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Human endothelial cell attachment to and growth on polypyrrole-heparin is vitronectin dependent

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Composite materials comprised of the electrically conducting polymer, polypyrrole, with a variety of biologically active molecules, e.g. proteins or polysaccharides, are emerging as a novel class of ''smart'' biomaterials. In the present work we have studied the utility of a heparin–polypyrrole composite as a substrate for human umbilical vein endothelial cell (HUVEC) growth. We found that the polymer composites were well suited to support cell attachment and growth; displaying low surface hydrophobicity (water contact angle of approximately 20°) and roughness, R_q, of approximately 10–12 nm. Doubling times for HUVEC on heparin–polypyrrole were greater than observed for gelatin-coated tissue culture polystyrene (44 and 36 h, respectively), however, the cells did proliferate to cover the polymer in an even monolayer. The initial mechanism of attachment and subsequent proliferation of HUVEC on heparin–polypyrrole was critically dependent on the presence of the serum adhesion glycoprotein vitronectin. Polymers that were composed of polypyrrole and sodium nitrate were more hydrophobic than heparin–polypyrrole and they did not support HUVEC growth. Given the relative ease with which these polymer composites can be electrochemically synthesized, the diverse range of cellular ''signal agents'', e.g. growth factors, that can be incorporated within them, and the high degree of control that can be achieved in the release–surface exposure of these agents, we suggest that polypyrrole composites could serve a useful role as ''smart'' biomaterials in the near future.

1. Introduction

Polypyrrole is an electrically conducting polymer that can be synthesized to contain a variety of (poly) anions [1, 2]. When polypyrrole is in its oxidized state, it exists as a polycation with delocalized positive charges created along its conjugated "back-bone", see [3] for review. In this state, the interaction of polypyrrole with a polyanion occurs in order to neutralize charge; a process also called doping. Electrical (or chemical) switching of the polymer to its reduced state extinguishes the positive charge on the polypyrrole and thereby alters the interaction of the polyanion within the material, leading to (in many cases) an alteration of the physical and chemical nature of the material. This attribute has permitted the use of polypyrrole composites in a diverse array of new technologies in fields ranging from chemical sensing and separation [3] to production of artificial muscle prototypes [4].

Previous studies from our group [1] and others [5–7] have identified polypyrrole as a novel substrate for the support of mammalian cell growth, thus indicating a potential utility for these polymers as "smart" biomaterials. The use of these electrically conducting polymers as biomaterials imparts a high degree of control to the material that has not been available in other polymers. We propose that by using dynamically controllable polymers, cellular growth at the material's surface may be selectively modulated. As a case in point, we have previously incorporated nerve growth factor (NGF) into polypyrrole composites and shown that the composites can be reduced

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(electrically) and thereby elicit the release of NGF from the substrate and subsequently promote nerve cell growth [2]. Other studies confirm that polypyrrole composites can be used to support nerve cell growth and neurite extension [6, 8].

Another potential use for polypyrrole composites may be as coatings for vascular stents and other prostheses [9]. Because several clinical advantages would be expected to result from encouraging reendothelialization of vascular prostheses, there is a clear need for new materials (or coatings) in this area [10]. It seemed plausible that a composite material based on polypyrrole could be developed to suit this need. Heparin is a sulfated polysaccharide, well known for its potent anticoagulant activity [11] and as a promoter of endothelial cell growth [12]. It is already known that the incorporation of heparin into polymer coatings enhances the haemocompatability of certain biomaterials, including intravascular stents [13]. The polyanionic nature of the heparin molecule predicts that it would be well suited for use in a polypyrrole-based biomaterial. This was recently shown to be the case with the resulting heparin–polypyrrole composites retaining their electrically conducting properties, even after autoclaving [9]. Furthermore, the quantity of free sulphate groups available to bind cationic dyes could be controlled reversibly by altering the redox state of the polymer [9].

The purpose of the present studies was to characterize the suitability of heparin–polypyrrole composites for use as substrates to support endothelial cell proliferation. This was first assessed by measuring key surface parameters that were known to influence cellular attachment to materials, i.e. surface hydrophobicity and roughness; and, second, by comparing the growth rates of endothelial cells on heparin–polypyrrole with standard culture conditions on gelatin-coated tissue culture polystyrene (gel-TCPS). By selectively removing the major serum adhesion glycoproteins, fibronectin (Fn) and vitronectin (Vn), from serum before addition to the cell culture medium, we were able to elucidate the mechanism of initial attachment of endothelial cells to heparin–polypyrrole. The results presented here define the nature of the interaction between endothelial cells and heparin–polypyrrole composites and support the notion that these materials could be used as stimulus responsive "smart" biomaterials in the near future.

2. Experimental procedure

2.1. Materials

Pyrrole (Merck) was purified by fractional distillation 130–1 °C and stored at −10 °C under N_2. Heparin sodium salt from porcine intestinal mucosa (used in polymer synthesis) and from bovine lung (used as an endothelial cell growth supplement) were from Sigma (Catalogue numbers H3149 and H4898, respectively). Milli-Q water (purified to 18 MΩ cm^2) was used in the preparation of all monomer solutions. Mylar film coated with gold was specially prepared by Courtaulds Intrex (CA, USA). All other reagents and solvents were of analytical grade and obtained through standard suppliers.

2.2. Polymer preparation

Polypyrrole-heparin or -$NaNO_3$ polymers were grown galvanostatically on gold-coated mylar films (9.5 cm^2) routinely at a current density of 0.5 mA cm^{-2}, which was applied for 4 min. Even polymer growth was achieved by applying a uniform parallel electrical field over the entire working electrode and by using a reticulated vitreous carbon counter electrode. Potentials generated during electrosynthesis were recorded as a chronopotentiogram using Ag–AgCl as a reference electrode. The monomer solution routinely contained 0.1 M pyrrole and 5 g l^{-1} heparin or 0.15 M $NaNO_3$ and was sparged with N_2 for approximately 10 min before use.

2.3. Autoclave conditions

After synthesis, polymers were sandwiched between two glass microscope slides and autoclaved at 121 °C (approximately 130 kPa) for 2 min without a drying cycle. This procedure was sufficient to kill *Pseudomonus sp.* that were applied directly to the test polymers.

2.4. Polymer reduction

Polymers were reduced by application of negative potential (−0.7 V) using the same three electrode cell as described for polymer synthesis and in an electrolyte of 0.15 M NaCl. Potential was applied for 90 s and samples were used either directly in experiments (in the case of cell attachment assays) or stored under N_2 in air-tight containers (to prevent oxygen induced re-oxidation of the polymers) for up to 4 h [for surface hydrophobicity and atomic force microscopy (AFM) analysis].

2.5. Isolation and culture of endothelial cells

Human umbilical vein endothelial cells (HUVEC) were isolated by collagenase dispersion as previously described [14] and maintained in Medium 199 (Sigma) supplemented with 20% (v/v) foetal bovine serum (FBS, Trace Biosciences, Melbourne, Australia), 1% (v/v) endothelial cell growth promoter (Starrate, Bethungra, Australia), 100 μg ml^{-1} heparin, 100 Units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. HUVEC were subcultivated after treatment with trypsin–ethylenediaminetetraacetic acid (EDTA) and grown on gelatin-coated tissue culture plastic (Type B gelatin from bovine skin used at 5% w/v and purchased from Sigma). Cells (used before passage 8) were then seeded directly onto heparin–polypyrrole films that had been autoclaved previously and placed in rectangular polystyrene multiwell plates.

2.6. Quantitation of cell attachment, growth and metabolic activity

The polymer composites with cells attached were removed from the tissue culture plates, fixed in Zamboni fixative for 20 min at 22 °C [15], rinsed thrice in 70% (v/v) ethanol in H_2O, stained with haematoxylin and

eosin, and finally viewed and photographed using an Olympus BH2 microscope and camera. Cells were counted in at least six randomly selected fields for each sample. Reduction of the tetrazolium dye (3-{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide, MTT) was also used as an index of cellular growth and metabolic activity [6, 16]. For this assay, cells were washed thrice with 37 °C Hanks' balanced salt solution (HBSS) then incubated for 3 h at 37 °C in a 5% CO_2 atmosphere in the presence of M199, 20% (v/v) FBS, 0.5 mg ml^{-1} MTT. Note that in studies that used FBS depleted of specific adhesion glycoproteins in order to investigate mechanisms of cellular attachment to the polymer surfaces (see below), the relevant depleted FBS was also used in the MTT assay. After 3 h, the cell supernatant was aspirated and the cells (containing the blue formazan crystals) were extracted into 1 ml 0.04 M HCl in isopropanol. After a 2 min centrifugation (10 000 *g*) to remove cellular debris, the absorbance of the supernatant was measured at 570 nm.

2.7. Preparation of Fn and Vn depleted sera

Where indicated, FBS used in the culture medium was treated to remove the adhesive glycoproteins Fn and/or Vn prior to use in cell culture experiments. Fn was removed by passage over a gelatin-Sepharose 4B (Pharmacia) affinity column [17]. Vn was removed by passing FBS over an affinity column consisting of anti-bovine Vn mAb A27 immobilized on Sepharose 4B [18]. A combination of these procedures was used to remove both Fn and Vn from FBS. Both affinity matrices were pre-equilibrated with serum-free culture medium prior to application of the FBS. The relative contents of Fn and Vn in the intact and depleted FBS were determined by enzyme-linked immunosorbent assay (ELISA) using anti-Fn mAb A12 or anti-Vn mAb A27 [18], and showed that the Vn or Fn content of the treated sera were less than 0.5% of that of the original FBS.

2.8. Polymer surface characterization

The surface hydrophobicity of the polymers was ascertained using a Ramé–Hart goniometer equipped with a constant temperature environmental chamber and micro syringe attachment. Each polymer was assessed in at least four different regions using a water (Milli-U, $\gamma_L = 72.8$ mJ m^{-2}) droplet of 3 µl. Samples were maintained at 25 °C.

AFM was used to determine surface roughness of the polymers. A Digital Nanoscope III scanning probe microscope was used in contact mode to scan areas of 10 and 1 µm^2, $n = 3$ for each sample. Polymers were air dried (or dried under a stream of N_2 gas for reduced samples). Data are given as the root-mean-square, R_q, of the *z*-values within the given area.

2.9. Statistical analysis

Statistical significance was determined using the two tailed *t*-test. A P value of < 0.05 was considered significant.

3. Results

In our previous studies we have described in detail the synthesis and electrochemical characterization of heparin–polypyrrole composites [9, 19]. Preliminary observations also indicated that HUVEC were able to attach to and apparently grow over heparin–polypyrrole [9]. Here we have studied the rates of HUVEC proliferation on heparin–polypyrrole and compared this to standard *in vitro* conditions on gel-TCPS. The period chosen to study cellular proliferation was between 24 and 96 h after seeding. This time-frame has previously been shown to be a good indicator of proliferative capacity [20]. From time, $t = 0$ to 24 h, HUVEC undergo a lag phase, proliferating slowly [12, 20], while beyond four to six days (using the present culture conditions) the proliferation rate declines due to contact inhibition. Fig. 1 shows that both the number of cells present and the amount of MTT reduced (observed over a 3 h period) increased significantly when cells were cultured on either gel-TCPS or heparin–polypyrrole for 96 h and compared with 24 h values. Doubling times (estimations based on cell number) were calculated to be approximately 36 h on gel-TCPS and approximately 44 h on heparin–polypyrrole. Taking both the cell number and metabolic

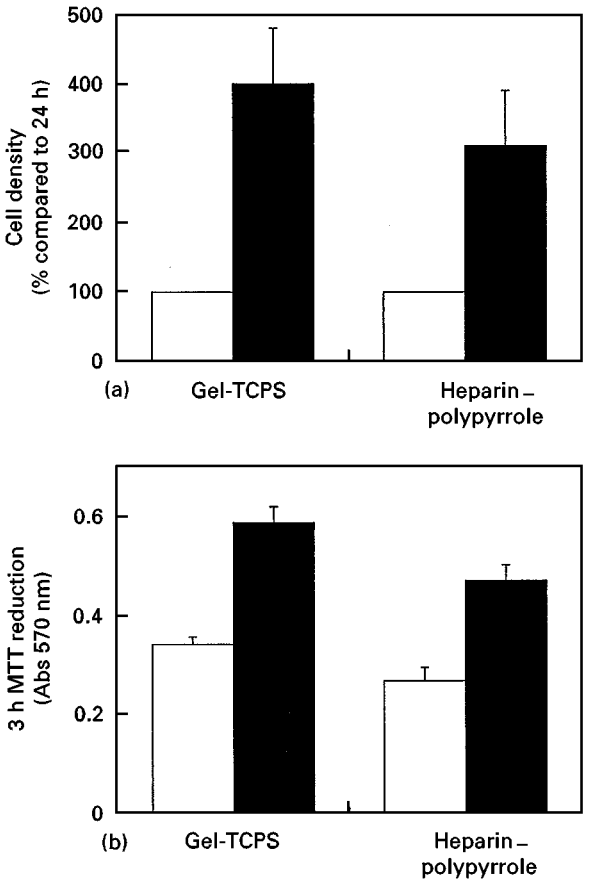

Figure 1 Proliferation of HUVEC on heparin–polypyrrole or gelatin-coated tissue culture polystyrene. HUVEC were seeded at an initial density of approximately 1×10^5 cells per well (or polymer). The number of cells present (a) or the amount of MTT reduced in a 3 h period (b) was then assessed after 24 h (white bars) and 96 h (black bars) *in vitro*. Values for cell density at 24 h are standardized to 100%. Data are means and SEM (where indicated by error bars) of three experiments each performed in duplicate.

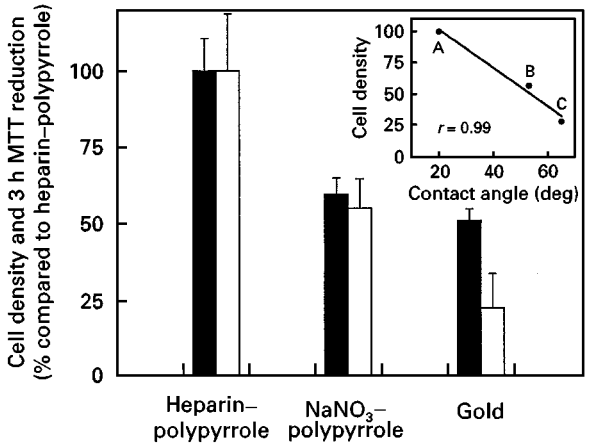

Figure 2 Attachment of HUVEC to polypyrrole composites or gold—effect of surface hydrophobicity. HUVEC were seeded at an initial density of approximately 3×10^5 cells per sample. The number of cells present (white bars) or the amount of MTT reduced in a 3 h period (black bars) was then assessed after 90 min. Values are standardized to the heparin–polypyrrole that was assigned a value of 100%. The inset shows the correlation of the cell density versus the hydrophobicity of the samples where A, B and C are heparin–polypyrrole, $NaNO_3$–polypyrrole and gold, respectively. Data are means and SEM (where indicated by error bars) of two experiments each performed in duplicate.

activity (MTT reducing capacity) into account, the rate of cell growth on heparin–polypyrrole was approximately 80% of that observed on gel-TCPS. The doubling times observed on either substrate are within literature values for HUVEC grown under different culture conditions, which can range from 17 to 60 h depending, for example, on the source and purity of media growth factor supplements and heparin [12].

The use of heparin in composite biomaterials has the obvious advantage that it can inhibit thrombus formation and thereby improve the materials' haemocompatability [13]. We also considered that heparin could itself impart properties on the polypyrrole that promote HUVEC attachment. We therefore compared HUVEC attachment and proliferation on polymer composites comprised of polypyrrole and either heparin (as a polyanion) or $NaNO_3$ (as a simple inorganic anion). HUVEC attachment to heparin–polypyrrole was almost two-fold higher than to $NaNO_3$–polypyrrole and approximately five-fold higher than to gold-coated Mylar (Fig. 2). This was demonstrated both by cell numbers present on each material and by MTT reduction. In the case of HUVEC attached to gold, the MTT reducing activity was higher, which may suggest that the cells were in some way activated when attached to gold. It is established that cellular activation, e.g. by inflammatory stimuli or cytokines, increases MTT reducing activity most likely via upregulation of mitochondrial dehydrogenase activity.

In order to ascertain which physical characteristics of the material surface could account for the better attachment of HUVEC to heparin–polypyrrole compared with $NaNO_3$–polypyrrole or gold we studied the surface hydrophobicity and roughness of each of the polymers. These parameters are known to influence endothelial cell attachment to biomaterials

TABLE I Surface hydrophobicity and roughness of polymers and gold film[a]

Material	Sessile contact angle[b] (θ°)	Roughness, R_q[c] (nm)
Gold-coated Mylar	65 ± 3	5.4 ± 0.1
$NaNO_3$–polypyrrole	53 ± 1	5.6 ± 0.5
$NaNO_3$–polypyrrole (reduced)	63 ± 6	5.1 ± 0.2
Heparin–polypyrrole	20 ± 1	11.8 ± 0.3
Heparin–polypyrrole (reduced)	32 ± 3	10.5 ± 0.2

[a] Polymers were synthesized electrochemically as described in Section 2. Representative samples both in oxidized and reduced states were then assessed for surface hydrophobicity using water in air contact angle measurements and surface roughness, the latter assessed by AFM.
[b] $n = 16$–32 determinations made on two–four samples of each material (mean ± SEM).
[c] n = three–four determinations made on each material (mean ± SEM).

[20, 21]. The air–water sessile contact angles of each of the test materials are given in Table I. Gold was the most hydrophobic surface, followed by $NaNO_3$–polypyrrole and heparin–polypyrrole, the latter having a contact angle of only 20°. When the number of cells attached to the materials was plotted versus the hydrophobicity of the materials, a strong inverse linear correlation was observed (Fig. 2, inset). This type of correlation has also been observed for the initial attachment of HUVEC to other materials of varying hydrophobicities [20, 22]. Tissue culture polystyrene (not gelatin-coated) exhibited sessile contact angles of 64 ± 1°, in close agreement with other literature values (e.g. 67°, [22]).

The polypyrrole composites containing heparin or $NaNO_3$ are redox active and can be switched between the oxidized and reduced states. We have already shown that this characteristic can be utilized in order to elicit the controlled release of signal agents, e.g. growth factors, from the biomaterial and thereby control cell growth [2]. In the case of heparin–polypyrrole, reduction results in enhanced surface expression of the polysaccharide [9]. If this dynamic property of the polymer were to be used in the future to trigger the release or altered surface expression of a growth-promoting signal agent, it would be ideal if cellular attachment was not dramatically altered when the polymer was in either redox state. The effect of polymer reduction on surface hydrophobicity and HUVEC attachment was therefore assessed. Polymer reduction led to an increase in the hydrophobicity of both the polypyrrole composites (Table I). However, this did not significantly decrease the initial attachment of HUVEC (assessed by direct counting and by MTT reduction) to the materials (data not shown). Table I also shows that reduction had very little impact on the surface roughness of the polymers (assessed by AFM), whereas there were significant differences in roughness when $NaNO_3$–polypyrrole was compared to heparin–polypyrrole; the latter being rougher. AFM also revealed that the polymers were homogeneous with respect to surface roughness, this is

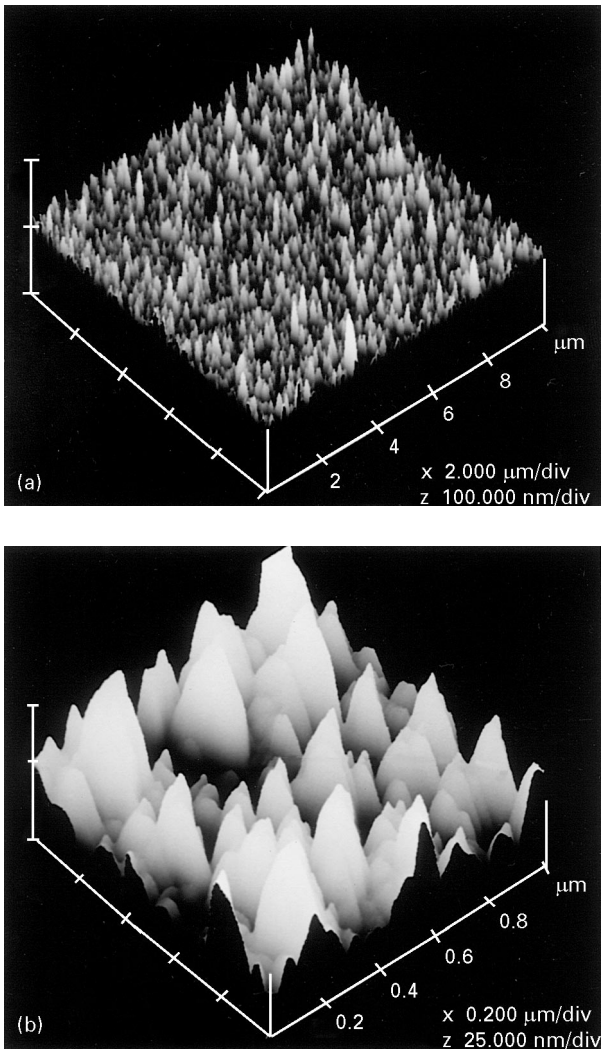

Figure 3 Surface topography of heparin–polypyrrole. Heparin–polypyrrole composite polymers were analysed using AFM in contact mode. Representative fields of 10 and 1 μm^2 are shown in (a) and (b) respectively. The scale bars on the *z*-axis represent 100 nm per division for (a) and 25 nm per division for (b).

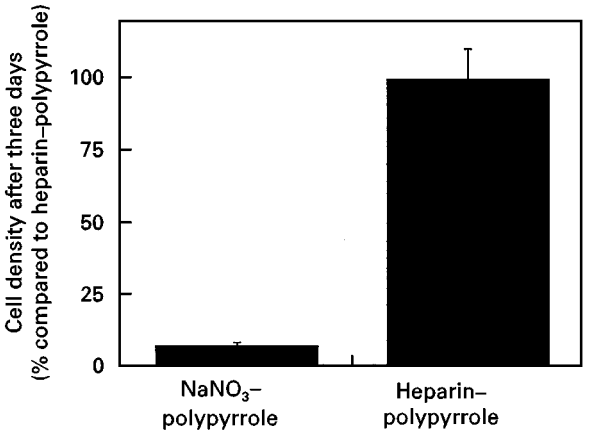

Figure 4 HUVEC growth on polypyrrole composites after three days *in vitro*. HUVEC were seeded at an initial density of approximately 1×10^5 cells per sample. The number of cells present on either heparin–polypyrrole or $NaNO_3$–polypyrrole was then assessed after three days. Values are standardized to the heparin–polypyrrole that was assigned a value of 100%. Data are means and SEM of a single experiment performed in duplicate and are representative of two experiments.

illustrated in the three-dimensional images shown (Fig. 3). Taken together, these data suggest that both the surface hydrophobicity and roughness contributed to the enhanced ability of cells to attach to and grow on heparin–polypyrrole. In addition, it appears that the modest increase in surface hydrophobicity observed when heparin–polypyrrole is switched to its reduced state is not sufficient to affect cell attachment adversely.

The data shown in Fig. 2 indicated that $NaNO_3$–polypyrrole may be a moderately useful material to grow endothelial cells on, however, after the initial attachment, HUVEC did not proliferate on this polymer. After three days in culture, there were polymer ten-fold more cells on heparin–polypyrrole compared with $NaNO_3$–polypyrrole (Fig. 4). The small number of remaining viable cells detected on $NaNO_3$–polypyrrole were not well spread and tended to grow on top of each other (data not shown).

We next investigated the mechanism of initial HUVEC attachment to heparin–polypyrrole and compared this with cellular attachment to either TCPS or gel-TCPS. When Fn was removed from the culture medium, there was no significant effect on initial cell attachment to heparin–polypyrrole, TCPS or gel-TCPS (Fig. 5). In sharp contrast, when Vn was removed from the medium, HUVEC attachment to heparin–polypyrrole was inhibited by approximately 70% and to TCPS by approximately 80% (Fig. 4). Although the effect of Vn removal on cell attachment was not as dramatic when the MTT assay was used to assess cellular attachment, the data showed that the observed inhibition in the number of cells attached was paralleled by lower MTT-reducing activity (Fig. 5). HUVEC attachment to gel-TCPS was not affected by removal of either (or both) Fn or Vn from the culture medium, suggesting that gelatin likely provides alternative cell-binding sites (Fig. 5). The removal of Fn from serum that was already depleted of Vn did not have any additional impact on HUVEC attachment to heparin–polypyrrole, suggesting that Vn was the most important glycoprotein for HUVEC attachment to this substrate (Fig. 5).

While up to one third of the cells appeared to be attached to heparin–polypyrrole via Vn-independent mechanisms, these cells were not well spread on the polymers (Fig. 6) and after a further 24 h *in vitro*, only a very small proportion of these cells remained attached and viable (Fig. 7). The deleterious effects on HUVEC attachment to heparin–polypyrrole that were observed in the absence of Vn were also accentuated after a 24 h period when HUVEC were seeded on TCPS (but not on gel-TCPS). Thus Vn appears to be essential for optimal attachment of HUVEC to TCPS [22] and to heparin–polypyrrole composites.

4. Discussion

The present studies show that composites of heparin–polypyrrole can be used as substrates for endothelial cell growth and, given the anticoagulant properties of the vascular endothelium [23], could therefore potentially serve a role as intravascular biomaterials or

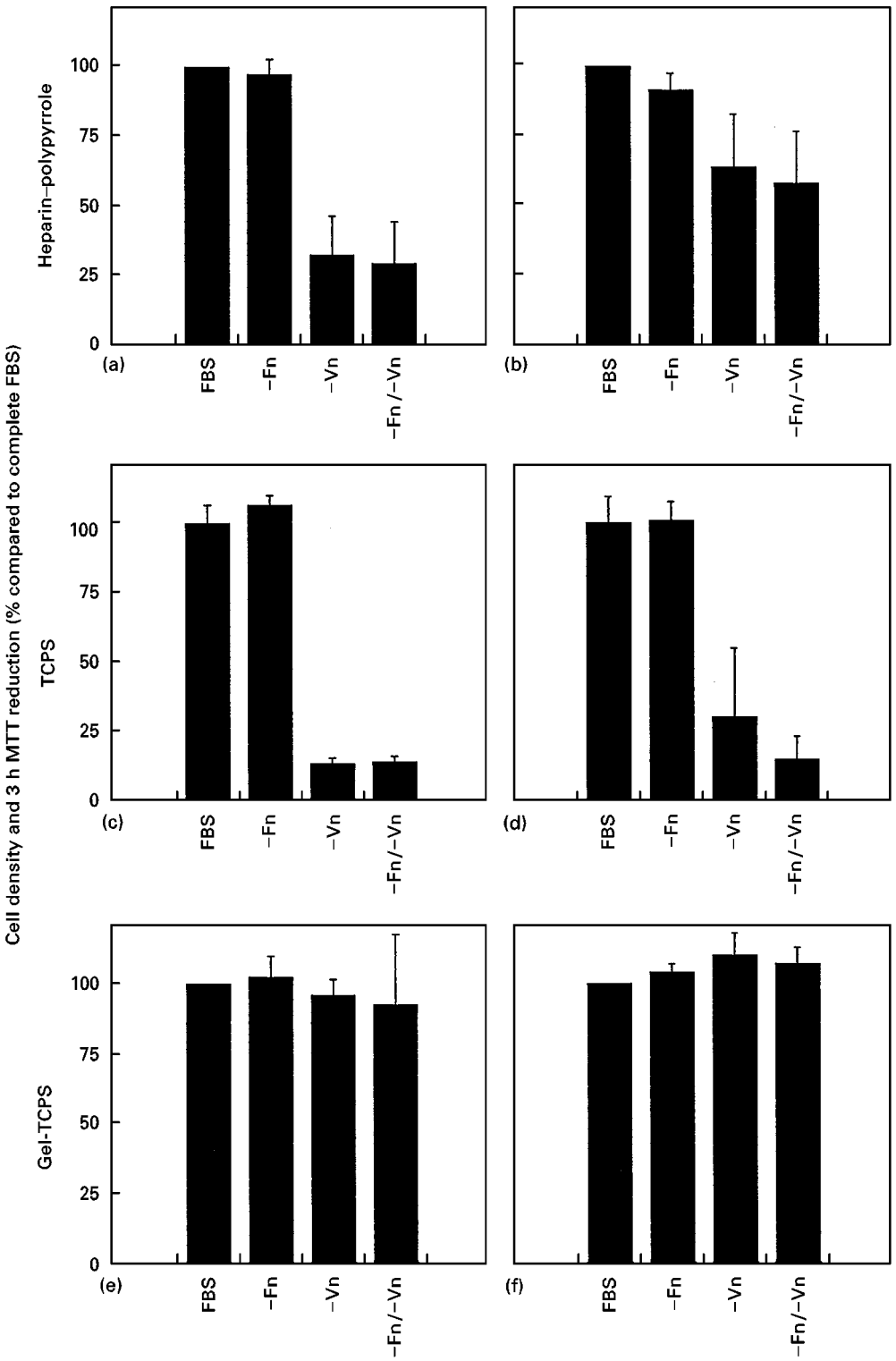

Figure 5 Requirement for vitronectin of HUVEC attachment to various surfaces. HUVEC were seeded at an initial density of approximately 3×10^5 cells on to heparin–polypyrrole. (a, b), tissue culture polystyrene (c, d) or gelatin-coated tissue culture polystyrene (e, f). The number of cells present (a, c and e) and the MTT reduced in 3 h (b, d and f) were assessed after an initial 90 min incubation to allow for attachment. The medium contained either intact foetal bovine serum (FBS), FBS depleted of: fibronectin (-Fn), vitronectin (-Vn) or both Fn and Vn (-Fn/-Vn). Values are standardized to the conditions that contained intact FBS, which was assigned a value of 100%. Data are means and SEM (or range) of three, four, one, one, two and two experiments performed in duplicate for (a) to (f), respectively.

material coatings. The growth rate of HUVEC on heparin–polypyrrole was somewhat slower than on gel-TCPS; the reasons for this are not entirely clear. We know that heparin can be detected within these composites, based on toluidine blue binding to sulfate groups associated with the polysaccharide [9], and we have evidence that at least some of its biologically activities are retained, e.g. heparin within heparin–polypyrrole composites binds thrombin but not unrelated proteins such as albumin [19]. It has been shown by others that heparin in solution can promote endothelial cell growth by enhancing the delivery of growth factor(s) to the cell or by inducing a conformational change in the growth factor that increases its

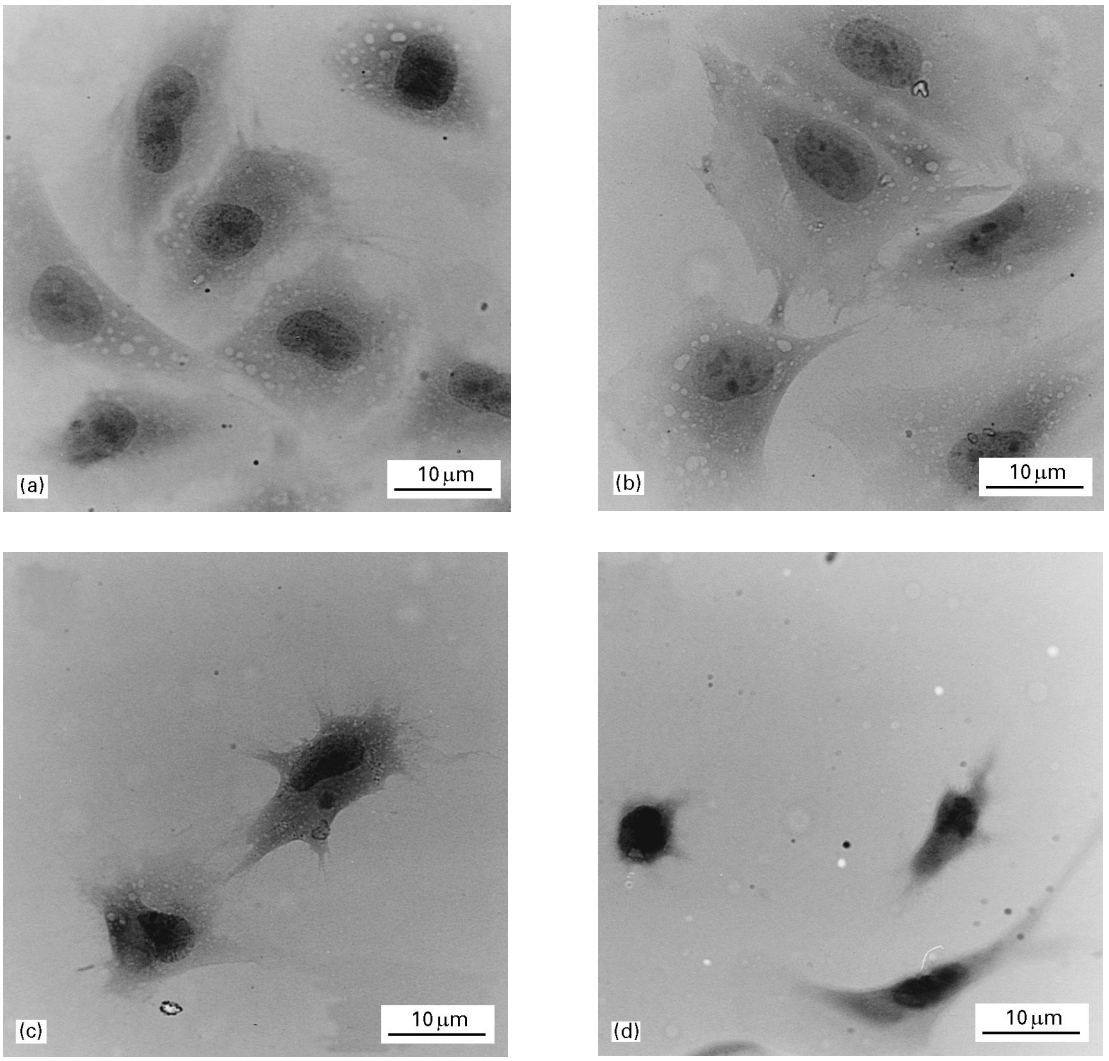

Figure 6 Morphology of HUVEC attached to heparin–polypyrrole in the presence or absence of adhesion glycoproteins. HUVEC were seeded at an initial density of approximately 3×10^5 cells on to heparin–polypyrrole in medium that contained either intact foetal bovine serum (a), FBS depleted of: fibronectin (b), vitronectin (c) or both Fn and Vn (d) and incubated for 90 min. The cells were then rinsed with Hanks' balanced salt solution and stained with haematoxylin and eosin before being photographed.

mitogenic activity [24]. It seems unlikely that heparin incorporated within the polymer composites, i.e. distal to the upper most surface upon which the cells are attached, would compete with heparin added to the growth medium (as a mitogen) as there is approximately 15 times more present in the medium. If part of the slower growth rate of HUVEC on heparin–polypyrrole is due to misdirection of growth factors, or if acceleration of proliferation is desired in future, it should be possible to incorporate additional growth factors within the polymer that could be released either electro- or biochemically, as previously suggested by us [1, 2].

The physical character of the heparin–polypyrrole surface was shown to be well suited for cell attachment. While direct binding of heparin by endothelial cells is well known [25] the dependence of Vn for cell attachment indicated that direct cell-heparin interactions did not contribute to the adhesion mechanisms. The surface hydrophobicity and roughness provided an environment upon which Vn could presumably bind and thereby facilitate cell attachment and spreading. The precise nature of the interaction between heparin–polypyrrole and Vn has not been defined here; however, the major mechanism for Vn binding is probably not via direct interaction with heparin. This is based on the knowledge that unless Vn is denatured to reveal an otherwise "cryptic" heparin-binding domain (located in an area rich in basic amino acids at the COOH terminus) only a small proportion of native Vn (12–25%) is able to bind heparin [26–28]. Secondly we have synthesized composites comprised of polypyrrole and poly(2-acrylamido-2-methyl-1-propanesulphonate) (as a polyanion) and find that these composites are also able to support endothelial cell growth [29]. On the other hand, if a small proportion (12–25%) of Vn binding to the heparin–polypyrrole was via a direct interaction with heparin, this would provide an additional means to enhance cellular attachment through a more specific chemical interaction and could possibly explain why there was no decrease in cell attachment to the reduced composites (which were more hydrophobic). In this case, reduction would have increased the hydrophobicity of the polymer but also increased the amount of surface-exposed heparin, thereby presenting additional Vn-binding sites.

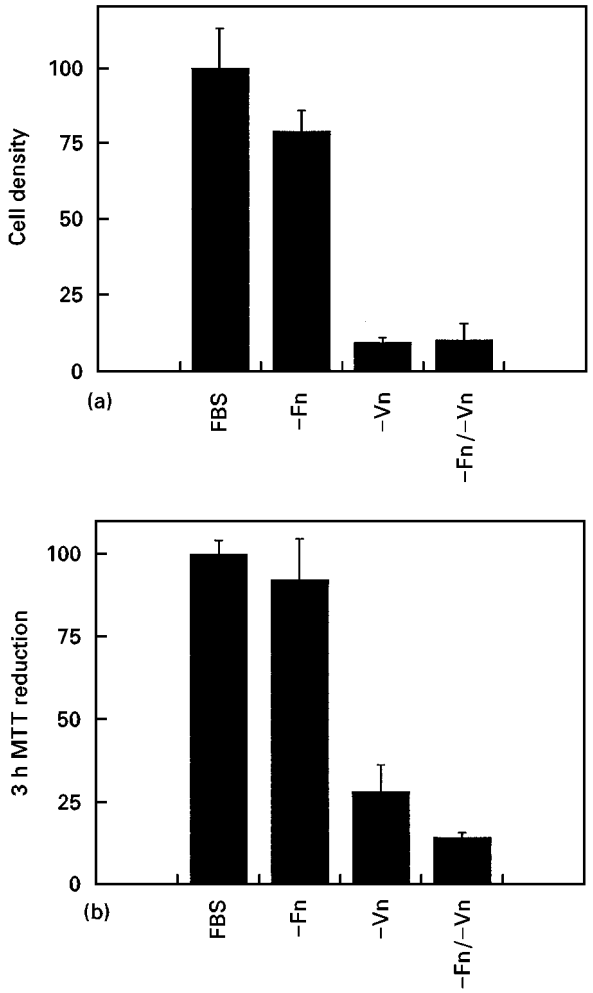

Figure 7 Requirement for vitronectin of HUVEC attachment to heparin–polypyrrole after 24 h *in vitro*. HUVEC were seeded at an initial density of approximately 3×10^5 cells on to heparin–polypyrrole and the number of cells present (a) and the MTT reduced in 3 h (b) was assessed after 24 h incubation. Conditions are as described in the legend to Fig. 5.

It is not entirely clear why the reduction of heparin–polypyrrole resulted in an increased surface hydrophobicity. In the neutral (dedoped) state, polypyrrole is more hydrophobic than in the oxidized state [30, 31]. However, if it is assumed that the surface exposure of heparin was responsible for decreasing the hydrophobicity of the polymer when compared with $NaNO_3$–polypyrrole, it would follow that reduction, which increases the amount of heparin exposed at the surface, should further decrease the surface hydrophobicity. The fact that this was not observed may indicate that the increased protrusion of heparin out of the surface of the polymer composite is not sufficient to overcome the increased hydrophobicity induced by the polypyrrole. Further studies are required to investigate this possibility.

Regardless of the mechanism involved, the increased hydrophobicity of the heparin–polypyrrole (change in the air–water contact angle of 12 °) was not sufficient to decrease the number of cells attaching in the first 90 min. This is mostly likely because the surface properties of heparin–polypyrrole influenced cell adhesion via Vn and the range of hydrophobicities measured both before and after polymer reduction (contact angles of 20–32 °) were within the ideal range for Vn binding. Other studies of HUVEC attachment to nitrogen-containing polymers that exhibit sessile contact angles of approximately 30 ° showed that HUVEC attachment in the first 90 min after seeding was close to maximal [22].

It is clear that Fn can also mediate binding of HUVEC to polymers (including polypyrrole) and to heparin-like microcarriers comprised of polystyrene sodium sulphonate if it is first adsorbed or covalently linked to the surface [7, 32]. The present data indicate that heparin that is present within a polypyrrole composite may lose its ability to bind to Fn, as this glycoprotein did not play a significant role in HUVEC attachment to the polymer. It should also be noted that when serum is used at concentrations of $> 2\%$, the absorbance of Fn to certain material surfaces (depending on the surface chemistry) is inhibited due to competition with other serum factors and Vn is the major adhesion protein for endothelial cell attachment [22]. If a cell-type-selective material is desired there may be some disadvantage in developing materials that are readily coated with Fn because a variety of cell types can bind to this glycoprotein. To increase endothelial cell specificity, it should be possible to incorporate specific peptide sequences that are selective for endothelial cell attachment. Of relevance, Hubble *et al.* have defined an endothelial cell specific sequence (REDV) that could be of potential benefit [33].

5. Conclusions

In summary, heparin–polypyrrole is a novel substrate capable of supporting human endothelial cell growth. Cellular attachment to heparin–polypyrrole is indirect, requiring the presence of the serum adhesion glycoprotein, Vn. Tissue implantation studies have shown that polypyrrole is biocompatible in animals [6, 8, 34], further indicating the potential utility of these polymers as biomaterials in humans.

Acknowledgements

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