DYNAMIC WHOLE BLOOD STUDY OF SILICONE MODIFIED WITH PEO-SILANE AMPHIPHILES

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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Blood-contacting medical devices rapidly adsorb plasma proteins that initiate clot (i.e. thrombus) formation. Antithrombotic drugs may reduce clotting and associated device dysfunction and ischemia, but put the patient at risk for hemorrhaging. Silicone, a common blood-contacting device material is highly prone to protein adsorption and subsequent clotting due to its extreme hydrophobicity. Poly(ethylene oxide) (PEO), a hydrophilic polymer, is highly protein resistant but its function when incorporated into silicone depends critically on its presence at the silicone-water interface. To enable rapid and substantial migration of PEO to the silicone surface, a PEO-silane amphiphile [α-$(EtO)_3Si(CH_2)_2$-$ODMS_{13}$-*block*-PEO_8-OCH_3] was prepared that bears the ability to substantially reduce fibrinogen adsorption on silicone, even at low concentrations. This work comprehensively evaluated the thromboresistance of a silicone modified with the PEO-silane amphiphile via its exposure to whole blood under dynamic conditions using a Chandler Loop. Clotting was evaluated in terms of occlusion time and thrombus formation for silicones modified with varying levels of the PEO-silane amphiphile. Results demonstrated that concentrations as low as 10 μmol of amphiphile per gram of silicone were able to significantly reduce platelet adhesion and prevent occlusion during the course of the study. This indicates that

the amphiphiles are useful in preventing protein adhesion and obviating clot formation, increasing the safety and lifetime of implantable blood-contacting devices.

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SECTION I

INTRODUCTION

Preventing thrombosis via PEO-additives

Blood-contacting devices such as hemodialysis catheters must be thromboresistant, or capable of preventing clotting, in order to preserve patency. Silicone is commonly used to prepare catheters based on its favorable mechanical properties.[1] Unfortunately, as a result of its hydrophobicity, silicone has a high affinity for protein adsorption making it very susceptible to thrombosis.[2] Consequently, patients utilizing silicone catheters or other medical devices composed of or coated with silicone must be placed on anti-thrombotic drugs to prevent clot formation. These drugs not only lead to an increased risk of bleeding complications, but also have demonstrated limited efficacy.[2] A silicone material or coating that is inherently resistant to thrombosis could improve the safety of implantable devices and obviate the need for anti-thrombotics.

Coatings made from poly(ethylene oxide) (PEO), a hydrophilic polymer, have shown the potential to resist protein adsorption by steric repulsion[3] and blockage of adsorption sites.[4] Molecular simulations have also shown that PEO chains strongly associate with water via hydrogen bonding, creating a repulsive "hydration layer" that physically excludes macromolecules.[4] While PEO's protein resistance has been seen on materials with physically stable surfaces (e.g. silica or gold), grafted coatings on polymers have largely failed to prevent protein adsorption.[5-7] This is likely due to PEO's tendency to enter to the bulk of the polymer, particularly after exposure to air.[8, 9] Therefore, in order to optimize PEO's protein resistance, PEO must be able to spontaneously rise to the water-surface interface from the polymer matrix.

In previous studies, a (PEO)-silane amphiphile (**Figure 1**) was prepared[10] to improve the thromboresistance of silicones by enhancing PEO migration to the surface. Unlike traditional non-amphiphilic PEO-silanes, this amphiphile includes a siloxane tether in order to enhance mobility of PEO within the silicone network and to the water-surface interface. When silicone was bulk-modified with this amphiphile and exposed to water, a remarkable increase in surface hydrophilicity and protein (fibrinogen) resistance was observed. This implies that the modified silicone has the potential to prevent clot formation. In this study, the thromboresistance of a silicone modified with the PEO-silane amphiphile was evaluated with a dynamic whole blood adhesion study using a Chandler Loop.

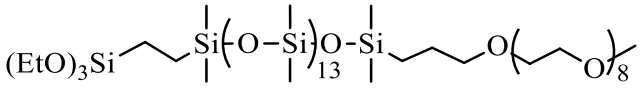

Figure 1: PEO-silane amphiphile.

Measuring coagulation

Model framework

Current methods of evaluating thromboresistance include measuring the partial thromboplastin time,[11-13] platelet adhesion,[12, 14-17] thrombin generation,[13, 15, 18] protein (e.g. fibrinogen) adsorption,[16, 19] and split products resulting from the coagulation cascade.[20] However, each poses serious limitations. While its results are discriminative among similar materials, fibrinogen adsorption is incomplete in comparison to thrombin generation, as it only measures one of the clotting proteins found in blood[13] and does not take the effect of protein conformation into account.[21] It has also been found that partial thromboplastin time is not a reliable test method, particularly when comparing materials of similar hemocompatibility.[13, 22] When evaluating

thromboresistance, researchers will often utilize two or more methods, where at least one is quantitative. A thrombin generation-based or platelet adhesion-based test that is consistent as well as discriminatory can act as the primary quantitative method in whole blood studies. Platelet adhesion is more commonly tested as it can be measured on the surface of the material as well as in solution. Static blood tests have been used for measuring platelet adhesion, but cannot accurately simulate thrombosis *in vivo*, which occurs with blood flow.[23, 24] Dynamic tests better mimic the circulatory system environment, making them desirable for predicting efficacy *in vivo*.[24]

Mechanical simulation

Constructs for dynamic blood tests include parallel plate[25, 26] as well as bioreactor flows.[27] A relatively new construct is the Chandler Loop (**Figure 2**), wherein a tube shaped into a loop is partially filled with blood that flows continuously while the loop is rotated. Once the blood is removed, the entirety of the tube's inner surface can be analyzed. This system, as a dynamic environment, better imitates the circulatory system relative to static conditions. With this setup, flow can be carefully tuned to match physiological shear rates and avoid turbulent flow.[28-31] Already in use for detecting thrombosis in stents,[32, 33] the Chandler Loop has also recently been demonstrated as effective for evaluating silicone systems.[34] It can thus be expected to provide results that are more representative of *in vivo* performance than static blood tests and be a viable construct for quantitative platelet adhesion tests.

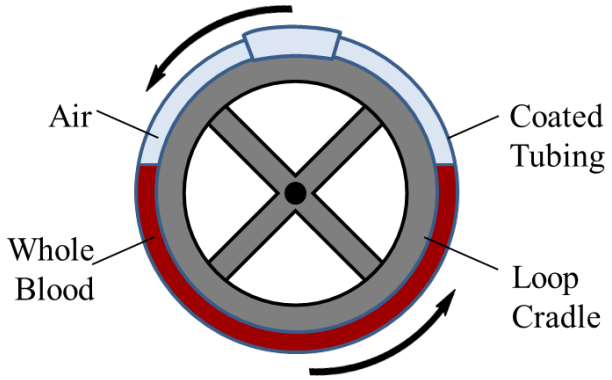

Figure 2: Chandler Loop

Data collection

The adherence of platelets or proteins on materials can be compared qualitatively via imaging with SEM[12, 35, 36] or confocal microscopy.[14, 37] For quantitative analysis, flow cytometry[28, 38, 39] as well as ELISA[28] and a large variety of colorimetric and fluorescence assays (e.g. for Factor X[27] or LDH[19]) may be used. Flow cytometry, in which platelets are counted as they pass between a laser and an electrical detector, reports platelet concentration with high precision but requires the platelets to be in solution.[40] A more direct approach could measure adhered platelets by measuring the concentration of proteins released into solution after membrane lysis. Lactate dehydrogenase (LDH) is expressed in nearly all living cells, as it catalyzes the conversion of lactate to pyruvate. A colorimetric LDH assay could be used as a simple and reliable method to quantify adhered platelets on a material surface.[19, 27]

SECTION II

METHODS

Materials

Vinyltriethoxysilane (VTEOS) and α,ω-bis-(SiH)oligodimethylsiloxane [$ODMS_{13}$; M_n = 1000–1100 g/mol per manufacturer's specifications; M_n = 1096 g/mol per ^{1}H NMR end group analysis; ^{1}H NMR (δ, ppm): 0.05–0.10 (m, 78H, SiCH_3), 0.19 (d, J = 2.7 Hz, 12H, OSi[CH_3]$_2$H) and 4.67–4.73 (m, 2H, Si*H*)], and Pt-divinyltetramethyldisiloxane complex (Karstedt's catalyst) in xylene were purchased from Gelest. Allyl methyl PEO [Polyglykol AM 450, M_n = 292-644 g/mol per manufacturer's specifications; M_n = 424 g/mol per ^{1}H NMR end group analysis; ^{1}H NMR (δ, ppm): 3.35 (s, 3H, OCH_3), 3.51–3.66 (m, 32H, OCH_2CH_2), 4.00 (d, J = 5.4 Hz, 2H, CH_2=CHCH_2O), 5.13–5.28 (m, 2H, CH_2=$CHCH_2O$) and 5.82–5.96 (m, 1H, CH_2=C*H*CH_2O)] from Clariant was utilized. RTV medical-grade silicone (MED-1137) was purchased from NuSil Technology (Carpinteria, CA), composed of α,ω-bis-(Si-OH)polydimethylsiloxane, silica (11-21%), methyltriacetoxysilane (<5%), ethyltriacetoxysilane (<5%), and trace amounts of acetic acid. Phosphate-buffered saline (PBS, without calcium and magnesium, pH = 7.4) and a Pierce™ LDH Cytotoxicity Assay Kit were purchased from Fisher. $RhCl(Ph_3P)_3$ (Wilkinson's catalyst) was obtained from Sigma-Aldrich. Organic solvents used were purchased from Sigma-Aldrich and were dried over 4 Å molecular sieves prior to use.

Chandler Loop design and construction

First, a stepper motor (Nema 17 – 12 V, 37 oz-in) paired with a driver (GE StepStick DRV8825) was combined with a voltage supply controller, consisting of an LCD Shield (SainSmart 1602)

user input interface linked to a microcontroller board (Arduino UNO R3). The controller was programmed to allow shaft rotation from 0 to 35 rpm in 5-rpm increments. A loop cradle was designed in SolidWorks and 3D printed to fit around the shaft of the stepper motor and extend radially outward. The entire apparatus (**Figure 3**) was secured by attaching the step motor to an L-shaped base that could then be bolted to a table.

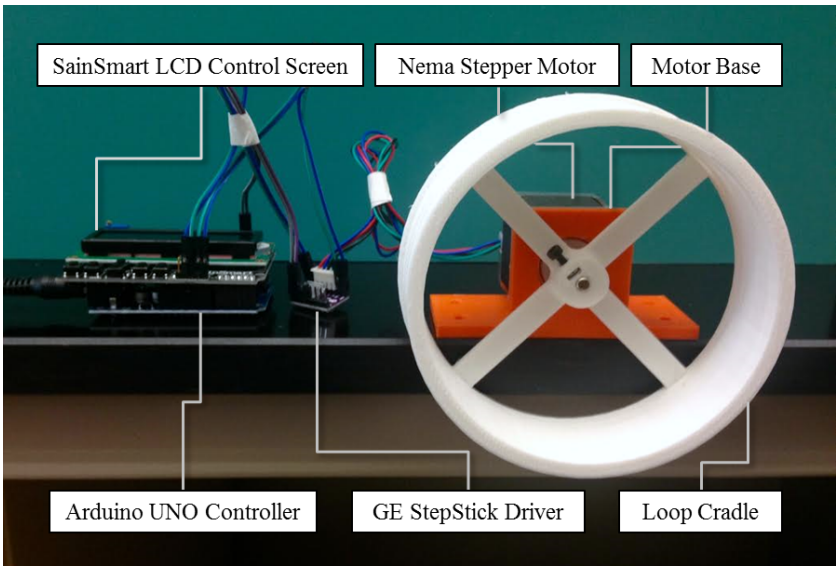

Figure 3: Finalized Chandler Loop construct.

Test material preparation

PEO-silane amphiphile synthesis

The PEO-silane amphiphile was synthesized according to the procedure described by Murthy, et al.[41] A triethoxysilane crosslinking group was first attached to one end of an oligodimethylsiloxane tether by performing a regioselective hydrosilylation reaction with VTEOS and $ODMS_{13}$ for 16 hours using the Wilkinson's catalyst. A Pt-catalyzed (Karstedt's)

hydrosilylation reaction was then performed for 8 hours between the product and allyl PEO monomethyl ether of length n = 8, yielding the final amphiphile (**Figure 4**).

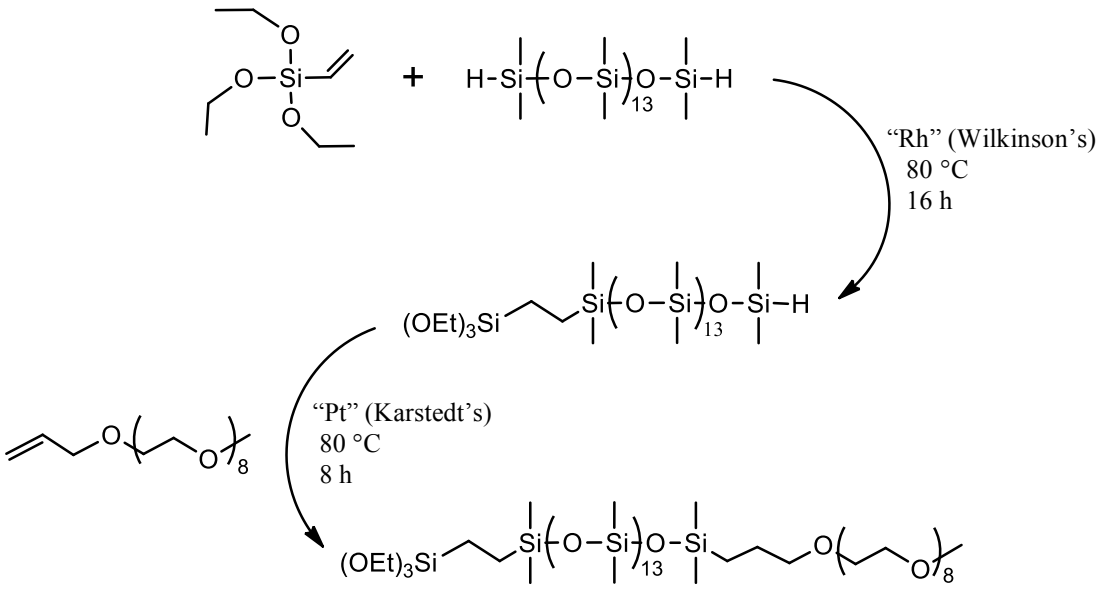

Figure 4: Synthesis of PEO-silane amphiphiles.

Modification of elastomer and application to tubing

The PEO-silane amphiphile was added to Nusil MED-1137 RTV silicone at 5, 10, and 50 μmol per gram silicone. The mixtures were each diluted to 25 wt% in ethyl acetate. Poly(ethylene vinyl acetate) (EVA) tubing (1/8 in inner diameter, 40 cm length, McMaster-Carr) was exposed to oxygen plasma (time = 180 sec; Harrick Plasma PDC-001) to provide surface hydroxyl groups for improved adhesion of the modified silicone coating (i.e. those containing the PEO-silane amphiphile) and the unmodified silicone coating. For a given solvent-based mixture, 2.5 mL was poured into the EVA tubing and rotated at 5 rpm on the Chandler Loop for 30 minutes. Excess mixture was drained and the coated tubing was allowed to cure overnight. This coating process

was repeated a second time. Finally, the tubing was rinsed once with 10 mL DI H_2O to remove trace amounts of ethyl acetate.

Data collection

Long-term occlusion study

Citrated bovine blood was obtained from the Texas A&M Vet Med Park and used within 30 minutes.[42] Recalcified blood (1.6 mL, blood:0.1 M $CaCl_2$ = 10:1, v/v) was poured into each coated tube and the blood-filled tubing was allowed to rotate in the Chandler Loop at 15 rpm. These conditions were determined to correspond to a strain rate of 272.5 s^{-1}.[31] Rotation was continued for a maximum of 24 hours or until clotting arrested blood flow.

Platelet adhesion testing

Citrated bovine blood was obtained from Texas A&M Vet Med Park and used within 30 minutes.[42] Recalcified blood (1.6 mL, blood:0.1 M $CaCl_2$ = 10:1, v/v) was poured into unused coated tubing and the blood-filled tube rotated in the Chandler Loop at 15 rpm for 15 minutes.[31] Excess blood was removed, and the tubing was gently rinsed with 10 mL PBS to remove unbound cells.

LDH assay

A LDH cytotoxicity assay was performed per standard protocols.[17] Three 2-cm sections of tubing were obtained from those used for platelet adhesion testing. Cells in these sections were lysed with 75 µL 0.7-1.0% poly(oxy-1,2-ethanediyl) for 45 minutes at 37 °C. From each section of tubing, 50 µL was then placed into an individual well in a 96-well microplate and 50 µL of the kit's reaction mixture was added for 30 minutes before adding the kit's stop solution. The absorbance of reaction products was measured with a microplate reader (Tecan Infinite® M200

PRO). The kit's LDH positive control assay was used with bovine serum albumin (BSA) to develop a standard curve to determine protein concentration in the solution.

SECTION III

RESULTS

The thromboresistance of silicones modified with varying levels of the PEO-silane amphiphile (5, 10, and 50 µmol per gram silicone) were compared to that of unmodified silicone using a Chandler Loop. First, the time for occlusion to occur was assessed (**Figure 5**).

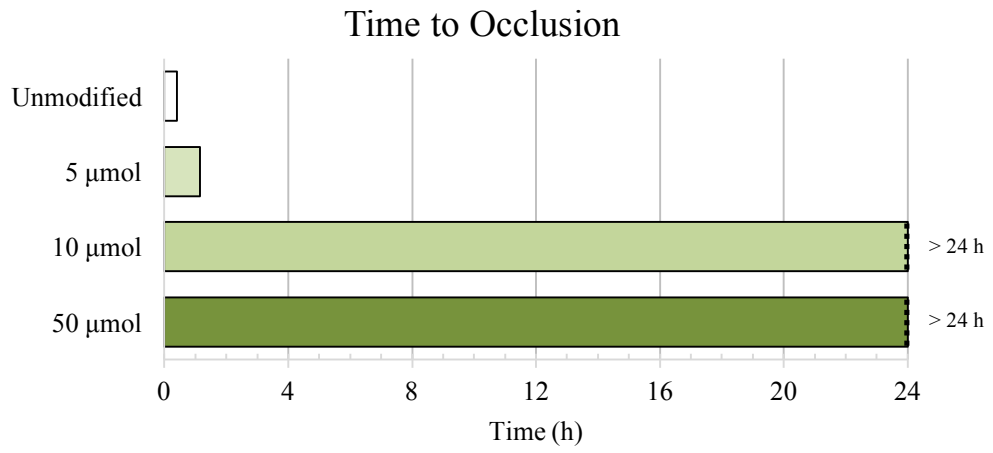

Figure 5: Loop occlusion times for variable PEO-silane amphiphile concentration in silicone.

The unmodified silicone coating was the least efficient, with an occlusion time of only 24.0 minutes. For the silicone modified with only 5 µmol (0.86 wt%) of the amphiphile, occlusion time was modestly increased to 68.5 minutes. Upon increasing the amphiphile concentration to 10 µmol (1.7 wt%) and 50 µmol (8.5 wt%), occlusion did not occur after 24 hours.

In order to understand the occlusion time results, protein adsorption resulting from platelet adhesion were performed for modified and unmodified silicones. **Figure 6** depicts relative

platelet adsorption quantified by LDH assay following 15 minutes of exposure to whole blood in the Chandler Loop.

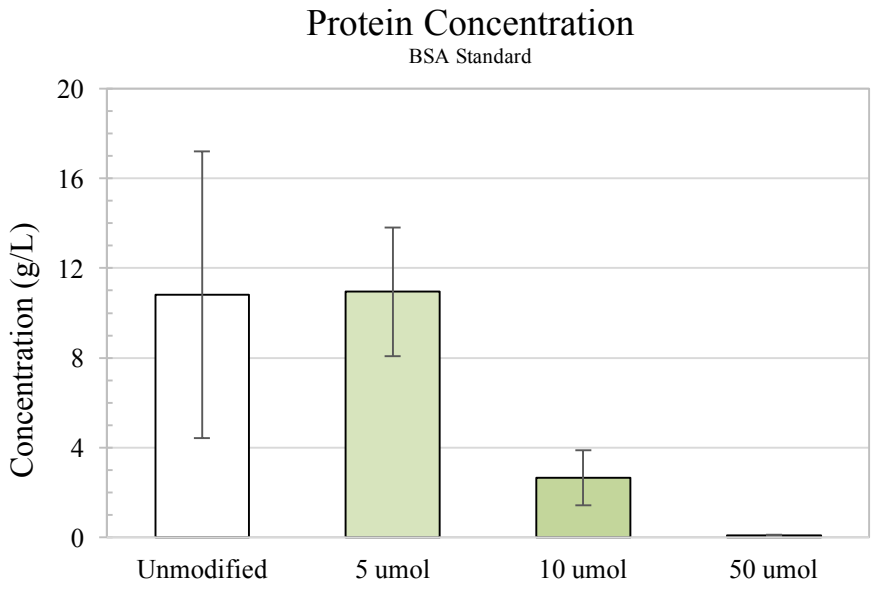

Figure 6: Protein concentration from platelets adhered to modified silicones.

Both the unmodified silicone and the silicone modified with 5 µmol PEO-silane amphiphile exhibited relatively high LDH concentration, indicating increased platelet adhesion. Notably, the unmodified silicone showed a significantly higher variance in protein concentration for different sections of the single loop. Protein concentration decreased substantially with increased concentration of amphiphile in the silicone. For silicones modified with 10 and 50 µmol amphiphile, protein concentration was 25 and <1% of that versus the unmodified silicone, respectively. A substantial and concentration-dependent decrease in LDH concentration was observed, indicating a correlated decrease in platelet adhesion.[19] Also, for the modified silicones,

protein adsorption was very consistent across the tubing. As predicted,[19] these protein adsorption results correlate with the occlusion time observations (**Figure 5**).

SECTION IV

CONCLUSION

Preventing the premature failure of implantable medical devices resulting from thrombosis using a material modification approach presents a noteworthy alternative to the use of anticoagulants. A PEO-silane amphiphile developed by Grunlan and co-workers has shown promise in improving silicone resistance to the adsorption of fibrinogen. However, testing with whole blood was required to assess thrombogenicity of the modified silicones. To create a testing environment that simulates flow *in vivo*, a Chandler Loop was constructed and used for a whole blood dynamic *in vitro* test. Results indicate that the amphiphile significantly improves thromboresistance of the silicone at concentrations as low as 10 μmol per gram of silicone (1.7 wt %). At a concentration of 50 μmol, platelet adhesion was nearly eliminated.

Future directions can be taken to optimize the *in vitro* test before moving into *in vivo* studies. Recommended work includes modifying the Chandler Loop to match the *in vivo* body temperatures, identifying the minimum viable concentration of amphiphile, and assessing long-term amphiphile efficacy *in vitro.* Additional testing should then be performed in an *in vivo* system. All of these will more comprehensively evaluate the functionality of the PEO-silane amphiphile in preventing protein adhesion.

REFERENCES

1. Colas, A.; Curtis, J., Silicone biomaterials: history and chemistry. *Biomaterials Science: an Introduction to Materials in Medicine* **2004,** *2*, 80-85.

2. Gulløv, A.; Koefoed, B.; Petersen, P., Bleeding complications in oral anticoagulant treatment. *Nordisk medicin* **1994,** *110* (4), 114-118.

3. Jeon, S.; Lee, J.; Andrade, J.; De Gennes, P., Protein—surface interactions in the presence of polyethylene oxide: I. Simplified theory. *Journal of Colloid and Interface Science* **1991,** *142* (1), 149-158.

4. Chen, S.; Li, L.; Zhao, C.; Zheng, J., Surface hydration: principles and applications toward low-fouling/nonfouling biomaterials. *Polymer* **2010,** *51* (23), 5283-5293.

5. Park, K.; Shim, H. S.; Dewanjee, M. K.; Eigler, N. L., In vitro and in vivo studies of PEO-grafted blood-contacting cardiovascular prostheses. *Journal of Biomaterials Science, Polymer Edition* **2000,** *11* (11), 1121-1134.

6. Sefton, M. V. G., C.H., Nonthrombogenic Treatments and Strategies. In *Biomaterials Science: an Introduction to Materials in Medicine*, 2nd ed.; Ratner, B. D., Ed. 2004; pp 456-470.

7. Du, Y. J.; Klement, P.; Berry, L. R.; Tressel, P.; Chan, A. K., In vivo rabbit acute model tests of polyurethane catheters coated with a novel antithrombin-heparin covalent complex. *Thrombosis and haemostasis* **2005,** *94* (2), 366-372.

8. Ratner, B. D.; Hoffman, A. S., Physicochemical Surface Modification of Materials Used in Medicine. In *Biomaterials Science: an Introduction to Materials in Medicine*, 2nd ed.; Ratner, B. D.; Hoffman, A. S.; Schoen, F. J.; Lemons, J. E., Eds. Elsevier Academic Press: New York, 2004; pp 201-218.

9. Delamarche, E.; Donzel, C.; Kamounah, F. S.; Wolf, H.; Geissler, M.; Stutz, R.; Schmidt-Winkel, P.; Michel, B.; Mathieu, H. J.; Schaumburg, K., Microcontact printing using poly(dimethylsiloxane) stamps hydrophilized by poly(ethylene oxide) silanes. *Langmuir* **2003,** *19* (21), 8749-8758.

10. Rufin, M. A.; Gruetzner, J. A.; Hurley, M. J.; Hawkins, M. L.; Raymond, E. S.; Raymond, J. E.; Grunlan, M. A., Enhancing the protein resistance of silicone via surface-restructuring PEO–silane amphiphiles with variable PEO length. *Journal of Materials Chemistry B* **2015,** *3* (14), 2816-2825.

11. Han, D. K.; Jeong, S. Y.; Kim, Y. H.; Min, B. G.; Cho, H. I., Negative cilia concept for thromboresistance: synergistic effect of PEO and sulfonate groups grafted onto polyurethanes. *Journal of Biomedical Materials Research* **1991,** *25* (5), 561-575.

12. Huang, N.; Yang, P.; Leng, Y.; Chen, J.; Sun, H.; Wang, J.; Wang, G.; Ding, P.; Xi, T.; Leng, Y., Hemocompatibility of titanium oxide films. *Biomaterials* **2003,** *24* (13), 2177-2187.

13. Van Oeveren, W.; Haan, J.; Lagerman, P.; Schoen, P., Comparison of coagulation activity tests in vitro for selected biomaterials. *Artificial Organs* **2002,** *26* (6), 506-511.

14. Falati, S.; Gross, P.; Merrill-Skoloff, G.; Furie, B. C.; Furie, B., Real-time in vivo imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse. *Nature Medicine* **2002,** *8* (10), 1175-1180.

15. Visser, M. R.; Tracy, P. B.; Vercellotti, G. M.; Goodman, J. L.; White, J. G.; Jacob, H. S., Enhanced thrombin generation and platelet binding on herpes simplex virus-infected endothelium. *Proceedings of the National Academy of Sciences* **1988,** *85* (21), 8227-8230.

16. Zhang, Z.; Zhang, M.; Chen, S.; Horbett, T. A.; Ratner, B. D.; Jiang, S., Blood compatibility of surfaces with superlow protein adsorption. *Biomaterials* **2008,** *29* (32), 4285-4291.

17. Tamada, Y.; Kulik, E. A.; Ikada, Y., Simple method for platelet counting. *Biomaterials* **1995,** *16* (3), 259-261.

18. Keuren, J. F.; Wielders, S. J.; Driessen, A.; Verhoeven, M.; Hendriks, M.; Lindhout, T., Covalently-bound heparin makes collagen thromboresistant. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2004,** *24* (3), 613-617.

19. Tsai, W. B.; Grunkemeier, J. M.; Horbett, T. A., Human plasma fibrinogen adsorption and platelet adhesion to polystyrene. *Journal of Biomedical Materials Research* **1999,** *44* (2), 130-139.

20. Frank, R. D.; Müller, U.; Lanzmich, R.; Groeger, C.; Floege, J., Anticoagulant-free Genius® haemodialysis using low molecular weight heparin-coated circuits. *Nephrology Dialysis Transplantation* **2006,** *21* (4), 1013-1018.

21. Tanaka, M.; Motomura, T.; Kawada, M.; Anzai, T.; Kasori, Y.; Shiroya, T.; Shimura, K.; Onishi, M.; Mochizuki, A., Blood compatible aspects of poly (2-methoxyethylacrylate)(PMEA)—relationship between protein adsorption and platelet adhesion on PMEA surface. *Biomaterials* **2000,** *21* (14), 1471-1481.

22. Seyfert, U. T.; Biehl, V.; Schenk, J., In vitro hemocompatibility testing of biomaterials according to the ISO 10993-4. *Biomolecular Engineering* **2002,** *19* (2), 91-96.

23. Chatterjee, M. S.; Denney, W. S.; Jing, H.; Diamond, S. L., Systems biology of coagulation initiation: kinetics of thrombin generation in resting and activated human blood. *PLoS Comput Biol* **2010,** *6* (9), e1000950.

24. Van Oeveren, W., Obstacles in haemocompatibility testing. *Scientifica* **2013,** *2013*.

25. Van Kruchten, R.; Cosemans, J. M.; Heemskerk, J. W., Measurement of whole blood thrombus formation using parallel-plate flow chambers–a practical guide. *Platelets* **2012,** *23* (3), 229-242.

26. Tseng, P.-Y.; Rele, S. S.; Sun, X.-L.; Chaikof, E. L., Membrane–mimetic films containing thrombomodulin and heparin inhibit tissue factor-induced thrombin generation in a flow model. *Biomaterials* **2006,** *27* (12), 2637-2650.

27. Browning, M.; Dempsey, D.; Guiza, V.; Becerra, S.; Rivera, J.; Russell, B.; Höök, M.; Clubb, F.; Miller, M.; Fossum, T., Multilayer vascular grafts based on collagen-mimetic proteins. *Acta Biomaterialia* **2012,** *8* (3), 1010-1021.

28. Krajewski, S.; Prucek, R.; Panacek, A.; Avci-Adali, M.; Nolte, A.; Straub, A.; Zboril, R.; Wendel, H. P.; Kvitek, L., Hemocompatibility evaluation of different silver nanoparticle concentrations employing a modified Chandler-loop in vitro assay on human blood. *Acta Biomaterialia* **2013,** *9* (7), 7460-8.

29. Mutch, N. J.; Moore, N. R.; Mattsson, C.; Jonasson, H.; Green, A. R.; Booth, N. A., The use of the Chandler loop to examine the interaction potential of NXY-059 on the thrombolytic properties of rtPA on human thrombi in vitro. *British Journal of Pharmacology* **2008,** *153* (1), 124-131.

30. McClung, W.; Babcock, D.; Brash, J., Fibrinolytic properties of lysine-derivatized polyethylene in contact with flowing whole blood (Chandler loop model). *Journal of Biomedical Materials Research Part A* **2007,** *81* (3), 644-651.

31. Touma, H.; Sahin, I.; Gaamangwe, T.; Gorbet, M. B.; Peterson, S. D., Numerical investigation of fluid flow in a chandler loop. *Journal of Biomechanical Engineering* **2014,** *136* (7), 071004.

32. Sinn, S.; Scheuermann, T.; Deichelbohrer, S.; Ziemer, G.; Wendel, H. P., A novel in vitro model for preclinical testing of the hemocompatibility of intravascular stents according to ISO 10993-4. *Journal of Materials Science: Materials in Medicine* **2011,** *22* (6), 1521-1528.

33. Tepe, G.; Wendel, H. P.; Khorchidi, S.; Schmehl, J.; Wiskirchen, J.; Pusich, B.; Claussen, C. D.; Duda, S. H., Thrombogenicity of various endovascular stent types: an in vitro evaluation. *Journal of Vascular and Interventional Radiology* **2002,** *13* (10), 1029-1035.

34. Mitchell, D. B.; Santone, K. S.; Acosta, D., Evaluation of cytotoxicity in cultured cells by enzyme leakage. *Journal of Tissue Culture Methods* **1980,** *6* (3-4), 113-116.

35. Moser, M.; Nieswandt, B.; Ussar, S.; Pozgajova, M.; Fässler, R., Kindlin-3 is essential for integrin activation and platelet aggregation. *Nature Medicine* **2008,** *14* (3), 325-330.

36. Okano, T.; Nishiyama, S.; Shinohara, I.; Akaike, T.; Sakurai, Y.; Kataoka, K.; Tsuruta, T., Effect of hydrophilic and hydrophobic microdomains on mode of interaction between block polymer and blood platelets. *Journal of Biomedical Materials Research* **1981,** *15* (3), 393-402.

37. Hermann, M.; Nussbaumer, O.; Knöfler, R.; Hengster, P.; Nussbaumer, W.; Streif, W., Real-time live confocal fluorescence microscopy as a new tool for assessing platelet vitality. *Transfusion Medicine and Hemotherapy* **2010,** *37* (5), 299-305.

38. Losi, P.; Lombardi, S.; Briganti, E.; Soldani, G., Luminal surface microgeometry affects platelet adhesion in small-diameter synthetic grafts. *Biomaterials* **2004,** *25* (18), 4447-4455.

39. Nguyen, K.; Su, S.-H.; Sheng, A.; Wawro, D.; Schwade, N.; Brouse, C.; Greilich, P.; Tang, L.; Eberhart, R., In vitro hemocompatibility studies of drug-loaded poly-(L-lactic acid) fibers. *Biomaterials* **2003,** *24* (28), 5191-5201.

40. Shapiro, H. M., Practical flow cytometry. John Wiley & Sons: 2005; pp 10-18.

41. Murthy, R.; Cox, C. D.; Hahn, M. S.; Grunlan, M. A., Protein-resistant silicones: incorporation of poly (ethylene oxide) via siloxane tethers. *Biomacromolecules* **2007,** *8* (10), 3244-3252.

42. Bowbrick, V. A.; Mikhailidis, D. P.; Stansby, G., The use of citrated whole blood in thromboelastography. *Anesthesia & Analgesia* **2000,** *90* (5), 1086-1088.