Supporting information

Antifouling and Rechargeable Antibacterial Polyurethane Modified with N-halamine for Dental Unit Waterlines

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EXPERIMENTAL SECTION

Materials

Anhydrous toluene, dibutyltin dilaurate (DBTDL), hexamethylene diisocyanate (HDI), anhydrous ethanol, glutaraldehyde and polyethylene glycol (PEG) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 5,5-Dimethylhydantoin (DMH), starch, potassium iodide, dibutylamine, 2-propanol, bromophenol blue, N,N-dimethylformamide, sodium thiosulfate, acetic acid, PBS buffer, HCl, and sodium hydroxide were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Bleach solution was purchased from Shanghai Jiameng Industrial Co., Ltd. (Shanghai, China). Bacterial Live/Dead Staining Kit was purchased from Beyotime Biotechnology (Shanghai, China).

Characterization

Physical and chemical properties of functionalized PU samples were characterized by ATR-FTIR (NEXUS-670 FTIR spectrometer, PerkinElmer, USA). The above samples were pasted onto a silicon wafer, and the surface morphology of the films was analyzed using field emission scanning electron microscopy (FSEM) (S-4800, Hitachi, Japan). Thermogravimetric analysis (TGA) was performed using a TGA 8000 thermogravimetric analyzer (PerkinElmer, Waltham, MA, USA). The hydrophilicity of each group of samples was detected using a contact angle tester (SL200B, Solon, China), and each group was measured three times. UV-vis spectra were collected using a Lambda 25 UV-vis spectrophotometer (Perkin Elmer, Waltham, MA).

Preparation of chlorinated PU-HDI-PEG-DMH

The PU-HDI-PEG film was dispersed in 50 mL of anhydrous toluene containing 2 g of DMH,

and the mixture was stirred under a nitrogen atmosphere at 70 °C for 12 h. After the reaction, the film was washed three times with anhydrous toluene and ethanol, respectively, and then dried under vacuum. The PU-HDI-PEG-DMH film was immersed in a 10% bleach solution at room temperature for 45 min with continuous stirring. Subsequently, the sample was thoroughly rinsed with deionized water, air-dried, and stored in a desiccator for 48 h to reach a constant weight.

Statistical analysis

The experiments were repeated for at least three times and data was shown containing sample size (n). The data are presented as mean ± standard deviation (SD). The significant differences were indicated as follows: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. The significant differences were analyzed using one-way ANOVA (between more than two groups). Statistical analysis was conducted using GraphPad Prism 8 Software.

Preparation of PU-HDI

Forty milliliters of anhydrous toluene was poured into a 250 mL single-neck round-bottom flask. Subsequently, 4 g of hexamethylene diisocyanate (HDI) and 0.04 g of dibutyltin dilaurate (DBTDL) were added to the flask. A polyurethane film was fully immersed in the toluene solution of HDI, and the reaction mixture was stirred under a nitrogen atmosphere at 50 °C for 12 h using a rotor. After completion of the reaction, the HDI-grafted polyurethane film was removed and thoroughly washed with anhydrous toluene to remove physically adsorbed HDI.

Preparation of PU-HDI-PEG

Forty milliliters of anhydrous toluene were introduced into a 250 mL single-neck round-

bottom flask, followed by the addition of 4 g of polyethylene glycol (PEG, Mw = 1000/2000/3000/10000/20000). The HDI-activated polyurethane film was immersed in the toluene solution of PEG, and the mixture was stirred under a nitrogen atmosphere at 60 °C for 12 h using a magnetic stir bar. After the reaction, the PEG-grafted polyurethane film was collected and thoroughly washed with ethanol to remove any physically adsorbed PEG.

BSA adsorption capacity determination

Cy5.5 (16.7 mg) and bovine serum albumin (BSA, 83.3 mg) were stirred at room temperature for 24 h, followed by dialysis and lyophilization. The resulting BSA-Cy5.5 conjugate was stored at 4 °C for later use. To prepare the incubation solution, 15 mg of BSA-Cy5.5 was dissolved in 15 mL of distilled water with thorough mixing. Then, 2 mL of the solution was added to each of the six wells of a 6-well plate. The samples were incubated at 37 °C in the dark for 4 h. After incubation, fluorescence measurements were performed by a confocal laser scanning microscopy (ZEISS LSM 700, Jena, Germany) on the six sample groups. Additionally, the ultraviolet absorbance of the incubation solution at 278 nm was measured to determine the residual BSA concentration and calculate the amount of BSA adsorbed.

The Δ absorbance was calculated using the following equation:

$$\Delta \text{ absorbance} = A_0 - A_t \quad (1)$$

Here, A_0 represents initial absorbance of the BSA-Cy5.5 incubation solution, and A_t represents absorbance of the residual incubation solution after 4 h of incubation.

Determination of Isocyanate Content

PU-HDI, PU-HDI-PEG, and PU-HDI-PEG-DMH films (each cut into 1.5 cm × 1.5 cm pieces) were individually placed in 250 mL Erlenmeyer flasks and immersed in 40 mL of toluene. Then, 0.1 mL of 0.01 M dibutylamine solution was added to each flask. After thorough mixing, 40 mL of isopropanol was added, and the solution was titrated with 0.0001 M hydrochloric acid. Bromophenol blue was used as the indicator (0.1% solution prepared by mixing 0.10 g of hydrochloride and water-insoluble bromophenol blue with 1.5 mL of 0.1 M sodium hydroxide, then diluting to 100 mL with water) to determine the titration endpoint. Each experiment was performed in triplicate, and the average value was recorded.

The number of isocyanate groups was calculated using the following equation:

$$\text{Number of isocyanate groups} = \frac{N_A \times \left(0.0001 \times 0.01 - 0.0001 \times V_{HCl} \times 0.001\right)}{2 \times 2.25} \quad (2)$$

Wherein, N_A is the Avogadro constant, and V_{HCl} is the volume of HCl consumed in titration (mL).

Determination of Chlorine Content

Both the unchlorinated and chlorinated films were separately dispersed in a mixture of 20 mL N,N-dimethylformamide (DMF) and 20 mL aqueous solution containing 1.0 wt% acetic acid. One gram of potassium iodide (KI) was added to each mixture, and the solutions were stirred at room temperature for 1 h under a nitrogen atmosphere. The released iodine was titrated with 0.000001

mol/L sodium thiosulfate solution. A blank titration under the same conditions was performed as a control.

The active chlorine content was calculated using the following equation:

$$\text{Active chlorine content} = \frac{N_A \times V_{ST} \times 0.001 \times 0.000001}{2 \times 2.25} \quad (3)$$

Where V_{ST} represent the volumes of sodium thiosulfate solution consumed in titrating the N-halamine-functionalized film (mL).

Evaluation of Chlorine Regeneration Performance

The N-halamine-functionalized PU films were treated with 0.1 M aqueous sodium thiosulfate solution at room temperature for 24 h to remove active chlorine. Subsequently, the films were rechlorinated by immersion in a 10% aqueous bleach solution for 45 min. After each chlorination cycle, the PU films were washed with distilled water and air-dried, followed by repeated dechlorination with sodium thiosulfate solution and rechlorination with 10% bleach solution. After different numbers of dichlorination-rechlorination cycles, the chlorine content of the resulting films was measured to comprehensively evaluate their regeneration capacity. Each test was performed in triplicate.

Determination of the inhibition zone

Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATCC 25922) were utilized to assess the antibacterial properties of the samples. The concentrations of *E. coli* and *S. aureus* were adjusted to 10^6 CFU/mL, and 100 μL of the bacterial

suspension was uniformly spread onto an agar plate. Samples of PU, PU-HDI-PEG, PU-HDI-PEG-DMH, PU-HDI-PEG-DMH-Cl, PU-HDI-PEG-DMH-Cl (post) and PU-HDI-PEG-DMH-Cl (2) (1.5 cm × 1.5 cm) were placed on the agar plate. Subsequently, the samples were incubated in a constant temperature incubator at 37 °C for 24 h. Afterward, the samples were removed, and the presence and size of the inhibition zone were observed.

Bacterial Live/Dead Staining Assay

Samples of PU, PU-HDI-PEG, PU-HDI-PEG-DMH, PU-HDI-PEG-DMH-Cl, PU-HDI-PEG-DMH-Cl (post) and PU-HDI-PEG-DMH-Cl (2) (1.5 cm × 1.5 cm) were placed on cell culture plate. Prepare a bacterial suspension in the logarithmic growth phase and adjust it to a certain concentration using liquid culture medium. In the cell culture plate, add 2 mL of the bacterial suspension to each well, then place the film samples into the wells, ensuring that the bacterial suspension completely submerges the films. After incubation at 37 °C for 24 h, carefully aspirate and discard the bacterial suspension from the wells. Rinse the surface of the films very gently with sterile PBS 2 times to wash away unattached planktonic bacteria. Add 300 μL working solution to each well to fully cover the film surface. Incubate at room temperature in the dark for 20 min. After staining, directly use sterile tweezers to remove the films, place them with the bacterial side facing downward onto glass slides, and cover with cover slips. Observe under a confocal laser scanning microscopy.

Bacterial morphology observation

Samples of PU, PU-HDI-PEG, PU-HDI-PEG-DMH, PU-HDI-PEG-DMH-Cl, PU-HDI-

PEG-DMH-Cl (post) and PU-HDI-PEG-DMH-Cl (2) (1.5 cm × 1.5 cm) were placed on cell culture plate. Prepare a bacterial suspension in the logarithmic growth phase and adjust it to a certain concentration using liquid culture medium. In the cell culture plate, add 2 mL of the bacterial suspension to each well, then place the film samples into the wells, ensuring that the bacterial suspension completely submerges the films. After incubation at 37 °C for 24 h, the samples were gently rinsed three times with 0.1 M phosphate buffered saline (PBS, pH 7.4) to remove non-adherent planktonic cells. The samples were immersed in a primary fixative solution (2.5% glutaraldehyde in 0.1 M PBS) and stored at 4 °C overnight to firmly preserve the cellular structures. The fixed samples were subjected to a graded ethanol series for dehydration to remove all water, which is incompatible with the SEM high-vacuum environment. The sequence was as follows: 30%, 50%, 70%, 80%, 90%, 95%, and 100% ethanol (twice). Each step lasted for 15 min at room temperature. Once the samples were dried, the bacterial topography on the sample surface was observed using a SEM.

Antibacterial test

The concentrations of *E. coli* and *S. aureus* were adjusted to 10^7 CFU mL^{-1}. 60 μL of the bacterial suspension was dropped onto the surface of the PU, PU-HDI-PEG, PU-HDI-PEG-DMH, PU-HDI-PEG-DMH-Cl, PU-HDI-PEG-DMH-Cl (post) and PU-HDI-PEG-DMH-Cl (2) (1.5 cm × 1.5 cm). The sample was then placed in a 37 °C incubator for 24 h. Afterward, the sample along with the bacteria and culture medium on its surface were collected into a centrifuge tube. The bacteria were shaken off the sample surface and diluted to a specific concentration. 100 μL of the

bacterial suspension was spread evenly on an agar plate and incubated in an incubator at 37 °C for 18 h. The number of bacterial colonies on the agar culture plates of each group was counted, and a pure PU sample was selected as the control group. The antibacterial rate is calculated as follows:

$$\text{Antibacterial rate} = (A - B)/A \times 100\% \quad (4)$$

Here, A represents the number of colonies on the PU sample in the control group, and B represents the number of colonies in the experimental group.

Statistical analysis.

The experiments were repeated for at least three times and data was shown containing sample size (n). The data are presented as mean ± standard deviation (SD). The significant differences were indicated as follows: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. The significant differences were analyzed using one-way ANOVA (between more than two groups). Statistical analysis was conducted using GraphPad Prism 8 Software.

Table S1 The isocyanate content of PU, PU-HDI, PU-HDI-PEG, and PU-HDI-PEG-DMH.

Sample	Titration consumption of HCl (mL)	-NCO (groups cm^{-2})	Relative content of -NCO (%)
PU	10.00	0	0
PU-HDI	8.75	$(1.67 \pm 0.07) \times 10^{16}$	100
PU-HDI-PEG	9.76	$(3.21 \pm 0.41) \times 10^{15}$	19.20
PU-HDI-PEG-DMH	9.97	$(4.01 \pm 0.01) \times 10^{14}$	2.40

Table S2 The chlorine content of PU-HDI-PEG-DMH-Cl and PU-HDI-PEG-DMH-Cl (2).

Sample	Titration consumption of $Na_2S_2O_3$ (mL)	Active chlorine content (atoms/cm^2)	Relative content of active chlorine (%)
PU-HDI-PEG-DMH-Cl	11.55	$(1.55 \pm 0.02) \times 10^{15}$	100
PU-HDI-PEG-DMH-Cl (2)	10.85	$(1.46 \pm 0.02) \times 10^{15}$	94.2

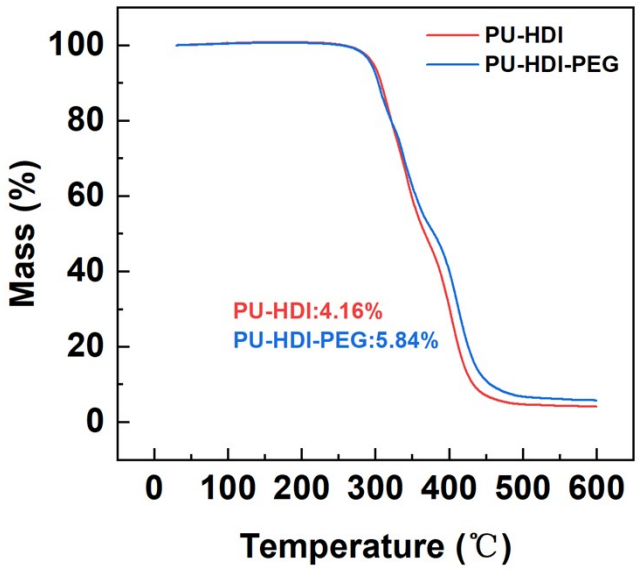

Figure 1. Thermogravimetric analysis (TGA) curves of PU-HDI and PU-HDI-PEG.