Surfactant-Free Stain Removal by a Sustainable Protein Nanofilm

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Article

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Abstract

The extensive use of detergents in modern life and industry has seriously impeded ecologically sustainable development. Facing this unresolved global challenge, we herein propose a CAW (coating at will) concept to endow virtually arbitrary surfaces with underwater superoleophobicity that supports the fast and easy removal of oily stains by mere use of water. The key selling point of this CAW concept is its ability to sustainably regenerate the coating throughout an infinite life cycle. The foundation of this concept is to make use of rapid amyloid-like aggregation of lysozyme (Lyz) conjugated with zwitterionic poly(sulfobetaine methacrylate) (pSBMA). The resultant phase-transitioned Lyz-pSBMA (PTL-pSBMA) could quickly prime versatile surfaces to afford a robust colourless ultrathin nanofilm on surfaces with high hydrophilicity. As a result, the hydrophilic PTL-pSBMA layer endows materials with excellent underwater superoleophobicity and provides outstanding detergent-free cleaning efficiency to remove oily stains (e.g., greater than 95% on silk surfaces and 99% on dishes). With excellent optical transparency, biocompatibility and negligible effects on wearing comfort, the PTL-pSBMA further showed extraordinary cost-effectiveness ($675/ton) and great savings on water and energy by 40%-50%. Overall, this work proposes an ingenious CAW design that breaks down the long-standing surfactant contamination barriers in the traditional detergent industry. Such surfactant-free water washing strategy holds great promise towards scale-up application to replace commercial detergents in the removal of common stains from fabrics and kitchenware surfaces, thereby greatly inhibiting the negative environmental pressures caused by surfactant emissions and providing a transformative response to ecosystems and water resource protection on Earth.

Keywords: amyloid-like protein, superoleophobicity, surfactant, zwitterionic polymer

1. Introduction.

As a major global challenge, ecological sustainable development is continuously challenged by the aggravated use of chemical surfactants in daily life and industrial production. For instance, the amount of surfactants reached 15.93 million tons in 2014, which is expected to increase to 24.19 million tons in 2022[1]. Among the numerous uses of surfactants, homecare detergents (e.g., laundry and kitchenware detergents) account for more than 40% of the global market[2-9]. As a result, the ever-increasing use of detergents has pressed the treatment of surfactant-containing wastewater to become a growing burden. For instance, wastewater treatment in 2017 consumed approximately 4% of all the electrical power produced in the United States[10,11]. It is further estimated that the electricity required for wastewater treatment will increase by 20% over the next 15 years in developed countries, leading to a significant climb in CO_2 emissions and energy consumption[12,13]. Moreover, in economically underdeveloped regions, household detergents are directly discharged into the ecological environment without pretreatment[14-16], resulting in the long-term accumulation of surfactants in the ecosystem. Such detergent invasion would have significant adverse effects on aquatic life, plants, animals and humans[17-19]. Regarding to the above-mentioned surfactant issue, although a few commercially available natural detergents have emerged[20-24], their low production yields, high production costs, poor cleaning efficiency, irritation to sensitive skin and damage to wool or silk fabrics have limited their scale-up applications[25].

In principle, the main function of detergents is to detach oily stains adhered onto solid surfaces by entrapping them through micellization and then dispersing them into water. In this process, the stronger intermolecular interaction between the detergent and the oily stain would replace the interaction between the oily stain and solid surface. In this regard, a superoleophobic surface that easily repels oil staining due to the ultralow intermolecular forces between the solid surface and oily stain would be beneficial to reduce detergent usage. However, few marketed examples or literature studies based on such recognition have been engineered on a large scale to significantly reduce detergent usage due to the following inevitable problems. First, in contrast to the formation of

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superhydrophobic surfaces, the fabrication of superoleophobic surfaces is more complicated and often requires the construction of delicate micro/nanotopography on a surface. Subsequently, nearly all of the as-prepared micro/nanoconstructed surfaces in the existing approaches are very easily recontaminated by particles and pollutants or re-abrased by external forces, which leads to a loss of the oil repellence capability in a short service period. Second, to fabricate superoleophobic surfaces, most materials need to be modified with a low surface energy layer after the formation of rough microstructures, which often involves the use of fluoro-containing compounds that are undesirable in food/life-related applications. Third, to overcome the interfacial adhesion weakness of conventional vapour deposition techniques, complex chemical reactions[26] are often required, which typically suffers from several disadvantages that restrain the commercial potential of these techniques, such as high cost, complex and unfriendly procedures and poor eco-compatibility. Moreover, classical surface priming methods, such as polydopamine (PDA) and polyphenol/Fe complex chemistry, typically impart surfaces with undesirable dark colour and rough topography, and the resultant coating has poor stability in alkaline pH environment[27-30].

In contrast to conventional postfunctionalization techniques on surfaces, we herein propose a distinctive coating at will (CAW) pathway to impart any kind of surface with underwater superoleophobicity that can be endurably maintained for an unlimited life cycle. The core feature of this CAW concept is that any target surface could be reformed to have autonomous underwater anti-oil staining properties via a rapid one-step aqueous coating with an antifouling hydrophilic biopolymer. This coating process can be repeated on demand at any time to regenerate the coating layer, and the long-lasting underwater oil repellent state could be stably maintained. Similar to normal washing, the whole coating and regeneration process could be simply achieved by the end user in a facile and friendly one-step aqueous soaking step that could be repeated for an unlimited number of times. As a result, oily staining on fabrics and kitchenware could be easily removed by simply rinsing with water without the addition of detergents, and the perfect oil-repellent state could be maintained for an infinite number of washing-staining cycles.

In the present work, we prove this CAW concept by making use of an amyloid-like protein-mediated interfacial adhesion system. The rapid amyloid-like aggregation of lysozyme (Lyz) conjugated with zwitterionic poly(sulfobetaine methacrylate) (pSBMA) was manipulated in water to afford a robust ultrathin nanofilm (PTL-pSBMA) on solid surfaces. This process was finished in minutes by simple dip coating and could instantly prime virtually any surface (e.g., metal, plastic, glass, ceramic) to become highly hydrophilic and superoleophobic under water through oil exclusion from the hydrophilic polymer chains. As a result, after soaking in or flushing with water, hydrophobic oily stains would be easily removed from the primed surfaces of fabrics (e.g., linen, flannel, cotton, vinylon, silk, modal cotton, and Dacron) and kitchenware (e.g., ceramic dishes), leading to a detergent-free cleaning procedure. Compared with normal detergent-based washing protocols, such a detergent-free cleaning mode has a lower required dosage, higher cleaning efficiency on fabrics and kitchenware and significant water and energy savings of at least 40~50%. In contrast to all other known approaches, this detergent-free washing mode is further highlighted by its ready-to-use regeneration feature, by which any kind of primed surface coated with the PTL-pSBMA nanofilm could be regenerated upon request by simply resoaking the surface in aqueous solution containing 0.005% w.t. PTL-pSBMA. By this coating regeneration process (CAW concept), the excellent detergency performance of PTL-pSBMA-modified fabrics could be well maintained for at least 200 cycles of washing-regeneration. In combination with its colourlessness, optical transparency and biocompatibility as well as good functional extendibility[31-34] (e.g., being easily endowed with antimicrobial properties), fast surface priming with PTL-pSMBA offers a universal strategy to create stealthy coatings on versatile material surfaces for autonomous stain resistance. In light of its extraordinary cost-effectiveness ($675/ton) and low recommended dosage (0.9 g/kg clothes), this method thus holds great commercial potential to transform current mainstream laundry detergent-based cleaning protocol for fabrics and kitchenware washing, which accounts for a large portion of global surfactants. This concept would open a door to develop a series of CAW-derived coating methods to greatly power the sustainable development of a green modern society with a low carbon footprint.

2. Results and Discussion.

To obtain the protein-polyzwitterionic polymer conjugate, zwitterionic poly(sulfobetaine methacrylate) (pSBMA) was first synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization (**Figure 1a**), and the resultant polymer product had a molecular weight of 4640 (degree of polymerization=17) and a polydispersity index (PDI) of 1.17, as analysed by ^{1}H NMR (**Figure S1**) and gel permeation chromatography[35] (GPC) (**Figure S2**). Then, a typical protein-polymer conjugate was obtained via a 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC)/N-hydroxysuccinimide (NHS)-mediated coupling reaction between the amine groups of native lysozyme and carboxyl groups of pSBMA (**Figure 1a**). By this process, one lysozyme molecule could attach to 1, 2, or 3 pSBMA graft chains, affording the protein-polymer conjugates Lyz-1pSBMA, Lyz-2pSBMA, and Lyz-3pSBMA, respectively (**Figure S3**). These products were properly characterized by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), Fourier transform infrared (FTIR) and corresponding X-ray photoelectron spectroscopy (XPS) spectra, reflecting an exact shift in the molecular mass of the protein after polymer grafting (**Figure 1b**) and displaying the characteristic signals for the sulfobetaine side chains in the FTIR (the symmetric S=O stretching at 1039 cm^{-1} in **Figure 1c**) and XPS (S_{2p} (167.5 eV) and N_{1s} (402.5 eV) in **Figure S4**) spectra of the Lyz-pSBMA conjugate[36,37]. The native bioactivity of lysozyme was highly preserved after polymer conjugation, further indicating a negligible influence of the conjugation process and polymer chain on protein conformation and bioactivity (**Figure S5**). For simplicity, the present work mainly used Lyz-2pSBMA (herein unless otherwise noted, Lyz-2pSBMA was referred as Lyz-pSBMA for simplicity) to complete the experiments.
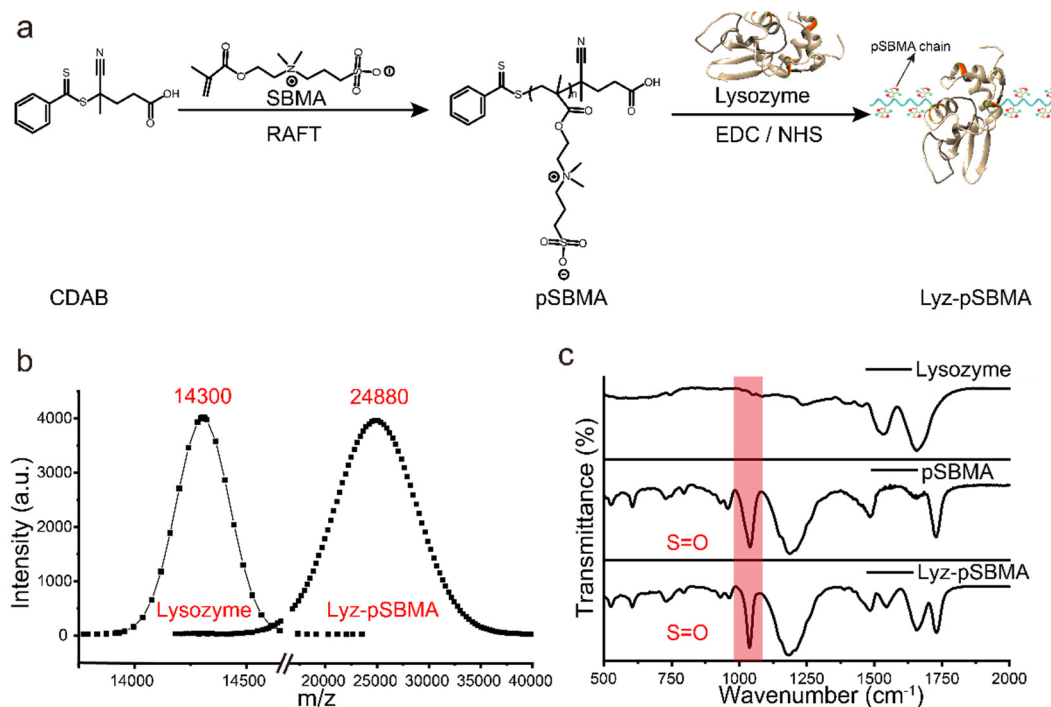
Figure 1. Synthesis and Characterization of Lyz-pSBMA. (a) Synthesis of Lyz-pSBMA. (b) MALDI-TOF spectra of lysozyme and Lyz-pSBMA. (c) FTIR characterization of Lyz-pSBMA and the characteristics of the S=O peaks.

Starting from the Lyz-pSBMA conjugate, amyloid-like aggregation of Lyz-pSBMA could be triggered after reducing the intramolecular disulfide bond in the protein to afford a conformal nanofilm coating on articles of daily use. Such amyloid-like aggregation, termed the protein phase transition, was pioneeringly developed by our group in 2012[38,39]. Typically, after reducing the intramolecular disulfide bond of Lyz-pSBMA with tris(2-carboxyethyl)phosphine (TCEP), the corresponding amyloid-like aggregation of Lyz-pSBMA rapidly formed a stable two-dimensional phase-transitioned Lyz-pSBMA nanofilm (PTL-pSBMA) on various substrates **(Figure 2a)**. During this aggregation process, the conformational transition of the proteins from an α-helix to a β-sheet was reflected by the signal enhancement from the β-sheet structure in the circular dichroism (CD) (216 nm) and Fourier transform infrared (1625 cm^{-1}) spectra of PTL-pSBMA **(Figures 2b, 2c, S6)**[40,41]. The time-resolved growth monitored by the aniline-1-naphthalene sulfonate (ANS) assay **(Figure 2d)** and thioflavin T (ThT) staining reflected the rapid amyloid-like aggregation driven by hydrophobic residue exposure and subsequent hydrophobicity-induced aggregation. In this process, the exposure of the free thiol groups by TCEP-induced disulfide bond reduction of Lyz-

pSBMA was also reflected by N- (1-Pyrenyl) maleimide (NPM) staining (**Figures S7, S8**)[42].

By the phase transition, the atomic force microscopy (AFM) and transmission electron microscopy (TEM) results then clearly showed the formation of nanoparticles approximately 20 nm in size from the protein oligomeric aggregation in the reaction solution (**Figure 2e, 2f; Figures S9, S10**). The nanoparticles in the resultant PTL-pSBMA emulsion showed excellent colloidal stability, since the size of these colloids did not continuously increase upon storage under ambient conditions for >56 days and the resultant emulsion remained clear without precipitation (**Figures S11, S12**). More importantly, the stock solution maintained high interfacial activity to reform the nanofilms on the substrate surface (**Figures S13, S14**). In addition to the colloidal stability, the PTL-pSBMA also presented good redispersion property. For instance, after the PTL-pSBMA nanoparticles were subjected to dialysis to remove unreacted reactants (e.g., TCEP) and freeze-dried (**Figure S15**), the resultant freeze-dried PTL-pSBMA powder could be quickly redispersed in water to reform a stable emulsion. When the nanofilm formation is desired, the emulsion can be simply utilized as the dipping solution to form the high-quality PTL-pSBMA nanofilm on solid surface (**Figure S16**). By this process, a dense PTL-pSBMA nanofilm with a water contact angle of 65° and a thickness of approximately 11-14 nm formed on virtually any material surface (**Figure S17**). This result suggests that at the scale-up/commercial application stage, PTL-pSBMA could possibly be offered to the consumer in a convenient powder form. This state noticeably enhances the ease of use, transportation convenience, and storage stability and corresponding shelf life of the product.
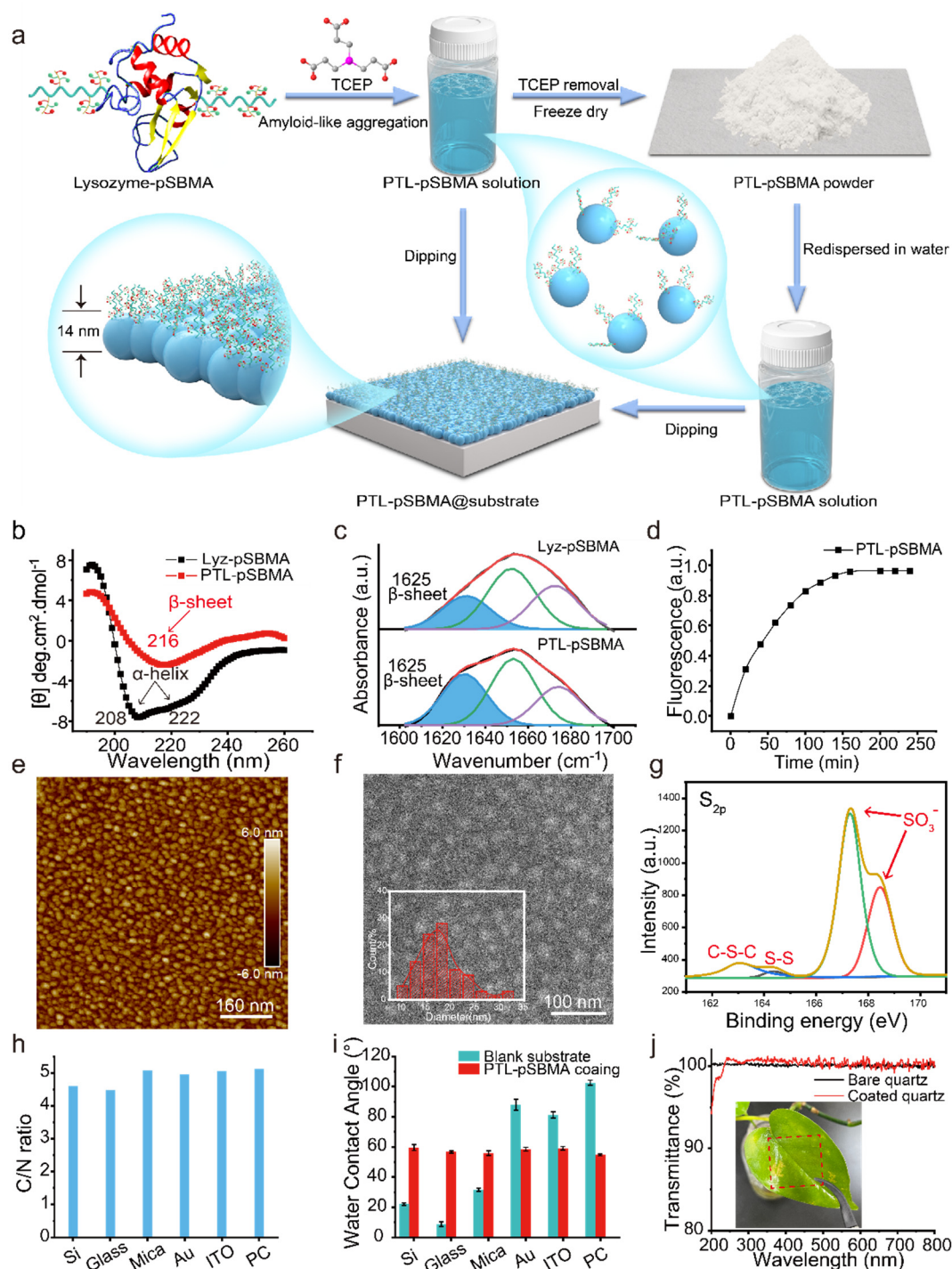
Figure 2. Synthesis and rapid adhesion of the PTL-pSBMA nanofilm on a substrate. (a) Schematic illustration of the formation of PTL-pSBMA nanofilms on substrates by dip coating in freshly prepared PTL-pSBMA emulsion without or with dialysis, freeze-drying and re-dispersion in water; (b) CD spectra of Lyz-pSBMA and PTL-pSBMA solutions; (c) FTIR characterization of the Lyz-pSBMA and PTL-pSBMA powders and the corresponding deconvolution of the amide I regions; (d) ANS fluorescence of the PTL-pSBMA solution; (e-i) the tests of AFM (e), TEM (f), S_{2p} XPS (g), C/N ratio from the XPS (h) and WCA (i) on the PTL-pSBMA nanofilm; j) the optical transmittance of PTL-pSBMA nanofilm-coated quartz glass.

229   After forming the PTL-pSBMA nanofilm on a given surface, the XPS signals

230   corresponding to the core electrons of S_{2p} (167.5 eV) and N_{1s} (402.5 eV) from the

231   zwitterionic sulfobetaine side chains further reflected that the polymer chains were

232   significantly exposed on the nanofilm surface (**Figure 2g**). The XPS signals also

233   indicated the simultaneous presence of anionic groups (carboxyl and sulfo) and cationic

234   groups (amine) on the nanofilm surface (**Figure S18**). In addition to the good water

235   dispersity and stable storage properties, PTL-pSBMA also exhibited high interfacial

236   adhesion activity (10 times higher than common detergents) (**Figure S19**) and

237   robustness under various harsh conditions, which further strengthens its use for scale-

238   up applications. First, simply dipping the target surface in the PTL-pSBMA solution for

239   just 1-5 min is enough to form a dense PTL-pSBMA nanofilm on various substances,

240   such as mica, silica wafers, glass, Au, polycarbonate (PC) and indium tin oxide (ITO).

241   The nanofilm on these substrates showed a dense monolayer of oligomers with a

242   consistent thickness (approximately 11-14 nm) (**Figure S20**), C/N atomic ratio from

243   the XPS spectra and water contact angle (approximately 60°) (**Figure 2h, 2i**) as well as

244   almost uniform 100% optical transmittance between 350 and 800 nm (**Figure 2j**). The

245   corresponding C_{1s} and N_{1s} signals in the XPS spectrum of the PTL-pSBMA nanofilm

246   further reflected the presence of a variety of functional groups, including amino,

247   guanidyl, carboxyl, hydroxyl, and grafted pSBMA, on the film surface (**Figure S21**),

248   which are responsible for the stable interfacial adhesion onto a variety of solid

249   surfaces[43]. Second, the PTL-pSBMA nanofilm did not show notable changes in surface

250   topography or film thickness (**Figures S22-S24**) after being subjected to stringent

251   treatments, such as extreme pH (2-11), organic solvents (dimethyl sulfoxide (DMSO),

252   acetonitrile, petroleum ether, and ethyl acetate), adhesive tape peeling and even plasma

253   irradiation, indicating the robustness of the PTL-pSBMA nanofilm under a wide range

254   of external conditions.

255   Based on the classical antifouling property of the pSBMA block, the good features

256   of the PLT-pSBMA layer were further highlighted by its excellent biofouling resistance,

257   which is another highly desirable property for functional textiles (**Figure 3a**). As

258   examined by quartz crystal microbalance with dissipation (QCM-D), in contrast to bare

259 Au, the PTL-pSBMA nanofilm showed superior resistance to nonspecific adsorption of

260 a series of biofluid mixtures (e.g., milk, foetal bovine serum [FBS], bacterial lysate),

261 adhesive proteins (e.g., β-lactoglobulin [β-Lg], fibrinogen [Fibri], human serum

262 albumin [HSA], concanavalin A [Con A], lysozyme [Lyz], horseradish peroxidase

263 [HRP], bovine serum albumin [BSA]), and extracellular matrix (ECM) components

264 (e.g., proteins, polyoses and carbohydrates) (**Figure 3b**)[44]. For example, the adsorption

265 capacity of the PTL-pSBMA film to milk and human serum albumin was as low as 10

266 ng/cm^2, while the adsorption capacity of blank Au to those substances is >1400 ng/cm^2

267 (**Figure S25**). The resistance to bacterial adhesion by the nanofilm was then directly

268 visualized by incubating the micropatterned PTL-pSBMA nanofilm (**Figure S26**) in

269 bacterial liquid (*E. coli*, *S. aureus*) for a certain culture period. In this way, the

270 micropatterned bacterial adsorption was observed on the blank area (**Figure 3c**) but not

271 the PTL-pSBMA-coated region (**Figure 3d-3f**). The clear contrast between blank and

272 the PTL-pSBMA-coated surfaces indicated the effective inhibition of nonspecific

273 bacterial adhesion on the PTL-pSBMA nanofilm. In addition to bacteria, the PTL-

274 pSBMA nanofilm also showed good resistance to the adhesion of typical fungi, such

275 as *Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum*, and *Candida albicans*

276 (**Figure 3g, Figure S27**)[45]. In addition to microbes, the nonspecific adsorption of

277 human platelets and a fibroblast cell line (L929) was also largely suppressed on the

278 PTL-pSBMA-modified surface (**Figure 3h, 3i**). Accordingly, the above results showed

279 the great potential of PTL-pSBMA-modified fabrics to prevent mildew and rot in

280 commodity articles, clothes, kitchenware and foods. By further doping PTL-pSBMA

281 with antibacterial peptides such as ε-polylysine (PL)[46,47], the bactericidal functions on

282 agar plates and in simulated sweat (65% RH, 25°C for 20 days) presented 99.9%

283 antibacterial activity towards *E. coli* (ATCC25922) and *S. aureus* (ATCC6538) (**Figure**

284 **S28-S31**)[48]. This result is promising for constructing self-bactericidal anti-staining

285 coatings on textiles and kitchenware.
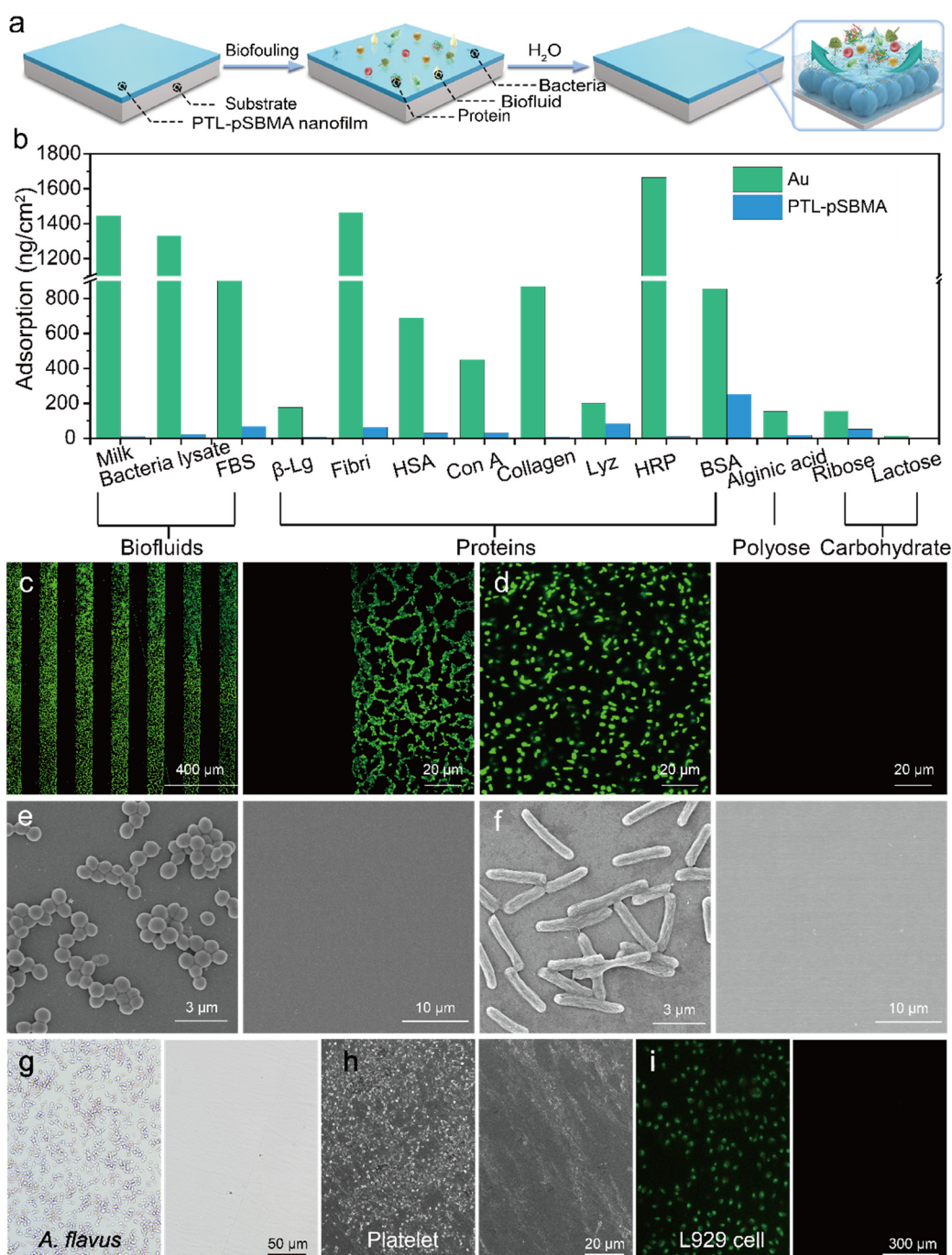
**Figure 3**. Antifouling property of the PTL-pSBMA nanofilm. (a) Schematic illustration of the formation of the PTL-pSBMA nanofilm and its antifouling property towards various fouling agents. (b) Biofluid, protein, polyose and carbohydrate adsorption on bare Au chips and PTL-pSBMA nanofilm-coated Au chips. (c) Laser scanning confocal microscopy (LSCM) images showing the patterned adherence of *S. aureus*. (d) *E. coli* cultured on bare glass and PTL-pSBMA@glass. FE-SEM images showing *S. aureus* (e) and *E. coli* (f) adherence to a bare silicon wafer (left) and the PTL-pSBMA nanofilm (right). (g) Optical microscopy images showing *A. flavus* adherence to the PS culture dish (left) and PTL-pSBMA nanofilm (right). (h) FE-SEM images showing the platelets adhered to the bare glass (left) and PTL-pSBMA nanofilm (right), and (i) LSCM images showing L929 cells adhered to the bare glass (left) and PTL-pSBMA nanofilm (right).

298   The robustness and universal adhesion capability of PTL-pSBMA highlighted its

299   great value for the development of functionalized fabrics and smart textiles. To date,

300   few universal methods can functionalize fabric surfaces without changing the original

301   properties of the fabric, such as its optical appearance, biocompatibility and

302   breathability. In this context, the present work offers a universal strategy to create

303   stealthy coatings on a variety of mainstream fabrics, such as linen, flannel, cotton,

304   vinylon, silk, modal cotton, and Dacron (**Figure 4a**). By using FITC-labelled Lyz-

305   pSBMA in the phase transition, the resultant PTL-pSBMA coating on the textile surface

306   was clearly visualized under a fluorescence microscope (**Figure 4b, 4c, S32**)[49]. In

307   contrast to conventional universal coatings, such as black or brown polydopamine or

308   polyphenol/Fe complexes, the PTL-pSBMA coating was optically transparent and had

309   a thickness of 14 nm. Such a thin proteinaceous coating with stealth optical properties

310   did not affect the wearing comfort of clothing, as the air and moisture permeability of

311   silk, cotton and Dacron after PTL-pSBMA modification were basically the same as

312   those of the unmodified fabrics **(Figure 4d, 4e)**. With the introduction of the

313   hydrophilic zwitterionic pSBMA polymer, the subsequent WCA test indicated a large

314   increase in hydrophilicity on the modified fabrics, since after modification, the WCAs

315   on silk, gunny and modal changed from 130°, 130° and 50° to 10.2°, 0° and 0°,

316   respectively (**Figure S33**). As a result, water-based ink droplets spread completely on

317   the PTL-pSBMA-coated silk surface while maintaining poor wetting on the pristine silk

318   surface (**Figure 4f, 4g**). The high hydrophilicity of PTL-pSBMA offered a surface

319   hydration layer on the modified fabric surface, which supported good underwater oil

320   repellence by blocking oil droplets from approaching the fabric surface. For this reason,

321   while Nile red-dyed dichloromethane (DCM) droplets completely diffused into pristine

322   silk cloth (**Figures 4h, S34**), such oil droplets exhibited an underwater oil contact angle

323   (OCA) >150° on the modified fabric surfaces (**Figure 4i, S35**). In addition to organic

324   solvents, edible oils such as rapeseed oil also showed OCAs>150° on a series of typical

325   fabric surfaces (**Figures 4j**). This underwater superoleophobic property of PTL-

326   pSBMA is also robust, as it did not change substantially after doping with different

327   concentrations of external molecules (e.g., antibacterial PL) (**Figure S36**).
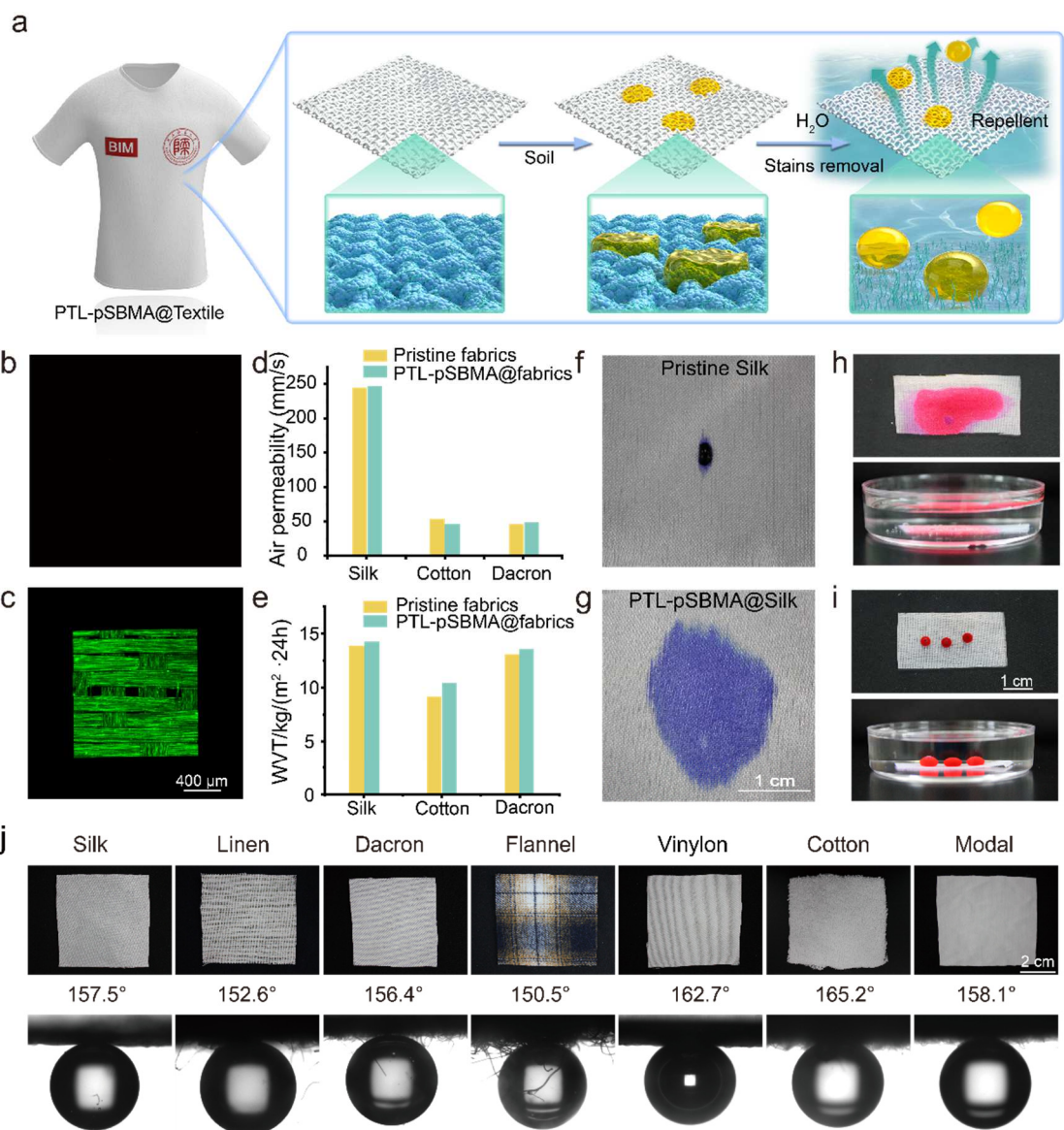
Figure 4. Surface modification of fabrics by PTL-pSBMA. (a) Schematic diagram of the surface modification of fabrics by PTL-pSBMA and stain removal with water. (b, c) LSCM images showing the PTL-pSBMA@fabrics modified by FITC labelled Lyz-pSBMA. (d, e) Air (d) and moisture (e) permeability tests before and after the PTL-pSBMA modification. (f, g) Photographs of pristine silk (f) and PTL-pSBMA@silk (g) after the deposition of an ink droplet. (h, i) Photographs of pristine silk (h) and PTL-pSBMA@silk (i) after dropping Nile red-dyed dichloromethane droplets under water. (j) Photographs of PTL-pSBMA-modified fabrics and the underwater oil contact angles.

The above results shed important light on easy oil removal from textile surfaces, since oil stains on fabric (i.e., clothes) is a big issue that consumes large amounts of water, detergent and energy during washing. In this context, the hydrophilicity enhancement and resultant superoleophobicity after attachment of the PTL-pSBMA nanofilm coating to the fabric surface is highly desirable and holds great promise to

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343 develop a detergent-free oil removal protocol by pure water washing, which would

344 significantly reduce the consumption of precious water and energy resources and

345 decrease the ecological impact from detergents (**Movie S1**). For this purpose, the ability

346 of PTL-pSBMA-modified fabrics to resist common stains, including chili oil, ketchup,

347 grass, and coffee, was then examined (**Figure S37**). In this test, the same amounts of

348 these four kinds of stains were applied to the surfaces of pristine fabrics and PTL-

349 pSBMA@fabrics (Dacron, vinylon, silk and cotton). The following washing steps were

350 divided into four groups: (1) the blank group consisting of pristine cloth with the four

351 stains followed by machine washing with water; (2) the PTL-pSBMA@fabrics group

352 consisting of the PTL-pSBMA-modified cloth with the four stains followed by machine

353 washing with water; (3) the DWL group consisting of pristine cloth with the four stains

354 followed by machine washing with dishwashing liquid (DWL); and (4) the LP group

355 consisting of pristine cloth with the four stains followed by machine washing with

356 laundry powder (LP). By photographing and determining the detergency and whiteness

357 retention of the fabrics[50], it was clearly observed that for water-soluble stains (e.g.,

358 ketchup and coffee), the detergency and whiteness retention in the PTL-pSBMA, DWL

359 and LP groups were basically the same, and all were greater than those in the blank

360 group (**Figures S38, S39**). This result indicated that with the use of the PTL-pSBMA

361 coating, simple machine washing by water without the use of detergent is enough to

362 achieve conventional detergent-based cleaning performance for water-soluble stains.

363 Further, by quantifying the detergency and whiteness retention of the four groups of

364 fabrics polluted with oil-soluble stains including chili oil and grass, it was then found

365 that the PTL-pSBMA-modified fabric group presented the same or even better cleaning

366 performance than the DWL and LP groups, and the values in these three groups were

367 ~4-fold enhancement from those in the blank group (**Figure 5 and Movie S2**). For

368 another control, the anti-oil stain performance of the fabrics after dip-coating in the PTL

369 (phase-transitioned lysozyme) or pSBMA was basically the same as that in the blank

370 group, and both were lower than that of PTL-pSBMA (**Figure S40**). This result further

371 proved that the phase transition of Lyz-pSBMA is a key step to support excellent oil

372 resistance for detergent-free water cleaning.
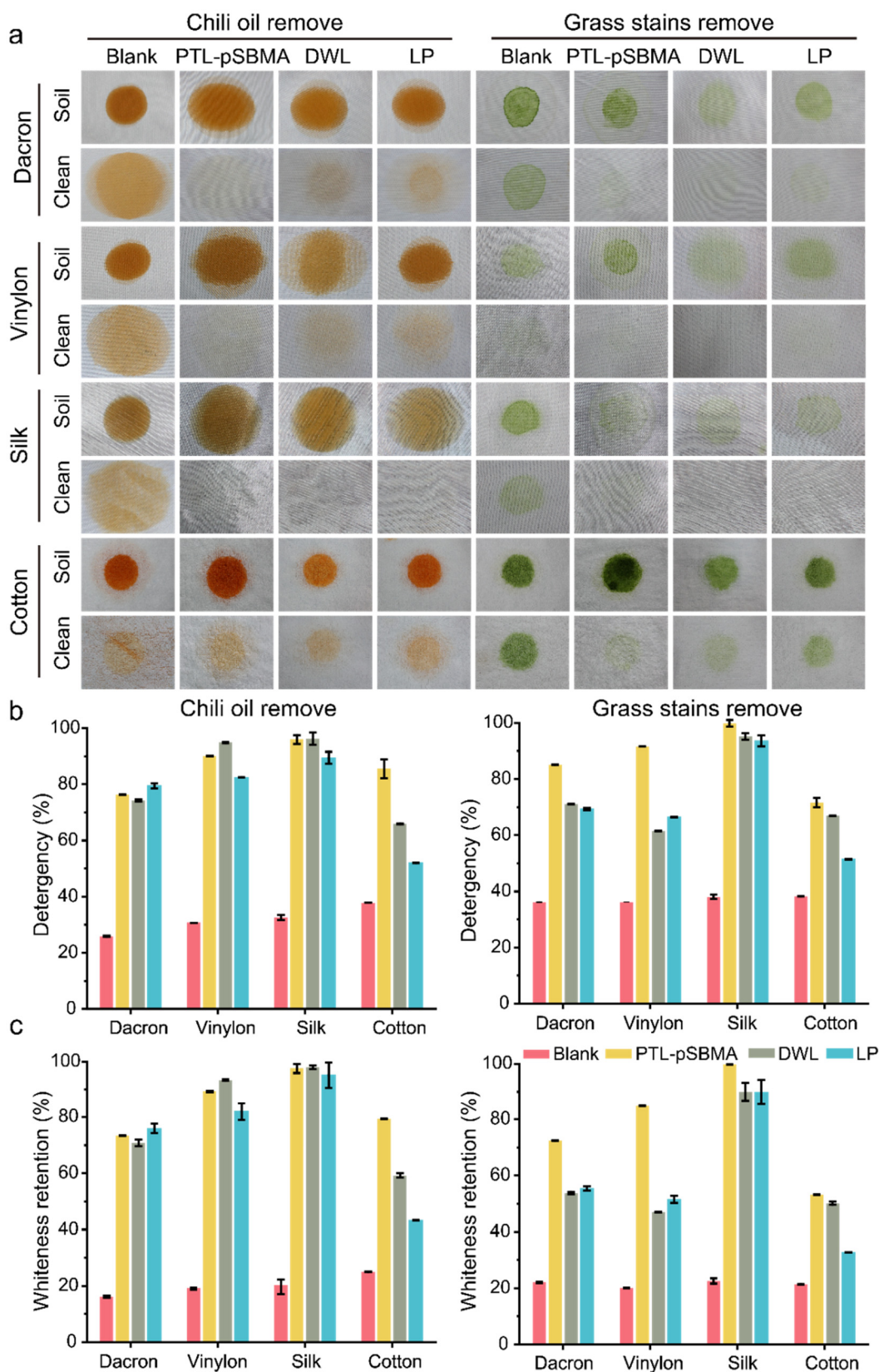
Figure 5. The stain resistance of PTL-pSBMA-modified fabrics. (a, b) Optical images showing removal of chili oil and grass stains from white Dacron, vinylon, silk and cotton fabrics in the groups of blank, PTL-pSBMA, conventional DWL and LP; (c, d) the corresponding detergency and whiteness tests to (a, b).

378   The scale-up application of the PTL-pSBMA technique is closely correlated to

379   three factors, including long-lasting oil stain resistance, application breadth and

380   additional biological impact on the ecosystem. First, the long-lasting oil stain resistance

381   of PTL-pSBMA-modified fabrics strongly depends on the easy adhesion and excellent

382   stability of the amyloid-like protein-polymer layer on the fabrics. In this regard, the

383   unique advantage of PTL-pSBMA compared with existing examples is its rapid filming

384   capability on versatile surfaces and robust interfacial adhesion stability, which thus

385   enable long-term oil stain resistance through circulating fabric surface modification.

386   For this purpose, the PTL-pSBMA-modified fabrics after oil staining were subjected to

387   treatment in a washing machine with water, and it was found that the oil resistance

388   could be well maintained after 20 washing-staining cycles (**Figure S41**). After that, the

389   fabric could be remodified with PTL-pSBMA by simple dip coating in the PTL-pSBMA

390   emulsion, and the refreshed PTL-pSBMA@fabrics again showed excellent oil

391   resistance after periodic washing-staining cycles. By this coating-regeneration process

392   (CAW concept), the excellent detergency performance of PTL-pSBMA-modified silk,

393   vinylon and Dacron was well maintained for at least 200 cycles of washing-

394   regeneration (**Figure 6**). Second, regarding the expendability of the application scope,

395   the PTL-pSBMA-supported detergent-free cleaning method of textiles was further

396   extended to clean kitchenware, such as plates, bowls and dishes. In the chili oil

397   resistance test, the cleaning efficiencies of the PTL-pSBMA-modified dishes (plastic,

398   stainless steel, ceramic and glass plates) were all above 99%, which were significantly

399   higher than those of the DWL and blank groups (**Figure 7 and Movie S3**). More

400   importantly, due to the ease of oil stain cleaning, the cleaning time (correlating to energy)

401   and water consumption in the PTL-pSBMA group were decreased by at least 50%

402   compared with those in the DWL group (**Figure S42**). These results indicated that PTL-

403   pSBMA could not only reduce the large dosage of detergent needed for kitchenware

404   cleaning but also significantly decrease the consumption of water and energy (more

405   than 50%) during the tableware oil cleaning process.
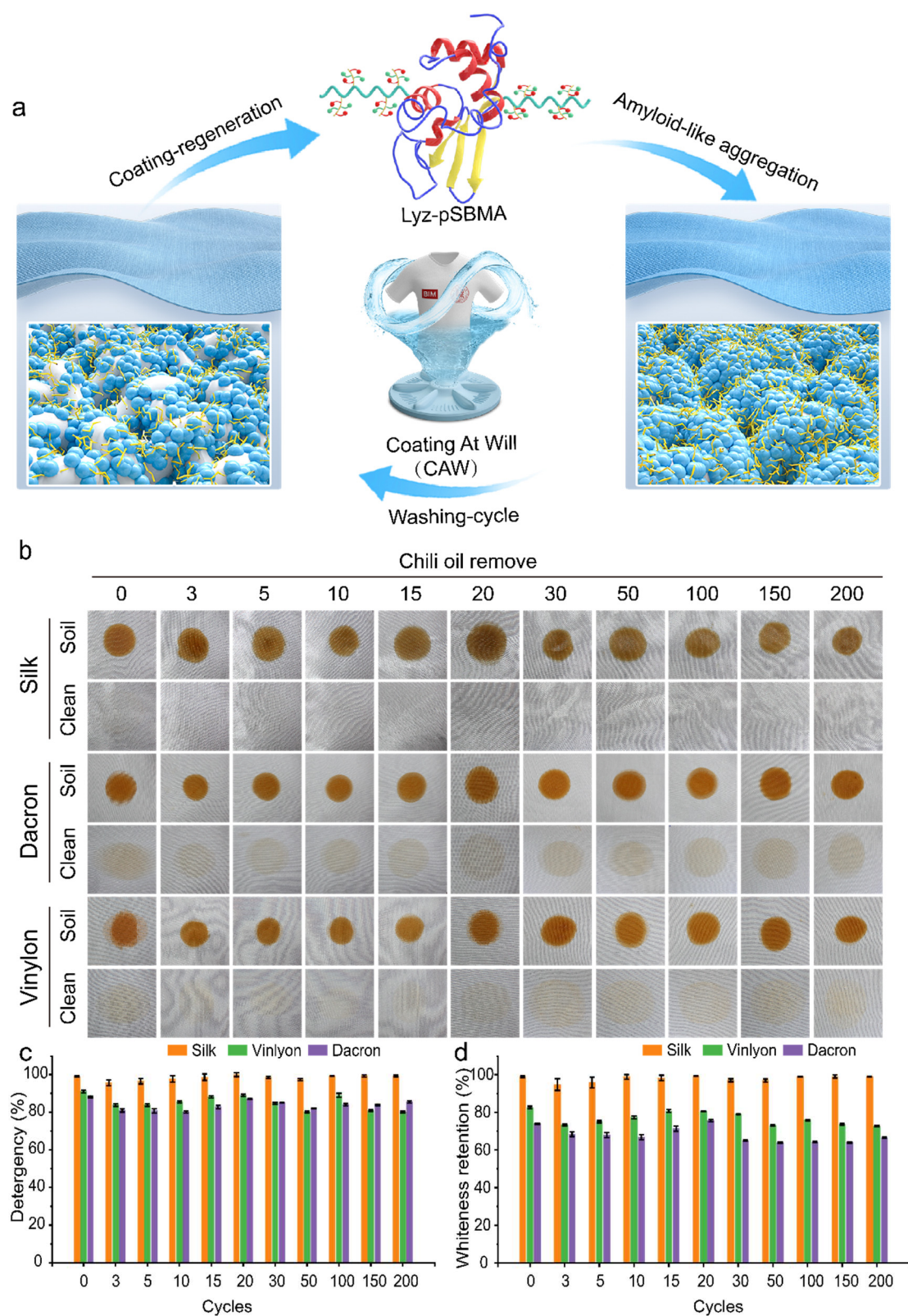
Figure 6. The CAW concept-based oil removal on the PTL-pSBMA-modified fabrics (a). Optical images to show the removal of chili oil from the CAW-based PTL-pSBMA-modified white Dacron, vinylon and silk fabrics for 0-200 cycles of washing and staining (b); (c, d) the corresponding detergency and whiteness retention of PTL-pSBMA@fabrics to (b).
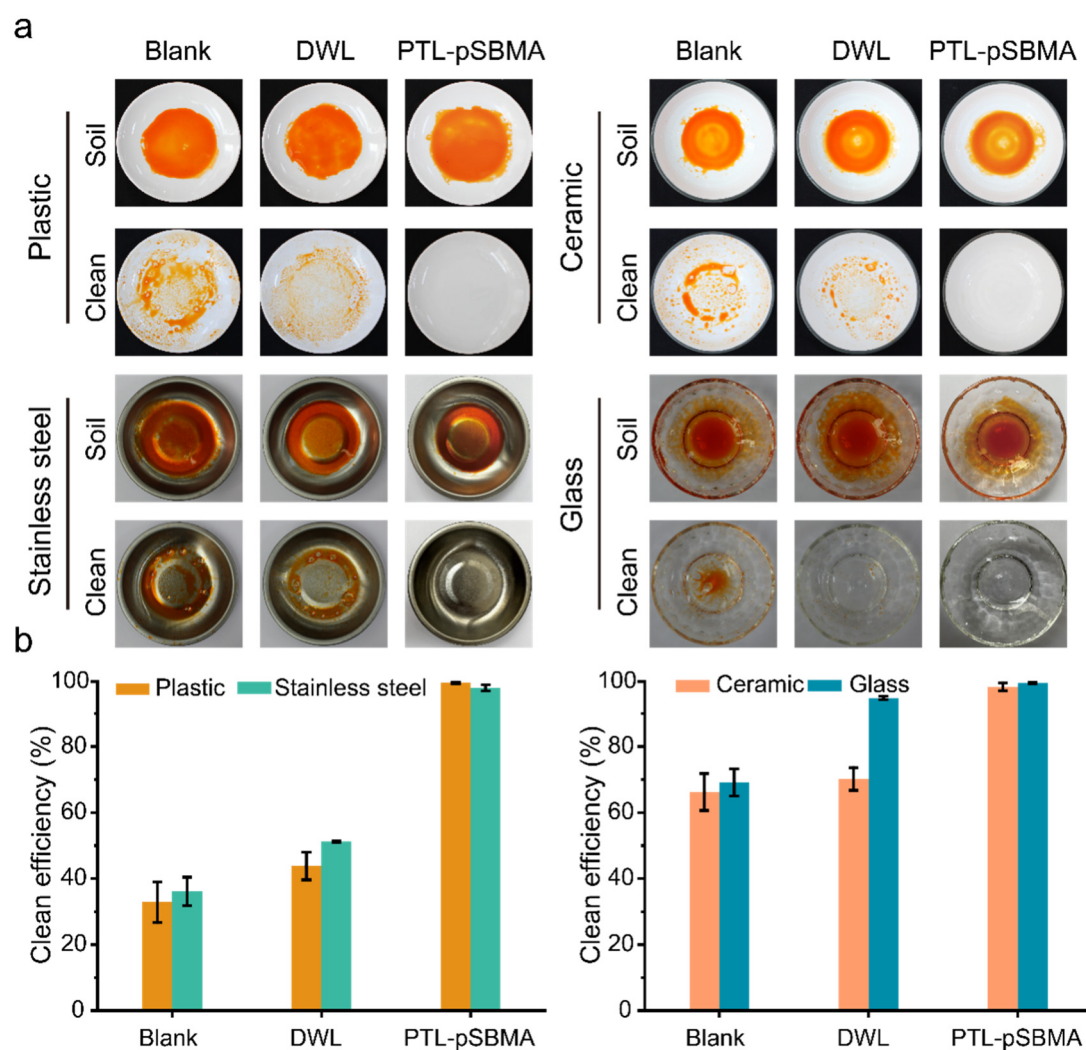
Figure 7. The optical images to show cleaning effect of PTL-pSBMA compared to DWL to remove chili oil from plastic, stainless steel, ceramic, and glass plates (a) and the corresponding cleaning efficiency values (b).

On the basis of the excellent oil resistance, a series of requirements for sustainable scale-up application are further explored. From the perspective of material cost and energy consumption, the cost to wash 1 kg clothes by PTL-pSBMA is approximately $0.675, which is much lower than those of plant enzymes and natural plant extract detergent (**Figure 8a**). Low reagent cost is further supported with a low washing dosage, as the consumption of PTL-pSBMA to wash clothes is only 2.5% of DWL or LP (**Figure S43, S44**)[51-53]. It was further determined that compared with conventional DWL and LP treatments, PTL-pSBMA utilization could reduce water consumption by 50% and electricity consumption by 40% (**Figure 8b**). Furthermore, due to the

426   proteinaceous nature and biocompatibility of betaine derivatives, the negligible

427   cytotoxicity of PTL-pSBMA was then reflected by the MTT method in L929 cells

428   (**Figure 8c**). Then, the biological friendliness of PTL-pSBMA was again confirmed by

429   classical behavioural and developmental evaluations of larval deformation in aquatic

430   creatures. This method is often utilized as a standard evaluation protocol for

431   commercial detergents. Based on the mortality (%) test for zebrafish, the LC50 (median

432   lethal concentration) for the test detergent samples were found to be 9.120, 0.105 and

433   0.048 g/L for PTL-pSBMA, LP and DWL, respectively (**Figure 8d**). This suggests that

434   DWL and LP are highly toxic to zebrafish, while PTL-pSBMA, which showed a rather

435   high LC50, had basically no toxic effects on zebrafish. In addition to aquatic animals,

436   the effect of PTL-pSBMA in water on plant growth was further evaluated, since

437   domestic wastewater often plays an important role in agricultural irrigation. By

438   photographing and measuring the photosynthetic rates after culturing aquatic lettuce

439   with PTL-pSBMA, DWL and LP at concentrations of 1 mg/mL and 2 mg/mL

440   respectively, it was found that the growth status of the plants in the groups of PTL-

441   pSBMA and blank (without the use of any PTL-pSBMA or other detergents) was

442   basically the same; in these groups, the leaves and roots were not withered, and the

443   photosynthetic rate was normal (**Figure 8e, 8f**). However, in the DWL and LP groups,

444   the leaves withered, roots turned brown, and photosynthetic rates decreased

445   significantly. This result indicated that in contrast to the notable toxicity of DWL and

446   LP, PTL-pSBMA had no significant influence on the growth of lettuce, proving its low

447   negative impact on the ecological environment. The biocompatibility of PTL-pSBMA

448   was further assessed based on a skin irritation test by directly applying the PTL-

449   pSBMA@fabrics to the rear skin of male mice. Similar to the results observed in the

450   blank group (pristine fabrics), neither erythema nor oedema was found after 1, 2, 3, 4,

451   and 5 days[54,55], and the haematoxylin and eosin (H&E) staining images further indicated

452   that there were no visible histopathological abnormalities in all tested regions covered

453   with PTL-pSBMA@fabrics (**Figure 8g-h**). Consequently, the principal irritation

454   indices of the PTL-pSBMA@fabrics were all negligible.
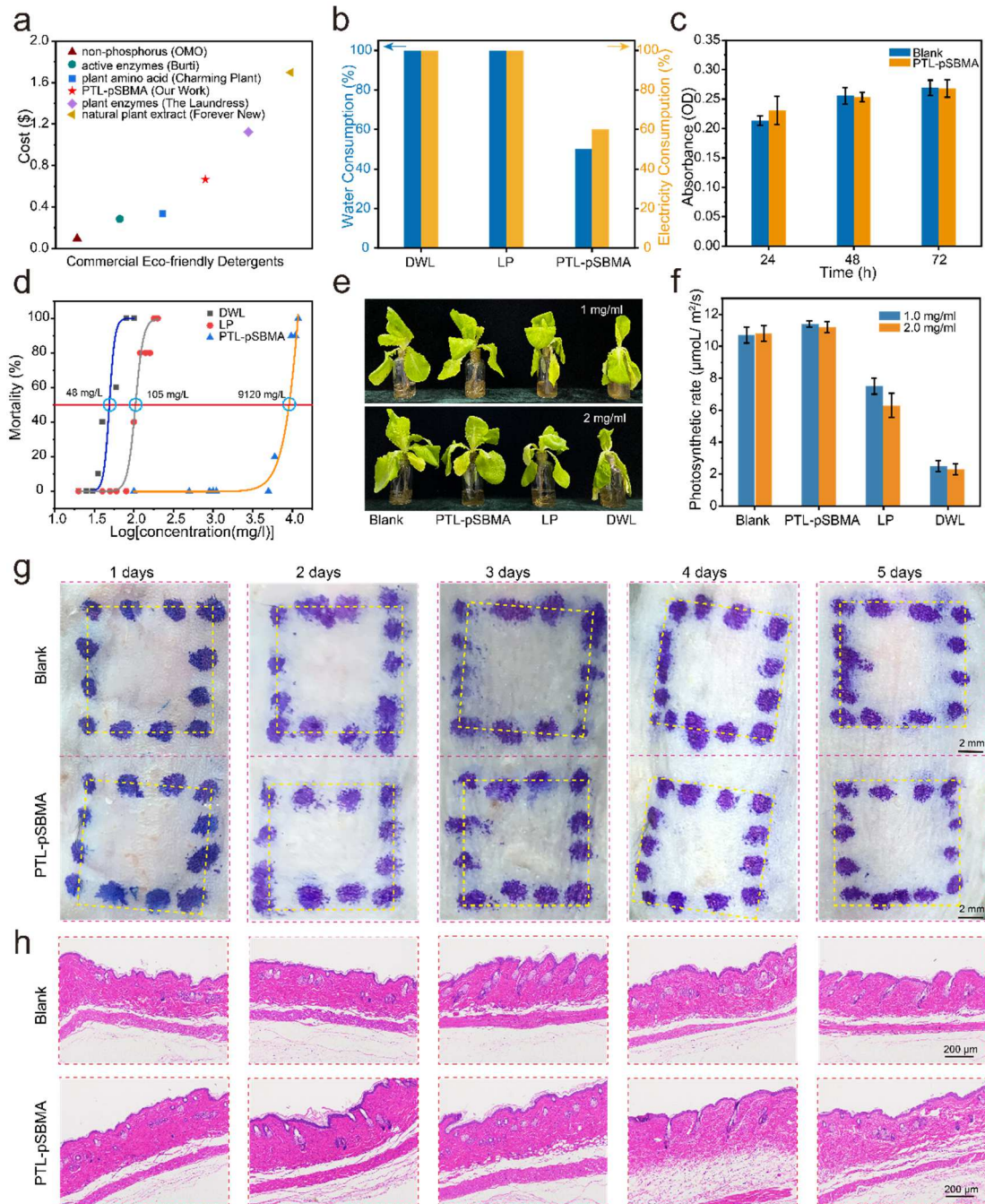
Figure 8. Economic and safety evaluation of PTL-pSBMA. (a) Cost comparison between PTL-pSBMA and commercial eco-friendly detergents; (b) the comparison of water and electricity consumption between PTL-pSBMA and commercial DWL and LP; (c) the cytotoxicity test of the PTL-pSBMA nanofilm; (d) the mortality test of zebrafish for the PTL-pSBMA, LP and DWL; (e, f) Photosynthetic rates of hydroponic lettuce treated with the groups of the PTL-pSBMA, LP and DWL after 48 h of cultivation at 1 and 2 mg/mL, respectively; (g, h) optical and H&E staining images showing mouse back contacting with the blank and PTL-pSBMA@fabrics for 1-5 days.

3. Conclusion.

In conclusion, the present work addressed the issue of aggressive detergent pollution impeding sustainable society development and endeavoured to develop a detergent-free cleaning concept for fabrics and kitchenware. This concept featured the manipulation of the amyloid-like aggregation of lysozyme-pSBMA, a protein-zwitterionic polymer conjugate. The resultant product, PTL-pSBMA, as a new type of surface-active substance, quickly primed virtually arbitrary surfaces (metal, glass, plastic, ceramic and fabric) at a low dosage (0.005% w.t.) to impart them with high hydrophilicity and corresponding excellent underwater superolephobicity. The resultant PTL-pSBMA coating on fabrics and kitchenware could autonomously remove oily stains (chili oil, grass stains, coffee and ketchup) by the hydrophilic polymer chains, leading to the facile water-induced removal of the oily stains without the addition of detergent. This method achieved a cleaning efficiency comparable to or even better than those of normal detergent-based cleaning, and the CAW (coating at will) concept was further proposed to regenerate the PTL-pSBMA-primed surface at any time by simple recoating with PTL-pSBMA. In this way, the detergent-free water cleaning mode supported by the PTL-pSBMA coating on fabrics and kitchenware can be maintained for an unlimited period (passing through at least 200 washing-staining cycles). Compared with the normal detergent-based cleaning mode, the water and energy consumption with the PTL-pSBMA system was reduced by 40%-50%. In further combination with its cost-effectiveness ($675/ton), low washing dosage, good optical transparency and biocompatibility, as well as excellent anti-biofouling properties, the PTL-pSBMA and corresponding CAW concept opens a door for household and industrial detergent-free cleaning with outstanding cleaning efficiency and ecological safety, providing a solid market-ready base to power the development of a sustainable modern society with a minimized ecological impact. In light of the great importance of surface wettability modulation in advanced technologies, it is further expected that our strategy may evoke great interest in a variety of relative fields to stimulate the development of sustainability science and technology, such as droplet manipulation, membrane separation, resource recycling and water protection.

Conflicts of Interest.

The authors declare no conflicts of interest.

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