

Electrospun polyurethane as an alternative ventricular catheter and in vitro model of shunt obstruction

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Abstract

Intracranial pressure and volume vary considerably between hydrocephalic patients, and with age, health and haemodynamic status; if left untreated, intracranial pressure rises and the ventricular system expands to accommodate the excess cerebrospinal fluid, with significant morbidity and mortality. Cerebrospinal fluid shunts in use today have a high incidence of failure with shunt obstruction being the most serious. Conventional proximal shunt catheters are made from poly(dimethyl)siloxane, the walls of which are perforated with holes for the cerebrospinal fluid to pass through. The limited range of catheters, in terms of material selection and flow distribution, is responsible in large part for their poor performance.

In this study, we present an alternative design of proximal catheter made of electrospun polyether urethane, and evaluate its performance in the presence of glial cells, which are responsible for shunt blockage. The viability and growth of cells on catheter materials such as poly(dimethyl)siloxane and polyurethane in the form of cast films, microfibrinous mats and porous sponges were studied in the presence of proteins present in cerebrospinal fluid after 48 h and 96 h in culture. The numbers of viable cells on each substrate were comparable to untreated poly(dimethyl)siloxane, both in the presence and absence of serum proteins found in cerebrospinal fluid. A cell culture model of shunt obstruction was developed in which cells on electrospun polyether urethane catheters were subjected to flow during culture in vitro, and the degree of obstruction quantified in terms of hydraulic permeability after static and perfusion culture. The results indicate that a catheter made of electrospun polyether urethane would be able to maintain cerebrospinal fluid flow even with the presence of cells for the time period chosen for this study. These findings have implications for the design and deployment of microporous shunt catheter systems for the treatment of hydrocephalus.

Keywords

Hydrocephalus, ventricular catheter, electrospun polyurethane, hydraulic permeability, cell viability, in vitro modelling

Introduction

Hydrocephalus is a neurological condition characterised by the accumulation of cerebrospinal fluid (CSF) in the ventricles or cavities of the brain, brought about by an imbalance in formation and absorption of CSF. Hydrocephalus is an incurable condition at present.¹ The most common treatment option available is implantation of a CSF shunt device, to divert the excess CSF to another part of the body, where it is absorbed. Although the mortality rate has decreased since their introduction in the 1950s, the incidence of shunt failure remains unacceptably high, which may be attributed in part to the lack of substantial investment in the advancement of the technology.^{2,3}

Ventricular shunts fail for many reasons, but the failure that causes the greatest impact is obstruction of the catheter, proximal occlusion being the most common.⁴ Clinical reports indicate that 20–30% of shunt failures in the first year of surgery are caused by obstruction.⁵ The main sources of obstruction are the glial cells, which invade the perforations (fenestrations) in the walls of the ventricular catheter, preventing the entry of the

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CSF.⁶ The obstruction in all cases necessitates replacement of the catheter, which, in turn, may lead to further complications and discomfort for the patient.⁷

Since shunt devices were introduced, several improvements in shunt design have been made, including alternative drainage locations, ventricular catheter placements, new valve and catheter assembly designs, and anti-siphon and on-off devices that bring about gravity-actuated changes in opening pressure and externally adjustable valves.⁸ Nevertheless, to date, there is no one valve that is proven to be superior.² Arguably one of the most promising avenues of research being explored at present involves creating a smart shunt in which the valve is capable of being controlled remotely. A shunt that can regulate CSF more effectively, and is less susceptible to malfunction, would have obvious benefits to the patient.^{5,9} Although such advances would represent a milestone in hydrocephalus shunt device development, a blocked catheter will inevitably lead to revision surgery.¹⁰

There is a lack of design criteria for the proximal catheter available at present.^{6,11,12} More than 80% of the perforations present in the proximal catheter are not in use at any given time, and they may become easily blocked.¹¹ Conventional catheters are made of poly(dimethyl)siloxane (PDMS), with few exceptions, and although there are studies which involve surface modification of PDMS to reduce the cell attachment,^{7,13,14} few, if any, studies have considered micro-porous materials for this application.

The aim of this study was to evaluate the hydraulic performance and cell growth characteristics of a micro-fibrous proximal catheter in comparison to conventional catheter materials.

Materials and methods

Catheter material preparation

The candidate catheter materials chosen for this study were medical-grade PDMS (Silastic® MDX4210, Dow Corning Corp., Midland, MI, USA; ImmobaSil®, Ashby Scientific Ltd, Coalville, UK) and polyurethane (*b₉*™ Series 'A', Biomer Technology Ltd, Runcorn, UK; Tecoflex® SG-80A, Thermedics Polymer Products, Woburn, CA, USA) in the form of cast films, microfibrous mats and porous sponges. A silicone hydrogel (Nelfilcon 'A', CIBA VISION Corp., Duluth, GA, USA) served as negative control.

The materials to be evaluated in contact with cells were prepared and secured by means of a short length of fluorinated polyethylene tubing in the bottom of 96-well polystyrene plates, with blank wells serving as positive control. Silastic® MDX4210 biomedical grade elastomer was prepared according to the manufacturer's

instructions. Approximately 20 µl of the combined solution was injected into the base of each well and allowed to cure at 50°C for 12 h before seeding with cells. ImmobaSil porous microcarriers were cut so as to fit the base of the each well. Polyurethanes Z1A1 and Z3A1 pellets were dissolved to a concentration of 15% w/w in dimethylformamide by mixing on a roller mixer for 48 h at room temperature and atmospheric pressure. Thereafter, each polyurethane solution was cast into glass petri dishes and the solvent removed at 65°C under a vacuum of 45 kPa for 48 h. The resulting cast films were detached from the petri dishes and soaked in deionised water for 24 h before use to ensure complete removal of all remaining solvent. The surface exposed to air was used so as to eliminate any variation attributed to the polymer in contact with the petri dish surface.¹⁵

Microfibrous polyurethane (electrospun polyether urethane, EPU) catheters were produced according to an electrospinning protocol described by Andrews et al.¹⁶ The morphology of the EPU material was determined by the scanning electron microscopy (Hitachi TM 1000 SEM Hitachi High-Technologies Europe GmbH, Krefeld, Germany), in which a small portion of the catheter was cut and affixed to a stub ready for imaging. Micrographs of both the inner and outer surfaces of the electrospun material were acquired and analysed to determine the pore size, diameter and alignment of the fibres.

Cell culture protocols

Primary astrocytes were isolated from 1- or 2-day-old neonatal rat pups that had been sacrificed according to UK Home Office guidelines. The cortices were separated from the brain, minced and suspended in phosphate-buffered saline (PBS). The minced samples were then sieved through 100-µm cell strainer using a sterile glass tissue grinder, followed by passage through a 70-µm cell strainer, and the samples were collected in a centrifuge tube. After a 5-min centrifugation at 800 rpm, the sample was resuspended in 5 ml Dulbecco's modified Eagle Medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 1% nonessential amino acids. The cell suspension was seeded at 3×10^5 cells/cm² in a tissue culture flask and incubated under a 5% CO₂ atmosphere at 37°C. After reaching 80% confluence, the flask was shaken at 60 rpm for 12 h to remove other types of cells. Immortalised mouse fibroblast (3T3) cells were established from NIH Swiss mouse embryo and cultured in identical growth medium.

Protein adsorption and cell seeding

The plates were sterilised under a UV lamp for 15 min before culture. Bovine plasma fibronectin (Fn) (2 g/ml)

(Invitrogen, Paisley, UK) was diluted in PBS to a final concentration of 4 µg/ml. Twenty micro litres of this solution was added to cover the surface of the material in each well and left in incubator for 1 h at 37°C. Any unbound Fn was removed by washing twice with PBS. The treated surface was then blocked by adding 20 µl of 1% w/v bovine serum albumin (BSA) (Sigma-Aldrich, Gillingham, UK) for 1 h at 37°C. After the time period for incubation the excess BSA was removed by washing twice with PBS. The surface was not allowed to dry out at any stage during the surface treatment process. NIH/3T3 cells or primary astrocytes were cultured under static conditions at a seeding density of 1.2×10^4 cells/cm² on samples of each material, which were secured to the base of a 96-well plate as before.

Fluorescence microscopy

Samples of the cast films and silicone foam (SF) were prepared as above and incubated with astrocytes for 96 h prior to imaging. The samples were washed with PBS twice, incubated with LIVE/DEAD® (Molecular Probes, Invitrogen) viability assay for 30 min, and again washed with PBS twice before viewing under a fluorescence microscope (Zeiss Apotome, Carl Zeiss Ltd, Welwyn Garden City, UK).

Cell viability assessment

Cell viability was determined quantitatively using an established viability assay (alamarBlue® ABDiserotec, Kidlington, UK). The alamarBlue (AB) assay exploits the natural ability of cells to induce an oxidation–reduction (REDOX) reaction that give rise to changes in fluorescence and colour of an indicator dye. Hence, an increase in cell numbers gives rise to progressively greater reduction, whereas inhibition of cell growth (or cell death) maintains the dye in its oxidised form and colour. AB was added to fresh medium to a final concentration of 10% by volume, and incubated with each sample for 24 h (6 h in the case of the perfusion study owing to the higher cell seeding density). Thereafter, the medium was transferred to the wells of a 96-well plate and read in a plate reader (Multiskan Ascent). The end time points in the case of the static tests were 48 h and 96 h, and immediately after seeding ($t=6$ h) and after 12 h of perfusion in the shunt obstruction model. The measured absorbance values were converted according to the calculations detailed in the manufacturer's instructions, and expressed as a percentage to indicate the amount by which the AB dye is reduced by the cells. The percentage reduction in AB was taken as a measure of the number of viable cells present on each surface and at each time point. The viability data presented for the perfusion studies are

expressed relative to the mean values for the after seeded catheter.

Quantification of EPU catheter obstruction

The hydraulic permeability of the electrospun form of polyurethane catheter material (EPU) in the presence of NIH/3T3 fibroblasts was taken as a measure of shunt obstruction in the in vitro model. The cells were seeded on the luminal surface of the microporous catheter at a seeding density of 3.5×10^4 cells/cm². The catheter sections were 4 cm in length and 3 mm in diameter. The catheter was then rotated on roller table at 37°C at 5% CO₂ for 6 h to achieve a uniform distribution of cells. After seeding, one catheter section was maintained in a static environment and used as a control group for this study.

Perfusion of the growth medium represented the flow of CSF for the purposes of this study. The medium was delivered by means of a syringe pump at a constant flow rate of 0.07 ml/min for the duration of each test (12 h). The chosen flow rate corresponds to flow of CSF through an actual ventricular catheter. The distal end of the catheter was blocked so that the medium permeated through the walls of the catheter. The resulting perfusate accumulated in the T-25 flask in which each catheter was secured (Figure 1). The samples subject to perfusion and those cultured under static conditions were incubated with medium containing AB for 6 h prior to measurement in the plate reader. Cell viability was assayed at two points: immediately after seeding ($t=6$ h) and after 12 h of perfusion ($t=24$ h). Thereafter, the perfusate was transferred to a 96-well plate and the syringe replenished with fresh medium for the remainder of the experiment (or until the next measurement point). The resulting viability data were expressed relative to the values recorded for the EPU catheters immediately after seeding (Figure 5).

The hydraulic permeability of each length of EPU catheter was measured at the beginning and conclusion of each test with the aid of a computer-controlled shunt testing apparatus based on a dynamic mechanical analysis system (Bose® Electroforce® 3200, Bose Corp., Eden Prairie, MN, USA). The apparatus recorded the pressure drop for a series of prescribed flow rates delivered by a syringe pump. The corresponding mass flow was quantified gravimetrically by means of a load cell.

Statistical analysis

The data presented throughout this work are expressed as mean values \pm standard error. All experiments were repeated in triplicate, with a minimum of six samples per material and/or treatment. Statistical significance was determined following student's *t*-test or one-way

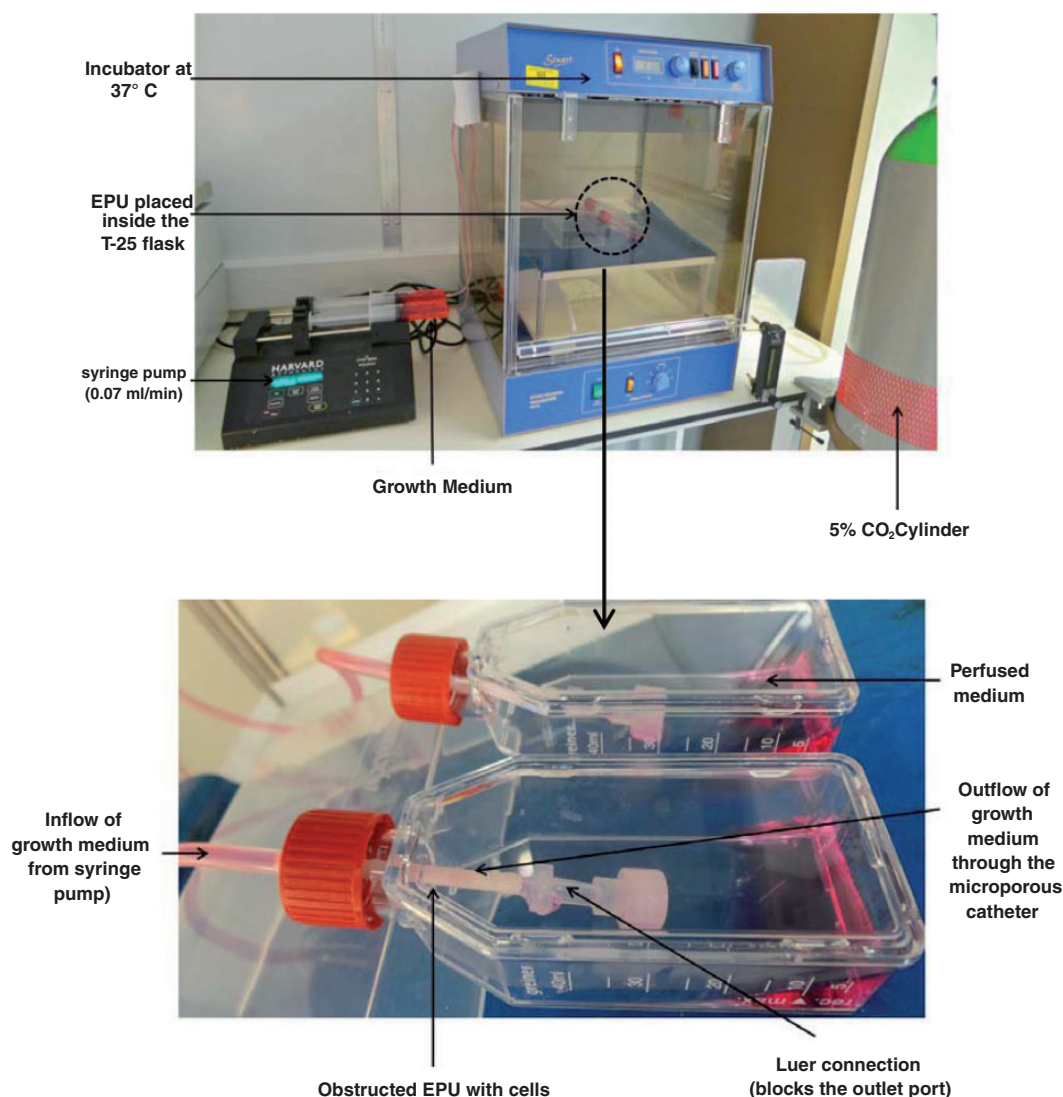


Figure 1. Schematic diagram of the shunt obstruction model. A syringe pump to delivered a constant flow of growth medium at a rate of 0.07 ml/min for 12 h through each EPU catheter, which was placed inside a T-25 flask. The T-25 flasks were placed inside an incubator maintained at 37°C and connected to a 5% CO₂ cylinder. The growth medium was not recirculated but collected inside the T-25 flask after passing through the walls of each catheter.

analysis of variance (ANOVA) with post hoc testing according to either Dunnett's or Tukey's procedure at a 95% confidence level (Minitab, software version 17). The analysis was performed on the data acquired using the plate reader (numbers of viable cells – as in Figure 4 and Table 2). Data were normalised in some cases with respect to the untreated PDMS samples (Table 1) and EPU catheter material after seeding (Figure 5).

Results

EPU characterisation

The morphology of the EPU catheter material was determined by scanning electron microscopy; both

inner and outer surfaces are shown in Figure 2. The plaques present on the inner surface of EPU are where some of the polymer has flowed onto the surface of the mandrel during the electrospinning process, whereas discrete fibres are seen in the outer surface. The fibre diameter is in the range of 2–5 µm, and the pore size 9–15 µm. The thickness of the material was ~150 µm.

Characterisation of cell growth under static culture conditions

Fluorescence microscope images of astrocytes after staining with Live/Dead® Assay after 96 h in culture on both treated and untreated samples are presented in Figure 3. In all cases, according to the results of

Table 1. The relative cell numbers on treated and untreated cast film polyurethane (PU) samples (Z1A1, Z3A1) in comparison to untreated PDMS after 48 h and 96 h in static culture (NIH/3T3 cells and astrocytes).

	48 h in culture						96 h in culture					
	Astrocytes			NIH/3T3 cells			Astrocytes			NIH/3T3 cells		
	BSA		Fn-BSA	BSA		Fn-BSA	BSA		Fn-BSA	BSA		Fn-BSA
	Untreated	Treated	Treated	Untreated	Treated	Treated	Untreated	Treated	Treated	Untreated	Treated	Treated
PDMS		○	+	○	–	+		○	+	○	○	+
Z1A1	○	○	○	○	○	○	○	○	○	○	○	○
Z3A1	○	○	○	○	○	○	○	○	○	○	○	○

Note: PDMS: poly(dimethyl)siloxane; BSA: bovine serum albumin.

+Denotes significantly ($p < 0.05$) greater cell numbers, –Denotes significantly fewer cells ($p < 0.05$) and ○ denotes no difference when compared to untreated PDMS (determined by ANOVA with Dunnett's post hoc test).

Table 2. Comparison of EPU catheter behaviour in the presence of NIH/3T3 fibroblasts and astrocytes.

Cell type	Cell viability (mean percentage reduction of AB \pm standard error)		Hydraulic permeability (mean \pm standard error; $\text{ml min}^{-1} \text{cm}^{-2} \text{mmHg}^{-1}$)	
	Post Seeding	Post Perfusion	No cells	Post Perfusion
Astrocytes	11.11 ± 0.71	16.56 ± 1.26	0.062 ± 0.003	0.040 ± 0.003
NIH/3T3	11.49 ± 0.97	19.01 ± 1.28	0.071 ± 0.002	0.029 ± 0.005

Note: EPU: electrospun polyether urethane (Tecoflex).

Cell viability measured in terms of percentage of reduction for both post-seeding and post-perfusion samples ($n = 3$).

Hydraulic permeability was determined both in the presence and absence of cells.

the viability assay, more cells were present on the Fn-BSA-treated samples in comparison to untreated samples, but the difference was significant only for PDMS (Table 1).

The numbers of NIH/3T3 cells cultured on untreated PDMS, cast and electrospun forms of polyurethane, and the SF were quantified according to the experimental protocol described in the Materials and methods section (cell viability assessment), and the results compared to those cells grown on plasma-treated tissue culture plastic (TCP) and silicone hydrogel controls (Figure 4). The numbers of cells after 48 h and 96 h in culture, expressed in terms of the percentage reduction in AB dye, is presented in Figure 4. The silicone hydrogel (H) was used as a negative control on the basis that this material would be less conducive to cell growth; TCP, on the other hand, served as positive control, this substrate having been plasma treated specifically to promote cell attachment and proliferation. As expected, the greatest reduction in AB was recorded for the positive control, whereas the negative control did not show an appreciable increase in cell numbers, even after 96 h in culture. In contrast, all other substrates show a marked difference in terms of the reduction in AB relative to the controls ($p < 0.05$) at both time points.

PDMS, Z1A1 and Z3A1 were prepared in well plates as before. The samples were then treated with proteins (either BSA or fibronectin adsorbed and blocked with albumin (Fn-BSA)) and seeded with either NIH/3T3 or astrocytes according to the above protocols. A comparison of cell viability for treated and untreated samples expressed relative to the untreated PDMS substrate, for NIH/3T3 and astrocytes after 48 h and 96 h in culture, respectively, is presented in Table 1. It is evident from the data presented in Table 1 that the numbers of cells present on the treated and untreated Z1A1 and Z3A1 are comparable to untreated PDMS for both cell types and time points. NIH/3T3 cells on BSA-treated PDMS at 48 h show significantly lower viability ($p < 0.05$) than other substrates, but at 96 h, there is no difference ($p = 0.163$). Fn-BSA-treated PDMS, on the other hand, supports significantly greater numbers of both cell types when compared to untreated PDMS at both time points.

Quantification of hydraulic permeability and shunt obstruction

Primary astrocytes and NIH/3T3 fibroblasts were seeded on the EPU catheters followed by perfusion in the apparatus as illustrated in Figure 1 according to the

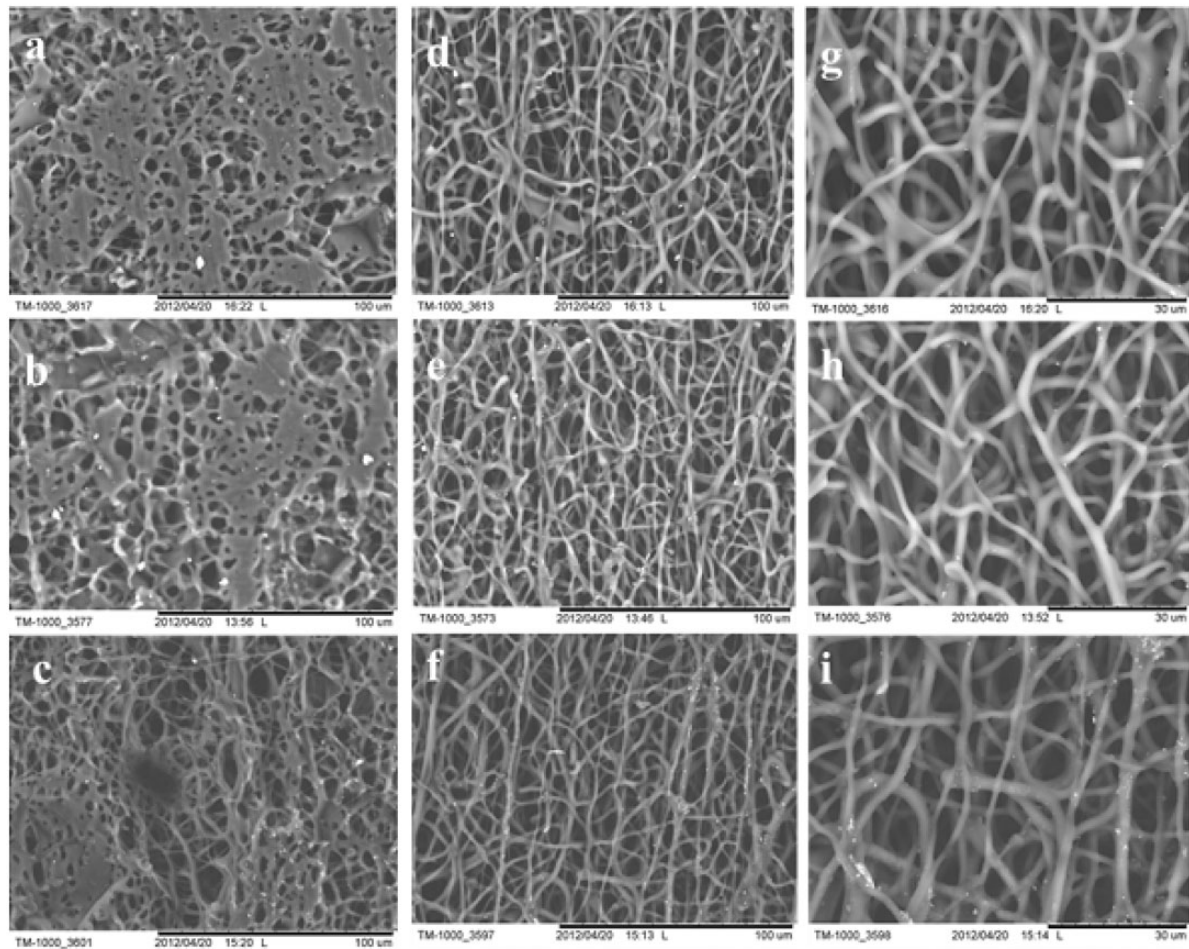


Figure 2. SEM micrographs illustrating the range of morphologies of the EPU catheters used in this study. Representative images of the inner (a–c) and outer (d–f) surfaces of the material are shown (scale bar 100 μm). Higher magnification images (g–i) show the outer surface with a scale bar of 30 μm .

methods described in the Materials and methods section. Catheter samples were incubated with AB-supplemented medium 6 h after seeding and again 12 h of perfusion. Cell viability was determined post seeding (after 6 h) and post perfusion (after 12-h perfusion and 6 h exposure to AB). The results are presented in Table 2. The numbers of cells present on the catheter material were no different for either cell type, either before or after perfusion (p value = 0.09). In all cases, the hydraulic permeability of each catheter at the end of the perfusion experiment revealed a significant decrease ($p < 0.05$) irrespective of the cell type (Table 2).

In a further series of experiments, the EPU catheters were first treated with fibronectin and blocked with albumin and then seeded with NIH/3T3 cells as described above. As a control group, a number of EPU catheters ($n = 3$) were seeded with cells and maintained under static conditions. The results of cell viability after seeding and at the end of perfusion/static EPU catheters are summarised in Figure 5. The

numbers of cells on the Fn-BSA treated and untreated samples increased significantly ($p < 0.05$) for both static and post-perfusion samples compared to the numbers immediately after seeding. Untreated and Fn-BSA-treated samples fail to show any significant change in cell numbers either during perfusion or after static culture.

At the conclusion of each test, the hydraulic permeability of all EPU catheters was quantified and compared with the values obtained for each sample beforehand. The results of this comparison are presented in Figure 6 in terms of hydraulic permeability before seeding (no cells present) and at the conclusion of each experiment. Despite a significant reduction ($p < 0.05$) in hydraulic permeability owing to cell obstruction under perfusion or static conditions, we calculate that an obstructed EPU catheter having dimensions equivalent to those of an actual ventricular catheter would still be capable of clearing CSF at a rate of 0.35 ml/min.

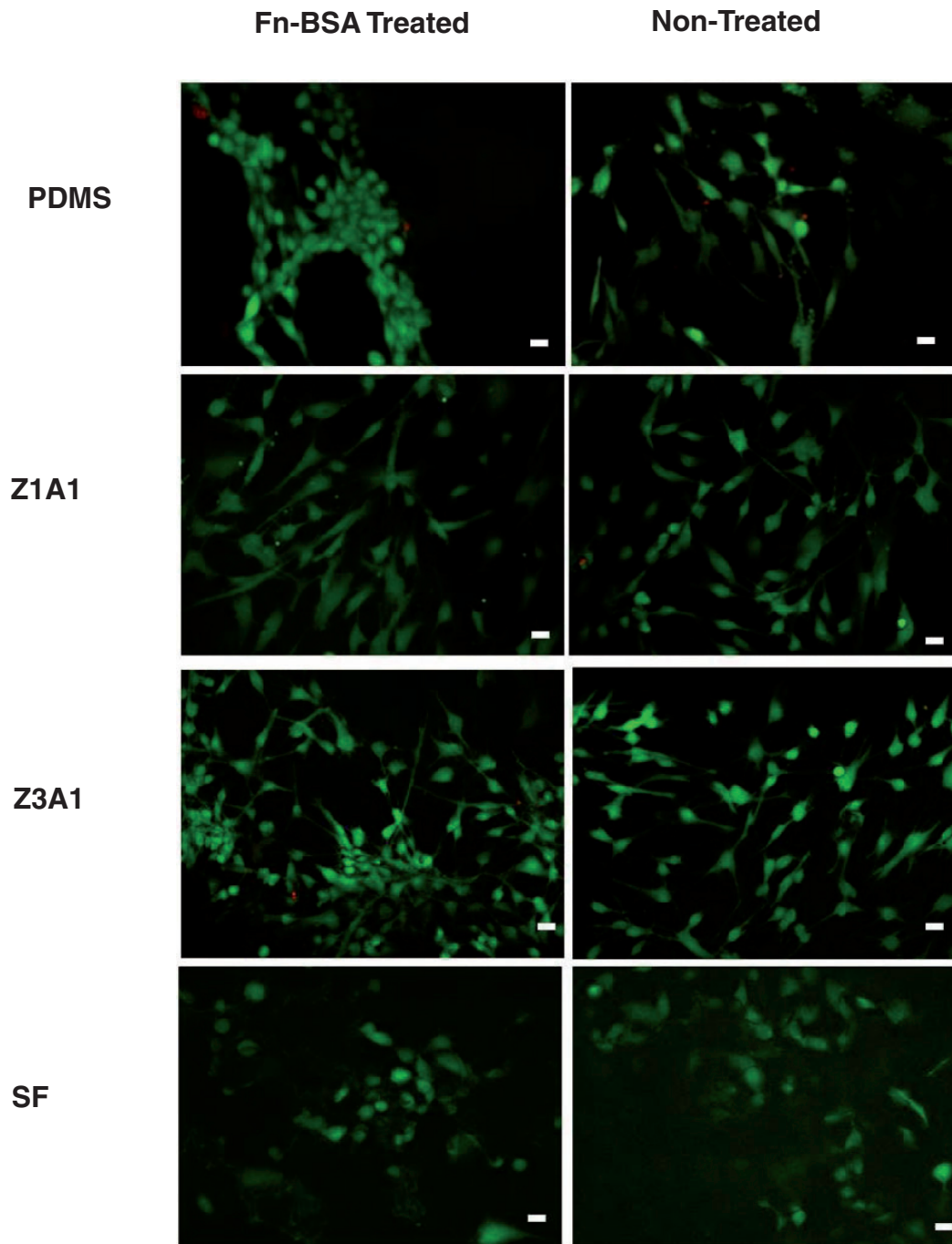


Figure 3. Fluorescence images of astrocytes taken after 96 h in culture on Fn-BSA-treated and untreated samples. Live cells are stained green (Calcein AM) and dead cells are stained red (ethidium homodimer – 1). Scale bars are 20 μm .

Discussion

CSF shunt technology, and the proximal catheter, in particular, has not benefitted from any major advancement in terms of materials and surface modification seen in other applications, for example, the cardiovascular system. Conventional proximal catheters are

made of PDMS, and are perforated with up to 40 discrete holes, each 0.5–1 mm in diameter. To our knowledge, there is no record that any other materials or forms have been considered for this application. In an effort to maintain a more uniform distribution of flow, however, other researchers have examined the effect of increasing the size and location of the perforations.

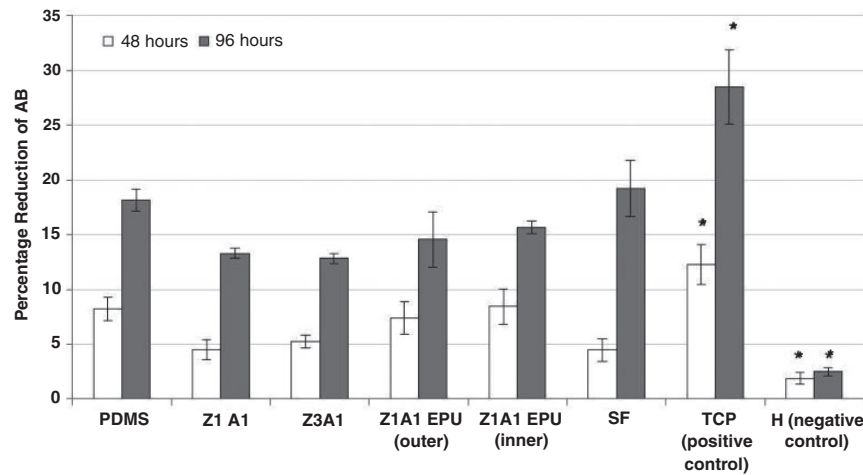


Figure 4. Mean percentage reduction of AB for NIH/3T3 cells grown on untreated PDMS, Z1A1, Z3A1, EPU (outer and inner surfaces), SF, positive control (tissue culture plastic, TCP) and negative control (H) after 48 h and 96 h in culture. Data represent mean \pm standard error mean and $n = 6$. (*) Indicates a significant difference ($p < 0.05$) in cell viability compared to the controls (relevant time point) determined by one-way ANOVA and Dunnett's post hoc analysis.

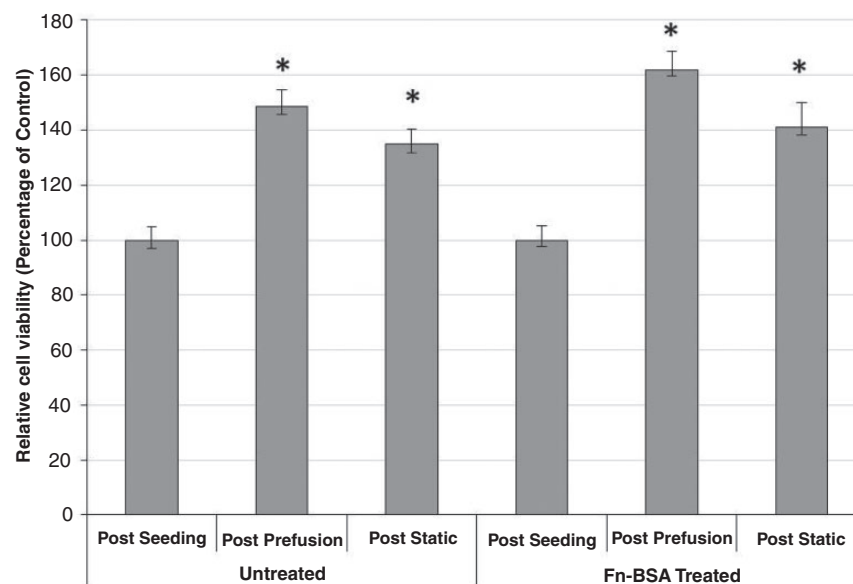


Figure 5. Relative cell viability after 12-h perfusion and static culture on untreated and Fn-BSA-treated EPU catheters. Data are expressed as a percentage relative to values obtained for control samples immediately after seeding (mean \pm standard error; $n = 8$). (*) Denotes a significant difference ($p < 0.05$) as determined by ANOVA and Tukey's post hoc analysis.

Lin et al.,¹¹ for example, suggested that by increasing the size of holes located closer to the tip of the catheter, the flow entering the catheter is distributed more uniformly along its length. Similarly, Thomale et al.⁶ demonstrated that the clinical performance of a catheter is improved if it has fewer, larger holes. Harris and McAllister¹² postulated that such a configuration gives rise to a better flow distribution. The results from the latter study show that larger holes generated less resistance and the lowest shear stress. In both studies,^{6,12} the

uniformity of flow was found to increase with increasing size of the perforations, while the overall area available to flow was maintained the same.

Materials consideration

The findings of this investigation support the hypothesis that polyurethane is a viable alternative to PDMS under static culture conditions (Table 1). The results also shed some light on how the cells behave on each

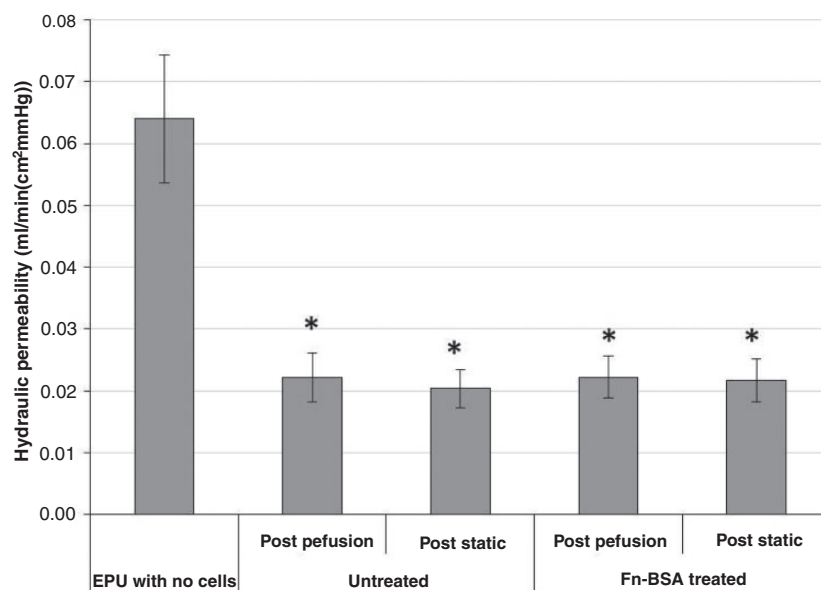


Figure 6. Hydraulic permeability compared with no cells present (before seeding) and 12 h after perfusion/static culture conditions for untreated and Fn-BSA-treated EPU catheters. Data points represent mean \pm standard error mean and $n=8$. (*) Denotes a significant difference ($p < 0.05$) in permeability after perfusion/static culture relative to the control (before seeding) as determined by ANOVA with Tukey's post hoc analysis.

substrate. While there was no significant difference between the cast film polyurethanes for both the time points, there was a significant difference on protein-treated PDMS after 48 h but not after 96 h in culture. These results suggest that, initially, the polyurethanes do not encourage cell proliferation to the same degree as PDMS, however cells do appear to adapt to grow on these surfaces after a longer period in culture; with more time, it is likely that cells grown on impermeable forms of PDMS and polyurethane will eventually populate the entire surface of the substrate. It follows that substituting polyurethane for PDMS may not offer any advantage or overcome the drawbacks of conventional catheters made of this material. The same could be said of the macroporous sponge forms, which do not show a significant difference to cast film PDMS at both time points (Figure 4).

The topography, roughness and charge of the material surfaces each play an important part in cell attachment.¹³ Reports in the literature suggest that the greater porosity and interconnectivity of microporous materials offer better nutrient and waste transport, and mimic more effectively the extra cellular matrix by providing structural and functional cues found in nature.^{17,18} At higher cell densities, however, cells are likely to become contact inhibited (on reaching confluence). The increased surface area of EPU in this context may impair such interactions, at least at the seeding densities and time points used in this study, which showed that cells on the porous forms of each material fared no better or worse than on the cast films. One

explanation for this observation might be the use of the unmodified EPU as a substrate. It is known that modifying the fibres with poly-D-lysine or similar compounds initiates better adhesion and cell survival;¹⁸ equally, the choice of polymer, which does not readily promote cell attachment without modification with extracellular matrix components.¹⁹

Cell growth on the surfaces examined as part of this study is a function of many factors, including cell type and seeding density, and the presence or absence of soluble factors in the medium such as growth factors and proteins. The interaction of the latter with the surface depends on adsorption kinetics and surface properties, including surface chemistry, charge, wettability, hydrogen bonding, ionic interaction, calcium bridges between carboxyl groups and time of exposure.²⁰ Is it surprising, therefore, that cell growth was the same for all substrates and forms, regardless of surface treatment, with the exception of Fn-BSA-treated PDMS at 48 h and 96 h, which supported greater numbers of cells than untreated PDMS or PU (Table 1; see also Figure 3). This result may be due to the relatively low protein concentrations used in this study, however.

The use of silicone hydrogel as a negative control surface in the present study points to the use of such materials as a way of minimising protein adsorption, through the use of superhydrophilic materials or coatings, for example, or by means of surface modification strategies like those proposed by Harris and coworkers,^{13,14} who plasma-treated PDMS catheter materials in order to render their surfaces more hydrophilic.

Hydraulic performance

The hydraulic performance of an EPU catheter material was evaluated in the presence of cells and proteins present in CSF. Here, the adhesion of immortalised 3T3 cells and astrocytes were compared under perfusion conditions for a set of untreated EPU catheters: there was no significant difference in terms of cell number (Table 2). In all subsequent experiments, therefore, the NIH/3T3 cell line was used instead of primary astrocytes. Treating the surface of each sample beforehand with fibronectin and albumin did not enhance cell adhesion or growth either (Figure 5). Likewise perfusion, which for NIH/3T3 cells at least, does not influence cell growth ($p > 0.05$), although it would be premature to infer from these findings that the flow of medium through the pores of the material does not have a bearing on cell proliferation. It may be that some cells proliferate more in some areas, while others are inhibited or removed under perfusion; this is unlikely, however, as there was no evidence of viable cells present either in the medium or on the surface of the T25 flask at the conclusion of the perfusion experiments.

Even though the initial seeding density was higher, it remains a distinct possibility that a higher seeding density and perfusion over an extended period could give rise to quantifiable differences between static and perfusion cultures. The only conclusion that we can draw at this stage is that the measure of hydraulic permeability reveals the contribution made by cells on the permeability of EPU catheters. Clearly, the physical presence of cells on the surfaces of the EPU material gives rise to a significant decrease in permeability, which would result in a reduction in CSF flow that had the measurement been made under pressure control.

An understanding of the behaviour of cells in the presence of flow is critical in this context. Cell behaviour is mediated by many factors, both chemical and physical. The need to study obstruction of catheter materials under realistic flow conditions was clearly outlined by Harris et al.²¹ Hence, cell adhesion and growth under physiological flow conditions *in vitro* takes us one step closer to understanding how these materials might perform within the ventricular microenvironment.

The number of viable cells on each sample of catheter material was the same overall regardless of the treatments or culture conditions, or hydraulic permeability. This result is surprising insofar as other studies have reported that porosity and size of pores have a bearing on the ability of ability fibroblasts and astrocytes to bridge the pores.²² When cell infiltration is limited by very small pore sizes, cell adhesion is

unaffected. On the other hand, large pores may not retain cells to the same extent. Indeed it has been shown that cells become quiescent if the pore diameter is more than 20 μm in electrospun polymers.²³

The model of shunt catheter obstruction showed a significant decrease in hydraulic permeability for all samples. The obvious inference is that the cells and cell debris have blocked the pores resulting in a reduction in flow through the catheter when subjected to a fixed pressure gradient. Remarkably, there was no difference noted in the blockage between perfusion or static, and Fn-BSA-treated and untreated samples. Although flow through the obstructed EPU was reduced almost threefold for all the samples, the catheter material remains permeable to flow. As the pressure gradient across the walls of the catheter rises, however, there would likely be a corresponding increase in flow entering the catheter. The extent to which cell obstruction affects the catheter permeability and CSF flow over longer periods remains to be shown, but any reduction in flow could be overcome in terms of catheter design, through an increase in available surface area, or the introduction of fenestration, for example.

The cell culture techniques employed in this study are a poor representation of the microenvironment present within the ventricular system of the brain. The choice of materials, cell types, seeding densities, time points and duration of study cannot be expected to replicate the complex interactions between an implanted device and host in a manner that can predict long-term performance or replicate failure modes. The present study made use of two cell types, primary astrocytes and an immortalised cell line, but explant studies confirm that other cell types are involved, for example, macrophages.⁶ The NIH/3T3 cell line was chosen for practical and cost considerations. Nevertheless, having established that the behaviour of the latter cells on the substrates used in this study was similar to astrocytes, these cells were considered a suitable model for shunt obstruction. In further studies, however, it is recommended that such experiments are conducted using cocultures of macrophages and astrocytes to replicate better the conditions present *in vivo*, and to elucidate the role of inflammatory cells and signalling involved in mediating the host response to the implanted materials. For longer term studies, consideration should be given to the use of animal models, where local and systemic responses and device performance can be studied along with adverse events.

Conclusions

An evaluation of alternative materials has been conducted under static and dynamic conditions representative of CSF shunt transport *in vivo*. The results show

that cell growth and viability are largely dependent on the material and form of the substrate, and to a lesser extent the culture conditions. This is the first study, to our knowledge, to consider electrospun materials as a ventricular catheter material. These preliminary results warrant further investigation since a relatively modest reduction in terms of the impact of cell overgrowth on catheter permeability may translate to a reduction in the complications associated with shunt catheter obstruction.

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Declaration of conflicting interests

None declared.

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