ADVANCED MATERIALS

# Supporting Information

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# Superdurable Coating Fabricated from a Double-Sided Tape with Long Term "Zero" Bacterial Adhesion

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# Supporting Information

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**This supporting information file includes**:

Experimental sections

Figure S1 to S11

Movie S1 to S6

**Experimental Sections**

***Materials.*** Polyurethane coupons were purchased from BioSurface Technology. Stainless steel strips were purchased from McMaster-CARR. All solvents and chemicals were purchased from Sigma-Aldrich and were used as received.

***Fabrication of DURA-Z tapes.*** DURA-Z tapes were fabricated by in-situ forming of a thin layer of PCBAA hydrogel on commercially available polypropylene liner. Briefly, 5 mg 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (I-2959) initiator and 30 mg N,N'-Methylenebis(acrylamide) (MBAA) crosslinker were dissolved in 10 ml DI water. 300 mg CBAA monomer was then dissolved in 300 μl initiator/crosslinker solution to prepare a pre-gel solution, which was transferred to between two polypropylene liners adhered to glass slides separated by a Teflon spacer (1 mm in thickness). UV was applied for a few minutes to crosslink the PCBAA hydrogel. DURA-Z tapes were obtained by removing the hydrogel layer along with the liners from glass slides.

***Fabrication of DURA-Z coating.*** The polyurethane (PU), stainless steel, glass and wood substrates were cleaned with alcohol and dried in air (wood and metal were pre-treated by sandpaper to obtain rough surfaces, and glass was pre-coated with a thin layer of EVO-stik glue). Then one drop of cyanoacrylate superglue (ACE Hardware Corp.) was applied onto the dried substrate, followed by pressing the DURA-Z tape (hydrogel layer facing down) onto the superglue for a few seconds. One hour was then allowed to completely solidify the superglue. The liner on top of the hydrogel was removed, and the glued coating was transferred to DI water for equilibrium. 20 mins later, the

DURA-Z coating was polished either by a small shovel or tweezer, leaving the surface with a thin layer of coating.

***Scanning Electron Microscope (SEM) imaging.*** All samples were dried in vacuum and then coated with nano-gold using an SEEVac Conductive IV sputter coater before imaged by a JSM - 6510LV SEM. For sectional imaging, all tested coupons (bare PU, superglue coated and DURA-Z coated substrates) were vertically cut into half using a sharp scalpel to expose the cross-section.

***Atomic-force Microscopy (AFM) imaging.*** AFM imaging of the bare PU, superglue coated and DURA-Z coated substrates was conducted on a Dimension 3100 AFM from VEECO. All samples were vacuum dried before imaging. The coating thickness and morphology were measured in the air through the tapping mode using silicon probes (VEECO) with a nominal frequency of 150 kHz. The AFM images were analyzed using Nanoscope software version 5.12 (VEECO).

***Infra-red (IR) spectroscopy.*** Bare PU, superglue coated and DURA-Z coated substrates were vacuum dried before testing. PCBAA hydrogel was freeze-dried before testing. All samples were characterized on a NICOLET 6700 IR (Thermo Electron Corporation) equipped with an attenuated total reflectance (ATR) accessory. For the PU surface, the peak at 3350 $cm^{-1}$ represented the stretching vibration of –OH. The peak at 2870 $cm^{-1}$ and 1200 $cm^{-1}$ represented the $–CH_2–$ and $–CH_3$ groups. The peak at 1710 $cm^{-1}$ represented the –CO– group. The peak at 1580 $cm^{-1}$ represented the –OCONH– group. The peak at 1450 $cm^{-1}$, 1500 $cm^{-1}$ and 1600 $cm^{-1}$ represent the conjugated –C–C–C=C– on the aromatic structure. For the surface with glue, the peak at 2350 $cm^{-1}$ was the characteristic peak of conjugated –CN group. The peak at 1780 $cm^{-1}$ represented the –OCO– group. The peak at 1270 $cm^{-1}$ represented the twist vibration of $–OCH_3$ group. The peak at 2900 $cm^{-1}$, 1320

$cm^{-1}$ and 1100 $cm^{-1}$ represented the $–CH_2–$ and $–CH_3$ groups. The peak at 850 $cm^{-1}$ represented the unreacted $=CH_2$ group. For the DURA-Z coating and PCBAA hydrogel, the peak at 1650 $cm^{-1}$ represented the –CO– group. The peak at 1510 $cm^{-1}$ represented the –OCONH– group. The big peak between 3000 $cm^{-1}$ – 3300 $cm^{-1}$ represented the combination of $–COO^-$ and quaternary ammonium groups. The peak at 2930 $cm^{-1}$, 1340 $cm^{-1}$ and 1070 $cm^{-1}$ represented the $–CH_2–$ and $–CH_3$ groups. The peak at 1390 $cm^{-1}$ was the characteristic peak for the symmetrical vibration of $–COO^-$ group.

***Contact angle.*** The water contact angle on bare PU, superglue coated and DURA-Z coated substrates was conducted using a KSV contact angle instrument equipped with a camera at room temperature and ambient humidity. 2 μl of water was dropped on different surfaces and water contact angle was calculated using the CAM2008 software.

***Durability tests in the aqueous environment.*** PBS shearing was created on a stirring plate by a 5-cm stirring bar at 1500 rpm in a beaker (D = 10 cm). The tested samples (DURA-Z coating on PU, glass, wood and stainless steel substrates) were firmly clamped and positioned at the inner wall of the beaker, and subjected to the continuous shear stress of 202 G. Body temperature (37 ℃) was controlled by the stirring plate and DI water was added to compensate the evaporation every day to maintain standard PBS concentration. In water flushing test, water was cycled by an electric pump with a flow rate of 42.8 ml/s. In the dry/wet stability test, the DURA-Z coating was completely dried under air flow and re-hydrated in water. 10 dry/wet cycles were conducted before protein adsorption test. Protein adsorption test was conducted on the samples before and after the durability challenge.

***Mechanical damage tests.*** For the knife-scratch test, DURA-Z coating on PU coupon was randomly cut by a sharp scalpel. The abrasion test was conducted on an INSTRON compressor equipped with

a precise loading detector. Sandpapers (Gator waterproof sanding 400 grit) were fixed on both sides of the compressor and the pressure was calculated by the loading divided by the area of PU coupon. One cycle of abrasion contains pushing and pulling the coupon for 1-cm displacement back and forth under a given pressure. The samples were subjected to 20 cycles of abrasion. Water wettability and protein adsorption tests were conducted on the samples before and after the mechanical damage challenge.

***Friction index measurement.*** The sandpaper was fixed on a ruler and the PU coupon with or without DURA-Z coating was placed on one end of the ruler, which was then slowly lifted up. Immediately when the coupon could slide down spontaneously, the lifting angle (θ) of the ruler was recorded. The friction index between the coupon and sandpaper was be calculated by tangent θ.

***Platelet adhesion test.*** The rats' platelet rich plasma (PRP) was collected and prepared as described previously.[1] The rat blood was collected and transferred to a centrifuge tube containing 1 mg/ml heparin sodium. The blood was then centrifuged for 10 min at 700 RCF. The resulting plasma in the top layer was collected and applied on PU coupon, superglue surface and DURA-Z coating overnight at 37 ℃. After PRP challenging, the sample surfaces were fixed in 2.5% glutaraldehyde, dehydrated in gradient ethanol and dried under vacuum. The adhered platelet was visualized and calculated under SEM.

***Protein absorption test (ELISA).*** Human fibrinogen (Fg, Sigma-Aldrich) adsorption on a variety of substrates (bare substrates, superglue coated and DURA-Z coated substrates) was measured using an enzyme-linked immunosorbent assay (ELISA). All samples were incubated with 1mg/ml Fg for 10 minutes at room temperature, followed by 5 washes with PBS buffer. They were then incubated with

1 mg/ml bovine serum albumin solution for 10 minutes at room temperature with 5 washes again with PBS buffer. The samples were removed from the fifth PBS wash and transferred to new wells. They were next incubated with a 1:200-dilution of horseradish peroxidase (HRP)-conjugated anti-fibrinogen (USBiological, Life Sciences) in PBS for 10 minutes, followed by another 5 washes with PBS buffer. After the fifth wash, the samples were transferred to new wells and SIGMAFAST OPD (Sigma-Aldrich) was added to each well. They were incubated in the OPD solution for 30 minutes in the dark. The supernatant was removed from each test well, transferred to a 96-well plate, and its absorbance at 490 nm was measured using a UV-VIS spectrometer (Thermo Scientific Multiscan Go). All samples were measured in triplicate.

***Biofilm resistance of DURA-Z coating.*** *E. Coli*, *S. Aureus* and *C. Albicans* bacteria were cultured for 24 h at 37 ℃ on agar plates (Luria-Bertani (LB) agar for *E. Coli*, Tryptic Soy (TS) agar for *S. Aureus* and Yeast Mold (YM) Agar for *C. Albicans*). One colony was picked and cultured in 20 mL of broth medium (Luria-Bertani (LB) broth for *E. Coli*, Tryptic Soy (TS) broth for *S. Aureus* and Yeast Mold (YM) broth for *C. Albicans*) overnight at 37 ℃ on a shaker (Standard Analog Shaker, VWR) at 200 rpm. The resulting culture was used to inoculate a second culture in 50 mL of broth medium until an optical density (OD) of 0.8 at 600 nm was reached. The bacteria were collected by centrifugation at 8,000 x g for 10 min at 4 ℃, washed three times with sterile PBS (pH 7.4) and finally suspended in broth to get a final concentration of $1.05\times10^9$ cells per ml (OD = 1.31 at 600 nm) for biofilm formation test. In the dynamic method, PU (stainless steel, wood, and glass) substrates, superglue coated substrates and DURA-Z coated substrates were placed in bacterial culture medium (broth medium) containing highly concentrated bacteria ($1.05\times10^9$ cells per ml) at

37 ℃ for 30 days under continuous shaking (300 rpm). The culture medium was gently refreshed every two days, and the bacterial density within the refreshed medium was kept at 1.05×109 cells per ml. In the static method, PU substrates, superglue coated substrates and DURA-Z coated substrates were stationarily placed in bacterial culture medium (broth medium) containing highly concentrated bacteria ($1.05\times10^9$ cells per ml) at 37 ℃ for 30 days. Bacteria were allowed to settle on the surface of the substrates through gravity. The culture medium was gently refreshed day by day, and the bacterial density within the refreshed medium was kept at $1.05\times10^9$ cells per ml. After long-term incubation, all substrates were rinsed with PBS for 30 mins (samples tested in the static method were also imaged without rinsing). After the challenging periods, the substrates were then immersed in the fix solution of 2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M sodium phosphate buffer and were then dehydrated in a gradient ethanol series and dried in vacuum before SEM imaging. Adhered bacteria density was counted by averaging 6 randomly selected areas (100 $\mu m^2$ each).

## References

[1] L. Kucuk, H. Gunay, O. Erbas, U. Kucuk, F. Atamaz, E. Coskunol, *Acta. Orthop. Traumato.* **2014**, 48, 449

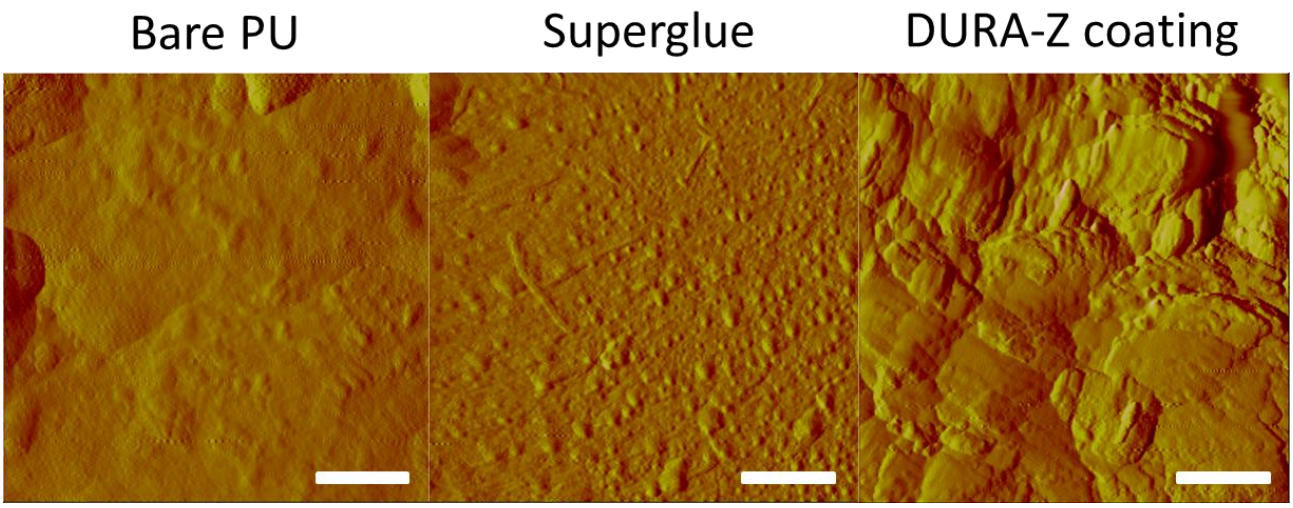


**Figure S1.** AFM images of bare PU substrate, superglue and DURA-Z coated PU substrates. Scale bar = 50 μm.

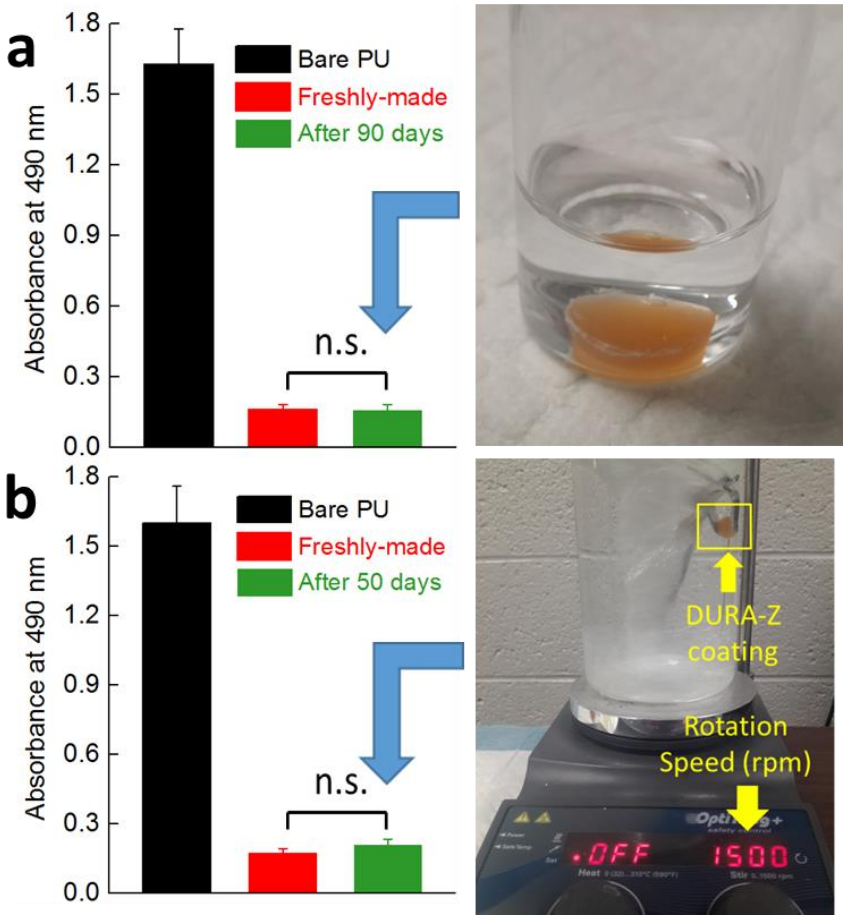


**Figure S2**. Antifouling property of DURA-Z coating after durability tests. (a) 3-month incubation in water and (b) 50 days exposure to PBS shearing at room temperature. Data are presented as mean of replicates (n=3) ± standard deviation. Statistical analysis: unpaired, two-tailed t-test, n.s.: no significant difference at $P > 0.05$, meaning the great anti-fouling property was retained.

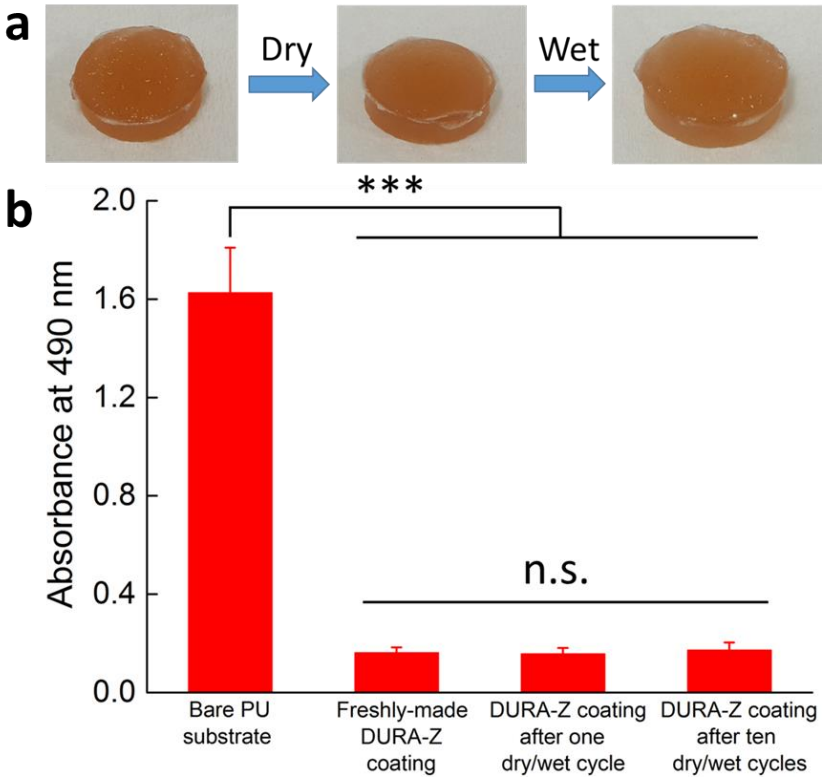


**Figure S3.** (a) Dry/wet cycle challenge on DURA-Z coating on PU coupon, DURA-Z coating was completely dried under air flow for 1h and then placed in water to re-hydrate. (b) anti-fouling property of DURA-Z coating after dry/wet cycles. All data are presented as mean of replicates (n=3) ± standard deviation. Statistical analysis: one-way ANOVA with Bonferroni multi-comparison. ***: $p < 0.0001$. n.s.: no significant difference, $p > 0.5$

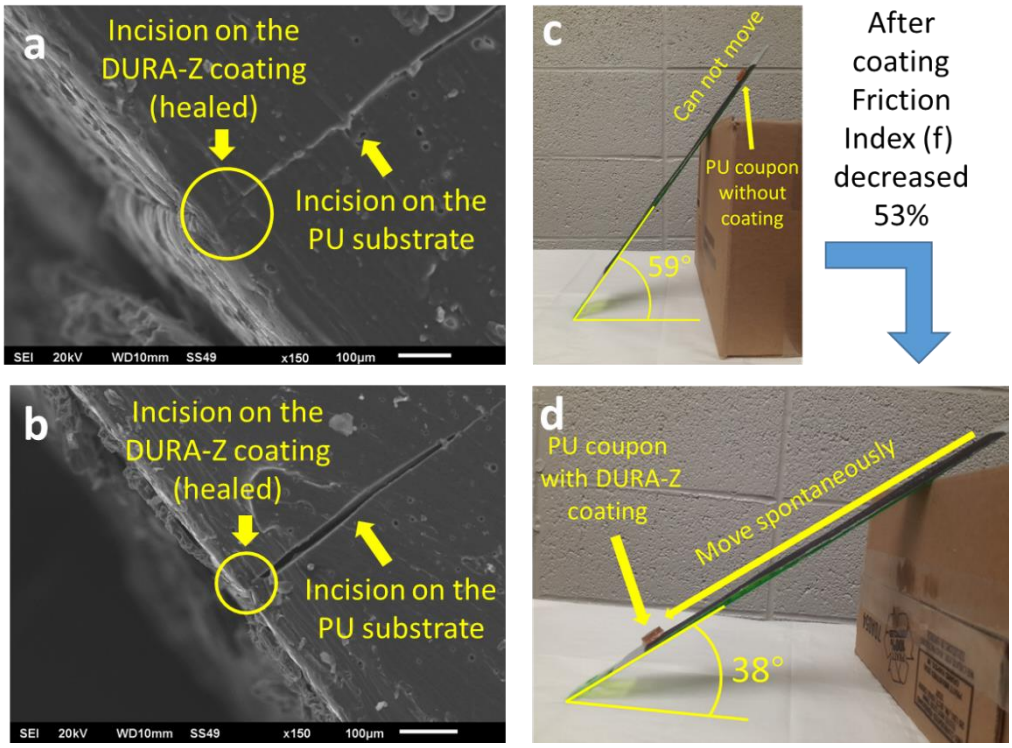


**Figure S4.** (a) and (b) DURA-Z coating was able to heal in water after knife scratch. (c) and (d) DURA-Z coating on PU substrate was able to decrease more than 53% in friction index against sand paper (without coating: $f$ = tan (59 °) = 1.664; with DURA-Z coating, $f$ = tan (38 °) = 0.781)

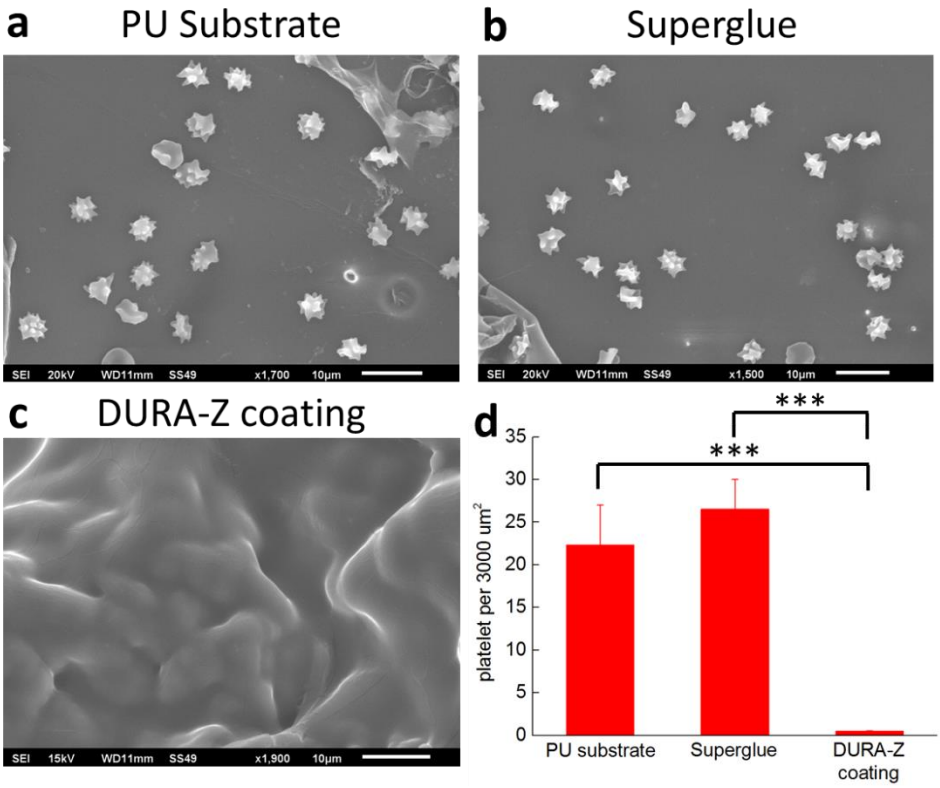


**Figure S5.** Platelet adhesion after platelet-rich plasma challenge on (a) PU substrate, (b) superglue surface and (c) DURA-Z coating, and (d) calculated platelet density on different surfaces. Data are presented as mean of replicates (n=3) ± standard deviation. Statistical analysis: one-way ANOVA with Bonferroni multi-comparison. ***: $p < 0.0001$.

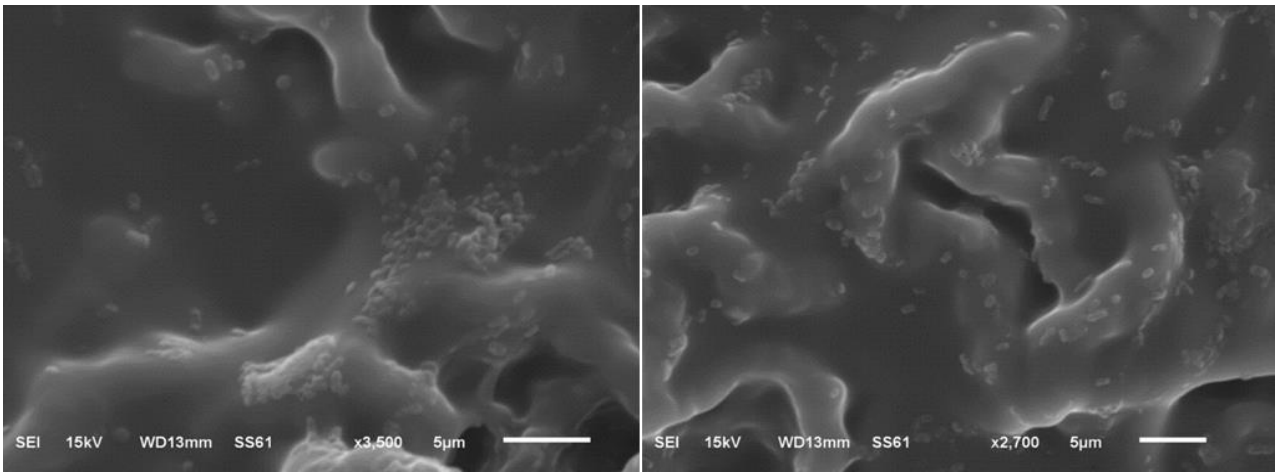


**Figure S6.** representative SEM images showing bacteria adhesion on DURA-Z coating after 30 days of culture with bacteria at static condition without any rinsing. Scale bar = 5 µm.

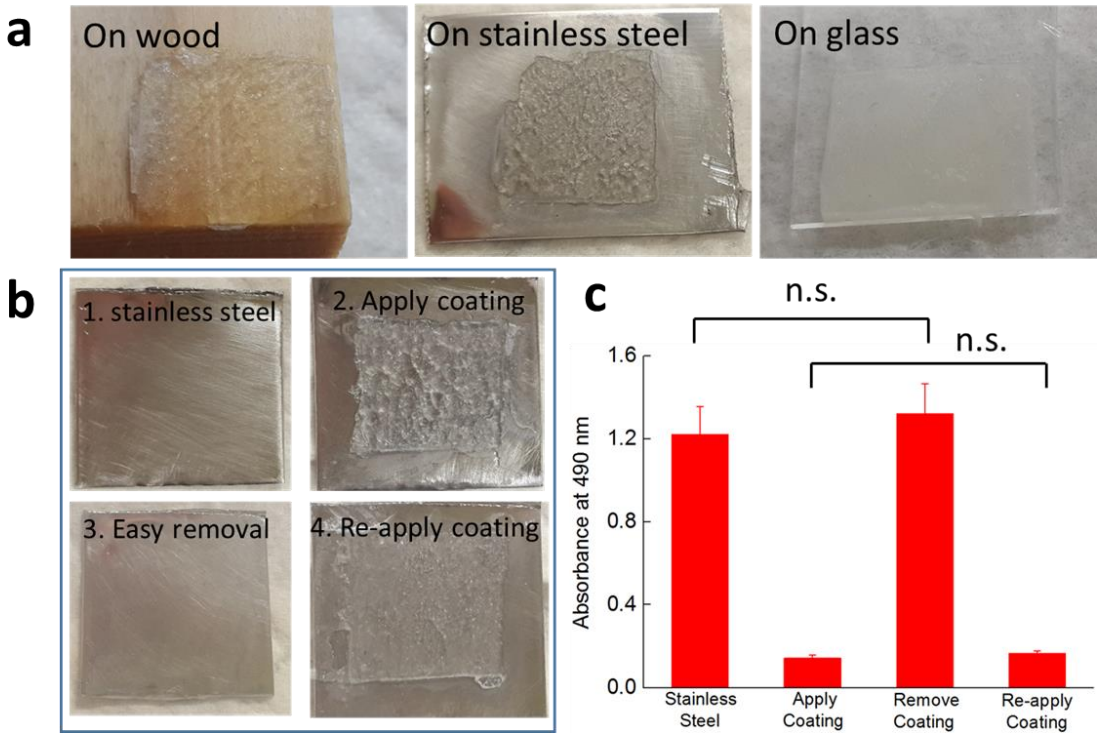


**Figure S7.** (a) DURA-Z coating on wood, stainless steel, and glass substrates. (b) Easy removal and re-application of DURA-Z coating on stainless steel substrate, and (c) corresponding antifouling property. All data are presented as mean of replicates (n=3) ± standard deviation. Statistical analysis: unpaired, two-tailed t-test, n.s.: no significant difference at $P > 0.05$.

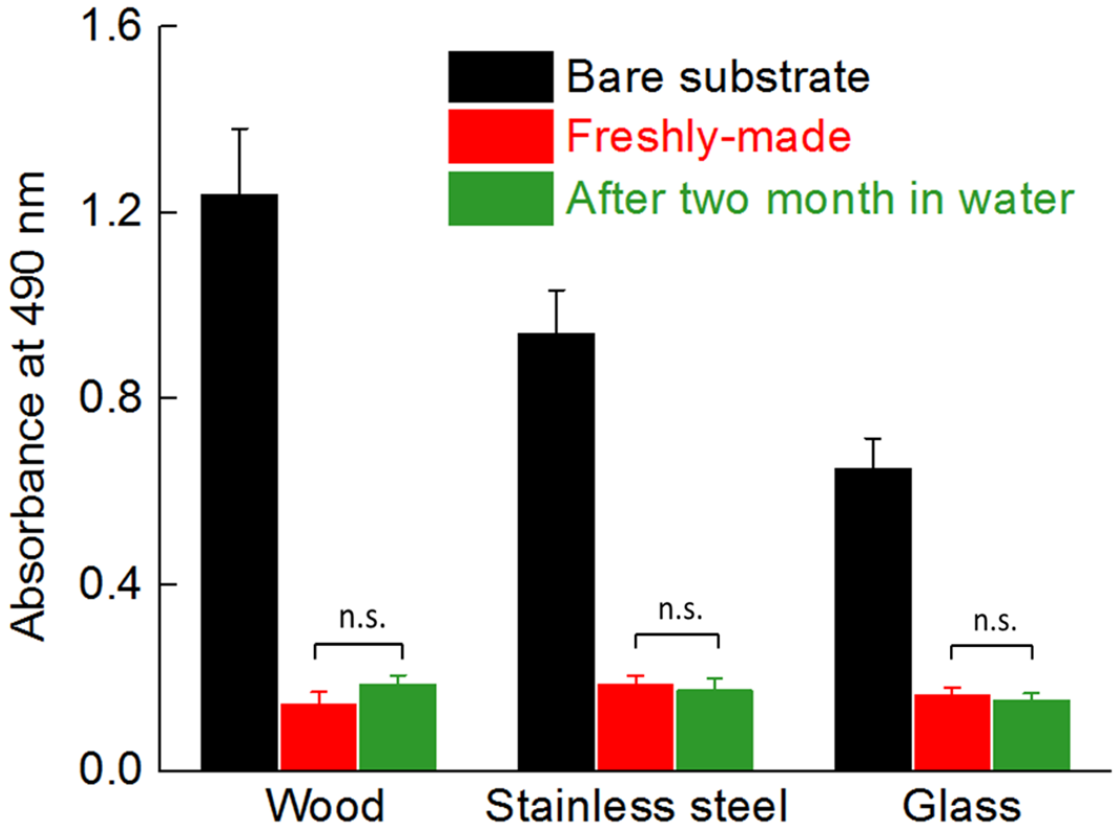


**Figure S8.** Antifouling property of DURA-Z coating on wood, stainless steel and glass substrates after two-month incubation in water. All data are presented as mean of replicates (n=3) ± standard deviation. Statistical analysis: unpaired, two-tailed t-test, n.s.: no significant difference at $P > 0.05$, meaning the great anti-fouling property was retained.

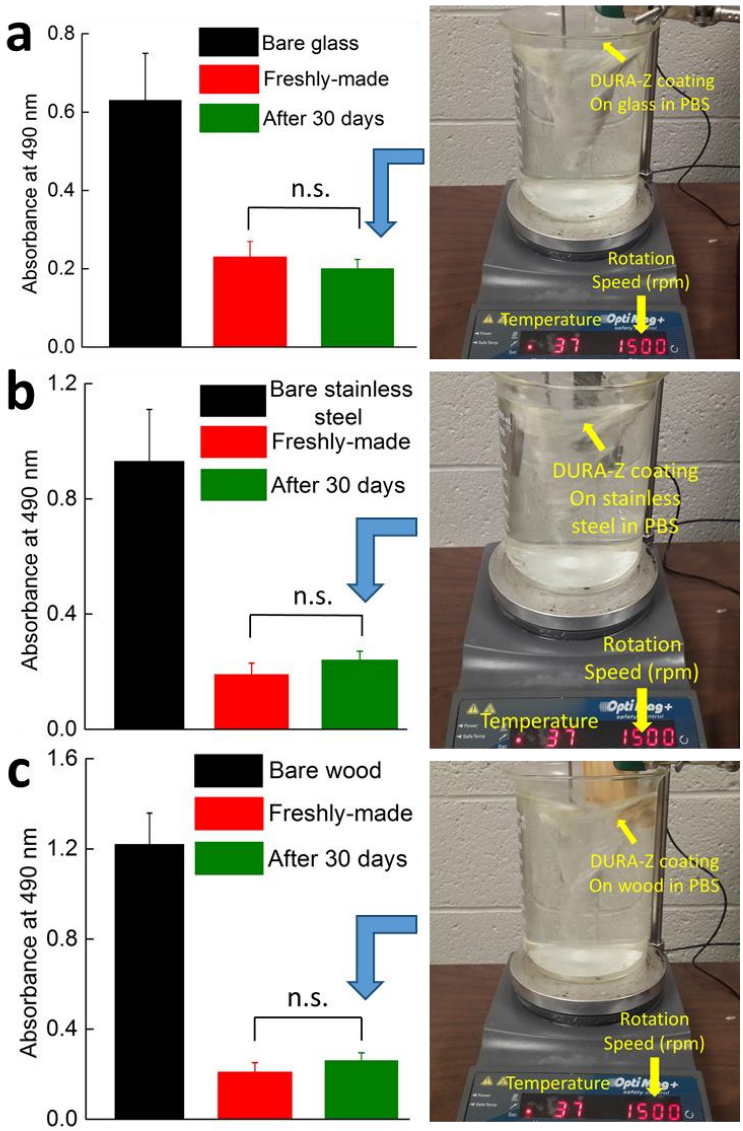


**Figure S9**. Anti-fouling property of DURA-Z coating on (a) glass, (b) stainless steel and (c) wood substrates after durability test of 30 days exposure to PBS shearing under body temperature, All data are presented as mean of replicates (n=3) ± standard deviation. Statistical analysis: unpaired, two-tailed t-test, n.s.: no significant difference at $P > 0.05$

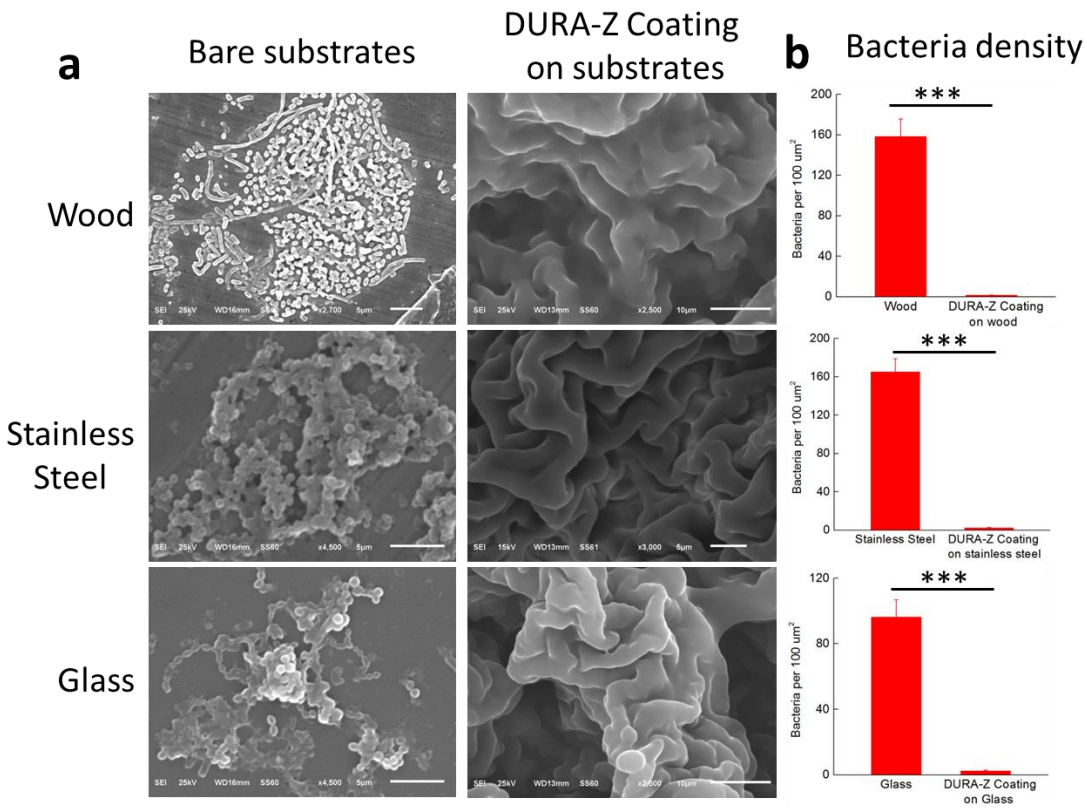


**Figure S10.** (a) representative SEM images of bacteria adhesion on wood, stainless steel, glass and DURA-Z coating on these substrates after 30 days of co-culture with bacteria at shaking condition, and (b) calculated bacterial density on wood, stainless steel, glass and DURA-Z coatings on these substrates. All data are presented as mean of biological replicates (n=6) ± standard deviation. Statistical analysis: unpaired two-tailed t-test, ***: $p < 0.0001$. scale bar = 10 μm.

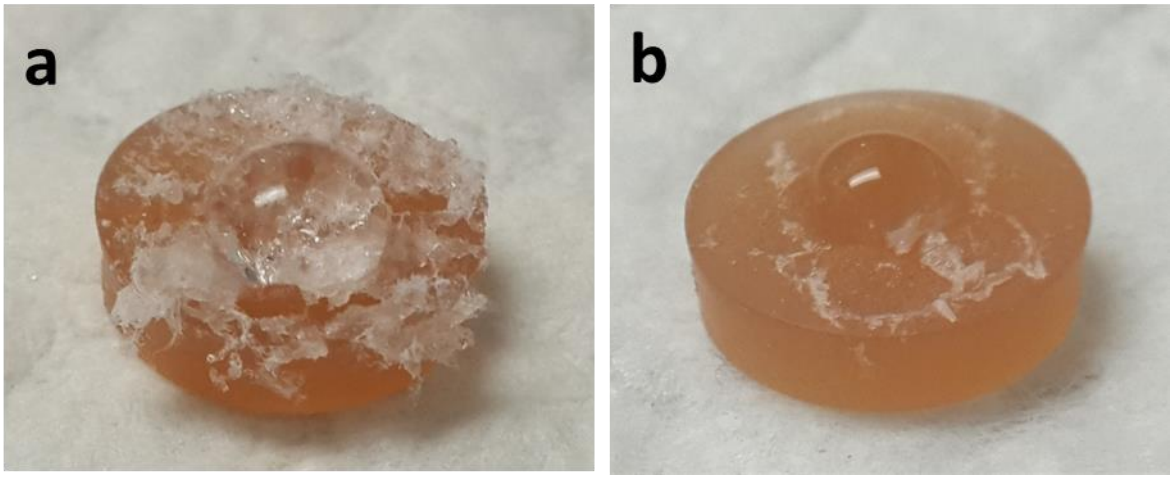


**Figure S11.** (a) PCBAA polymer powder and (b) PCBAA water solution were glued on PU substrates. The coated surface showed hydrophobic nature after incubation in water, as indicated by the non-spread water droplet.

**Movie S1**

Fabrication of DURA-Z coating on polyurethane substrate. The water-drop spreading test was conducted on DURA-Z coated and uncoated substrates.

**Movie S2**

Fabrication of DURA-Z coating on stainless steel substrate. The water-drop spreading test was conducted on DURA-Z coated and uncoated substrates.

**Movie S3**

Knife-scratch test on DURA-Z coating. The water-drop spreading test was conducted before and after the knife-scratch challenge.

**Movie S4**

Abrasion test on DURA-Z coating. The coupon with both sides coated was moved back and forth (displacement = 1 cm) for 20 circles under 570 kPa pressure between two sandpapers. The water-drop spreading test was conducted on both sides of the coating before and after the abrasion challenge.

**Movie S5**

Abrasion test on uncoated PU substrate. Manpower was not able to move the coupon under 50 kPa pressure between sandpapers.

**Movie S6**

Easy removal of DURA-Z coating from stainless steel substrate by immersing the DURA-Z coating in acetone for one hour.