Cytoskeleton reorganization of spreading cells on micro-patterned islands: a functional model

BY Y. LOOSLI1,2,3,*, R. LUGINBUEHL3 AND J. G. SNEDEKER1,2

1Laboratory for Orthopedic Research, Department of Orthopedics, University of Zurich, Forchstrasse 340, 8008 Balgrist, Switzerland
2Institute for Biomechanics, Department of Mechanical Engineering, ETH Zurich, 8093 Zurich, Switzerland
3RMS Foundation, 2544 Bettlach, Switzerland

Predictive numerical models of cellular response to biophysical cues have emerged as a useful quantitative tool for cell biology research. Cellular experiments in silico can augment in vitro and in vivo investigations by filling gaps in what is possible to achieve through ‘wet work’. Biophysics-based numerical models can be used to verify the plausibility of mechanisms regulating tissue homeostasis derived from experiments. They can also be used to explore potential targets for therapeutic intervention. In this perspective article we introduce a single cell model developed towards the design of novel biomaterials to elicit a regenerative cellular response for the repair of diseased tissues. The model is governed by basic mechanisms of cell spreading (lamellipodial and filopodial extension, formation of cell–matrix adhesions, actin reinforcement) and is developed in the context of cellular interaction with functionalized substrates that present defined points of potential adhesion. To provide adequate context, we first review the biophysical underpinnings of the model as well as reviewing existing cell spreading models. We then present preliminary benchmarking of the model against published experiments of cell spreading on micro-patterned substrates. Initial results indicate that our mechanistic model may represent a potentially useful approach in a better understanding of cell interactions with the extracellular matrix.

Keywords: cellular modelling; spreading; stress fibres; focal adhesions; mechanotransduction

1. Introduction

Since cellular mechanisms involve processes at the molecular level that render them unobservable in live cell experiments with most analytical techniques, numerous key aspects of cell behaviour remain unknown. Biophysics-based numerical models provide a tool to simulate subcellular processes and can be used

*Author for correspondence (yloosli@ethz.ch).

One contribution of 13 to a Theme Issue ‘The virtual physiological human: computer simulation for integrative biomedicine I’.
to systematically probe mechanisms that underlie tissue and organ homeostasis or human disease (Mogilner et al. 2006). The work focuses on a subset of these mechanisms that regard interaction between cells and the extracellular matrix (ECM). While the reliance of a cell on matrix cues to guide its behaviour is by now widely appreciated, many of the basic mechanisms that govern this information exchange are yet to be elucidated.

In vivo, cells depend on the ECM to provide both biochemical cues (e.g. cytokines) and biomechanical cues (e.g. anchorage-dependent mechanical stress) to guide their behaviour. Given the wide range and complexity of potential cues that the ECM can present to a cell, a similarly wide range of scientific disciplines is required to understand cell–matrix interactions. Biochemistry dictates the reactions between cellular receptors and substrate bound ligands that enable anchorage of the cell (Hynes 1987, 2002); polymer physics underlie cytoskeletal modelling and remodelling (Peskin et al. 1993; Deng et al. 2006); biomechanical principles govern cell and matrix deformation in response to endogenous and exogenous mechanical stimuli (Chicurel et al. 1998; Addae-Mensah & Wikswo 2008; Broussard et al. 2008). This last class of cellular cues, mechanical signals, has received increasing attention as it has steadily become clear that mechanical signals play a central role not only in enabling cell behaviours like migration and mitosis, but also in integrating contextual information and triggering state changes like cellular differentiation or apoptosis. The following sections briefly outline some key aspects of mechanical forces (and force transduction) as they relate to cell behaviour.

As early as the nineteenth century, Wolff proposed that bone tissue adapts its geometrical structure according to the loads that are placed on it. One hundred years later Perren and Pawel (Perren 1979) hypothesized that cells mediate bone healing according to fluid flow and hydrostatic pressure. Bringing this theory forward, Carter et al. (1998) and Prendergast et al. (1997) proposed quantitative models that predicted progenitor cell differentiation as functions of the nature of applied mechanical stimulus. Today it is well accepted that processes of mechanical signal transduction (mechanotransduction) are critical to a wide range of specific biological responses, yet elucidation of the underlying mechanisms is still ongoing (Shieh et al. 2006; Waldman et al. 2007; Allori et al. 2008; McMahon et al. 2008).

Cells contain many proteins that are potentially involved in translating mechanical stimulation into biochemical signals that induce downstream processes. For example ‘stretch-activated ion channels’ are large transmembrane proteins that regulate ion flow and consequently intracellular ion concentration in response to applied load. The classic examples of which are the hair cells of the inner ear which transduce mechanical vibrations to a neural signal. Many less intensively studied mechanotransductive proteins are associated to adhesion sites and/or the cytoskeleton (CSK). Among other mechanisms, such proteins possess specific cryptic receptor binding sites which activate or release signalling enzymes in response to mechanical load (Vogel & Sheetz 2006).

Given that key top-level cellular behaviours like differentiation probably rely on cytoskeletal arrangement (focal adhesion (FA) and actin distribution, CSK pre-tension), identifying the rules that govern FA formation and actin distribution of the CSK is essential. The complexity of these processes is daunting, and our understanding of them is still in its infancy (Gieni & Hendzel 2008).
Nonetheless, relentless advancement in available engineering techniques and cell biology methods allow for improving our ability to postulate and test proposed mechanisms of cell behaviour (Discher et al. 2009).

Among other things, this review discusses how new methods combining molecular biology and cell imaging with techniques such as micro-fabrication and nano-patterning have allowed unprecedented insight into CSK mechanobiology. In the following sections, we will introduce and discuss one such experimental approach: ‘controlled cell spreading’ on micro-fabricated adhesive substrates. Controlled cell spreading experiments offer unique insights into the evolution of cellular adhesions and cytoskeletal elements, and with the help of models that can mimic such experiments we hope to advance our understanding of the rules that govern these central processes.

2. Biological underpinnings

(a) Spreading

Cells change their shape from a spherical to a more flattened disc-like appearance when coming into contact with a solid quasi-two-dimensional surface. This process is called spreading. As spreading cells adhere to a substrate, signalling is initiated that affects various physiological functions such as cell migration (Lauffenburger & Horwitz 1996; Woodhouse et al. 1997), morphogenesis (Gumbiner 1996), differentiation (McBeath et al. 2004), growth (Folkman & Moscona 1978) or tumour metastasis (Woodhouse et al. 1997). Initial spreading is accompanied by formation of cellular adhesions and small actin bundles that are later remodelled into mature FAs and reinforced stress fibres (SFs). These phenomena are to some extent dictated by the ‘spreading history’ or time-dependent sequence of initial adhesion formation and maturation (Zimerman et al. 2004; Théry et al. 2006). In addition to FA formation, spreading is characterized by increased area of the cell/ECM interface, which is regulated by, among other factors, the matrix or surface stiffness (Engler et al. 2004; Discher et al. 2005; Yeung et al. 2005). For instance fibroblasts adopt a more spread configuration on stiffer substrates, e.g. a 25 per cent increase in the projected surface area was observed as substrate modulus increased from 14 to 30 kPa (Lo et al. 2000), or disconnect from the matrix and enter apoptosis if the substrate is too soft (Ingber & Folkman 1988).

Maximum spreading is reached after a cell has passed through a number of intermediate spreading steps. Based on observations of fibroblasts, Döbereiner et al. (2004, 2006) and Dubin-Thaler et al. (2008) recently described three distinct spreading phases with rapid inter-phase transitions as depicted in figure 1: (i) early spreading, cells flatten until they reach a similar cross-sectional area as in its initial spherical shape, (ii) intermediate spreading, cells rapidly increase their contact surface and initiate contractile forces, and (iii) late spreading, cells optimize their surface with increased adhesion and contractile cytoskeletal pre-tension. Various theories attribute the early spreading phase to different driving mechanisms. For instance, non-specific spreading mechanisms have been proposed for which the process is dictated by force/energy imbalances between cell-substrate binding and deformation of a weakened cortical shell.
Figure 1. Schematic of the evolution of cell spreading from a non-contacting state through to post-spreading activity (here migration). (a) Early spreading: cell flattens and initiates non-specific contact. (b) Intermediate spreading: cell generates adhesions and long actin bundles are formed. (c) Late spreading: cell reinforcement through rearranging adhesions and actin bundles configuration. (d) Migration: one possible process that follows spreading.

(Cuvelier et al. 2007). Other groups have proposed that this phase is driven by active CSK remodelling with a breakdown of cortical actin that reduces cell stiffness combined with local polymerization of protruding actin filaments near the cell/ECM interface (Cai et al. 2006; Chamaraux et al. 2008). Transition to the intermediate phase of spreading has been associated with the activation of focal adhesion kinase (FAK). FAKs co-localize with APR 2/3, a protein that initiates actin meshwork polymerization and contractile forces, the engines of the characteristic cellular surface increase that is associated with this phase (Serrels et al. 2007). In the intermediate phase, cells initiate specific adhesions (particularly underneath lamellipodia and filopodia) that facilitate their own anchorage and, eventually, provide stability for the molecular motors. These motors initiate local contractile forces within the lamellipodia that are possibly powered by non-sarcomeric contraction (Verkhovsky & Borisy 1993). The late phase is characterized by a global reinforcement of the CSK and its anchors to the substrate. As will be discussed in later sections, contractile forces are apparently necessary for the recruitment of proteins crucial to the maturation of the adhesive anchors; cells recruit actin bundles to form SFs, contractile forces are generated in the SFs via molecular motors associated to actin bundles (Reinhart-King et al. 2005), and this in turn results in increased size of the anchoring adhesion. The process is driven by imbalances between the extracellular forces (e.g. ECM/substrate deformation) and intracellular forces (e.g. CSK pre-tension). It is disputed which cellular objectives rule the tuning of the balance (Ghibaudo et al. 2008): specifically whether cells seek to maintain a constant deformation or a constant tension. Regardless, experiments have shown that
cell area decreases in the absence of contractile forces (Wakatsuki et al. 2003), implying that actin polymerization alone, without contractile forces, is not sufficient to ensure spreading.

(b) Controlled cell spreading

In vivo, cells reach the late spreading phase and an eventual equilibrium with a consistent morphology and cytoskeletal organization depending on their phenotype (Gumbiner 1996). In contrast, cells spreading in vitro on material surfaces are not static even if the materials are coated homogeneously with sufficient ligands to saturate cell receptors. After termination of the final spreading phase, cells start to orient themselves by generating robust, mature

Phil. Trans. R. Soc. A (2010)
SFs which align to bring the cell into a polarized configuration (cell spatial segregation). Polarization may be subsequently followed by either migration associated with a constantly remodelling CSK that systematically alters cell morphology and drives the cell forward, or alternatively cells continue in their cycle (e.g. G1 phase), which also results in a constant reorganization of the CSK (Assoian & Klein 2008). If the substrate is not homogeneous or continuous, however, and cells are confined to micro-patterned islands (from 600 to 1400 μm²), they adopt reproducible shapes and yield a ‘steady state’ behaviour (Singhvi et al. 1994). Controlled spreading provides the ability to systematically and reproducibly characterize CSK evolution at discrete time points, and micro-patterned surfaces have thus been used to study attainment of cell morphology and the basic mechanisms of CSK organization (Kevin Parker et al. 2002; Chen et al. 2003; Cuvelier et al. 2003). Théry et al. (2006) further advanced this principle by strategic investigation of SF and FA configuration using human epithelial cells spreading on concave and convex curvatures. They engineered geometries with thin adhesive areas in T, U, Y or V shapes as depicted in figure 2. After staining actin and vinculin (adhesion proteins), they observed a common behaviour of cellular FA distribution and SF orientation: the highest vinculin concentrations were found along the periphery of adhesive areas, and actin bundles were concentrated along non-adherent edges of concave-shaped areas. These useful results have since been used in recent modelling studies and these are later discussed in more detail.

(c) Cellular adhesion and FA plaques

Cell adhesion molecules (CAMs) are central to mechanotransduction, connecting the internal cell skeleton to the cell surroundings. Most CAMs are transmembranous proteins and belong to the immunoglobulin superfamily, integrins, cadherins, lectin-like CAMs or homing-like receptors (Hynes 1987, 2002; Albelda & Buck 1990). Integrins act as receptors to ECM ligands (collagen, fibronectin, laminin, etc.) on the extracellular side, and on the cytoplasmic side they interact with numerous proteins (paxillin, actinin, talin, etc.) that in turn bind directly or indirectly to the CSK (Zaidel-Bar et al. 2007). The adhesion process is a highly dynamic assembly that typically evolves from a single point of adhesion into a large mature adhesion area by sequentially recruiting proteins. How cells orchestrate this process is only partially understood. For example, Talin is an actin-binding protein that is part of this process and that is known to be important with regard to mechanotransduction. Talin attaches to the cytoplasmic domains of integrins that recruit other proteins such as paxillin, actinin, tensin and zyxin (Giannone et al. 2003). In tension, talin also changes its conformation exposing a binding site for vinculin, which induces the reorganization of the CSK (Del Rio et al. 2009). Such changes in molecular conformational are enabled through cell contractility mainly powered by the actin machinery.

The bridging of integrins and the actin meshwork is a key step in promoting FA maturation (Frame & Norman 2008) with tensile force at the FA being well known to increase FA size to maintain a constant stress at the adhesion (5.5 nN μm⁻²; Balaban et al. 2001). Prior to maturation, nascent adhesions (focal complexes) with length smaller than 1 μm are generally localized in the membrane underneath filopodia and lamellipodia (actin-based membrane protrusions that

Phil. Trans. R. Soc. A (2010)
Cytoskeleton reorganization of spreading cells

will be discussed later in more detail). The focal complexes located beneath lamellipodia have a limited lifetime (of the order of minutes, the time required by the lamellipodium to move forward), while those in filopodia have higher likelihood of evolving into longer-lived mature FAs (Zaidel-Bar et al. 2003; Schäfer et al. 2009). Focal complexes are made of hundreds of molecular components linked together by many more interconnections (Zaidel-Bar et al. 2007). They can reach lengths of 10 μm, and are generally positioned at the inner rim of lamellipodia to provide anchorage to contractile SFs (Nobes & Hall 1995; Zamir & Geiger 2001; Goffin et al. 2006; Schäfer et al. 2009). FAs are active assemblies; they not only ensure the mechanical link between the CSK and the ECM, but they also sense and translate local mechanical stimuli (Gumbiner 1996; Giancotti & Ruoslahti 1999; Hynes 2002). Such pathways regulate crosstalk between FA and actin mediated signalling and are important to mechanotransduction (Chen 2008; Geiger et al. 2009).

(d) Actin machinery

Actin-based elements are central to cellular motility and affect both intra- and intercellular processes. Rheological properties of the whole cell are regulated by tuning intracellular tension of actin bundles (Trepat et al. 2007) and the stiffness of the actin cortex (Van Citters et al. 2006). On the intercellular level, actin is involved in processes from embryonic development, to tissue organization and remodelling, to apoptosis (Burridge & Chrzanowska-Wodnicka 1996; Pollard & Borisy 2003).

Actin is present in cells in its globular form (G-actin) and in its filamentous form (F-actin). It is assumed that G-actin generates protrusive forces when it polymerizes against the membrane and that these forces deform the lipid layer (i.e. the ‘ratchet’ mechanism; Peskin et al. 1993; Pollard & Borisy 2003). Polymerization occurs, among other places, at the leading edge of the cell (section of the membrane undergoing directed protrusion). Here G-actin is polymerized at the growing tip of an actin filament while depolymerization occurs at the opposite end of the filament that is embedded within a dense actin meshwork (lamellipodia). The availability of G-actin for new filament formation at the leading edge is ensured by the so-called process of ‘treadmilling’ in which depolymerizing F-actin feeds a monomer flow from the rear of the lamellipodia towards the front of the cell where the filament grows (Small et al. 1993).

(i) Lamellipodia and filopodia

Lamellipodia are wide flat protrusions (1–5 μm breadth and only 0.2 μm thickness) formed by an actin network, whereas filopodia are finger-like extensions of the membrane powered by tight, parallel F-actin bundles with a width of 0.1–0.3 μm and lengths up to 10 μm (Small et al. 2002; Mattila & Lappalainen 2008). Two potential (and complementary) mechanisms underlie formation of filopodia, which are used by the cell to explore the environment (Wood & Martin 2002; Gupton & Gertler 2007). The first one is based on filopodia being issued from the lamellipodium whereas in the second filopodia generally nucleate at tips of previous entities (Mattila & Lappalainen 2008). If mechanical and chemical properties of ECM or a solid surface are satisfactory, focal complexes form near the tip of a filopodium (Schäfer et al. 2009). Once a lamellipodium has
reached these nascent adhesions, they generally mature into stable FAs (Schäfer et al. 2009). Thus the purpose of such filopodia is to guide and anchor the lamellipodia as they advance. If a filopodium is not stabilized by FAs it will eventually buckle and fold laterally to form actin bundle contractile bridges (Nemethova et al. 2008). These bundles, or so-called transverse arcs, are not anchored in the ECM and are either moved towards the cell centre before eventual depolymerization or alternatively can be involved in the formation of mature SFs if the cell sends an appropriate polarization signal (Hotulainen & Lappalainen 2006; Senju & Miyata 2009).

In contrast to filopodia, lamellipodia occupy a zone spanning only a few micrometres behind the leading edge, where most F-actin is polymerized into a dense meshwork stabilized by other molecules (Arp 2/3, α-actinin and filamin; Small et al. 2002). This network provides the required anchoring to resist protrusive forces that allow a quasi-continuous extension of the leading edge.

(ii) Stress fibres

SFs are the third major actin-based component in addition to the actin-mesh powered lamellipodia and the protruding actin-bundle filopodia (Ridley et al. 2003). SFs are large bundles of 10–30 actin filaments held together by α-actinin and cross-linked by myosin, which uses ATP as an energy supply to move directionally on the SFs to induce sliding between actin filaments (Pellegrin & Mellor 2007). This motion results in filament length changes that in turn generate contractile force on SFs with anchored extremities. SFs are generally divided into three groups (Hotulainen & Lappalainen 2006; Naumanen et al. 2008): (i) SFs that are connected at both extremities to FAs—these are known as ventral SFs (vSFs) and are essential to cell contractility; (ii) dorsal SFs (dSFs) are linked to a transverse arc at one end and to an FA at the other; and (iii) transverse SFs (tSFs) are located under lamellipodia and connected to FAs via dorsal SFs. Senju & Miyata (2009) proposed that interplay between dSFs and transverse arcs is at the origin of vSFs. The common action of vSF and actin myosin exerts contractile forces that have been measured in the range of 25–50 nN for fibroblasts and 150 nN for myofibroblasts (Balaban et al. 2001; Goffin et al. 2006). A recent study by Deguchi et al. (2006) investigated the mechanical properties of isolated single SFs and described a maximum tensile strain of 2 per cent (±0.6%), an averaged ultimate force of 380 nN (±210 nN), and an elastic modulus of 1.45 MPa. Furthermore, they observed that vSFs are subjected to a substantial pre-tension with lengths decreasing to 83 ± 11 per cent of the initial length after detachment of one end. However, more recent in vivo investigation of SFs estimated elastic moduli to be substantially lower (230 kPa) than the isolated fibres (Lu et al. 2008). The large difference in these reported values is typical and indicates the difficulty in measuring mechanical properties of biopolymers. Therefore, such results must be considered with due caution.

(iii) Limitations of current experimental methods

While observations from experimental cell biology and biophysics can offer valuable insight into fundamental cellular processes, modern experimental setups allow ‘top-level’ behaviours to be observed but often leave the underlying driving
principles and mechanisms to only be hypothesized. As a tool for testing the validity of such hypotheses numerical models can play an important role in understanding and explaining experimental outcomes. The following sections focus on mechanically based single cell models to describe cell spreading and cytoskeletal reinforcement.

3. Single cell spreading models

Many numerical (and/or theoretical) models of single cell rheology have been developed to describe the constitutive behaviour of cells regarding their ‘flow’ in response to applied boundary conditions. For instance in the finite element model of Guilak & Mow (2000), the cell was represented as a biphasic, two-dimensional structure. Other rheological models have incorporated quasi-dynamic processes in an attempt to capture aspects of living cells (Ingber 1997; Trepat et al. 2007). These models are useful in describing the passive/pseudo-active rheological behaviour of cells, and have been thoroughly reviewed elsewhere (Lim et al. 2006; Stamenović 2008; Vaziri & Gopinath 2008). The present article rather focuses on models that have been introduced to represent initial cell/substrate interaction and cytoskeletal organization which is likely to be a key function of downstream cell behaviours. First, we introduce cell spreading kinetics models, where the cell surface is described as a function of time. We then examine biochemical–biomechanical models of cytoskeletal architecture and reinforcement in response to mechanical stress induced by cell contractility. Finally, we present our own functional model of cell spreading, which is based on biophysical rules governing actin dynamics and FA modelling/remodelling.

(a) Kinetics of spreading

Three decades ago, a first attempt to describe cell spreading was performed by modelling time evolution of cell area (Bardsley & Aplin 1983). Bardsley and Aplin tried to relate the spreading dynamics of a cell population to the laws of chemical kinetics but without notable success. Twenty years later, Frisch & Thoumine (2002) compared initial cell spreading with surface wetting of liquid drops. Such phenomena were already extensively investigated and simulated (Tanner 1979; Leger & Joanny 1992) incorporating the critical parameters of liquid viscosity, surface tension and substrate adhesion. Considering cells as viscous droplets surrounded by a membrane under tension and neglecting a nuclear contribution, Frisch and Thoumine computed cell radius as a function of time and compared their predictions with experimental data. Comparison showed an underestimation of spreading velocity at early time points (up to 1 h) followed by an overestimation of the velocity in later stages. The authors attempted to model the whole spreading process with a single set of equations, despite the markedly different mechanisms involved in each spreading step. Using a similar model (a membrane-bound viscous cortical shell and membrane that encloses a fluid cytoplasm) but opting for a direct computation of the contact diameter (circular contact assumption), Cuvelier et al. (2007) predicted...
initial spreading by balancing adhesive energy with viscous dissipation. They further proposed two mechanisms of viscous dissipation, depending upon the stage of the spreading phase. Early damping that determines the kinetics of cell spreading is attributed to viscous flow in the cortical shell. Later, whole cell rheology dominates dissipation (Cuvelier et al. 2007). Common to all these passive paradigms the main parameters that regulate spreading are the properties of the cortical actin (thickness and cortical-shell viscosity) with actin (de)polymerization being assumed to have a negligible effect on both the spreading kinetics and adhesive energy. Treating actin processes to be time independent is perhaps an oversimplification since it is known that adhesion initiation triggers a breakdown of the cortical actin and cortical structures (Döbereiner et al. 2005). Despite this simplification, the overall agreement of the model with experimental data is fairly good, and makes possible an estimation of initial spreading kinetics with a simple model at extremely low computational cost. The Cuvelier model focuses mainly on the initial phase of spreading and claims to demonstrate that substrate adhesion starts with a non-specific binding phase that is dominated neither by actin polymerization nor diffusion of adhesive receptor through the membrane. Thus, it is not dependent on either cell type or substrate (as long as non-specific binding is possible).

Although modelling a cell as a passive entity that lacks both actin activity and specific binding can successfully mimic initial spreading of isotropic cells, these assumptions seem to be inappropriate for later isotropic spreading phase or anisotropic cell spreading. Chamaraux et al. (2005, 2008) focused on ameobian cells with podosome-like adhesions (blebs extensions) at the leading edge that result in an anisotropic spreading process. Such membrane protrusions are different from either filopodia or lamellipodia extensions. Blebs extensions are created by local weakening of the connection between the lipid membrane and the actin cortex. This weakening allows for formation of bubble-like protrusions driven by intracellular pressure (Boulbitch et al. 2000; Paluch et al. 2005, 2006; Yoshida & Soldati 2006). This phenomenon is essential in the Chamaraux spreading model since it assumes that intracellular pressure pushes the membrane onto the substrate, leading to novel adhesion regions that in turn trigger polymerization of basal F-actin structures connected to the membrane. This model was proposed to simulate quite specialized cells exhibiting a pseudo-pod, and is therefore not suited for cells that spread via lamellipodia. Further, actin activity differs between these spreading mechanisms: in blebbing cells, actin polymerization occurs underneath the cell body to support newly covered regions, whereas lamellipodial spreading actin polymerization acts rather to deform the membrane (Pantaloni et al. 2001).

Thus without considering actin dynamics, several different models of cell spreading kinetics have been able to predict spreading area as a function of time. Nonetheless spreading is not only characterized by surface increase. The CSK reorganizes constantly during spreading, even in the final stage (reinforcement) when cell surface area is relatively stable. Hence such models offer limited insight into the numerous signalling pathways that are related to cellular morphological changes and the associated force exchange between the cell and its substrate (Stamenović & Ingber 2002; Chen et al. 2003; Chen 2008; Stamenović 2008).
(b) Cell reinforcement models

Cells reinforce their CSK in the last spreading phase by dynamically rearranging SFs and FAs (Chen et al. 2003; Bershadsky et al. 2006; Hirata et al. 2007). This phase has important implications for mechanotransduction, and can precede key changes in cellular state (e.g. migration, differentiation). Hence being able to understand how cells regulate this process is highly relevant (Chen 2008). Based on earlier works (Deshpande et al. 2006, 2008), Pathak et al. (2008) developed a two-dimensional model driven by a coupling of biochemical rules (signal propagation and integrin diffusion) and biomechanical rules (SF contractility) that yielded realistic predictions of previously published distributions of SFs and FAs following reinforcement of cells spread on micro-patterned islands (Théry et al. 2006). The similarities between simulation and experiments (averaged actin/myosin cartography) were striking: FAs at island corners were faithfully reproduced, as was the presence of high density SF at non-adherent edges. In contrast to experimental data, predicted adhesions were distributed nearly homogeneously along the adhesive edges. Using a similar modelling approach, SF distribution in response to a superimposed substrate strain has also been simulated (Wei et al. 2008). Since both models were based on a continuum approach, investigation of subcellular evolution of single SFs or FAs (formation, growth, fusion, etc.) was not possible. Furthermore, the models employed deterministic methods that resulted in symmetrical SF and adhesion distributions that do not faithfully represent living cells which are driven by non-deterministic processes. Finally, these models focused only on reinforcement, and did not account for initial phases of spreading and the potential role that spreading history may have on end-stage CSK arrangement.

(c) Discrete spreading model using divided medium

The divided medium strategy is based upon modelling structures as discrete ‘grains’ whose interactions are simulated using springs (Jean 1999). Divided medium models have been proposed to investigate the dynamic organization of the CSK in response to stimuli (Milan et al. 2007), and have been employed to model active spreading in three dimensions (Maurin et al. 2008). Maurin and co-workers modelled the whole cell with three different types of grains (membrane, cytosol and nucleus), which were interconnected by springs with stiffness set for each CSK component. Some membrane peripheral grains were assigned adhesive capacities to mimic integrin activity. The authors proposed that polymerization of microtubules (relatively high bending stiffness CSK elements) drives spreading and that actin filaments are only involved in maintaining structural integrity (i.e. the tensegrity theory; Ingber 1991). Using this model, predicted three-dimensional shape evolution was confirmed by experimental data, despite the debatable physiological mechanism of microtubule powered spreading. While microtubules have been described to bear contractile loads (Stamenović et al. 2002) and to influence adhesion and actin filament activity (Palazzo & Gundersen 2002; Deschesnes et al. 2007), there appears to be little support in the literature for the mechanisms hypothesized by Maurin (Maurin et al. 2008). Therefore model predictions must be considered with caution, though the methods used are interesting regarding their potential for incorporating the concept of cellular tensegrity within simulation of CSK remodelling.
4. A novel predictive model of CSK reorganization

Based on the shortcomings of the models mentioned above, we present here a novel approach for simulating CSK evolution over the course of spreading. Our model was developed to provide a prediction of post-spreading FA and SF distribution. The FA/SF architecture is necessary input for eventual computation of the force balance between the CSK and the ECM (not addressed in the present work). This force balance could later be implemented as feedback for guiding post-spreading reorganization of the CSK, for instance to gain insight into substrate-dependent CSK reinforcement. Force balance mediated at the FAs is an important vector for transduction of mechanical stimuli to the cell (Chen 2008) with critical downstream consequences for top level processes (e.g. apoptosis (Ingber & Folkman 1988), durotaxis (Lo et al. 2000b; Lazopoulos & Stamenović 2008), differentiation (Engler et al. 2006), etc.). For the modeller, predicting the consequences of tensile forces acting at FAs requires one to quantify not only tension of each individual SF but their directions as well. Thus an accurate prediction of CSK architecture is critical to this goal and is delivered as output by the algorithm described below. Since spreading history appears to be an essential factor in end-stage CSK configuration, we included a sequential, rule-based functional model that is capable of predicting CSK and adhesion formation and remodelling in spreading cells. The next sections describe the modelling concept and compare first results against experimental observations of spreading cells on micro-patterned islands.

(a) Algorithm description

We propose an iterative spreading algorithm that is conceptually illustrated in figure 3 and described as pseudo-code in figure 4. This CSK reorganization paradigm is driven by two weakly coupled processes that occur in parallel: continuous lamellipodial extension and local filopodial protrusion. These membrane activities are both thought to rule CSK and adhesion reorganization during the active spreading phase, with lamellipodial extension believed to be the dominant spreading mechanism (Small et al. 2002). Lamellipodial extension is the result of actin meshwork polymerization that continually pushes the membrane outward. The model adopts leading edge protrusion velocity distributions described from prior experimental observations (Dubin-Thaler et al. 2008), with an iteratively stochastic advance/retraction (mean $+1.7\mu m$; $\sigma = 1\mu m$) of the leading edge perimeter in a normal direction. In our model, the lamellipodium is defined as the surface encompassing the substrate area bounded by the leading edge at two consecutive iterations. Nascent (unstable) adhesions are generated beneath this area if there is a presence of binding ligand (Zaidel-Bar et al. 2003; Choi et al. 2008).

The main function of the algorithm is to distribute nascent FAs and determine which of these (and their associated actin filaments) should be selected for maturation/reinforcement. The algorithm currently considers three mechanisms for FA maturation: (i) lamellipodia retraction occurs that would otherwise leave a nascent adhesion outside the cell body (Zaidel-Bar et al. 2003), (ii) membrane tension spanning two FAs exceeds a certain force threshold...
(Balaban et al. 2001; Bischofs et al. 2009), and (iii) the cell leading edge advances until it encompasses a nascent FA at the protruding tip of an existing filopodium (Schäfer et al. 2009).

FA maturation induced by lamellipodial retraction has been described as a force independent process (Zaidel-Bar et al. 2003). While experimentally well characterized, it is to date not well understood. In contrast, the separate mechanism of tension induced adhesion maturation is clearly force regulated, inherently involving actin SFs that are recruited to the FA. Here, bending SFs support the membrane and this is balanced by actomyosin contractility at the anchoring FAs (Bischofs et al. 2009). This mechanism is modelled in the present algorithm by assuming that membrane tension is uniformly distributed along transverse SFs that span neighbouring FAs at the cell leading edge. The algorithm uses an elastic hull technique to define the outer perimeter of the FA point cloud, and interconnects neighbouring adhesions on this perimeter by line segments of defined length. When the spanning distance exceeds a certain length (described below in more detail), the tension of the SF is sufficient to induce maturation of the FA and reinforcement of the SF.

Thus, nascent adhesions at the perimeter of the cell leading edge support the membrane through bridging actin bundles. Such actin bundles have been experimentally observed to form by diverse mechanisms including...
membrane | $[n \times 2]$ array with location $(x,y)$ of all “$n$” adhesions defining the membrane perimeter; arranged into correct order (consecutive adhesion)

FX | $[n \times 2]$ array with location $(x,y)$ of all “$n$” unstable (immature) adhesions

newPotentialFX | $[n \times 2]$ array with location $(x,y)$ of “$n$” newly created (and inherently unstable) adhesions formed beneath the lamellipodia within the current iteration

newFX | $[n \times 2]$ array with location $(x,y)$ of the “$n$” newly created (and inherently unstable) adhesions underneath the lamellipodia in this iteration that fall on an adhesive region of the substrate

FA | $[n \times 2]$ array with location $(x,y)$ of all “$n$” mature adhesions

newFA | $[n \times 2]$ array with location $(x,y)$ of all “$n$” mature adhesions created in this iteration

SF | $[n \times 4]$ array with location $(x1,y1,x2,y2)$ of the mature adhesions interconnected by the “$n$” stress fibres

stableFilopodia | $[n \times 4]$ array of “$n$” stable filopodia with endpoint (origin, tip) locations $(x1,y1,x2,y2)$. stableFilopodia are associated with a mature FA

unstableFilopodia | $[n \times 4]$ array of “$n$” unstable filopodia with endpoint (origin, tip) locations $(x1,y1,x2,y2)$

cellArea | Scalar corresponding to the cell area

cellAreaVariation | Scalar corresponding to the iterative change in substrate area covered by the cell

Figure 4. Pseudo-code describing the predictive model of cytoskeleton reorganization. Note that italicized words proceeded by ‘%’ represent comments, and that boldface type is used to represent variables.

Phil. Trans. R. Soc. A (2010)
Cytoskeleton reorganization of spreading cells

Filopodial buckling (Nemethova et al. 2008) and non-sarcomeric actomyosin contraction (Verkhovsky & Borisy 1993). As mentioned above, these supporting SFs are tensioned resulting in loading on their anchoring adhesions, for which the maturation process is force regulated. The present algorithm implements force-regulated FA maturation based on experimental evidence that membrane bridges spanning more than 5 μm between adhesions require counterbalancing actin-bundle tension that falls above a threshold (more than 10 nN) sufficient to induce FA maturation (Galbraith et al. 2002; Bershadsky et al. 2006). This force threshold is determined by the interrelationships between membrane tension, actin-mysosin contractility, and the FA size required to anchor them (reaching areas larger than 1 μm²; Balaban et al. 2001; Bischofs et al. 2009). In the current model, we thus implemented an actin bridge length threshold of 5 μm beyond which the actin-bundle tension was assumed to be large enough to trigger fibre maturation, making the actin bundle persistent, and fixing it along with its associated adhesion (which was previously considered to be nascent or ‘unstable’).

Finally, the lamellipodia protrusions are complemented by secondary filopodial activity. Based on experimental observations, we modelled filopodium formation as a stochastic process with ‘stable filopodia’ being defined both as those formed close (less than 0.5 μm) to a mature FA or those extending an already existing (stable) filopodium (Nemethova et al. 2008; Schäfer et al. 2009). Filopodia outwardly protrude (mean +10 μm; σ = 2 μm) from the cell body and generate a non-mature adhesion at their tip. When these nascent filopodial-formed adhesions are later overtaken by the advancing leading edge of the cell, they then mature into stable adhesions. Additionally, an actin filament is formed that connects the anchoring adhesions at the filopodium origin and tip (Schäfer et al. 2009). Finally, a subsequent filopodium is then nucleated from the now stable adhesion at the tip of the previous filopodium. The process is illustrated schematically in figure 3.

To summarize, the algorithm predicts cell morphology, the FA distribution and SF layout based on an iterative process. This process involves force balance between the ‘elastic’ convex hull of the membrane and the reciprocal recruitment of SFs and FAs to support the hull. Although there is no explicit force computation in the present form of the algorithm, force balance is indirectly incorporated; it is assumed that any segment of the membrane perimeter that is supported by FAs spaced greater than 5 μm will require a counterbalancing SF tension above a biophysical threshold that initiates maturation of the anchoring adhesions. The algorithm also involves spatial interactions between the advancing front of the cell leading edge and the filopodial extensions that protrude from the actin meshwork. The model is deemed to reach a steady state (convergence) when the cell area remains constant over 10 iterations or if the cell area has reached an imposed physical limitation (here set to 1200 μm²). The described algorithm was implemented in MATLAB R2009 (Mathworks Inc., Natwick, MA, USA) according to the pseudo-code presented in figure 4.

(b) Initial comparison between in vitro and in silico experiments

As mentioned above, the proposed paradigm mainly focuses on cell shape, adhesion formation and maturation, and the resultant SF/FA architecture at the end-stage of the spreading process. To evaluate the predictive ability of the
model, we employed published controlled spreading experiments of epithelial cells seeded on micro-patterned substrates (Théry et al. 2006). This dataset has been used by other authors for similar purposes (Pathak et al. 2008). In the original experiments, constraining the cell to an island forced the cells to a steady state after spreading, and the cells were then stained to reveal adhesive proteins (vinculin) and actin. As in the experiments, we defined geometrical ligand covered surfaces (Y- and V-shaped island) and used them as input to the model (figure 5a,d). The model was then assessed for its ability to faithfully reproduce the experimental arrangement of actin bundles and FAs.

Visual comparison of the model against the benchmark data revealed a close similarity in CSK architecture (figure 5b,c,e,f). Bundles running along non-adhesive regions did not cross each other. Distribution and clustering of adhesions was also similar, with large adhesions predicted to reside at the corners where large fibres are supported. Small discontinuous adhesions were successfully predicted to form along external edges (green arrows). Highly oriented adhesions
on the internal edges were also successfully predicted (blue arrows). The algorithm did fail, however, to fully predict the preference for sparse adhesion formation at curved regions of the geometries, indicating that this process is driven by other mechanisms that are not yet included in the algorithm (i.e. enhanced lamellipodial activity in convex regions (James et al. 2008)). Lacking explicit consideration of membrane induced bending of SFs, the simulated cell morphologies also did not capture the curvature of the large actin bundles spanning non-adhesive regions of the substrate (white arrows in figure 5).

5. Discussion

We described a novel model of cell spreading driven by top-level rules that are based upon formation of FAs and SF reinforcement dynamics. The model can apparently mimic cellular spreading (cytoskeletal organization of SFs and FAs) on different adhesive shapes, and is based on a limited number of biophysical parameters that have been experimentally determined. A perfect likeness between experiment and simulation was not expected nor observed given the stochastic modelling approach that was employed to model lamellipodial and filopodial movements (to simulate these similarly stochastic cellular processes). Further, the model seems to confirm that the end-stage cytoskeletal configuration reflects earlier cell spreading history, as indicated by the non-intersecting actin filament lattice that spans non-adhesive regions. Although the preliminary simulations presented here do indicate the potential promise of this approach for modelling cell spreading, the model is considered to be a prototype. Accordingly, there are limitations that must be acknowledged.

First, the impact of filopodial spreading was limited by the relatively small dimensions of the confining adhesive islands. To more effectively assess the effects of filopodial activity (which should have greatest impact in spanning distances longer than 5μm), larger dimension test substrates will have to be simulated and compared with experimental results. Experiments and simulations are currently in progress in this regard. The model also contains a greatly simplified force balance between membrane tension and the supporting SFs and adhesions. Later versions of the model will explicitly incorporate this force balance, as well as the consequences for morphology (curvature) of the cell membrane. Finally, the preliminary model we present must be tested with regard to sensitivity of the model output as a function of the biophysical parameters that drive its behaviour. To this end, quantitative metrics for describing FA and SF location as a function of substrate topology must be developed. This work is also ongoing.

6. Conclusion and outlook for integrated multi-scale simulations

In the present work we have described cell spreading, and introduced selected models that have been developed to replicate it. Our own model was built on biophysical mechanisms as interpreted from recent experimental evidence. In contrast to some other recent cell spreading modelling approaches, our model
is based on a limited number of functional rules (such as the threshold at which an FA becomes stable, and actin bundles thus become anchored) and takes into account active subcellular processes (FA and SF maturation). While the model development is still in its preliminary stages, we view the ability of the algorithm to successfully replicate CSK arrangement (size and orientation of the actin network) as a crucial first step to providing input to later models. These models will include an internal force balance of the cell and computation of the resultant force acting at individual FAs. This will enable more detailed investigation of FA mediated mechanotransduction, and eventually implementation of the model within a multi-scale framework to predict cell–biomaterial interactions.

Models use simplifying assumptions to make a problem tractable, often with as few variables as possible. These simplifications can be made in the interest of intuitiveness, or computational expense. However, they are often made because the underlying mechanisms defy our comprehension. This forces one to make a reasonable ‘black box’ (phenomenological) approximation of the relationships between stimulus and response. Predictive models of tissue evolution (modelling and remodelling in growth, disease and repair) have been no exception, with many approximations attempting to link global tissue or organ response to chemical, electrical and mechanical stimuli at the cellular level (McNamara et al. 1992; Lacroix & Prendergast 2002; Isaksson et al. 2008, 2009; Checa & Prendergast 2009). In contrast, the model we have described lays a foundation for more mechanistic modelling of cell response to mechanical stimuli—by explicitly representing subcellular coupling between the single cell CSK and the ECM. We believe this added complexity is necessary to understand (and eventually simulate) the nature of cell–biomaterial interaction that determines the success or failure of a therapeutic biomaterial in a healing environment. It must be noted that our single cell model itself employs simplifying phenomenological rules to mimic subcellular behaviours like the formation and maturation of FAs. While these rules are predicated on experimental evidence and yield plausible predictions of cell-level behaviour, there is a possibility to model the molecular level processes as well (e.g. implementing a ratchet polymerization model for membrane protrusion). In any case, the degree of model complexity one invokes need only match the complexity required to achieve a robust and relevant predictive output.

The model we propose thus provides a framework for scalability in either metric direction with expansion of our single cell model into three dimensions and integration within multi-scale models of tissue differentiation that link macro-scale stimulus to cell/molecular mediated tissue level changes. In doing so, it might enable insight into the driving factors behind these processes, and for ultimately predicting the performance of engineered biomaterials.

References


Cytoskeleton reorganization of spreading cells


**Phil. Trans. R. Soc. A** (2010)
Cytoskeleton reorganization of spreading cells


