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CARDIOVASCULAR DEVICES WITH MULTIFUNCTIONAL HYDROGEL COATINGS: FIGHTING INFECTION AND THROMBOSIS AT THE SAME TIME

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Abstract:

Thromboembolic and infectious consequences resulting from the use of cardiovascular devices continue to be prevalent and cause a considerable amount of morbidity and mortality. Up until now, there hasn't been a plan that successfully tackles both problems at once. There are many surface modification techniques that have been suggested, such as liquid-impregnated surfaces, heparin- and silver-impregnated surfaces, however they all have drawbacks and restrictions. Here, it is demonstrated that bacterial adhesion to medical-grade tubing is reduced by 95% by including an ultrathin and mechanically strong hydrogel layer. Additionally, it is shown that the hydrogel layer significantly lowers blood clot formation and adherence to the tubing without compromising the blood's inherent clotting capabilities using a mix of in vitro and in vivo studies. In an in vivo porcine model, the adherence of clots to the tubing walls is reduced by over 90% (in vitro model), leading to an approximately 60% increase in the device occlusion time (time before closure due to clot formation). Because of these passive coating's advantageous properties, it is a promising surface material candidate for medical devices that interface with blood diseases, such as cancer, end-stage renal disease, cardiovascular disease, and the care of critically ill patients in the intensive care unit (ICU).

Keywords: Hydrogel coatings, blood-contacting devices, thrombosis, bacterial adhesion, cardiovascular implants.

Introduction:

problems associated with catheters patients who rely on these catheters are probably fragile and/or critically sick (e.g., in the ICU, patients with serious infections, cancer), making them expensive and difficult to treat. Apart from the heightened The morbidity and mortality linked to

some medical procedures, such as urinary and central venous catheterization, along with the resulting care expenses, place a substantial strain on the health care system overall.

Although significant efforts have been made, infectious and thromboembolic complications associated with the use of cardiovascular medical devices continue to represent a significant, unmet clinical challenge, particularly given the growing number of chronically ill patients who rely on these devices. These complications have also been reported for other blood-contacting devices, including stents, prosthetic bypass grafts, dialysis equipment, ventricular-assisted devices (VADs), artificial heart valves, and extracorporeal membrane oxygenation (ECMO) equipment.[17–22].As of right now, there are no commercially available products that effectively treat complications from infection and thrombosis. The majority of technologies that are sold commercially tackle infections-related issues. These methods include the use of antibiotic-coated catheters, antibiotic-impregnated catheters, and antisep-tic wound dressings. [7,13,23–28] These technologies demonstrate a slight decrease in bacteremia, but only for specific patient types and short-term catheter use.

None of these methods produce a surface for the device that inherently reduces bacterial adherence and, as a result, lessens the production of bacterial films. Additionally, there is little evidence of a clinical benefit for long-term uses and no improvement in other side effects of catheter use, such as thrombosis. [23, 24] A different set of technologies is available to treat thrombosis and the ensuing device blockage. These methods involve binding bioinert polymers or bioactive molecules, like heparin, corn trypsin inhibitor, or thrombomodulin, to the surface of the device. [6,10,29–32] Clinical trial results have been inconsistent, despite encouraging pre-clinical data; only a few heparin-coating technologies have demonstrated clinical benefits. [3,5,6,30]

Hydrogel Coatings for Blood-Contacting Devices: A Potential Solution to Infections and Thrombosis

Technologies that target both infections and thrombosis are quite rare. One method entails incorporating a nanotextured surface that is entrained with perfluorinated liquids.[33–35] This surface repels blood components as well as bacteria, but it hasn't been applied in clinical settings probably because of worries about fluorine-containing fluids being present in bloodstream. A more sophisticated method was first developed in the 1980s to lessen friction and irritation for urinary catheters. It was marketed as a “hydrogel-coating” technology even though it only entailed surface polymer grafting and not a 3D polymer network. The second approach involves photografting hydrophilic polymers onto the device surface.[7,36–40]. With names like Biocath and Lubri-sil (Bard), Flocath (Teleflex), Serpia (Braun), Convey and HydroPlus (Boston Scientific), and HydroPicc (Access Vascular), among others, the majority of major brands now offer "hydrogel-coated" catheters.[41–43] Despite these catheters demonstrating clinical

performance and being adopted by healthcare professionals, there are concerns about adverse effects resulting from coating failure and delamination, as these grafted coatings are typically tens of nanometers thick.[41,42] The evidence led the FDA to issue a safety communication in 2015 and a labeling guidance in 2019 to address these complications.[44,45] Based on the available clinical data and published literature, it is clear that the development of a thrombosis-resistant and bacteria-repellent surface would be a significant advancement.

in the rapidly expanding field of critical care and cardiovascular devices. The mechanical, antibacterial, and thrombosis-resistant characteristics of ultrathin hydrogel films applied to medical device surfaces have all been described in this work. Our lab has recently developed a method that exhibits robust adhesion of difficult hydrogel materials, which has the potential to improve the interfacial properties of elastomer-based medical interfaces [46–48]. Medical-grade Ty-gon (polyvinyl chloride) tubing, a common medical device used for blood management in dialysis and cardiopulmonary bypass settings, was used in the experiments covered here. This was done to ensure that the blood only comes into contact with one material because the blood would be completely contained inside the tubing. Any testing involving catheters would necessitate the collection of blood in an external vessel made of a different substance, which would have complicated the study. The coated surfaces exhibited low coefficients of friction and remained unchanged for up to 12 hours when fluid flowed at physiological rates. When compared to uncoated surfaces, there is a noticeable decrease in the adherence of bacteria and fibrin. Furthermore, we demonstrated that the hydrogen coating lowered blood clot formation and adhesion to the device surface without affecting the blood's inherent clotting capacity using a battery of in vitro testing and flow loop experiments. Additionally, we showed the superior patency rates of PVC tubings coated in hydrogel that were inserted into the systemic circulation (arterial to arterial conduit) in pigs. These preliminary findings suggest that hydrogel coatings for blood-contacting devices may reduce the risk of infection and thrombosis-related consequences in cardiovascular devices, which could eliminate the need for individual technologies designed to address these clinical issues.

Characterization and Durability of Ultra-Thin Hydrogel Coatings for Blood-Contacting Devices

The hydrogel coatings were made in the same manner as before. outlined.[48] This technique was used because it made it possible to coat cylindrical objects (such as catheters and tubing) on both the inside and outside surfaces. The medical surfaces in this work were cleaned and then immersed in an ethanol-based benzophenone solution, in accordance with established procedures to generate the coatings. After the solution was eliminated, the activated surfaces were treated with an aqueous pregel solution that contained N,N-dimethylacrylamide (DMAA) and a biocompatible photoinitiator (Irgacure 2595). The hydrogel layer was then polymerized on the surface by UV radiation after this monomer was

selected to prevent the toxicity issues associated with acrylamide, the monomer employed in earlier studies. An ultra-thin (≈10 μm) hydrogel layer was evenly placed on the device following a thorough rinsing of the surplus polymers and byproducts, as schematically depicted in Figure 1a for Tygon CPB tubing. In comparison to the standard OD of venous catheters (5-7 mm) or the ID of blood-management tubing (0.75–1.5 cm), the layer is extremely thin. Figure 1b displays macro pictures of pristine and coated (inner lumen only) tubing to illustrate that the hydrogel coating was consistent and, because of its micrometer-sized dimensions, had no effect on the overall dimensions of the device. The cross-sectional microscopy images are displayed in Figure S1 (Supporting Information). A prior paper has more information on the adhesion mechanism, manufacturing procedure, and chemical makeup of the hydrogel coating.[48]

It was established what the hydrogel layer's elastic modulus was. Previously, the value E was approximately 30 kPa [48], which is two orders of magnitude less than the E = 6 MPa of the virgin Tygon substrate. As previously described, the water-containing hydrogel layer reduced the surface's coefficient of friction (COF), which was measured here using a rheometer with a steel parallel plate geometry. [47,48] We were able to characterize the COF of both.

layer of interpenetrated hydrogel. The continuous shearing would have worn down weaker coatings, causing the COF to rise to the same level as the untreated substrate. It is noteworthy that these loading conditions were higher than what was finally seen in real-world applications. Additionally, the coating could tolerate pressures (≈100 mmHg) and physiologically comparable flow rates (≈1.5 L min−1). Saline was continuously circulated through a coated tube segment for up to 12 hours using the arrangement seen in Figure 2b. The sessile drop method was used to measure the surface water contact angle at specific time points, and fluorescent microscopy was used to evaluate the coating thickness. The clean sample's observed contact angle was more than 80° because the pristine Tygon surface is hydrophobic. Following the application of the approximately 10 μm hydrogel coating, a reduction in the measured contact angle (less than 40°) was seen. The hydrogel coating's robustness and integrity were demonstrated by the fact that, even after continuous flow for up to 12 hours, neither the contact angle nor the coating thickness changed.

Blood Compatibility of Ultra-Thin Hydrogel Coatings: Reduced Thrombosis and Minimal Hemolysis:

Because stasis encourages coagulation,[3,15,16,32,56] studies were carried out with the blood phase maintained in a continuous state to reduce this confounding factor and more precisely describe the relationship between the blood and surface coating. As seen in the films in the Supporting Information, the first test used a straightforward method to

introduce flow: pushing a column of blood back and forth inside tubes until clotting occurred. When a macroscopic thrombus blocked the tube's lumen and there was no visible blood movement upon inverting the tubing, this was referred to as the occlusion time. The outcomes of multiple testing are displayed in Figure 3c. The clotting times for coated and pristine tubes were the same as the control (blood in an Eppendorf tube) in the absence of movement (i.e., stasis).

The measured clotting time in coated tubing was longer than in clean tubing when the blood was disturbed. Given that the tests were conducted on different days, the data dispersion most likely reflected variability in the blood samples utilized. Furthermore, it is significant to note that, despite the fact that flow within the tubing was still detected, the five highest coated tests were unilaterally halted at t = 25 minutes. Upon closer examination, it was discovered that the tubing still contained uncoagulated blood and that, in contrast to the clean tube tests, the thrombi were not adhered to the tubing surface.
All things considered, these were extremely encouraging findings indicating that the coating would be able to enhance the functionality of blood-contacting devices passively—that is, without the need for an anticoagulant like citrate or heparin. Although the use of heparin-containing surfaces (such Medtronic Cortiva bioactive coatings or GORE CARMEDA) has been suggested as a way to lessen thrombosis issues, the introduction of heparin also has certain negative side effects, like the possibility of bleeding and heparin-induced thrombocytopenia. Furthermore, in patients with aberrant coagulation, this local anticoagulant characteristic might have unpredictable effects and is difficult to regulate.

Overall, these heparin-coated surfaces have a minimal and, at most, transient clinical impact. The results shown in Figure 3c could have been explained by the hydrogel coating interfering with the blood's coagulation cascade through binding, denatured components, or cell lysis. To rule out these scenarios, two experiments were conducted. Prior to being collected and pelleted in a centrifuge, whole blood samples were incubated under gentle agitation on top of pristine, coated tubing. In Figure 3d, the samples were compared to an untreated bovine blood negative control and a hemolyzed positive control (hypotonic hemolysis) positive control. The supernatant was examined using a spectrophotometer at the greatest absorption frequencies for oxygenated hemoglobin (540 and 576 nm). (Figure 3d). Comparing the measurements to the negative control (baseline), they confirmed that the coating did not result in any significant hemolysis. Hemoglobin would have been released into the supernatant of the red blood cell as a consequence of hemolysis, which would have produced absorbance values that were higher.

Secondly, the impact of the hydrogel coating on the blood clotting process was measured using optical thromboelastography (OTEG) [58–60]. This technique is schematically depicted in Figure 3e and involves analyzing the autocorrelation function of a reflected laser beam to create an OTEG graph, which is displayed at the bottom of the

Section of Experiments

Hydrogel Coatings on Tygon Surfaces: The experimental procedure was as follows: the medical-grade (U.S. Plastic Corp.) flat surface or tubing was cut to the desired size, cleaned with isopropanol and DI water, and fully dried with a nitrogen stream. All chemicals were purchased from Millipore-Sigma and used as received, unless otherwise indicated. After that, the vices are exposed to air plasma treatment (PDC-001, Harrick Plasma, <350 torr, 18 W) for 1-2 minutes, and then they are immersed in a 10% ethanol benzophenone solution for 3-5 minutes. After removing the devices from the solution and blotting off any excess ethanol with tissue, they are submerged in a hydrogel precursor solution that has been previously degassed (20–30 weight percent N,N-dimethylacrylamide, 0.5 weight percent Irgacure I-2959 in water). At least 5 mm of precursor solution should be present above the face of FF. The inner lumen of tubing should be fully filled with the precursor solution. Following that, the devices were exposed to UV (UVP CL-1000, λ = 365 nm) for 45 minutes in order to form the hydrogel layer that interpenetrated the device surface. After removal, the polymerized solution was disposed of as waste. Before testing, the devices were properly cleaned with DI water for three days (immersed in water, shaker-mounted for flat surfaces, and attached to a peristaltic CBP (VWR, variable flow pump) for tubing).

Evaluating Antibacterial and Thrombogenic Properties of Ultra-Thin Hydrogel Coatings:

applying a fluorescein solution (a hydrophilic dye) and using a fluorescent microscope (Nikon Eclipse LV100ND) to image it. With the use of built-in tools (NIS-Elements program), the coating thickness was quantified.
Bioengineered Bacterial Adhesion: An E. A green fluorescent protein (GFP)-expressing coli strain was cultivated in Luria-Bertani broth (LB broth) for an entire night at 37 °C. This strain has been utilized in earlier papers [47, 48]. Placed on top of fish samples (1 cm x 1 cm), a 1 mL aliquot of bacteria culture was diluted in 10 mL of new LB broth and incubated for 4 hours at 37 °C (static condition). Using a VWR peristaltic pump and 3/8 ID tubing, a scaled-down version of the apparatus shown in Figure 2b was assembled for the flow condition. The bacterial solution flowed through the tube for eight hours at 37 °C. Following the incubation period, the samples were removed, gently washed twice in phosphate buffered saline (PBS) to get rid of any bacteria that were free-floating, and then examined under an epifluorescence microscope (Nikon Eclipse LV100ND). Image J was used to quantify the amount of bacteria present on the samples.

Fibrin Deposition: Using previously described procedures[31,32], the wells of a 24-well Corning plate were blocked for 30 minutes with 1 weight percent bovine serum albumin (BSA) in PBS, and then they were washed with PBS. In order to prevent overall clotting, heparinized whole human blood (0.25 U mL−1) spiked with fluorescently tagged fibrinogen (Alexa Fluor 488 conjugated human fibrinogen, ThermoFischer Scientific) was added to the blocked wells containing pristine and coated fibrin samples (1 × 1 cm). The samples were placed on an orbital shaker at room temperature and incubated for increasing time intervals. The samples were carefully removed from the well, cleaned with normal saline (0.9% sodium chloride), and fixed

for 1 hour in 0.1 M phosphate buffer containing 2.5% glutaraldehyde. Subsequently, they were imaged using an upright confocal microscope (SP8, Leica). Using ImageJ, the photos were examined and quantized. Blood Hemolysis: After 5 minutes of mild agitation, citrated bovine blood samples (about 3 mL) were put to the inner lumen of pristine and coated tubing (3/8″ ID). As-received blood and lysed blood (20% DI water added) were the two controls used. After that, the blood was placed into Eppendorf tubes and centrifuged (VWR minicentrifuge) for two minutes. Using a UV–vis spectrophotometer (BioMate 3S, Thermo Scientific) at two wavelengths, 540 and 576 nm, which correspond to the maximum, the supernatant was put in disposable cuvettes for analysis. absorbance of oxygen-containing hemoglobin.

Optical thromboelastography: The Nadkarni group's researchers custom-built the apparatus utilized for these experiments, as previously detailed. [51–53] These articles also include information on the data collecting and processing techniques. Human blood that has been citrated was placed for three minutes inside spotless and coated tubing (3/8″ ID), then recalculated using 60 μL mL−1 blood in a 0.2 M calcium chloride solution in saline. A 100 μL portion of blood was extracted and stored in a silicone blood collection cartridge at 37 °C until the level of coagulation was determined. Blood was recalcified and examined in control samples without coming into contact with any tubing. Using specially designed MATLAB techniques, the OTEG curves were fitted to determine the parameters.

Evaluating Blood Clot Formation in Ultra-Thin Hydrogel Coated Tubing Under Static and Flow Conditions

Tests for Blood Flow Inside Tubing: Two distinct kinds of tests were performed. The first form was recalcitranting 3 mL of citrated bovine blood with 0.5 M calcium chloride in saline (24 μL per mL blood) and putting the mixture within pris-tine and coated tubing (about 9′ length and 3/8″ ID). Prior to being fully cleaned with saline solution, the tubing was sterilised using ethanol and UV light. As seen in the videos (in the Supporting Information), the tubing was moved back and forth until a clot developed or twenty-five minutes had passed. The blood column stopped moving, and this was recorded as the clotting time. A CBP roller pump was used to agitate the blood for the second type of tests (Cobe Century). Here, twenty sections of 3/8″ ID immaculate and coated tubing were filled with approximately 25 mL of recalcified blood, and the ends were connected into a loop by securing a short piece of 3/4″ tubing. Both before and after filling with blood, the tubing was weighed. Up until a control sample (left in an Eppendorf tube) clotted, a 0.25L min−1 flow was set up. After the contents of the loop were transferred into a petri dish, the clot was put in a weigh boat and given weight. After being lightly cleaned with regular saline, the tubing was weighted.

Conclusion:

In this work, we introduce a groundbreaking ultra-thin hydrogel coating for blood-contacting devices that tackles both thrombosis and bacterial adhesion. Our findings, based on a combination of in vitro and in vivo experiments, suggest significant promise for this technology in combating the critical issue of blood-related complications arising from cardiovascular implants. This innovation has the potential to revolutionize cardiovascular devices by offering a single surface modification that eliminates the need for separate anti-thrombotic and anti-bacterial treatments, leading to improved patient outcomes and reduced healthcare costs.

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