Biointerphases (2012) 7:39
DOI 10.1007/s13758-012-0039-5

ARTICLE

Dorsal and Ventral Stimuli in Cell–Material Interactions: Effect on Cell Morphology

José Ballester-Beltrán · Myriam Lebourg · Patricia Rico · Manuel Salmerón-Sánchez

Received: 22 April 2012 / Accepted: 15 May 2012

Abstract Cells behave differently between bidimensional (2D) and tridimensional (3D) environments. While most of the in vitro cultures are 2D, most of the in vivo extracellular matrices are 3D, which encourages the development of more relevant culture conditions, seeking to provide more physiological models for biomedicine (e.g., cancer, drug discovery and tissue engineering) and further insights into any dimension-dependent biological mechanism. In this study, cells were cultured between two protein coated surfaces (*sandwich-like* culture). Cells used both dorsal and ventral receptors to adhere and spread, undergoing morphological changes with respect to the 2D control. Combinations of fibronectin and bovine serum albumin on the dorsal and ventral sides led to different cell morphologies, which were quantified from bright field images by calculating the spreading area and circularity. Although the mechanism underlying these differences remains to be clarified, excitation of dorsal receptors by anchorage to extracellular proteins plays a key role on cell behavior. This approach—sandwich-like culture—becomes therefore a versatile method to study cell adhesion in well-defined conditions in a quasi 3D environment.

Electronic supplementary material The online version of this article (doi:10.1007/s13758-012-0039-5) contains supplementary material, which is available to authorized users.

J. Ballester-Beltrán · M. Lebourg · P. Rico · M. Salmerón-Sánchez (✉)
Center for Biomaterials and Tissue Engineering, Universitat Politècnica de València, Valencia, Spain
e-mail: masalsan@fis.upv.es

M. Lebourg · P. Rico · M. Salmerón-Sánchez
CIBER de Bioingeniería, Biomateriales y Nanomedicina, Valencia, Spain

1 Introduction

Cells in multicellular organisms live within tissues, where they are surrounded by the extracellular matrix (ECM), a complex fibrous matrix that provides mechanical support as well as specific biochemical and biophysical signals able to direct cell function [1–3]. Since the natural habitat of most living cells is a tridimensional (3D) mesh surrounding them, culturing cells on bidimensional (2D) surfaces imposes an unnatural environment totally different from the natural ECM. Changes in cell behavior as a way to self-adaptation to the situation occur [4, 5]. For instance, fibroblasts spread on 2D surfaces [6], whereas they adopt a bipolar shape in vivo; moreover, spindle-like shape similar to in vivo is recovered if cells are cultured in 3D collagen gels [7, 8] and tissue-derived matrices, [9] suggesting that morphological alterations are related to the dimensionality of the surroundings. Likewise, the more physiologically relevant 3D environment is increasingly preferred when doing research on cellular processes in vitro, including matrix secretion, cell differentiation, morphogenesis, cancer research and drug development [10–13].

We hypothesize that the excitation of dorsal receptors in cells attached on 2D surfaces could be the link covering the gap between 2D and 3D cell-materials interactions. It has been suggested that the lack of dorsal interaction induces changes in cell morphology and cytoskeletal organization through a calcium signaling pathway [14]. In addition, it has been shown that cell phenotype, functionality and physiology can be drastically altered by exciting dorsal and ventral receptors [15–18]. So, although it can be argued that double-layer cultures might not provide a truly 3D environment, since cells are not isotropically stimulated, the use of this approach permits to engineer a large variety of experimental designs, with different material substrates

Published online: 02 June 2012

and coating proteins, in a robust way (Fig. 1) in environments closer to the in vivo 3D one. This strategy could be useful to engineer biomimetic tissues, directing physiological cell phenotypes and functionality [19]. Moreover, the double layer configuration might shed some light in fundamental cell biology studies, such as the existence of focal adhesions in 3D [20–22]. Dorsal and ventral stimulation strategies, so-called sandwich-like cultures, represent therefore an interesting approach to engineer tissues in a quasi-3D environment.

We have developed a new sandwich-like methodology to investigate the role of dorsal and ventral stimuli on cell morphology, using different material substrates (Fig. 1a). Concretely, we have used as upper substrate thin poly(ethyl acrylate) film and different bottom substrates: (i) spincoated poly(ethyl acrylate) on which FN assembles spontaneously into fibrillar networks in a physiological way [23]; (ii) then, we have used FN adsorbed on glass, which allows cells to reorganize FN at the material interface [24, 25]; (iii) finally, we have investigated the role of topological

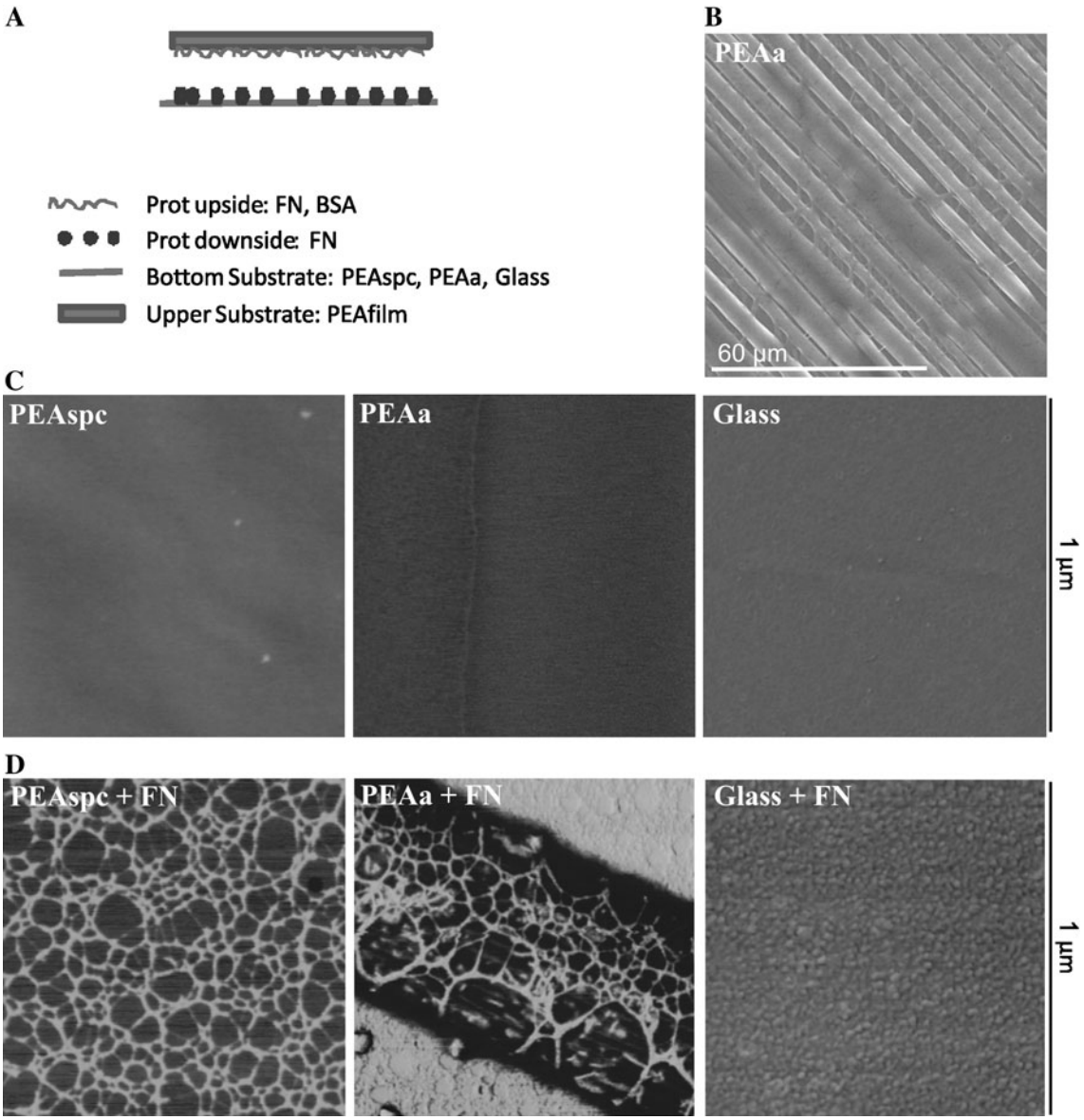

Fig. 1 Model and substrates for sandwich-like cultures. **a** Combination of different substrates and coating proteins offers a wide range of experimental designs. **b** Morphology of aligned PEA fibers as observed by SEM. **c** Representatives images of the substrates surfaces as shown by AFM. **d** Fibronectin distribution on the substrates surfaces as observed by AFM: fibrillar on plain and electrospun PEA (*left, center*) and globular on glass (*right*)

cues making use of aligned fibers obtained via electrospinning. These substrates were coated with different proteins (fibronectin or albumin) and assembled in a sandwich-like configuration. As a widely studied model of cell adhesion and migration in 2D and 3D, NIH3T3 fibroblasts were used; such cells do not naturally display apical–basal polarity, thus allowing to observe specific effect of anchorage of cells on either one or both sides and the influence of its temporal course.

2 Materials and Methods

2.1 Materials

Polymer sheets of ethyl acrylate (EA) (Sigma-Aldrich, Steinheim, Germany) 0.4 mm of thickness were obtained by radical polymerization of a solution of EA using 0.2 wt% benzoin (98 % pure, Scharlau, Barcelona, Spain) as photoinitiator. The polymerization was carried out up to limiting conversion. After polymerization, low molecular-mass substances were extracted from the material by drying in vacuum to constant weight. Rounded samples were cut from the polymerized film to be used as the top substrates of the sandwich. PEA films were washed in an ultrasonic bath for 5 min and hydrated overnight in Dulbecco's phosphate buffered saline (DPBS, Invitrogen) the day before cell culture.

2.2 Spin Coater

Thin films of poly(ethyl acrylate) (PEAspc) were prepared by making use of a spin-coater (Brewer Science, Rolla, USA). PEA was dissolved in toluene at a concentration of 2.5 wt%. Spin casting was performed on glass coverslips at 2,000 rpm for 30 s. Samples were dried under vacuum at 60 °C before use.

2.3 Electrospinning

Electrospun fibers of PEA were collected as described elsewhere [26]. Briefly, PEA 1 % Benzoin was dissolved in hexafluoroisopropanol (HFIP, Sigma) at 20 mg/mL. Polymer solution was electrospun at a constant feed rate of 900 μL/h using a programmable syringe pump (New Era Pump Systems, Wantagh, NY, USA) with a voltage of 12.5 kV (Glassman High Voltage, High Bridge, NJ, USA) and a collector distance of 20 cm. In order to obtain aligned fibers (PEAa) the polymer solution was electrospun onto a rotating drum (rotating at 900 rpm, equivalent to a linear speed of 337.5 cm/s) where glass coverslips were stuck.

2.4 Scanning Electron Microscopy

The electrospun fibers were characterized by scanning electron microscope (SEM) (JEOL JSM 6300, JEOL Ltd., Tokyo, Japan) at 15 kV.

2.5 Protein Adsorption

Fibronectin (FN) from human plasma (Gibco) at 20 μg/mL in DPBS or heat-denatured Bovine Serum Albumin Fraction V (BSA) (Roche) at 10 mg/mL in water were adsorbed on the different substrates by immersing the sample in the protein solutions for 1 h. After adsorption, samples were rinsed in DPBS to eliminate the non-adsorbed protein

2.6 Atomic Force Microscopy

Atomic force microscopy (AFM) was performed in a NanoScope III from Digital Instruments (Santa Barbara, CA) operating in the tapping mode; the Nanoscope 5.30r2 software version was used for image processing and analysis. Si-cantilevers from Veeco (Manchester, UK) were used with force constant of 2.8 N/m and resonance frequency of 75 kHz. The phase signal was set to zero at a frequency 5–10 % lower than the resonance one. Drive amplitude was 200 mV and the amplitude setpoint (Asp) was 1.4 V. The ratio between the amplitude setpoint and the free amplitude (Asp/A0) was kept equal to 0.7.

2.7 Cell Culture

NIH3T3 fibroblasts (European Collection of Cell Cultures) were maintained in DMEM medium with 10 % Calf Serum (Thermo Scientific) and 1 % penicillin–streptomycin (Lonza). Prior to seeding on the substrates, samples (both the top and the bottom ones) were sterilized by UV exposure for 30 min and then coated with FN or BSA as explained. Then, 7,000 cells/cm^2 were seeded in serum free conditions on the different bottom surfaces placed in a multi-well dish. Afterwards a film of PEA was gently laid over the bottom substrate either immediately (*sandwich 0-y*) or after 3 h of culture (*sandwich 3-y*). A highly concentrated cellular suspension was used in order to avoid cell loss after laying the upper substrate. Also, for the *sandwich 3-y*, excess of medium on the bottom surface was removed before laying the film of PEA. After assembling the sandwich a gentle pressure of approx. 10^3 Pa was applied for 3 min on the top surface to facilitate the initial stability of the system. Finally, pressure was released and the medium replenished. Sandwich-like cultures were then maintained at 37 °C in a humidified atmosphere under 5 % CO_2. For longer cultures (24 h) medium was changed by serum-containing medium after 3 h of culture.

Finally, samples were fixed with 10 % formalin solution (Sigma) at 4 °C for 1 h, rinsed with DPBS and observed in a Leica DMI6000 inverted microscope.

2.8 Live/Dead Assay

Viability of cells was measured by live/dead assay (Invitrogen) and analyzed by fluorescence microscopy (Leica DMI 6000). Viability is given as the percentage of living cells.

2.9 Image Processing

All image processing and analysis was done using Adobe Photoshop CS5 and ImageJ. Briefly, brightness and contrast were modified in bright field images in order to define the cell shape with Adobe Photoshop CS5. Thereafter morphology was quantified by calculating cell area and circularity ($4\pi \times \text{area/perimeter}^2$), which corresponds to a value of 1 for a perfect circle using ImageJ software of at least 20 cells for each condition.

2.10 Statistical Analysis

Results are shown as average ± standard deviation. All experiments were performed in triplicate unless otherwise noted. Results were analyzed by one-way ANOVA. If treatment level differences were determined to be significant, pair-wise comparisons were performed ($n \geq 20$). Statistically significant differences are depicted with the following signs. Let sandwich x–y stand for x-hours of ventral contact and y-hours of dorsal contact, then * is for the significance of y comparing among equal total culture times, † for the significance of y comparing for the same x. ϴ shows significance comparing with control for the same culture time and ¥ for conditions with same total culture time but different y (i.e. cells cultured in sandwich 3–3 and sandwich 0–6 adhere similarly on the ventral FN-coated substrate but not to the upper substrate).

3 Results and Discussion

We have checked cell viability within the sandwich-like system at the longest time (1 day). By doing so, we intend to rule out any diffusion problem of the culture medium through the system. As expected, most cells remain viable during the experiment (viability higher than 80 % after 1 day of culture) since a permeable material, PEA, is used as the top surface of the sandwich construct (the diffusion coefficient of water in PEA is $D \sim 3.4 \times 10^7$ cm^2/s) [27].

To address the effect of the initial ventral material interaction before dorsal stimulation, sandwich-like cultures were established either immediately after cell seeding—to prevent any preferential role of ventral receptors—or after 3 h of 2D culture, to permit initial cell adhesion on material surfaces using ventral receptors. Cell culture within sandwiches was maintained up to 24 h to study the time evolution of cell morphology. Hereafter, a two variable nomenclature will be used to easily identify each culture condition: *sandwich x–y*; where *x* stands for the time (hours) of ventral stimulation and *y* for the time (hours) in full sandwich-like culture.

3.1 Dorsal and Ventral Stimulation Using Material-Driven FN Fibrils

We have used FN-coated poly(ethyl acrylate), PEA, for both dorsal and ventral stimulation as FN organizes into interconnected physiological-like fibrils upon adsorption on this material. The fibrillar organization of FN upon passive adsorption on PEA was named material-driven fibrillogenesis, since the assembled FN fibrils on PEA share some similarities with cell-assembled FN matrices [23]. In addition, the resulting fibrillar FN structure on PEA recapitulates the native structure of FN matrices and displays enhanced biological activity [23, 26]. Figure 1c shows the FN network assembled upon adsorption on PEA observed with AFM in comparison with globular organization of FN on glass. FN organization on electrospun PEA fibers is also shown.

Figure 2 shows representative images for cell morphology after different ventral and dorsal stimulations using the material-driven FN network on PEA. Quantifications in terms of spread area and circularity are also included. Overall, it is observed that, regardless the *sandwich x–y* condition, cells spread less compared with 2D control ($p < 0.05$ for all conditions). Since cell volume cannot change that much, this behavior is likely to involve cell spreading in the z-direction, rather than flat spreading as it happens on 2D substrates [28]. Cell morphology is altered, with a significant rounding for 0-y conditions, whereas *sandwich 3-y* substrates show no significant difference with the corresponding controls. Nevertheless, within *sandwich 3–21* cells are significantly rounder than *sandwich 3–3*, suggesting that cells tend to evolve towards a more rounded morphology when sandwiched. This can be also seen in the reduction of cell size, as it is shown by the statistical difference between cell area of *sandwiches 3-y* with control at all times. It seems though that once the cell has started the ventral adhesion process, cell spreading on the material surface provokes morphological changes that are only partially reversible upon dorsal stimulation, as shown by the significant differences both in area and circularity with the samples that were sandwiched at time 0, before cell spreading (shown in Fig. 2). That is to say that comparing systems with the same total culture time, but

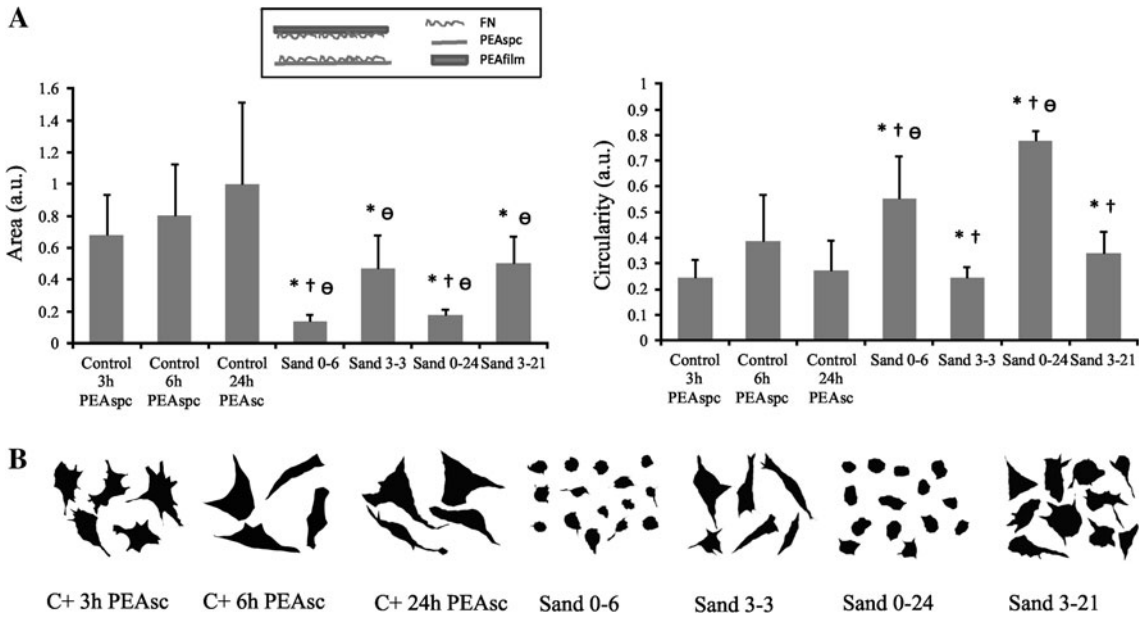

Fig. 2 **a** Cell area and circularity for cells within sandwiches including FN-PEAspc (ventral) and FN-PEA film (dorsal) as sketched in the *inset*. **b** Representative cells outlined from contrast phase microscopy for each one of the conditions

different ratio of dorsal–ventral contact, quite different morphological parameters are obtained, e.g. *sandwich 0–6* and *sandwich 3–3* (the same happens for *sandwich 0–24* and *sandwich 3–21*). The time evolution for the different systems has been built from Fig. 1 and is better shown in Figure S1.

3.2 Effect of Topological Cues at the Ventral Material Interface

Seeking to quantify the relative influence of ventral versus dorsal stimuli, we have investigated cell behavior in an asymmetric system: cells receive topological inputs from the ventral side, as the FN network has been assembled on electrospun PEA fibers (Fig. 1) [29], while the material induced FN network on plane PEA remains as dorsal stimulus. Figure 3 shows the different *sandwich x–y* configurations; cells on aligned electrospun fibers (2D) have been included as control systems.

Cells tend to align and spread under the strong geometrical input coming from the fibers (2D). After 3 h, cells are already aligned and circularity does not change anymore as a function of time (2D). Our results show that upon dorsal stimulation, cells do not continue the interaction with the underlying fibers as in the 2D situation (compare e.g. *sandwich 3–3* and *sandwich 3–21,* Fig. 3), which is somehow frozen in. In addition, this experiment suggests that signaling coming from the dorsal stimuli diminishes the strength of the inputs coming from the ventral topological cues: cells are not able to align on the fibers if the sandwich is assembled from the very beginning. By contrast, cells remain in a rounded-like morphology, with high circularity regardless the underlying ventral topological cues (*sandwich 0–6* and *sandwich 0–24*, Fig. 3). Moreover, even when ventral adhesion on the electrospun fibers is allowed for 3 h before dorsal stimulation, more rounded cells are equally obtained (*sandwich 3–3* and *sandwich 3–21*, Fig. 3). The time evolution of the morphological parameters has been included for this configuration in Figure S2 for easy reading.

3.3 Effect of FN Reorganization at the Ventral Material Interface

Hydrophilic glass is known to be a material surface on which FN adopts a globular-like conformation (Fig. 1c), in such a way, that the strength of interaction between the material surface and the protein is low enough for cells to reorganize the adsorbed FN layer at the cell-material interface [30]. We intend to investigate whether this property confers additional degrees of freedom for cells stimulated on both sides using the sandwich construct. To do so, we have used FN-coated glass as the ventral surface while the material-assembled FN network on PEA was maintained as the dorsal stimulus. Figure 4 shows cell morphology and its quantification for different *sandwich x–y* configurations and the corresponding 2D controls (glass). Cells remain less spread, with lower area and higher circularity, upon simultaneous dorsal and ventral stimulation (0-y) compared to the 2D control. In the case of 3-y sandwiches, cells have significantly lower areas at all times, but circularity is reduced and not different from

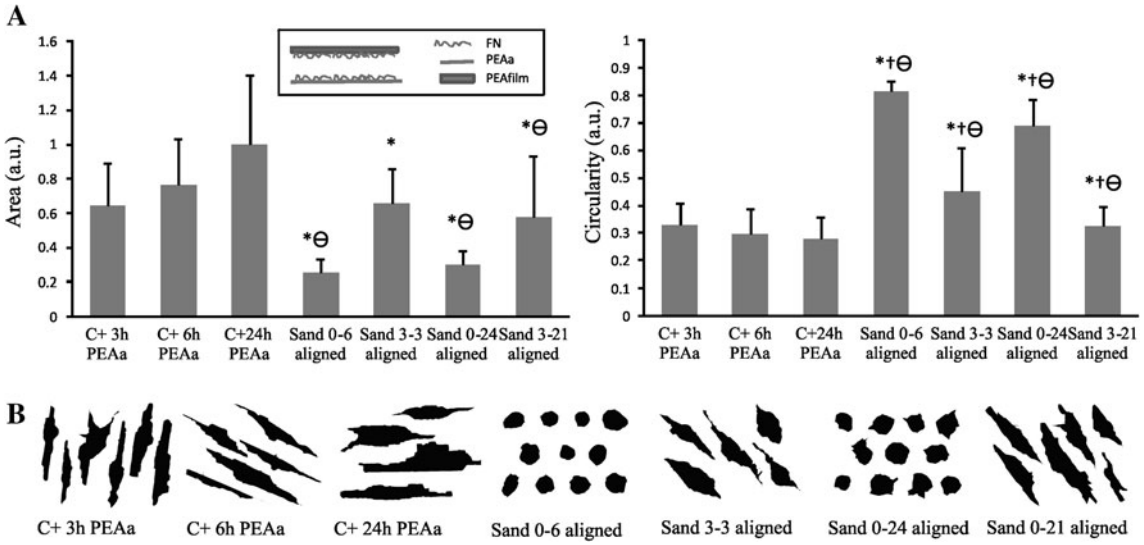

Fig. 3 **a** Cell area and circularity for cells within sandwiches including FN-PEAa (ventral) and FN-PEA film (dorsal) as depicted in the *inset*. **b** Representative cells outlined from contrast phase microscopy for each condition

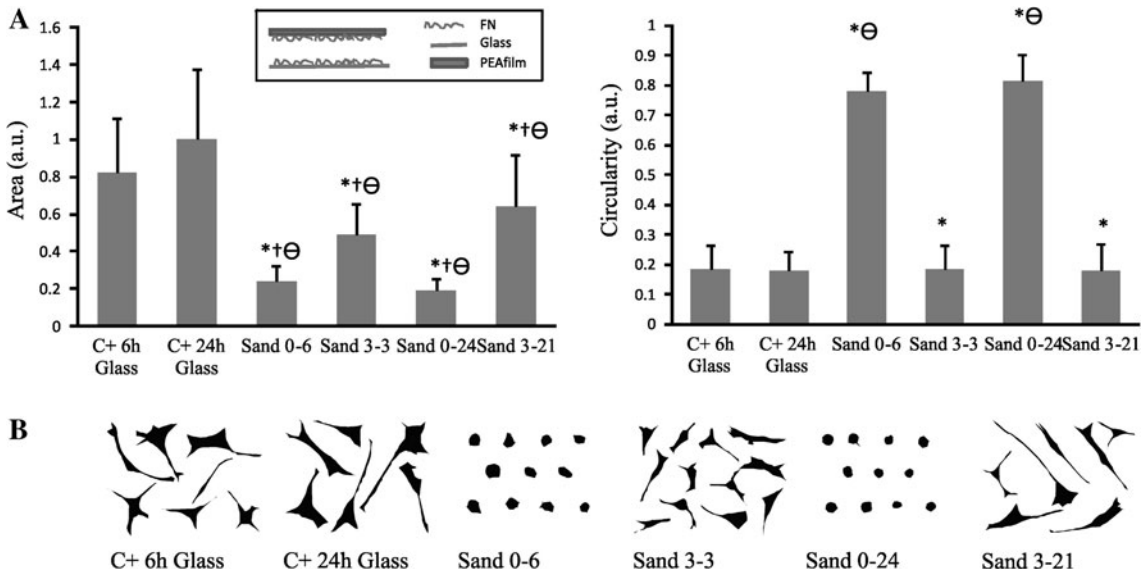

Fig. 4 **a** Cell area and circularity for cells within sandwiches including FN-Glass (ventral) and FN-PEA film (dorsal) as sketched in the *inset*. **b** Representative cells outlined for each one of the conditions as shown by contrast phase microscopy

controls, neither it rises with time as was the case in the PEA sandwiches. As before, cells are somehow frozen in after receiving dorsal stimuli, even if the underlying surface (FN on glass) is now much more mobile. That is to say, the ability to reorganize the underlying (ventral) FN on glass surface does not change the effect of dorsal stimuli after sandwich assembly, nor prevents the inhibition of normal 2D spreading on glass. This is easily observed comparing results for *sandwich 0–6* and *sandwich 0–24* (or *sandwich 3–3* and *sandwich 3–21*). Likewise, although cells are able to reorganize the ventral FN layer, activation of dorsal receptors after initial spreading (3-y) does not revert the attained spreading; e.g. very different morphology (and parameters) are obtained for *sandwich 3–21* and *sandwich 0–24*; or the other way around, morphological parameters for *sandwich 3–21* stay as for *sandwich 3–3* rather than evolve to *sandwich 0–24*. The time evolution for the different systems has been built from Fig. 4 and it is better shown in Figure S3.

3.4 Effect of Non-Adhesive Dorsal Contact

Previous experiments have shown the preferential role that dorsal stimulation plays on the cell-material interaction,

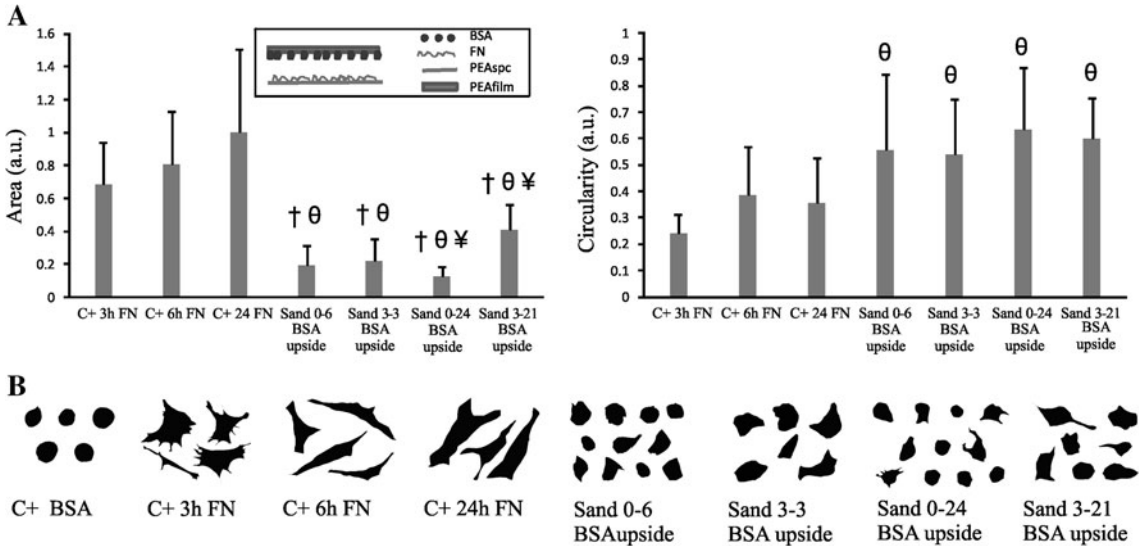

Fig. 5 **a** Cell area and circularity for cells within sandwiches including FN-PEAspc (ventral) and BSA-PEA film (dorsal) as depicted in the *inset*. **b** Representative cells outlined from contrast phase microscopy for each condition

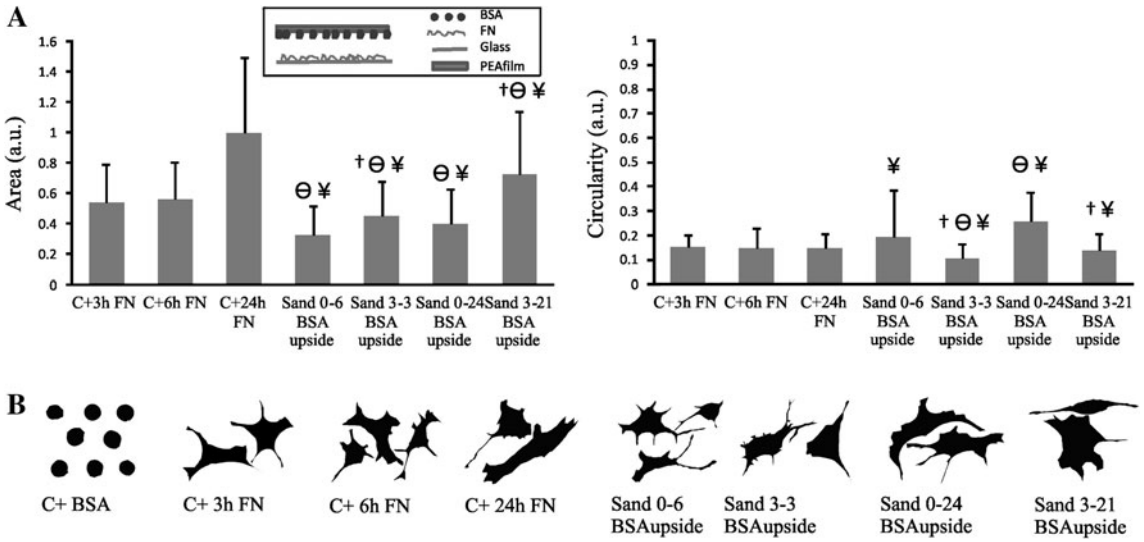

Fig. 6 **a** Cell area and circularity for cells within sandwiches including FN-Glass (ventral) and BSA-PEA film (dorsal) as sketched in the *inset*. **b** Representative cells outlined for each one of the conditions as shown by contrast phase microscopy

regardless the nature of the ventral interaction: neither the existence of topological cues nor the ability to reorganize the ventral layer of proteins disturbs the dominant role of dorsal sensing. We want to clarify now whether this phenomenon is a consequence of a biological cell-protein-material interaction or, by contrast, its origin must be sought as a mere physical interaction through direct contact. To do so, we have used BSA to coat the upper part of the sandwich construct (instead of FN). BSA is known to be a non-adhesive protein on which cells cannot adhere and spread. By contrast, cells remain in a rounded-like morphology on BSA-coated materials [31–33]. Experiments have been done using both FN-coated glass and FN assembled fibrils on PEAspc as substrates for ventral cell adhesion. Figure 5 shows the experimental results for PEAspc. Strikingly, even if cells cannot adhere to the dorsal BSA-coated material, their behavior does not follow the expected one for a cell spreading on a material surface (2D). Had this happened, both spread area and circularity should be the same for cells within the sandwiches and the 2D controls, which is not the case. Moreover, cells within *sandwich 3–3* should behave as in *sandwich 0–6*, since both situations involve 6 h of ventral interaction without any biological dorsal stimulation (idem for *sandwich 3–21* and *sandwich 0–24,* Fig. 5). The situation is slightly different when cells are seeded on FN-coated glass as the

ventral surface. Even if values obtained for the spread area do not reach the 2D situation, cells spread more than if FN were used as dorsal stimuli (Figs. 4, 6). Correspondingly, cell circularity remains quite the same for every spatial condition (sandwich or 2D) and much lower than the values calculated for FN as the dorsal interacting protein (Fig. 4), where true dorsal biological stimuli (integrin-mediated) must occur. That is to say, the role of merely dorsal physical stimuli is strongly linked to the properties of the surface on which cells interact ventrally. On a mobile layer of ventral FN (adsorbed on glass), cells do not feel the physical (BSA-coated PEA) perturbation coming from the dorsal side; which is not so evident when cells are seeded on a more stable (less mobile) physiological-like FN fibrils (on PEAspc). The time evolution for the different systems has been built from Figs. 5 and 6, and it is better shown in Figure S4 and S5 respectively.

3.5 Relevance and Limitations of the Model

Nutrient, oxygen and waste diffusion are important factors to take into account in cell culture, being more important therefore in 3D scaffolds, hydrogels, multi-layer and sandwich cultures. That is the reason why we use thin poly ethyl acrylate (PEA) films with a water diffusion coefficient of $D \sim 3.4 \times 10^7$ cm^2/s as upper substrates in our sandwich-like model. Cell viability was above 80 % during the experiment, showing that oxygen and nutrient diffusion is not a limiting factor in this system. This issue could become relevant at longer times or to transport molecules (such as growth factors) with higher molecular weight, that do not diffuse throughout the polymer. Considering the temporal framework of our experiments, as well as the absence of growth factors in the medium, we assume that our results observed in the sandwich-like cultures must be ascribed to the dorsal interaction, disregarding any diffusion problems that deprive cells from nutrients or other important chemical cues. Nevertheless, it must be taken into account that permeability of the upper substrate is a critical issue in the design of the sandwich-like system, and using less permeable materials as upper substrates could lead to a gradient of cell death, from the centre to the periphery, as happens in tumors. In fact, sandwich-like cultures with limited diffusion has been studied as an approach to supplement multicellular spheroids as tumor analogues [34–36].

Important differences with 3D environment include the lack of isotropy, limitation of cell mobility to the x–y plane, mechanical properties of the substrates and absence of a physiological nanofibrillar environment (although this is somehow mimicked by the fibrillogenesis of fibronectin on PEA). As a result, the round morphology observed in sandwiches 0-y is different from the spindle-like morphology observed in fibronectin-coated acrylamide sandwiches [14] or even in natural ECM [9]. These differences might be sought in the mechanical modulus of the substrates (acrylamide substrates have lower stiffness), although protein composition also seems to be important as cells cultured in 3D collagen gels do not always display the characteristic spindle-like morphology [9]. So, even if our sandwich-like system is not a truly representation of a 3D situation, it is a useful model beyond 2D systems, and it allows a controlled and versatile tuning of the substrates and composition, more difficult to tailor in standard 3D environments such as Matrigel or collagen gels.

The own nature of this system enables a wide range of possibilities. Such model enables to study the effect of external pressure on cell behavior, by simply changing the weight applied; substrates with varying chemistries and mechanical properties can be used. It could also be possible to study the role of cell–cell interaction by seeding both sides of the sandwich system with cells. Furthermore, several conditions can be studied at the same time such as the influence of the pressure on cell–cell interaction. Therefore sandwich-like cultures could become an important system for deciphering cell response under well-defined conditions and later, to translate this knowledge to multi-layer approaches used nowadays on tissue engineering and to 3D environments if a good agreement is achieved (between sandwich and 3D). Representative contrast phase images of cells in several conditions have been included as supplementary material (Figure S6).

4 Conclusions

The use of sandwich-like cultures has shown to be a robust tool to investigate the role of dimensionality in cell-materials interactions. It allows the combination of different adhesive proteins and geometrical inputs in both dorsal and ventral sides.

Overall, initial dorsal and ventral stimuli inhibit cell spreading and give rise to rounded-like cell morphology. However, if dorsal stimuli are applied once cells have already started (ventral) spreading on a material surface, cell stop spreading and somehow freeze into the attained morphology. By contrast, cell retraction into a rounded morphology is not observed as time goes by. Moreover, stimulation of dorsal receptors is strong enough to inhibit the geometrical inputs coming from the ventral side (e.g. alignment of cells along electrospun fibers). Strikingly, cell behavior in 3D environments might not be only a consequence of integrin-mediated adhesion to the surrounding matrix, as BSA-covered top-substrates elicit the same response as FN-coated one. That is to say, not only protein–protein interaction should be considered to explain

cell behavior in 3D environments, but also the role of a pure mechanical-contact interaction must be considered.

Further studies are needed to get more insights into the role of dimensionality in cell behavior using this sandwich-like approach, where the mechanism for cell adhesion must be elucidated. The origin of cell behavior due to mere mechanical dorsal contact might be sought in the need for cell to adhere and build up ventral focal adhesions, in dependence on how protected cells feel their membrane surface. However, this would mean the existence of additional cellular mechanotransduction mechanisms to explore the environment other than integrin mediated ones.

Acknowledgments The support of the project MAT2009-14440-C02-01 and FPU program AP2009-3626 is acknowledged. CIBER-BBN is an initiative funded by the VI National R&D&i Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund.

References

1. Folkman J, Moscona A (1978) Nature 273:345–349
2. Watt FM, Jordan PW, O'Neill CH (1988) Proc Natl Acad Sci USA 85:5576–5580
3. Weiss P (1959) Rev Mod Phys 31:449–454
4. Lewis WH, Lewis MR (1926) In: Cowdry EV (ed) General cytology. University of Chicago Press, Chicago
5. Weiss P (1959) Rev Mod Phys 31:11–20
6. Bard JB, Hay ED (1975) J Cell Biol 67:400–418
7. Elsdale T, Bard J (1972) J Cell Biol 54:626–637
8. Grinnell F (2003) Trends Cell Biol 13:264–269
9. Cukierman E, Pankov R, Stevens DR, Yamada KM (2001) Science 294:1708–1712
10. Hutmacher DW (2010) Nat Mat 9:90–93
11. Lutolf MP, Hubbell JA (2005) Nat Biotech 23:47–55
12. House DJM, Elstad K, Socrate S, Kaplan DL (2012) Tissue Eng Part A 5–6:499–507
13. Rimann M, Graf-Hausner U (2012) Curr Opin Biotech 23:1–7
14. Beningo KA, Dembo M, Wang YL (2004) PNAS 101:18024–18029
15. Dunn JC, Yarmush ML, Koebe HG, Tompkins RG (1989) FASEB J 3:174–177
16. Ryan CM, Carter EA, Jenkins RL, Sterling LM, Yarmush ML, Malt RA, Tompkins RG (1993) Surgery 113:48–54
17. Knop E, Bader A, Boker K, Pichlmayr R, Sewing KF (1995) Anat Rec 242:337–349
18. Guaccio A, Guarino V, Alvarez-Pérez MA, Cirillo V, Netti PA, Ambrosio L (2011) Biotechnol Bioeng 108:1965–1976
19. Gong YY, Xue JX, Zhang WJ, Zhou GD, Liu W, Cao Y (2011) Biomaterials 32:2265–2273
20. Fraley SI, Feng Y, Krishnamurthy R, Kim DH, Celedon A, Longmore GD, Wirtz D (2010) Nat Cell Biol 12:598–604
21. Kubow KE, Horwitz AR (2011) Nat Cell Biol 13:3–5
22. Fraley SI, Feng Y, Wirtz D, Longmore GD (2011) Nat Cell Biol 13:5–7
23. Salmerón-Sánchez M, Rico P, Moratal D, Lee T, Schwarzbauer J, García AJ (2011) Biomaterials 32:2099–2105
24. Altankov G, Groth T (1994) J Mat Sci Mat Med 5:732–737
25. Altankov G, Grinnell F, Groth T (1996) J Biomed Mat Res 30:385–391
26. Ballester-Beltrán J, Cantini M, Lebourg M, Rico P, Moratal D, García AJ, Salmerón-Sánchez MJ (2012) Mat Sci Mat Med 1:195–204
27. Gallego Ferrer G, Monleón Pradas M, Gómez Ribelles JL, Pissis P (1998) J Non-Cryst Soli 235–237:692–696. http://www.sciencedirect.com/science/article/pii/S0022309398005730
28. Chaudhuri O, Parekh SH, Lam WA, Fletcher DA (2009) Nat Methods 6:383–387
29. Gugutkov D, González-García C, Rodríguez Hernández JC, Altankov G, Salmerón-Sánchez M (2009) Langmuir 25:10893–10900
30. Altankov G, Groth T, Krasteva N, Albrecht W, Paul D (1997) J Biomat Sci Polym E 8:721–740
31. Curtis ASG, Forrester JV (1984) J Cell Sci 71:17–35
32. Zelzer M, Albutt D, Alexander MR, Russell NA (2012) Plasma Process Poly 9:149–156
33. Tamada Y, Ikada Y (1993) J Colloid Interf Sci 155:334–339
34. Hlatky L, Alpen EL (1985) Cell Tissue Kinet 6:597–611
35. Hlatky L, Alpen EL, Yee MK (1986) Radiat Res 1:62–73
36. Hlatky L, Sachs RK (1988) Alpen EL 2:167–178