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Probing Cellular Traction Forces by Micropillar Arrays: Contribution of Substrate Warping to Pillar Deflection

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Abstract

Quantifying cellular forces relies on accurate calibrations of the sensor stiffness. Neglecting deformations of elastic substrates to which elastic pillars are anchored systematically overestimates the applied forces (up to 40%). A correction factor considering substrate warping is derived analytically and verified experimentally. The factor scales with the dimensionless pillar aspect ratio. This has significant implications when designing pillar arrays or comparing absolute forces measured on different pillar geometries during cell spreading, motility or rigidity sensing.

Keywords

traction force; mechanotransduction; cell motility; rigidity sensing

Generation of mechanical forces is central for regulating the attachment of cells to a substrate, for cell spreading and migration (for reviews see 1–6). In turn, cells sensitively respond to physical parameters of their environment, e.g. geometry or rigidity 7–17 and even malignancy is promoted by crosslinking of extracellular matrix fibers which increases the stiffness of the matrix 18. Via micron-sized cell adhesion sites, cells can locally apply up to several nN of force 19, 20. The force is generated via the cytoskeletal motor protein myosin II which pulls on actin filaments 21, 22 that are coupled via adaptor proteins to transmembrane integrins which anchor cells to the outside world 23, 24. Proteins that are part of the force-bearing physical connection linking the cytoskeleton to the outside can act as mechano-chemical signal converters 6, 16, 25–27. To elucidate the detailed underpinning mechanisms that control mechanotransduction processes, accurate knowledge of the forces that cells apply via adhesions to substrates is required.

Over the last ten years, a variety of experimental methods has been employed to quantify cellular forces 28, 29, such diverse as atomic force microscopy (AFM) 30, 31, optical traps 32, 33, flat elastic substrates (traction force microscopy) 19, 34–37, or elastic substrates with arrays of micro or nanoscopic pillars (see Fig. 1 A) 14, 38–46. They are based on measuring force-induced deformations of the sensor and converting them into actual force values via its elastic properties. For small deformations, the force F is assumed to be proportional to the deformation δ (Hooke's law).

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Supporting Information Available. Detailed materials and methods, a theoretical derivation of the tilting coefficient, additional figures and a movie accompany this article. This material is available free of charge via the Internet at <http://pubs.acs.org>.

$$F = k \cdot \delta \quad (1)$$

Accurate force calculations require a proper calibration of the sensor's stiffness (spring constant k) and need to be corrected for possible crosstalk between adjacent measurement sites. In this paper we theoretically and experimentally address these important issues in the context of elastic pillar substrates.

Pillar arrays for cellular studies are typically made of poly(dimethylsiloxane) (PDMS) and characterized by pillar dimensions and spacing (Fig. 1 B). The spring stiffness of a pillar is determined by the combination of the material's Young's modulus E and the absolute dimensions (height L , diameter D) and typically lies in the range 1 to 200 nN/ μ m. In most experimental studies, only bending of a bottom-fixed pillar is taken into account to describe the deflection in response to a lateral force at the pillar top (Fig. 1 C) by the bending formula⁴⁷:

$$F = k_{bend} \cdot \delta = \frac{3\pi E D^4}{64 L^3} \delta \quad (2)$$

This expression for the spring constant of bending k_{bend} is also commonly used to design appropriate pillar dimensions for a desired stiffness. More sophisticated analyses considered contributions from pillar shear^{48, 49}, non-linearities⁴⁹, deviations from an ideal cylindrical geometry⁵⁰, viscoelastic material properties⁵¹, and different referencing methods that either incorporate or correct for the lateral displacement of the pillar base^{48, 52}. Surprisingly, only one study recognized that the flexible substrate on which the pillar is anchored is warped by the torque acting at the pillar base (Fig. 1 C)⁵³. The authors suggested that this term would add to the total deflection of the pillar but they did not quantify its contribution. Let us consider two limiting cases to illustrate extremes: a soft pillar on a rigid substrate will deflect without affecting the substrate, versus a stiff pillar on a soft substrate where the substrate accounts for 100% of the deflection.

In the following, an analytical expression is introduced how the forces acting on a pillar top can be calculated from the deflection of an elastic pillar that explicitly takes into account the warping of an underlying elastic substrate of the same material. Using finite element simulations and a macroscopic pillar model mimicking experimentally significant pillar aspect ratios it is shown that substrate warping beneath individual pillars causes a tilting of the pillar axis and substantially contributes to their total deflection. The implications of our findings for the correct calculation of forces and the design of pillar arrays are discussed.

Theoretical Description of Pillar-Base Tilting caused by Substrate Warping

Consider a cylindrical pillar of height L and diameter D whose base is sealed to a flat substrate of the same material and centered at the coordinate origin (Fig. 1 B). The elastic material is characterized by a homogenous Young's modulus E and the Poisson ratio ν (that describes the ratio between transverse compression to axial strain under uniaxial loading). These variables can be adjusted by the experimentalist and are given in bold letters throughout the equations. In linear elastostatics, the pillar deflection in the direction of a lateral force F that is applied to the pillar top is described by bending and shear deformation of the pillar⁴⁷

$$\delta_{pillar} = \delta_{bend} + \delta_{shear} = (k_{bend}^{-1} + k_{shear}^{-1})F \quad \text{with} \quad k_{bend} = \frac{3EI}{L^3}, k_{shear} = \frac{KGA}{L} \quad (3)$$

where $A = \pi R^2$ the area of the circular pillar cross section with radius R , $I = \pi R^4/4$ is the 2nd moment of inertia, $G = E/2(1 + \nu)$ is the shear modulus, and $K = (6 + 6\nu)/(7 + 6\nu)$ is Timoshenko's shear coefficient⁵⁴.

The contributions of an elastic substrate to the deflection of the pillar top are additive and will be described as (see Fig. 1 C)

$$\delta_{total} = \delta_{pillar} + \delta_{substrate} = \delta_{bend} + \delta_{shear} + \delta_{tilt} + \delta_{base}. \quad (4)$$

The term δ_{base} stands for the lateral displacement of the pillar base which is usually subtracted experimentally by the top-base reference method^{48, 52} so that the total displacement becomes $\delta_{total}^* \equiv \delta_{total} - \delta_{base}$. The additional displacement δ_{tilt} of the pillar top arises from the warping of the substrate and a subsequent tilting of the pillar axis, for which we now derive an analytical expression.

When a force F acts at the pillar top, a torque $M = L \cdot F$ occurs at its bottom (Fig. 1 B) that induces normal stresses as described by the flexure formula⁴⁷.

$$\sigma_z = -x \frac{M}{I} = -\frac{x}{R} \sigma_{max} \quad (5)$$

Shear stresses that do not significantly alter the normal stresses are neglected⁴⁷. Inserting the term for the torque into equation (5) and solving for the maximum stress σ_{max} at the rear edge yields

$$\sigma_{max} = 8 \frac{L F}{D A} \quad (6)$$

The antisymmetric stress profile (Eq. (5)) causes a warping of the substrate beneath the pillar base. This deformation leads to a tilting of the pillar base and axis (Fig. 1 C) by an angle Θ that is proportional to the stress σ_{max} and inversely proportional to the Young's modulus E of the elastomer.

$$\Theta = T_{tilt}(\nu) \cdot \sigma_{max} / E \quad (7)$$

The hereby introduced *proportionality factor* $T_{tilt} \equiv T_{tilt}(\nu)$ for the tilting of the base, in the following called '*tilting coefficient*', depends per definition only on the *shape* of the warping profile and thus on the Poisson ratio. An analytical expression for this tilting coefficient is derived from first principles in the Supplementary Information and yields

$$T_{tilt}(\nu) = a \frac{(1+\nu)}{2\pi} \left\{ 2(1-\nu) + \left(1 - \frac{1}{4(1-\nu)} \right) \right\} \quad (8)$$

Here, the dependence on the Poisson ratio originated from the mixed boundary conditions for infinite/half-infinite media. The multiplicative constant a arose from the averaging over the warping profile and can be interpreted as a standardized slope. For the description of the results from numerical simulations, it was used as free fitting parameter (see Fig. 2 C) and resulted as $a = 1.3$.

The tilting of the pillar axis causes a displacement $\delta_{tilt} = L \cdot \tan \Theta$ of the pillar top. For small deflections $\tan \Theta \approx \Theta$, we insert equations (6) and (7) and obtain

$$\delta_{tilt} = 8T_{tilt}(\nu) \frac{L}{D} \frac{L}{E} \frac{F}{A} = 8T_{tilt}(\nu) \left(\frac{L}{D}\right)^2 \frac{4}{\pi} \frac{F}{ED}. \quad (9)$$

In summary, the *total displacement of the pillar top* and its three major components from bending, shear and base tilting can be written as

$$\delta_{total}^* = \delta_{bend} + \delta_{shear} + \delta_{tilt} = \left(\frac{16}{3} \left(\frac{L}{D}\right)^3 + \frac{7+6\nu}{3} \frac{L}{D} + 8T_{tilt}(\nu) \left(\frac{L}{D}\right)^2 \right) \frac{4}{\pi} \cdot \frac{F}{ED}. \quad (10)$$

Note that all contributions scale with the applied force F normalized to the material's Young's modulus E and the pillar diameter D . In contrast, the *relative* contributions $\delta_i/\delta_{total}^*$ i.e. to which percentage each mechanism contributes to the total deflection, are solely determined by the aspect ratio L/D of the pillar and the Poisson ratio ν of the elastomer.

Quantifying the Substrate Contribution to Deflection of Microscopic Pillars by Numerical Simulations

Numerical simulations were performed to investigate the behavior of an elastically anchored pillar. Finite element modeling was used to implement various pillar geometries, parameter values and boundary conditions.

First we asked to which extent the substrate contributes to the deflection at the pillar top. Figure 2 A shows the ratio between the deflection of an elastically anchored pillar δ_{total} compared to the deflection of a pillar δ_{pillar} firmly clamped to an inelastic substrate. The pillar on the elastomer was substantially more deflected than the pillar alone. The *additional* deflection increased from 10% to 50% for decreasing pillar aspect ratios, from 10 to 1. When the displacements that had been determined relative to the unstrained geometry were compared with those determined relative to the position of the pillar bottom in the strained state, small differences showed up at very small aspect ratios that originated from the lateral displacement δ_{base} of the substrate by the shear force. For the rest of the paper, the displacement of the pillar top will be corrected for that lateral substrate shift as it is done in experiments where the position of the pillar top is evaluated relative to the position of the pillar bottom (top-base referencing method 48–52).

Next it was tested whether our analytical description for the warping-induced tilting of the pillar base (see previous section) can explain the observed additional deflection. Therefore, a linear profile of normal stresses $\sigma_z = -x/R \cdot \sigma_{max}$ (see Eqs. (5)–(6)) was directly applied at the bottom of an unloaded pillar (Fig. 2 B). The resulting deflection of the pillar top was 55 nm, in comparison to a difference of 57 nm between the top-loaded pillars with and without elastic substrate. Moreover, the strain profile in z direction at the pillar bottom nearly perfectly resembled that of the top-loaded pillar. We conclude that the additional pillar deflection is mainly caused by the torque acting at the pillar bottom.

To obtain a quantitative expression for the conversion of the warping profile into a tilting angle, the average incline of the pillar bottom evoked by the stress profile was calculated. This angle was then multiplied by E/σ_{max} to obtain the characteristic tilting coefficient T_{tilt} that depended on the Poisson ratio alone (see Eq. (7)). Figure 2 C shows that the tilting coefficient for small

Poisson numbers lies around 0.57, then decreases with increasing Poisson ratio and reaches a value of 0.47 for incompressible materials at $\nu = 0.5$. This dependency was well fitted by equation (8) with $a = 1.3$. Our simulation results may also be compared to the analytical result for the deformation of the free elastic half-space derived by Merkel and colleagues⁵³: their calculations imply a tilting factor of $T_{\text{tilt}} = 0.51$ for $\nu = 0.5$ which is in excellent agreement with our result when the changed boundary conditions due to the presence of the pillar are taken into account (Suppl. Fig. S1 D). Notably, boundary effects due to finite pillar height did not affect the tilting coefficient as long as the pillar aspect ratio was larger than 0.5 (Suppl. Fig. S1). The finite substrate thickness dampened the warping only when the substrate layer was thinner than the pillar diameter (Suppl. Fig. S2), in contrast to lateral displacements on flat elastic substrates that are influenced by the substrate thickness up to $60 \mu\text{m}$ ³⁵. In summary, if the substrate is thicker than $2 \mu\text{m}$ and pillars are taller than their radius, the substrate warping is well characterized by the derived tilting coefficient.

Finally, it was investigated how the substrate contribution to the total pillar deflection depends on the Poisson ratio of the material. The simulations show that substrate warping contributed more to the total deflection at small Poisson numbers and that this effect was most pronounced at smaller pillar aspect ratios (Fig. 2 D). The data were well described by the analytical model together with the tilting coefficient derived in the previous section without any other fitting parameter. We conclude that our simplified model provides a convenient quantitative description of the substrate contribution to the deflection of an individual pillar.

Experimental Validation using Force Measurements on PDMS Pillars

A central finding of our derivation is that the *relative* contribution of substrate warping to pillar deflection is scale-free: it depends on the aspect ratio of the pillar but not on its absolute values (Eq. (10)). Therefore it is possible to validate our derivation with millimeter-sized pillar models that also reduce unwanted contributions from surface defects that are intrinsic to microfabrication processes and errors from direct force measurements. Macroscopic PDMS models of different stiffness (1 MPa, 2.2 MPa, 3M Pa) comprising pillars with aspect ratios from 2 to 9 were fabricated (see Supplementary Information), and a micromanipulator and a MEMS force sensor were used to manipulate and measure the pillars with high accuracy (Fig. 3 A, see Supplementary Movie). To derive the spring constant of the pillar, either the slope of the experimentally determined force-deflection curve or the optical top-base method was used (Fig. 3 B, see Methods and Supplementary Material online).

Figure 3 C shows a double-logarithmic plot of the spring constant versus the aspect ratio of the pillars for three samples with different material stiffness. The results from the two evaluation methods were identical within their experimental errors. The good agreement justified the used correction for local deformations around the sensor tip (Suppl. Fig. S3). The pillar stiffness followed a $(L/D)^{-3}$ dependence (*dotted lines*) at large aspect ratios as expected for pure bending. Towards shorter pillars, the measured spring constants increasingly deviated from this trend indicating that the pillars deflected more than extrapolated from the tall pillars as expected from the formulas derived above.

To quantify the reduction of the effective pillar stiffness and for a comparison with the theory, the measured spring constants was rescaled by the values that were predicted by the bending formula (Eq. (2)) together with the independently measured Young's moduli (see Methods). As a result, the measurements from the different samples followed the same trend that reached a plateau at large aspect ratios and decreased towards small aspect ratios (Fig. 3 D). The plateau was consistent with predominant bending of the pillar. The decline at smaller aspect ratios was well described by the joint action of bending, shear and tilting (Fig. 3 D, *solid line*) but not by bending or bending and shear alone. In conclusion, substrate warping substantially contributed

to the pillar deflection in our experimental test system and its contribution was well described by our analytical approach.

Implications of Substrate Warping for Micropillar Studies

Elastic micropillars are typically anchored to an elastic substrate of the same material. The preceding paragraphs quantitatively analyzed how the warping of elastic substrates results in an additional tilting of the pillar axis that can change significantly the conventionally assumed force-deflection relationship or “effective” pillar elasticity. The substrate contribution critically depends on the aspect ratio L/D of the pillar (Fig. 2 D): it is around 10% for tall pillars but reaches up to 40% for short pillars. Considering that variations of the pillar dimensions^{14, 39, 41, 43, 55} are the most efficient way by which the pillar stiffness can be tuned over the two orders of magnitude (see Eq. (2)) needed to mimic the range of substrate rigidities sensed by cells¹³, a proper correction for the warping effect is necessary for the comparison of results derived from different laboratories on different pillar arrays^{14, 39, 41, 43, 55}, as well as for the design of pillar arrays with defined effective rigidities. In the following, central aspects and consequences of the substrate warping effect are discussed and data from the existing literature is reevaluated.

How to calculate the “Effective” Spring Constant or Stiffness of a Pillar on a Warping Substrate

Bending, shear, and the substrate-induced tilting of the pillar are independent and add up to the total displacement of the pillar top (Fig. 4 A). The analytical analysis showed that the ratio between the individual contributions depends mainly on the pillar aspect ratio as summarized in Fig. 4 B (see Eq. (10)). Three regimes can be distinguished: For very short pillars shear dominates, for tall pillars bending dominates, whereas at intermediate aspect ratio (up to $L/D \approx 5$) the substrate substantially contributes via pillar base tilting. This substrate contribution makes the pillar effectively softer than would be expected for the isolated pillar and leads to an effectively reduced spring constant. The most convenient way to calculate the effective spring constant k of an elastically founded pillar is to use the spring constant of pure bending (Eq. (2)) and to multiply with a correction factor:

$$k = corr \cdot k_{bend} \quad \text{with} \quad corr = \frac{\delta_{bend}}{\delta_{bend} + \delta_{shear} + \delta_{tilt}}. \quad (11)$$

The validity of this procedure can be proven by inserting Eq. (11) into Eq. (1) and using the identities $F = k_{bend}\delta_{bend}$ and $\delta \equiv \delta_{bend} + \delta_{shear} + \delta_{tilt}$. The terms for the individual deflections that enter the correction factor can be taken from equation (10) and yield

$$corr = \frac{\frac{16}{3}\left(\frac{L}{D}\right)^3}{\left(\frac{16}{3}\left(\frac{L}{D}\right)^3 + \frac{7+6\nu}{3}\frac{L}{D} + 8T_{tilt}(\nu)\left(\frac{L}{D}\right)^2\right)} \quad (12)$$

with the tilting coefficient T_{tilt} from equation (8) ($a = 13$, see also Fig. 2 C). Note that the material's Young's modulus and the absolute dimension dropped out by the division. The correction factor for a certain pillar geometry can be determined by this formula, or visually from Fig. 4 B, and is tabulated for selected parameter values in Table 1.

Apart from theory, the correction factor also emerged directly from our measurements with macroscopic pillars: Dividing the measured spring constant by the spring constant of bending

(that was calculated based on the measured Young's modulus) is equivalent to the definition of the correction factor (Eq. (11)). The experimental data (Fig. 3 D) proof quantitatively that the correction factor depends on the pillar aspect ratio but not on the Young's modulus. Ideally, one would also like to calibrate micron-sized pillars experimentally to account directly for the substrate warping and also for unknown surface versus volume effects or an imperfect geometry. However, experimental errors are usually larger than for macroscopic measurements (Fig. 3 C) because the smaller dimensions entail a less accurate manipulation and readout of pillar deflection and force. In this case it is recommended to measure pillar dimensions and bulk material properties and to calculate the effective spring constant by equation (11). Note that already during the design of pillar arrays it is important to consider the warping effect to guarantee that appropriate pillar dimensions are chosen to achieve a desired effective rigidity, e.g. mimicking flat elastic substrates⁴³.

Recalibration of Micropillar Force Data from the Literature

The results from studies that have used an experimental calibration of pillars remain untouched because the base-induced tilting already entered the experimentally determined spring constant (see Fig. 3 C). If substrate warping occurred but was neglected in the force calculation, however, the pillar spring constant and thus the derived force were overestimated. This systematic error can be corrected for by using the effective spring stiffness of the elastically founded pillar (Eq. (11)) together with Hooke's law:

$$F = corr \cdot k_{bend} \cdot \delta \quad (13)$$

Among published micropillar force data, the studies of Ladoux and colleagues^{41, 43} are of special interest because they compared forces obtained from pillars exhibiting different aspect ratios. They varied the pillar length with the aim to investigate how cells adapt their forces to different substrate rigidities. On substrates with low rigidity, they found a linear increase in the forces that MDCK cells or fibroblasts applied to the pillars, whereas the forces reached a plateau on rigid substrates (Fig. 4 C). Since the pillar aspect ratio was not constant, each pillar geometry requires to be corrected by a different factor to account for the warping effect. The forces deduced from the deflection of tall pillars (low rigidities) were overestimated less severely than those involving short pillars (high rigidity). The recalibrated forces are smaller than the published forces, with -15% and -45% for the pillar aspect ratios 4.8 and 1.4, respectively (Fig. 4 C; see also Suppl. Fig. S4). Since the force still plateaus, the corrections therefore do not change the central conclusion of the authors that a maximal force exists by which cells can pull on the pillars. However, the maximum plateau force was decreased as much as from 60 nN to 35 nN (Fig. 4 C) when correcting for substrate warping. This example illustrates the importance of taking substrate warping into account, and the equations provided will allow to quantitatively compare forces measured by different arrays and laboratories.

Force evaluations from pillar arrays such as from the above example are based on the assumption that deflections of pillars act independent from neighboring pillars, in contrast to flat elastic substrates that have a global coupling of lateral deformations. Obviously, the surface of the elastic base also gets deformed *around* a force-loaded pillar (see Fig. 2 B) which in principle constitutes a coupling between individual pillars. The relative error in the force determination can be shown to scale inversely with the pillar aspect ratio and the center-to-center distance r_{cc} according to $\sim 0.1(r_{cc}/D)^{-3}(L/D)^{-1}$ (I.S., unpublished results). Importantly, this error is smaller than 5 % in most commonly used pillar arrays and the crosstalk is negligible.

In conclusion, microfabricated elastic pillar substrates of various geometries found widespread applications to address many fundamental questions in cell biology regarding the mechanoregulation of cell functions. This includes the underpinning of cell migration^{36, 46},

⁵⁶ or the interplay between force and focal adhesion maturation ^{19, 57}, and whether nuclear deformation are affiliated with mechanotransduction processes ^{58, 59}. Furthermore, pillar arrays can also be used for a variety of screening assays, including the discrimination between carcinogenic and normal cells ^{45, 60}. Our analytical expressions presented here will allow for a proper force calibration of pillars, and for a more rational design of pillar arrays.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Elastic pillar deflections induced by lateral force. (A) Fibroblasts on micropillar array. Overlay of a DIC image with fluorescence from a DiI membrane stain. (B) Relevant parameters of the system: the pillar has height L , diameter D , Young's modulus E , and Poisson ratio ν . A force F directed in the positive x direction causes a deflection δ_{total} of the pillar top and induces a momentum $M = F \cdot L$ at its bottom. (C) The total deflection comprises contributions from bending and shear of the pillar, as well as tilting and a lateral displacement of the base beneath the pillar.

Figure 2.

Substrate contributes to total deflection through pillar base tilting as revealed by finite element simulations. (A) Comparing deflections of pillars with elastic (1) or fixed base (2) for a shear force F applied to the top and for different pillar aspect ratios L/D . Deflections were determined absolute (circles) or relative to the pillar bottom (diamonds). (B) A stress profile σ_z at the pillar bottom (3, Equ. (5)) caused a similar warping of the base as in 1 (right graph) and accounted for most of the substrate contribution (left schematic). (C) Tilting coefficient $T(v)$ connecting the shape of the warping profile (right) to its mean angle Θ . Fitting to equation (8) (red line) yielded $a = 1.3$. (D) Equation (10) reproduced the substrate-mediated additional deflection over the full range of relevant pillar aspect ratios and Poisson numbers.

Figure 3.

Force-deflection measurements of macroscopic PDMS pillars using a MEMS force sensor. (A) Micrographs of the pillar top before (*upper*) and during (*lower*) the manipulation by the force sensor. The position of the force sensor was controlled by a micromanipulator and adjusted to about 100 μm below the pillar top. *Insets*: image of the pillar base. (B) Data evaluation. The spring constant of the pillar was determined either directly from the force-distance curve (*upper*) with a correction for the local action of the sensor (see Methods) or by using the maximum deflection from the micrographs and the corresponding force (*bottom*). (C) Double-logarithmic plot of the pillar spring constant for three PDMS samples of different rigidity. On each sample, pillars with 1 mm diameter and a height ranging from 2 to 9 mm

were investigated. Dashed lines indicate a $(L/D)^{-3}$ dependence as it is expected for bending only. (D) Pillar spring constants of all samples after rescaling with the bending formula. The pillars are up to 40% softer at small aspect ratios than predicted by the bending formula. The data are well described by equation (12) with $a = 1.3$. Error bars result from Gaussian error propagation of error bars in (B) and from the s.d. of the measured material's Young's modulus.

Figure 4.

Importance of pillar base tilting for the correct derivation of cellular forces. (A) Mechanical equivalent of a pillar on an elastic substrate: serial connection of springs for bending, shear, and tilting along with their qualitative dependence on the pillar aspect ratio. (B) Individual contributions of bending, shear, and tilting to total pillar deflection for a Poisson ratio of 0.45. Calculations based on bending only can be corrected for the neglected tilting and shear by rescaling the derived force value with a correction factor (see also Table 1). (C) Reevaluation of literature data: maximum forces exerted by fibroblasts. Original data (*open symbols*) were taken from Fig. 2 in reference ⁴³ and both the spring constant and the force were multiplied by the correction factor (*full symbols*) according to the aspect ratio of the respective measurements (see Eqs. (11)–(13)). Reference ⁴³ did not specify the pillar diameter for each data point, thus we assumed a diameter of 2 μm (rather than 1 μm) for all pillars. With 1 μm , the correction would even have been more pronounced. Straight lines connect respective data points, dashed lines indicate plateau forces.

Table 1

Multiplication factor for the correct calculation of the pillar spring constant and of forces according to equation (12).

		Pillar aspect ratio L/D									
		1.5	1.7	2	2.5	3	4	5	6	8	10
Poisson ratio ν	0.3	0.56	0.60	0.65	0.71	0.75	0.81	0.84	0.87	0.90	0.92
	0.4	0.56	0.60	0.65	0.71	0.76	0.81	0.85	0.87	0.90	0.92
	0.5	0.57	0.61	0.66	0.72	0.77	0.82	0.86	0.88	0.91	0.93