J. Biomater. Sci. Polymer Edn, Vol. 17, No. 1–2, pp. 199–212 (2006)

Also available online - www.vsppub.com

Polyaniline, an electroactive polymer, supports adhesion and proliferation of cardiac myoblasts

PAUL R. BIDEZ III [1], SHUXI LI [3], ALAN G. MACDIARMID [2], EVERALDO C. VENANCIO [2], YEN WEI [3] and PETER I. LELKES [1,*]

[1] *School of Biomedical Engineering, Science, and Health Systems, Drexel University, Commonwealth Hall 7-721, 3141 Chestnut Street, Philadelphia, PA 19104, USA*
[2] *Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, PA 19104, USA*
[3] *Department of Chemistry, Drexel University, 3141 Chestnut Street, Philadelphia, PA 19104, USA*

Received 10 February 2005; accepted 20 July 2005

Abstract—Conductive polymers, such as polypyrrole, have recently been studied as potential surfaces/matrices for cell- and tissue-culture applications. We have investigated the adhesion and proliferation properties of H9c2 cardiac myoblasts on a conductive polyaniline substrate. Both the non-conductive emeraldine base (PANi) and its conductive salt (E-PANi) forms of polyaniline were found to be biocompatible, *viz.*, allowing for cell attachment and proliferation and, in the case of E-PANi, maintaining electrical conductivity. By comparison to tissue-culture-treated polystyrene (TCP), the initial adhesion of H9c2 cells to both PANi and E-PANi was slightly reduced by 7% ($P < 0.05$, $n = 18$). By contrast, the overall rate of cell proliferation on the conductive surfaces, although initially decreased, was similar to control TCP surfaces. After 6 days in culture on the different surfaces, the cells formed confluent monolayers which were morphologically indistinguishable. Furthermore, we observed that E-PANi, when maintained in an aqueous physiologic environment, retained a significant level of electrical conductivity for at least 100 h, even though this conductivity gradually decreased by about 3 orders of magnitude over time. These results demonstrate the potential for using polyaniline as an electroactive polymer in the culture of excitable cells and open the possibility of using this material as an electroactive scaffold for cardiac and/or neuronal tissue engineering applications that require biocompatibility of conductive polymers.

Key words: Conductive polymer; biocompatibility; H9c2 cardiac myoblasts; tissue engineering.

*To whom correspondence should be addressed. Tel.: (1-215) 895-2219. Fax: (1-215) 895-4983. E-mail: pilelkes@drexel.edu

INTRODUCTION

Many industrial polymers and composites, such as polyethylene, carbon fiber, silicone rubber and polytetrafluoroethylene (PTFE), are increasingly used in a variety of biomedical applications [1]. Particularly, electrically conductive polymers, such as polyaniline, polythiophene and polypyrrole, which are well characterized in terms of their chemical and physical properties [2], have also gained attention as electroactive substrates for the culture of electrically excitable cells, such as neuronal or muscle cells [3].

Conductive polymers, in particular polypyrroles, have been studied as potential electroactive surfaces for diverse cell- and tissue-culture applications, with the implicit assumption that electrical currents through these surfaces can control the shape and function of anchorage-dependent cells [4]. For example, when grown in the absence of electrical stimulation on polypyrrole, acetylcholine-stimulated catecholamine secretion of neural-tube-derived adrenal chromaffin cells was found to equal that of cells grown on collagen substrates [5]. Electrical stimulation enhanced nerve growth factor (NGF)-induced neuronal differentiation of adrenal medullary chromaffin cell-derived PC12 pheochromocytoma cells grown on polypyrrole. Specifically, a brief period of electrical stimulation (100 mV for 2 h) potentiated NGF-induced neuritogenesis, as assessed by the length of neurons in cells subjected to electrical stimulation [3]. More recently, Schmidt and co-workers [6] found that electrical stimulation of polypyrrole resulted in altered fibronectin adsorption to the conductive surface, which in turn enhanced neuritogenesis in PC-12 cells.

Recent *in vivo* studies showed that both the conductive (E-PANi, emeraldine salt) and nonconductive (PANi, emeraldine base) forms of polyaniline, a commercially available polymer with electroactive properties [7], did not provoke inflammatory responses in a rodent model, suggesting good tolerance and bio-/histo-compatibility [8, 9]. To date, to the best of our knowledge, no studies have been published evaluating the biocompatibility, *viz.*, lack of cytotoxicity of polyaniline *in vitro*, especially in its emeraldine salt form. Indeed, some prior preliminary studies using cultured fibroblasts had suggested innate cytotoxicity of the conductive, emeraldine salt of polyaniline (data not shown). We recently reported that PC12 pheochromocytoma cells exhibited limited adhesion and proliferation on untreated, non-conductive polyaniline and showed that that the biocompatibility of PANi films could be significantly enhanced by grafting adhesive peptides, such as YIGSR [10, 11].

In the present study, the biocompatibility of thin films of polyaniline, in both conductive and nonconductive forms, was evaluated by assessing the adhesion and proliferation of H9c2 rat cardiac myoblasts. Surface resistivity of the emeraldine salt form of the polymeric films was determined before and for up to 100 h after exposure to cell-culture medium. Since the conductive properties of PANi rely on the presence of available dopant protons, assessment of the duration of conductivity under physiological conditions was of interest. Our data suggest that for the cell

type tested, polyaniline, in both its PANi and E-PANi forms, is biocompatible, i.e., allowing for cell attachment and proliferation similar to tissue-culture-treated polystyrene, and that a significant level of electrical conductivity, albeit decreasing over time, is maintained for at least 100 h in a physiologic environment.

MATERIALS AND METHODS

Polyaniline films

For all experiments thin films were prepared from saturated solutions (approx. 4 wt%) of polyaniline (Aldrich, Milwaukee, WI, USA) in n-methyl-pyrrolidone (NMP, Fluka, Buchs, Switzerland). This solution was filtered through glass wool. Aliquots (35 μl) of the PANi solution were applied to 13-mm Thermanox™ (Nalgene Nunc, Rochester, NY, USA) tissue-culture discs, forming a single thin layer and dried in a vacuum oven at 50°C and under 250 mmHg vacuum. E-PANi films were generated by doping the PANi-coated discs with 1 M HCl for 15 min, followed by three washes with incomplete (no additives) cell-culture medium (Dulbecco's Modified Eagle's Medium (DMEM, Cellgro, Herndon, VA, USA). Native (emeraldine base) PANi-coated discs and uncoated tissue-culture-treated polystyrene (TCP) plates (Corning, Big Flats, NY, USA) were used as non-conductive samples and as controls, respectively. For adhesion and proliferation studies, and for assessing long-term conductivity, discs were sterilized in 70% ethanol placed in 24-well plates and held in position by 13-mm Viton rubber O-rings (Great Lakes Rubber, Glendale, WI, USA), which had previously been shown to be compatible with cell culture [12].

Cells

H9c2 rat myoblast cells (ATCC CRL-1446; American Type Culture Collection, Rockville, MD, USA), a generous gift from Dr. Yasha Kresh, Drexel University College of Medicine, were maintained (and all experiments were performed) in DMEM with 4.5 g/l glucose, 4 mM L-glutamine, 25 units/ml penicillin/streptomycin (Cellgro), and 10% fetal bovine serum (Hyclone, Logan, UT, USA). Tissue culture incubators were set for 5% CO_2 and 37°C. To prevent differentiation into myocytes by over-confluence, H9c2 myoblasts were split every 4 days at a 1:5 ratio upon reaching approx 80% confluence.

Myoblast adhesion and proliferation

Cell adhesion and proliferation were measured with a continual fluorescence assay, using alamarBlue™ (AB, Biosource, Alameda, CA, USA), as described previously [12, 13]. For details, see below.

Adhesion. Cells were seeded onto unmodified tissue-culture-treated Thermanox™, PANi and E-PANi-coated Thermanox™ discs at approx. 90% confluence

or 1.1×10^5 cells/cm^2. After 15 min for attachment in a tissue-culture incubator and 3 min of slight agitation on a gyrating platform (Belly Dancer™ setting 8, SPI Supplies, West Chester, PA, USA), supernatants were removed and plated in other empty tissue-culture-treated polystyrene wells of the same 24-well plates. Fresh medium was added to the wells where the supernatants had been removed and the plates were incubated for an additional 2 h in order to allow the cells that were transferred with the supernatants to adhere to the new wells. After 2 h, the medium was removed from all wells and replaced with fresh complete medium containing 10% AB. All plates were returned to the incubators for another 3 h to allow for the cell-mediated reduction of the non-fluorescent AB compound to its fluorescent species [12, 13]. After this last incubation period, all the supernatants were again transferred to yet another 24-well plate. AB fluorescence was read in a fluorescent plate reader, as described previously [12] (Millipore Cytofluor 2350, excitation filter 530 ± 20 nm, emission filter 590 ± 20 nm). AB fluorescence values from the original wells and the fluorescence signals originating from the cells that had been transferred with the first supernatants were added. These data represent the total number of cells plated. The number of cells attached was determined as a percentage of the AB fluorescence of the cells attached after 15 min divided by the AB fluorescence from the total number of cells seeded.

Proliferation. Cells were seeded on TCP, PANi and E-PANi discs at 10^4 cells/cm^2, and allowed to proliferate for up to 200 h. At 24, 48, 100, 150 and 200 h the medium was exchanged with an equal volume of 10% AB containing complete medium and the plates were returned to the incubator. After 3 h of incubation, the AB-containing medium was aspirated and set aside for reading in a plate reader. The cells were fed with fresh medium without AB and placed back in the incubator until the next time point. AB fluorescence in the supernatants was determined as above. For each set of experiments, the data were normalized to the maximal fluorescence of H9c2 cells growing on control TCP.

Surface resistivity

In order to determine the surface resistivity of the E-PANi substrate after exposure to cell culture media, PANi and E-PANi discs were incubated in DMEM at 37°C and 5% CO_2 for the same time intervals as described in the proliferation assay, removed, quickly washed in distilled water and dried in air. Surface resistivity (expressed as Ohm per square) of the electrically conductive and non-conductive polyaniline discs was measured *via* a standard four-point probe technique, as described previously [11]. Current inputs, delivered *via* an EG&G PAR Model 173 constant current source (Princeton Applied Research, Oak Ridge, TN, USA), ranged from 1 μA to 100 μA and voltage measurements were made on an RMS multimeter (B&K Precision, Yorba Linda, CA, USA).

Microscopy

Cytoskeletal architecture and surface morphology of the cells seeded on the various substrates were determined *via* fluorescence light microscopy and scanning electron microscopy (SEM), respectively. H9c2 cells were seeded at low (clonal) density on each substrate and analyzed at 1, 3, 6 and 24 h. Samples for SEM were fixed with 2% paraformaldehyde/2% glutaraldehyde in 0.1 M phosphate-buffered saline, dehydrated in graded ethanol, dried in a critical point dryer (CPD 7501, SPI Supplies) and visualized by SEM (XL30 ESEM, FEI/Philips, Eindhoven, The Netherlands).

For light microscopy, the cells were cultured for up to 144 h, fixed with 2% gluteraldehyde/2% paraformaldehyde, permeabilized with Triton 1×, stained with 5 μg/ml rhodamine-phalloidin (Sigma) and visualized on a Leica DMRX microscope using a Leica 300F camera (Leica Microsystems, Bannockburn, IL, USA).

Atomic force microscopy was performed using a DI Dimension 3000 AFM (Veeco Instruments, Woodbury, NY, USA) scanning probe microscope on 10 × 10 μm areas.

Statistics

Statistical analyses were performed using the one-way ANOVA ($P < 0.001$) and Tukey post-test ($P < 0.05$). All data reported are means ± standard error of the means ($\sigma_{\bar{x}}$) All data sets had six independent replicates and each were run in triplicate ($n = 18$), except for surface resistivity, which was measured in triplicate ($n = 3$).

RESULTS

Cell attachment

In preliminary studies we determined that, upon 15 min incubation, approx. 70% of the H9c2 cells attached to the TCP control surface (data not shown). In Fig. 1, cell attachment, as determined by the AB response, is reported as the percentage of cells adhered after 15 min incubation, normalized to the total cell number seeded. Under these standard conditions, H9c2 cell attachment to control TCP substrate was 69.3±1.0%, while cell adhesion to native PANi and conducive E-PANi surfaces was slightly lower, amounting to 65.4 ± 0.3% and 63.6 ± 0.5%, respectively. Statistical analysis revealed that the adhesion to both E-PANi and PANi substrates, while not significantly different from each other, was statistically different by approx. 7% ($P < 0.05, n = 18$) from TCP controls (Fig. 1).

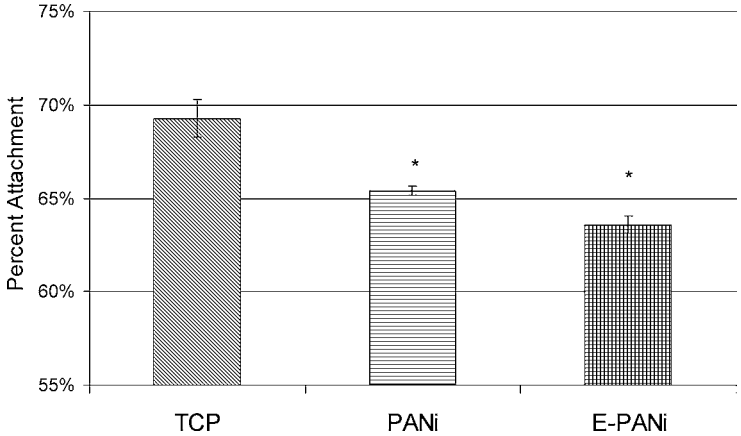


Figure 1. H9c2 cell adhesion on PANi, E-PANi and TCP controls. Cells (10^5/well) were allowed to attach for 15 min and then shaken on a Belly Dancer™ gyrating platform for 3 min, speed 8, as detailed in Materials and Methods. Supernatants containing unattached cells were removed and re-plated. Numbers of attached and total seeded cells were determined using the AB fluorescent assay. Normalized cell attachment was calculated by dividing the fluorescence signal from the initially attached cells by the fluorescence of the total number of cells plated. Data are expressed as means ± standard error of the mean ($\sigma_{\bar{x}}$, $n = 18$) Asterisks denote statistical difference between control TCP and both E-PANi and PANi ($P < 0.05$, one-way ANOVA, Tukey post-test).

Cell proliferation

H9c2 Myoblasts were plated at low density (10^4 cells/cm^2) and allowed to proliferate for up to 200 h. Relative cell numbers were assessed by the AB assay and normalized to TCP control, as detailed in Materials and Methods (Fig. 2). Cells on the E-PANi substrate exhibited a significantly extended lag phase of growth compared to the PANi and TCP, which were indistinguishable in terms of their lag phases. In the first phase of cell proliferation, between 48 and 100 h post seeding, the doubling time of h9c2 cells was 24 ± 5 h when grown on TCP and PANi and 28 ± 8 h when grown on E-PANi (Fig. 3). These doubling times were statistically indistinguishable. After the first 100 h, the doubling time of the cells on the E-PANi surface was significantly ($P < 0.05$) shorter (54 ± 11 h) than that of cells growing on PANi (78 ± 20 h) and TCP controls (93 ± 25 h). The cells, while initially growing more slowly on E-PANi, eventually caught up and reached equivalent cell numbers to those on the TCP controls by 200 h.

Surface resistivity

In line with previous reports, surface resistivity of non-conductive PANi was greater than 10 MΩ per square [2], the limit of the equipment employed in this study. Prior to the partial de-doping with DMEM culture medium, conductive samples of E-PANi had an initial surface resistivity of 130 Ω per square (data not shown). After partial de-doping, resistivity increased to 2 kΩ per square. During the time-course

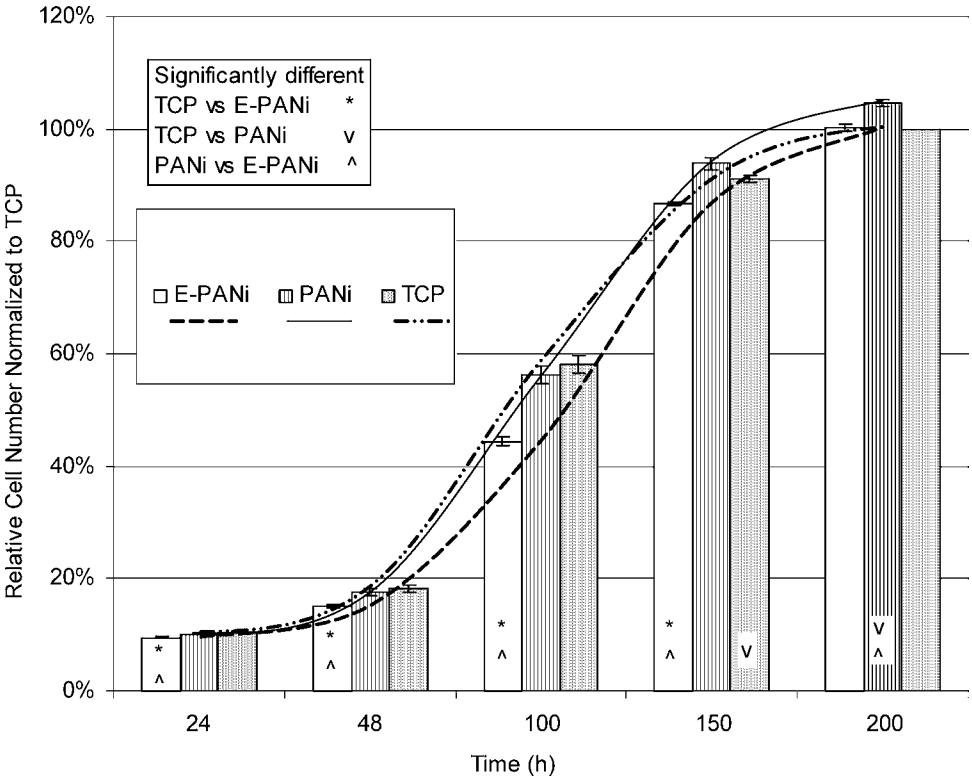


Figure 2. H9c2 myoblast proliferation on PANi, E-PANi and TCP controls. Cells were seeded at 10^4 cells/cm^2 and allowed to proliferate for up to 200 h. Cell numbers were assessed by measuring AB fluorescence at the time points indicated. The data are normalized to the maximal fluorescence signal of cells growing on Thermanox™ tissue culture plastic (TCP) control surfaces and expressed as means $\pm\ \sigma_{\bar{x}}$ ($n = 18$). The symbols (*, V and ^) denote statistically significant differences between various groups, as indicated in the figure ($P < 0.05$, one-way ANOVA, Tukey post-test). For details see Materials and Methods.

incubation in DMEM at 37°C and 5% CO_2, the resistivity gradually increased by greater than 3 orders of magnitude, reaching approx. 6 MΩ per square at 100 h (Fig. 4). After 100 h of continual exposure to a physiological culture medium the resistivity of the dried film was equivalent to that of non-doped PANi, i.e., higher than 10 MΩ per square.

Microscopy

The scanning electron micrographs in Fig. 5 showed comparable morphologies for cells grown on both PANi and E-PANi. Cells readily attached and spread in similar fashion on both PANi and E-PANi substrates in the first hour. By 24 h, the cells had assumed the typical morphology of cultured H9c2 myoblasts. The morphology of cells growing on TCP was at all time points indistinguishable from that of cells growing on the polyanilines (data not shown).

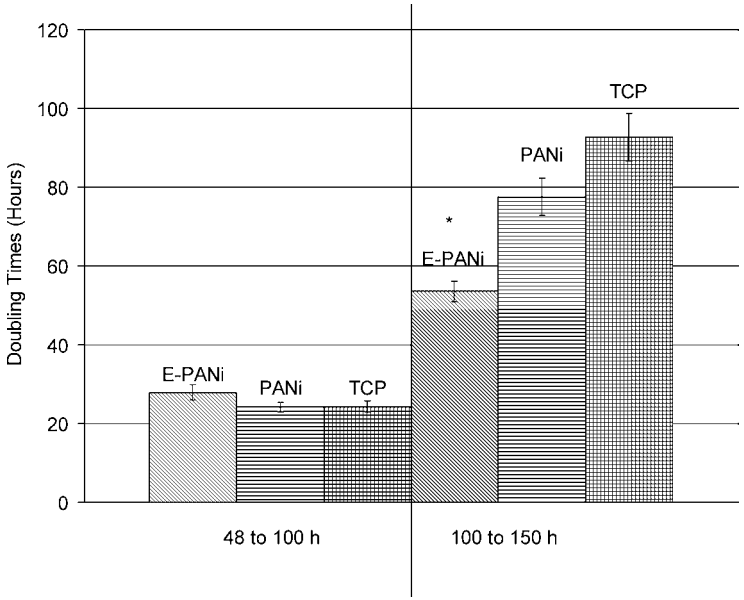


Figure 3. H9c2 cell population doubling (PD) times. PD times were calculated for an early stage (48–100 h) *versus* a later stage (100–150 h) of H9c2 proliferation, well before the cells in the monolayer reached confluence. Data are presented as means $\pm\ \sigma_{\bar{x}}$ ($n = 18$). Asterisk denotes significant difference from other samples during the same time period ($P < 0.05$, one-way ANOVA, Tukey post-test).

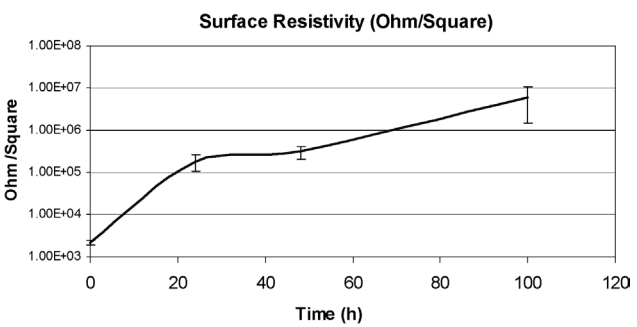


Figure 4. E-PANi surface resistivity. The surface resistivity of E-PANi was measured by the standard four-probe method. Samples of activated E-PANi were washed three times with DMEM and then subsequently incubated in DMEM at 37°C and 5% CO_2 for the same time intervals as the proliferation experiment. Error bars represent $\pm\ \sigma_{\bar{x}}$. For details see Materials and Methods.

To further assess the morphology and cytoskeletal architecture of H9c2 cells on different substrates, the cultures were fixed at various time points, permeabilized and stained with rhodamine-phalloidin, a specific stain for F-actin [14]. Light microscopic images confirmed a similar degree of cell spreading during the first hour (Fig. 6). At 1 h the cells on all substrates appear rounded. We noted that the perimembranal microfilamentous web appeared to be more punctate in cells seeded

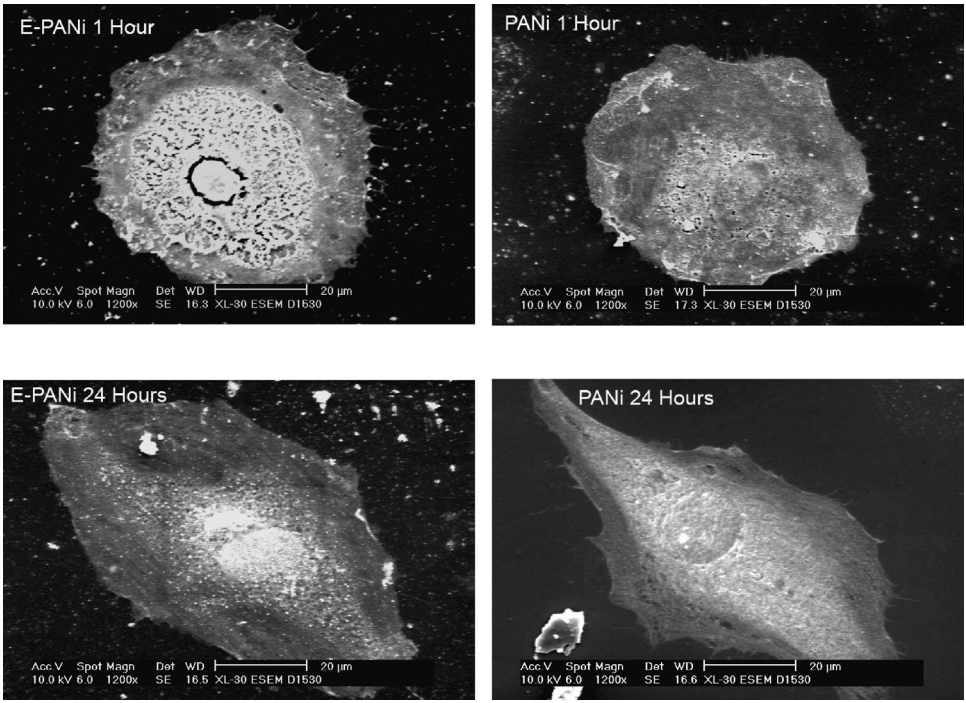


Figure 5. Scanning electron micrographs of H9c2 myoblasts growing on PANi and E-PANi for 1 and 24 h. Cells were seeded at low (clonal) density and incubated for up to 24 h. The samples were processed for scanning electron microscopy and visualized as detailed in Materials and Methods.

on TCP than on PANi or E-PANi. By 24 h individual cells appeared all similarly elongated, irrespective of the substrates on which they were growing. After 144 h, no difference in the cytoskeletal architecture or histotypic morphology was noted. On all substrates tested, the cells formed nearly identical monolayers in terms of cell density and organization of their F-actin stress fibers.

Two of the physical parameters of the films, thickness and surface roughness, were determined microscopically. Film thickness was assessed by SEM *via* an edge-on view of several samples (Fig. 7). The average film thickness, for both doped and undoped PANi was similar, amounting to approx. 265 nm. The samples appeared smooth in these edge-on views, with a few randomly occurring areas of elevation. Surface roughness was determined by atomic force microscopy, interrogating multiple 10×10 μm areas. On average the RMS roughness of the polyaniline sample was found to be approx. 1.10 nm, slightly less than that of the per definition smooth, tissue-culture treated polystyrene control substrate, which was on average 1.60 nm (Fig. 8).

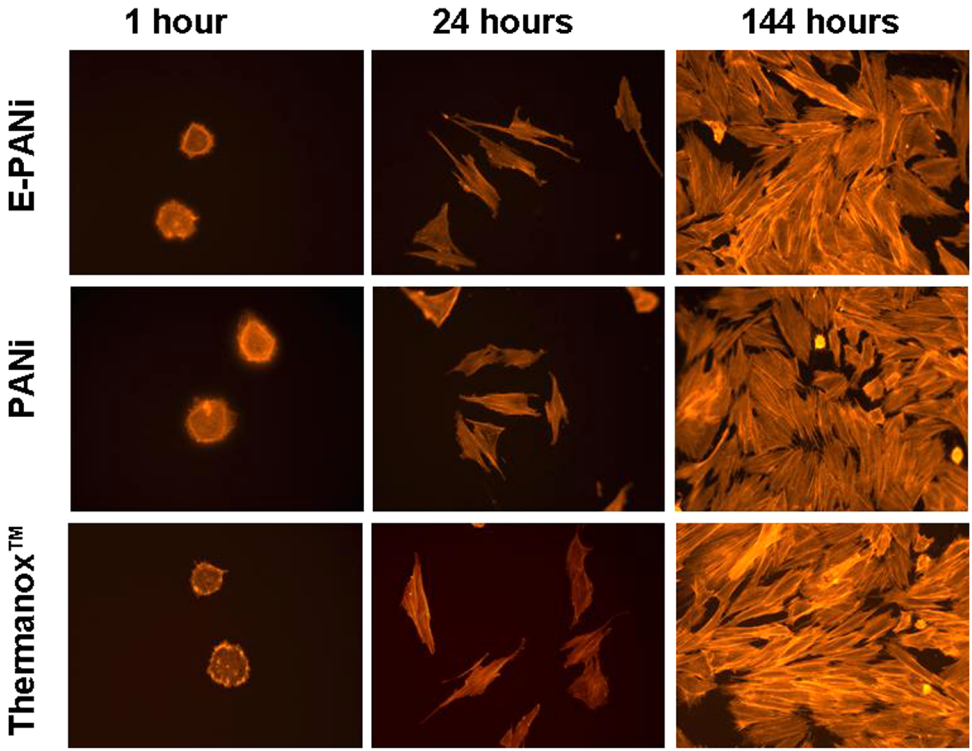


Figure 6. Microfilament arrangement of H9c2 cells growing on E-PANi, PANi and Thermanox™. Myoblast cells were seeded at low density (clonal) and cultured for up to 144 h, as detailed in Materials and Methods. Cells were stained with the F-actin-specific stain rhodamine-phalloidin and visualized on a Leica DMRx fluorescence microscope. All images were captured with Leica 300FX camera using a 20× fluorescence objective. This figure is published in colour on http://www.ingenta.com

DISCUSSION

In order for any polymer to be useful in tissue engineering it must be biocompatible, *viz.* not leach toxic substances that my cause harm locally or systemically, or otherwise interfere with the physiological responses of the cells that come in contact with these polymers. Cell attachment experiments demonstrated that H9c2 cardiac myoblasts attached readily, within 15 min, and in similar numbers, within 7% of controls, to both conductive and non-conductive forms of polyaniline (PANi). Subsequent cell proliferation experiments demonstrated that cells seeded on both the native and the electroactive form of PANi remained viable and proliferated at comparable rates, albeit with a slight delay, in comparison to tissue-culture-treated polystyrene (TCP).

Cells on PANi and TCP exhibited identical lag and log phases of growth, suggesting that polyaniline may be a suitable electroactive/conductive substrate for particular cell-culture and tissue-engineering applications, such as cardiac tissue engineering. We want to emphasize, however, that our observation that H9c2

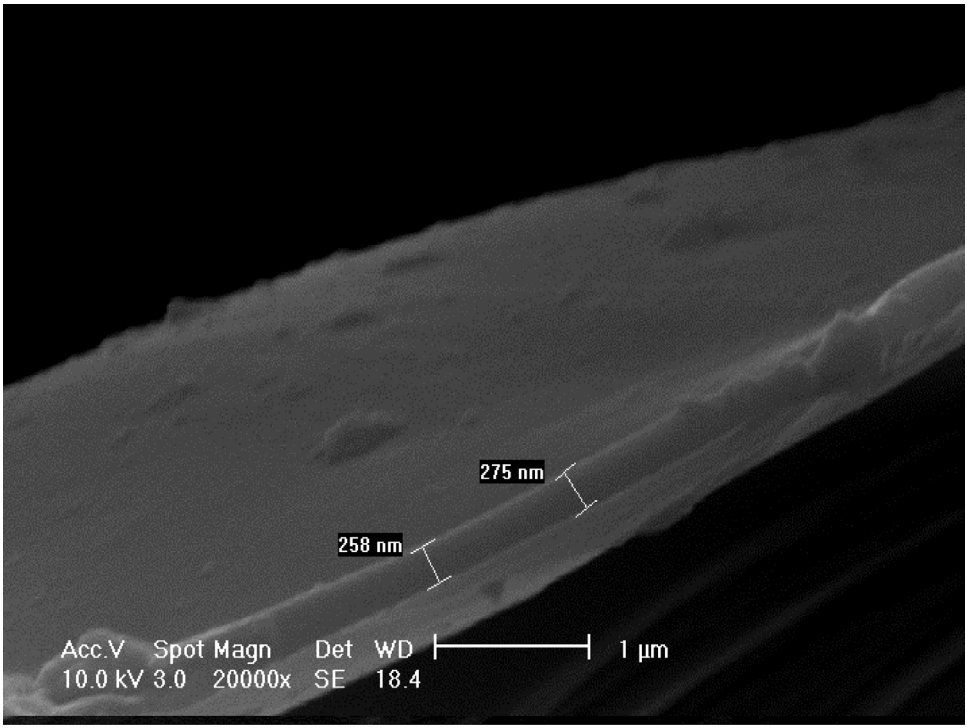
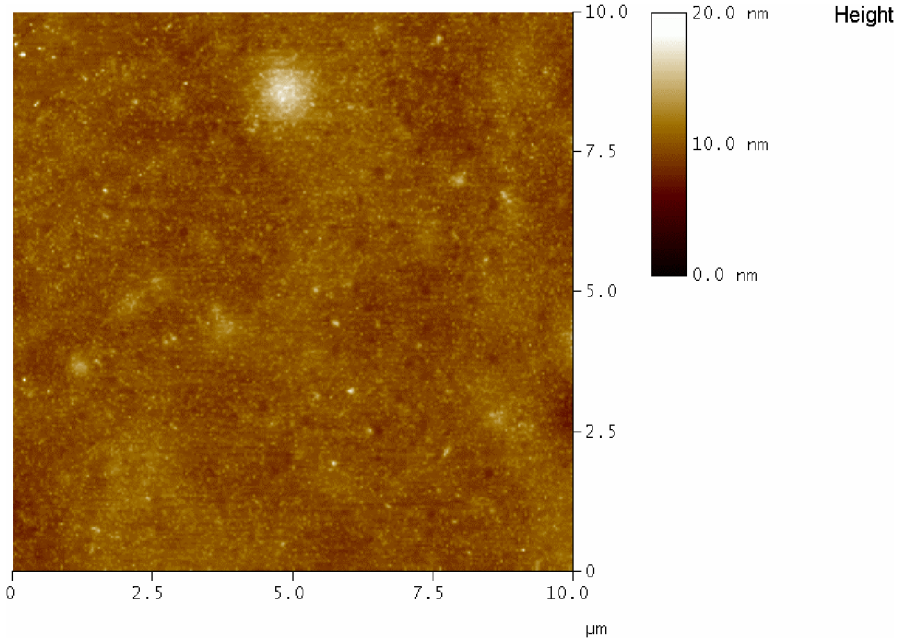


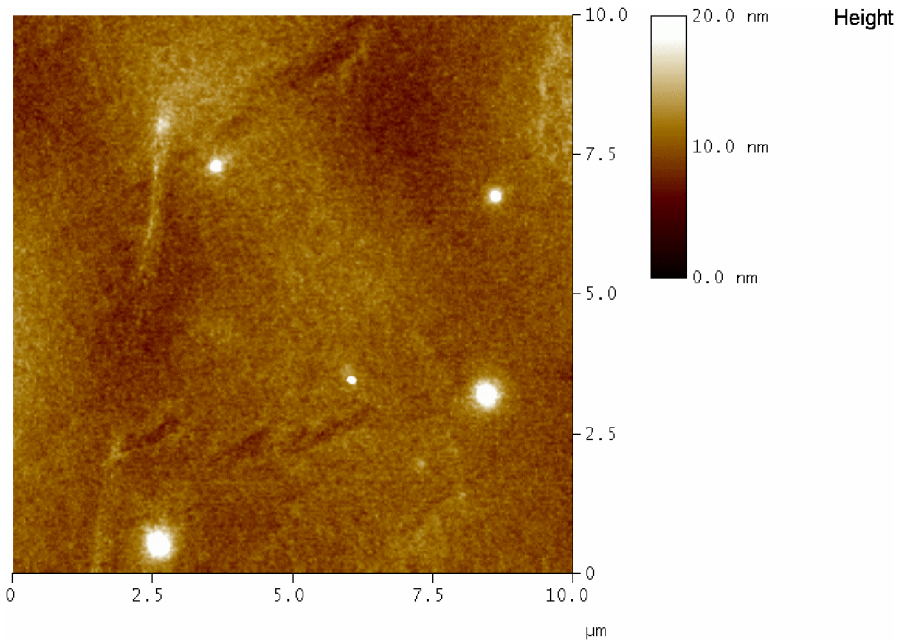
Figure 7. Representative scanning electron micrograph of a PANi film, edge-on view, for determining thickness. For details see Materials and Methods.

cells grow equally well on TCP and PANi may not be generalized to all cell types. Previous work in our lab demonstrated that, compared to TCP, PC12 adhesion to thin PANi films was decreased, but could be enhanced by covalently grafting bioactive peptides to the PANi surface [10, 11]. Neuroectoderm-derived PC12 cells may be more sensitive to the physicochemical properties of a substrate than other cell types [15]. While our previous findings with PC12 cells contrast the results reported here for cardiac H9c2 myoblasts, we recently saw a similar degree of adhesion and growth of murine embryonic fibroblasts on submicron-sized fibrous scaffolds containing PANi (data not shown). At this stage we do not have a straightforward explanation for these, probably cell-specific, discrepancies. While these findings will have to be corroborated with yet other cell types, our results suggest that the biocompatibility of conductive polymers might be restricted and cell-specific. Hence, and as caveat, we suggest that the biocompatibility of a biomaterial needs to be evaluated and ascertained for each cell type under investigation.

H9c2 cells growing on E-PANi exhibited a slightly prolonged period of delayed growth (lag phase) most probably due to the fact that in spite of the extensive 'curing' and washing after doping, small amounts of residual acid dopants apparently continue to leak for quite some time from the polymer surface. Once the dopant



(A)



(B)

Figure 8. (A) Representative AFM image of a PANi film applied on a Thermanox™ TCP cover slip, as detailed in Materials and Methods. (B) Representative AFM image for the Thermanox™ TCP substrate. This figure is published in colour on http://www.ingenta.com

dissipated (after approx. 100 h), the cells growing on E-PANi proliferated rapidly and reached a plateau similar to the cells on TCP control. The slope of the line (Fig. 2) as the cells on the E-PANi reached 200 h indicates that had the experiment run longer the cells on the E-PANi would have likely achieved the same endpoint as those on PANi. Doubling times were slightly prolonged for cells growing on E-PANi between 48 and 100 h. The slower growth of the cells may again be attributed to the possibility of small amounts of acid leaching from the surface. By contrast, the subsequent growth rate of the cells on E-PANi between 100 and 150 h is significantly ($P < 0.05$) faster than on the two other substrates. These results probably reflect the fact that the cells on TCP and PANi may approach confluence by 100 h and, hence, slow down their proliferation, while the cells on E-PANi, being further away from confluence, still have room to proliferate. We infer from these observations that at or around 100 h in culture the dopant in E-PANi has dissipated and the surface has become essentially non-conductive. This latter notion was verified by the conductivity experiments which show no conductivity when exposed to cell culture medium for 100 h or more (Fig. 4). We surmise that at this time point the former conductive surface has become more conducive to cell proliferation.

As described previously [3] relatively short (2 h) stimulation times during the first 24 h were effective in achieving increases in nerve growth factor (NGF)-induced PC12 cell neuritogenesis, as compared to controls, which were treated with NGF alone and not exposed to electrical stimulation. Continued electroactivity of the substratum beyond 24 h, such as used in the experiments by Schmidt *et al.* [3], is easily achievable with this polyaniline system (see Fig. 4). In our hands, stimulation of cells growing on electrically conducting PANi surfaces is feasible for up to 100 h in culture. These experiments, beyond the scope of this paper, are currently ongoing.

The work described here demonstrates the potential usefulness of PANi as an electroactive conductive polymer in cell-culture experiments and biotechnological applications, such as tissue-engineering or biosensors research. In both these instances biocompatibility and short-term electrical conductivity are important. Polyaniline may additionally be useful in studying the effects of electrical stimulation on excitable cells and tissues, such as cardiac myocytes. Future work will concentrate on demonstrating the functionality of the substrate with respect to the effects of electrical stimulation on cell function, specifically in neuronal and cardiac tissue engineering applications.

Acknowledgements

We are grateful to Dr. Yasha Kresh, Drexel College of Medicine for his generous gift of H9c2 cardiac myoblasts. This study was supported, in part, by a NASA Graduate Student Research Fellowship (NAG9-1380) to P.B., by grants-in-aid from NTI, the Nanotechnology Institute of Southeastern Pennsylvania (P.I.L., Y.W. and A.G.M.) and by a Drexel Synergy grant (P.I.L.).

REFERENCES

1. S. Ramakrishna, J. Mayer, E. Wintermantel and K. W. Leong, *Composit. Sci. Technol.* **61**, 1189 (2001).
2. A. G. MacDiarmid, W. Jones Jr., I. Norris, J. Gao, A. Johnson Jr., N. Pinto, J. Hone, B. Han, F. Ko, H. Okuzaki and M. Llaguno, *Synth. Metals* **119**, 27 (2001).
3. C. Schmidt, V. Shastri, J. Vacanti and R. Langer, *Proc. Natl. Acad. Sci. USA* **94**, 8948 (1997).
4. J. Wong, R. Langer and D. Ingber, *Proc. Natl. Acad. Sci. USA* **91**, 3201 (1994).
5. T. Aoki, M. Tanino, K. Sanui, N. Ogata and K. Kumakura, *Biomaterials* **17**, 1971 (1996).
6. A. Kotwal and C. Schmidt, *Biomaterials* **22**, 1055 (2001).
7. A. G. MacDiarmid, *Curr. Appl. Phys.* **1**, 269 (2001).
8. S. Kamalesh, P. Tan, J. Wang, T. Lee, E. Kang and C. Wang, *J. Biomed. Mater. Res.* **52**, 467 (2000).
9. C. Wang, Y. Dong, E. Kang and K. Neoh, *Synth. Metals* **102**, 1313 (1999).
10. E. Guterman, S. Cheng, K. Palouian, P. Bidez, P. Lelkes and Y. Wei, *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **43**, 766 (2002).
11. Y. Wei, P. Lelkes, A. G. MacDiarmid, E. Guterman, S. Cheng, K. Palouian and P. Bidez, in: *Contemporary Topics in Advanced Polymer Science and Technology*, Q.-F. Zhoi and S. Z. D. Cheng (Eds), p. 430. Peking University Press, Beijing (2004).
12. V. Nikolaychik, M. Samet and P. Lelkes, *J. Biomater. Sci. Polymer Edn* **7**, 881 (1996)
13. P. Lelkes, E. Ramos, V. Nikolaychik, B. Unsworth and T. Goodwin, *In Vitro Cell. Dev. Biol.* **33**, 344 (1997).
14. P. I. Lelkes, J. E. Friedman, K. Rosenheck and A. Oplatka, *FEBS Lett.* **308**, 375 (1986).
15. P. I. Lelkes and B. R. Unsworth, in: *Methods in Tissue Engineering*, A. Atala (Ed.), p. 371. Academic Press, San Diego, CA (2002).