A Novel Thromboresistant Hydrogel Material for Vascular Access

Matthew M. Mannarino, Ph.D., Daniel Donahue, Michael Bassett, and James Biggins
Access Vascular, Inc., Bedford, MA

The peripherally inserted central venous catheter (PICC) has become increasingly popular over recent years as an alternative for venous access over traditional central venous lines due to their ease of insertion, low insertion related complications, reduced cost, and placement primarily by nurses or vascular access teams.[1] However, despite these advantages, PICCs are more than twice as likely to experience catheter-related thrombus leading to dysfunction, infection, loss of central venous flow, or upper extremity deep vein thrombosis than other central venous access devices (CVADs).[2] Due to the significance of potential complications along with the increase in acute care patients receiving PICCs, methods to prevent or reduce catheter-related thrombus are of paramount importance to clinicians.

Shortly after a catheter is placed into the blood stream, blood proteins (such as fibrinogen and collagen) and host cells (such as platelets) begin to deposit on the device surface, leading to the formation of adherent material on and around the catheter surface. The magnitude and rate of the biological response is dependent upon the materials used in the construction of the catheter as well as the effect of shape/design affecting blood flow. Poor protein resistance is often associated with surface hydrophobicity, which generates a high surface energy that the body relieves via protein adsorption.[3] Proteins undergo a conformational change to associate their hydrophobic domains with the biomaterial surface and their hydrophilic domains with the biological environment to create a substantial reduction in surface energy.[4] Catheters used for vascular access are commonly prepared from polyurethanes (TPUs) or silicones that provide flexibility, durability, and strength;[5] however, these polymeric materials are hydrophobic and therefore susceptible to non-specific protein adsorption.[6] Significant research has been conducted to improve the protein resistance of polymeric biomaterials by using coatings, films, and direct surface modification as well as bulk modification with surface modifying additives;[7] however, these modifications are often transient and are not durable solutions for protein resistance in long-term vascular implants, masking the underlying issue.

The HydroPICC™ Technology

Access Vascular, Inc.'s HydroPICC™ device is constructed of a proprietary combination of biocompatible polymers to create a high-strength hydrogel material. Hydrogels inherently possess low interfacial tension, which has been shown to resist thrombus adhesion. The surface of the HydroPICC device contains large extended, hydrophilic polymer chains that provide a steric barrier to repel protein adsorption. For decades, the medical device industry has focused on grafting hydrogels or hydrophilic polymers to surfaces of more durable biomaterials in an effort to decrease their thrombogenicity. Access Vascular, Inc. has engineered around this problem by developing a unique hydrogel composition that combines the superior mechanical properties of polyurethanes with the intrinsically low thrombogenicity of hydrogels into a single bulk material.

Assessment of Thromboresistance *in vitro*

Quantification of the thromboresistance of the HydroPICC material was evaluated in an *in vitro* blood loop and an *in vivo* ovine study. Thrombus accumulation and platelet adhesion were assessed by Thrombodyne, Inc. (Salt Lake City, UT), using an established *in vitro* blood flow loop model.[8] HydroPICC devices were hydrated in sterile saline for approximately 24 hours prior to testing along with TPU samples comprising of PowerPICC (Bard Access Systems, Inc.) and BioFlo PICC (Navilyst Medical, Inc.); single lumen samples were cut to 15 cm prior to mounting in the blood flow loop. The proximal lumen opening was occluded with epoxy, simulating a locked catheter. Fresh bovine blood was collected by cardiac puncture and heparin was added to achieve a 0.75 U/mL concentration. Autologous platelets were purified, labelled with 111-Indium, then added back to the original blood. Samples were inserted into the blood flow loop of 1/4 inch (6.4 mm) inner diameter polyvinyl chloride tubing for approximately 120 minutes. Blood was kept at 37 °C and pumped at 200 mL/min through the loop using a peristaltic pump for the duration of testing to simulate physiological blood flow across the device. Samples were initially checked for thrombus accumulation after 45 minutes in the blood flow loop and removed after 60-120 minutes. At the end of the test, the devices were rinsed with saline and placed in a gamma counter (Perkin Elmer, Wizard 3) for analysis. Each experiment consisted of three (3) independent flow systems (corresponding to the three groups being compared) circulating blood from the same animal; this enabled simultaneous comparisons without cross-over effects. Twelve (N=12) replications of the experiment were run with blood from a different animal used in each replication.

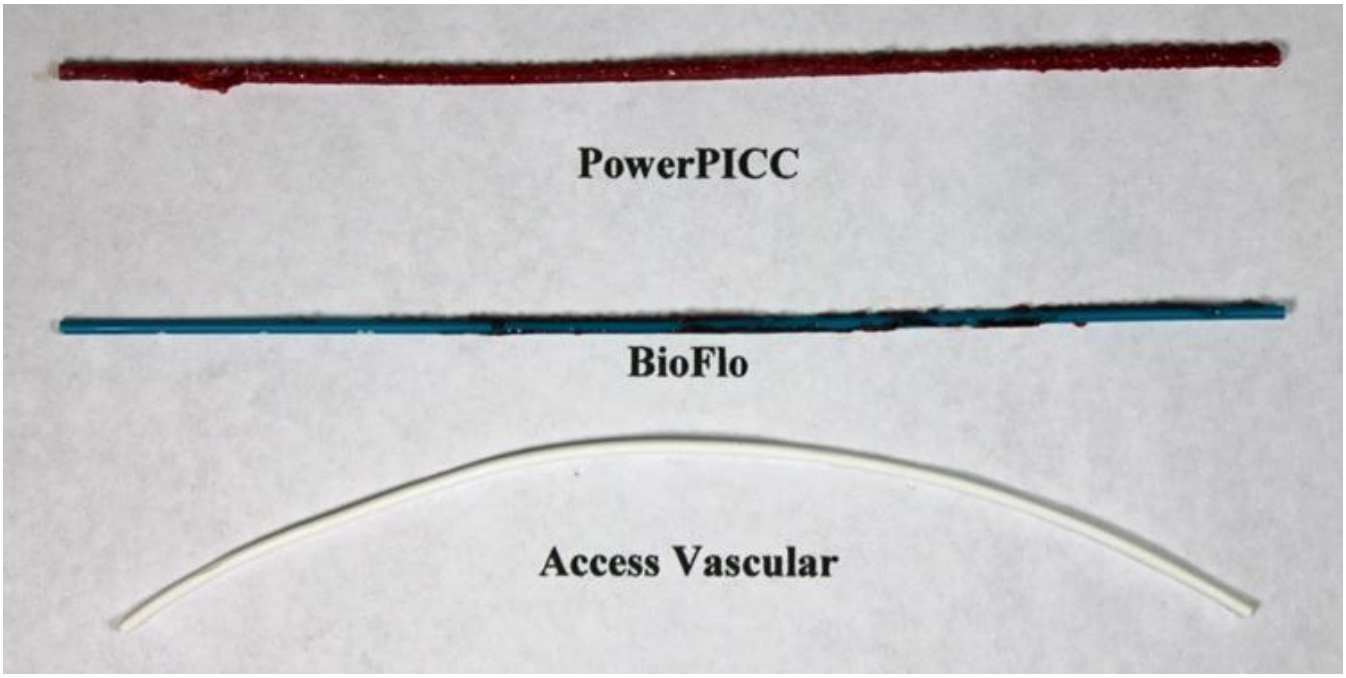

Figure 1. Representative optical image of thrombus accumulation on PowerPICC polyurethane (top), BioFlo fluoro-oligomer modified polyurethane (middle), and Access Vascular HydroPICC (bottom) catheters after removal and rinsing from *in vitro* blood loop.

The *in vitro* blood loop model provides a valuable assessment of inherent device thrombosis characteristics. The hematological parameters (e.g., hemodynamics, anticoagulation) in this *in vitro* model are more controlled than in *in vivo* models, thus enabling direct semi-quantitative evaluation of thrombogenicity. Extraneous dynamic parameters (e.g., vessel geometry, animal physiology, activity, variable hemostasis and homeostasis, and infection) that can confound *in vivo* assessments can be eliminated in the *in vitro* blood loop model. This allows the thromboresistance evaluation to be focused on the device surface properties/chemistry, with other parameters remaining relatively constant. The *in vitro* blood loop model allows for the isolated quantification of platelet adhesion, as platelet adhesion is a fundamental and critical step in thrombus formation, its quantification is a conservative measure of the thrombus accumulation. A representative optical image of a set of paired samples after exposure to the *in vitro* blood loop model are shown in **Figure 1**, qualitatively indicating a significant amount of thrombus accumulation on the two TPU samples, while the HydroPICC devices exhibited only a minimal amount of thrombus accumulation. Because some hematological parameters cannot be consistently controlled between experimental groups, the radiation counts for the experimental HydroPICC and Bioflo were normalized to the radiation counts for the PowerPICC control samples for each paired group. The HydroPICC and BioFlo devices were found to exhibit a statistically significant reduction of thrombus formation compared to the PowerPICC control based on a paired, two-sided t-test (p-values of 0.017 and 0.035, respectively). The HydroPICC was also found to exhibit a statistically significant decrease in thrombus accumulation when compared to BioFlo (p-value of 0.033). A plot of the normalized thrombus accumulation for the two TPU control samples as compared with the HydroPICC device is shown in **Figure 2**, data shown includes experiments with outliers removed. When compared to PowerPICC, a fluoro-oligomer modified TPU catheter (Bioflo) exhibited a 71±30% reduction in thrombus accumulation, while the HydroPICC device exhibited a 97±2% reduction in thrombus.

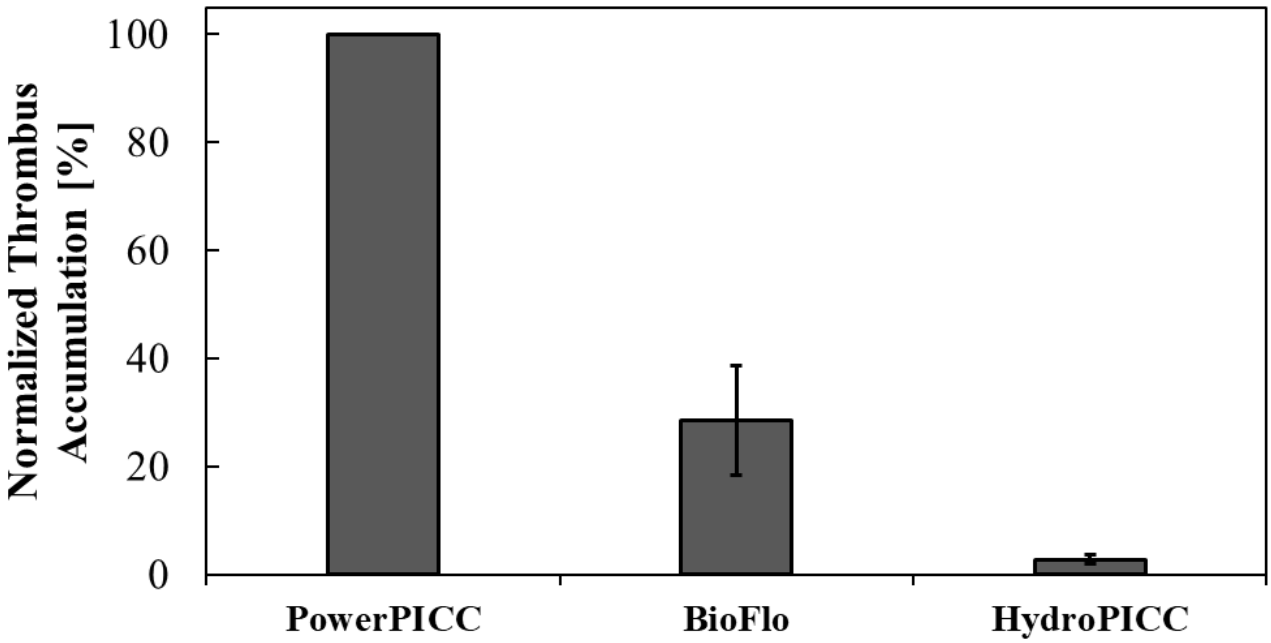

Figure 2. Normalized thrombus accumulation of PowerPICC polyurethane, BioFlo fluoro-oligomer modified polyurethane, and HydroPICC after removal and rinsing from *in vitro* blood loop.

Evaluation of HydroPICC Technology *in vivo*

Seven (n=7) 4F single-lumen HydroPICC kits (AVI p/n PICC-141) were used for insertion in an ovine jugular model. Four Polypay sheep, 70-85 kg in weight, were obtained by Pine Acres Rabbitry Farm (PARF) for the purpose of implantation. The use of all the animals in this study was with the review and approval of the Institutional Animal Care and Use Committee (IACUC) under an approved protocol. PARF is licensed by the USDA and is AAALAC accredited. The ovine jugular model was chosen for testing because it provides a fair representation of human vasculature. Ovine blood also preserves any susceptibility to microbial challenges relating to human pathogens, making it suitable for safety evaluations.[9] Unlike the canine model, the ovine model is very comparable to human coagulation profiles in terms of clotting time, clot formation time, maximum clot firmness, and maximum lysis.[10] Canine blood differs markedly in fibrinolytic activity, making fibrin-rich thrombus very rare[11]. The ovine model, however, may be better suited for the evaluation of venous access devices due to the increased mechanical fragility of ovine blood cells compared to humans.[12] Due to their increased fragility but comparable clotting effects, the ovine model makes a great tool for the accelerated evaluation of implants that induce flow dynamic changes, such as increased shear due to vessel occlusion by a catheter.

An over-the-wire (OTW) version of the Seldinger technique was used for the insertion of each HydroPICC device. HydroPICC test articles were hydrated prior to insertion for at least 10 minutes in sterile saline. Initial vein access was achieved using an introducer needle. A guide wire was then inserted into the needle, and then the needle replaced with a vein dilator advanced over the guidewire. A nick was made at the insertion site with a scalpel to ease the advancement of the dilator. The target vessel was imaged under fluoroscopy to evaluate the implant length of the catheter via guidewire approximation. The HydroPICC device was then trimmed to length with a scalpel. The guidewire was reinserted and the dilator was removed; the catheter was then advanced over the wire to approximately 1 cm proximal to the juncture of the jugular veins as determined by fluoroscopy. The guidewire was then removed. Each catheter was flushed and locked with heparinized saline (100 U/mL) and the clamp on the extension tubing was engaged. Sterile injection plugs were securely placed on each catheter luer hub. The catheter was positioned such that the tip of the suture wing penetrated just below the skin; and two drops of Loctite 435 cyanoacrylate adhesive were placed at the entry site and held in place for 15-20 seconds. The catheter was then secured via the suture wing to the skin of the animal using 3-0 non-braided prolene monofilament suture material at the two suture wing eyelets and the suture groove on the body of the suture wing. A chlorhexidine patch (Biopatch™) was cut in half and placed at the exit site and a Tegaderm™ film dressing was placed over the catheter and exit site.

Flushes and patency checks were performed every day over the course of the study. Animal observations and bandage checks (observe for integrity, contamination/moisture, bleeding, etc.) were performed at least once daily. Just prior to euthanizing, a 2000 IU/kg dose of heparin was administered to each animal to assure no postmortem coagulation. Limited necropsy was performed with macroscopic observations and photographic documentation. The defined veins were incised longitudinally, taking care not to dislodge the in-dwelling catheter. The luminal surface of the implanted veins was gently rinsed with

saline to remove any residual liquid blood. Macroscopic photos of the incised veins were obtained, including the level of device insertion into the vein to the level of catheter tip location. Relative thrombus scores were evaluated on a 0-5 scale commonly used for assessing nonanticoagulated venous implant (NAVI) models as shown in **Table 1**.[13] A thrombogenicity score of ≥3 would be considered a failed device. Vessel patency was also assessed on a semi-quantitative scale from 0 (100% patent) to 4 (100% occluded). Retain samples from each excised catheter with attached tissue were fixed in formalin and sent to Charter Preclinical Services for hematoxylin and eosin (H&E) staining and histopathologic analysis.

Table 1. Thrombogenicity Scoring of Explanted Devices

Thrombus score	Description of thrombus formation
0	Minimal to nonexistent-thrombus observed to cover ≤1% of device surface
1	Minimal-thrombus on 2-10% of device surface
2	Mild-thrombus observed to cover 11-25% of device surface
3	Moderate-thrombus observed to cover 26-50% if device surface
4	Extensive-thrombus observed to cover 51-75% of device surface
5	Severe-thrombus observed to cover 76-100% of device surface

The lubricious nature of the proprietary hydrogel material made insertion of every HydroPICC device smooth and easy, with minimal to no resistance upon insertion without a tearaway sheath. The cyanoacrylate adhesive was found to be useful in keeping the catheter in-place during suturing and maintaining position for up to 28 days after implantation. No significant changes in clinical observations or animal body weight were noted over the course of study. Patency checks were conducted daily and all three (3) 14-day cohort PICCs were patent (flushing & aspirating) at term and all four (4) 28-day cohort PICCs were patent (flushing & aspirating) at term. Results of thrombosis scoring are provided in **Table 2** and photographs of explanted jugular and catheters are shown in **Figure 3** (14-day and 28-day cohort). A small fibrin sheath was observed in every explanted catheter that extended from the subcutaneous tunnel into the main body of the catheter; however, for all test articles except 3993R, this sheath extended no more than 10-15% of the total length of the catheter. This sheath was observed to be fragile in nature and lacked any attachment to the catheter itself, it simply slid off with minimal force during explantation. The main bodies of all the test articles showed no signs of thrombosis and were functional at term. Histopathologic analysis of the catheter samples found very minimal fibrin accumulation and tissue inflammation for all seven explanted devices, with an overall decrease in fibrin from the 14-day to the 28-day cohort.

Table 2. Thrombosis Evaluation of Explanted HydroPICC Devices.

Vessel ID	Cohort	Device Score	Notes
3994L	14-day	1	Minimal thrombus at entry site
3994R	14-day	2	Mild thrombus extending ~2-3 cm from entry site
3995L	14-day	1	Minimal thrombus extending ~1-2 cm from entry site
3993L	28-day	1	Minimal thrombus at entry site
3993R	28-day	3	Very thin, non-adherent thrombus along ~50% of device surface
3997L	28-day	1	Minimal thrombus extending ~1-2 cm from entry site
3997R	28-day	1	Minimal thrombus at entry site

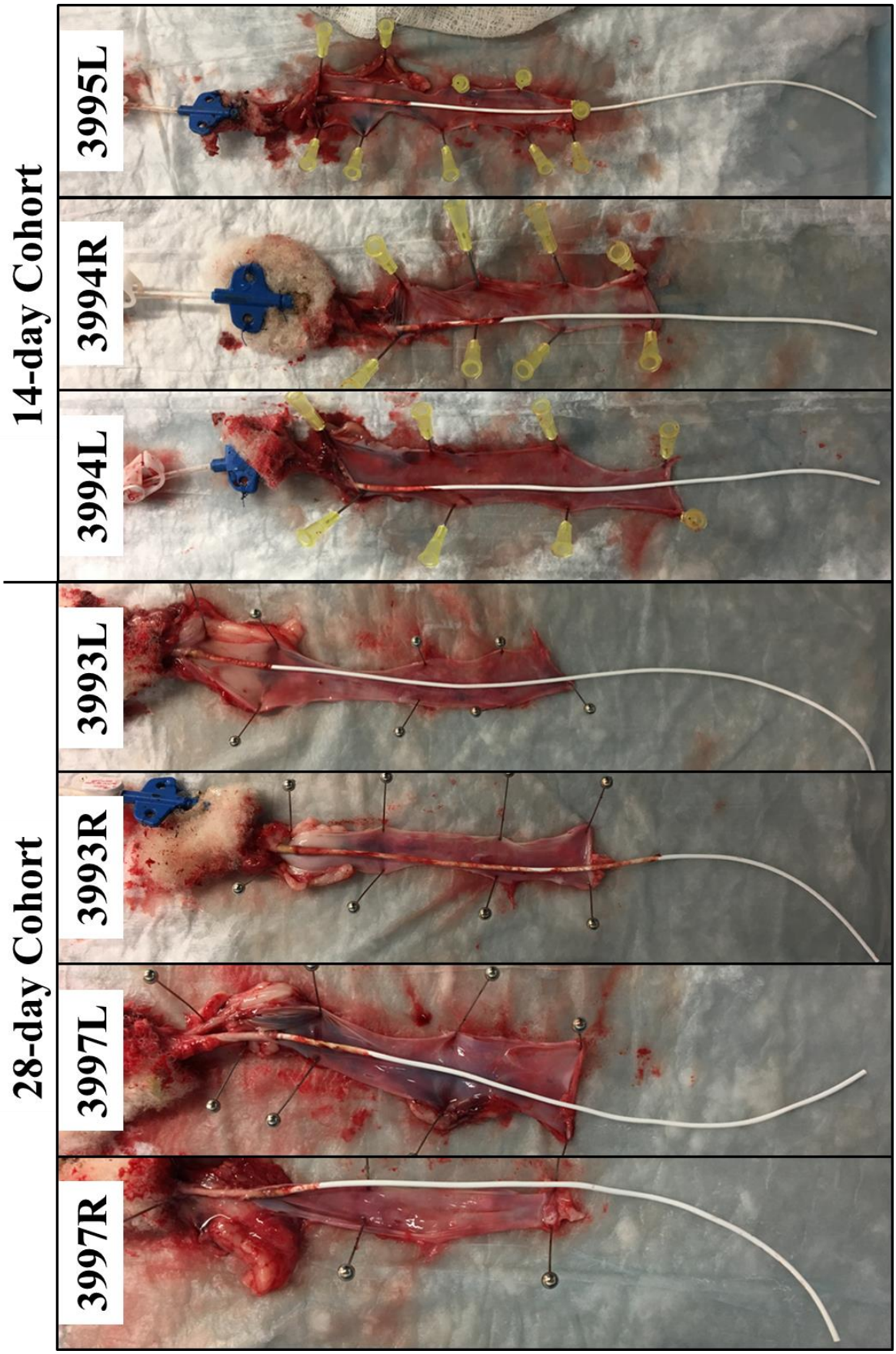

Figure 3. Optical images of explanted HydroPICC devices from 14 & 28-day cohort.

Conclusions

The HydroPICC technology represents a new method to reduce catheter-related thrombus by fabricating catheters from an inherently non-thrombogenic bulk hydrogel. Thromboresistance of the HydroPICC technology was also evaluated in an *in vitro* blood loop model, with results showing up to an average of 97% reduction in platelet adhesion as compared to conventional TPU catheters and a 64% reduction when compared to a thromboresistant TPU catheter. Seven (n=7) HydroPICC devices were implanted into ovine jugular vessels for 14-day and 28-day survival time-points. All devices were patent (flushing & aspirating) at term; three out of the four 28-day cohorts exhibited no visible thrombus on the distal 2/3 of the device and no tissue on any of the seven implants was found to be substantially adherent to the catheter. Based on the *in vitro* and *in vivo* testing, the HydroPICC technology is shown to be a viable class of high strength thromboresistant hydrogels to replace conventional polyurethanes and silicones in vascular access and other blood-contacting medical device applications.

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