissue organization.

The main mean of communication is release and capture of biochemical molecules. In cell migration, this leads to *chemotaxis*, that is directed movement along a chemical gradient [1]. Many different cell types employ chemotaxis, for example neutrophils homing in for pathogens and growth cones during neural development. In the context of tissue organization, chemotaxis is involved for example in morphogenesis, angiogenesis, and wound healing. By using the lock-and-key principle of ligand-receptor binding, cells can exchange very specific information. When cells are suspended in solution, they can capture signalling molecules which are distributed according to the physical laws of diffusion and reaction. In contrast, when cells adhere to an extracellular structure, in addition they can bind biochemical ligands which are attached to external surfaces, for example to the plasma membrane of other cells or the proteins and sugars of the ECM. Therefore, in cell adhesion, the biochemical information is supplemented by several additional degrees of information, including spatial distributions of ligands which are not determined by diffusion, and the topographical and mechanical properties of the structure the cell attaches to.

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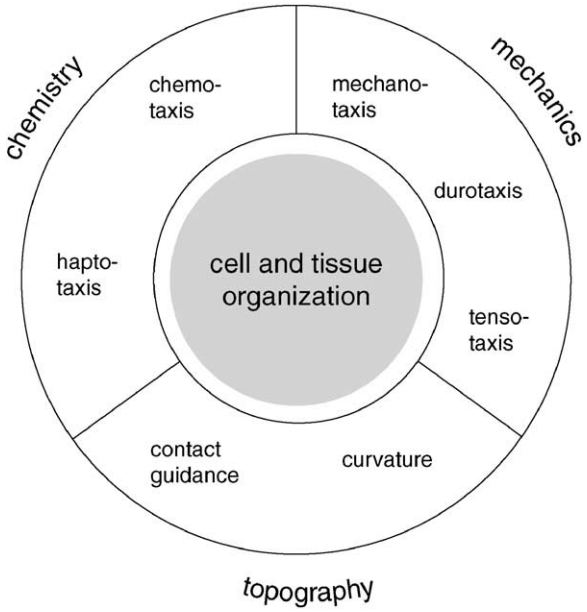

Fig. 1. The organization of cells and tissues results from cell behaviour that integrates different kinds of input signals from the environment. Different terms have been coined in this context and are explained in the main text. They can be group into three categories, namely chemistry, topography and mechanics [18–20].

Weiss was the first to observe that cells preferentially orient along ECM-fibres, an organization principle he termed *contact guidance* [2,3]. Moreover, he observed that tissue explants condense the collagen gel between them into aligned parallel fibre bundles, which then guide cell migration out of the explants. Contact guidance therefore could serve as a large-scale organization principle in tissue development by guiding motile cells along aligned ECM-bundles. Although Weiss associated contact guidance with differential adhesiveness and interfacial tensions [3,4], the term has now gained a strong topographical connotation. In 1976, Dunn demonstrated that cells react to surface curvature and prefer to align along the axis of minimal curvature, where minimal distortion of the cytoskeleton occurs [5,6]. This mechanism also favors orientation of cells along fibre bundles. Contact guidance also manifests itself as cell alignment along microfabricated grooves [7]. During recent years, it has also been shown that even topographical features in the nanometer range influence cell behaviour [8]. It is important to note, though, that contact guidance through elongated topographic features provides only a bidirectional cue for cell migration. In contrast, a unidirectional cue can originate from spatial variations in adhesiveness, for example by immobilized ligand gradients (*haptotaxis*) [9]. Contact guidance and haptotaxis were among the first cell organizing principles discovered for adherent cells that attributed a role to physical cues in the environment.

In addition, it has long been known, especially in the medical and bioengineering communities, that certain cell functions are strongly determined by mechanical stimuli. For example, endothelial cells in blood capillaries [10], osteocytes in bone [11] and alveolar cells of type II in lung [12] only function properly when subject to a certain level of strain indicating proper body functioning (namely the one resulting from blood pulsation, body movements and breathing, respectively). A widely used method to study these effects is the use of cyclically stretched substrates (typically with a physiological frequency of 1 Hz) [13]. These studies showed that cyclic mechanical forces induce progression through the cell cycle. In regard to changes to the cytoskeleton, it was found that cells tend to orient away from the direction of stretch [14,15], presumably because they try to avoid the perturbations arising from the passive deformations. In order to study the underlying mechanotransduction processes in more detail, different experimental techniques have been used to apply external forces to single cells in a localized and well-controlled way, including magnetic twisting cytometry (see article by Dobson in this issue) [16] and use of micropipettes [17]. In these studies, forces were applied to integrin-based cell-matrix contacts, and the main effect found was contact upregulation, that is structural reinforcement and increased signalling. In general, a large body of evidence now demonstrates that external forces trigger different signalling cascades determining cell behaviour.

Cells not only react in a specific way to external forces, they also use actively generated internal forces in order to explore the properties of their environment (*active mechanosensing*) [21]. Using fibronectin-coated beads held in an optical trap, it was shown that cells strengthen integrin-mediated contacts to the beads in proportion to the force restraining it [22]. The authors concluded that this mechanism might allow cells to navigate in the extracellular matrix in response to its mechanical resistance, a phenomena they termed *mechanotaxis*. During recent years, this notion has been confirmed by several other experiments. In particular, the sophisticated use of elastic substrates has shown that cells sense even purely elastic features in their environment. Elastic substrates for the study of cell traction have been introduced in the early 1980s by Harris and coworkers [23,24], who found that anchorage-dependent cells like fibroblasts show a remarkable degree of mechanical activity and that they react to mechanical changes in their environment caused by traction of other cells. In another early study with elastic substrates, it was shown that fibroblasts actively sense and align parallel to the axis of stretch of prestrained elastic substrates [25]. More recently, it was found that cells more strongly upregulate cytoskeleton and cell-matrix adhesion on stiffer substrates [26,27] and locomote in favor of stiffer or strained substrates, a phenomenon which has been termed *durotaxis* [28,29]. The underlying process is active mechanosensing through cell-matrix contacts, which have been shown to transduce force biochemically [30–33]. Conceptually, durotaxis should be distinguished from cell migration in favor of strained substrates, because in general, rigidity and prestrain are not equivalent [21]. Therefore, the later effect might be termed *tensotaxis*, a term which has

been proposed to describe the migration of ectodermal cells in favour of strained regions in *Xenopus* embryos [34]. A similar phenomenon has been observed for neutrophil migration in a physiological hydrogel [35].

While traditionally much attention has been focused on the role of biochemical determinants of cell organization, the results described above clearly show that physical cues like topography, forces or the mechanical properties of the environment might be equally important for cellular decision making. Recent advances in materials science (including microcontact printing, soft lithography, microfluidics, and nanotechnology) now provide tools to study the physical determinants of cell organization in much more detail than formerly possible. Moreover, they allow to design new artificial and biomimetic environments for cells, which open up new perspectives for biomedical applications, in particular in tissue engineering [36]. It is important to note that these tools allow not only for better control, but also for quantification of cell adhesion, for example of spreading area as a function of ligand density and substrate rigidity [26]. There are two main reasons why quantification is essential in this field. First, it allows to single out the relevant mechanisms from a large list of possible explanations which emerge from the intrinsic complexity of biological systems. Second, quantification might be essential for guiding the design process for artificial cellular environment, because there are too many degrees of freedom as to succeed simply by trial-and-error. Here, we discuss recent developments regarding physical determinants of cell organization in soft media, with a special emphasis on the role of quantification and modelling. In Section 2, we start by describing the experimentally observed cell behaviour on elastic substrates. In Section 3, we discuss the underlying mechanosensory processes at integrin-mediated cell-matrix adhesions. In Section 4, we describe recent progress in quantification and modelling of physical determinants of cell organization in soft media. Finally, we briefly conclude in Section 5.

2. Cell behaviour on elastic substrates

A systematic study of the role of substrate elasticity for cell organisation requires new technologies to create substrates with well-defined mechanics in combination with accurate measurement methods to quantify their local mechanical properties [37]. In the pioneering work by Harris and coworkers [23,24], the wrinkling pattern resulting from cell traction on thin films of polydimethylsiloxane (PDMS) were used to estimate the order of magnitude of mechanical activity of cells for the first time. Recent years have seen the development of new protocols for elastic substrates, which now allow for detailed quantitative analysis of cell behaviour as a function of the mechanical properties of their environment [38,39]. In particular, using elastic substrates which do not wrinkle under cell traction allows to use linear elasticity theory in order to calculate cellular traction patterns with high spatial resolution (*traction force microscopy*) [40–43]. Today, three different material systems are commonly used as model substrates to study the effects of substrate elasticity on cell organization: agarose gels, polyacrylamide (PAAM), and polydimethylsilicone (PDMS). All these materials are synthetic polymer gels and usually are used as thick films (thickness around 100 μm). By adjusting the degree of cross-linking, their mechanical properties can be tuned within and beyond the physiologically relevant rigidity ranges of sub-kPa (nerve tissue) up to several MPa (pressurized arteries). Usually, agarose, PAAM and PDMS are used in the stiffness ranges below, around and above1 kPa, respectively. In order to promote cell adhesion, the gel surfaces might have to be modified. Since these surfaces often are resistant to protein absorption from solution, this has to be done by covalent modification. This stepwise procedure allows variation of mechanical properties independently from surface chemistry, in contrast to materials like collagen gels, for which rigidity and ligand density cannot be varied independently. In order to measure the local mechanical properties of elastic substrates, one possibility is a spatial scan with an atomic force microscopy and extraction of the local Young modulus by using the Hertz-model from contact mechanics [26].

In 1997, it was reported for the first time that substrate compliance influences cell behavior [27]. It was observed that cell morphology changed remarkably when reducing the substrate rigidity of a PAAM gel. Later, it was shown that substrate compliance regulates growth and apoptosis of normal, but not of cancer cells [44]. In Fig. 2, we show the typical change of fibroblast morphology from a round unspread cell on a very soft PAAM substrate to a well spread cell morphology with several distinct adhesion sites on a stiffer substrate [45]. Note the reorganization of the actin cytoskeleton, which on very soft substrates is localized beneath the cell membrane in a cortical shell (similarly to non-adherent cells). On stiffer substrates, long straight actin bundles (*stress fibres*) form, which run straight through the cytoplasm. This distinct change in morphology was first observed for endothelial cells and fibroblasts [27] and recently a similar observation

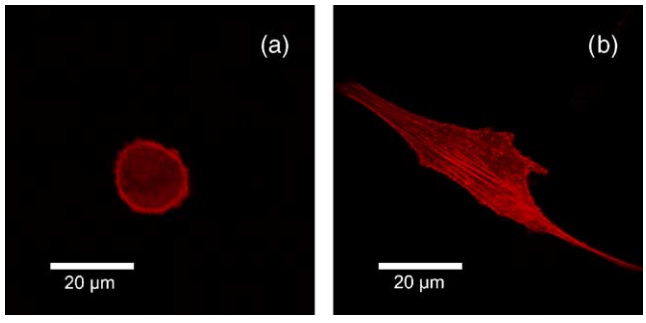

Fig. 2. Cell morphology on elastic substrates depends on substrate rigidity [45]. (a) Fibroblasts on a very soft PAAM gel are round and actin localizes beneath the cell membrane to form a cortical shell similar to non-adherent cells. (b) On stiff substrates cells spread and actin organizes to form stress-fibers typically connecting different adhesion sites.

has been reported for vascular smooth muscle cells (VSMC) [26]. Durotaxis, that is migration in favour of stiffer regions on the substrate, has been reported for the first time in 2000 [28]. In the vicinity of a step gradient in rigidity, fibroblasts migrated from the soft to the stiff side. However, cells on the stiff part did not cross over to the soft side, but rather reoriented by 90° to move along the interface. Moreover, by gently pulling or pushing the substrate with a micro-needle, the direction of locomoting cells could be reversed through the effect of substrate strain (tensotaxis).

During recent years, more progress has been made through the application of micro-fabrication techniques to control the mechanical properties of the substrate on the micron scale. For example, it is now possible to modulate substrate compliance of elastic substrates on a micron scale using a combination of photopolymerization and micropatterning/microfluidics tools, which allows for spatial control of the degree of gel crosslinking [29]. With this technique, durotaxis has now been observed for VSMCs on a continuous radial gradient substrate, including the accumulation of cells on the stiff parts of the gel. Spatial modulation of substrate rigidity can also be accomplished by pouring a soft gel on top of a topographically structured stiffer gel. Cells aligned along the stiffer lines when lines were suffciently separated [46]. Taken together, these experimental results provide strong evidence that cells respond to the purely elastic properties of their environment. While more systematic studies are clearly needed, it appears that many cell types show similar responses to substrate stiffness.

Interestingly, similar observations as the ones reviewed here for elastic substrates have been reported numerous times also for tissue cells in physiological hydrogels. In 1979, Bell and coworkers introduced three-dimensional collagen assays as model systems for studying tissue equivalents [47]. For fibroblasts in collagen gels, they not only found that traction considerably contracts the gel, but also reported orientational effects: cells align along the direction of pull between fixed points and parallel to free surfaces. When a collagen gel is stretched uniaxially, cells polarize in the direction of principal strain [48]. Moreover, cells align in a nose-to-tail configuration, thus forming strings running in parallel to the direction of external strain. If a collagen gel is cut perpendicular to the direction of tensile strain and if cells are present in sufficient numbers, they round up and reorient parallel to the free surface introduced [49]. Although the situation in hydrogels is much more complicated than the situation on synthetic elastic substrates due to the competition of contact guidance and durotaxis/tensotaxis, these observations suggest that very similar principles govern cell behaviour in these two cases [21].

3. Mechanotransduction at cell-matrix contacts

A growing body of evidence now suggests that cell behaviour in soft media is determined by mechanotransductory events localized to cell-matrix contacts [30–32]. Although, a recent study has shown that cell-matrix contacts are somehow different in morphology and composition in collagen gels as compared to elastic substrates [50], it is likely that the same physical processes are at work in both instances. Cell-matrix contacts are based on transmembrane proteins from the integrin family, which bind to e.g. the RGD-motif presented by several extracellular ligands, including collagen, fibronectin, vitronectin, and laminin. On the cytoplasmic side, they can connect to the actin cytoskeleton via linker proteins like talin, α-actinin, filamin, and tensin. In addition, more than 50 other kinds of proteins assemble at the interface between membrane and cytoskeleton, forming the cytoplasmic plaque which provides both structural integrity to the cell-matrix contact and triggers signalling pathways which influence cell behaviour and fate. In particular, signalling molecules like tyrosine kinases (for example focal adhesion kinase) and phosphatases localize to focal adhesions. Cell-matrix adhesions are highly dynamic structures, as evidenced by the fast turnover times revealed by fluorescence measurements [51]. For example, integrins have been shown to turn over within 5–10 min. Since integrins connect the extracellular matrix and the actin cytoskeleton, they can transmit internal forces to the environment and external forces to the cell. According to their location, size and maturation, cell-matrix contacts are classified as focal complexes, focal adhesions and fibrillar adhesions [52]. Focal complexes are small ($<1\,\mu m^2$) contacts based on integrin clustering close to lamellipodia. If initial clustering is stabilized by the properties of the extracellular environment, focal complexes can mature into focal adhesions, in which case integrin packing density can increase two- or three-fold [51]. Focal adhesions connect to the actin cytoskeleton and contractile force builds up that is actively generated by myosin II molecular motors interacting with the actin cytoskeleton. In mature adhesion, the most prominent feature of the actin cytoskeleton are stress fibres, which act like little cellular muscles. Focal adhesions often elongate in the direction of the attached stress fibres, which corresponds to the direction of internal force [33]. Fibrillar adhesions are streak-like contacts rich in tensin which originate from focal adhesions and move centripetally towards the perinuclear region, possibly due to growth at the leading edge and dissociation at the trailing edge. Together with the actin cytoskeleton, the different kinds of cell-matrix adhesions are tightly regulated by the members of the Rho-family of small GTPases, in particular Rho, Rac and Cdc42, which are related to the formation of stress fibres, lamellipodia and filopodia, respectively [53]. The maturation of focal complexes into focal adhesions correlates with the downregulation of Rac signalling and the upregulation of Rho signalling. The main downstream targets of Rho are Rho-associated kinase (ROCK), which activates myosin II contractility, and mDia1, which regulates both actin polymerization and growth of microtubules [17].

Only recently has it become possible to correlate forces and aggregation at focal adhesions, again by using elastic

substrates [33,42]. In the early studies by Harris and coworkers, an inverse relationship between the degree of cellular motility and the magnitude of overall cellular forces had been observed [24]. The authors estimated that fibroblasts exert 100–1000 times larger forces than actually needed for cell locomotion and concluded that these large cellular forces must be required to fulfill the cell's specific function in the organism. Using a new variant of traction force measurements, involving micro-patterned elastic substrates, individual forces exerted at single focal adhesions could be resolved [33,42]. An example for the resulting force pattern is shown in Fig. 3. It was found that stationary fibroblasts typically exert forces of 10 nN at mature focal adhesions, with force being proportional to contact size with a constant of proportionality of 5.5 nN/μm^2. In fact this linear relation persisted even in the case where actomyosin contractility was perturbed by administering the drug 2,3-butanedione monoxoime (BDM), which interferes with actomyosin contractility by inhibiting myosin ATPase [83]. A similar value for the stress constant has been found for smooth muscle cells plated on a bed of flexible microneedles [54]. Since adherent cells can have up to hundreds of focal adhesions, the overall force exerted by the cell can amount to 1 μN. Since typical forces produced by molecular motors are in the pN-range, there must be up to 10^6 myosin II molecular motors contributing to overall cell traction. Recently, it has also been shown that the same correlation between force and contact size exists if forces are applied from the outside: using micropipette manipulation it was shown that focal adhesions grow if the same magnitude of force is applied to them which usually is provided by actomyosin contractility to mature focal adhesions [17]. Therefore, one can conclude that focal adhesions act as mechanosensors which convert mechanical force into biochemical signalling.

In Fig. 4, we show schematically that focal adhesions might be regulated by a positive feedback loop. Integrin clustering in the plasma membrane is accompanied by the assembly of the cytoplasmic plaque and might lead to Rho-activation. Rho in turn activates myosin II molecular motor activity (through ROCK) and F-actin polymerization (through mDia1), leading to increased tension in the actin cytoskeleton. This tension is transmitted back to the focal adhesion, where it leads to anisotropic cluster growth in the direction of force by an unknown mechanotransductory mechanism. In order to avoid unlimited growth of focal adhesions, there must be some additional mechanism which interferes with this feedback loop and which might be provided by microtubules, which have been shown to be targeted to focal adhesions, possibly delivering a dissociation signal [55]. Until the positive feedback loop is terminated, it can be modulated by both internal and external factors, including substrate rigidity. In particular, it is important to note that elastic elements are present both in the extracellular environment and in the cellular structures. In fact it is very likely that internal elasticity (depicted as springs between cytoplasmic plaque and actin cytoskeleton in Fig. 4) is used by the cells to calibrate the way extracellular rigidity (depicted as springs between substrate and cell) is sensed.

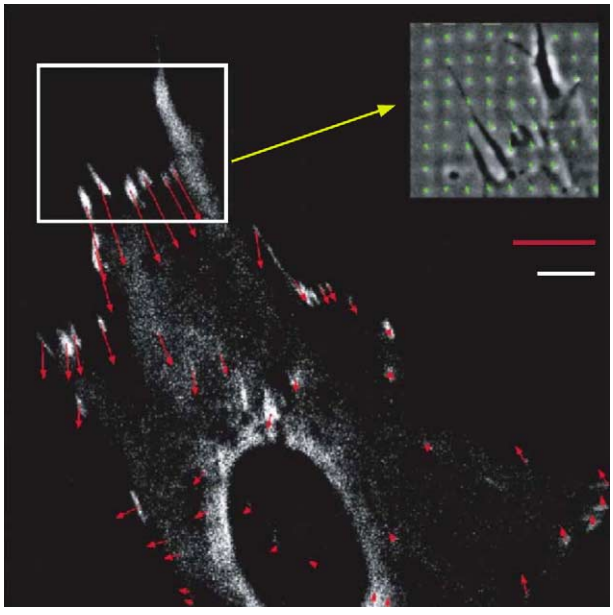

Fig. 3. Forces (red) at single focal adhesions (white, fluorescently marked by GFP-vinculin) can be calculated from the displacement of micro-patterned elastic substrates (green, inset) under cell traction [33,42]. Bars: forces (red), 30 nN; space (white), 4 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

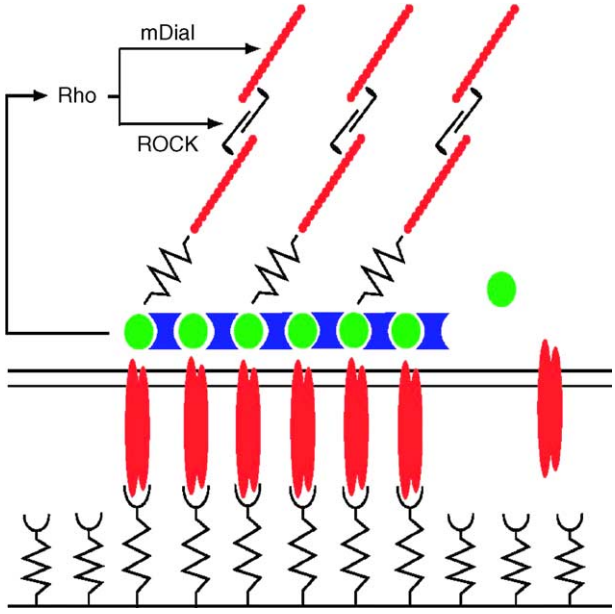

Fig. 4. Cell organization in soft media results from active mechanosensing through focal adhesions. In this schematic representation, a focal adhesion is shown which grows under force from left to right. Activation of the small GTPase Rho through integrin clustering, increased tension in the actin cytoskeleton and focal adhesion growth under force might lead to a positive feedback loop which is modulated by intra- and extracellular rigidity (depicted by springs).

One key step in the positive feedback loop at focal adhesions is the transduction of force into signalling, whose nature has not been clarified yet. From a physical point of view, there are many different processes which might be used for the mechanosensor in focal adhesions. The best known examples for mechanotransduction are sensory systems. In particular, two out of the five human senses (touch and hearing) are based on mechanotransduction. Mainly supported by mutant studies for worm and fly, it is well known that these systems are based on the mechanical opening of ion channels [56]. Although there is some evidence for the role of ion channels in mechanotransduction at cell-matrix adhesions as well [57], most evidence seems to point towards a mechanism acting inside the contacts themselves. In particular, diffusion of small ions is too fast to explain the local nature of the mechanosensitive behaviour of cell-matrix adhesions. Moreover, it has been shown that mechanosensitivity of cell-matrix adhesions remains intact even for detergent-treated cells without ion channels [58]. Several different mechanisms have been suggested for this mechanotransduction event, including protein rearrangement due to mechanical loading and conformational changes in specific molecules. Theoretical modelling indeed predicts that elastic deformations inside the focal adhesion can lead to sufficient increase in density at the leading edge of the growing contact [59,60]. This model can also account for the effects of matrix rigidity and offers a natural explanation for the stick-slip motion of fibrillar adhesions. A crucial assumption of this model, which has to be tested experimentally in the future, is that tension is applied only to the inner area of the adhesion, leading to elastic deformations of the outer area (compression at the leading edge, stretching at the trailing edge). Another appealing explanation for the mechanosensor is the notion that specific molecules (integrins or cytoplasmic plaque members) undergo conformational changes under force. For example, a recent structure determination of vinculin has shown that in solution, it exists in an autoinhibited form. Activation corresponds to a large conformational change and seems to require simultaneous binding of different partners, for example PtdIns(4,5)P2 and tensin [61]. It is easily conceivable that this activation might be facilitated by force in the protein network of the cytoplasmic plaque. Indeed a similar effect has been shown to be at work in fibrillogenesis, when fibronectin fibrils self-assembly after exposure of cryptic binding sites by force [62,63]. With a measured stress constant for focal adhesions of 5.5 nN/μm^2, force on individual molecules in mature focal adhesions might well be in the range of a few pN [33], which is known to be sufficient for mechanical opening-up of biomolecular bonds [64]. Moreover, little is known about the details of the force distribution in focal adhesions. Therefore, it might well be that force on single bonds might reach larger values due to collective rupture processes inside the contacts, which could localize force onto smaller regions [65]. In particular, bonds at the trailing edge of a focal adhesion are more likely to break due to their greater age, thereby resulting in force localization to the leading edge, where conformational changes then can lead to increased growth. At this point, detailed experimental studies (possibly involving force-sensitive fluorescence probes) are required to gain more insight into the processes inside focal adhesions.

4. Quantification and modelling

Recent years have seen a strong increase in quantitative studies of cell adhesion as a function of physical determinants in the environment. For example, quantitative analysis of cell spreading and adhesion is crucial to understand how cells react to competing cues from their environment. A recent study nicely demonstrated that ligand concentration and substrate compliance are orthogonal determinants. A systematic quantification of spreading area of VSMCs as a function of collagen density and rigidity on PAAM gels showed that cells react strongly to changes in collagen density or stiffness, but not on soft substrates, with the crossover occurring at an extracellular stiffness around 10 kPa [26]. In order to study the effect of inhomogeneous ligand presentation, microcontact printing can be used to create adhesive islands of different shapes on rigid surfaces. Pioneered by the groups of Ingber and Whitesides, this method has been used to show that adhering cells adopt the given shapes and that shape determines if cells grow and divide or if they switch on apoptosis [66,67]. The same collaboration has reported that cells on adhesive islands extend lamellipodia and filopodia preferentially from the corner regions [68,69]. Recently, microcontact printing of adhesive patterns has also been used to show that spreading cells can bridge non-adhesive regions only up to 25 μm and that mature spreading requires at least 15% area coverage by ECM-ligand, independent of the pattern geometry [70]. Combing inhomogeneous ligand presentation with elastic substrates showed that cellular traction is largest at the corner regions [71] Recently, a new techniques has been developed to control ligand presentation even on the nanometer scale [72]. It was found that cell-matrix adhesion was stabilized only if the distance between different RGD-ligands was smaller than 73 nm, possibly because larger separations do not allow for stabilization of integrin clusters. In the future, simultaneous control of ligand presentation and rigidity will lead to completely new environments for cell adhesion. Another recent development in the quantitative analysis of cell adhesion is the finding that the dynamics of cell spreading proceeds in well-defined phases, with contractile phases possibly used to explore the mechanical properties of the extracellular environment [73,74].

Quantification of experimental results also makes it possible to compare directly with biophysical modelling. As already mentioned, first attempts have been made to theoretically understand the mechanosensor at focal adhesions by calculating the potential effects of force on the internal state of focal adhesions [60,65]. In the following, we now show that one does not need to understand all details of active mechanosensing at focal adhesions in order to predict cell

organization in soft media. Anchorage-dependent cells constantly assemble and disassemble focal adhesions in order to react dynamically to changes in the mechanical properties of their environment. In particular, they often change position and orientation in response to anisotropic mechanical properties of their environment. In this context, one would like to have a predictive model, which not only would contribute to a better understanding of many physiological situations, but also would be of large practical value for application in tissue engineering. Whereas the role of contact guidance and haptotaxis in tissue organization have been theoretically addressed in coupled transport equations for cell and fiber degrees of freedom [75–77], there exists little theory for elastic effects. Recently a new model has been introduced which relates the way mechanical information is gathered at cell-matrix adhesions to effective cell behaviour in soft media [21,78].

In order to introduce the essential idea of this new model, let us consider a cell adhering to a deformable medium through several mechanosensitive contacts. Each contact experiences a different microenvironment, which for the time being we characterize by a single spring constant K. In order to understand how K feeds into the positive feedback loop at focal adhesions (compare Fig. 4), we first note that build-up of force is more efficient on stiffer substrates [21]. In detail, the cell has to invest the energy $W = F^2/2K$ in order to build up the force F. Therefore, the larger K (the stiffer the spring), the smaller W (the less energy has to be invested). The same argument can be made in dynamical terms: if the cell invested the constant power L into stretching the spring, then it took the time $t = F^2/2KL$ to build up the force F. Therefore, the larger K (the stiffer the spring), the smaller t (the faster the process of building up force). A molecular basis of this mechanism might be that stabilization of focal adhesions depends on reaching a certain threshold in force, for example the force needed to produce sufficient density changes at the contact rim [59,60] or sufficient force to expose cryptic binding sites of specific molecules within a cluster which shares force between different bonds [65]. This line of reasoning can now be turned into an extremum principle: given a choice of different spring constants K a cell can pull on, we hypothesize that those contacts will eventually determine cell organization which correspond to the largest K-value. Since the spring constant K is inversely related to the elastic energy $W = F^2/2K$, we conclude that the cell effectively tries to minimize the quantity W. Defining the extremum principle in terms of W rather than in terms of K has two advantages. First, this principle can be easily generalized to the case of adhesion to a continous environment (see below). Second, minimizing W corresponds to maximizing effective stiffness, a term which comprises not only extracellular stiffness proper, but also its stiffening due to prestrain. Indeed experiments have shown that stiffness and prestrain have the same effect on cell behaviour, namely upregulation of contacts and the cytoskeleton [28].

For adhesion to a continuous environment, for example to an elastic substrate, deformation is not localized and thus we cannot use the simple notion of an array of different spring constants K. Instead we now have to turn to continuum elasticity theory. Synthetic elastic substrates are described by isotropic linear elasticity theory, that is they are characterized by two elastic constants, for example Young's modulus E and the Poisson ratio. The situation is more complex for adhesion to the extracellular matrix, which in the future might be described by the same kind of non-linear elasticity which recently has been suggested also for cytoskeletal elasticity [79]. In the present model, however, we restrict ourselves to isotropic linear elasticity, which can be regarded as a first approximation also for the propagation of strain and stress in more complicated materials. Then the elastic energy which has to be invested by the cell into the surrounding medium is $W = (1/2) \int \mathrm{d}V\, C_{ijkl} u_{ij}\, u_{kl}$, where the integral runs over the volume filled with elastic material, C_{ijkl} is the elastic constants tensor involving E and V, and u_{ij} is the strain tensor (summation over repeated indices is implied). Regarding our extremum principle, the quantity W now has to be minimized under two constraints. First, the strain tensor has to satisfy the elastic equations in the presence of the cellular traction pattern. Here, we make the simplest assumption possible, namely that the cellular force pattern arising from actomyosin contractility can be described by a pinching force pair, that is by two forces of equal, but opposite magnitude. This model can be interpreted as one stress fiber connecting two focal adhesions and satisfies the fundamental requirement that overall force should vanish because cells are in mechanical equilibrium most of the time [80]. In condensed matter physics, such an object is called an *anisotropic force contraction dipole*. In general, the concept of force dipoles has been used before to model the elastic interactions of defects in condensed matter, for example hydrogen in metal [81]. The second constraint for minimization is that the strain tensor has to satisfy the correct boundary conditions. In particular, for elastic material of finite size, one approach is to implement free boundaries (the sample surface can deform as it likes, but normal stress has to vanish at the boundary) or another is to have clamped boundaries (displacement has to vanish at the boundary, but stress can have arbitrary values).

This model can be solved exactly for different geometries and boundary conditions of interest [21,78]. Fig. 5 schematically shows our results for some of these situations. Since contacts prefer to grow in the direction of maximal effective stiffness in their environment, for a cell close to a clamped boundary, the perpendicular orientation is most favorable (Fig. 5a), because a clamped boundary effectively corresponds to a stiff environment. Our calculations show that this effect is virtually independent of the value for the Poisson ratio. Moreover, our calculations can be used to predict in quantitative detail which differences in effective stiffness the cell senses in different positions. For a free boundary, the situation is reversed as compared to the case of a clamped boundary and the parallel orientation is most favorable (Fig. 5b), because a free surface effectively corresponds to a soft environment. Orientation due to a free surface

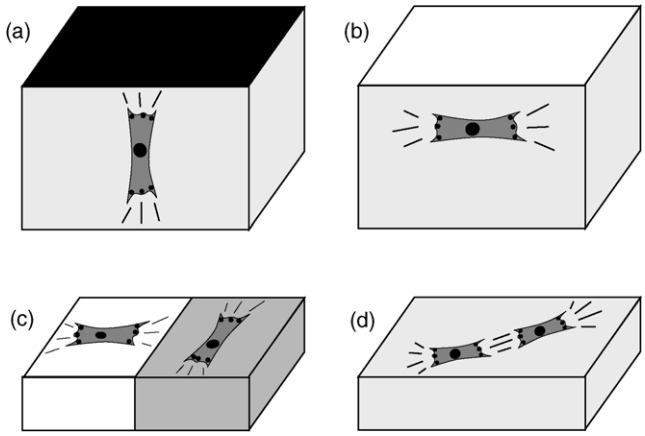

Fig. 5. Cell organization in soft media can be predicted by an extremum principle in linear elasticity theory which states that cells in soft media position and orient in such a way that they sense maximal effective stiffness in their environment. (a,b) For clamped and free boundaries, optimal orientation is perpendicular and parallel, respectively. (c) Corresponding orientation effects occur on elastic substrates. (d) Elastic interactions of cells lead to parallel alignment.

is therefore an aversion response. Experimentally, the predicted orientation response to the nature of the boundaries has been observed numerous times for mechanically active cells in hydrogels [47–49]. Regarding biomedical applications, our model predicts for example that cells close to metal implants should adopt a perpendicular orientation and that this orientation might be altered by using soft implant surfaces. Our model also explains the migration behaviour of fibroblasts on elastic substrates (Fig. 5c) where orientation on the soft and stiff regions should be perpendicular and parallel, respectively, exactly as observed experimentally [28]. Moreover, our model also makes interesting predictions for elastic interactions of cells: because polarized cells stiffen the surrounding environment along their long axis, they are expected to line up in parallel (Fig. 5d). In fact this mechanism implies a positive feedback loop involving several cells: the more cells line up in the same direction, the larger becomes the input signal for other cells to adopt the same direction. In the future, our predictions should be tested in experiments. For example, upregulation of contacts due to anisotropic mechanical cues in the environment can be investigated using arrays of flexible microneedles of different stiffness [54], while elastic interactions of cells might be tested on continuous elastic substrates.

5. Conclusion

In this paper, we have discussed recent advances in elucidating physical determinants of cell organization in soft media. Much progress has recently been made by introducing new tools from materials science into cell biology, which leads not only to improved understanding of physiological processes, but also to new perspectives for designing new environments for cells and tissues. Moreover, quantification allows the use of theoretical and computer modelling, which is essential to achieve a future systems level understanding of cell adhesion. The use of fluorescence constructs combined with data from elastic substrates, controlled ligand presentation, microfluidics, and colloidal probe spectroscopy will in the future lead to large amounts of data, which cannot be understood simply by rules of thumb, but have to be analysed in a quantitative way. For this purpose, new theoretical concepts have to be developed and to be tested against the experimental data. Therefore, an exciting new field has emerged for interdisciplinary work involving concepts from biology, physics, bioengineering, and materials science.

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