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Review

# Cell Form and Function: Interpreting and Controlling the Shape of Adherent Cells

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**Beautiful images of animal cells cultured on surfaces are ubiquitous in biological research, but these shapes also carry valuable information about the cells and the organism that they came from. Cell morphology is an emergent property of the cellular phenotype as well as of the physiological and signaling state of the cell. Many functional changes in cells cause stereotypical changes in cellular morphology, and some changes in shape can also cause characteristic changes in cellular phenotype. Thus, controlling cell shape through substrate engineering may emerge as another mechanism to modulate cell function for human health. This review summarizes current understanding of the morphology–phenotype connection, and surveys progress in the effort to interpret and control cell morphology.**

## Highlights

Cell shape and morphology can provide a complex readout of cell state or phenotype.

Some changes in shape can change cell state through mechanotransduction and nuclear shape changes.

Morphological analysis may be useful to screen cancer cell populations and stem cells.

Patterned substrates and hydrogels can be used to modulate cell function by controlling cell shape.

## The Relevance of Cell Morphology

Scientists and laypersons alike are fascinated by biological form. Life abounds with examples: the intricate patterns of a leaf vein, the geometric arrangement of sepals and petals in a flower, and the bizarre universe of bacterial shapes, to mention only a few. Scientists have always wondered how these shapes arose and whether they performed a function. A little over a century ago, D'Arcy Wentworth Thompson published his famous book, *On Growth and Form*, where he argued that physical and mathematical laws are needed to explain biological forms. While D'Arcy Thompson was perhaps the first to postulate that cell shape has a physical and mechanical basis, shape was being used as a diagnostic marker for disease even earlier. As far back as the late 19th century, aberrant cell shapes found in smears of bodily fluids and tissue samples were recognized as a marker for many types of disease, leading to the establishment of the field of diagnostic cytology [1]. By the middle of the 20th century, aberrant cell and nuclear shapes in samples obtained from tumors using fine-needle aspiration were being used to diagnose cancer. However, how cells acquired their characteristic shapes, and the relation between shape and function, was not well understood. The study of cell morphology received another boost at the turn of the century when cell shape was discovered to affect cell division and stem cell differentiation, and biophysical mechanisms for shape determination began to be formulated (Box 1).

In the past 5 or 6 years the study of cell morphology has matured, driven on one hand by the interest in morphological screening using imaging, and on the other by several groundbreaking studies that provided substantive correlative evidence that cell morphology is sensitive to specific cellular changes in a reproducible way. Technological developments, especially in the field of nanopatterning and hydrogels, have also developed to an extent that it is now possible to talk about controlling cell shape rather than merely interpreting it. Finally, several experimental and theoretical advances have provided clues and insights into the mechanism by which cell shape affects, and is affected by, cellular phenotype. Cell shape is now understood to be an

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### Box 1. Seminal Studies of Cell Shape

The first biophysical model for cell shape determination was probably that put forward by Donald Ingber and coworkers, based on the tensegrity hypothesis, inspired by the visionary architect Buckminster Fuller. According to this hypothesis, the shape of a cell is determined by a mechanical balance arising from contractile actin forces acting on a rigid tensed scaffolding formed by the microtubules [87]. Early models were not built using a computer, but using elastic cords and poles, and they helped to show that an unsupported tensegrity structure is spherical, like cells in suspension, because of stress minimization. Adhesion to a stiff substrate leads to spontaneous flattening, and adhesion to a malleable substrate deforms it [88]. Proteins in tensegrity structures may have altered thermodynamic properties under stress, and mechanical forces could be transmitted directly from the cell membrane to the nucleus via intermediate filaments [12,89].

Cell shape has been invoked to explain contact inhibition of growth [90] because crowded cells are more compact. Shape is also involved in anchorage-dependence of cell growth because constraining adhesion led to spherical cells with lower rates of DNA synthesis [91], corresponding to lower total spread area [88]. Observations of nuclear flattening in proliferating lens epithelial cells, in contrast to the rounded nuclei of non-proliferating cells [92], suggested presciently that nuclear shape may play a causal role.

The development of microcontact printing of adhesive proteins on micron-sized islands was a major advance, allowing direct manipulation of cell spread area, and shape [93]. Cell spreading could be controlled by culturing cells on various-sized micron-scale islands, which affected both cell proliferation [94] and cell differentiation [95]. By culturing MSCs on fibronectin 'islands' of different sizes in mixed differentiation media, a seminal paper reported that adipogenesis occurred only on small islands, osteogenesis on large islands, and intermediate-sized islands supported both [96]. MSC differentiation into adipocytes, osteoblasts, and chondrocytes requires culturing at the appropriate densities, and cells retained a memory of the initial plating density for at least 1 week. Cytoskeletal tension and RhoA regulate the switch between osteogenic and adipogenic fates [96,97]. Later work showed that MSCs are sensitive to the shape of the islands, specifically to the curvature and aspect ratio, which correlated with cytoskeleton tension and increased osteogenesis. High cytoskeletal tension was associated with upregulation of the MAP kinase pathway and RhoA and ROCK proteins. By contrast, cells on rounded, adipogenesis-favoring, shapes showed inhibition of these pathways [8].

Another seminal paper demonstrated that MSCs are sensitive to the mechanical properties of the substrate [98], inspiring a research program for controlling differentiation using substrate properties (reviewed in [99]). Intriguingly, MSCs also appeared to be sensitive to disorders in adhesion patterns in the substrate [100]. Although cell shape has not been extensively studied as a causal factor in these cases, the evidence discussed in this review strongly suggests that modulation of shape may be a significant way in which substrate properties affect MSC fate.

emergent property of the subtle interplay of genetics with physics, although the fundamental principles that determine cell shape are still not understood, which is the background and rationale for the current review.

## Mechanisms Connecting Cell Morphology to Cell State

Cells adhere to substrates through protein complexes, called focal adhesions, that signal downstream through multiple pathways, leading to the activation of the Rho and Rac proteins that are regarded as master regulators of the cytoskeleton. These processes in cell adhesion and spreading have been extensively reviewed [2–4]. The interplay of focal adhesion formation with Rho and Rac signaling is a major theme in biophysical studies of cell spreading (reviewed in [5]) and in some mathematical models of cell shape [6,7]. One mechanism by which matrix- and shape-dependent signals are transduced is through changes in actomyosin tension that have been shown to change the fate of multipotent stromal cells (MSCs, often called mesenchymal stem cells) [8]. Studies of cells spreading on micropatterned substrates have shown that cells often develop thick, tensile, actomyosin cables to bridge large adhesive gaps [9]. The tension state of the cell is communicated to the nucleus through mechanotransductive proteins such as Yap/Taz [10], the LINC complex, and lamin-A [11], and affects cell fate [10,12,13]. Some motile cells, such as fish keratinocytes, may acquire specific shapes arising from the mechanical requirements of motility [14,15].

Other than direct sensing of the tension state of the cell through cytoskeletal cables, mechanistic links have been suggested between changes in cell and nuclear morphology. Shivashankar and collaborators [16–20] have shown that different cell geometries lead to changes in actin structure and nuclear morphology [21], and significant differences in gene expression, histone acetylation, nuclear morphology, and volume were reported between cells cultured on adhesive islands of different sizes and geometries [22]. Changes in nuclear volume and geometry may affect gene expression by changing the intermingling of chromosome territories [17], and the same group then showed that changes in cell geometry lead to repositioning of chromosome territories [20] and changes in gene expression [18,20]. These ideas are also consistent with recent developments in understanding the spatial organization of chromosomes (reviewed in [23,24]).

A perinuclear actin cap was shown to regulate the shape of the nucleus of cells cultured on micropatterns [25]. This perinuclear cap was absent in embryonic stem cells (ESCs) as well as in induced pluripotent stem cells, suggesting that control of nuclear shape and volume may be an important part of transcriptional control in stem cell differentiation [26]. Actomyosin fibers also play a significant role in controlling nuclear shape, as shown in [27] where it was reported that changes in cell shape changed actin filament organization, which then affected nuclear shape and orientation. Nuclear elongation was accompanied by a significant decrease in nuclear volume, and a simple mechanical model predicted a force-dependent increase in the effective Young's modulus of the nucleus, and this was directly related to the extent of chromatin condensation. Nuclear shape and volume may also play important roles in the role of shape in cell proliferation (Box 1). In one study, cell spreading, nuclear volume in G1, as well as DNA synthesis and hence proliferation, were strongly correlated [28]. Another paper isolated the role of shape by culturing cells on differently shaped islands with the same area, and found that cells of different shapes had different proliferation rates, and the nuclear shape was strongly correlated with proliferation [29]. Interestingly, this latter paper reported that calcium signaling may also link the cytoskeleton with cell and nuclear shape. Cell shape-dependent dynamics of calcium waves have also been reported [30].

Finally, adherent cells have been reported to sense changes in shape through shape-related changes in the distribution of signaling proteins on the cell membrane. Iyengar and collaborators [31] hold that cell shape information is transduced through both tension-dependent signals as well as tension-independent signals, specifically signaling through integrin proteins. They showed that some apparent cellular responses to changes in actomyosin-driven tension were in fact also determined by an independent contribution from integrin signaling. In turn, integrin proteins may sense shape through curvature-dependent formation of signaling microdomains [32]. Some of these mechanisms of sensing cell shape, in particular the role of curvature-sensing proteins, have been recently reviewed [33].

Cells become rounder when they divide [34], and the information stored in cell shape was assumed to be lost at each cell division. However, combining experiments with mathematical modeling [35] showed that a zebrafish cell type retains a memory of its previous shape even after cell division. Mathematical modeling suggested that the memory mechanism was the persistence, despite mitotic rounding, of the shape-dependent asymmetric spatial organization of an important protein. While this effect has yet to be demonstrated in human cells, if it does exist it would imply that materials that control cell shape could have effects that persist beyond the timescale of cell contact and adhesion.

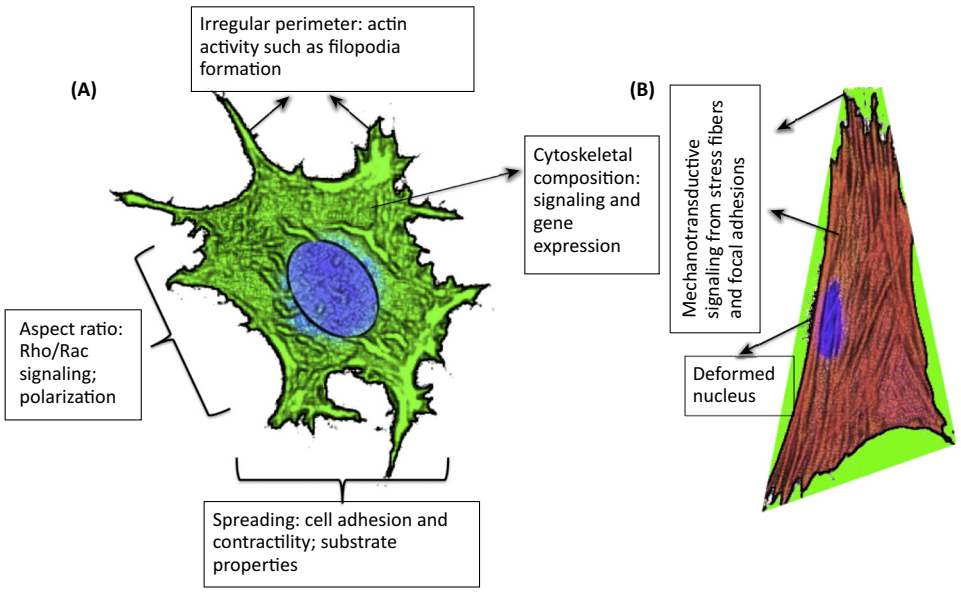


Figure 1. Cell Shape Encodes and Can Affect Cell State. (A) Some aspects of cell morphology that reflect some aspects of cell state. (B) Modulation of cell shape by an adhesive island (green) leads to changes in state as a result of focal adhesion signaling, mechanotransduction, and nuclear shape changes.

The multiplicity of links between cell morphology and cell state, some of which are summarized in Figure 1, lead to the question of identifying quantitative metrics to measure morphology for capturing different aspects of cell state for scientific and medical applications.

## Measuring Cell Morphology

Cell morphology can be visualized using both phase-contrast [36] and fluorescence imaging, and the cell outline is identified by using a segmentation algorithm that identifies the cell boundary. Cellular actin organization, the organization of other cytoskeletal components, or of the intracellular organelles or other structures, can be captured using intensity-based greyscale images (Figure 2). Shape quantifiers can be broadly divided into two main classes: descriptive measures or basis function expansions. Descriptive measures include user-defined attributes of shape or texture, such as cell area, perimeter, aspect ratio, circularity, fractal dimension of the perimeter, roughness of the texture, polarization of the texture, and so on. Basis function expansions are based on expansion of the cell shape or the cell perimeter in some appropriate orthonormal series, and the coefficients of the expansion represent the shape of the cell. The advantage of the basis function method is that the series representation is, in theory, a complete description of the cell shape. However, changes in coefficients are often not easy to interpret. The two most common basis function expansions used are Fourier series and Zernike polynomials. Two alternative ways of using Fourier series are to separately decompose either the $x$ and $y$ coordinates, or the $\rho$ and $\theta$ coordinates, of every pixel in the cell boundary. On the other hand, Zernike polynomials are intrinsically 2D, and the coefficient of the basis function in this expansion, called the Zernike moments, are used as the descriptor of the cell image. The Zernike moments have the advantage of being naturally rotation-invariant [37], although some mathematical transformations can also render Fourier coefficients rotationally invariant. Although most applications are on fixed cells, live cell imaging can yield morphological dynamics that may potentially carry more information.

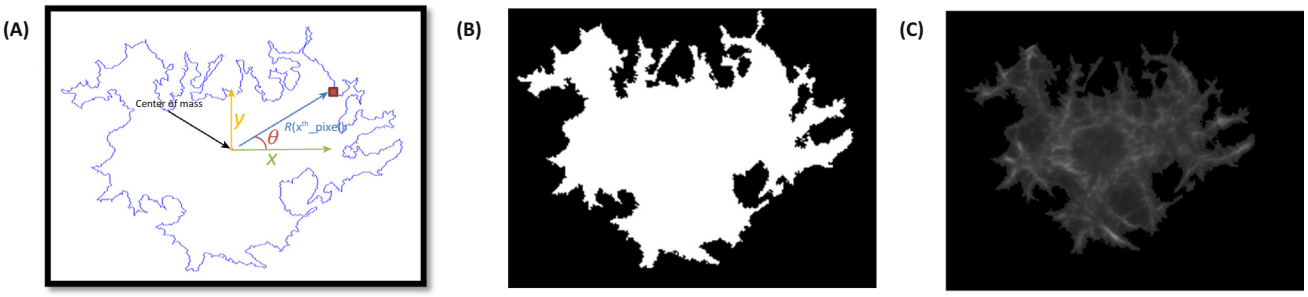


Figure 2. Representation of Cell Shape. (A) 2D outline: position of each pixel in the boundary is recorded in polar or Cartesian coordinates. (B) Binary image in which the cell is labeled white and the background black. (C) Greyscale intensity plots showing the internal distribution of actin.

A recent review of shape quantifiers [36] argues for three desirable properties. The first is fidelity, in other words not discarding information or adding distortion to the signal. The second is that the quantifiers should capture biologically important variations, while the third is that each quantifier should be individually meaningful and interpretable. However, different shape measures are often relevant for different comparisons, and in practice retaining a large set of shape measures may be the best practice. In addition, some shape measures such as the Zernike moments are hard to interpret intuitively but can have good discriminating power. Pincus and Theriot [36] compared Fourier expansion, Zernike moments, and principal component analysis (PCA) and independent component analysis (ICA) representations of the shape data of three different types of cells. Their overall conclusion was that the PCA representation of shape appears to perform the best. However, they were strongly limited by the choice of only a 16-component feature set. Methods such as Zernike moments require including ~30–50 moments to yield greater fidelity and discrimination power [38].

The development of publicly available software platforms for morphological image analysis makes the measurement of hundreds of morphological parameters very straightforward. In particular, the freely available software platform CellProfiler [39], with the integrated analysis package, CellProfiler Analyst [40], offers image processing, a large list of quantitative features that can be automatically extracted from the images, and data-analysis and machine-learning capabilities. CellProfiler Analyst enables scientists who are not experts in machine learning to use methods such as random forest classification, support vector machines, and AdaBoost [40]. Other open-source software packages performing similar tasks include CellCognition [41] and Advanced Cell Classifier [42]. Scientists unfamiliar with data analysis of morphological data can benefit from an excellent recent review [43]. An important development in morphological analysis is the widespread use of machine-learning methods to carry out predictive classification and model the relation between morphology and phenotype; many of the papers discussed in this review have used these machine-learning methods.

## Interpreting Cell Morphology: Some Important Applications

A seminal paper from Perrimon and collaborators is an early example that combined machine-learning methods with morphometrics [44]. They included 249 gene-overexpression treatment conditions, imaged over 12 000 cells, and used machine-learning tools to link cell signaling with shape in a *Drosophila* cell line. A related paper [45] demonstrated a definite quantitative relationship between morphology and signaling pathways for Rho GTPase regulators. Another seminal paper [46] classified the morphologies of *Drosophila* cells into five discrete classes, and suggested that transitions between these classes were 'switch-like' rather than gradual. Gene

knockdowns led to enrichment of specific classes rather than to the emergence of new ones, suggesting that stochastic gene expression in control cells leads to a diverse population of cell shapes [46]. Another study [47] used RNAi screens to find Rho GTPase regulators controlling the frequency of the above-mentioned discrete cell shape classes, and identified specific proteins that appear to regulate specific types of cell shapes. Whether the cellular morphologies of mammalian cells are discrete or a continuum has not yet been settled.

These studies contributed to developing classification methods using 'high-content' images of cells [48], also including DNA and organelle stains, as well as fluorescently tagged proteins, which have been used to screen biologically active molecules [49,50]. One significant application of high-content imaging is in high-throughput screening of small molecules for drug development because cellular morphology is relatively sensitive to physiology. Small molecules that are being investigated for cytotoxicity can be screened rapidly and sensitively using morphological changes characteristic of apoptosis. High-content imaging has been reviewed recently [51], and we will therefore not discuss it in detail. However, even without 'high-content' images, morphological differences and changes have been used for screening cells, as reviewed at length recently [52]. We supplement the description in this earlier review by emphasizing two applications in greater detail below.

### Morphology Identifies Cancer Phenotypes

Abnormal changes in morphology are used in diagnostic cytology, and the challenge before morphological screening is whether it can improve on current practice. Detecting morphological changes due to metastatic transformation is an area where these methods may contribute significantly. Ren and coworkers [53] showed that characteristic changes in shape characterize cells after undergoing induced epithelial-to-mesenchymal transition (EMT), a cellular transformation associated with metastasis [54]. Prasad and collaborators studied whether cell shape is correlated with metastatic potential by measuring shape features of eight osteosarcoma cell lines, four highly metastatic and four with low metastatic ability [38,55]. Using descriptive morphological measures [55], as well as Zernike moments [38], they showed that shape changes in metastatic osteosarcoma cells were of two types that appeared to be stereotypical and were also predictive. In particular, a neural network could predict the metastatic capacity of cell lines using morphological markers with ~99% accuracy for a related pair of cell lines, and, even more remarkably, with ~90% accuracy for the six cell lines from type I [38]. Another comprehensive empirical study by the group of Wirtz [56] used thousands of images of 11 pancreatic ductal adenocarcinoma (PDAC) cell lines, as well as 10 breast cancer cell lines, to develop a high-throughput imaging and computer vision and analysis system. However, in contrast with other work, this study did not find specific morphological signatures of metastasis, except for a lower degree of heterogeneity, suggesting that PDAC cells may not show many shape changes. Nevertheless, morphological information could identify metastatic cells. Morphological information may also identify chemoresistance, as shown by two papers [57,58] that find significant differences between the shapes of chemoresistant and chemosensitive colon cancer cell lines.

Using imaging and data analysis, coupled with genomic tools and RNAi screens, Bakal and collaborators have provided compelling evidence that cell morphology can identify non-trivial aspects of the cancer cell state [44–47,59–61]. Sailem and coworkers [60] showed that a quantitative and predictive relationship between cancer gene expression, cell shape, and tumor clinical phenotypes could be determined, and they identified subsets of genes, or 'morphological metagenes', whose expression level correlated with shape features and, significantly, with tumor grade [60]. In another fascinating study, the same group found that

the activation of the major transcription factor nuclear factor-κB (NF-κB) displayed complex (i.e., both linear and non-linear) correlations with many morphological parameters [59]. Similarly, a measure of the activation of Yap/Taz, an important mechanotransductive transcription factor, was also strongly correlated with morphological parameters [47,61]. Melanoma cells spontaneously shift between amoeboid and mesenchymal shapes when plated in 3D collagen matrices, and a quantitative analysis of these shape changes led to the identification of different transition paths in shape space and associated protein regulators [46]. Thus, in the future it may be possible to detect complex signaling and genetic events by using only label-free morphological analysis.

### Morphology Predicts the Phenotype of Stem Cells

Lineage commitment of MSCs can also be predicted by changes in morphology. Treiser and coworkers [62] analyzed the morphometric descriptors of the actin cytoskeleton, using 43 quantitative morphological measures, and could identify signatures of osteogenesis and adipogenesis as early as 24 h after treatment. By contrast, conventional end-point assays require weeks in culture. When applied to surface-directed osteogenic differentiation, their methods identified two morphological populations corresponding to the osteogenic and non-osteogenic populations. Seiler and coworkers [63] measured seven morphological features that could identify myogenic differentiation but not other fates. However, the small number of features considered in this study may have been a limitation. Matsuoka and coworkers used cell shape to non-invasively predict osteogenic differentiation potential of human MSCs, and trained a machine-learning algorithm to predict osteogenic differentiation of MSCs from new patients with excellent accuracy [64]. In a more recent paper, Marklein and coworkers [65] also examined the morphological changes consequent upon osteogenic induction of MSCs from several human donors, using CellProfiler [40]. They found that MSC morphology after 3 days was highly correlated with mineralization at 35 days. In particular, a specific subset of seven morphological features was highly predictive of mineralization. After adding additional data, they found that a standard marker of osteogenesis, alkaline phosphatase expression, was a poor predictor of mineralization, while morphological models showed 88% and 92% accuracy with only a few parameters. Shape may play an important role *in vivo* for maintaining the stem cell niche because MSCs cultured on small adhesive islands maintained the multipotent stem cell phenotype more reliably than on smooth surfaces [66].

Brief mention of two other applications will be useful. First, activated macrophages can be detected by morphological changes [67], and Pavillon and coworkers [68] suggest that morphological parameters may be picking up some information related to the Raman spectra of the cells. Second, MSCs also have immunosuppressive roles *in vivo*, and morphological features can predict immunosuppressive capacity [69].

## Controlling Cell Shape: Methods and Applications

Patterned substrates may control cell shape directly (as with fibronectin islands of different shapes) by providing contact guidance cues (as with microgrooves) or by controlling the size and distribution of focal adhesions through their effect on both shape and modulation of focal adhesion signaling. Patterned substrates have been reviewed extensively [70–72], notably including their possible role in cell-based therapies [73]. We briefly highlight some recent work relevant to the control of cell shape. Adhesive islands of different geometries affect lineage choice by stem cells, as discussed previously. Contact guidance has been deployed extensively in attempts to control axonal growth from neurons [74], with applications in neural prostheses. Multiple studies have shown that neurons use contact guidance cues to determine

polarization and axon growth. More remarkably, neuronal markers are upregulated when MSCs as well as ESCs were grown on nanogrooved surfaces [75,76].

Nanopatterned substrates that modulate the size and shape of adhesive spots exercise control over cell shape and function by constraining focal adhesions. A detailed study of this effect, reported by Slater and coworkers [77], showed that small spots <100 nm in diameter changed the distribution of focal adhesions and led to enhanced migration, as well as a very dynamic morphology, compared with larger spots (~200 nm and 400 nm) and smooth controls. Nanopatterned surfaces have been widely used to control osteogenic differentiation of mesenchymal stem cells, as reviewed previously [71,78].

One exciting advance in exerting fine control over cell shape by patterning surfaces is based on use of computer-guided laser ablation rather than on lithography masks, allowing greater control over the position, size, and geometry of adhesive spots. This technique has been applied to create biomimetic patterns by printing patterns and shapes derived from labeled focal adhesions or cell images [79]. This technique was able to recapitulate the patterns corresponding to a typical adipocyte, and, when treated with mixed-differentiation media, the adipocyte-mimetic surface enhanced adipogenesis [80]. Cell-mimetic patterned substrates therefore can be used for much subtler control of differentiation outcome than simple geometric patterns.

Could methods to control cell shape actually be used to control the architecture of tissue? Preliminary results suggest that they can. Sarkar and colleagues [81] cultured a breast epithelial cell line (MCF-10A) on square, triangular, and rectangular islands that were micropatterned on substrates of different degrees of stiffness. They found that asymmetric and elongated shapes reduced the strength of cell–cell junctions compared to cells on square patterns, even on softer substrates that otherwise showed stronger cell–cell junctions compared to stiffer substrates. Thus, combining substrate stiffness with geometric patterning, by printing extracellular matrix (ECM) proteins on hydrogels [82,83], may enable subtle control over tissue properties.

## Concluding Remarks and Future Perspectives

It has been often remarked that form and function are closely related in biological organisms, and this may be universal for self-organized structures. This review has focused on one aspect: the form and function of mammalian cells. These forms carry information that can be very valuable. Thus, it may be possible in future to detect and characterize invasive cells, cancer stem cells, chemoresistant cells, and other clinically relevant subtypes from cancer biopsies. Morphological methods may be able to predict whether stem cells in a given state are appropriate for the intended purpose in stem cell therapy. Morphological analysis may help to select immunosuppressive cells from a population or remove sperm with subtle defects for *in vitro* fertilization. All cell-based analyses and therapies must contend with cellular heterogeneity, and morphological analysis may provide a complex readout of the ensemble of cell states.

These applications are already nascent in the field but are also limited by the lack of mechanistic information linking morphology to state. For example, an exciting question here is whether perturbations in specific pathways can be detected through morphological changes. Recent studies suggest that the answer is yes, but much more work would be needed for practical applications.

### Outstanding Questions

What changes in cell state are unambiguously reflected in changes in morphology? Many changes in state lead to changes in morphology, but is the mapping unique or many-to-one? Can specific changes in gene or protein expression be identified from morphological changes alone?

Can we predict cell morphology from chemomechanical models of the cell? Are cell shapes the result of chemical and mechanical principles that can be mathematically expressed and simulated?

Which of the many genetic and signaling perturbations in cancer and metastatic cells be identified in patients from morphology alone? Can the morphological space inhabited by a collection of tumor cells from a biopsy predict the properties of the cancer?

To what extent does heterogeneity of cell shape in a culture reflect cellular heterogeneity, and to what extent is it random? Can shape be used as a single-cell readout of cellular heterogeneity?

Can cancer stem cells be detected from morphological analysis? If differentiation of stem cells can be predicted from morphology, then can dedifferentiation of cancer cells be similarly predicted?

Can shape changes be used as a readout for biosensing of biologically active chemicals? Biologically active small molecules can change morphology. Can environmental toxins be detected using these changes?

Cell morphology is dynamic and changes over time. What additional information can we obtain from dynamics of morphological changes of live cells?

What are the mechanisms by which morphology affects cell state? Deeper understanding is required of the mechanisms by which cell morphology is determined, and of how they can change cell phenotype.

An emerging challenge is the vast quantities of morphometric data that will soon be generated by high-throughput imaging systems, especially as live cell imaging becomes more common. Machine-learning methods will become more and more important for formulating and testing novel hypotheses. New methods will also need to be developed to deal with dynamic morphological data, and the timescales at which morphological effects are important will need to be studied in more detail. Machine-learning is not limited to classification and can be used to build predictive structure–function models [84] which can overcome some of the limitations of traditionally reductionist mathematical models.

Patterned substrates have not only helped to drive research on cell morphology but also provide a means to control it. Some of the intriguing effects of some types of patterns, for example of types of disorder, are likely to be at least partly caused by changes in shape. Specific patterns on implants or implantable biological devices may help tissue regeneration or prevent unwanted scar tissue formation *in situ*. Much of the work on cell shape is in 2D, and for screening purposes 2D assays have definite advantages in that they are cheaper, simpler, and more high-throughput than 3D assays. However, for tissue engineering it is important to understand the interplay between morphology and function in a 3D biomimetic environment [85], as well as to develop methods of controlling 3D cell shape, for example by changing the microstructure of collagen hydrogels as reported here [86]. Could hydrogel microstructure promote or inhibit tissue or cancer cell growth through effects on cell shape, possibly synergistically with chemical means?

Though much remains to be done (see Outstanding Questions), the interpretation and control of cell morphology is poised to profoundly enhance both biological understanding and biomedical engineering.

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