Published in final edited form as:

Thin Solid Films. 2017 February 28; 624: 181–186. doi:10.1016/j.tsf.2017.01.017.

Elasticity patterns induced by phase-separation in polymer blend films

Joanna Raczowska^{a,*}, Szymon Prauzner-Bechcicki^b, Paweł Dłubczyński^a, and Renata Szydłak^c

^aThe Marian Smoluchowski Institute of Physics, Jagiellonian University, Łojasiewicza 11, 30-428 Kraków, Poland ^bThe Henryk Niewodniczański Institute of Nuclear Physics, Polish Academy of Sciences, Radzikowskiego 152, 31-342 Kraków, Poland ^cChair of Medical Biochemistry, Jagiellonian University Medical College, Kopernika 7, 31-034 Kraków, Poland

Abstract

Systematical studies on the impact of the thickness of thin films composed of *polystyrene (PS)* or *poly(ethylene oxide) (PEO)* on the effective elasticity of polymer-decorated soft *polydimethylsiloxane* substrate were performed. For both investigated polymer films, elasticity parameter was determined from force-displacement curves recorded using atomic force microscopy. *Effective stiffness* of supported film *grows monotonically with film thickness*, starting from the value comparable to the elasticity of soft support and reaching plateau for polymer layers thicker than 200 nm. In contrary, for films cast on hard support no significant thickness dependence of elasticity was observed and the value of elasticity parameter was similar to the one of the substrate. Based on these results, *non-conventional method* to produce *elasticity patterns* of various shapes and dimensions induced by *phase-separation* process in symmetric and asymmetric PS:PEO blend films on soft support was demonstrated. *Elevated PS domains were characterized by elasticity parameter 2 times higher than lower PEO matrix*. In contrary, adhesion force was increased more than 3 times for PEO regions, as compared to PS areas.

Keywords

Elasticity patterns; Thin films; Polymer blend; Phase separation; Thickness-dependent elasticity; Polystyrene; Poly(ethylene oxide); Polydimethylsiloxane

1. Introduction

Thin polymer films have large variety of applications, ranging from biomedicine to electronics [1,2] as protective and functional coatings. They enable to design innovative devices simply by combining the attributes of bulk material with desired properties of covering polymer layer, such as wettability, non-fouling, biocompatibility, adhesion, lubrication or friction modification. Thin polymer films are very attractive as biomaterial coatings, giving the possibility to control the surface properties in the way enabling its

*Corresponding author. joanna.raczowska@uj.edu.pl (J. Raczowska).

integration with host tissue, due to a great versatility in the chemical groups that can be incorporated [3,4]. Mostly investigated factors influencing cellular response include surface topography [5–12], chemistry [13–18] and wettability [19–21]. However, it has been shown that also mechanical properties of the substrate play an important role for adhesion and proliferation of cells, which can be enhanced or prohibited, depending on subtle changes of substrate elasticity [22–28]. Cells are able to react to external elasticity and even generate internal forces, enabling detection of extracellular environmental properties [29,30]. Physical signals are transduced into biochemical changes [29,31–34], most probably through complex mechanisms [34–36], including conformational changes or unfolding of distinct subsets of proteins [31,33]. Cellular sensing and responding to altered external elasticity is involved in numerous biological processes, such as aging, cardiovascular disease, wound healing [30,37–40] and oncogene activity [41–43]. Therefore, it is important to remember, that thin polymer layer may change also mechanical properties of the substrate [44–47], influencing its interactions with cells which can sense the effective stiffness of rigid objects that are not in direct cellular contact, up to several microns [48].

There are numerous experimental techniques enabling determination of physico-chemical properties of supported thin polymer layers, including atomic force microscopy (AFM)-based force spectroscopy [49–57], nanoindentation [46,58–60], acoustic waves measurements or buckling-based metrology [44]. In case of indentation experiments, the measured parameters are usually combination of film and substrate properties. Therefore several theoretical methods have been proposed, to describe properly such a complex substrate-coating system [56,58, 60–62].

In this paper we propose non-conventional method to prepare elasticity patterns by coating soft polydimethylsiloxane (PDMS) substrate with thin polymer blend films composed of poly(ethylene oxide) (PEO) and polystyrene (PS).

For this purpose, we performed systematical studies on the impact of the thickness of decorating layer on effective elasticity of soft substrate (Young modulus $E = 1.67$ MPa [63]) covered with thin polymer film.

For both investigated polymer films, their stiffness measured using AFM-based force-displacement curves grows monotonically with film thickness, starting from the value comparable to the substrate elasticity and reaching plateau for polymer layers thicker than 200 nm.

Motivated by these results, we have fabricated thin polymer films of binary PS:PEO mixture, where phase separation process leads to the formation of specific patterns of surface topography reflecting phase domains. Thus, we were able to fabricate patterns of elasticity, reflecting the surface topography, traced with AFM. Proposed method gives the possibility to produce elasticity patterns of desired shapes and dimensions, simply by proper adjustment of polymer mixture composition and casting conditions [64,65]. Moreover, the range of accessible elasticities may be tuned by the initial stiffness of supporting PDMS layer. Preferential adhesion of cells to lower, softer areas is strengthened by properties of the polymers chosen for the study, which exhibit significant difference in cell adhesion ability

and are commonly used to fabricate substrates for guided cell adhesion [66,67]. This enables not only precise positioning of cells, essential for potential applications in cell-based biosensors and microarrays [68,69] but also basic studies on understanding how do single cells sense their mechanical environment [36,70].

2. Experimental details

2.1. PDMS substrate preparation

The PDMS mixture was prepared using commercially available Sylgard 184 (Dow Corning) with the base:curing agent mass ratio of 10:1. The PDMS substrates were prepared on the 25 mm round coverslip glass using a spin-coating technique, resulting in PDMS films with thickness of ~60 μm . The spinning speed was 500 rpm.

2.2. Thin polymer films

Polymer solutions of polystyrene (PS, Sigma Aldrich) and polyethylene oxide (PEO, Polymer Standard Service) in chloroform were used to prepare thin polymer films. The drop of solution was placed on PDMS or Si substrate and spun-cast (KW-4A, Chemat Scientific) with spinning speed ω varying from 1000 to 8000 rpm. To control thickness of films in a broader range, three polymer concentrations were used, i.e. 10, 20 and 30 mg/mL.

To prepare polymer blend films, PS:PEO binary mixtures (1:1, 2:1 and 3:1 w:w) were dissolved in chloroform ($c_p = 10$ and 30 mg/mL) and spun-cast onto PDMS substrate ($\omega = 3000$ rpm).

2.3. Film thickness measurements

The thin film thickness was measured by spectroscopic ellipsometer (SENTECH SpectraRey/3) equipped with micro spot. Due to optical transparency of PDMS substrate, direct measurements of thin films cast onto soft support were not possible. Therefore, for each sample its counterpart on SiO_x substrate was prepared and used for film thickness evaluation.

The ellipsometric angles Ψ and Δ were determined for wavelengths λ in a spectral range between 320 and 800 nm. The measurement were taken at angles of incidence and detection of 70° . The polymer layers were modeled as Cauchy layer of thickness d with an refractive index $n(\lambda)$ of the form:

$$n(\lambda) = n_0 + n_1 \lambda^{-2} + n_2 \lambda^{-4}$$

Layer thickness and parameter n_0 , n_1 , n_2 were varied numerically in order to achieve the best agreement between the model and the measured values $n(\lambda)$ and $\Psi(\lambda)$. The thickness of silicon oxide layer on the top of substrate were measured independently and accounted for in the model.

2.4. Force–displacement curves

Commercially available AFM system AFM-5500 from Keysight (USA) was used to measure force-displacement curves. The PDMS substrates were mounted in the AFM stage and the AFM probe (PPP-FMR, Nanosensors) was brought close to the surface. The nominal spring constant for PPP-FMR (Nanosensors) cantilevers is 2 N/m and the tip radius less than 10 nm. To determine actual spring constant force-distance curves on reference, infinitely hard Si support were collected, resulting in spring constant equal to 0.97(3) N/m.

Force curves (i.e. dependencies between the applied load force and relative displacement) were collected within a grid of 8×8 points separated by 100 nm termed here a stiffness map with constant loading rate equal to 290 nN/s. Each map was recorded in three different places on the studied surface.

From the recorded approach part of the force curve, a slope was determined using a linear regression and expressed in N/m. The slope was used as ‘elasticity parameter’ to measure sample stiffness.

2.5. Elastic properties imaging

Imaging of local stiffness properties of a substrate was performed using Nanowizard IV AFM (JPKSPM Data Processing 6.0.032, Germany). Imaging was done using quantitative imaging (QI) mode, which gives the possibility to collect a force-distance curve at each pixel of scanned area. As a result map of mechanical properties is collected for given substrate. Representative AFM micrographs $10 \times 10 \mu\text{m}^2$ (64×64 pixels) size were recorded. All collected force distance curves were analyzed in order to get information about the distribution of substrate stiffness at microscale area ($100 \mu\text{m}^2$). For all QI experiments the top value of the guaranteed tip radius (10 nm) was assumed. QI measurements were performed in air, at ambient conditions with fixed probing rate, equal to 0.1 s per line.

2.6. AFM

Topographic images were recorded on randomly chosen regions of the sample surface. Measurements were carried out at room temperature in air using commercially available Agilent 5500 system (Keysight) working in non-contact mode. They were performed with non-coated super sharp silicon probes.

3. Results and discussion

3.1. Film thickness

Polymer film thickness h may be controlled by spin-casting conditions, mainly by total polymer concentration in solution (c_p) and spinning speed ω . According to the theoretical eq. [64], final thickness of spin-coated film $h \sim \frac{c_p}{\sqrt{\omega}}$. For the investigated PS and PEO layers, film thickness measured using ellipsometry grows linearly with $\frac{c_p}{\sqrt{\omega}}$ (Fig. 1) in accordance with theoretical predictions [64].

Linear relation between measured film thickness and experimental conditions of spin-casting, given by formula:

$$h = -69(14)[nm] + 946(43) \left[nm \cdot \frac{mL}{mg} \cdot rpm^{1/2} \right] \cdot \frac{c_p}{\sqrt{\omega}},$$

enables repeatable preparation of polymer layers with pre-defined thickness.

3.2. Polymer films elasticity

Thin polymer films are commonly used as coatings modifying desired surface properties of the bulk material, such as wettability, non-fouling, biocompatibility, adhesion, lubrication or friction modification. However, coverage with polymer layer changes the mechanical properties of the substrate simultaneously [44–46], especially for hard film on a soft support. This effect can have great consequences for potential applications of polymer coatings in biomedical field as cells are very sensitive to substrate elasticity [28,71–73] and can sense the effective stiffness of rigid objects that are not in direct cellular contact, up to several microns [48].

Therefore we have performed systematic studies on film thickness dependent elasticity of soft PDMS substrate ($E = 1.7$ MPa [63]) decorated with stiffer polymer layers composed of PS ($E = 3.4$ GPa [44,49]) or PEO ($E = 0.2$ – 0.6 GPa [74,75]). The polymer substrates were characterized by the presence of strong attractive forces observed during the load. This made the widely applied Hertz model unable to be used [76]. To overcome, the elasticity of polymer substrate was quantified by slope determined from the approach part of the force-displacement curve recorded using AFM, defined as ‘elasticity parameter’ and expressed in N/m as sketched schematically in Fig. 2a.

Typical force curves, recorded for thin and thick polymer layers cast on soft PDMS substrate (Fig. 2b and c) show the slope of the linear part of the curves significantly smaller for thin films, as compared to thick ones. To analyze quantitatively the impact of film thickness on effective elasticity of the substrate, the slope of the curves recorded for each polymer coating was determined using linear regression. The results, calculated for PS and PEO layers are presented in Fig. 3a and b, respectively. For PS film (Fig. 3a), the initial elasticity parameter, recorded for films of 50 nm, equals approximately to 0.31(2) N/m and is comparable with the value recorded for pure PDMS substrate (0.28(2) N/m). Then, the elasticity parameter grows linearly with film thickness, to reach 0.76(2) N/m for PS layer of 200 nm and remains constant for thicker films. Observed results are in good agreement with the literature data [29–30], recorded for ultrathin PS layers on soft substrate.

For PEO layer the overall characteristic is slightly different (Fig. 3b). The initial value of elasticity (0.32(3) N/m) is maintained up to film thickness of 125 nm, then grows linearly to 0.59(2) N/m at 200 nm and do not exhibit any noticeable changes for thicker films. Observed differences in the course of elasticity parameter changes may be related to the bulk stiffness of decorating polymer, reduced by one order of magnitude for PEO, as compared to PS.

To verify the substrate-dependence of this effect, polymer films were cast additionally on substantially harder Si substrates ($E = 130$ to 188 GPa [77]). For both polymer coatings, measured elasticity parameters, presented as green diamonds in Fig. 3, do not show any significant thickness-dependent changes. However, for films thicker than 200 and 300 nm for PEO and PS, respectively, slight reduction of elasticity parameter can be observed, from the value comparable with Si stiffness ($0.97(3)$ N/m) to $0.91(1)$ N/m for thicker films. Similar effect of polymer-specific enhancement of the effective modulus for very thin polymer films has been observed by P. Chung et al. [46].

3.3. Patterns of elasticity induced by phase-separation

Phase separation in thin polymer films, initiated by evaporating solvent during spin-coating process, may lead to formation of lateral phase-domain structure. This structure is very often reflected by surface topography as a consequence of different solidification rates of different phases, varied with solubility parameter [78] or glass temperature [79, 80]. Moreover, the shape and dimensions of resulting morphology may be controlled by blend composition and total polymer concentration in solution, respectively [64,81], as presented in Fig. 4.

Regular island-like domains observed for symmetric PS:PEO blend (Fig. 4a and d) evolve into large-scale elongated structures (Fig. 4b and e) for slightly asymmetric blend and round hole-like structures for films with strongly excessive PS material (Fig. 4c and f) in accord with theoretical predictions [81]. Analogously to film thickness, lateral dimensions of observed domains scale linearly with total polymer concentration and significantly larger structures are observed for higher c_p values (Fig. 4d–f) [64].

This effect, together with thickness-dependent elasticity of polymer films, should enable formation of various elasticity patterns, induced by phase-separation process in polymer blend film. To verify this hypothesis symmetric PS:PEO blend film on PDMS substrate was complexly analyzed. Resulting film topography, recorded using AFM (Fig. 5a) depicts elevated islands composed of PS, surrounded by PEO matrix shallower by approximately 150 nm (Fig. 5b) [82].

To investigate whether such small height difference between phase-separated domains leads to any noticeable modification of surface mechanical properties, the quantitative imaging (QI)-AFM mode was employed. Resulting stiffness and adhesion maps of PS:PEO blend films are presented in Fig. 6.

The comparison of all recorded images show direct correspondence between topography (Fig. 6a), stiffness (Fig. 6b) and adhesion (Fig. 6b) of examined PS/PEO blend film. Measured adhesion force is more than 3 times higher for PEO regions, as compared to PS areas (Fig. 6c). As adhesive properties of the surface depend strongly on the roughness [83–88] this effect may be related to the difference in surface roughness of both polymers, determined by root-mean-square (rms) value, equal to $0.46(28)$ nm and $2.27(11)$ nm for PS and PEO, respectively. In contrast, elasticity parameter show inverse tendency and is approximately twice higher on PS islands as compared to PEO matrix. This enables production of substrates with elasticity patterns of desired shape and dimensions which can be applied for precise positioning of cells.

4. Conclusions

In this paper systematical studies on the impact of the thickness of decorating layer on the effective elasticity of thin films composed of PS or PEO cast on soft substrate were performed. For both investigated polymer films, their stiffness derived from AFM force-displacement curves grows monotonically with film thickness, starting from the value comparable to the PDMS elasticity and reaching plateau for polymer layers thicker than 200 nm. Although the overall characteristic was similar, the exact values of elasticity were polymer specific. In contrary, for films cast on hard support no significant thickness dependence of elasticity was observed.

Inspired by these results, we have fabricated thin polymer films of binary PS:PEO mixture, where phase separation process leads to the formation of specific patterns of surface topography reflecting phase domains. Elasticity and adhesion maps, recorded using QI mode of AFM, show that mechanical properties mirror exactly the topography of surface, enabling production of elasticity patterns induced by phase-separation. The shape and dimensions of the pattern may be tuned by polymer blend composition and casting conditions whereas the range of accessible elasticities may be controlled by the initial stiffness of supporting PDMS layer. Moreover, preferential adhesion of cells to lower areas may be additionally strengthened by the significant difference in cell adhesion ability of both blend components. This gives a powerful tool to study the stiffness-dependent interactions between cells and their environment, crucial for numerous cellular processes. Moreover, it can be used for precise positioning of cells, applicable for cell-based microarrays and biosensors.

Acknowledgments

This work was financially supported by the project National Science Centre agreement number UMO-2013/09/D/ST5/03859. The research was partly carried out with the equipment purchased thanks to the financial support of the European Regional Development Fund in the framework of the Polish Innovation Economy Operational Program (contract no. POIG.02.01.00-12-023/08).

References

1. Griesser, H., editor. Thin Film Coatings Biomater. Biomed. Appl. first. Woodhead Publishing; Cambridge: 2016.
2. Geoghegan, M., Hadzioannou, G. Oxford Master Series in Physics. first. Vol. 22. Oxford University Press; Oxford: 2013. Polymer electronics.
3. Vendra, VK., Wu, L., Krishnan, S. Polymer thin films for biomedical applications. In: Kumar, CSSR., editor. Nanostructured Thin Films and Surfaces. first. Wiley; Weinheim: 2010. p. 1-51.
4. Vadgama P. Surface biocompatibility. Annu. Rep. Prog. Chem., Sect. C: Phys. Chem. 2005; 101:14–52. <http://dx.doi.org/10.1039/b408906p>.
5. Gallego-Perez D, Higuera-Castro N, Denning L, DeJesus J, Dahl K, Sarkar A, Hansford DJ. Microfabricated mimics of in vivo structural cues for the study of guided tumor cell migration. Lab Chip. 2012; 12:4424–4432. <http://dx.doi.org/10.1039/C2LC40726D>. [PubMed: 22936003]
6. Tzvetkova-Chevolleau T, Stéphanou A, Fuard D, Ohayon J, Schiavone P, Tracqui P. The motility of normal and cancer cells in response to the combined influence of the substrate rigidity and anisotropic microstructure. Biomaterials. 2008; 29:1541–1551. <http://dx.doi.org/10.1016/j.biomaterials.2007.12.016>. [PubMed: 18191193]

7. Nikkhah M, Strobl JS, Schmelz EM, Roberts PC, Zhou H, Agah M. MCF10A and MDA-MB-231 human breast basal epithelial cell co-culture in silicon micro-arrays. *Biomaterials*. 2011; 32:7625–7632. <http://dx.doi.org/10.1016/j.biomaterials.2011.06.041>. [PubMed: 21764441]
8. Nikkhah M, Edalat F, Manoucheri S, Khademhosseini A. Engineering microscale topographies to control the cell-substrate interface. *Biomaterials*. 2012; 33:5230–5246. <http://dx.doi.org/10.1016/j.biomaterials.2012.03.079>. [PubMed: 22521491]
9. Tanaka M. Design of novel 2D and 3D biointerfaces using self-organization to control cell behavior. *Biochim. Biophys. Acta Gen. Subj.* 2011; 1810:251–258. <http://dx.doi.org/10.1016/j.bbagen.2010.10.002>.
10. Ramalingam M, Tiwari A. Spatially controlled cell growth using patterned biomaterials. *Adv. Mater. Lett.* 2010; 1:179–187. <http://dx.doi.org/10.5185/amlett.2010.9160>.
11. Mitchel JA, Hoffman-Kim D. Cellular scale anisotropic topography guides Schwann cell motility. *PLoS One*. 2011; 6 <http://dx.doi.org/10.1371/journal.pone.0024316>.
12. Kim D, Han K, Gupta K, Kwon KW, Suh KY, Levchenko A. Mechanosensitivity of fibroblast cell shape and movement to anisotropic substratum topography gradients. *Biomaterials*. 2009; 30:5433–5444. <http://dx.doi.org/10.1016/j.biomaterials.2009.06.042>. [PubMed: 19595452]
13. Blackstone BN, Willard JJ, Lee CH, Nelson MT, Hart RT, Lannutti JJ, Powell HM. Plasma surface modification of electrospun fibers for adhesion-based cancer cell sorting. *Integr. Biol. (Camb)*. 2012; 4:1112–1121. <http://dx.doi.org/10.1039/c2ib20025b>. [PubMed: 22832548]
14. Li C, Glidle A, Yuan X, Hu Z, Pulleine E, Cooper J, Yang W, Yin H. Creating “living” polymer surfaces to pattern biomolecules and cells on common plastics. *Biomacromolecules*. 2013; 14:1278–1286. <http://dx.doi.org/10.1021/bm4000597>. [PubMed: 23495918]
15. Richter C, Reinhardt M, Giselsbrecht S, Leisen D, Trouillet V, Truckenmüller R, Blau A, Ziegler C, Welle A. Spatially controlled cell adhesion on three-dimensional substrates. *Biomed. Microdevices*. 2010; 12:787–795. <http://dx.doi.org/10.1007/s10544-010-9433-2>. [PubMed: 20480241]
16. Wu CC, Yuan CY, Ding SJ. Effect of polydimethylsiloxane surfaces silanized with different nitrogen-containing groups on the adhesion progress of epithelial cells. *Surf. Coat. Technol.* 2011; 205:3182–3189. <http://dx.doi.org/10.1016/j.surfcoat.2010.11.036>.
17. Nie Z, Kumacheva E. Patterning surfaces with functional polymers. *Nat. Mater.* 2008; 7:277–290. <http://dx.doi.org/10.1038/nmat2109>. [PubMed: 18354414]
18. Dinca V, Palla-Papavlu A, Paraico I, Lippert T, Wokaun A, Dinescu M. 2D spatially controlled polymer micro patterning for cellular behavior studies. *Appl. Surf. Sci.* 2011; 257:5250–5254. <http://dx.doi.org/10.1016/j.apsusc.2010.09.113>.
19. Dewez J, Lhoest J, Detrait E, Berger V, Vincent L, Schneider Y, Bertrand P, Rouxhet PG. Adhesion of mammalian cells to polymer surfaces: from physical chemistry of surfaces to selective adhesion on defined patterns. *Biomaterials*. 1998; 19:1441–1445. [PubMed: 9794515]
20. Sun K, Xie Y, Ye D, Zhao Y, Cui Y, Long F, Zhang W, Jiang X. Mussel-inspired anchoring for patterning cells using polydopamine. *Langmuir*. 2012; 28:2131–2136. <http://dx.doi.org/10.1021/la2041967>. [PubMed: 22085048]
21. Fuard D, Tzvetkova-Chevolleau T, Decossas S, Tracqui P, Schiavone P. Optimization of poly-dimethyl-siloxane (PDMS) substrates for studying cellular adhesion and motility. *Microelectron. Eng.* 2008; 85:1289–1293. <http://dx.doi.org/10.1016/j.mee.2008.02.004>.
22. Sun Y, Jiang LT, Okada R, Fu J. UV-modulated substrate rigidity for multiscale study of mechanoresponsive cellular behaviors. *Langmuir*. 2012; 28:10789–10796. <http://dx.doi.org/10.1021/la300978x>. [PubMed: 22742430]
23. Palchesko RN, Zhang L, Sun Y, Feinberg AW. Development of polydimethylsiloxane substrates with tunable elastic modulus to study cell mechanobiology in muscle and nerve. *PLoS One*. 2012; 7:e51499. <http://dx.doi.org/10.1371/journal.pone.0051499>. [PubMed: 23240031]
24. Guvendiren M, Burdick JA. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. *Nat. Commun.* 2012; 3:792. <http://dx.doi.org/10.1038/ncomms1792>. [PubMed: 22531177]

25. Kloxin AM, Benton JA, Anseth KS. In situ elasticity modulation with dynamic substrates to direct cell phenotype. *Biomaterials*. 2010; 31:1–8. <http://dx.doi.org/10.1016/j.biomaterials.2009.09.025>. [PubMed: 19788947]
26. Gray DS, Tien J, Chen CS. Repositioning of cells by mechanotaxis on surfaces with micropatterned Young's modulus. *J. Biomed. Mater. Res. A*. 2003; 66:605–614. <http://dx.doi.org/10.1002/jbm.a.10585>. [PubMed: 12918044]
27. Bhagat AAS, Jothimuthu P, Papautsky I. Photodefinable polydimethylsiloxane (PDMS) for rapid lab-on-a-chip prototyping. *Lab Chip*. 2007; 7:1192–1197. <http://dx.doi.org/10.1039/b704946c>. [PubMed: 17713619]
28. Prauzner-Bechcicki S, Raczkowska J, Madej E, Pabijan J, Lukes J, Sepitka J, Rysz J, Awsiuk K, Bernasik A, Budkowski A, Lekka M. PDMS substrate stiffness affects the morphology and growth profiles of cancerous prostate and melanoma cells. *J. Mech. Behav. Biomed. Mater.* 2015; 41:13–22. [PubMed: 25460399]
29. Schwarz US, Bischofs IB. Physical determinants of cell organization in soft media. *Med. Eng. Phys.* 2005; 27:763–772. <http://dx.doi.org/10.1016/j.medengphy.2005.04.007>. [PubMed: 15951217]
30. Mason, BN., Califano, JP., Reinhart-King, CA. Matrix stiffness: a regulator of cellular behavior and tissue formation. In: Bhatia, SK., editor. *Engineering Biomaterials for Regenerative Medicine*. first. Springer-Verlag; New York: 2012. p. 19–38. <http://dx.doi.org/10.1007/978-1-4614-1080-5>
31. Chowdhury F, Na S, Li D, Poh Y-C, Tanaka TS, Wang F, Wang N. Material properties of the cell dictate stress-induced spreading and differentiation in embryonic stem cells. *Nat. Mater.* 2010; 9:82–88. <http://dx.doi.org/10.1038/nmat2563>. [PubMed: 19838182]
32. Ladoux B, Nicolas A. Physically based principles of cell adhesion mechanosensitivity in tissues. *Rep. Prog. Phys.* 2012; 75:116601. <http://dx.doi.org/10.1088/0034-4885/75/11/116601>. [PubMed: 23085962]
33. Winer, JP., Chopra, A., Kresh, JY., Janmey, PA. Substrate elasticity as a probe to measure mechanosensing at cell-cell and cell-matrix junctions. In: Wagoner Johnson, A., Harley, B., editors. *Mechanobiology of Cell-Cell and Cell-Matrix Interactions*. first. pringer-Verlag; New York: 2011. p. 11–23. <http://dx.doi.org/10.1007/978-1-4419-8083-0>
34. Lange JR, Fabry B. Cell and tissue mechanics in cell migration. *Exp. Cell Res.* 2013; 319:2418–2423. <http://dx.doi.org/10.1016/j.yexcr.2013.04.023>. [PubMed: 23664834]
35. Schwarz US, Safran SA. Elastic interactions of cells. *Phys. Rev. Lett.* 2002; 88:048102. <http://dx.doi.org/10.1103/PhysRevLett.88.048102>. [PubMed: 11801175]
36. De Santis G, Lennon AB, Boschetti F, Verheghe B, Verdonck P, Prendergast PJ. How can cells sense the elasticity of a substrate? An analysis using a cell tensegrity model. *Eur. Cell. Mater.* 2011; 22:202–213. [PubMed: 22048898]
37. Assoian RK, Klein EA. Growth control by intracellular tension and extracellular stiffness. *Trends Cell Biol.* 2008; 18:347–352. <http://dx.doi.org/10.1016/j.tcb.2008.05.002>. [PubMed: 18514521]
38. Kumar S, Weaver VM. Mechanics, malignancy, and metastasis: the force journey of a tumor cell. *Cancer Metastasis Rev.* 2009; 28:113–127. <http://dx.doi.org/10.1007/s10555-008-9173-4>. [PubMed: 19153673]
39. Ng MR, Brugge JS. A stiff blow from the stroma: collagen crosslinking drives tumor progression. *Cancer Cell.* 2009; 16:455–457. <http://dx.doi.org/10.1016/j.ccr.2009.11.013>. [PubMed: 19962663]
40. Zhu Y, Qiu H, Trzeciakowski JP, Sun Z, Li Z, Hong Z, Hill MA, Hunter WC, Vatner DE, Vatner SF, Meininger GA. Temporal analysis of vascular smooth muscle cell elasticity and adhesion reveals oscillation waveforms that differ with aging. *Aging Cell.* 2012; 11:741–750. <http://dx.doi.org/10.1111/j.1474-9726.2012.00840.x>. [PubMed: 22639979]
41. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart- King CA, Margulies SS, Dembo M, Boettiger D, Hammer DA, Weaver VM. Tensional homeostasis and the malignant phenotype. *Cancer Cell.* 2005; 8:241–254. <http://dx.doi.org/10.1016/j.ccr.2005.08.010>. [PubMed: 16169468]
42. Saez A, Ghibaudo M, Buguin A, Silberzan P, Ladoux B. Rigidity-driven growth and migration of epithelial cells on microstructured anisotropic substrates. *Proc. Natl. Acad. Sci. U. S. A.* 2007; 104:8281–8286. <http://dx.doi.org/10.1073/pnas.0702259104>. [PubMed: 17488828]

43. Zaman MH, Trapani LM, Sieminski AL, Siemeski A, Mackellar D, Gong H, Kamm RD, Wells A, Lauffenburger D, Matsudaira P. Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:10889–10894. <http://dx.doi.org/10.1073/pnas.0604460103>. [PubMed: 16832052]
44. Stafford CM, Vogt BD, Harrison C, April RV, Re V, Recei M, June V. Elastic moduli of ultrathin amorphous polymer films. *Macromolecules.* 2006; 39:5095–5099.
45. Ao Z, Li S. Temperature- and thickness-dependent elastic moduli of polymer thin films. *Nanoscale Res. Lett.* 2011; 6:1–6.
46. Chung PC, Glynos E, Green PF. The elastic mechanical response of supported thin polymer films. *ACS Macro Lett.* 2016; 5:439–443.
47. Liao Y, Zhou Y, Huang Y, Jiang L. Measuring elastic–plastic properties of thin films on elastic–plastic substrates by sharp indentation. *Mech. Mater.* 2009; 41:308–318. <http://dx.doi.org/10.1016/j.mechmat.2008.10.008>.
48. Buxboim A, Rajagopal K, Brown AEX, Dennis E. How deeply cells feel: methods for thin gels. *J. Phys. Condens. Matter.* 2010; 22:1–19. <http://dx.doi.org/10.1088/0953-8984/22/19/194116>.
49. Nie H-Y, Motomatsu M, Mizutani W, Tokumoto H. Local elasticity measurement on polymers using atomic force microscopy. *Thin Solid Films.* 1996; 273:143–148. [http://dx.doi.org/10.1016/0040-6090\(95\)06807-4](http://dx.doi.org/10.1016/0040-6090(95)06807-4).
50. Motomatsu M, Nie H-Y, Mizutani W, Tokumoto H. Scanning force microscopy application to polymer surfaces for novel nanoscale surface characterization. *Thin Solid Films.* 1996; 273:304–307. [http://dx.doi.org/10.1016/0040-6090\(95\)06792-2](http://dx.doi.org/10.1016/0040-6090(95)06792-2).
51. Zaitseva AV, Rudoy VM, Dement'eva OV, Kartseva ME. Study of polystyrene surface local mechanical properties by the atomic force microscopy. *Mater. Sci.* 2002; 20:37–43.
52. Shulha H. Some aspects of AFM nanomechanical probing of surface polymer films. *Eur. Polym. J.* 2004; 40:949–956. <http://dx.doi.org/10.1016/j.eurpolymj.2004.01.021>.
53. Du B, Tsui OKC, Zhang Q, He T. Study of elastic modulus and yield strength of polymer thin films using atomic force microscopy. *Langmuir.* 2001; 17:3286–3291.
54. Dimitriadis EK, Horkay F, Maresca J, Kachar B, Chadwick RS. Determination of elastic moduli of thin layers of soft material using the atomic force microscope. *Biophys. J.* 2002; 82:2798–2810. [http://dx.doi.org/10.1016/S0006-3495\(02\)75620-8](http://dx.doi.org/10.1016/S0006-3495(02)75620-8). [PubMed: 11964265]
55. Rezende CA, Lee L-T, Galembeck F. Surface mechanical properties of thin polymer films investigated by AFM in pulsed force mode. *Langmuir.* 2009; 25:9938–9946. <http://dx.doi.org/10.1021/la9010949>. [PubMed: 19705889]
56. Domke J, Radmacher M. Measuring the elastic properties of thin polymer films with the atomic force microscope. *Langmuir.* 1998; 14:3320–3325.
57. Cappella B. Mechanical properties and adhesion of a micro structured polymer blend. *Polymers (Basel).* 2011; 3:1091–1106. <http://dx.doi.org/10.3390/polym3031091>.
58. Zhao M, Xiang Y, Xu J, Ogasawara N, Chiba N, Chen X. Determining mechanical properties of thin films from the loading curve of nanoindentation testing. *Thin Solid Films.* 2008; 516:7571–7580. <http://dx.doi.org/10.1016/j.tsf.2008.03.018>.
59. Chen S, Liu L, Wang T. Investigation of the mechanical properties of thin films by nanoindentation, considering the effects of thickness and different coating – substrate combinations. *Surf. Coat. Technol.* 2005; 191:25–32. <http://dx.doi.org/10.1016/j.surfcoat.2004.03.037>.
60. Zhao M, Chen X, Xiang Y, Vlassak JJ, Lee D, Ogasawara N, Chiba N, Gan YX. Measuring elastoplastic properties of thin films on an elastic substrate using sharp indentation. *Acta Mater.* 2007; 55:6260–6274. <http://dx.doi.org/10.1016/j.actamat.2007.07.045>.
61. Silbernagl D, Cappella B. Mechanical properties of thin polymer films on stiff substrates. *Scanning.* 2010; 32:282–293. <http://dx.doi.org/10.1002/sca.20196>. [PubMed: 20718012]
62. Antunes JM, Fernandes JV, Sakharova NA. On the determination of the Young ' s modulus of thin films using indentation tests. *Int. J. Solids Struct.* 2007; 44:8313–8334. <http://dx.doi.org/10.1016/j.ijsolstr.2007.06.015>.
63. Raczkowska J, Prauzner-Bechcicki S, Lukes J, Sepitka J, Bernasik A, Awsiuk K, Paluszkievicz C, Pabijan J, Lekka M, Budkowski A. Physico-chemical properties of PDMS surfaces suitable as

- substrates for cell cultures. Appl. Surf. Sci. 2016; 389:247–254. <http://dx.doi.org/10.1016/j.apsusc.2016.07.009>.
64. Lawrence CJ. The mechanics of spin coating of polymer films. Phys. Fluids. 1988; 31:2786–2795. <http://dx.doi.org/10.1063/1.866986>.
 65. Raczkowska J, Rysz J, Budkowski A, Lekki J, Lekka M, Bernasik A. Surface patterns in solvent-cast polymer blend films analyzed with an integral-geometry approach. Macromolecules. 2003; 36:2419–2427.
 66. Saltzman, M. Cell interactions with polymers. In: Lanza, RP., editor. Princ. Tissue Eng. second. Elsevier; Worcester, Massachusetts: 2000. p. 221–235.
 67. Welle A, Chiumiento A, Barbucci R. Competitive protein adsorption on micro patterned polymeric biomaterials, and viscoelastic properties of tailor made extracellular matrices. Biomol. Eng. 2007; 24:87–91. <http://dx.doi.org/10.1016/j.bioeng.2006.05.027>. [PubMed: 16861035]
 68. Gonzalez-Macia L, Morrin A, Smyth MR, Killard AJ. Advanced printing and deposition methodologies for the fabrication of biosensors and biodevices. Analyst. 2010; 135:845–867. <http://dx.doi.org/10.1039/b916888e>. [PubMed: 20419231]
 69. Hwang H, Kang G, Yeon JH, Nam Y, Park J-K. Direct rapid prototyping of PDMS from a photomask film for micropatterning of biomolecules and cells. Lab Chip. 2009; 9:167–170. <http://dx.doi.org/10.1039/b810341k>. [PubMed: 19209351]
 70. Jiang L, Sun Z, Chen X, Li J, Xu Y, Zu Y, Hu J, Han D, Yang C. Cells sensing mechanical cues: stiffness influences the lifetime of cell-extracellular matrix interactions by affecting the loading rate. ACS Nano. 2016; 10:207–217. <http://dx.doi.org/10.1021/acs.nano.5b03157>. [PubMed: 26701367]
 71. Discher DE, Janmey P, Wang Y. Tissue cells feel and respond to the stiffness of their substrate. Science. 2005; 310:1139–1143. [PubMed: 16293750]
 72. Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material's perspective. Curr. Opin. Biotechnol. 2013; 24:948–953. <http://dx.doi.org/10.1016/j.copbio.2013.03.020>. [PubMed: 23611564]
 73. Park JY, Yoo SJ, Lee EJ, Lee DH, Kim JY, Lee SH. Increased poly(dimethylsiloxane) stiffness improves viability and morphology of mouse fibroblast cells. BioChip J. 2010; 4:230–236. <http://dx.doi.org/10.1007/s13206-010-4311-9>.
 74. Nie H-Y, Motomatsu M, Mizutani W, Tokumoto H. Local modification of elastic properties of polystyrene-polyethyleneoxide blend surface. J. Vac. Sci. Technol., B Microelectron. Nanometer Struct. 1995; 13:1163. <http://dx.doi.org/10.1116/1.588229>.
 75. Jee A-Y, Lee H, Lee Y, Lee M. Determination of the elastic modulus of poly(ethylene oxide) using a photoisomerizing dye. Chem. Phys. 2013; 422:246–250. <http://dx.doi.org/10.1016/j.chemphys.2012.12.028>.
 76. Lekka M, Gil D, Pogoda K, Duli ska-Litewka J, Jach R, Gostek J, Klymenko O, Prauzner-Bechcicki S, Stachura Z, Wiltowska-Zuber J, Oko K, Laidler P. Cancer cell detection in tissue sections using AFM. Arch. Biochem. Biophys. 2012; 518:151–156. <http://dx.doi.org/10.1016/j.abb.2011.12.013>. [PubMed: 22209753]
 77. Hopcroft MA, Nix WD, Kenny TW. What is the Young's modulus of silicon? J. Microelectromech. Syst. 2010; 19:229–238. <http://dx.doi.org/10.1109/JMEMS.2009.2039697>.
 78. Walheim S, Böltau M, Mlynek J, Krausch G, Steiner U. Structure formation via polymer demixing in spin-cast films. Macromolecules. 1997; 30:4995–5003. <http://dx.doi.org/10.1021/ma9619288>.
 79. Raczkowska J, Bernasik A, Budkowski A, Sajewicz K, Penc B, Lekki J, Lekka M, Rysz J, Kowalski K, Czuba P. Structures formed in spin-cast films of polystyrene blends with poly(butyl methacrylate) isomers. Macromolecules. 2004; 37:7308–7315.
 80. Budkowski A, Bernasik A, Cyganik P, Raczkowska J, Penc B, Bergues B, Kowalski K, Rysz J, Janik J. Substrate-determined shape of free surface profiles in spin-cast polymer blend films. Macromolecules. 2003; 36:4060–4067.
 81. Tanaka H. Hydrodynamic interface quench effects on spinodal decomposition for symmetric binary fluid mixtures. Phys. Rev. E. 1995; 51:1313–1329. <http://dx.doi.org/10.1103/PhysRevE.51.1313>.

82. Zemła J, Lekka M, Raczkowska J, Bernasik A, Rysz J, Budkowski A. Selective protein adsorption on polymer patterns formed by self-organization and soft lithography. *Biomacromolecules*. 2009; 10:2101–2109. [PubMed: 19586049]
83. Ramakrishna SN, Clasohm LY, Rao A, Spencer ND. Controlling adhesion force by means of nanoscale surface roughness. *Langmuir*. 2011; 27:9972–9978. <http://dx.doi.org/10.1021/la201727t>. [PubMed: 21721580]
84. Çolak A, Wormeester H, Zandvliet HJW, Poelsema B. Surface adhesion and its dependence on surface roughness and humidity measured with a flat tip. *Appl. Surf. Sci.* 2012; 258:6938–6942. <http://dx.doi.org/10.1016/j.apsusc.2012.03.138>.
85. Budhe S, Ghumatkar A, Birajdar N, Banea MD. Effect of surface roughness using different adherend materials on the adhesive bond strength. *Appl. Adhes. Sci.* 2015; 3:20. <http://dx.doi.org/10.1186/s40563-015-0050-4>.
86. Persson BNJ, Gorb S. The effect of surface roughness on the adhesion of elastic plates with application to biological systems. *J. Chem. Phys.* 2003; 119:11437–11444. <http://dx.doi.org/10.1063/1.1621854>.
87. Cheng W, Dunn PF, Brach RM. Surface roughness effects on microparticle adhesion. *J. Adhes.* 2002; 78:929–965. <http://dx.doi.org/10.1080/00218460290010584>.
88. Biazar E, Heidari M, Asefnejad A, Montazeri N. The relationship between cellular adhesion and surface roughness in polystyrene modified by microwave plasma radiation. *Int. J. Nanomedicine*. 2011; 6:631–639. <http://dx.doi.org/10.2147/IJN.S17218>. [PubMed: 21698084]

Fig. 1.
Thickness of PS (black full square) and PEO (red empty square) layers prepared with different casting conditions.

Fig. 2. Schematic determination of elasticity parameter (a) and typical force curves recorded for thin (b) and thick (c) PS (black lines) and PEO (red lines) films on soft PDMS substrate.

Fig. 3. Thickness-dependent elasticity of PS (a) and PEO (b) film on soft (black solid squares) and hard (green open diamonds) support.

Fig. 4. Phase separation in PS:PEO blend films controlled by blend composition (rows) and total polymer concentration (columns).

Fig. 5. AFM image of PS/PEO blend film topography (50 × 50 μm, a) with corresponding profile (b).

Fig. 6. QI-AFM image of topography of PS/PEO blend film ($10 \times 10 \mu\text{m}$, a) and corresponding elasticity (b) and adhesion (c) maps.