

~~Sustainable alternative to hemocompatible fossil-~~
~~based polymer brushes~~ Green solvent-based,
antifouling polymer brushes demonstrate excellent
hemocompatibility

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ABSTRACT

The utilization of medical devices is crucial for patient care, yet even with the best biomaterials, it can lead to infections, and unwanted activation of blood coagulation, potentially being life-threatening. While hydrophilic polymer brushes are the best surface coatings to mitigate these issues, their reliance on depleting fossil raw materials underscores the urgency for seeking bio-based alternatives. In this work, we introduce new bio-based polymer brushes of a green solvent-based monomer, prohibiting that providing protection against protein adsorption, bacterial colonization and blood clot formation at the same level as fossil-based polymer brushes. The bio-based polymer brushes are composed of *N,N*-dimethyl lactamide acrylate (DMLA), can be polymerized in a controlled manner and show strong hydrophilicity as determined by thermodynamic analysis of the surface tension components. Moreover, The contact of various challenging protein solutions including human blood plasma results in repellency on the poly(DMLA) brushes. Furthermore, the poly(DMLA) brushes completely prevent the adhesion and colonization of *Escherichia coli*. Remarkably, when in contact with blood upon blood contact, the poly(DMLA) brushes successfully prevent the formation of a fibrin network and leukocyte adhesion on the surface. Compared to the gold standard of polymer brushes of *N*-hydroxypropyl

methacrylamide (HPMA), the poly(DMLA) brushes provide equal antifouling properties, but due to the lack of hydroxyl groups in the poly(DMLA) brushes, they demonstrate no activation of the complement system in blood. While showcasing excellent antifouling properties similar to *N*-hydroxypropyl methacrylamide (HPMA) polymer brushes as one of the best antifouling coatings, the absence of hydroxyl groups in the poly(DMLA) brushes prevents activation of the complement system in blood. We envision the bio-based polymer brushes to highly contribute to the future of sustainable hemocompatible coatings for blood-contacting medical devices.

Introduction

In this work, we introduce polymer brushes composed of a green-solvent based monomer as an sustainable alternative to the best antifouling and hemocompatible fossil-based coatings. These properties are of paramount importance in medicine technology. The contact of bodily fluids with artificial surfaces commonly leads to the adsorption of proteins, which affects its functions and acts as a biological transducer that informs the biological counterpart of the presence of the material, unfortunately often causing deleterious reactions.¹ Particularly in case of blood, the contact with any artificial surface causes the activation of coagulation due to adsorption of

proteins.² For instance, when albumin, a highly abundant blood protein, adsorbs onto surfaces, it undergoes a conformational change that exposes domains facilitating integrin binding, thereby promoting platelet activation.³⁻⁴ Similarly, in the contact activation system, coagulation factor XII (FXII) unfolds upon adsorption, generating its activated form (FXIIa), which triggers the intrinsic coagulation pathway, initiating thrombus formation.⁵ Additionally, contact between blood and artificial surfaces can activate the complement system. For instance, the adsorption of complement component 3 (C3) causes its cleavage into fragments C3a and C3b, instigating a cascade of reactions, which increases fouling and activates an immune response.⁶ On top of that, adsorbed proteins can form a conditioning film promoting bacterial adhesion and biofilm formation, thereby contributing to bacterial infections.

To address these challenges, a number of nanoscale coatings have been developed. The first prerequisite is that the coating minimizes the driving force for protein adsorption. Primarily, the coating has to be hydrophilic to reduce the interfacial energy with water and should introduce physical barriers.⁷⁻¹⁸ Among these strategies, hydrophilic polymer brushes have emerged as the most effective system for preventing non-specific adsorption of proteins, cells, bacteria, and blood components.¹⁹⁻²⁸ This effectiveness stems from the high retention of surface-bound water

molecules by the polymer chains, restricting protein-brush interactions.²⁹ Moreover, hydrophilic polymer brushes generate an entropic barrier against protein adsorption. When a protein approaches the surface, it leads to compression of the polymer chains. This compression results in a reduction of conformational entropy, which turns adsorption thermodynamically unfavorable.³⁰ Importantly, this entropic barrier operates not only at the molecular level but also extends to a mesoscopic scale.³¹⁻³³ To date, the best antifouling polymer brushes are based on *N*-hydroxypropyl methacrylamide (HPMA) and zwitterionic carboxybetaines or sulfobetaines^{23, 31, 34-42} However, there are still challenges that have to be addressed. As an example, the hydroxyl groups in poly(HPMA) have been shown to trigger the complement system, potentially inducing undesired immune responses and inflammatory reactions.^{36, 42} Additionally, despite their exceptional antifouling capabilities, even the most advanced antifouling polymer brushes face a restricted long-term outlook due to their reliance on chemicals derived primarily from fossil resources. In 2016, over 90% of plastic production relied on fossil fuels as feedstocks.⁴³ However, considering that fossil oil and gas reserves are projected to be depleted by around 2040 and 2042 respectively,⁴⁴ there is a pressing need to address resource limitations, environmental concerns, financial incentives, and regulatory pressures. This has driven the exploration of sustainable alternatives in

the realm of polymer development.⁴⁵⁻⁵⁴ Regarding bio-based polymer brushes, efforts have yielded innovations like poly(α -methylene- γ -butyrolactone) brushes sourced from tulips,⁵⁵ poly(cholesteryl methacrylate) brushes,⁵⁶ poly(cysteine methacrylate) brushes⁵⁷, and poly(lactic acid) brushes⁵⁸⁻⁵⁹. Nevertheless, the investigation into bio-based polymer brushes with antifouling properties comparable to their fossil-based counterparts is still relatively limited. Given this existing gap, the application of current bio-based polymer brushes is fraught with challenges as they may cause surface-associated complications such as blood coagulation or bacterial colonization.

In this work, we report the synthesis and characterization of hydrophilic ~~bio-based~~ polymer brushes composed of a green solvent-based monomer that are on par with the best antifouling hemocompatible fossil-based polymer brushes (**Figure 1**). The ~~bio-based~~ polymer brushes were formed by grafting *N,N*-dimethyl lactamide acrylate (DMLA), which is derived from lactic acid through the green biosolvent *N,N*-dimethyl lactamide, commercially available as Agnique® AMD 3L.^{53-54, 60} DMLA possesses inherent hydrophilicity and, in contrast to HPMA, lacks a hydroxyl group. The latter suggests that DMLA is less likely to activate the complement system upon contact with blood, mitigating potential concerns in this regard. We performed for the first time the

synthesis of poly(DMLA) brushes with photo-induced single electron transfer-living radical polymerization (SET-LRP) with few ppm of copper catalyst. Brushes were grafted from gold and silicon model surfaces as well as poly(ethylene) (PE) and poly(4-methyl-1-pentene) (PMP). PE is utilized for catheters and implants while PMP is the materials of oxygenator's membranes. The growth of the brushes was confirmed with X-ray photoelectron spectroscopy (XPS), spectroscopic ellipsometry and contact angle goniometry. The polymerization kinetic displayed a linear regression between the dry thickness and time, indicating a highly controlled tailorable system. Varying concentrations of DMLA led to different reaction rates, yielding thicknesses up to 250 nm in 25 min. The analysis of the surface tension components of poly(DMLA) and poly(HPMA) revealed that while both brushes were hydrophilic and had nucleophiles, the **bio-based poly(DMLA)** brushes display stronger interactions with water, which resulted in excellent repellency to proteins, undiluted human blood plasma (BP) and *E. coli*. Moreover, the poly(DMLA) brushes demonstrated improved repellency to human blood components and prohibited the deposition of fibrin clots. The investigation of the activation of the complement system in blood showed a superior performance of poly(DMLA) brushes compared to fossil-based poly(HPMA) brushes, which was also manifested by less leukocyte adhesion on poly(DMLA)

brushes. We envision the novel bio-based polymer brushes to pave the route to more long-term sustainable coatings for medical devices without sacrificing performance.

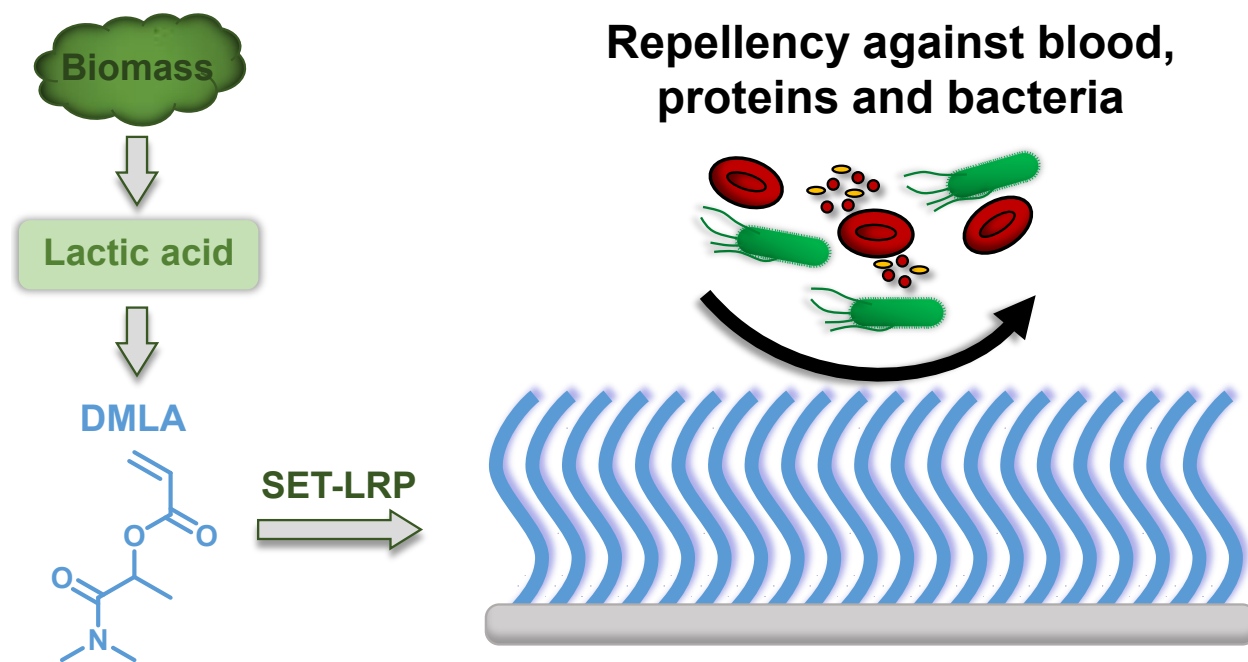


Figure 1. Concept of the bio-based antifouling hemocompatible polymer brushes. DMLA is derived from lactic acid, which is obtained through fermentation of biomass. The green solvent-based bio-based monomer is utilized to generate poly(DMLA) brushes, providing excellent repellency against proteins, bacteria and blood components.

Experimental Section

Materials

All chemicals used in the experiments were purchased and used without further purification unless stated. Copper(II)bromide (CuBr_2 , 99%), phosphate buffered saline tablets (PBS), human serum albumin (HSA, $\geq 98\%$), lysozyme (Lys), hexamethyldisilazane and citrated human BP were purchased from Sigma Aldrich. Dimethyl sulfoxide (DMSO, $>99.9\%$) and ethanol (EtOH, $>99.8\%$) were acquired from VWR International GmbH. Water used in the experiments was purified by a Millipore Milli-Q system. Tris(2-dimethylaminoethyl)amine (Me_6TREN , 99+%) was acquired from ThermoFisher GmbH. Silicon wafers were purchased from Siegert Wafer GmbH. SPR gold chips were purchased from Cenibra GmbH (BioNavis) or Xantec bioanalytics GmbH. PE and PMP substrates were cut from polymer films (150 mm x 150 mm x 0.5 mm for PMP and 150 mm x 150 mm x 1 mm for PE) purchased from Goodfellow Cambridge Limited. 11-(trichlorosilyl) undecyl 2-bromo-2-methylpropanoate and ω -mercaptoundecyl bromoisobutyrate were synthesized as previously described.^{20, 61}

Synthesis of DMLA

N,N-dimethyl lactamide (11.0 mL, 90 mmol), acrylic acid (7.4 mL, 108 mmol), triethylamine (39.0 mL, 280 mmol) and propylphosphonic anhydride solution (68.0 mL, 114 mmol) were added to a rounded-bottomed flask charged with 2-methyltetrahydrofuran Me-THF (100 mL). The reaction mixture was stirred for 24 h at room temperature under argon. Next, the solvent was removed under vacuum and the crude was purified by vacuum distillation in the presence of hydroquinone (5% w/w). Finally, the obtained colorless oil was filtered through a small column of basic alumina to remove hydroquinone traces to obtain DMLA (12.9 g, yield 84 %). ¹H-NMR (401 MHz, CDCl₃) δ 6.46 (dd, *J* = 17.4, 1.4 Hz, 1H), 6.18 (dd, *J* = 17.3, 10.4 Hz, 1H), 5.87 (dd, *J* = 10.4, 1.4 Hz, 1H), 5.47 (q, *J* = 6.7 Hz, 1H), 3.07 (s, 3H), 2.97 (s, 3H), 1.47 (d, *J* = 6.8 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 170.06, 165.65, 131.69, 127.81, 67.00, 36.77, 35.92, 16.63. HRMS (HESI-Orbitrap) calculated for [M + Na]⁺ C₈H₁₃NNaO₃⁺ (*m/z*): 194.0787; found: 194.0789

Synthesis of poly(DMLA) brushes

For the synthesis of the polymer brushes, a self-assembled monolayer (SAM) of initiator of 11-(trichlorosilyl) undecyl 2-bromo-2-methylpropanoate on silicon substrates, PE and PMP, and ω-mercaptopundecyl bromoisobutyrate on gold substrates were formed according to previous literature procedures.²⁰ Details can be found in the Supporting Information. DMLA (0.24 g,

1.39 mmol, 0.348 M, or 1.21 g, 7.04 mmol, 1.76 M) was dissolved in DMSO (4 mL). A stock solution of CuBr₂ (8.7 mg, 3.9 μmol) and Me₆TREN (62.5 μL, 23.4 μmol) was prepared in DMSO (10 mL) and an aliquot (0.178 μL) was added to the monomer solution. The monomer solution was degassed by bubbling with N₂ for 30 min while stirring. The substrates were degassed by purging with N₂ for 30 min. The polymerization solution was transferred to the vials and the polymerization was conducted by irradiation with UV-light using a nail-curing device (Salon Edge, four 9-W-lamps, λ_{max} = 365 nm) while keeping them at room temperature by fanning. After the irradiation times, the vials were opened and the polymerization was quenched by adding DMSO. The substrates were cleaned by rinsing with ethanol and Milli-Q water, followed by drying in a N₂ stream.

Thermodynamic analysis

The components of the surface tension were obtained from the contact angles of three different fluids (water, formamide, and α-bromonaphtalene) onto the brushes using the van Oss acid-base approach⁶²⁻⁶³. Further information can be taken from the Supporting Information.

Investigation of protein adsorption

Protein adsorption on was determined with surface plasmon resonance (SPR) on a MP-SPR Navi™ 210A VASA (BioNavis). The system was used with the SPR-Navi control software. The data were analyzed with SPR-Navi data viewer. The substrates were contacted with sample solutions in buffer at a rate of $10 \mu\text{L}\cdot\text{min}^{-1}$ for 60 min. The unspecific adsorption was calculated from the difference of baseline before and after sample injection.

Assessment of bacterial adhesion

A culture of *Escherichia coli* (DSM 18039) was prepared in Luria-Bertani (LB) broth at 37 °C and 180 rpm in the dark for 16 h using bacterial cells stored in 25% (v/v) glycerol. Bacterial suspension with starting OD₆₀₀ of 0.1 ($\sim 8 \cdot 10^7 \text{ CFU}\cdot\text{mL}^{-1}$) was prepared in fresh LB broth. Each of the UV-sterilized polymeric samples was immersed upside down in 4 mL of bacterial suspension inside of a 50 mL Falcon® tube and cultivated for 24 h at 37°C and 180 rpm in the dark. After the incubation, bacterial suspension was replaced with 5 mL of PBS buffer. Samples were washed by adding and removing 5 mL of PBS buffer three times. The samples were fixed with 3% glutaraldehyde solution in PBS. The samples dried by incubation with 50%, 60%, 70%, 80%, 90% and 100% ethanol twice for 15 minutes, followed by drying with hexamethyldisilazane.

Static blood experiments

Blood from three healthy donors was collected anonymously (approved by the Ethics Committee at the RWTH Aachen Faculty of Medicine, number: EK088/20) and anticoagulated with heparin ($0.75 \text{ IU} \cdot \text{mL}^{-1}$). Bare and coated PMP coins ($d = 1$) were glued into 48-well plates and contacted with 0.5 mL of fresh ~~not-pooled~~ blood. The blood was not pooled as this can hide effects stemming from large variability of blood depending on the donor. The samples were incubated at 37°C for 2 h and subsequently rinsed with 0.9% NaCl solution and PBS, followed by fixation with glutaraldehyde (2% in MilliQ water) for 2 h at 4°C . Subsequently, the samples were dried by incubation with ethanol 50%, 60%, 70%, 80%, 90% and 100% (twice) for 15 minutes to analyze with FESEM. For further investigation of C5a concentrations in blood, blood was collected after contact with the substrates and anticoagulated with EDTA ($10 \text{ mmol} \cdot \text{L}^{-1}$), followed by centrifugation at 2500 rcf for 20 min at 4°C . The plasma was stored at -80°C until utilization for a C5a micro ELISA (DRG International).

Results and Discussion

Synthesis and characterization of ~~bio-based~~ poly(DMLA) brushes

The poly(DMLA) brushes were grafted by photo-induced SET-LRP from the silicon substrates from different substrates, including silicon (Figure 2A), gold, PE and PMP. The exploration of various substrates is driven by the demand for tailored substrates to suit specific analytical techniques, such as ellipsometry, and fouling measurements with SPR. Furthermore, these substrates hold promise for diverse applications, such as their potential use in blood-contacting devices or implants that come into contact with proteins or bacteria. To ensure their comparability, it is essential that the grafting densities and coating thicknesses under study remain similar. Kinetic studies and fouling measurements of different substrates (Supporting Information Figure S1 and S2) have confirmed the similarity in grafting densities and the analogous resistance to fouling, substantiating the uniformity in coating thicknesses. Silicon surfaces serve as a basic model for studying polymerization kinetics, while the use of gold surfaces is essential for surface plasmon resonance (SPR) analysis. Additionally, we conducted hydrophilicity and bacterial adhesion assessments on PE due to its wide use in medical devices such as venous and urinary catheters, and orthopedic implants, all susceptible to bacterial infections. To evaluate the interaction of

poly(DMLA) brushes with blood, we selected PMP, commonly employed in extracorporeal membrane oxygenators, which directly interface with blood.

The kinetics of the photo-induced SET-LRP of poly(DMLA) brushes were assessed by the evolution of the dry thickness over time, determined with ellipsometry (Figure 2B, Supporting Information Table S1). The polymerization was carried out in DMSO with minute amount of copper catalyst (8 ppm of CuBr_2) and a monomer concentration of either 0.348 M or 1.76 M. For both conditions, a linear growth of the polymer brushes over time was observed, hinting that the polymerization proceeds controlled. A dry thickness of 39.6 ± 1.1 nm was obtained after 25 min of irradiation with $[\text{DMLA}]_0 = 0.348$ M. A five-fold increase in monomer concentration (1.76 M) resulted in a dry thickness of 256.3 ± 21.1 nm after the same irradiation time, which is equivalent to a roughly six-fold increase of thickness compared to the lower concentration. Thus, the polymerization conditions can be tailored to yield the desired polymer brush thickness in a controlled manner without the need to increase the amount of catalyst. Moreover, we observed similar growth of the poly(DMLA) brushes on PMP (Supporting Information Figure S1). The successful grafting of the poly(DMLA) brushes on silicon substrates was confirmed with XPS (Figure 2C and D). The C1s XPS spectrum of the coated silicon substrate shows the predominance

of a peak at 285.0 eV from C-C and C-H bonds from the polymeric backbone. Moreover, additional bands at 285.9 eV, 287.1 and 288.6 eV stem from C-N, C-O, and O=C-O groups in poly(DMLA). Furthermore, the N1s spectrum of coated silicon substrates yields a peak at 400 eV, which stems from the O=C-N group of the polymer. Polymers of DMLA are soluble in water, methanol, acetone, chloroform, dichloromethane, dimethyl sulfoxide, and dimethyl formamide.

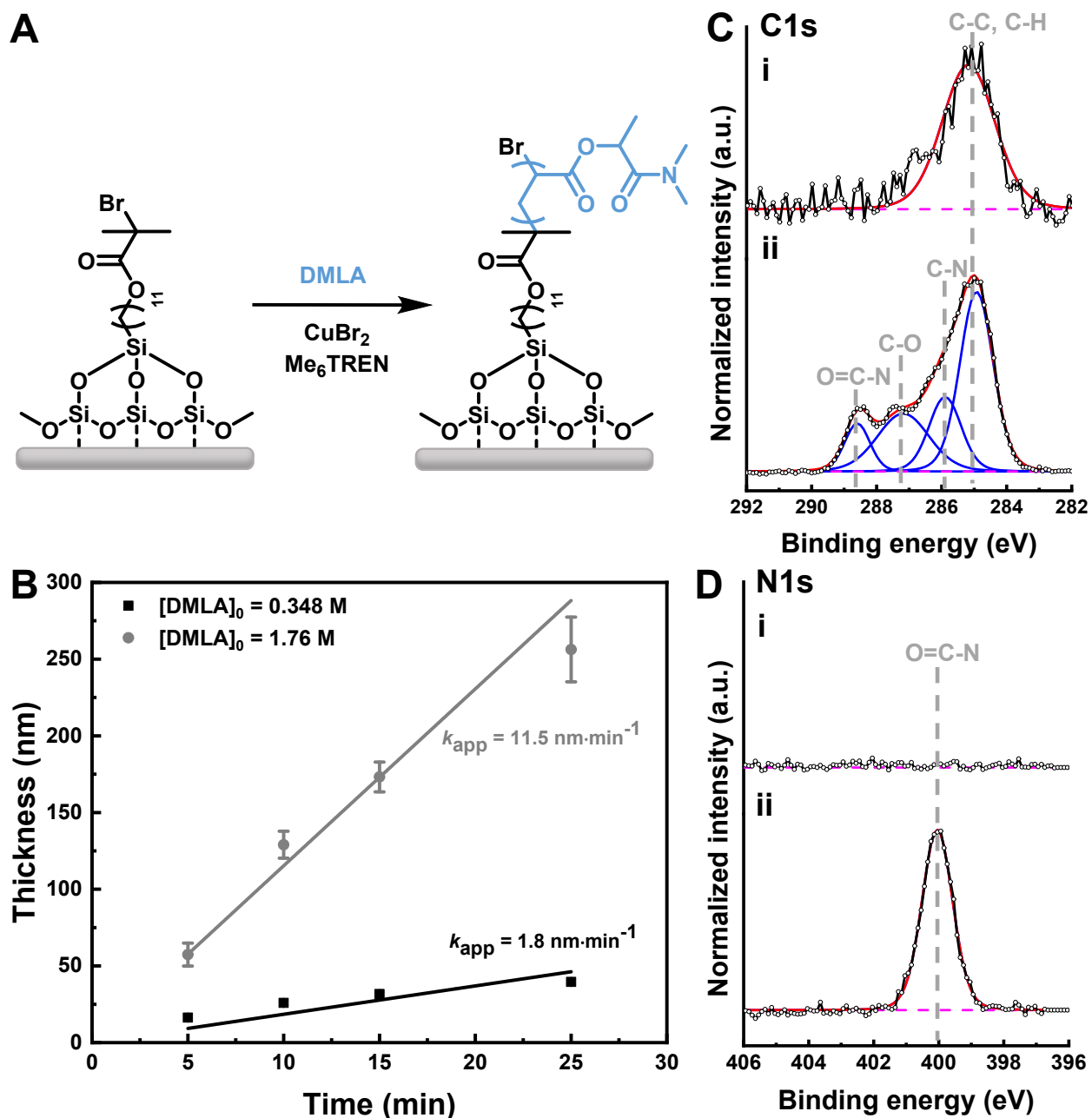


Figure 2. A) Scheme of poly(DMLA) brush synthesis from silicon substrates. B) Kinetics of the photo-induced SET-LRP of poly(DMLA) brushes in DMSO with a monomer concentration of 0.348 M (black squares) and 1.76 M (blue grey dots). ($n = 3$) C) C1s spectrum of i) bare silicon

substrates and ii) silicon substrates coated with poly(DMLA) brushes. D) N1s of i) bare silicon substrates and ii) silicon substrates coated with poly(DMLA) brushes.

We performed a thermodynamic analysis of the surface tension components based on the van Oss acid-base approach to assess the hydrophilicity and surface energy of the poly(DMLA) brushes, and compared them with those of antifouling polymer brushes of fossil-based HPMA (Table 1).^{14, 63} We determined the static contact angles (θ) of two polar (water and formamide) and one nonpolar (α -bromonaphthalene) liquid on poly(DMLA) brushes with different thicknesses and poly(HPMA) brushes. All brushes showed water contact angles significantly lower than 90°, being highly wettable by water. Noteworthy, poly(DMLA) brushes with a higher thickness exhibited even lower water contact angles than poly(HPMA) brushes. The surface tension components such as the Lewis base parameter (γ), Lewis acid (γ^+) parameter and the hydrophobicity (ΔG^H) were determined by using the contact angles of the different fluids on the surfaces (Supporting Information Equations S1-S4). All coatings show a predominance of γ compared to γ^+ , indicating that the brushes are electron donors. ΔG^H is the Gibbs free energy when two surfaces adhere across water. Higher values of $\Delta G^H > 0$ indicate that the surfaces prefer to interact with water. All coatings possess values of ΔG^H of larger than zero, suggesting that the brushes are hydrophilic.

Among them, the poly(DMLA) brushes with a thickness of 30 nm show lower values for ΔG^H than poly(HPMA) brushes with the same thickness. However, upon increasing the thickness of the poly(DMLA) brushes to 140 nm, larger values for ΔG^H than poly(HPMA) brushes are obtained. This higher hydrophilicity might be associated with a stronger water barrier to fouling. We calculated the γ^{LW}/γ known as Chen Qi ratio which was found to correlate with the amount of bacterial adhesion based solely on thermodynamic considerations.⁶⁴⁻⁶⁵ All coatings show similarly low Chen Qi ratios, which is associated with the ability of a coating to reduce or prevent bacterial adhesion. While a thermodynamic analysis of the surface tension components provides insights into certain coating characteristics, a complete characterization would entail the investigation of surface potential and interaction with water molecules.

Table 1. Static contact angles ($n = 3$), surface tension components, hydrophobicity and Chen Qi ratio of poly(HPMA) brushes and poly(DMLA) brushes on PE.

	Static contact angle (°)	Surface tension components (mJm ⁻²)		Hydrophobicity (mJm ⁻²)	Chen Qi ratio
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	θ_w	θ_F	θ_B	γ^{LW}	γ^+	γ^-	γ^{AB}	γ_L	ΔG^H	CQ
HPMA ₃₀	34.8±1.5	35.2±6.2	26.1±4.9	40.8	0.2	46.6	5.4	46.2	27.1	0.9
DMLA ₃₀	34.4±1.7	24.6±1.0	12.0±2.9	44.3	0.5	40.0	8.5	52.8	14.4	1.1
DMLA ₁₄₀	24.3±6.7	28.5±2.4	14.2±2.1	43.9	0.1	53.7	4.7	48.6	35.4	0.8

HPMA₃₀, DMLA₃₀ and DMLA₁₄₀: poly(HPMA) and poly(DMLA) brushes with a thickness of 30 nm and 140 nm; Static water contact angles in water (θ_w), formamide (θ_F), and α -bromonaphthalene (θ_B); γ^{LW} : Lifshitz-van der Waals component, γ^+ : Lewis acid parameter, γ^- : Lewis base parameter, γ^{AB} : Lewis acid-base component, γ_L : Surface energy ($\gamma_L = \gamma^{LW} + \gamma^{AB}$).

Antifouling properties

Following the thermodynamic studies, we assessed the ability of the poly(DMLA) of 30 nm and 140 nm to repel proteins (**Figure 3A**). Firstly, we studied the unspecific adsorption of FXII at physiological concentration (30 $\mu\text{g}\cdot\text{mL}^{-1}$). This zymogen becomes activated upon adsorption to surfaces and sets the initiation of coagulation by the contact system.⁵ The adsorption of FXII in a control sample (Au) was $\Gamma_{\text{FXII}} = 45 \text{ ng}\cdot\text{cm}^{-2}$, which is sufficient to set the beginning of coagulation. On the other hand, both nanoscale coatings of poly(DMLA) brushes completely prevented the fouling from FXII on par with poly(HPMA) brushes, suggesting the potential of the poly(DMLA) brushes to prevent activation of coagulation via the intrinsic pathway.

Moreover, we assessed the interaction of the coatings with two charged single protein solutions as models of positively and negatively charge proteins. Human serum albumin (HSA), which is negatively charged, rapidly adsorbed on gold ($\Gamma_{\text{HSA}} = 63 \text{ ng}\cdot\text{cm}^{-2}$). The thinner poly(DMLA) brushes were already capable of reducing the fouling by 81% ($\Gamma_{\text{HSA}} = 12 \text{ ng}\cdot\text{cm}^{-2}$). On the other hand, no fouling was observed on the thicker poly(DMLA) brushes (140 nm) as for poly(HPMA) brushes. Lysozyme (Lys) was used as the cationic model protein. Similarly to HSA, Lys rapidly adsorbed on gold ($\Gamma_{\text{Lys}} = 69 \text{ ng}\cdot\text{cm}^{-2}$), while poly(DMLA) brushes reduced its adsorption to negligible levels ($\Gamma_{\text{Lys}} = 2 \text{ ng}\cdot\text{cm}^{-2}$) and even undetectable ($\Gamma_{\text{Lys}} = 0 \text{ ng}\cdot\text{cm}^{-2}$) for the thin and thicker brushes and again at the same level as poly(HPMA) brushes ($\Gamma_{\text{Lys}} = 0 \text{ ng}\cdot\text{cm}^{-2}$). We subsequently assessed the resistance of the surfaces to undiluted human BP, one of the most challenging bodily fluids due to its rich protein and biomacromolecule content, which tends to adsorb onto surfaces and may promote secondary adsorption events. Gold substrates exhibited significant fouling ($\Gamma_{\text{BP}} = 212 \text{ ng}\cdot\text{cm}^{-2}$). However, this fouling was notably reduced by 55% to $\Gamma_{\text{BP}} = 96 \text{ ng}\cdot\text{cm}^{-2}$ when coated with 30 nm thick poly(DMLA) brushes. Impressively, increasing the thickness of poly(DMLA) brushes to 140 nm resulted in a remarkable 98% reduction in unspecific BP component adsorption, achieving a level of $\Gamma_{\text{BP}} = 5 \text{ ng}\cdot\text{cm}^{-2}$ comparable to the

repellency achieved with poly(HPMA) brushes ($\Gamma_{BP} = 1 \text{ ng}\cdot\text{cm}^{-2}$). As it was already determined that the hydrophilicity of the brushes is similar on both brush thicknesses, the improved repellency of BP on the thicker poly(DMLA) brushes is most likely attributable to the increased entropic barrier and therefore the steric repulsion against the highly diverse components of BP upon increasing the polymer chain length. The adsorption of proteins on a polymer brush surface would result in an unfavorable loss of entropy, thus longer polymer chains provide an improved steric shielding of the surface. Therefore, a thickness of 140 nm was selected for subsequent experiments. These findings underscore the exceptional repellent properties of poly(DMLA) brushes, which rival the performance of the best fossil-based antifouling polymer brushes. This demonstrates their potential to replace such coatings in sustainable future research endeavors.^{20-21,}

²⁵⁻²⁶ Furthermore, we compared the non-specific protein adsorption on bare gold with that on PMP-modified gold and PMP-modified gold with poly(DMLA) brushes with a thickness of 140 nm (Supporting Information Figure S1). The similar results indicate a comparable performance of the poly(DMLA) brushes on polymeric substrates.

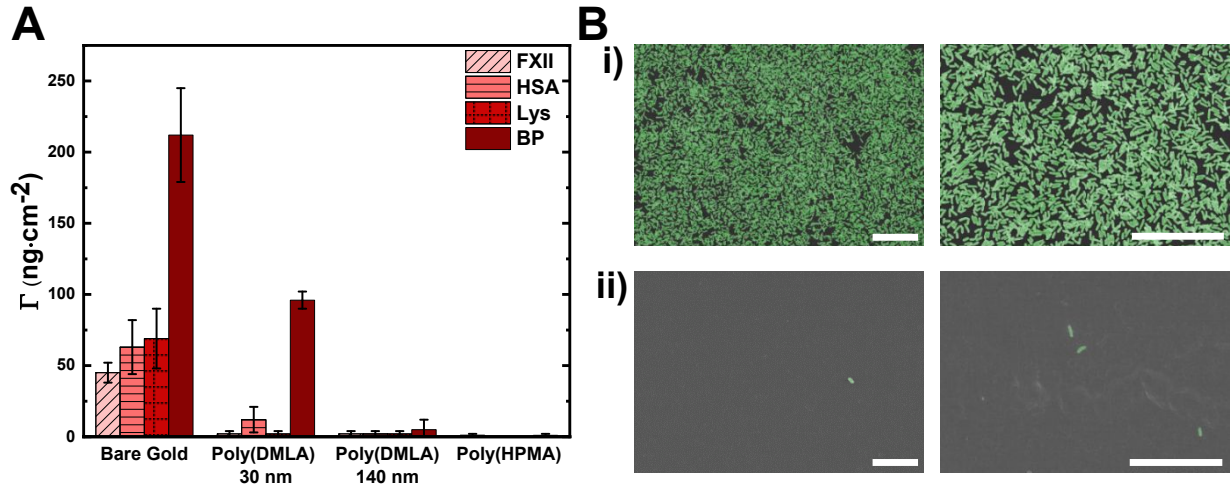


Figure 3. A) Fouling of FXII ($30 \mu\text{g}\cdot\text{mL}^{-1}$ in PBS), HSA ($5 \text{ mg}\cdot\text{mL}^{-1}$ in PBS), Lys ($1 \text{ mg}\cdot\text{mL}^{-1}$ in PBS), and undiluted human BP on bare gold sensor slides and gold sensor slides coated with poly(DMLA) brushes with thicknesses of 30 nm and 140 nm and poly(HPMA) brushes. ($n = 3$) B) FESEM images of (i) bare PE and (ii) PE coated with poly(DMLA) brushes with a thickness of 140 nm after 24 h of exposure to *E. coli*. The images were false colored to enhance visualization. (scale bar = 20 μm)

Furthermore, we studied the potential of poly(DMLA) brushes to protect PE surfaces from bacterial colonization. PE is a polymeric material utilized mainly for urinary catheters and orthopedic implants and is particularly susceptible to infections due to bacterial colonization as its initial stage. Bare PE and PE coated with poly(DMLA) brushes were exposed to *E. coli* for 24 h to study the potential of poly(DMLA) brushes to suppress colonization, the initial stage of bacterial

infections (Figure 3B and Supporting Information S24). FESEM images of bare PE revealed a high coverage of the surfaces with *E. coli*, whereas the coated PE showed barely any bacteria present on the surface. Concluding, the poly(DMLA) brushes successfully prohibit the adhesion and colonization of *E. coli* on PE, proving strong repellency of bacteria, which is essential to prevent bacterial infections on medical devices.

Compatibility with blood

Having established the remarkable antifouling characteristics of poly(DMLA) brushes, our next objective was to assess their compatibility with human blood, specifically their capacity to prevent surface-induced coagulation activation. To assess this, we chose PMP as a substrate, as it is utilized for extracorporeal membrane oxygenators – devices that oftentimes fail due to their lack of hemocompatibility. incubated both Bare PMP and PMP coated with poly(DMLA) brushes were incubated with slightly heparinized human blood ($0.75 \text{ IU} \cdot \text{mL}^{-1}$) for 2 h at 37°C under static conditions, as illustrated in **Figure 4A** and compared it to poly(HPMA) brushes. The utilization of heparin was necessary, as blood coagulation typically commences immediately upon withdrawal from the circulatory system, potentially confounding the assessment of surface performance. The

low dose of heparin employed in this study served to delay the onset of coagulation, allowing for a focused analysis of surface effects. After incubation, the blood was collected and the rinsed substrates were analyzed by FESEM. The experiments were carried out in triplicates with not pooled blood of three healthy donors.

PMP, the material of choice for extracorporeal oxygenator membranes, was found to serve as a nucleation site for blood coagulation, as illustrated in Figure 4B. After a 2-hour incubation, a firmly attached macroscopic clot formed on the surface of the PMP substrate (Figure 4B). Further microscopic examination using FESEM revealed the development of a fibrin network and the strong adhesion of leukocytes, both indicative of the surface's role in initiating blood coagulation (Figure 4C, Supporting Information S32 and S43). This underscores the potential for surface coatings to enhance hemocompatibility and, consequently, the performance of this polymeric substrate. Specifically, the application of poly(HPMA) and poly(DMLA) brushes demonstrated a marked improvement, with minimal macroscopic thrombus formation observed in the case of the former and none in the latter, as visually depicted in Figure 4B. A more detailed examination through FESEM analysis revealed that surfaces coated with poly(DMLA) brushes remained free of fibrin networks and leukocytes, in contrast to poly(HPMA) brush-coated surfaces where some

leukocyte adhesion was observed without the formation of a fibrin network (Figure 4C, Supporting Information S32). But why did leukocytes adhere to poly(HPMA) brushes and not to poly(DMLA) brushes if both repel proteins? Complement system is part of body's defense system, which can also become activated by surfaces⁶⁶ and cause increased inflammatory response and activation of coagulation while promoting leukocyte binding. For assessing complement system activation, we quantified C5a concentration in the blood, a pivotal marker released into the blood stream during activation (Figure 4D). C5a functions as a mediator between the complement system and the coagulation pathway in blood, thereby significantly contributing to thrombus formation through the extrinsic pathway (triggered by leukocyte activation).⁶⁶ We discovered that poly(HPMA) brushes, one of the most antifouling coatings available, caused a two-fold increase in C5a concentration in blood compared to PMP and poly(DMLA) brushes. This difference in activation of this biomarker, may be associated with the presence of hydroxyl groups in poly(HPMA).^{36, 42} This informs that complete repellency of proteins is not a perfect proxy for the blood compatibility of a surface.

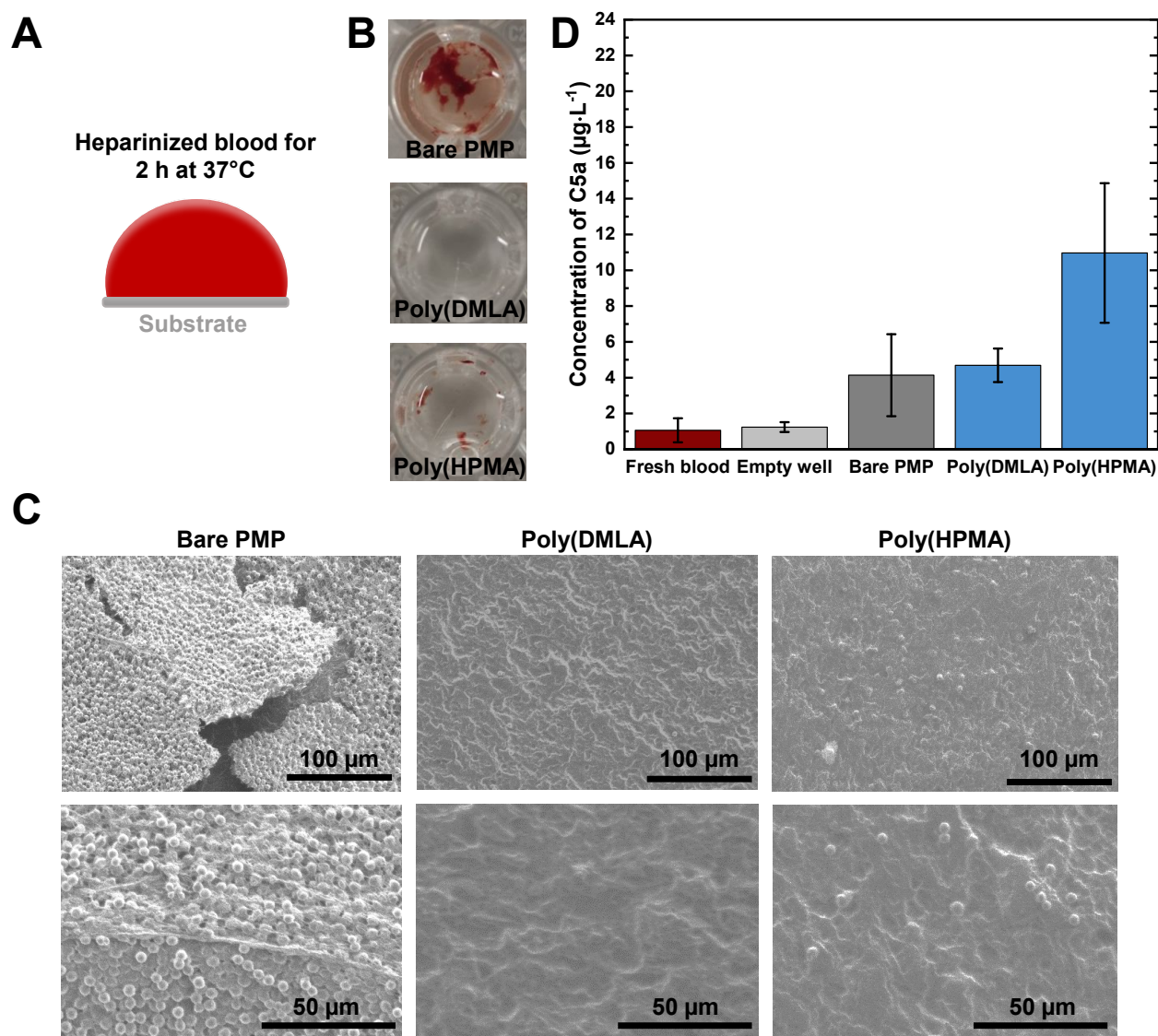


Figure 4. A) Schematic depiction of static incubation of the substrates with heparinized full human blood for 2 h at 37°C. B) Macroscopic images of the substrates after static blood contact with visible clot formation on bare PMP. C) FESEM images of bare and coated PMP substrates after static blood contact for donor 1. D) Concentration of complement activation marker C5a after blood contact with the substrates (n=3).

Conclusions

We introduced novel polymer brushes that combine their green solvent based ~~bio-based~~ origin with outstanding antifouling properties on par with those of fossil-based polymer brushes for the first time. The polymer brushes could be grown in a controlled manner and showed strong hydrophilicity as demonstrated by thermodynamic analysis of the surface tension components. Challenging the ~~bio-based-novel~~ polymer brushes with FXII, charged single protein solutions, and BP proved their superior repellency, being at the same level as antifouling fossil-based polymer brushes. Moreover, the ~~bio-based~~ polymer poly(DMLA) brushes showed an almost complete repellency against *E. coli*, demonstrating their potential for coatings for biomedical devices. Remarkably, upon exposure to whole human blood, the ~~bio-based~~ polymer poly(DMLA) brushes demonstrated their prevention of thrombus formation on the surface, at the same level as fossil-based polymer brushes. Moreover, the comparison of the activation of the complement system revealed a superior performance of poly(DMLA) brushes compared to fossil-based poly(HPMA) brushes. We envision our coating to significantly contribute to the future of medical device coatings and aid suffering patients even after depletion of resources.

ASSOCIATED CONTENT

Supporting Information. Supporting information is available free of charge.

Experimental details and data (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. The synthesis of DMLA was conducted by M.P., while the synthesis of the polymer brushes, characterization and determination of antifouling properties was performed by J.E.. The static blood experiments were conducted by J.E., F.R. and O.G.. The analysis of the concentration of C5a in plasma was conducted by J.E. and L.W.. Data analysis was carried out by J.E., M.P., L.W. and C.R.-E.. Conceptualization and strong intellectual contribution was provided by G.L. and C.R.-E..

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ABBREVIATIONS

BP, blood plasma, DMLA, *N,N*-dimethyl lactamide acrylate, *E. coli*, *Escherichia coli*, EDTA, Ethylenediaminetetraacetic acid, FESEM, Field emission scanning electron microscopy, FXII, coagulation factor XII, HPMA, *N*-hydroxypropyl methacrylamide, HSA, Human serum albumin, Lys, Lysozyme, PBS, Phosphate buffered saline, PE, poly(ethylene), PMP, poly(4-methyl-1-

pentene), SET-LRP, single electron transfer-living radical polymerization, SPR, surface plasmon resonance, XPS, X-ray photoelectron spectroscopy

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