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A bioinspired omniphobic surface coating on medical devices prevents thrombosis and biofouling

Daniel C Leslie[1–4,9], Anna Waterhouse[1,3,5,9], Julia B Berthet[1,3,5], Thomas M Valentin[1,3], Alexander L Watters[1,3], Abhishek Jain[1,3,4], Philseok Kim[1,2], Benjamin D Hatton[1,2,8], Arthur Nedder[6], Kathryn Donovan[6], Elana H Super[1], Caitlin Howell[1,2], Christopher P Johnson[1,2], Thy L Vu[1,2], Dana E Bolgen[1], Sami Rifai[1], Anne R Hansen[3,5], Michael Aizenberg[1], Michael Super[1,3,4], Joanna Aizenberg[1,2,7] & Donald E Ingber[1–4]

Thrombosis and biofouling of extracorporeal circuits and indwelling medical devices cause significant morbidity and mortality worldwide. We apply a bioinspired, omniphobic coating to tubing and catheters and show that it completely repels blood and suppresses biofilm formation. The coating is a covalently tethered, flexible molecular layer of perfluorocarbon, which holds a thin liquid film of medical-grade perfluorocarbon on the surface. This coating prevents fibrin attachment, reduces platelet adhesion and activation, suppresses biofilm formation and is stable under blood flow *in vitro*. Surface-coated medical-grade tubing and catheters, assembled into arteriovenous shunts and implanted in pigs, remain patent for at least 8 h without anticoagulation. This surface-coating technology could reduce the use of anticoagulants in patients and help to prevent thrombotic occlusion and biofouling of medical devices.

Countless lives have been saved by implantable medical devices, (artificial hearts, ventricular assist devices, pacemakers, cardioverter-defibrillators and central lines) and extracorporeal devices that flow whole human blood outside the body through indwelling catheters and external circuits during cardiopulmonary bypass, hemodialysis and extracorporeal membrane oxygenation[1,2]. However, the need to co-administer soluble anticoagulant drugs, such as heparin, during many of these procedures, substantially reduces their safety and hampers their effectiveness[3,4]. Without systemic anticoagulation, these extracorporeal and indwelling devices can rapidly occlude due to thrombosis because clots form when fibrin and platelets in the flowing blood adhere to the surfaces of these artificial materials[5]. Unfortunately, heparin causes morbidity and mortality through post-operative bleeding, thrombocytopenia, hypertriglyceridemia, hyperkalemia and hypersensitivity[6], and its use is contraindicated in several patient populations[7]. In fact, most drug-related deaths from adverse clinical events in the United States are due to systemic anticoagulation[8].

The pressing clinical need to prevent blood clotting while minimizing administration of anticoagulant drugs has led to the search for biomaterial surface coatings that can directly suppress blood clot formation. The most successful approach to date has been to chemically immobilize heparin on blood-contacting surfaces to reduce thrombosis and lower anticoagulant administration[9,10]. Although this approach has been widely adopted, major limitations persist because the surface-bound heparin leaches, resulting in a progressive loss of anticoagulation activity[4,11] and the use of heparin-coated materials has not led to a drastic reduction in the clinical use of soluble heparin[12]. Some high-flow dialysis treatments can be carried out without heparin in subsets of patients with high bleeding risks, but even in this patient population, half are forced to switch to heparin bolus dialysis within the first year of treatment[2]. Due to these limitations, other nonthrombogenic, hydrophilic material coatings have been explored, including PHISIO (Sorin)[13], Trillium (Medtronic)[14], poly-2-methoxyethyl acrylate (PMEA) polymer[15] and sulfobetaine[16]. Extensive human clinical evaluation of these various alternative surface coatings is currently underway; however, no benefit has been shown to date when compared to existing heparin-coated materials[17,18].

Based on the limited clinical utility of these strategies for reducing thrombosis of extracorporeal circuits, we explored a recently described, slippery, liquid-infused, porous surface (SLIPS) approach. SLIPS was inspired by the *Nepenthes* pitcher plant, which uses a layer of liquid water to create a low friction surface that prevents attachment of insects[19]. The SLIPS technology creates omniphobic slippery surfaces by infiltrating porous or roughened substrates with various liquid perfluorocarbons (LPs) that prevent adhesion to the underlying substrate through formation of a stably immobilized, molecularly smooth, liquid overlayer[20]. However, existing medical-grade materials, such as polycarbonate, polysulfone and polyvinyl chloride

[1]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts, USA. [2]School of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts, USA. [3]Harvard Medical School, Boston, Massachusetts, USA. [4]Vascular Biology Program, Boston Children's Hospital, Boston, Massachusetts, USA. [5]Division of Newborn Medicine, Boston Children's Hospital, Boston, Massachusetts, USA. [6]Animal Research, Boston Children's Hospital, Boston, Massachusetts, USA. [7]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts, USA. [8]Present address: Department of Materials Science and Engineering, University of Toronto, Ontario, Canada. [9]These authors contributed equally to this work. Correspondence should be addressed to D.E.I. (don.ingber@wyss.harvard.edu).

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(PVC), have highly smooth surfaces. Thus, to create nonadhesive, antithrombogenic surfaces that might be useful for clinical medicine in the near-term, we set out to modify the SLIPS technology so that it can be applied to these smooth surfaces. This was accomplished by covalently binding a flexible molecular perfluorocarbon layer, or tethered perfluorocarbon (TP), on the material surface and then coating it with a mobile layer of an LP (perfluorodecalin) that has been used extensively in medicine for applications such as liquid ventilation[21,22], ophthalmic surgery[23] and as an US Food and Drug Administration (FDA)-approved blood substitute[24] (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm074920.htm). We refer to this antithrombogenic bilayer composed of the TP with an LP coating as a tethered-liquid perfluorocarbon (TLP) surface. Here, we show that the TP retains the free LP as a thin mobile liquid layer applied to virtually any medical-grade material surface, even when the surface is in contact with a flowing, immiscible fluid, such as blood (**Fig. 1a**). Importantly, the TLP surface coating also effectively repels whole blood, resists adhesion of blood components and bacteria, and reduces thrombosis *in vitro* and *in vivo* without dangerous anticoagulants.

RESULTS

TLP surface coating repels whole blood

To test the antiadhesive properties of the TLP coating method, we examined surface adhesion of fresh whole human blood on an acrylic surface with or without a TLP coating (tethered perfluorohexane and liquid perfluorodecalin) that was sloped at an angle of 30 degrees. Blood droplets immediately adhered to the control uncoated acrylic surface and left a trail of blood components over the time course of 5 s (**Fig. 1b**, top, **Supplementary Fig. 1** and **Supplementary Movie 1**). In contrast, when the same surface was coated with TLP, the blood droplet almost immediately slid off the surface (<0.3 s), and, remarkably, there was no evidence of any residual blood trail (**Fig. 1b**, bottom, **Supplementary Fig. 1** and **Supplementary Movie 2**). We quantified blood adhesion to surfaces by measuring the minimum angle required to cause a droplet to slide ('sliding angle') (**Fig. 1c**). Control uncoated surfaces and surfaces that were modified by coating with either the TP or LP layer alone all exhibited considerable blood adhesion, even when the surface was tilted to 90 degrees (**Fig. 1c**). In contrast, the minimum angle to cause blood sliding on the TLP acrylic surface was 0.6 ± 0.2 degrees. Importantly, this same TLP surface coating was equally effective when applied to multiple other smooth medically relevant plastics, glasses and metals, including polycarbonate, PVC, polysulfone, polyethylene, polypropylene, polyethylene terephthalate (PET), polyimide, polystyrene, borosilicate glass, polydimethylsiloxane (PDMS), titanium, silicon, fluorinated ethylene propylene and polytetrafluoroethylene (**Fig. 1d** and **Supplementary Fig. 2**). The TLP-coated smooth surfaces containing a thin, molecular film of LP immobilized through interactions with a covalently coupled TP layer were also equally as effective as roughened or porous materials into which a larger volume of LP was passively infused, as described in the original SLIPS coating technology; SLIPS-coated expanded PTFE (e.SLIPS) and nanostructured boehmite (B.SLIPS) (**Fig. 1d**) have previously been shown to repel crude oil and ice, as well as anticoagulated animal blood[20,25,26]. Additionally, the TLP-coated, smooth, medical-grade materials showed no blood adhesion after the greater challenge of immersion in human blood, which was in stark contrast to the control (**Supplementary Movie 3**).

To further illustrate how slippery the TLP coating is, we tested the ability of TLP-coated acrylic to repel the adhesion of a living gecko, which was previously shown to be unaffected by surface coatings on acrylic[27]. The stable adhesion of the LP to the TP is maintained by van der Waals attractive forces. Thus, we tested whether the gecko, which evolved to provide maximum van der Waals forces over 17 times their body weight on a vertical surface[27], could overcome the TLP coating. Geckos remained attached to control acrylic surfaces oriented at up to 90 degrees inclination (**Supplementary Movie 4**); however, the geckos were unable to hold on when the acrylic surface was coated with TLP, and thus, they slipped down before the angle approached vertical (**Supplementary Movie 5**).

Reduced adhesion and activation of blood components

Material-induced thrombosis is mediated through adhesion and activation of two major blood components, fibrinogen and platelets,

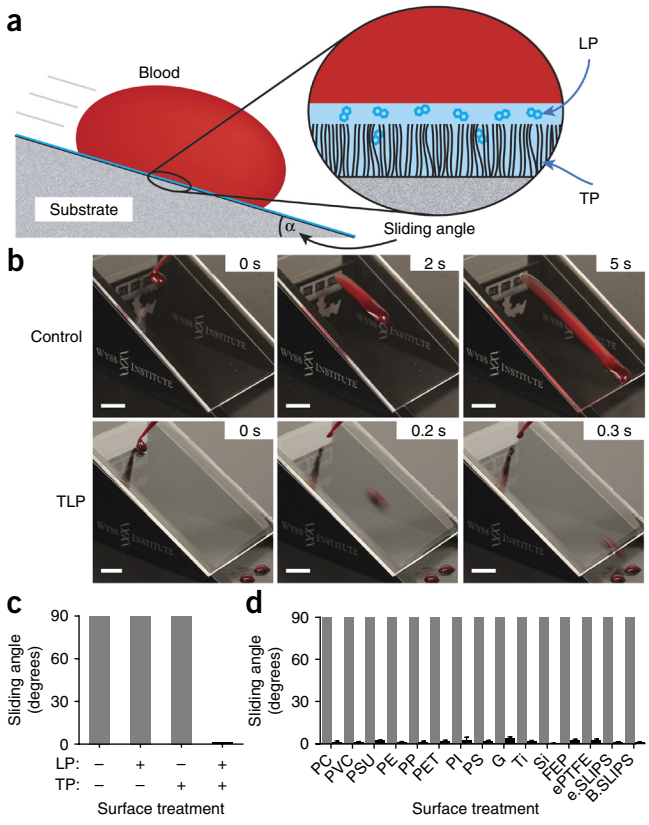

Figure 1 TLP-coated surfaces repel whole human blood. (**a**) Schematic of blood repellency on TLP surfaces showing the TP bound to a substrate through plasma activation and silane treatment, which then allows a stable film of LP to adhere to the surface. (**b**) Surfaces without TP or LP (–TP/–LP; control) show adhesion of a blood droplet (50 µl, 3.2% sodium citrate) on the 30-degree angled surface, low velocity and residence over 5 s (upper panels). When TLP is applied to the surface, a blood droplet (50 µl, 3.2% sodium citrate) is immediately repelled and slides down the surface at an incline of 30 degrees within 0.3 s (lower panels). Scale bars, 1 cm. (**c**) Graph showing the minimum angle that allowed whole blood (5 µl droplet, 3.2% sodium citrate) to slide on the different surface treatments (mean ± s.d., $n = 3$). (**d**) TLP can be applied to a wide range of materials with a low whole-blood sliding angle (black bars) compared to control surfaces (gray bars) comprising polycarbonate (PC), PVC, polysulfone (PSU), polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polyimide (PI), polystyrene (PS), borosilicate glass (G), titanium (Ti), silicon wafer (Si), fluorinated ethylene propylene copolymer (FEP), polytetrafluoroethylene (PTFE), expanded polytetrafluoroethylene (ePTFE), Slippery Liquid Infused Porous Surface (SLIPS) (e.SLIPS) and boehmite SLIPS (B.SLIPS) (mean ± s.d., $n = 3$).

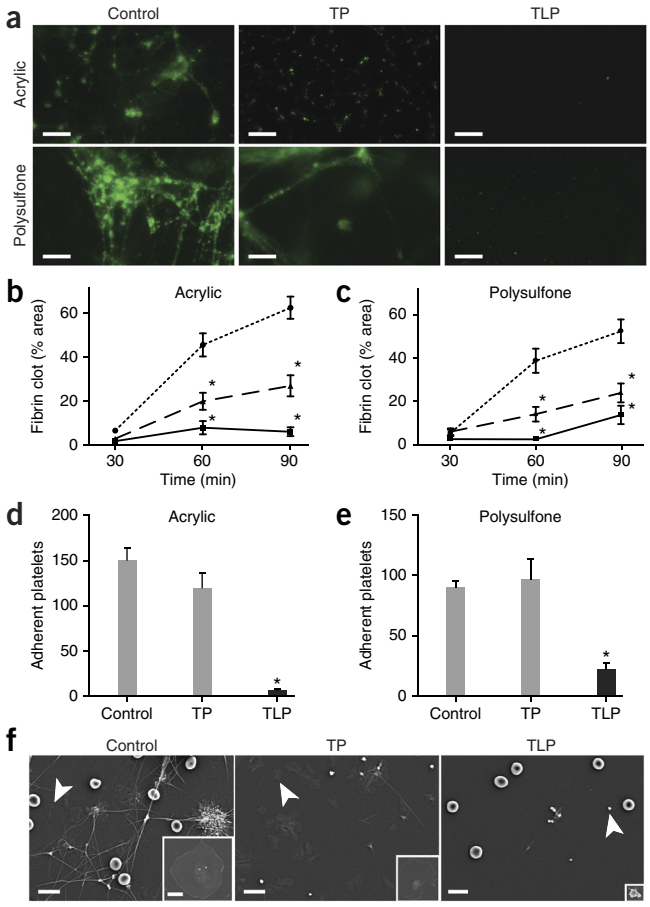

Figure 2 Whole blood interactions with TLP surfaces. (**a**) Fluorescent micrographs of acrylic (upper panels) or polysulfone (lower panels) pieces (11 × 8 mm) after 90-min incubation with fresh human blood containing heparin (0.25 U/ml) and fluorescent fibrinogen (150 μg/ml) showing polymerized fibrin networks on the control (left), decreased network formation on TP (middle) and punctate staining with minimal network formation on TLP (right). Scale bars, 50 μm. (**b**) Graph showing a reduction of percent fibrin-covered area on TLP (full line) acrylic compared to control (dotted line) and TP (dashed line). Percentages quantified using ImageJ (*$P < 0.05$ compared to control, two-way ANOVA, s.e.m.). (**c**) Graph showing a reduction of percent fibrin-covered area on TLP (full line) polysulfone compared to control (dotted line) and TP (dashed line) (*$P < 0.05$ compared to control, two-way ANOVA, s.e.m.). (**d**,**e**) Graph showing a 27-fold reduction in platelet adhesion (number per 150 μm^2) on TLP acrylic compared to control (**d**) and about fourfold reduction on TLP polysulfone (**e**) (*$P < 0.05$, one-way ANOVA, s.e.m.). (**f**) Scanning electron micrographs of acrylic surfaces after 30-min incubation shows reduced platelet adhesion on TLP compared to control and TP. Scale bars, 10 μm. Insets show platelet morphology, white arrowheads show platelets. Inset scale bar, 2 μm. All data are from experiments with three separate donors, two technical replicates, eight (**b**,**c**) or four (**d**,**e**) images per replicate.

within minutes of surface contact. Cleavage of fibrinogen to fibrin results in the formation of polymerized fibrin networks that form the backbone of the clot, which becomes intertwined with aggregates of platelets that are simultaneously activated by either interacting directly with the material surface or with surface-bound proteins[5]. We exposed smooth plastic surfaces to fresh, whole human blood that contained a low dose of heparin (0.25 U/ml) to prevent immediate clotting (compared to 5–7 U/ml clinical dosage). Even in the presence of heparin, control acrylic and polysulfone surfaces formed dense fibrillar networks of polymerized fibrin during the 90-min study (**Fig. 2a**). Whereas surfaces coated with a TP layer alone (without the LP layer) reduced network formation, adherent fibrils of polymerized fibrin were still detected. In contrast, coating the acrylic surface with the TLP bilayer completely prevented adhesion of fluorescently labeled fibrin in whole blood. Similarly, TLP-coated polysulfone showed only minimal punctate fibrin staining with rare thin fibrils (**Fig. 2a**). Quantitation of these results confirmed that TLP coating of the acrylic and polysulfone substrates decreased fibrin adhesion and polymerization relative to controls by six- and fourfold, respectively ($P < 0.05$), and by three- and twofold relative to surfaces coated with the TP layer alone (**Fig. 2b**,**c**). The reduced adhesion and polymerization of fibrin on the surfaces coated only with the TP layer is important because it suggests that TLP coatings will significantly suppress clot formation, even if the LP layer is displaced after extended exposure to blood. Furthermore, we confirmed the reduced coagulation on TLP surfaces exposed to whole blood by showing that D-dimer production, which is a quantitative marker of fibrin polymerization, was significantly ($P < 0.05$) lower on the TLP coating than on control surfaces (**Supplementary Fig. 3**).

To evaluate effects on platelet adhesion, we analyzed surfaces by scanning electron microscopy (SEM) after they were exposed to whole human blood for 30 min. These studies revealed that coating acrylic and polysulfone surfaces with TLP reduced platelet adhesion by 27- and fourfold, respectively, compared to control surfaces ($P < 0.05$, **Fig. 2d**,**e**). Furthermore, although nearly all platelets adherent to control materials and surfaces coated with TP alone exhibited a flattened or spread morphology consistent with platelet activation, most platelets found on surfaces coated with TLP were round, suggesting that they remained in a resting, nonactivated state (**Fig. 2f** and **Supplementary Fig. 4**). Taken together, these results indicate that TLP surfaces reduce fibrin polymerization and suppress both adhesion and activation of platelets, hence confirming their reduced thrombogenicity *in vitro*.

Suppression of thrombosis under flow *in vitro*

To be medically relevant, the TLP surfaces must remain stable and functional when exposed to physiological shear stresses. When we exposed the surfaces to a constant shear strain rate (1,000 s^{-1}) using a rheometer, we found that LP was displaced from acrylic surfaces that were coated with LP alone after 1 min of exposure to shear, resulting in blood adhesion even when the surface was tilted to 90 degrees. In contrast, TLP-coated acrylic surfaces continued to effectively repel blood (sliding angle < 3 degrees) even after 16 h exposure to the same amount of fluid shear (**Fig. 3a**). Also, these TLP surfaces were stable when exposed to a range of shear rates (250–2,000 s^{-1}) for 10 min with no change in sliding angle (**Supplementary Fig. 5**). Thus, the TLP coating remained stable under hemodynamic fluid shear conditions for extended time periods *in vitro* because of the higher affinity of LP for the immobilized TP than for blood or water.

We then flowed fresh whole human blood (0.25 U/ml heparin at 50 ml/h) through either control or TLP-coated medical-grade PVC tubing (1/16″ inner diameter (ID)). After rinsing, the remaining adherent proteins were digested off the tubing surface and quantified using a bicinchoninic acid (BCA) assay (**Fig. 3b**). These studies revealed that coating the tubing with TLP resulted in a threefold decrease in protein adhesion compared to control tubing (0.8 ± 0.2 versus 2.3 ± 0.5 μg/cm^2, respectively; $P < 0.05$) (**Fig. 3b**). Free hemoglobin levels were not significantly different from those measured in baseline blood samples, indicating that the TLP surfaces caused no hemolysis with a single pass of flowing blood (**Supplementary Fig. 6**).

We then challenged the TLP coating by testing it in a microfluidic device to mimic the physiological microenvironment of blood flowing through a vessel. Fresh whole human blood (0.5 U/ml heparin) was

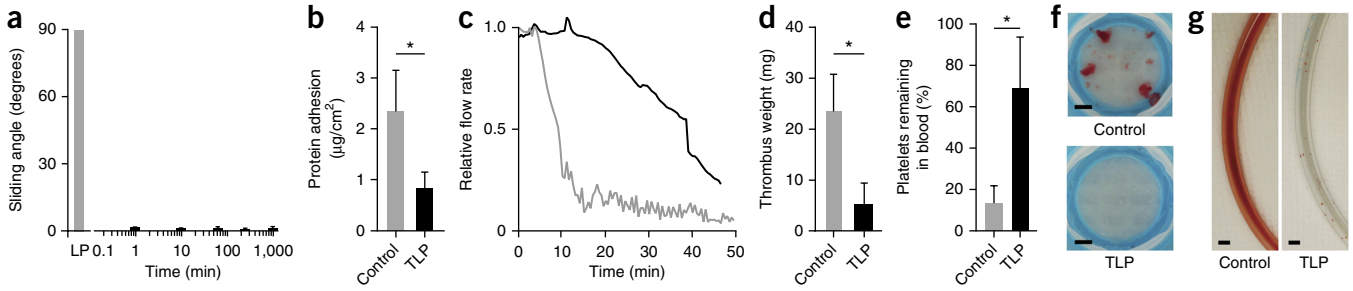

Figure 3 Stability and thrombogenicity of TLP surfaces. (**a**) The low blood sliding angle on TLP acrylic discs (black bars) remained stable over 1,000 min under a constant shear rate of 1,000 s^{-1}, whereas LP acrylic discs (gray bar) failed and had a 90-degree sliding angle after 1 min (s.d.). (**b**) Decreased protein adhesion on TLP PVC, measured by BCA assay, after whole human blood flow (0.25 U/ml heparin) through 1/16″ ID PVC tubing for 20 min at 50 ml/h. (*$P < 0.05$, paired student's *t*-test, s.d.) (**c**) Maintenance of whole human blood flow (0.5 U/ml heparin at 1,250 s^{-1}) in TLP microfluidic channels (PDMS) over 50 min (black line), whereas LP channels occluded after ~15 min (gray line). (**d**) Reduced thrombus weight in TLP medical-grade PVC tubing (black bar) compared to control (gray bar, *$P < 0.05$, unpaired, two-tailed student's *t*-test, s.d.) after blood was pumped in a closed loop at 3 L/h for 2 h. (**e**) Increased percentage of platelets remained in the blood after exposure to TLP tubing (black bar) compared to control tubing (gray bar, *$P < 0.05$, unpaired, two-tailed student's *t*-test, s.d.) after the blood was pumped in a closed loop at 3 L/h for 2 h. (**f**) Photographs of filtered thrombi after blood was pumped in a closed loop at 3 L/h for 2 h in control (upper image) and TLP medical-grade PVC (lower image). Scale bars, 5 mm. (**g**) Photographs of control (left) or TLP (right) cardioperfusion tubing sterilized with ethylene oxide after it had been exposed to porcine blood for 2 min. Scale bars, 5 mm. All data are from experiments with three separate donors.

infused under a constant pressure with an initial wall shear of 1,250 s^{-1}. Blood flowed without occlusion for longer in TLP devices than in LP devices (**Fig. 3c**), as shown by a significant 2.5-fold increase in average clotting time ($P < 0.05$), which corresponds to the time required for the occlusion to reduce flow by half (**Supplementary Fig. 7**). This indicates that overall thrombosis was significantly reduced on TLP surfaces, as we expected from the reduced fibrin polymerization and suppressed adhesion and activation of platelets that we observed in our earlier studies (**Fig. 2**).

To explore whether this surface-coating technology might be useful for extracorporeal circuits in the clinic, the TLP coating was applied to large-diameter (1/4″ ID), medical-grade PVC cardioperfusion tubing. No macroscopic or microscopic differences were observed with the application of TP to this tubing (**Supplementary Fig. 8a**,**b**). Distinct droplets of blood could also be seen moving along the TLP surface without adhesion, whereas blood adhered to surfaces that were coated with LP alone (**Supplementary Fig. 8c**). As extracorporeal circuits require peristaltic pumps to drive blood flow, we pumped fresh whole human blood (0.25 U/ml heparin) at 3 liters (L)/h through a closed loop (1/4″ ID perfusion tubing) system linked with polycarbonate connectors. These studies showed that there was greatly reduced formation of visually detectable clots within the lumen of the tubing in the TLP-coated PVC loops (**Fig. 3d**,**f**). This correlated with a reduction in thrombus weight by about fourfold after 2 h compared to uncoated control loops ($P < 0.05$, **Fig. 3d**), and the finding that blood platelet counts remained about threefold higher in blood exposed to TLP-coated loops than uncoated loops ($P < 0.05$, **Fig. 3e**) is indicative of reduced platelet adhesion and/or aggregation. Although there was some loss (28 ± 21%) of platelets in this experiment, the blood traveled through the pump 360 times, the equivalent of passing through 150 m of tubing. This is far harsher than extracorporeal circulation, and hence, a similar result is not to be expected to occur *in vivo*. Importantly, although the use of peristaltic pumps can sometimes mechanically abrade the tubing where they interface, this did not impair the antithrombogenic properties of the TLP surface. Furthermore, the TLP coating was consistent along the entire length of a 120″ (10 ft.) segment of cardioperfusion tubing (**Supplementary Fig. 9a**), as verified by similar tilt angles and a high percentage of fluorine atoms throughout (**Supplementary Table 1**). We also found that the TP was stable for 12 months when applied to cannulae and perfusion tubing (**Supplementary Fig. 9b**), and ethylene oxide sterilization of the TP-coated perfusion tubing did not alter the surface's ability to repel blood when coated with LP before use (**Fig. 3g**). This coating method therefore provides an effective method to create antithrombogenic surfaces on existing medical-grade materials, which is amenable to rapid commercial translation.

Reduced thrombosis *in vivo*

To examine the antithrombogenic properties of TLP-modified, medical-grade materials *in vivo*, commercially available polyurethane cannulae, polycarbonate connectors and PVC cardiopulmonary perfusion tubing were assembled into an arteriovenous (AV) shunt and modified with the TLP surface coating. We then analyzed its ability to support blood flow for 8 h in a porcine femoral AV shunt model (**Fig. 4a**), either with low heparin (30 U/kg compared to the 300 U/kg used for conventional systemic heparinization[28]) or without any systemic heparin anticoagulation. In the low heparin group, real-time flow measurements revealed that flow rates remained between 10 and 21 L/h in animals implanted with either uncoated control or TLP-modified shunts, although we observed increased flow rate variability in animals with the control (noncoated) materials (**Fig. 4b**). Importantly, all of the TLP-coated circuits also remained patent for 8 h even in the absence of any heparin, maintaining a flow rate of 11 to 18 L/h; in contrast, complete occlusion occurred in four out of five uncoated circuits in control animals ($P < 0.05$; **Fig. 4c**). In animals that did not receive heparin, two animals in the TLP group and one in the control group developed similarly low mean arterial pressures. Nevertheless, despite the increased thrombogenic potential from these decreased flow rates, the circuits remained patent in the TLP animals, whereas the circuit became fully occluded in the control animal.

Analysis of circuits from both heparin and no-heparin groups after completion of the study revealed that TLP-modified circuits exhibited a marked decrease in occlusive thrombosis in both the arterial and venous sections after 8 h of flow (**Fig. 4d**). Total thrombus weight in TLP-coated circuits was reduced by twofold compared to controls in the heparin and no-heparin groups ($P < 0.05$; **Fig. 4e**). To determine the extent of occlusion throughout the circuits post-explant, we measured lumen areas using computerized image analysis to calculate percent occlusion. In the heparin group, control circuits had 7 ± 3% occlusion, whereas there was no detectable occlusion

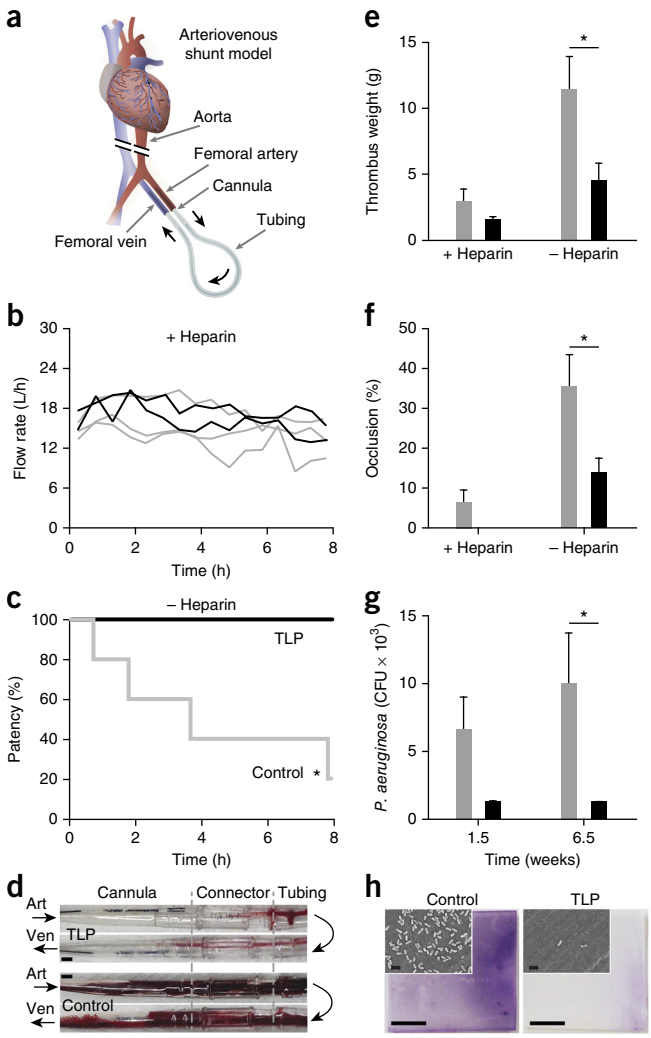

Figure 4 Thrombogenicity of TLP-coated circuits in a porcine arteriovenous shunt model. (**a**) Schematic of the porcine arteriovenous shunt model showing placement of the cannulae in the femoral artery and vein connected by tubing. Black arrows indicate direction of blood flow driven by the heart. (**b**) Real-time measurements of flow rate (10 s average) at the midpoint of the circuit showing that in heparinized animals (+ heparin), flow rate is maintained for 8 h in both control (gray lines, $n = 3$) and TLP (black lines, $n = 2$). (**c**) Kaplan-Meier curve of patency from real-time flow-rate measurements in nonheparinized animals (– heparin) revealed 4 out of 5 control circuits (gray line, $n = 5$) occlude, whereas TLP circuits (black line, $n = 4$) remain patent for 8 h ($^*P < 0.05$, Fisher's exact test). (**d**) Photographs of polyurethane cannulae, polycarbonate connectors and PVC tubing of TLP (top) and control (bottom) circuits after 8 h of blood flow. Arrows indicate direction of blood flow through arterial (Art) or venous (Ven) cannula. Increased thrombus is visible in the control circuit. Scale bars, 5 mm. (**e**) Thrombus weight in the TLP circuits (black bars) is reduced under both + heparin and – heparin conditions compared to control circuits (gray bars) ($^*P < 0.05$, two-way ANOVA with Bonferroni's multiple comparisons test, s.e.m.). (**f**) Percent occlusion of circuits showing that TLP (black bar) has minimal occlusion compared to control (gray bars) in the presence or absence of heparin ($^*P < 0.05$, two-way ANOVA with Bonferroni's multiple comparisons test, s.e.m.). (**g**) Biofilm formation on control PVC (gray bars) and TLP PVC (black bars) for 1.5 ($^*P < 0.05$, two-way ANOVA with Bonferroni's multiple comparisons test, s.e.m.) and 6.5 weeks. (**h**) Crystal violet–stained *P. aeruginosa* on polyethylene control (left) and TLP (right) surfaces after culturing for 16 h. Scale bars, 5 mm. Insets show SEM images. Scale bars, 2 µm.

in circuits coated with TLP (**Fig. 4f** and **Supplementary Fig. 10**). Moreover, even in the absence of heparin, there was a 2.5-fold reduction in lumen occlusion (from 35 ± 8% to 14 ± 4%) in TLP-coated circuits compared to controls ($P < 0.05$; **Fig. 4f** and **Supplementary Fig. 10**). These data confirm our *in vitro* results by showing that the TLP coating significantly reduced the thrombogenicity of medical-grade plastics, including widely used PU, polycarbonate and PVC, when in direct contact with whole blood flowing at high, physiologically relevant rates (10–21 L/h) for at least 8 h *in vivo*. Importantly, in these studies, the TLP coating was as effective at maintaining shunt blood flow with no heparin as control circuits in heparinized animals (**Fig. 4b**,**c**). Blood flow was also maintained without producing significant differences in hematologic parameters, pathology or histology (**Supplementary Note** and **Supplementary Table 2**), and there was minimal leaching of LP into the blood. In the few blood samples where it was detected (4 of 19 samples from three animals), free LP levels were at or near the limit of detection (0.07 µg/ml blood or ~4 µg LP/kg body weight), which is over six orders of magnitude lower than levels of LP that have been used clinically without toxicity (5.6 g LP/ kg body weight)[24,29].

TLP coating inhibits biofilm formation

Given the potent ability of the TLP coating to prevent surface adhesion, we also explored whether this surface-coating method could prevent adhesion of microorganisms and subsequent biofilm formation on medical materials, which can lead to clinical infections and increased risk of sepsis in patients with indwelling medical devices. In fact, when *Pseudomonas aeruginosa* bacteria were grown in TLP-coated loops of PVC medical tubing for up to 6.5 weeks, there was an eightfold reduction in biofilm formation compared to control tubing ($P < 0.05$) (**Fig. 4g**). The TLP coating also suppressed biofouling on other materials (PET and acrylic), as indicated by significantly reduced bacterial adhesion of *P. aeruginosa* and *Escherichia coli* relative to control surfaces ($P < 0.05$; **Fig. 4h** and **Supplementary Fig. 11**); these results are similar to those obtained by infusing a roughened surface with LP using the SLIPS technology[30]. Thus, the TLP coating not only displayed antithrombogenicity, it also exhibited potent antibiofouling capabilities. Other antibiofouling surface modification methods (e.g., sulfobetaine modification) have also been shown to reduce microbial attachment and biofilm formation *in vitro*, but only over the course of 24 h[16].

DISCUSSION

The TLP coating that we described in this study can be applied to a wide range of different, commercially available, medical-grade materials to create surfaces that are both antithrombogenic and antibiofouling. TLP-coated surfaces resisted adhesion of fibrin and platelets, suppressed biofouling and reduced thrombosis when the surfaces came in contact with flowing whole human blood driven by a peristaltic pump *in vitro*. Importantly, TLP-coated AV shunts also retained their ability to prevent occlusive thrombus formation for at least 8 h *in vivo* when implanted as a flowing vascular circuit in pigs.

Sulfobetaine surface coatings have been previously shown to reduce thrombus formation when applied to medical-grade materials in the presence of slightly reduced heparin concentrations (0.7 to 1 U/ml compared to 5–7 U/ml used clinically). When a sulfobetaine-coated catheter was inserted into a canine jugular vein in the absence of anticoagulants for 4 h, clot formation was reduced on its outer surface, but the surface of the inner lumen was not studied[16]. Sulfobetaine surface coatings also have not been shown to withstand mechanical

pumping. In contrast, in our *in vivo* model, arterial blood flowed through the lumen of the surgically implanted shunt tubing under a much higher pressure and shear rate (ten times higher than in the canine venous model), and thrombosis was reduced for twice as long (8 h versus 4). In addition, in our *in vitro* studies, the TLP coating prevented coagulation in blood containing one-fourth the heparin dose used in the sulfobetaine coating study (0.25 U/ml versus 1 U/ml), and we did this in blood flow circuits driven by a peristaltic pump, which is crucial for clinical medical devices that use extracorporeal circuits (gravity flow was used in the other studies). In addition, we successfully created antithrombogenic surfaces by applying the TLP coating to 20 different, medically relevant biomaterials, whereas the sulfobetaine coating method for thrombus reduction has only been demonstrated with polyurethane[16]. Importantly, the TLP process is the first nonheparin coating to show reduced thrombogenicity *in vivo* on the inner lumen of tubing, which is most clinically relevant for use in extracorporeal circuits.

The reduction in thrombosis we obtained with the TLP-coated circuit is similar to that obtained with other heparin-based coatings in porcine and bovine cardiopulmonary bypass models, but heparin-based coatings can leach into blood and require exposure of the blood to extremely high heparin doses (>50 U/kg)[31,32]. The TLP coating more closely resembles silicone liquid thin films, which were previously used *in vitro* to delineate blood coagulation mechanisms without anticoagulants for short durations[33]. However, silicone liquid thin films do not prevent platelet adhesion or activation[34] and have not been shown to reduce thrombosis *in vivo*. Hydrophobic surfaces (e.g., ePTFE, which is a porous perfluorinated solid material) are clinically approved as vascular grafts[35]; however, these fail to improve the performance of medical devices[36] because they contain trapped air that can be thrombogenic[5].

To our knowledge, there is no other known medical material coating that can effectively suppress occlusive thrombosis *in vivo* under high pressure and high-shear arterial flow in the complete absence of heparin. Thus, as the TLP coating does not leach anticoagulant activity into the blood, it could potentially offer a new way to prevent thrombosis without the complications of heparin anticoagulant therapy.

The TP we used was covalently coupled to the surface by the same silane chemistry that is used in dental adhesives, but other reactive groups could be used to generate TP layers[25]. The LP perfluorodecalin used here has been previously included in an FDA-approved blood substitute; however, we have found that a variety of LPs, medical grade and nonmedical grade, can also repel blood when integrated into a TLP coating (**Supplementary Fig. 12**). An important caveat for future clinical application of this technology is to avoid use of LPs that evaporate at body temperature, which can induce gaseous emboli formation and considerable toxicity[37]. Additionally, it is likely that the surfaces with the TP layer will be sterilized and stored dry, and then the LP will be added to the circuit shortly before use, allowing blood to flow through these TLP-coated circuits. This is consistent with the saline priming step that is currently used clinically with extracorporeal circuits, and so it should be easily integrated into these protocols.

Another advantage of the TLP coating method is that it relies on the use of a low-pressure plasma surface modification procedure commonly used for commercial modification of materials[38]. This can be applied to virtually any material regardless of the complexity of its geometry, without altering the bulk properties of the material[39]. The treatment is also ideal for temperature-sensitive materials, as the plasma occurs at approximately ambient temperature[38], and for medical devices and implants with complex shapes because the plasma permeates tortuous paths and surface features down to the microscale[40]. This is a great advantage relative to other surface coatings, such as sulfobetaine, which uses a peroxide to activate the surface that can generate bubbles, resulting in some areas being left untreated that can become thrombogenic[41].

This TLP coating represents the first surface coating to reduce thrombosis under physiological arterial flow *in vivo* without the use of soluble anticoagulants, and in tubing that experiences peristaltic pumping *ex vivo* using reduced levels of anticoagulation (0.25 U/ml heparin). Thus, it could be employed to coat materials used for various types of extracorporeal circuits, and it might potentially be valuable for coating indwelling devices, including total artificial hearts and ventricular assist devices, as well as needles, Vacutainers, sutures and blood storage bags. Importantly, although there are commercially available surface-coating technologies that partially reduce either blood thrombosis or bacterial adhesion, the TLP coating prevents both. Because the TLP coating technology also prevents biofouling, it opens up the possibility of creating a new class of medical materials and devices lined by antithrombogenic and antibiofouling surfaces that do not require co-administration of antiplatelet, anticoagulant or antibiotic medications when implanted in patients. This would reduce the need for systemic heparinization and antibiotic drug treatments to prevent related morbidity and fatalities, which would greatly decrease healthcare costs.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

D.C.L., A.W., J.B.B., T.M.V., A.L.W., A.J., M.A., M.S., J.A. and D.E.I. designed the research. D.C.L., A.W., J.B.B., T.M.V., A.L.W., A.J., P.K., B.D.H., E.H.S., D.E.B. and S.R. performed experiments. D.C.L., A.W., J.B.B., T.M.V., A.L.W., A.J., E.H.S., M.A., M.S., J.A. and D.E.I. analyzed data. C.H., C.P.J., T.L.V., M.A. and J.A. designed, performed and analyzed the PFD leaching study. D.C.L., A.W., J.B.B., A.N., K.D., D.E.B., A.R.H., M.S. and D.E.I. designed, performed and analyzed the *in vivo* study. D.C.L., A.W., A.L.W., M.A., M.S., J.A. and D.E.I. wrote the paper with input from all authors.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Modification of surfaces with TP. Samples were briefly exposed (40 s, unless stated otherwise) to low-pressure (150 to 250 mTorr) radio-frequency (13.56 MHz) oxygen plasma at 100 Watts in order to gently activate the surface to react with the TP silane in a PE-100 plasma system (PlasmaEtch). Immediately following plasma activation, samples were immersed in a liquid silane solution (5% v/v tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane (Gelest, Morrisville, PA) in anhydrous ethanol (Sigma, St. Louis, MO)) for 1 h at room temperature. Treated samples were rinsed with anhydrous ethanol (Sigma, St. Louis, MO), distilled, deionized water (Milli-Q Water Purification System, Millipore), and three times with pure ethanol (Koptec, King of Prussia, PA). Rinsed samples were gently blown dry with compressed nitrogen and gently heated in an oven with desiccant at 60 °C overnight at atmospheric pressure. Medical-grade cardiopulmonary perfusion tubing (Sorin Group, Arvada, CO) was exposed to oxygen plasma for 3 min; 8 French (Fr) pediatric arterial cannulae (polyurethane and polycarbonate connectors) (Bio-Medicus, Medtronic, Minneapolis, MN), monitoring lines (Smiths Medical, St. Paul, MN) and four-way stopcocks (Smiths Medical, St. Paul, MN) were exposed to oxygen plasma for 2 min. Other materials treated with oxygen plasma for 40 s were: poly (methyl methacrylate) (PMMA), polysulfone (PSU), polypropylene (PP), polytetrafluoroethylene (PTFE), polyethylene terephthalate (PET), ultra-high molecular weight polyethylene (UHMW PE), polycarbonate (Goodfellow, Coraopolis, PA), polystyrene (BD Biosciences, Durham, NC), PVC, fluorinated ethylene propylene (FEP), perfluoroalkoxy (PFA), titanium, polyimide, stainless steel (McMaster-Carr, Robbinsville, NJ), glass cover slip (VWR, Radnor, PA), polydimethylsiloxane (Dow Corning, Midland, MI), Aeos HyFlex expanded polytetrafluoroethylene (ePTFE) (Zeus), silicon wafer (Ted Pella, Redding, CA). Atomic force microscopy revealed minimal change in surface roughness after TP coating (3.4 ± 1 nm) compared to control acrylic (2.0 ± 0.2 nm) (mean ± s.d., $n = 3$).

Sliding angle of surfaces. The angle at which a droplet of liquid begins to move across a surface (sliding angle) was measured for the samples using a manual goniometer (Thor Labs GN05/M). Samples were dip-coated in perfluorodecalin (PFD) (FluoroMed, APF-140HP (sterile, high purity), Round Rock, TX) immediately before measurement and the sample was placed on top of the leveled goniometer. The amount of PFD on the surface was 4–6 µl/cm^2 as measured by an analytical balance after dip coating. For tilting at 30 degrees, 200 µl of citrated whole human blood (see Human blood samples[11]) was placed gently onto surfaces in ~50 µl droplets. For quantification of sliding angle, a 5-µl droplet of citrated whole human blood was gently placed on the surface. The sample was then tilted until the droplet was observed sliding along the surface. For samples that did not slide by 15 degrees, a custom built setup smoothly tilted the sample to 90 degrees. Samples still adherent at 90 degrees were noted as a sliding angle of 90 degrees. Sliding angle measurements were obtained on TLP acrylic with alternate LPs: perfluorohexane (PFH, Sigma), perfluorooctane (PFO, Sigma), 1-bromoperfluorooctane (PFOB, Oakwood Products), perfluoroperhydrophenanthrene (Vitreon, FluoroMed APF-215HP (sterile, high purity)), perfluorotripentylamine (FC-70, HamptonResearch), perfluorotributylamine/perfluorodibutylmethylamine (FC-40, Santa Cruz Biotechnology), 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethyl-hexane (HFE-7500, 3M), perfluoropolyether oils (Krytox 101, Krytox 103, food grade Krytox FG-40, Dupont).

Elemental analysis methods. X-ray photoelectron spectroscopy (XPS) was performed on a Thermo Scientific K-Alpha X-Ray Photoelectron Spectrometer (Thermo Scientific). Samples were prepared with TP treatment as described above. PVC tubing was stored under vacuum before XPS analysis to accelerate outgassing of plasticizers. Auto-Analysis scans (XPS displacement tolerance of 8eV, Auger displacement tolerance of 12eV) were performed and analyzed using the Thermo Scientific Avantage Data System v5.915 (Thermo Scientific). Three TP samples and one control sample were tested. Three points were selected on each sample with a spot size of 30 µm. Five survey scans (binding energy range from −4 to 1350 eV) were averaged at each point to identify potential elements on the sample surface. Based on the potential elements identified during the survey scans, higher resolution individual elemental analysis scans were then performed for each elemental range and averaged over 10 scans. XPS confirmed the highly fluorinated surface layer (TP) on acrylic, polycarbonate, polysulfone and PVC tubing (**Supplementary Table 1**).

Gecko adhesion. Study approval was obtained from Boston Children's Hospital Institutional Animal Care and Use Committee (protocol number 13-10-2552). One eyelash-crested (*Rhacodactylus ciliatus*) gecko was placed inside an acrylic cylinder and tilted slowly from horizontal to vertical. This was repeated with the gecko inside a TLP-treated acrylic cylinder and the tilting was stopped when the gecko slid to the bottom of the cylinder. This was repeated a total of three times with the gecko allowed to recuperate for 1 week between experiments. Videos are representative of the three trials with the same results.

Surfaces under shear. Surfaces were exposed to shear using a rheometer (TA Instruments, Model AR-G2) with a 40-mm diameter, 2-degree angle cone-and-plate setup (TA Instruments, Model 513406.905) and Peltier plate (TA Instruments, Model 531051.901). Acrylic sheets were cut into 40-mm diameter discs using a laser cutter (Epilog Legend 36EXT). These discs were then aligned with the cone platen, and stuck to the bottom Peltier plate platen with adhesive (3M, St. Paul, MN). The samples were then lubricated with just enough PFD to cover the surface of the acrylic disc (~500–600 µl of PFD). Approximately 2 ml of 35% v/v glycerol (Sigma, St. Louis, MO) in water was applied on top of the lubricated acrylic disc. The cone platen was then lowered 50 µm above the acrylic disc, and the excess PFD and glycerol was then pushed out from between the platens. This excess fluid was removed to allow a meniscus of glycerol solution to form between the two platens. Solvent rings were then placed around the setup to minimize evaporation of the liquids. For studies with shear rates below 500 s^{-1}, a 10-min conditioning step was performed at 500 s^{-1} to ensure the excess PFD was removed.

Scanning electron microscopy. Samples that had been in contact with blood or bacteria were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (Electron Microscopy Sciences, Hatfield, PA) for 1 h, 1% osmium tetroxide in 0.1 M sodium cacodylate (Electron Microscopy Sciences, Hatfield, PA) for 1 h, dehydrated in ascending grades of ethanol, and chemically dried with hexamethydisilazane (Electron Microscopy Sciences, Hatfield, PA) in a desiccator overnight before being mounted and sputter-coated with a thin layer of gold/palladium and imaged on a Zeiss Supra55VP microscope.

Human blood samples. Approval for studies involving human subjects was obtained from Harvard University Faculty of Medicine Committee on Human Studies (protocol number M20403-101). Whole human blood was obtained from healthy volunteers with informed consent who were nonsmokers and had not taken aspirin for the 2 weeks before donation. Blood was drawn by standard venipuncture into no additive Vacutainers (Becton Dickenson). A discard tube was drawn first, then heparin (1,000 U/ml) (APP Pharmaceuticals, Schaumburg, IL) was added to subsequent tubes to a final concentration dependent on the assay. Assays were based on ISO-10993-4 for evaluation of medical devices[42].

Whole blood adhesion assay. Wells of a 24-well plate were blocked with 1% (w/v) bovine serum albumin (BSA) (Sigma A3803, St. Louis, MO) in saline for 30 min and rinsed with saline. Samples (11 × 8 mm) of 100-µm thick polysulfone or 1/16″-thick acrylic were control, TP or dip coated in PFD to generate TLP. Samples were incubated in blocked wells with heparinized whole human blood (0.25 U/ml to prevent immediate clotting while retaining the ability of blood components to be activated by surfaces). Sample order was randomized for incubation with blood. Fluorescently labeled fibrinogen (150 µg/ml, Invitrogen, Carlsbad, CA, 90% clottable fraction) was added to the blood, which was then incubated with samples for increasing time points on an orbital shaker. D-dimer concentration was measured in blood from one donor by enzyme-linked immunosorbent assay (ELISA) after 60 min (Sekisui Diagnostics, LLC, Stamford, CT) according to the manufacturer's instructions. Acrylic and polysulfone samples were washed three times in normal saline (0.9% sodium chloride; Baxter Healthcare, Cambridge, MA) and fixed for 1 h with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer before imaging with a Hamamatsu 9100-02 EMCCD camera on a Zeiss Axio Observer Z1 fluorescent microscope using a 20× objective and Metamorph software.

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Quantification was carried out using ImageJ (http://imagej.nih.gov/ij/) on eight unadjusted images from each sample. The images from each experiment were converted to binary images and generated into a stack. The threshold was manually set to 6 for all images, and the area covered by fibrin was extracted from the measurements. A maximum fibrin clot area of 60–70% was quantified due to the fibrillar nature of the thrombus on the surface and imaging on the surface focal plane. Each experiment was done with two replicates per donor and repeated with three donors. Representative images in **Figure 2a** were converted to green and brightness and contrast was uniformly adjusted to all for clarity. Samples from the 30-min time point were processed for SEM as described above. Scanning electron micrographs from four areas per sample were obtained, and a blinded researcher counted the number of adhered platelets. Nonactivated platelets were differentiated from activated platelets by their round or dendritic morphology, as defined by Goodman[43]. Nonactivated platelets were subsequently counted, identified by rounded morphology with zero flattened protrusions. The number of nonactivated platelets was divided by total adherent platelets per field of view, and multiplied by 100% to obtain the percent activated platelet value.

Blood flow in small-diameter medical-grade tubing. TP monitoring lines (1/16″ ID by 48″ length) were primed with 3 ml of PFD. Control and TLP tubing were subsequently primed with saline. Human heparinized whole blood (0.25 U/ml) at room temperature was drawn through the monitoring line at a flow rate of 60 ml/h by a syringe pump (Harvard Apparatus, Holliston, MA) by withdrawal. This flow rate corresponds to a shear strain rate of 40 s^{-1} and was maintained over 20 min. Blood was collected into a 20-ml syringe (Becton Dickinson, Franklin Lakes, NJ) containing a 1:9 volume of 3.8% sodium citrate (Ricca Chemical Company, Arlington, TX). Tubing was rinsed with 20 ml normal saline at a flow rate of 60 ml/h. Blood samples were collected into EDTA vacutainers (Becton Dickinson, Franklin Lakes, NJ) for 18-parameter complete blood count using the VetScan HM2 Hematology System (Abaxis, Union City, CA). Tubing was incubated with buffer composed of 0.04 U/ml plasmin (Hematologic Technologies Inc., Essex Junction, VT), normal saline, 6.25 mM $CaCl_2$, and 4.68 mM $MgCl_2$ for 1 h at 37 °C. Samples were then centrifuged at 200 g for 10 min to sediment red blood cells, and the total protein concentration was measured in the supernatant by a BCA protein assay following manufacturer's protocol (Thermo Fisher Scientific, Waltham, MA). The mass of protein was then divided by the area of the inner lumen of the tubing (60.8 cm^2) to determine the protein adsorbed/cm^2. Further, we measured adsorption of physiological levels of bovine serum albumin (50 mg/ml) to test the hypothesis that this assay reflects the adhesion of abundant plasma proteins that are less relevant to thrombus formation. We detected very small amounts of albumin bound to the surface (0.035 ± 0.002 μg/cm^2) that were barely above background levels. Each paired (TLP versus control) experiment was done with three donors.

Blood flow in microfluidic channels. TP-treated and control microfluidic PDMS microdevices with microchannels 200 μm wide by 75 μm tall were primed with LP. Human heparinized whole blood (0.5 U/ml) was flowed in the constant pressure mode with a calculated initial wall shear rate of 1,250 s^{-1} (50 dynes/cm^2) by means of a syringe pump (PHD Ultra CP, Harvard Apparatus) with disposable pressure sensor (PendoTECH). Clotting times were derived by finding the time for flow to reduce to half its initial value, from a sigmoidal decay fitted curve (n = 3 donors). Because the microfluidic device required more extensive time for setup, we used a slightly higher heparin dose to prevent coagulation before TLP exposure before the analysis was carried out. This dose (0.25 U/ml) was still very low in comparison to that commonly used in standard *in vitro* coagulation assays (5 to 15 U/ml).

Blood flow in large diameter medical-grade tubing. TLP PVC tubing (1/4″ ID by 15″ length) was primed with 10 ml of PFD. Control and TLP tubing were subsequently washed with saline. PVC tubing was connected with control or TLP 1/4″ ID polycarbonate barbed connectors and filled with human heparinized whole blood (0.25 U/ml). Blood was pumped at a flow rate of 3 L/h (shear rate of 250 s^{-1}) by a peristaltic pump (Cole Parmer, MasterFlex L/S, Vernon Hills, IL) for 2 h. Blood was filtered through a pre-weighed 40 μm cell strainer and air-dried before thrombus weight was obtained. Blood was collected into EDTA Vacutainers for CBC analysis at the time of venipuncture, and again after being pumped through the tubing and filtered through the cell strainer. Reported values of the percentage of platelets remaining in blood were calculated as the platelets in the sample collected after pumping divided by the platelets in the sample collected before tubing multiplied by 100%. Each experiment was done with three donors.

Porcine arteriovenous shunt model. Study approval was obtained from Boston Children's Hospital Institutional Animal Care and Use Committee (protocol number 12-06-2202) and conducted in an AAALAC-accredited USDA registered facility. A total of 15 female Yorkshire swine weighing 24–35 kg (3–4 months old) were used in this study. Animals were randomly assigned to control or TLP groups (no blinding was performed as this was not possible due to the application of LP immediately before implantation of the shunt). No power analysis was performed to determine sample size. One group of animals received 30 U/kg heparin at the time of circuit placement (n = 3 control and n = 2 TLP). One animal in the TLP group was excluded as the TLP treatment was performed outside the prespecified parameters specified above; the pressure gauge on the plasma system was found to be out of calibration. The second group received no heparin (n = 5 control and n = 4 TLP). The control group received unmodified extracorporeal materials and the TLP group received an extracorporeal circuit in which all the materials had been treated with TP, sterilized with ethylene oxide and lubricated with 10 ml sterile LP (as received from Fluoromed) and drained within 10 min before implantation. The amount of PFD on the circuits at implantation was 0.8–1.2 g.

Animals were anesthetized with intramuscular injections of atropine (0.04 mg/kg), telazol (4.4 mg/kg) and xyalzine (2.2 mg/kg) and maintained on isoflurane (1.5–2.5%) and oxygen (1.2 liter/min) delivered through a 7-mm endotracheal tube using a positive pressure ventilator. The animal was placed in the supine position and a 6 Fr percutaneous sheath catheter was placed in the left femoral artery for pressure monitoring. A 20-g intravenous cannula was placed in the left marginal ear vein for administration of drugs and a 10 Fr Foley catheter was placed for urinary drainage. The AV shunt was established via cutdown of the right femoral artery and vein and insertion of 8 Fr pediatric arterial cannulae (Bio-Medicus, Medtronic, Minneapolis, MN). The cannulae were connected to the extracorporeal circuit, which consisted of 25″ of ¼″ ID perfusion tubing (Sorin Group) with a 1/4″ barbed connector (Sorin Group) and large bore four-way stopcock (with rotating male luer lock, Baxter) placed 3″ from the venous cannula. The perfusion tubing was filled with 1 U/ml heparinized saline during placement, which was fully drained before establishing circuit flow. Shunt implantation time was greater than two times the activated clotting time. The heart rate, pulse rate, arterial pressure, oxygen saturation, CO_2 level, temperature and respiratory rate were monitored throughout the experiment. Vitals were maintained at physiological levels and body temperature was maintained at 37 °C by means of a heat mat. After 8 h, animals were given 300 U/kg heparin and euthanized with an intravascular lethal dose of Fatal Plus.

***In vivo* blood sampling and flow measurements.** At baseline (before AV cannulation), time 0 h (immediately after arteriovenous circuit flow was established), time 3.5 h, and time 7.5 h, blood samples (20 ml) were taken for complete blood count (CBC), blood gas and chemistry profiles, and clotting times (PTT, APTT, ACT) (EDTA, heparin and citrate Vacutainers, respectively). Flow was measured in the midpoint of the perfusion tubing using a clamp-on tubing flow sensor connected to a TS410 flow meter module (Transonic, Ithaca, NY) for 15 min after circuit flow was established and every 30 min thereafter for ~15 min each time. Occlusion was measured post-explant by taking photographs of cross-sections of the cannulae and tubing and digitally determining the area of the lumen and area of the thrombus to calculate the percent occlusion.

Histology. Organ samples (lung, liver, kidney, spleen and brain) were fixed in 10% neutral buffered formalin (Electron Microscopy Sciences, Hatfield, PA) for 24 h at room temperature. Tissue was processed and stained with hematoxylin and eosin (H&E) at Boston Children's Hospital Histopathology services. No evidence of thrombi or microemboli was found in either control or TLP lung sections.

Gas chromatography/mass spectrometry (GC/MS). Blood samples (2 ml) were extracted with methyl nonafluoroisobutylether (HFE) (Miller-Stephenson) and analyzed by gas chromatography/mass spectrometry[44]. The limit of detection was determined by multiplying the s.d. of the baseline response by 3; this was converted into a clinical dose by multiplying the limit of detection (LOD) by 60 ml of blood/kg of body weight.

Biofouling assay. Control and TP samples of acrylic (11 × 8 mm, 1/16″ thick) were sterilized with ethanol (pure ethanol, 200 proof) and allowed to air dry in a biological laminar flow hood for 30 min. Samples were transferred to 24-well plate wells and sterile LP was added to TP samples. LP was removed and all samples were immediately incubated with 10^5 CFU of *E. coli* (ATCC 8739) in RPMI (Invitrogen, Carlsbad, CA) for 48 h at 37 °C. After culture, samples were washed three times in PBS and assayed for biofilm formation using crystal violet (Becton Dickenson, Sparks, MD). Samples were incubated with 0.1% crystal violet for 1 h and washed six times with distilled water. The crystal violet was solubilized with 10% acetic acid for 10 min before 100 µl was transferred to a 96-well plate and the absorbance measured using a plate reader at 590 nm. The inoculum and cultures of the control and TLP samples after 48 h were confirmed to be viable by plating.

PE and PET samples (1″ × 1″) were incubated with *P. aeruginosa* (a clinical isolate from Brigham and Women's Specimen Bank (protocol number M20403-101)) overnight. Samples were washed in PBS, stained with crystal violet for 15 min, washed with distilled water three times and photographed or fixed for SEM. Bacterial adhesion under continuous flow was tested using a modified Chandler loop setup. The *P. aeruginosa* bacteria were cultured in RPMI Media (Life Technologies, Carlsbad, CA) at 37 °C. Control and TLP loops (3-mm inner diameter) were filled with *P. aeruginosa* cultures (10^5 CFU/ml) and incubated at 25 °C. 1.5 weeks and 6.5 weeks after initial inoculation, two 1-cm segments of tubing were assayed for biofilm formation. To measure bacterial adhesion on the tubing surface we used a novel FcMBL fusion protein[45]. Briefly, the carbohydrate recognition domain of Mannose Binding Lectin (MBL) was fused to the Fc domain of human IgG and recombinantly expressed in Chinese hamster ovary cell lines. FcMBL was conjugated with horseradish peroxidase (FcMBL-HRP) using the Lightning Link-HRP Antibody Labeling Kit (Novus Biologicals, Littleton, CO) and used as a detection antibody for ELISA-based detection of *P. aeruginosa* on tubing segments. Tubing was washed in Tris-buffered saline, 0.1% Tween 20 (TBS-T) (Boston BioProducts, Ashland, MA) supplemented with 5 mM $CaCl_2$ (Boston BioProducts, Ashland, MA), followed by incubation with FcMBL-HRP in 3% BSA in TBS-T 5 mM $CaCl_2$. The colorimetric reaction was done with the Pierce 1-Step TMB substrate (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol, and absorbance at 450 nm was measured. Bacterial titers were established by comparison to a standard curve of *P. aeruginosa*. The culture removed from the loops after 6.5 weeks was confirmed to be viable by plating.

Statistical analysis. Data are expressed as mean ± s.d. for **Figures 1** and **3** and mean ± s.e.m. for all other data. *In vitro* assay sample size was predetermined with three separate donors to account for biological variability. The *in vivo* patency data are shown as a Kaplan-Meier curve (**Fig. 4c**). Data were statistically analyzed by paired, two-tailed student's *t*-test (**Fig. 3b**), unpaired, two-tailed student's *t*-test (**Fig. 3d**,**e** and **Supplementary Figs. 3**,**7**,**11**), Fisher's exact test (**Fig. 4c**), one-way analysis of variance (ANOVA) (**Fig. 2d**,**e** and **Supplementary Fig. 4**) and two-way analysis of variance (ANOVA) with Bonferroni post-hoc analysis for multiple comparison (**Fig. 2b**,**c** and **4e**–**g**). $P < 0.05$ was considered significant (as indicated in the figures by an asterisk), although caution is necessary due to the small sample sizes and therefore the normality assumption cannot be tested. Paired analysis was only used for experiments that used blood from a single venipuncture on both control and experimental surfaces. Prism version 6.00 (GraphPad Software) was used for statistical analysis.

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