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N.B.: When citing this work, cite the original article.

Original Publication:

Karl Svennersten, Maria H. Bolin, Edwin W.H. Jager, Magnus Berggren and Agneta Richter-Dahlfors, Electrochemical modulation of epithelia formation using conducting polymers, 2009, Biomaterials, (30), 31, 6257-6264.
http://dx.doi.org/10.1016/j.biomaterials.2009.07.059

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Postprint available at: Linköping University Electronic Press
http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-20545

*Title Page

Electrochemical modulation of epithelia formation using conducting polymers

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Abstract

Conducting polymers are soft, flexible materials, exhibiting material properties that can be reversibly changed by electrochemically altering the redox state. Surface chemistry is an important determinant for the molecular events of cell adhesion. Therefore, we analyzed whether the redox state of the conducting polymer PEDOT:Tosylate can be used to control epithelial cell adhesion and proliferation. A functionalized cell culture dish comprising two adjacent electrode surfaces was developed. Upon electronic addressing, reduced and oxidized surfaces are created within the same device. Simultaneous analysis of how a homogenous epithelial MDCK cell population responded to the electrodes revealed distinct surface-specific differences. Presentation of functional fibronectin on the reduced electrode promoted focal adhesion formation, involving $\alpha_v\beta_3$ integrin, cell proliferation, and ensuing formation of polarized monolayers. In contrast, the oxidized surface harbored only few cells with deranged morphology showing no indication of proliferation. This stems from the altered fibronectin conformation, induced by the different surface chemistry of the PEDOT:Tosylate electrode in the oxidized state. Our results demonstrate a novel use of PEDOT:Tosylate as a cell-hosting material in multiple-electrode systems, where cell adhesion and proliferation can be controlled by electrochemical modulation of surface properties.

1. Introduction

Cell adhesion, a central process in tissue morphogenesis, involves cell-cell and cell-matrix interactions. These interactions are crucially important for epithelia, which are the tissue that lines many surfaces throughout the body. Proliferation of epithelial cells depends on their adhesion to a surface [1-3]. *In vivo* this may be the basal lamina, whereas protein-coated synthetic materials are used as substrates *in vitro*. Basolaterally located integrins mediate binding to extracellular matrix (ECM) proteins like fibronectin (Fn) located in the basal lamina [4]. As Fn and other ECM proteins also are present in serum and, accordingly, the cell culture medium, these proteins form a surface-adsorbed coating when cells are cultivated *in vitro* [5].

The interaction between cellular integrins and Fn is known to depend on the surface energy, charge, and chemistry [6, 7] of the underlying surface. Insight of these parameters' influence on the complex cell adhesion process is valuable in biomaterial development for tissue engineering. Self-assembled monolayers have been used to create cell culture surfaces with highly defined surface chemistry [7], whereas studies addressing the role of defined micro- and nano-structured topography have been performed using micro-fabrication technology [8]. The latter involves etching techniques not commonly available in a cell biology lab. Soft lithography, on the other hand, does not require advanced equipment and can be used to pattern ECM proteins to surfaces on a µm scale [9]. Similarly, inkjet bio-printing has been demonstrated as a useful approach to bio-functionalize surfaces [10].

Conducting polymers belong to a class of polymers that conduct electricity like metals. The polymer backbone is carbon based, similar to biological materials. The developing field of organic bioelectronics refers to the use of conducting polymer-based electronic devices in biological applications [11]. Poly-3,4-ethylenedioxy thiophene (PEDOT) and polypyrrole (PPy) are conducting polymers commonly used in bio-applications, whose

conducting properties can be further enhanced in the presence of doping ions, such as toluenesulfonate (Tosylate). Application of a negative potential to a PPy electrode alters the morphology of bovine endothelial cells hosted on the electrode, via an unknown molecular mechanism [12]. PPy has also been used as cell-hosting material for neurons. Electronic addressing of PPy increase the formation of neurite extensions, an effect that was correlated to an increase of adsorbed Fn to the electrically stimulated PPy surface [13, 14].

We hypothesize that the reversibility of the redox reactions [15] can be utilized to obtain dynamic control of cell-surface interactions. To this end, a functionalized cell culture dish with multiple PEDOT:Tosylate electrodes was developed. An objective was to analyze whether the redox reaction of the conjugated polymer electrodes affects adsorbed ECM proteins, and whether any consequences of the electrochemical switch are transmitted to the epithelial cells. Hence, a detailed molecular analysis of cell adhesion and proliferation is reported.

2. Materials and Methods

2.1. Preparation of functionalized cell culture dishes

To produce electronically functionalized cell culture dishes, conducting polymer films were chemically polymerized inside polystyrene petri dishes (Ø= 60 mm, Corning) or on flat substrates of poly(ethyleneterephtalate) (PET). A solution with 3,4-ethylenedioxy thiophene (EDOT) (Aldrich) and 20% oxidant iron(III)p-toluenesulfonate ($Fe(OTS)_3$) (Aldrich) in butanol were mixed 1:24 (v/v) before 0.028 g/ml basic inhibitor pyridine was added. The solution was spin coated inside the petri dishes at 1200 rpm for 120 s [16-18] or barcoated on flat PET foils. A homogenous film was formed by moving a coating rod at 150 mm/s along the substrate. The substrates were heated at 40°C for 20 min to facilitate chemical polymerization and to evaporate non-reacted residues. Residual iron tosylate was removed by sequential washes in butanol, isopropanol, and deionized (DI) water; thereafter the films were

blow dried. Polypropylene rings were glued, using Sylgard 184™, to the barcoated films to define a well for cell culturing experiments. The 20% solution of $Fe(OTS)_3$ was chosen as it resulted in optimal optical properties for further cell experiments. The films were separated in two electrodes by a non-conducting break. Electrical leads were connected to the electrodes with conducting copper tape. The tape was applied such that it would not be in contact with the electrolyte. Barcoated films were switched with 1.0 V using a Keithley 2602 or a Hewlett Packard E3632A DC power supply. Spin-coated Petri dishes were switched using a 1.5 V AA battery to enable convenient addressing inside the cell incubator.

When indicated, electrodes were pre-coated with bovine Fn (5 µg/cm^2) (Sigma). Fn diluted in PBS was dispensed onto switched or un-switched surfaces**,** and was incubated at room temperature (> 45 min).

2.2. Characterization of polymer films

Cyclic voltammetry was performed using a Gamry Reference 600 potentiostat operated with Gamry PHE200 software. The three electrode setup was based on an intact 27 cm^2 PEDOT:Tosylate cell culture dish, half of which (13.5 cm^2) was working electrode while the other half was counter electrode. Experiments were performed at room temperature using either PBS or sDMEM as electrolytes. A Princeton K0265 Ag/AgCl reference electrode was used. A scan rate of 40 mV/s was used, and the potential on the working electrode was swept from -0.8 to 0.4 V versus the reference electrode after measuring the open circuit potential for 10 s. Recordings were analyzed using Gamry Echem Analyst 5.50 software.

2.3. Cell culturing procedures

MDCK cells (ATCC, no. CCL-34), was propagated in DMEM (Sigma) supplemented with 10% FBS, 2% Hepes buffer, L-Glutamin (0.3 g/l) and penicillin;streptomycin (100 U/ml;100 µg/ml) (Sigma). When cells were cultured under serum free conditions, 2% Serum

Replacement 1 (Sigma) was used. Cells were detached from the cell culturing flask with Trypsin-EDTA (0.5 g/l trypsin, 0.2 g/l EDTA in PBS) and washed once in DMEM, before they were seeded in functionalized cell culture dishes at a density of 1.0 – 3.0 x 10^5 cells/ml. When indicated, RGD peptide (20 mg/ml in 0.1 N acetic acid, Sigma) was added to the MDCK cell suspension (1.0 – 3.0 x 10^5 cells/ml DMEM) to a final concentration of 1 mg/ml. The cell suspension containing the RGD peptide was immediately added to the functionalized dish, and electrodes were continuously biased throughout the 24 h incubation in a humidified 37ºC, 5% CO_2 cell incubator.

2.4. Preparation of specimens for microscopic analysis

Cells were incubated in the functionalized Petri dishes for 24 h on electrodes constantly biased using 1.5 V, with the potential of the battery tested before and after the incubation. Barcoated samples were biased using 1.0 V with continuous monitoring throughout the 24 h incubation showing a stable potential and a diminishing current. After incubation, areas of approx. 6-8 cm^2 were excised from the Petri dish, whereas the barcoated samples were cut in 1.5 cm^2 squares. All specimens, including cells cultivated on control coverslips, were gently washed with PBS, pH 7.4, fixed in freshly prepared 0.4 or 4.0 % paraformaldehyde in PBS, pH 7.4, for 15 min in room temperature according to suppliers instructions for the individual antibodies. Actin filaments were stained with TRITC-phalloidin (1 µg/ml). Nuclei were stained with Hoechst 33258 (1 µg/ml). The primary antibodies used were: anti-zonula occludens-1, ZO-1 (Zymed); Mouse monoclonal Clone JBS5 to Integrin alpha 5 (Abcam); Mouse Anti-human integrin $\alpha_v\beta_3$ monoclonal antibody (Chemicon); Monoclonal Anti-Talin Clone 8D4 (Sigma); Monoclonal Anti-Vinculin Clone hVIN-1 Mouse Ascites Fluid (Sigma). Secondary antibodies used were goat anti-rabbit-Cy3 (Zymed) and goat anti-mouse-FITC (Abcam).

2.5. Immunofluorescence microscopy and quantification of cell numbers

Immunofluorescence image acquisition was performed using an Eclipse E400 fluorescence microscope (Nikon) equipped with a Hamamatsu C4742-98 digital camera and Wasabi image capture software (Hamamatsu). An UltraVIEW RS-3 laser confocal system with microlens-enhanced Nipkow discs (CSU21; Perkin Elmer) mounted on a Zeiss Axiovert microscope and a Zeiss LSM 510 confocal laser scanning microscope were used for confocal microscopy. The ImageJ plugin "Nucleus counter" (U. S. National Institutes of Health) was used for quantitative analysis of the number of nuclei. Ten visual fields on each electrode were captured straight across the specimen using the 20x objective. Data are displayed as mean ± standard deviation, n = 3- 5, paired students T-test was used to test for significance, p-value < 0.05 was considered significant.

2.6. Measurement of fibronectin adsorption

Potential was applied for one hour to either uncoated or Fn coated surfaces in sDMEM. Fn adsorption was measured as previously described [19]. In brief, polyclonal rabbit anti-Fn (Sigma) detected with an Alexa Fluor 680-tagged secondary antibody were used. Binding was quantified as pixel intensity using an IR scanner.

3. Results

3.1. PEDOT:Tosylate in complex electrolytes

Functionalized cell culture dishes, with two electrode surfaces present in the same device, were prepared by applying PEDOT:Tosylate using spin- or bar-coating procedures. When a potential (1.0-1.5 V) is applied in the presence of an electrolyte, the device functions as a two-electrode electrochemical cell. The potential and associated current drives the reduction and

oxidation reactions occurring at the negative and positive electrode, respectively (Fig. 1a and Equation 1). The electronic switch can be monitored visually as the PEDOT polymer exhibits electrochromic properties at optical wavelengths (Fig. 1b).

$$PEDOT^{+}:Tosylate^{-} + e^{-} + M^{+} \Rightarrow PEDOT^{0} + M:Tosylate \qquad (1)$$

When the potential and current had driven the redox reaction to completion, a distinct drop of the current was observed (Fig 1c). The remaining leakage current stayed in the nA range throughout the entire length of the experiment.

To analyze whether the device is fully functional with complex electrolytes such as cell culture media, cyclic voltammograms were recorded in two standard solutions, i.e phosphate buffered saline (PBS) and supplemented Dulbecco´s Modified Eagle´s Media (sDMEM). One of the PEDOT:Tosylate electrodes was set as working electrode, the other as counter electrode and an Ag/AgCl reference electrode was used. We found that the redox peaks appeared at their normal positions, i.e. the reduction peak at -0.4 V and the oxidation peak at -0.2 V vs Ag/AgCl [15]. The peaks correlate to the color changes in the polymer, suggesting the feasibility of using the electrochromic effect to monitor the redox reaction (Fig. 1c).

3.2. Cell viability on redox surfaces

Next, we analyzed whether epithelial cell adhesion and proliferation can be modulated using the electrochemical surface switch. Immediately (< 1 min) after seeding Madin Darby canine kidney (MDCK) cells into the functionalized dish, electrodes were biased at 1.5V. After 24 h incubation, electrodes were prepared for immunofluorescence microscopy analysis of adherent cells. When staining actin with TRITC-phalloidin and cell nuclei using Hoechst 33258, large numbers of MDCK cells were found to adhere to the reduced electrode, whereas the oxidized electrode hosted only few cells (Fig. 2a). No cells were found on the non-

conducting interface. Detailed morphological analysis demonstrated the reduced electrode's bio-compatibility since actin stress fibers, commonly regarded as a major hallmark of cell adhesion [20], were observed (Fig. 2b). Due to their epithelial origin, adherent MDCK cells form polarized monolayers [21]. This specialized epithelial phenotype was also clearly observed in cells located on the reduced PEDOT:Tosylate electrode when cells were stained using an antibody specific for the tight junction protein zona occludence-1, ZO-1 (Fig. 2c).

A markedly different pattern was observed on the oxidized electrode. Very few cells remained on this surface (Fig. 2a). Actin staining revealed that the cytoskeleton of these cells was completely deranged. Instead of well-organized, elongated stress fibers, actin formed a defined ring along the cell periphery (Fig. 2d). This prompted us to analyze whether the difference in cell numbers on the two electrodes could be explained by the fact that different surface chemistries are introduced on the reduced versus oxidized electrode as voltage is applied, and that this in turn affects the cell viability. Microscopic analysis of Hoechst stained cells was performed to determine the number of cells in meta- and telo-phase as a measure of proliferation (Fig. 2e). We found that the percentage of cells in meta- and telo-phase on the reduced electrode was approximately the same as that of cells cultivated on control glass coverslips (Table 1). A 3-4-fold relative decrease in proliferating cells was found on the oxidized electrode. In contrast, a striking, 200-fold increase of cells with fragmented chromatin, indicative of cell death, was observed on the oxidized electrode (Fig. 2e). This is in sharp contrast to cells on the reduced area and control glass coverslips, only showing 0.1% cells with fragmented chromatin.

To investigate whether cell death occurs as a result of the inability of cells to adhere to the oxidized electrode surface, MDCK cells were next seeded onto non-biased PEDOT:Tosylate electrodes and allowed to adhere and proliferate during 24 h. Electrodes were then biased with 1.5 V for the continuation of an additional 24 h incubation before cell

proliferation was analyzed. Both the reduced and oxidized electrodes now demonstrated large numbers of adherent cells (Fig. 2f). The percentage of cells in meta- or telo-phase was the same on the oxidized and reduced electrodes and on glass coverslips. Importantly, this scheme reduced the fraction of cells with fragmented chromatin on the oxidized electrode from 20.0% to 0.1%, i.e. similar to what is observed on the reduced electrode and control glass coverslips (Table 1). Collectively, this suggests that once cell adhesion is established to non-biased PEDOT:Tosylate electrodes, switching their redox state does not affect cell viability.

3.3. Fibronectin in redox-specific cell responses

When adding cell culture medium to a dish, serum proteins immediately adsorb to the surface. To investigate whether these may form a scaffold that mediates cell adhesion to the electrode surfaces, experiments were performed under serum-free conditions. Cultivation of cells on biased, functionalized dishes showed a reduced number of cells on the electrodes as compared to cells grown in the presence of serum (Fig. 3a). Furthermore, no significant difference in the number of cells between the electrodes was observed under serum-free conditions. This suggests an important role for serum in mediating the surface switch-induced difference in cell adhesion. As Fn is a major component amongst the serum proteins, its effect on cell adhesion was analyzed by coating the functionalized dish with Fn prior to seeding MDCK cells in serum-free medium. Quantification of cell numbers after incubation showed that Fn coating indeed restored the cells' preference for the reduced electrode (Fig. 3a).

Fn contains a specific sequence motif, the RGD-domain, which acts as a binding site for cellular Fn receptors. We tested the specificity of the cell-Fn interaction by cultivating cells in the presence of a soluble RGD-peptide. Quantification showed that the molecular block of the cellular Fn receptors reduced the total number of attached cells, and again, the difference in cell numbers between the electrodes was abolished (Fig. 3a).

It is currently unknown whether the redox state of the electrodes affects the adsorption of Fn to the surface, an effect that indirectly would affect cell adhesion. To investigate this, half of the electrode area on each side of the non-conducting interface of the functionalized dish was pre-coated with Fn. Immediately after cell seeding, electrodes were biased, which created a dish with four functionalized surface areas (Fig. 3b). Quantification of cell adhesion after 24 h revealed a preference for the reduced electrode over the oxidized, whether or not it had been pre-coated with Fn (Fig. 3c). Also, a marked increase in cell numbers was found on both the reduced and oxidized electrodes that were pre-coated with Fn, as compared to their counterparts that had only been exposed to sDMEM.

Polyclonal anti-Fn antibodies combined with an Alexa Fluor 680-tagged secondary antibody were used to analyze the relative density of Fn on surfaces that were either exposed to sDMEM or pre-coated with Fn. An increased amount of bound anti-Fn was found on the reduced electrode compared to the oxidized when pre-coated with Fn (Fig. 4). This was in contrast to the electrode surfaces that had solely been exposed to sDMEM. The anti-Fn binding was considerably lower in the latter experiment and no difference was observed between the electrodes.

3.4. Cellular focal adhesion on electrode surfaces

Cellular interaction with Fn is mediated by the Fn-receptor $\alpha_5\beta_1$ integrin and the vitronectin receptor $\alpha_v\beta_3$ [22], both interacting with the RGD-domain. We investigated the cellular expression pattern of these integrins in MDCK cells after cultivation in sDMEM on biased electrodes for 24 h. In control experiments, cells were cultivated on glass. Sections of the reduced and oxidized electrodes as well as from the glass were prepared for confocal microscopy after staining with antibodies against the α_5 subunit or the $\alpha_v\beta_3$ heterodimer. No expression of the α_5 subunit was observed in cells on any of the surfaces, suggesting that

MDCK cells are unable to express the $\alpha_5\beta_1$ integrin. This finding is corroborated by previous publications, which report the absence of the Fn receptor in MDCK cells and renal epithelial tubular cells [23, 24]. In contrast, the $\alpha_v\beta_3$ integrin was selectively expressed in cells on the reduced electrode and on glass, where they formed a punctuated pattern typical for focal adhesion complexes (Fig. 5). Further immunohistochemical characterization revealed a similar pattern for the structural proteins talin and vinculin known to connect the integrins to the actin cytoskeleton within the focal adhesions. Cells on the oxidized electrode showed a markedly different morphology (as previously demonstrated), and a diffuse staining pattern for the integrins. Together with the diffuse, cytoplasmic distribution of talin and vinculin and the previously demonstrated fragmented chromatin, these data further strengthen the hypothesis that the electrochemical switch induces a hostile surface on the oxidized electrode.

4. Discussion

The material science community is continuously producing new, advanced materials with numerous features aiming to improve the material's compatibility in biomedical applications. To explore the full potential of such materials, they must, already at an early stage, be integrated with the biological experiments, to ensure that the material and the device are developed under the constraints set by the biological system. In this study, we have functionalized a cell culture dish with a thin film of the polymer PEDOT:Tosylate to provide control of physical parameters at the liquid-surface interface. This is achieved by applying low potential, leading to reduction and oxidation of defined areas within the dish. We demonstrate that the redox state can be used to direct cell adhesion and proliferation to defined areas within the cell culture dish.

Based on the results reported in this manuscript, we propose a model that explains the differential effects of the reduced and oxidized surfaces on cell adhesion and

viability (Fig. 6). The reduced surface promotes adhesion and proliferation of MDCK cells; formation of tight junctions, actin stress fibers, and large, macro-molecular focal adhesion complexes containing the $\alpha_v\beta_3$ integrin, talin and vinculin, are readily observed. This is in sharp contrast to the oxidized electrode, on which cell death occurred. The difference could be linked to the functional presentation of the surface-adsorbed ECM-protein Fn. While the reduced electrode presents functional Fn for cell adhesion, the oxidized electrode interferes with the presentation of Fn, making the RGD-domain inaccessible for integrin binding.

The functionalized cell culture dish contains two adjacent, electronically isolated PEDOT:Tosylate electrodes in a common electrolyte. This allows for direct comparison of cell behavior on the reduced and oxidized electrode within the same device, in contrast to previously reported three-electrode systems. Whereas such systems offer detailed control of the working electrode potential, they are limited to only one active cell-hosting surface, commonly represented by PPy [12-14]. Although the two-electrode system lacks such control of the potential, this is compensated for by monitoring the electrochromic properties of PEDOT:Tosylate [25]. We show this by correlating the color changes to the redox peaks of the cyclic voltammogram. The color provides an easy way to monitor the status of the surfaces throughout the experiment. Because of the uncomplicated power supply used to operate the two-electrode system and the proven stability of PEDOT:Tosylate in aqueous solutions [26], the two-electrode system can be used in experiments performed during several days within a cell culture incubator.

Electric fields have been shown to affect various cell activities [27]. The nature of an electric field on polymer electrodes, in analogy to that created by well-defined Helmholtz layers on metal electrodes, has to our knowledge never been described. A complicating factor is that instead of being adsorbed to the surface, ions migrate into the polymer bulk where dipoles are formed. When potential is applied to our device, the resulting

current drops as electrochemical reactions are completed. At this stage, the electrochemical cell has reached equilibrium, and ion flow into the polymer stops. From here and onwards, an electric field is unlikely to reach outside the polymer where cells are located. Whereas we can not rule out an effect of the electric field on cells during the short duration of electrochemical reaction, any such effect can be disregarded for the majority of the 24 h experiments.

The surface chemistry has been shown to be of importance for the interaction of adsorbed Fn and cellular integrin receptors. Using self-assembled monolayers (SAMs) of alkanthioles with terminally presented CH_3, OH, COOH, or NH_2, it was demonstrated that the $\alpha_5\beta_1$ integrin binds to Fn presented on OH-, COOH-, and NH_2-SAMs, whereas $\alpha_v\beta_3$, the integrin expressed by MDCK cells, is more specific, binding to Fn on COOH-SAMs only [7]. This sensitivity to surface chemistry may explain the different preferences of MDCK cell adhesion to the reduced and oxidized surfaces. Failure to adhere to a surface determines the cells to a pathway termed “home-less cell death” [20, 21]. This is likely to occur on the oxidized surface, since cell death can be prevented when cells are first allowed to adhere to the surface before it is electronically addressed.

It is clear from the above results and the results from Grinnel et al [28] that Fn is presented differently depending on surface chemistry. They analyzed cell adhesion and binding of polyclonal anti-Fn antibodies and correlated this to detection of bound, radiolabeled Fn. Here, we electronically oxidize a surface pre-coated with Fn and find that less polyclonal anti-Fn antibodies bind as compared to a reduced surface. Thus, Fn may have desorbed from the oxidized surface, alternatively, changed into a conformation no longer accessible for binding. We find that the binding of polyclonal anti-Fn antibodies to adsorbed Fn is much higher on pre-coated surfaces than on surfaces that have only been switched in sDMEM, even though the Fn concentration in sDMEM and the coating solution is similar. Others have shown that when Fn is adsorbed from solutions of 10% serum, competitive

binding of other serum proteins interferes with the adsorption of Fn [28]. Still, this should represent an adequate amount of Fn to mediate cell adhesion and proliferation, as the Fn concentration in sDMEM and the pure Fn solution is circa 1000-fold higher than the sufficient concentration reported for cell adhesion (12 ng/cm^2, [28]). In our experiments, the difference in binding of anti-Fn antibodies to the pre-coated redox surfaces correlates well to the difference in cell adhesion. When sDMEM is the sole source of Fn, this effect is not seen. The latter is unexpected, as these surfaces exhibit a clear difference in the number of adhered cells. It is known that serum proteins, like albumin, are important for Fn to adopt a proper conformation [28] and these proteins may help to restore enough of the conformation of Fn to allow for anti-Fn antibody binding. Cell adhesion ought to be more sensitive to conformational changes of Fn. This is because cells only bind to the RGD-motif, whereas polyclonal anti-Fn antibodies bind several epitopes. Taken together, this suggests that the difference in cell adhesion and proliferation in favor of the reduced electrode occur because oxidation of the PEDOT:Tosylate electrode is accompanied by an altered surface chemistry and charge which cause a distortion of the Fn conformation.

5. Conclusion

The device presented here represents a functionalized cell culture dish with two adjacent PEDOT:Tosylate electrode surfaces that become reduced and oxidized, respectively, when electronically addressed. Molecular cell characterization reveals that the reduced polymer promotes cell proliferation in contrast to the oxidized electrode. The latter induces alteration of the Fn conformation, which severely compromises cell adhesion and viability. Collectively, this work shows that cell adhesion and proliferation can be controlled by electrochemical modulation of surface parameters.

Acknowledgments

We thank M Rydén-Aulin for help in preparing the manuscript, S Strömblad, and S Plantman for discussions. The project was funded by Swedish Foundation for Strategic Research (SSF) as part of the 'Strategic Research Centre for Organic BioElectronics' (A.R.D. and M.B.). The Organic Electronics group at Linköping University in Norrköping is a member of the COE@COIN SSF-funded project. Funding from Karolinska Institutet to K.S. is greatly appreciated.

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Captions

Fig. 1. The functionalized cell culture dish, consisting of two electrochemically active PEDOT:Tosylate electrodes. (A) Schematic description of the electrochemical device. The cathode (left) is reduced and the anode (right) is oxidized. The redox process is accompanied by an ion flow into/out of the polymer matrix. A^- denotes an arbitrary anion and M^+ an arbitrary cation. (B) Photograph of the switched cell culture dish, showing the electro-chromism: the reduced electrode is deep purple and the oxidized is light blue. The dashed line indicates the space that separates the two electrodes. (C) Characterization of the current versus time at 1.0 V measured every second for 18 h. (D) Cyclic voltammetry of the PEDOT:Tosylate cell culture dish at a scan rate of 40 mV/s.

Fig. 2. Morphology of cells cultivated in functionalized cell culture dishes, as illustrated by actin staining (TRITC-phalloidin, red) and nuclear morphology (Hoechst 33258, blue). (A) MDCK cells stained for actin and nuclei on the biased PEDOT:Tosylate surface. Left - reduced surface; middle - non-conducting interface (arrow heads); right - oxidized surface. (B) Actin stress fibers in MDCK cells on the reduced surface. (C) A polarized monolayer of cells is formed on the reduced electrode, as illustrated by immuno-staining of the tight junction protein ZO-1 (red). (D) Actin staining in cells on oxidized surface. (E) Actin staining and nuclear morphology illustrate MDCK cells in telo-phase (left), meta-phase (middle) and with fragmented chromatin (right). (F) Cells cultivated on a non-biased surface for 24 hours before biasing and then cultured for an additional 24 hours. Staining as in A.

Fig. 3. Effect of adsorbed Fn on MDCK cell growth. (A) The mean number of cells ± SD on the reduced (black) and oxidized (white) surfaces when cells are cultivated in sDMEM; serum free DMEM; serum free DMEM on surfaces pre-coated with Fn; and sDMEM medium with RGD-peptide. Each data point represent the number of cells on 10 visual fields using the 20x objective. (B) Schematic of the 4-square PEDOT:Tosylate cell culture dish used in C. It is divided in two electrode areas (left/right) and the top half has been pre-coated with Fn (dashed area). (C) Effect of Fn coating on cell growth using the method of A and the dish of B. * = Student's paired t-test with a p-value < 0.05.

Fig. 4. Anti-Fn antibody binding to reduced (black) and oxidized (white) surfaces. Y-axis display pixel intensity from an acquired IR-scan of the fluorescent secondary anti-rabbit antibody. Dashed bars indicates that surfaces have been pre-coated with Fn. Surfaces where biased for one hour at 1.0 V in sDMEM.

Fig. 5. Actin staining and focal adhesion complexes in MDCK cells studied with confocal microscopy. Actin is stained with TRITC-phalloidin (red) and components of the focal adhesion complex are stained with antibodies recognizing, from left to right, integrin α_5 subunit, integrin $\alpha_v\beta_3$ heterodimer, talin, and vinculin (green). Cells are grown on glass, reduced or oxidized PEDOT:Tosylate. Scale bar = 20 µm

Fig. 6. A schematic drawing of the proposed mechanism for cell interaction with the reduced and oxidized PEDOT:Tosylate surfaces, respectively.

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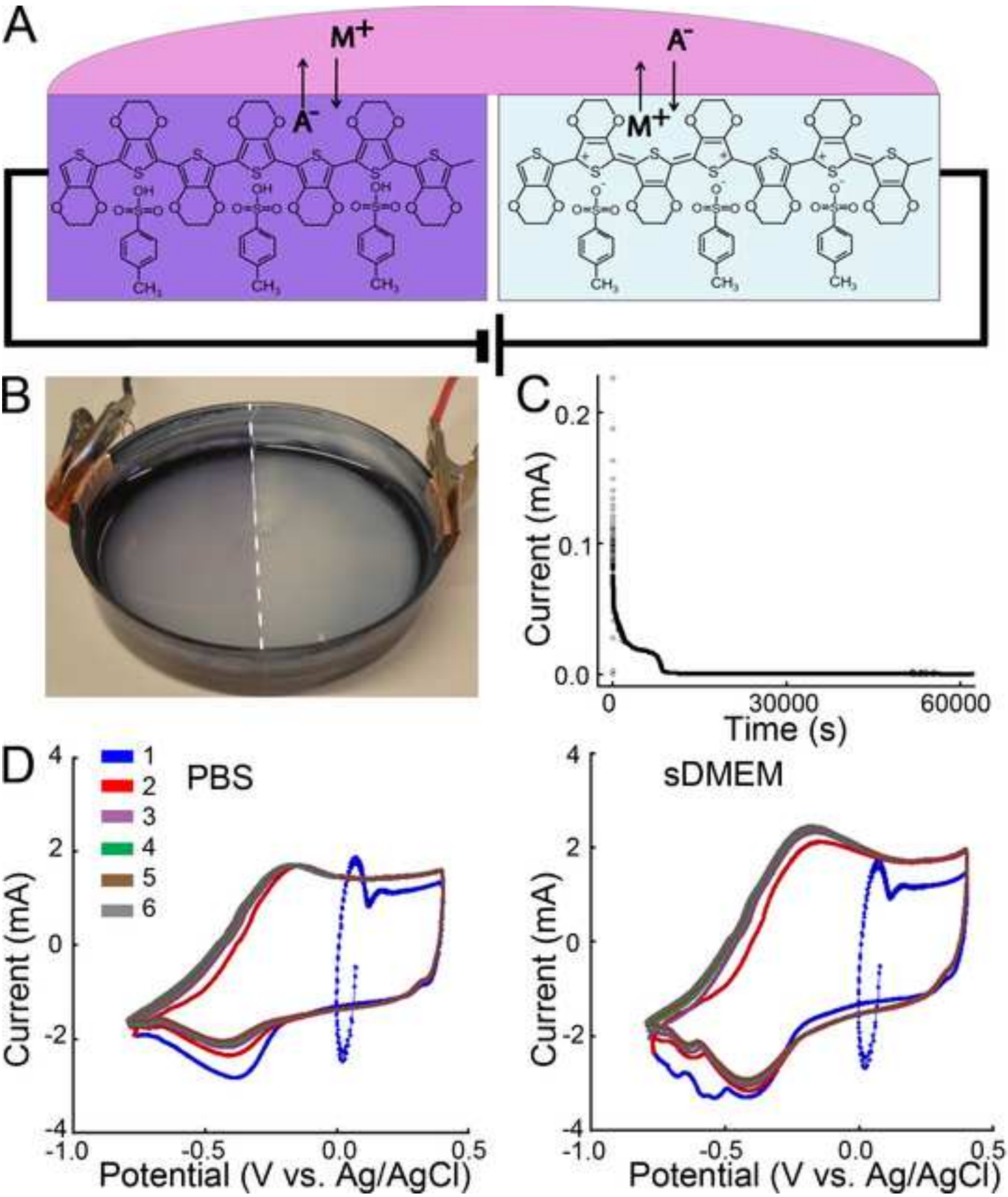

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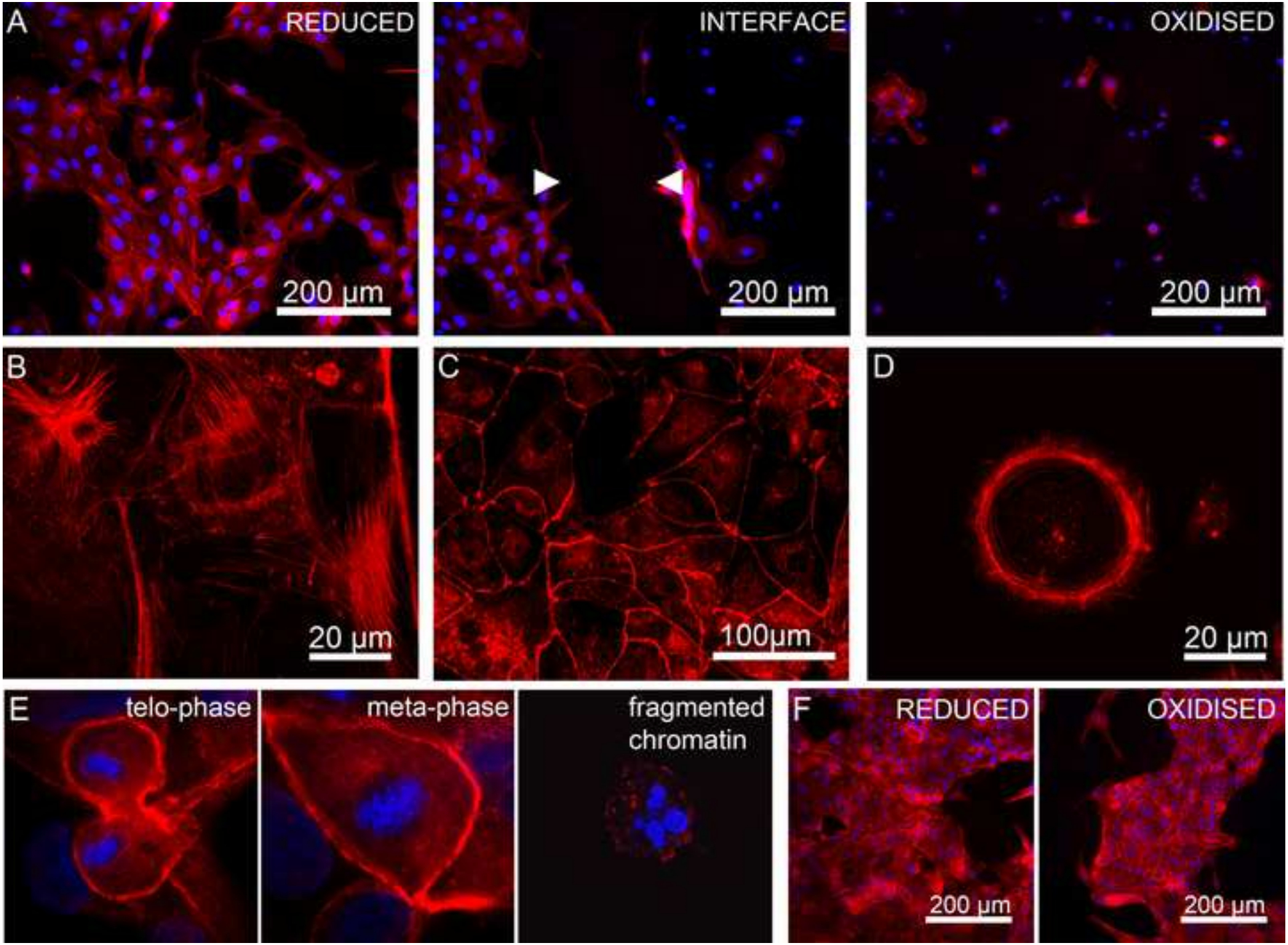

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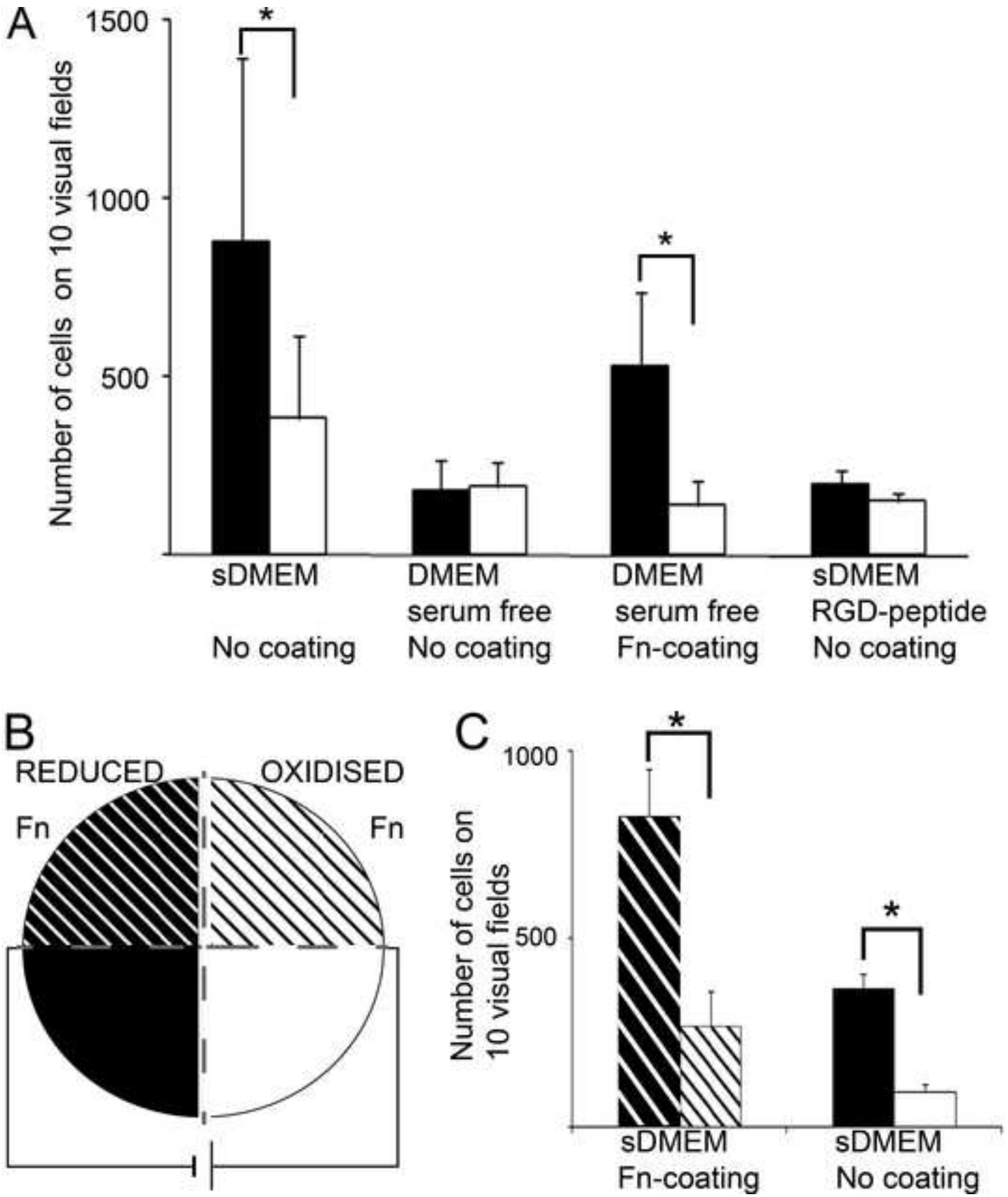

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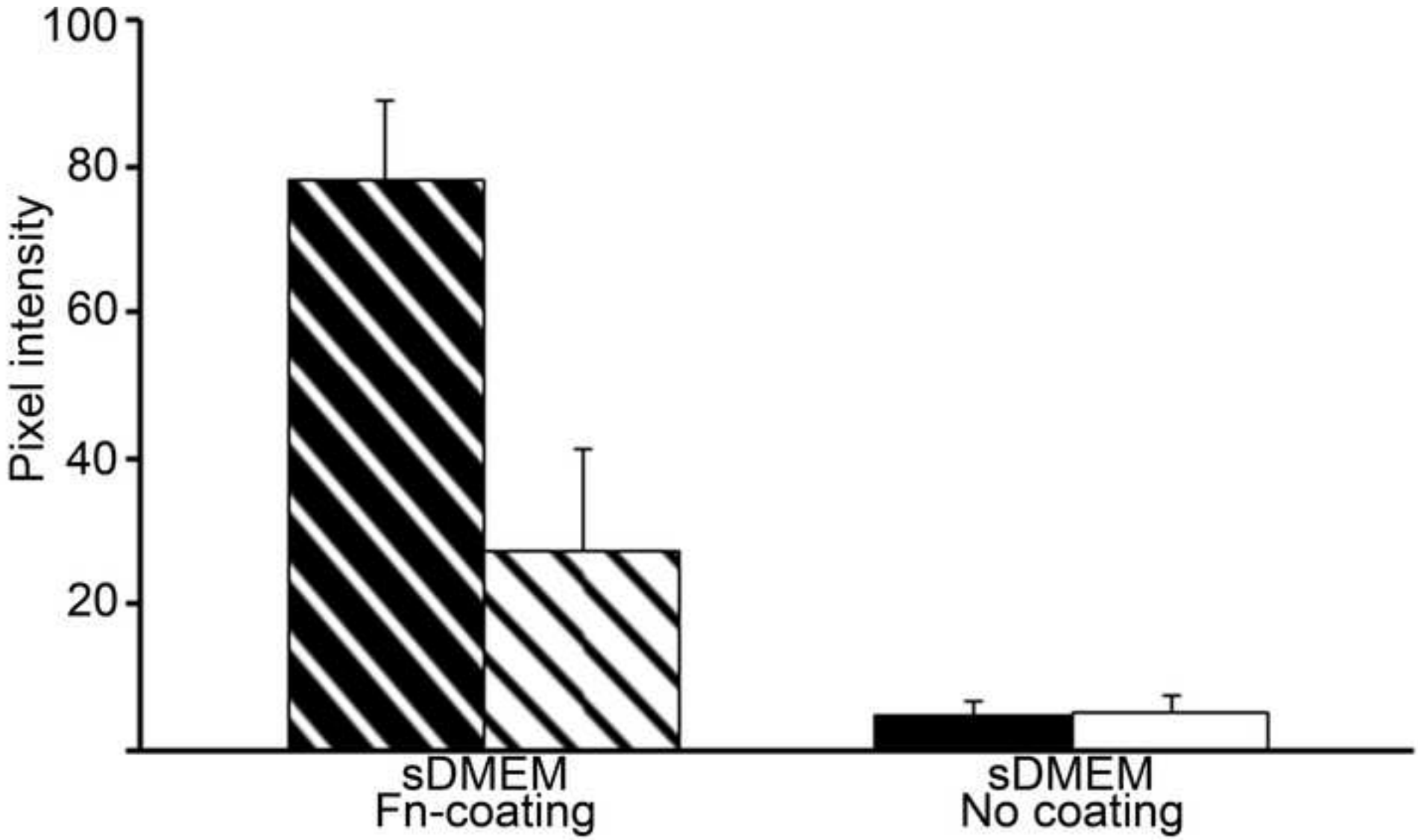

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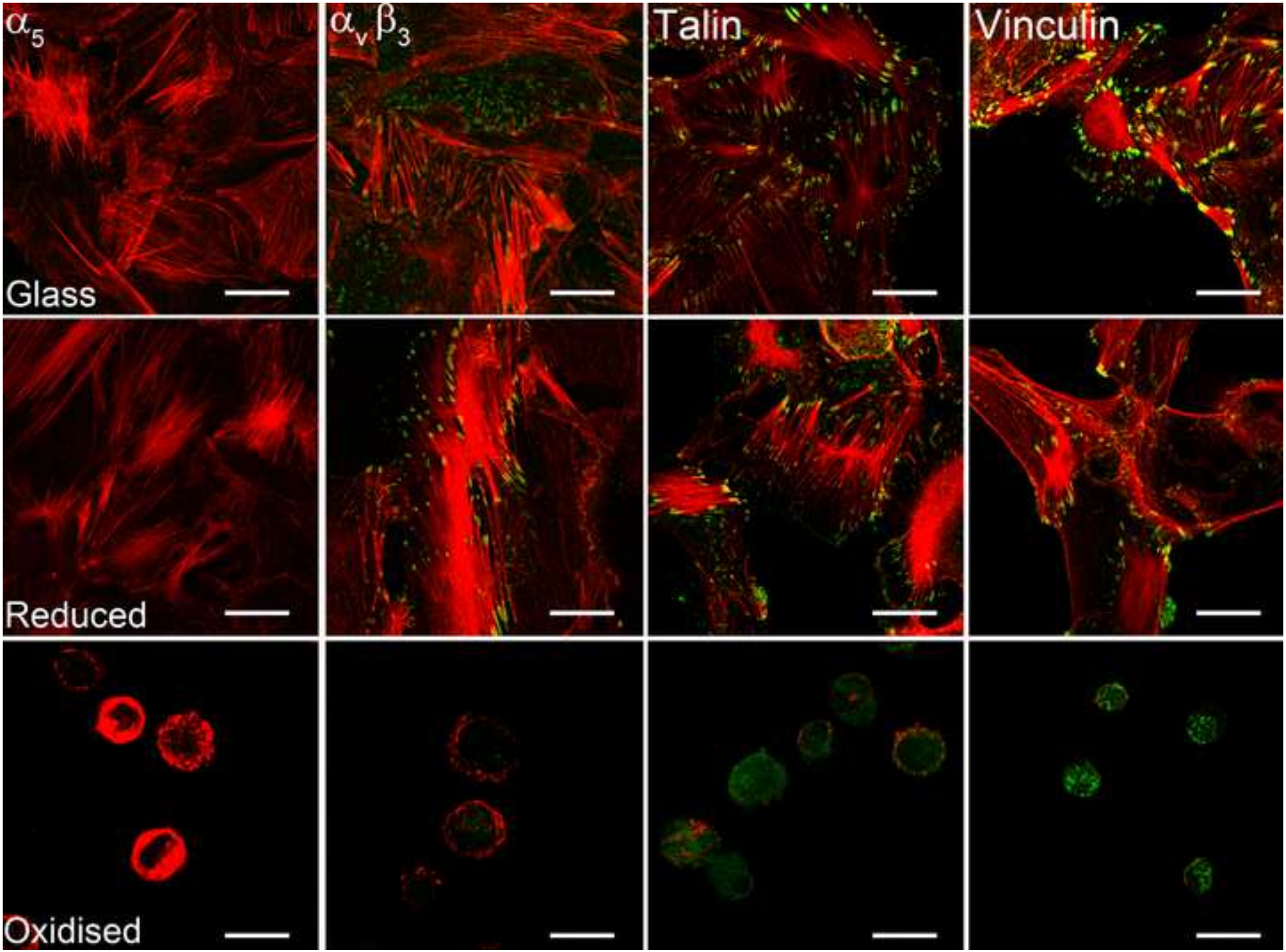

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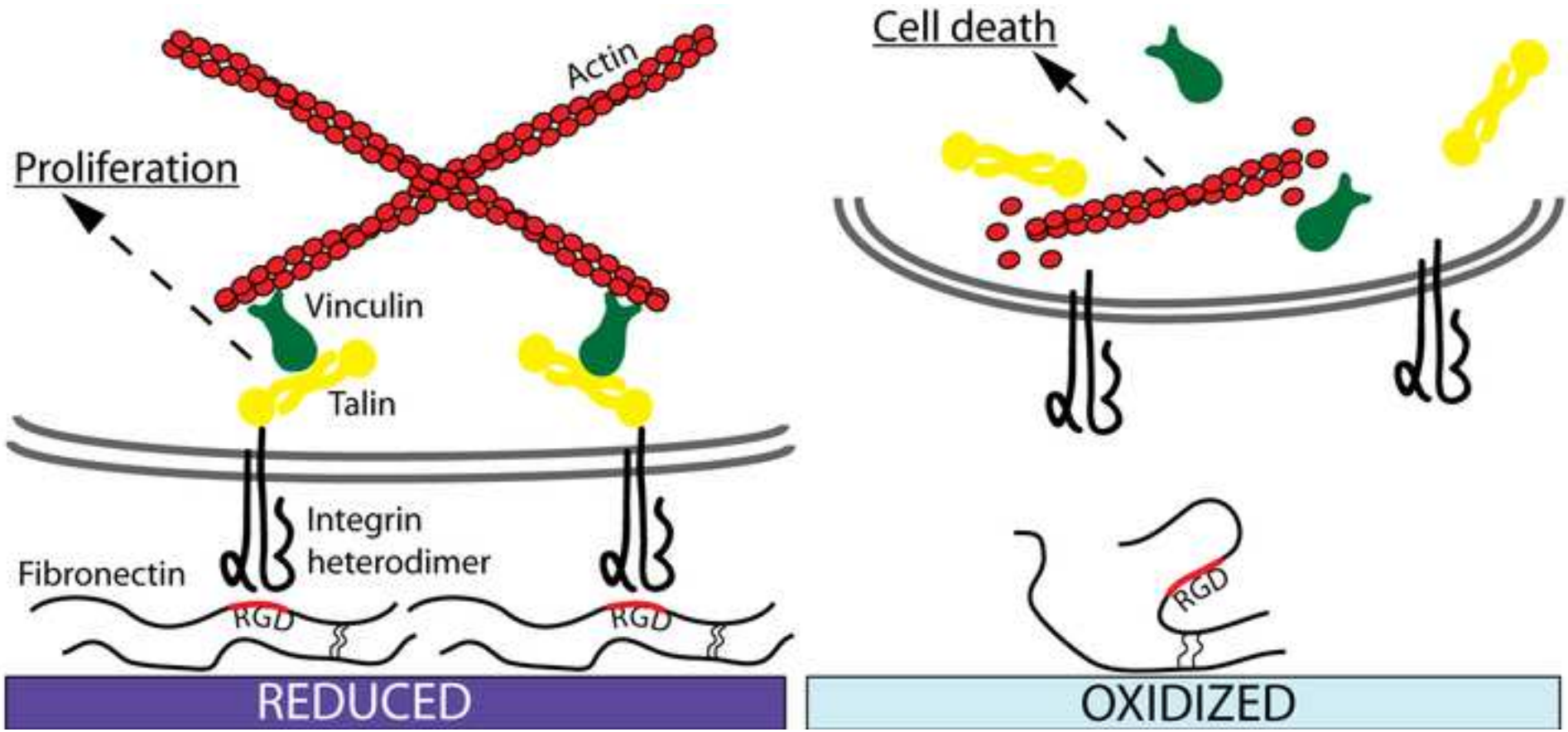

Table

Table 1 Viability of MDCK cells on switched PEDOT:Tosylate electrodes (% MEAN ± SD)

Viability	**Switch before adhesion**		**Adhesion before switch**		**Glass**
	Reduced	Oxidized	Reduced	Oxidized	
Meta- and telophase	3,1 ± 2,3	0,8 ± 0,3	2,9 ± 0,3	2,1 ± 1,1	2,4 ± 0,6
Fragmented chromatin	0,1 ± 0,2	20,0 ± 9,9	0,01 ± 0,01	0,1 ± 0,1	0,1 ± 0,1