Supporting Information

Rational Chemical Engineering in Natural Protein Derived Functional Interface

Arpita Shome\textsuperscript{a}, Adil M. Rather\textsuperscript{a}, Aindrila Ghosal\textsuperscript{a}, Bibhas K. Bhunia\textsuperscript{b}, Biman B. Mandal\textsuperscript{b}, Uttam Manna\textsuperscript{a,c,*}

\textsuperscript{a} Department of Chemistry, Indian Institute of Technology Guwahati, Amingaon, Kamrup, Assam 781039, India
\textsuperscript{b} Biomaterial and Tissue Engineering Laboratory, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Amingaon, Kamrup, Assam-781039, India.
\textsuperscript{c} Centre for Nanotechnology, Indian Institute of Technology-Guwahati, Amingaon, Kamrup, Assam 781039, India

Correspondence and requests for materials should be addressed to U. M. (email:umanna@iitg.ac.in)
Experimental Section:

Materials: Bovine Serum Albumin (MW \( \sim \) 66.5 KDa, Faction V), dipentaerythritol penta-acrylate (5Acl, MW \( \sim \) 524.21 Da), pentyamine, hexylamine, heptylamine, octylamine, decylamine, octadecylamine, 3-(dimethylamino)1-propylamine, rhodamine 6G, methylene blue, fluorescein, tetracycline hydrochloride, aspirin and phosphate buffer saline (PBS) capsules (pH 7.4, 0.01 M) were procured from Sigma-Aldrich (Bangalore, India) and absolute ethyl alcohol (CAS 64-17-5, Lot 17030799) was purchased from TEDIA Company (United States of America). Methanol (CAS 67-56-1) was purchased from RANKEM (Maharashtra, India). Reagent grade THF was purchased from RANKEM (Maharashtra, India). Cotton was purchased from a local medical shop in Guwahati city (Assam, India). Aluminum wire used for submersion of loaded cotton in PBS buffer was purchased from a local electrical shop in Guwahati city (Assam, India).

General considerations: Glass vials (Borosil) used for preparing the coating solutions were washed with acetone and ethanol prior to use. The Zeta potential (\( \zeta \)) and Dynamic Light Scattering (DLS) analysis was carried out using Zetasizer Nano ZS90 (model no. ZEN3690). Release profile of tetracycline and aspirin was monitored by a Perkin Elmer Lambda 25 UV/Vis spectrophotometer. Contact angle measurements were taken using a KRUSS Drop Shape Analyser-DSA25 with an automatic liquid dispenser at ambient conditions. FTIR spectra were recorded with a Perkin Elmer instrument at ambient conditions using KBr pellets. Scanning electron microscope images were obtained using a Sigma Carl Zeiss scanning electron microscope (samples were coated with a thin layer of gold prior to imaging). Digital pictures were acquired using a Canon Powershot SX420 IS digital camera. Fluorescence microscopic images of the superhydrophobic cotton before and after loading of rh-6G, methylene blue and fluorescein were acquired using an AX10 observer Z1 & AXio Cam MRCS, Carl Zeiss, Germany. Milli-Q grade water was used for all experiments.

Synthesis of chemically reactive biomacromolecular coating and post chemical modifications:

In order to achieve, chemically reactive biomacromolecular (bovine serum albumin; BSA) on the fibres of medical cotton, standard desolvation with ethanol solvent was associated. Briefly, medical cotton of dimension (1.8cm \( \times \) 1.8cm \( \times \) 0.8cm) was immersed in an aqueous solution of Bovine Serum Albumin (BSA) of concentration 10 mg/ml and kept for 3 hours with continuous agitation. Thereafter, 6ml ethanol was gradually added to this BSA solution that is with submerged selected fibrous substrate. The transparent aqueous solution of BSA turned into milky dispersion, and the fibrous substrate was left in this milky solution for 1 hour, with continuous and mild agitation. Subsequently, the cotton was washed thoroughly twice (10 mins) with ethanol. Next, chemically reactive and covalent cross-linking was introduced to the BSA coating by immerging the coated substrate in the 5Acl solution (1.325g in 10ml) in methanol for 3 hours, after which the cotton was washed with methanol to remove the unreacted and loosely adhered 5Acl molecules. The residual chemical reactivity was characterized with FTIR analysis. For the appropriate and desired post chemical modulations, this chemically reactive BSA coating on the fibrous substrate was exposed to primary amine containing selected small molecules, including pentyamine (30 mg/mL), hexylamine (30 mg/mL), octylamine(30 mg/mL), decylamine (30 mg/mL) and octadecylamine (5 mg/mL). The material was washed with THF and left to air dry.

Physical and Chemical Durability Tests

Various severe and harsh physical and chemical abrasion tests were performed on the BSA derived superhydrophobic cotton to ensure its utility at the practical settings. The detailed explanation of various tests are discussed as below:

(1) Finger Wiping Test:

In this particular durability test, the BSA derived superhydrophobic cotton was immobilized on the microscopic glass slide using adhesive tape. Next, the cotton was rubbed with the right hand index finger for multiple (10) times. Then, the anti-wetting property was examined on the abraded surface through visual inspection and contact angle measurement.

(2) Tissue Paper Wiping Test:
The tissue paper wiping test was performed in similar to that of the finger wiping test. Here in this test, the tissue paper was used instead of finger. Then, the anti-wetting property was examined on the abraded surface through visual inspection and contact angle measurement.

(3) Scissor Cutting Test:

In this test, a thick and large (5 x 4 x 2 cm³) piece of BSA derived superhydrophobic cotton was used for the demonstration. The selected cotton piece was cut with a sharp edged scissor at one end and which was considered as the first cycle of cutting. The anti-wetting property was examined after each cycle of cutting with the beaded water droplet through visual inspection and the contact angle measurement.

(4) Chemical Durability Test:

In this particular durability test, the protein derived superhydrophobic cotton was exposed to various harsh aqueous chemical conditions including extremes of pH (1, 12), surfactant (SDS, DTAB), artificial sea water, river (Brahmaputra, Assam India) water continuously for seven days. Next, the anti-wetting property was examined through visual inspection and the contact angle measurement. Artificial sea water was prepared by mixing MgSO₄ (0.325g), MgCl₂ (0.226g), CaCl₂ (0.112 g) and NaCl (2.673g)) in 100 ml deionized water.

Post-loading and release of drug molecules:

Ethanol solvent assisted temporary and reversible switching of water wettability was strategically exploited in post-loading of various small molecules—having different structures and functions. Selected bioactive drug molecules—that are aspirin and tetracycline were loaded from its ethanolic solution. The medical cotton—having biomolecular functional coatings was soaked in the respective ethanolic solution of selected dye/drug molecules, and then left to air dry in a dark place. The deposition of dye/drug molecules were examined through fluorescence images and the anti-wetting property was re-validated through digital images and contact angle measurements. To perform the release study of the post loaded drug molecules from the BSA derived functional coatings that modified with ODA, the respective drug loaded functional cotton was incubated in PBS buffer (pH=7.4) at 37°C and UV absorptions spectrophotometer was used to monitor the release of the drug molecules. The aliquot was collected at different time intervals, and UV/Vis absorption was recorded at wavelengths 265 nm and 385 nm for aspirin and tetracycline, respectively to determine the concentration of the drug released. The release of aspirin and tetracycline from BSA coated cotton that modified with amine containing other small molecules was carried out in the same manner.

Disc-diffusion assay:

Disc diffusion method was performed to determine the antibacterial activity of released tetracycline from the matrix. In brief, *Staphylococcus aureus* MTCC (gram +ve bacteria) 3160 and *Escherichia coli* MTCC 40 (gram -ve bacteria) (MTCC, IMTECH, India) were used for this antibacterial assessment. 100 µl of bacterial suspension (10⁸ CFU/ml) was spread over the surface of solidified nutrient-agar plate (Himedia, India) using a sterile glass L-spreader followed by drying for 10 min under sterile laminar air flow. Prior to the test, the concentration of released tetracycline was adjusted to 180 µg/ml. Thereafter, sterile paper discs (6 mm) were soaked in released tetracycline solution followed by placing them on the bacterial lawn. On the other side, freshly added tetracycline having the same concentration (180 µg/ml) and sterile PBS (pH 7.4) were used as positive (+ve) and negative (-ve) control, respectively. Test plates were incubated at 37 °C for 48 h. The antibacterial activity of the released tetracycline against the test bacteria was measured as “zone of inhibition” using a Vernier calipers.
Figure S1. (A-F) Digital images (A, C, E) and water contact angle images (B, D, F) of beaded water droplets on BSA coated cotton (A, B), after 5Acl treatment (C, D), after octadecylamine treatment followed by washing with THF and water (E, F) respectively. (G-H) Digital image (G) and contact angle image (H) of beaded water droplet on BSA immobilized cotton after direct ODA treatment of the BSA coated cotton followed by washing with THF and water.
Figure S2. (A-D) Digital images (A, C) and water contact angle images (B, D) of beaded water droplets on BSA coated cotton after octylamine treatment (A, B) and octanol treatment (C, D) respectively.
Figure S3. (A-H) Digital images (A-D) and contact angle images (E-H) of beaded water droplet (dyed blue) on superhydrophobic cotton that are loaded with rhodamine 6G with concentrations 0.1mg/ml (A-E), 0.5mg/ml (B-F), 1.0mg/ml (C-G) and 1.5mg/ml (D-H). Table S2 accounting for the amount of dye loaded for different concentrations along with the advancing contact angle and contact angle hysteresis.
Figure S4. (A-H) Digital images (A-D) and contact angle images (E-H) of beaded water droplet (dyed blue) on superhydrophobic cotton loaded with fluorescein with concentrations 0.1mg/ml (A-E), 0.5mg/ml (B-F), 1.0mg/ml (C-G) and 1.5mg/ml (D-H). Table S2 accounting for the amount of dye loaded for different concentrations along with the advancing contact angle and contact angle hysteresis.

| Concentration of Fluorescence (mg/mL) | Amount of drug loaded (mg) | θ Adv (°) | θ Hys (°) |
|--------------------------------------|---------------------------|-----------|-----------|
| 0.1                                  | 0.12                      | 158.2±0.9 | 7.2±1.5   |
| 0.5                                  | 0.60                      | 157.3±0.8 | 7.6±1.1   |
| 1.0                                  | 1.20                      | 156.4±0.9 | 8.0±0.2   |
| 1.5                                  | 1.80                      | 155.8±1.2 | 8.7±0.9   |
Figure S5. (A-H) Digital images (A-D) and contact angle images (E-H) of beaded water droplet (dyed green) on superhydrophobic cotton loaded with methylene blue with concentrations 0.1mg/ml (A-E), 0.5mg/ml (B-F), 1.0mg/ml (C-G) and 1.5mg/ml (D-H). Table S3 accounting for the amount of dye loaded for different concentrations along with the advancing contact angle and contact angle hysteresis.

| Concentration of Methylene Blue (mg/mL) | Amount of dye loaded (mg) | $\theta_{Adv}$ (°) | $\theta_{Hyst}$ (°) |
|----------------------------------------|--------------------------|--------------------|---------------------|
| 0.1                                    | 0.12                     | 158.8±0.2          | 7.8±0.2             |
| 0.5                                    | 0.60                     | 156.7±1.2          | 8.3±0.4             |
| 1.0                                    | 1.20                     | 154.6±1.1          | 8.8±0.6             |
| 1.5                                    | 1.80                     | 152.4±1.3          | 9.2±1.7             |
Figure S6. (A-H) Bright field microscopic images (A-D) and fluorescent images (E-H) of bare cotton (A-E) and superhydrophobic cotton loaded with small dye molecules—that are rhodamine-6G (B-F), fluorescein (C-G) and methylene blue (D-H).