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Evaluation of polymer and self-assembled monolayer-coated silicone surfaces to reduce neural cell growth

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Abstract

The development of silicone catheters has improved the treatment of hydrocephalus. Unfortunately, the functionality of the catheters used for the treatment of hydrocephalus is compromised by cell obstruction. In this study silicone surfaces coated with biopolymers (heparin and hyaluronan) and self-assembled monolayers (SAM) (octadecyltrichlorosilane—OTS and fluoroalkylsilane—FAS) were employed to investigate the effect of these coatings on astrocyte and choroid plexus cell growth in vitro. Compared to unmodified silicone, FAS surfaces significantly reduced ($p<0.05$) astrocyte proliferation, heparin ($p<0.001$) and hyaluronan ($p<0.001$) surfaces significantly increased astrocyte growth, while no significant difference was observed on OTS surfaces. A similar trend was observed for choroid plexus cell growth on heparin ($p<0.05$) and hyaluronan ($p<0.05$) coatings, however, no significant reduction in cell growth was observed on FAS- or OTS-coated surfaces compared to silicone. Low cell growth may be attributed to hydrophobicity of the surfaces (FAS $112.2\pm2.6^{\circ}$, OTS $102.2\pm1.3^{\circ}$). Contact angle measurements confirmed the stability of the hydrophobic and hydrophilic properties of all the coatings on the silicone surfaces for 30 days. Surface roughness did not play an important role on cell growth. Silicone shunts coated with SAMs may be suitable for future clinical applications to improve the treatment of hydrocephalus.

Keywords: Cell growth; Self-assembled monolayer coatings; Polymers; Silicone catheters; Hydrocephalus shunts

1. Introduction

Silicone shunts have been used for 50 years for the treatment of hydrocephalus. Continuous research and improvements have been made to the shunt system over this time. Nevertheless, the problems of shunt complication and failure still remain a serious issue, often requiring several surgical procedures to re-establish a functioning shunt. According to a retrospective study on cerebrospinal fluid shunting in the United States that reviewed the Nationwide Inpatient Sample (NIS) database for the year 2000, 40.7% of all procedures considered in the study were shunt malfunctions. The total cost related to the shunt procedures (primary and secondary) was $1.1 billion [1]. The most common shunt complications are obstruction and infection [2–4] leading to mortality and morbidity in the treatment of hydrocephalus [4–7]. Retrospective analyses of the charts of 94 children between the years 1993 and 2003 concluded that the most common complication was shunt blockage (30%) and the most frequent area of blockage was within the intraventricular component (90%) [8]. Various tissues and materials can lead to proximal shunt obstruction. Epithelial cells of the choroid plexus [2,9–11] and astrocytes are especially capable of

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proliferation and have been shown to fill the holes and lumen of the catheter [11]. The only remedy, to overcome the after effects of shunt blockage is removal of the old blocked shunt and replacement with a new shunt. A potential solution to this problem may be to coat the silicone surface of the shunts with biocompatible polymers, possibly having additional antimicrobial properties that considerably reduce the growth of cells and bacteria. There are no consistent data available from adequately sized, randomized and controlled trials for coated shunts. Clearly more research is needed on the cost-effective coatings of shunts.

Various studies with polymer coatings have been attempted to reduce cell growth on the implant surface. Heparin, a well-known anti-coagulant, is a natural polysaccharide and frequently used as a surface coating agent to improve blood-material compatibility. Studies by Zareie et al. [12] using heparin-coated silicone peritoneal catheters and Lev et al. [13] using heparin-coated stents demonstrated that coated surfaces had a lower failure rate compared to uncoated surfaces. When implanted for 5 days in rats, the catheters coated with heparin were reported to be less adhesive compared to conventional catheters. The drop out rate was 57% (8/14 animals) for regular silicone catheters because of omental wrapping around the tip of the catheter. This was compared to heparin-coated catheters, which had a dropout rate of only 20% (3/15 animals) [12]. Hyaluronic acid (HA), which has a heparin-like property, is used in hydrophilic coatings for a variety of medical devices, including catheters to improve biocompatibility and reduce cell and bacterial adhesion [14]. Pavesio et al. [15] concluded that the covalent binding of hyaluronan to the surface of biomedical materials could yield an anti-adhesive surface that resists adhesion of proteins, bacteria and cells. Another biocompatible polymer is *N*-octadecyltrichlorosilane [$CH_3(CH_2)_{17}SiCl_3$] (OTS), which is useful for its hydrophobic properties. Prior research with LRM55 cells cultured on OTS and *N*-(3-(trimethoxysilyl)-propyl)-diethylenetriamine (DETA, hydrophilic) coatings on silicon substrate for 6 h demonstrated that less cells adhered to OTS compared to DETA [16]. Perfluorodecyltrichlorosilane [$CF_3(CF_2)_5(CH_2)_2SiCl_3$] (FAS) is another polymer with hydrophobic properties and has been used by previous researchers to study cell adhesion in relation to hydrophobicity. Stenger et al. [17] have been able to direct the polarity of embryonic hippocampal neurons by manipulating the patterns of aminosilane self-assembled monolayers (SAM) on a background of FAS. Neurons avoided the FAS-coated portions of the pattern, suggesting that FAS reduces cell growth. Thus, previous studies on polymer coatings have demonstrated that coating the implant surface with polymers can reduce cell growth, which may minimize shunt obstruction.

Although previous studies have demonstrated that biopolymer coatings such as heparin, hyaluronan, OTS, and FAS improve the blood compatibility of medical devices, there are no recent systematic studies of silicone coatings that evaluate brain cell proliferation. Currently there are no polymer-coated shunts for hydrocephalus in the market, which in theory would have reduced cell growth thereby reducing blockage of the shunt. Therefore for this study, we used heparin, hyaluronan, OTS and FAS as coating materials for silicone. We hypothesize that coating the silicone catheter with SAMs or biopolymers would reduce cell growth on the shunt surface thereby reducing the chances of shunt obstruction and failure.

2. Material and methods

Silastic silicone sheets (thickness: 0.015 in) were obtained from Dow chemicals (Midland, MI) and cut into disks (diameter: 21 mm per sample). OTS (97.5%) was purchased from United Chemical Technologies (Bristol, PA). Heparin and HA were purchased from Sigma (St. Louis, MO). FAS (97.5%), 1-3-dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (98% water-soluble carbodiimide, WSC), and 4- azidoaniline hydrochloride (97%) were obtained from Aldrich Chemicals (St. Louis, MO). All chemicals were used without further purification.

2.1. Coating

2.1.1. Surface modification of silicone with OTS

Silastic silicone disks were cleaned by immersion in pure ethyl alcohol in a Branson 2200 ultrasonic cleaner for 5 min, and dried with nitrogen. The silicone disks were then treated by a plasma cleaner (Harrick Scientific, PDC-32G) for 5 min. Subsequently, the plasma-treated disks were placed together with OTS in a glass container and placed into a sealed chamber at 10^{-3} Torr at room temperature for 4 h. The silicone disks were kept in the sealed chamber with OTS for an additional 12 h.

2.1.2. Photo-immobilization of heparin and hyaluronan on OTS modified silicone

Heparin, WSC, and 4-azidoaniline hydrochloride at a weight ratio of 2.35:1.29:1 were dissolved in deionized water to make a 0.5% solution. The pH of the solution was adjusted to 4.70–4.75 using 2.3 N NaOH and 0.1 N HCL solutions, and then stirred at 4 °C for 24 h. A 0.2% aryl azido-modified hyaluronan solution was prepared by the same method except that the weight ratio was 42:28:17.06. All the reactions were carried out in a dark room. The OTS on silicone samples was illuminated with a mercury vapor UV lamp (175 W, Regent Lighting, Burlington, NC) for 10 min at a distance of 10 cm in the presence of the aryl azido-modified heparin and hyaluronan solution. The samples were then rinsed by immersion and washing with deionized water for 48 h.

2.1.3. Surface modification of silicone with FAS

Silicone disks were prepared by plasma cleaning as described above. FAS was then deposited on the silicone surface by chemical vapor deposition for 5 min under a vacuum of 10^{-3} Torr. The samples were then maintained in the sealed chamber for an additional 4 h.

2.2. Surface characterization

2.2.1. Contact angle measurement

Contact angles of silicone and modified silicone samples were determined by a NRL contact angle goniometer (Model 100, Ramehart,) at ambient pressure. A water droplet of approximately 20 μl was placed on the substrate and the contact angles were measured on both sides of the droplet. Three droplets were placed at various spots on the substrate surfaces and the average readings were recorded.

2.2.2. Atomic force microscopy measurements

All surfaces roughness measurements were acquired with a Nanoscope IV controller (Digital Instruments) and Dimension 3100 scanning probe microscope scanner (Veeco Instrument, Santa Barbara, CA). Height images of 10 μm × 10 μm samples were taken in ambient air using ultra-sharp silicon NSG 10 cantilevers (NT-MDT Co., Moscow, Russia) with a resonance frequency of about 300 kHz operating in tapping mode and a scan rate of 0.5 Hz. Integral and proportional gains were approximately 0.4and 0.7, respectively. The radius of the tip was about 10 nm. Measurements of root-mean-square (RMS) roughness were performed at five separate points (center, four corners) on each sample.

2.2.3. Stability test

Stability of OTS/silicone, FAS/silicone, heparin/OTS/silicone, and hyaluronan/OTS/silicone was tested in saline solution (0.9% NaCl) at 37 °C for 0, 5, 10, 20, and 30 days. Disks of modified samples were immersed in 10 ml of distilled water and were maintained at 37 °C in a water bath. Contact angles of the silicone disks were measured at the predetermined time interval to evaluate relative stability of the coatings.

2.3. Astrocyte and choroid plexus cell culture on silicone samples

Seven gravid rats (18 days gestation; Harlan Co., Indiana) were housed in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) with access to food and water until the day of surgery. Approximately 12 pups were obtained from each dam.

2.3.1. Techniques and assays

2.3.1.1. Isolation of brain tissue. Primary cultures were prepared from the brains of the 1–2-day old Sprague-Dawley rat pups (Harlan Inc., IN). The pups and dam were euthanized in a carbon dioxide chamber. The pups were washed thoroughly with 70% ethanol. Under a laminar flow hood, the skin was opened at the midline of the decapitated head, cutting from the base of the skull to the mid-eye area using micro-dissecting scissors. The skull was cut at the midline fissure, without cutting into the brain tissue. The brain was then released from the skull cavity by using a micro spatula.

2.3.1.2. Tissues cultures techniques. (a) *Astrocyte culture*: The extracted brains were transferred to a 100 cm Petri dish with Hanks Balanced Salt Solution (HBSS) under the laminar flow hood. The meninges were gently peeled from the brains under the microscope using forceps. The tissue was then minced, incubated and shaken in HBSS (Sigma, St. Louis, MO) containing 0.25% trypsin–EDTA (Sigma, St. Louis, MO) for 45 min at 37 °C. One milliliter of DNAse (12.5 μg/ml, Sigma, St. Louis, MO) was then added and the tissue was washed with HBSS containing 10% fetal bovine serum (FBS, Sigma, St. Louis, MO) and spun for 10 min at 275*g* to inhibit trypsin action. The tissue was re-suspended in 10 ml HBSS and titrated with a 10 ml pipette. The volume was brought to 50 ml with HBSS and the cells were centrifuged at 1100 rpm for 10 min. After removing the supernatant, the pellet was re-suspended in RPMI-1640 (Sigma, St. Louis, MO) supplemented with 10% FBS and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin, Sigma, St. Louis, MO) following which cells were seeded in 75 cm^2 flasks (Falcon, USA) at the density of 9×10^5 cells/cm^2. The cultures received exchanges with fresh medium twice a week. After 7 days the cells were shaken for 18 h on an orbital shaker to remove loosely adherent cells. The enriched astrocytes were seeded on the silicone samples secured with teflon rings (Zatkoff Seals & Packings, MI, USA.) in 12-well tissue culture dishes (Falcon, USA) with a density of 6×10^4 cells/cm^2. Cultures were maintained at 37 °C in 5% CO_2. Pups from two different rats were used to perform two sets of experiments. First set of experiments consisted of five different cell cultures performed on different days, while the second set of experiments consisted of six different cell cultures performed on the same day. Visual inspection of the cell growth was performed using an inverted microscope (Olympus, NY).

(b) *Choroid plexus culture*: Choroid plexuses were obtained from the lateral ventricles of 1–2-day old pups and kept in warm culture media. The tissue was rinsed twice in phosphate-buffered solution (PBS, pH = 7.3, Gibco BRL Co., Rockville, MD) supplemented with HEPES (*n*-2-hydroxyl-ethylpiperazine-*n*0-2-ethane sulfonic acid, 15 mM, Gibco BRL Co., Rockville, MD) and then incubated in PBS containing pronase (1 mg/ml, Gibco BRL Co., Rockville, MD) for 25 min at 37 °C. Digested plexuses were recovered by centrifugation at 1*g* force and washed twice with PBS. The supernatant containing mostly single nonepithelial cells was discarded and the large clumps of epithelial cells were shaken for 45 min in 0.25% trypsin (Gibco BRL Co., Rockville, MD) containing 12.5 ug/ml DNAse (Gibco BRL Co., Rockville, MD). The supernatant was withdrawn and 10% FBS was added. This mixture was kept on ice. Fresh trypsin solution was added to the tissue. Cells were collected in conical tubes and centrifuged at 800*g* for 5 min, supernatant was removed and pellets were resuspended in culture media consisting of Dulbecco's Modified Eagle Medium (DMEM/F-12 (1:1)) supplemented with 10% (v/v) FBS, 2 mM glutamine, 25 mg/ml gentamicin, 5 mg/ml insulin, 5 mg/ml transferrin, 5 ng/ml sodium selenite, 10 ng/ml epidermal growth factor, 2 mg/ml hydrocortisone, and 5ng/ml basic fibroblast growth factor (Gibco BRL Co., Rockville, MD). Epithelial cells were further enriched by differential attachment on tissue culture treated dishes for 90 min. After incubation at 37 °C, supernatants were collected and cells were seeded on silicone samples secured with Teflon rings in the 12-well plates (Falcon, USA) at a density of 2.4×10^4–6×10^4 cells/cm^2. Experiments were performed in duplicate for choroid plexus cells.

2.3.2. Cell attachment and viability

The number of astrocytes and choroid plexus cells attached to the silicone samples and the cell viability was assessed by trypan blue exclusion and counting with a hemocytometer. After 1 week (astrocyte) or 2 weeks (choroid plexus) the cells were washed with 1 ml HBSS. After aspiration of HBSS, cells were removed by trypsinization with 0.1 ml trypsin–EDTA in 1 ml HBSS, centrifuged at 1000 rpm for 5 min, and the pellet was suspended in fresh 0.5 ml media after discarding the supernatant. Viable cells were identified by trypan blue exclusion and counted on a hemocytometer. This was used to determine the number of viable adherent cells on each sample.

2.4. Statistical analyses

To examine the mean difference in the adhesion of astrocytes and choroid plexus cells, a one-factor analysis-of-variance model (ANOVA) was employed using statistical Package for the Social Sciences (SPSS) to analyze cell counts. Pair-wise comparisons were examined using a Bonnferoni correction to control for multiple tests of hypotheses. Statistical significance was considered achieved at a two-tailed *p*-value $\leqslant 0.05$. Results are presented with 95% confidence intervals.

3. Results

3.1. Contact angle measurements

A higher surface/water contact angle is an indication of a more hydrophobic surface. Bare silicone exhibited an average contact angle of $107.4 \pm 1.3^\circ$ (Fig. 2). However, upon plasma treatment, a 0° contact angle was obtained indicating the surface is fully oxidized with terminal hydroxyl groups. With OTS and FAS coated on the plasma-treated silicone, the contact angles increased to $102.2 \pm 1.3^\circ$ and $112.2 \pm 2.6^\circ$, respectively. Upon incorporation of heparin and hyaluronan, the surfaces became hydrophilic with contact angles of $55.3 \pm 1.8^\circ$ and $55.3 \pm 3.9^\circ$, respectively.

3.1.1. Contact angle measurements over-time

To determine the stability of the coatings, contact angles of the coated silicone surfaces were measured as a function of time (Table 1). For OTS on silicone, average contact angles of $102.2\pm1.3^\circ$, $101.1\pm2.8^\circ$, $99.5\pm2.7^\circ$, $101.7\pm2.1^\circ$, and $100.8\pm2.8^\circ$ were observed at 0, 5, 10, 20, and 30 days, respectively. Likewise, the contact angle measurements for FAS coated on silicone were $112.2\pm2.6^\circ$, $113.7\pm1.6^\circ$, $112.0\pm1.3^\circ$, $109.5\pm2.2^\circ$, and $112.6\pm1.8^\circ$ at 0, 5, 10, 20, and 30 days, respectively. Heparin and hyaluronan also exhibited stable contact angle measurements over the 30-day period (Table 2).

3.1.2. AFM measurements

Unmodified silicone exhibited the roughest surface among all the samples with an average RMS value of 153.8 ± 46.1 (Table 3). Surface coatings improved the surface roughness of silicone to some extent. FAS coating exhibited significantly a smoother surface (81.1 ± 26.4, $p<0.05$) compared to bare silicone. OTS (88.8 ± 12.5, $p<0.05$) and hyaluronan (96.8 ± 18.2, $p<0.05$) also improved the surface roughness of silicone. However, no significant difference was observed between the surface roughness of heparin (104.0 ± 20.7) and unmodified silicone.

3.2. Cell growth

3.2.1. Astrocyte

Astrocytes grew well on all the samples. In the absence of any silicone sample in the polystyrene 12-well plate, the cells showed a characteristic tendency to attach to the bottom of the well with a homogeneous distribution yielding the highest number of cells. Cells exhibited the star-like characteristics of glial cells (Fig. 1) with the intricate pattern of cytoplasmic processes. Due to the opaqueness of the silicone samples, visual inspection of cell growth on silicone samples was difficult. Hence, a polystyrene 12-well plate surface was used as a control to determine the stage of confluence for cell cultures.

Cell counts provide an indirect measure of cell growth, since attachment to a substrate is a requirement for prolonged survival in culture. The numbers of growing cells were expressed as a % of cell counts (mean + SD) on polystyrene 12-well plates, which was assigned the arbitrary value of 100.0% for individual experiments. Visual

Table 1
Contact angle measurements of silicone surfaces over-time

	Average contact angle (deg) ±standard deviations			
	OTS/silicone[a]	FAS/silicone	Heparin/OTS/silicone	Hyaluronan/OTS/silicone
Control	102.2 ± 1.3	112.2 ± 2.6	55.3 ± 1.8	55.3 ± 3.9
5 days	101.1 ± 2.8	113.7 ± 1.6	58.2 ± 4.2	54.7 ± 1.6
10 days	99.5 ± 2.7	112.0 ± 1.3	57.0 ± 4.0	54.4 ± 2.8
20 days	101.7 ± 2.1	109.5 ± 2.2	53.0 ± 2.5	56.7 ± 1.8
30 days	100.8 ± 2.8	112.6 ± 1.8	55.2 ± 3.2	54.5 ± 2.9

[a]Contact angle measurement of uncoated silicone was 107.4 ± 1.3.

Table 2
Astrocyte and choroid plexus percent cell proliferation on silicone surfaces

Cell type	Average cell counts ±standard deviations					
	Polystyrene 12-well plate[a]	Silicone	FAS/silicone	OTS/silicone	Heparin/OTS/silicone	Hyaluronan/OTS/silicone
Astrocyte ($n=5$)	100 ± 0.0	23.0 ± 6.4	7.6 ± 3.6	15.4 ± 4.8	40.0 ± 22.1	20.4 ± 10.8
Astrocyte ($n=6$)	100 ± 0.0	29.2 ± 9.6	15.7 ± 2.8	16.7 ± 5.6	70.1 ± 9.8	61.6 ± 7.2
Choroid plexus	100 ± 0.0	19.0 ± 5.8	21.4 ± 7.3	24.4 ± 7.8	55.6 ± 15.1	55.4 ± 18.7

[a]Arbitrary value of 100.0% assigned to the polystyrene 12-well plate for the individual cultures.

Table 3
Surface Roughness measurements of silicone and modified silicone surfaces

Average RMS values±standard deviations				
Silicone	FAS/silicone	OTS/silicone	Heparin/OTS/silicone	Hyaluronan/OTS/silicone
153.8 ± 46.1	81.1 ± 26.4	88.8 ± 12.5	104.0 ± 20.7	96.8 ± 18.2

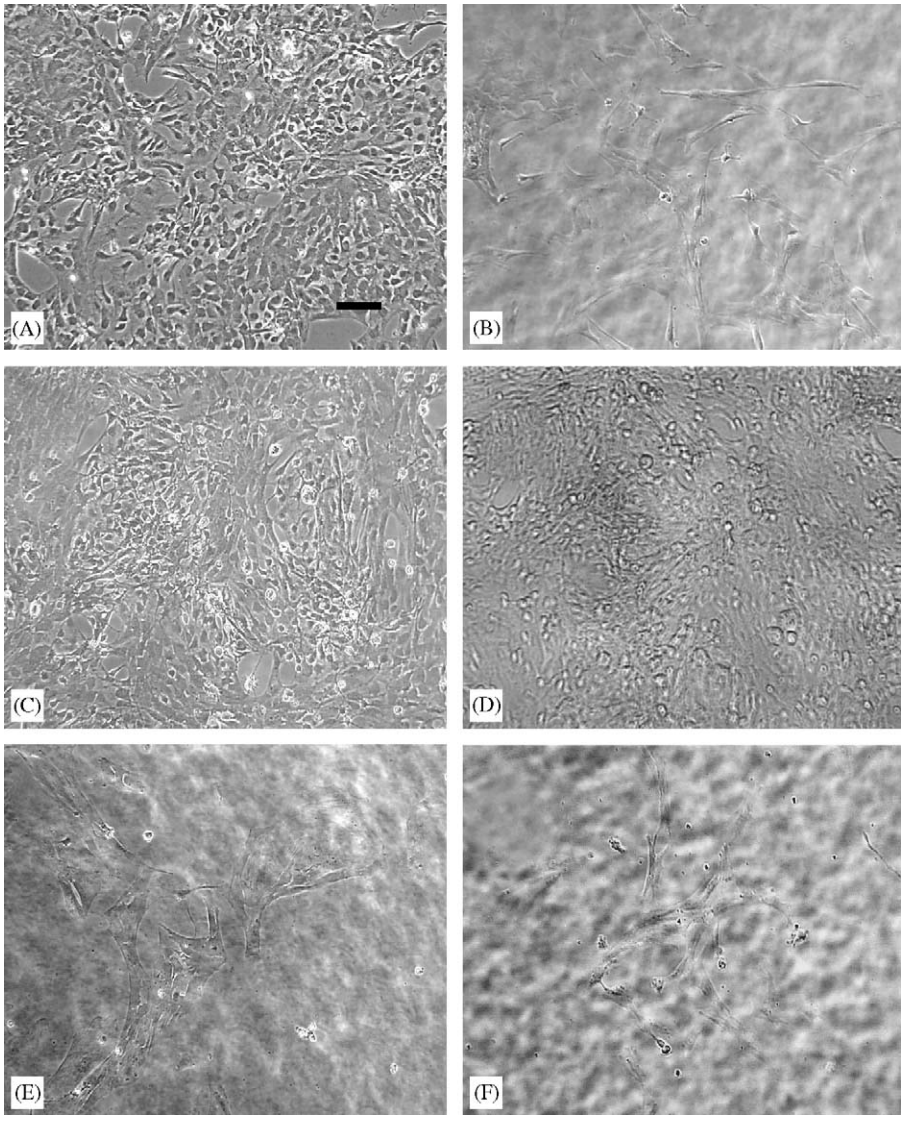

Fig. 1. Astrocyte growth on unmodified and modified silicone samples. Astrocytes attached better to polystyrene 12-well plates (panel A), compared to uncoated silicone (panel B) with scattered cells (pictures taken midway between center and edge of the plate). Heparin (panel C) and hyaluronan (panel D) coatings had greater cell growth compared to FAS (panel E) and OTS (panel F) coatings. The bar (panel A) corresponds to 10 μm.

inspection revealed that silicone, FAS- and OTS-coated surfaces exhibited less astrocyte proliferation compared to polystyrene, heparin- and hyaluronan-coated surfaces (Fig. 1). Cell counts confirmed this pattern (Table 2). Compared to unmodified silicone ($23 \pm 6.4\%$, range 15–31%), heparin-modified surfaces exhibited an increase in astrocyte growth ($40.0 \pm 22.1\%$, range 24–78%), while hyaluronan-coated surfaces ($20.4 \pm 10.8\%$, range 8–32%) depicted almost equal cell growth. FAS ($7.6 \pm 3.6\%$, range 5–14%) and OTS ($15.4 \pm 4.8\%$, range 13–24%) surfaces exhibited lower cell growth compared to unmodified silicone. A similar trend was observed for heparin-, FAS- and OTS-modified surfaces for another set of experiments (six experiments/ time point). Compared to unmodified silicone ($29.2 \pm 9.6\%$, range 19–43%), heparin surfaces significantly increased cell growth ($70.1 \pm 9.8\%$, range 58–80%, $p < 0.001$), while FAS had a significant decrease in cell growth ($15.7 \pm 2.8\%$, range 12–20%, $p < 0.05$). Hyaluronan surfaces exhibited a significant increase in cell growth ($61.6 \pm 7.2\%$, range 53–72%, $p < 0.001$) compared to uncoated silicone. No significant decrease in cell growth was observed on OTS surfaces compared to unmodified silicone.

3.2.2. *Choroid plexus*

Similar to astrocytes, choroid plexus epithelial cell growth was greatest on polystyrene (termed control; Table 2). An increase in choroid plexus cell counts on heparin ($55.6 \pm 15.1\%$, range 22–112%) and hyaluronan-coated surfaces ($55.4 \pm 18.7\%$, range 11–104%) was observed compared to other silicone surfaces. Lower cell numbers were observed on FAS ($21.4 \pm 7.3\%$, range 5–48%) and OTS-coated surfaces ($24.4 \pm 7.8\%$, range 8–50%). In addition, cell counts for uncoated silicone surfaces

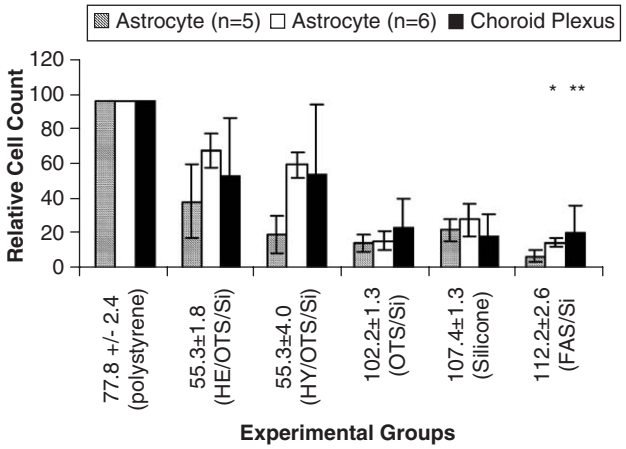

Fig. 2. Effect of surface coatings on astrocyte and choroid plexus epithelial cell growth. The numbers of growing cells are expressed as a % of cell counts (mean + SD) on polystyrene 12-well plates. The numbers on the *x*-axis represent the contact angle measurements of the silicone samples and the polystyrene surface. Self-assembled monolayer-coated surfaces with hydrophobic properties exhibited the lowest cell growth compared to surfaces with hydrophilic properties. Pups from two rats were used for two sets of experiments. $N = 5$ represents the first set of experiments which consisted of five different cell cultures performed on different days. $N = 6$ represents the second set of experiments which consisted of six different cell cultures performed on the same day. (*Significantly lower astrocyte cell growth on FAS compared to unmodified silicone, heparin, and hyaluronan coatings; $p < 0.05$, **significantly lower choroid plexus cell growth on FAS compared to heparin and hyaluronan coatings; $p < 0.05$.)

($19 \pm 5.8\%$, range 7–41%) were almost equal to cell counts for FAS-coated surfaces.

Statistical analyses of choroid plexus cell culture data indicated that the cell counts on FAS ($p < 0.05$), OTS ($p < 0.05$), and uncoated silicone ($p = 0.001$) surfaces were significantly lower compared to the polystyrene well plate. No significant differences in cell counts were observed between heparin-coated ($p > 0.05$), hyaluronan-coated ($p > 0.05$), and polystyrene surfaces (Fig. 2).

4. Discussion

Hydrophobicity of a material surface is probably one of the reasons for reduced cell growth. As previously demonstrated, modification of silicon prosthetic devices with the hydrophobic coating of OTS might provide the necessary control of astrocyte cells on the device surface [16]. To our knowledge, only one report has been published pertaining to cell growth on FAS-coated silicone surfaces. Embryonic hippocampal neurons, cultured on aminosilane SAM patterns of FAS, changed their polarity and avoided the FAS-coated portions of the pattern [17]. These interesting findings suggest indirectly that FAS can reduce cell growth. Our results indicated that the hydrophobic coatings of FAS and OTS minimized astrocyte and choroid plexus cell proliferation, suggesting that hydrophobic surface coating may reduce cell adhesion and thereby prevent cell migration into the shunt lumen.

A number of studies suggest that hydrophilic coatings can also reduce cell growth [12,15,18]. A previous in vivo study demonstrated that heparin-coated peritoneal catheters had a lower failure rate compared to uncoated catheters [21]. Those results conflict the findings of the current study. It may be that a heparin-coated silicone surface promotes adhesion and proliferation of central nervous system cells, while heparin is less adhesive for cell types located in the peritoneum. We also cannot exclude the possibility that there may be differences between in vivo and in vitro systems. Earlier work also indicates that hyaluronan, another hydrophilic polymer, can provide an anti-adhesive surface for the inhibition of cell and bacterial adhesion [15]. Polyhydroxyalkanoates (PHA) treated with lipase followed by a hyaluronan coating increased the hydrophilicity of the surface, thereby reducing mouse fibroblast cell adhesion. The results also indicated that the hydrophobicity of lipase on PHA proved favorable for the growth of L929 cells [18]. However, the present study demonstrates that astrocyte and choroid plexus cell growth on hyaluronan coatings was greater than the cell growth on hydrophobic coatings.

In addition to hydrophobic/hydrophilic characteristics, surface charge, surface energy, and topography can also influence cellular growth [19]. In vitro assessment of osteoclast cells on materials with different surface energies demonstrated that fewer osteoclast cells adhered to carbonated hydroxyapatite (surface energy: $9 \pm 5\,mJ/m^2$) compared to hydroxyapatite (surface energy: $44 \pm 2\,mJ/m^2$), or to natural calcium carbonate (surface energy: $58 \pm 0.5\,mJ/m^2$) [20]. Similar results were observed in the present study, with fewer astrocytes and choroid plexus cells adhering to surfaces with low surface energies. It was found that cell proliferation on FAS (surface energy: $\sim 8\,mJ/m^2$) [21] and OTS (surface energy: $\sim 21\,mJ/m^2$) [22] surfaces was less compared to heparin (contact angle: $55.3 \pm 1.8^\circ$) and hyaluronan (contact angle: $55.3 \pm 3.9^\circ$) surfaces. Specific surface energies for heparin and hyaluronan are not available, but since surface energy is the inverse of contact angle measurements, we can infer that heparin and hyaluronan have higher surface energies than FAS and OTS. Thus, surface characteristics play an important role in cell adhesion and proliferation.

Surface roughness has been shown to affect cell adhesion and proliferation of osteoblast-like MG-63 cells on titanium surfaces [24,25]. Previous work also demonstrated that smooth surfaces of titanium increased periodontal cell adhesion [23]. In the present study for astrocytes and choroid plexus cells, the surface roughness did not play an important role in cell adhesion and growth.

Earlier reports also indicate that variations in cell growth could be the result of serum proteins adsorbed to the material surface via integrins, a family of cell receptors [26]. Integrins recognize and bind specifically to certain proteins adsorbed on the biomaterial surface, thus forming a focal contact between cells and biomaterial surfaces [27]. It is well established that integrin binding varies according to

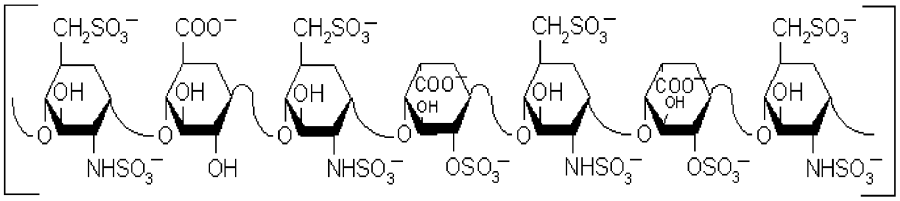

Fig. 3. Chemical formula of heparin.

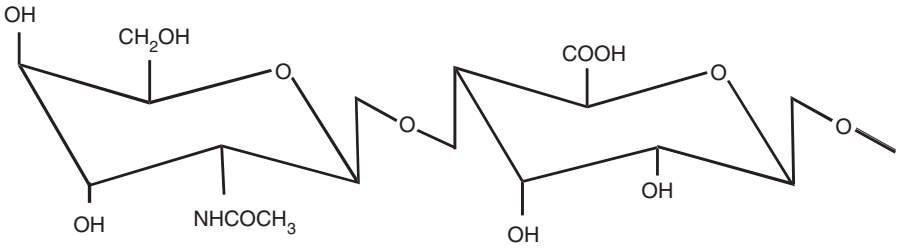

Fig. 4. Chemical formula of hyaluronan.

the characteristics of the surface. Surfaces with amine groups have been reported to show more vinculin (integrin) binding compared to surfaces with CH_3 (hydrophobic) groups. Also, talin (integrin) binding was reported highest among OH (neutral hydrophilic) groups compared to surfaces with COOH, CH_3, and NH_2 functional groups [28]. Therefore, the identification of specific surface properties and specific protein binding to which cellular function can be directly correlated is valuable in designing implant materials for biomedical applications. On our heparin and hyaluronan-coated silicone samples there were negatively charged groups (Fig. 3 [29], Fig. 4). Also, heparin surfaces have sulfate groups. The negatively charged groups were possibly more favorable for astrocyte and choroid plexus cell growth may be due to good interactions with the functional groups found in proteins. In contrast, FAS- and OTS-modified surfaces are neutral SAMs with low surface energies. This could be a possible reason for reduced cell growth due to less interaction between these SAMs and integrin. It is not known which functional groups in amino acids interacted with the functional groups on the modified silicone surfaces for astrocyte and choroid plexus cell proliferation. Nevertheless, it could be said that the underlying surface chemistry that produced hydrophobicity, low surface energy, and specific functional groups may have impacted our results and minimized cell growth on FAS and OTS.

Apart from the characteristics of the polymers to reduce cell growth, stability of the polymer coatings is very important for central nervous system implants, since shunt catheters and neural prostheses are implanted in the brain for a long period of time. The stability test results of the polymers in the present study indicated that the coatings were stable for 30 days. This durability indicates that the polymers are promising for long-term use in vivo.

Further in vivo studies are required to delineate anti-cell effects of FAS and OTS coatings on silicone. In vitro studies do not provide pulsatile flow of cerebrospinal fluid as well as intracranial pressure changes, two critical features of hydrocephalus. Further, to our knowledge, the specific effect of pulsatility on astrocyte and choroid plexus cell proliferation into the CSF drainage holes of the shunt is unknown. In vivo studies with polymer-coated shunts may provide insight into the effect of pulsatile flow of cerebrospinal fluid on brain cell proliferation.

5. Conclusion

The results of the present study indicate that statistically significant differences in cell counts were found between the hydrophilic and hydrophobic polymers when they were chemically coated on silicone. Within 2 weeks of culture, the hydrophilic coatings (heparin and hyaluronan) exhibited an increase in epithelial cells of the choroid plexus and astrocytes compared to unmodified silicone (Fig. 2). In contrast, the hydrophobic coatings, FAS and OTS, reduced astrocyte growth compared to unmodified silicone. Choroid plexus cell growth on FAS and OTS coatings was similar to that on unmodified silicone surfaces. In addition, contact angle measurements of heparin, hyaluronan, FAS, and OTS coatings suggested that the coatings were successfully applied on the silicone surface and were stable for 30 days. Overall, these results suggest that hydrophobic coatings on silicone could be an effective application to minimize shunt blockage due to cell proliferation, thus improving the standard treatment for hydrocephalus.

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