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Multifunctional Polyurethane Sponge for Polymerase Chain Reaction Enhancement

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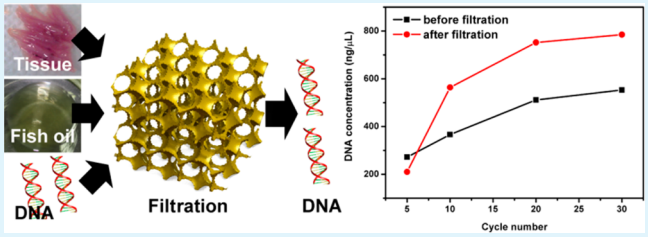
Supporting Information

ABSTRACT: Selective filtering of target biomaterials from impurities is an important task in DNA amplification through polymerase chain reaction (PCR) enhancement and gene identification to save endangered animals and marine species. Conventional gene extraction methods require complicated steps, skilled persons, and expensive chemicals and instruments to improve DNA amplification. Herein, we proposed an alternative method for overcoming such challenges by imparting secondary functionality using commercially available polyurethane (PU) sponges and cost-effective fabrication approaches through polydopamine and polysiloxane coatings. The porous, highly flexible, and chemically modified superhydrophilic and superhydrophobic PU sponges allow large surface areas and mechanically stable frames for effective extraction of genomic DNA through selective filtering of fish tissues and oils. Furthermore, these chemically modified PU sponges allow separation of genes and improvement of PCR for DNA amplification for the identification of fish species. The combination of a simple fabrication method and functionalized PU sponges could be a useful platform for PCR enhancement and gene-based identification of species for practical applications.



KEYWORDS: *polyurethane, filter, superhydrophobic, superhydrophilic, PCR, DNA*

1. INTRODUCTION

Effective identification of genomic information about endangered species, especially marine animals and fishes, is considered an important task to scientists and engineers because of their ecological, educational, and scientific value.[1−3] These challenges can be met by identification and categorization of species through polymerase chain reaction (PCR) of genomic DNA and merging of materials science, nanotechnology, and biotechnology. Currently, the extraction of DNA from tissues and amplification through PCR are the most common and effective methods for identifying genomic information about species.[4,5]

Analysis of genetic information is commonly initialized from direct extraction of DNA from tissues or cells. During DNA extraction, heterogeneous biological mixtures such as tissue fragments and 0.7−15.5 wt % fish oil from the various fish species are considered as major inhibitors in decreasing the level of PCR amplification; therefore, they should be removed.[6−8] In particular, mechanical filters are only capable in partial isolation of genomic DNA from tissue fragments while oil remains in the mixture. Furthermore, conventional extraction of DNA from target species often requires complicated and labor-intensive pretreatment procedures, excessive time, and low yields. Because of these limitations, a simple, practical, and inexpensive material for selective recovery of DNA is highly desirable.

Several studies have employed three-dimensional (3D) structural fabrication technologies, especially in microfabrication methods, including rapid prototyping-based, photomask-based, and micromold-based methods.[9,10] These techniques control the macro- or microscale polymeric pattern or porous 3D features. Although precise and easily controlled dimensions are possible, these approaches still require expensive equipment and facilities that lead to increased fabrication costs. More recently, glass or polystyrene beads have been used to prepare a variety kinds of hierarchically porous structures.[11] However, the complicated steps are required to synthesize uniform beads, and removal processes for forming the 3D matrix have become a major bottleneck in large-scale processes. Additionally, it has not been clearly determined whether the mechanical and chemical reliability and scalability of the materials are sufficient to meet those requirements.

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At present, a commercially available 3D porous polyurethane (PU) sponge has been considered as a new candidate material because of its low density, good elasticity, large surface area, and low cost. With these advantages, it plays the role of a core template in the fabrication of the superhydrophobic or superhydrophilic sponges for water−oil separation and selective adsorption of chemical solvents.[12−15] Although some work has been conducted in the past using superhydrophobic PU foam to recover different kinds of oils or chemical solvents from aqueous mixtures, to the best of our knowledge, the porous structural benefits of PU have rarely been applied in practical filtering of biomaterials, including DNA and tissues.[16−18] Nevertheless, its hierarchical and high porosity with great mechanical durability make this material suitable for usage in both mechanical and chemical filters.[19,20] Moreover, easy surface modification of PU with both organic and inorganic materials helps to control of its wettability.[21,22]

Taking those challenges and demands into consideration, herein, we propose a simple but effective way to produce an advanced multifunctional composite by adopting polyurethane foam and a thin layer coating of both organic and inorganic substances. Following acid etching of a PU frame, bio-inspired polydopamine (PDA) coating was conducted to increase hydrophilicity while methyltrichlorosilane was added to produce more hydrophobic PU sponges. Superhydrophobic and superhydrophilic PU sponges provide numerous advantages, including elasticity, mechanical stability, 3D porous structures, easy access, and commercial availability. The signature porosity of functionalized PU can be employed as both mechanical and chemical filters to separate tissue fragments and adsorb fish oil. Accordingly, the performance of as-prepared multifunctional sponges was investigated using real fish and a PCR method to identify fish species.

2. EXPERIMENTAL SECTION

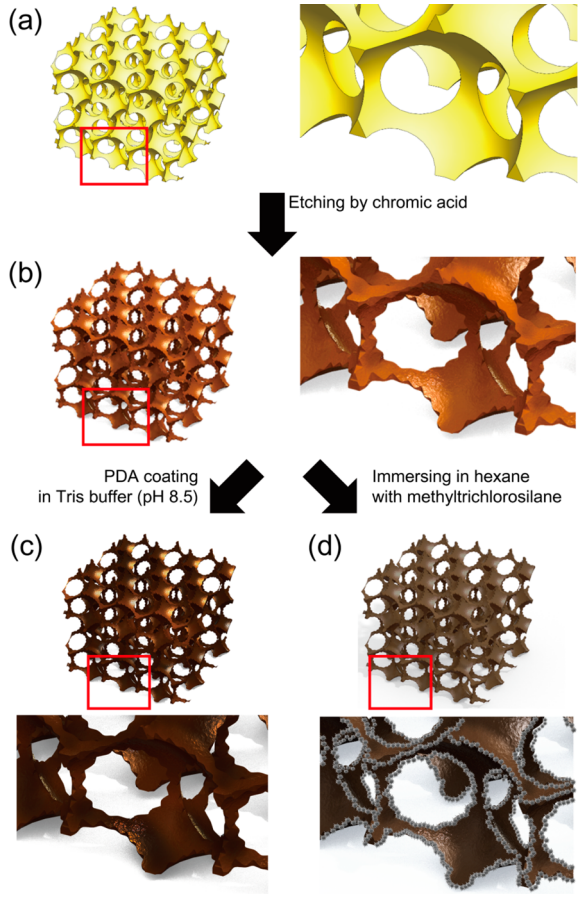
2.1. Materials. Chromium(VI) oxide (CrO_3, ≥99.0%), sulfuric acid (H_2SO_4, 95.0−98.0%), hexane (95.0%), methyltrichlorosilane (≥97%), dopamine hydrochloride (DA), and tris(hydroxymethyl)-aminomethane (Tris) were purchased from Sigma-Aldrich. The PU sponge (1000 mm × 1200 mm × 2400 mm and 30−45 kg m^{-3}) was obtained from EPONGE Co., Ltd., and ethanol (99.5%) was acquired from Samchun Pure Chemical Co., Ltd. 2× Prime STAR Max DNA Polymerase was purchased from Takara Bio Inc. Triply distilled (DI) water with a resistivity of >18 MΩ cm was used to prepare all solutions.

2.2. Fabrication of Superhydrophobic and Superhydrophilic PU Sponges. The PU sponge was washed with DI water and ethanol under ultrasound irradiation for 30 min. After sonication, the sponge was dried in the oven at 60 °C for 12 h. We next prepared the chromic acid solution that is composed of 4 g of chromium(VI) oxide, 2 g of sulfuric acid, and 40 mL of DI water. The PU sponge was placed in chromic acid solution for 2 min and then rinsed with DI water several times. This etched sponge was used as a template for secondary coatings to produce superhydrophobic and superhydrophilic PU sponges.

The superhydrophilic PU sponge was obtained by direct PDA coating. First, the etched PU was dipped in a mixture of DA and Tris buffer solution (pH 8.5, 10 mM) to initiate polymerization of DA. After polymerization, the PU surface was covered with PDA and washed with DI water several times, after which it was dried at 60 °C for 6 h.

The superhydrophobic sponge was fabricated through polysiloxane coating. Briefly, the etched sponge was placed in a mixture of methyltrichlorosilane dissolved in hexane (1 g/50 mL) for 30 min while being magnetically stirred. After polysiloxane coating, the PU sponge was rinsed with ethanol and DI water several times and dried at 60 °C for 6 h. All fabrication procedures are schematically illustrated in Scheme 1.

Scheme 1. Overall Procedures for PU Sponge Surface Modification Using Chromic Acid, PDA, and Polysiloxane[a]



[a](a) Pristine PU sponge, (b) chromic acid-treated PU sponge, (c) PDA-coated PU sponge, and (d) polysiloxane-coated PU sponge.

2.3. Genomic DNA Extraction. Genomic DNA (gDNA) was isolated by scrubbing the fish flesh from *Trachurus japonicas* (also known as saurel) several times using a cotton swab to remove flesh from its body. The flesh was placed in a test tube and then harshly shaken for gDNA extraction. Three different PU sponges were applied for pretreatment of the solution. A pristine PU sponge was employed to compare with surface-modified sponges as a filter. The PDA-coated PU sponge was immersed to remove the oil, and subsequently, the polysiloxane-coated PU sponge carefully filtered tissue sediments and separated them from the gDNA solution. The leftover solution was further investigated through PCR for DNA amplification.

2.4. Target DNA Amplification. The specific sites of the genes were amplified by PCR on a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA) in a 30 μL solution containing genomic DNA, primers (0.25 μM each), and 2× Prime STAR Max DNA Polymerase. The primer sequences were designed to have similar G/C contents (50%) and a T_m of ∼60 °C. Forward primer 5′-CAACCAACCACAAAGA-CATTGGCAC-3′ and reverse primer 5′-ACTTCAGGGTGACCGA-AGAATCAGAA-3′ were used to amplify target DNA samples. The PCR was programmed for an initial 60 s at 94 °C, followed by 29 cycles of 2 s at 94 °C, 5 s at 47 °C, and 2 s at 72 °C, and then a final extension for 2 min at 72 °C. After amplification, the PCR products were further analyzed by agarose gel electrophoresis.

2.5. Instruments. A field-emission scanning electron microscope (FE-SEM, S-4800, Hitachi) was employed to investigate the morphology of the sponges. The wettability of sponges over different

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chemical treatments was analyzed by contact angle (CA) measurement (Phoenix 300 Plus, Surface Electro Optics). The chemical characteristics of sponges were observed by Fourier Transform infrared spectroscopy microscopy (FT-IR microscope, Bruker Opticks) and X-ray photoelectron spectroscopy (XPS, MultiLab 2000, Thermo). XPS data were analyzed using a Thermo MultiLab 2000 system. An Al Mgα X-ray source at 200 W was used with a pass energy of 20 eV and a 45° takeoff angle in a 10^{-7} Torr vacuum analysis chamber. Each FT-IR spectrum was recorded from 4000 to 12 scans at a resolution of 2 cm^{-1}. A PCR thermal cycler (C1000 Touch Thermal Cycler, Bio-Rad), a submarine electrophoresis system (Mupid-2plus, Advance), and a compact gel documentation system (Slite 140, Avegene) were utilized to confirm the performance of sponges for amplification and analysis of DNA.

3. RESULTS AND DISCUSSION

3.1. Preparation of Superhydrophobic and Superhydrophilic PU Sponges. The superhydrophobic and superhydrophilic PU sponges were prepared through acid treatment and secondary surface coating using polysiloxane and PDA coating methods.[23,24] In conventional processes, a piece of commercially available sponge was cleaned and etched using a chromic acid mixture. Typical SEM images of sponges before and after chromic acid processing are shown in panels a and b of Figure 1, respectively. The mainframe surface of the pristine

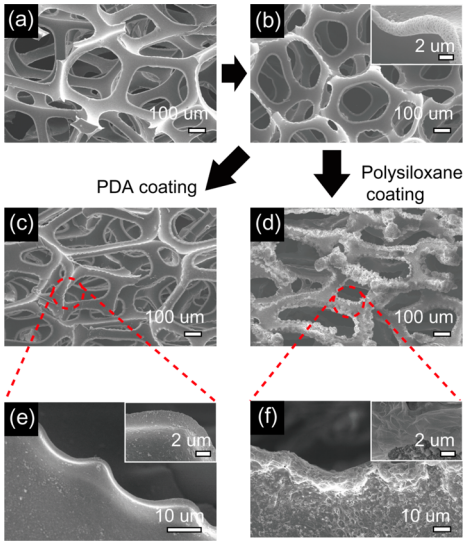

Figure 1. SEM images of (a) the pristine PU sponge, (b) the chromic acid-treated PU sponge, (c and e) the PDA-coated PU sponge, and (d and f) the polysiloxane-coated PU sponge at different magnifications.

PU form presented a smooth and flat surface. However, after acid etching, the PU surface became rougher and formed nanoscale pores over the main polymeric frames (inset of Figure 1b).

As-prepared etched sponges were further converted into superhydrophobic and superhydrophilic surfaces using polysiloxane and PDA coatings, respectively (Figure 1c–f). The superhydrophilic modification was prepared by simply dipping the etched sponge into a DA solution to form thin PDA layers through polymerization of DA. After coating, no significant changes in surface roughness changes were observed over the frame (Figure 1c,e). For the construction of superhydrophobic layers, the etched PUs were immersed in a methyltrichlorosilane/hexane mixture for secondary polysiloxane coating. During this process, random formation of polysiloxane layers over the polymeric frame resulted in the construction of hierarchical macro-, micro-, and nanoscale structures (Figure 1d,f). This rough surface, which was similar to a lotus leaf, helped to reduce the surface energy.[25,26]

Surface modifications of sponges were also confirmed through CA measurement. Initially, the CA of the pristine PU was ~110° (Figure 2a), while that of etched PU was 65°

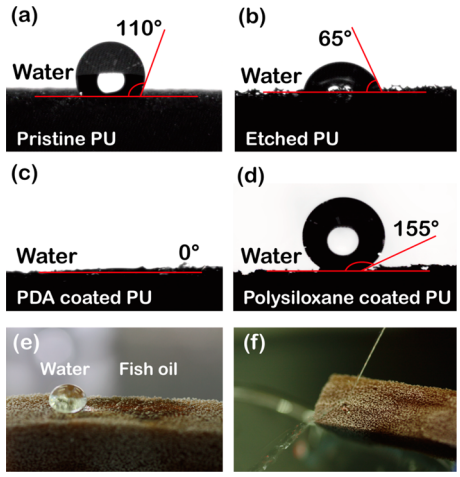

Figure 2. CA images of (a) pristine PU, (b) acid-treated PU, (c) PDA-coated PU, and (d) polysiloxane-coated PU for water. (e) Water droplet and fish oil on a polysiloxane-coated PU sponge. (f) Water being sprayed on a polysiloxane-coated PU sponge.

(Figure 2b). After adhesive PDA layers had been coated over the PU frames, the sponge showed excellent water adsorption capability and its CA is almost 0° (Figure 2c). The superhydrophobic sponge exhibited a CA of 155°, and all sides of this sponge repelled water but absorbed oil. This result confirmed the superhydrophobic and oleophilic characteristics caused by the benefits of both hierarchical structures and coating material (Figure 2d–f). Thus, the entire surface and inside of the sponge also repelled water while absorbing oil (Figures S1–S3 of the Supporting Information).

3.2. Surface Modification of PU. To understand the secondary surface coating of PU, we investigated chemical and structural changes by FT-IR and XPS (Figure 3 and Figure S5 of the Supporting Information). Initially, the pristine PU sponge was merged in a chromic acid solution that is mixture of chromium(IV) oxide and sulfuric acid. This acid etching dramatically changed the chemical constitution of the polymer; therefore, a limited extent of surface etching was beneficial to the surface wettability. The etching process of polyurethane usually occurs via the following mechanism. Initially, the oxidation reaction occurs in the tertiary C–H bonds of polyurethane. The H of tertiary C–H bonds is then oxidized to briefly convert into chromium(IV) ester as an intermediate state and finally form an alcohol.[27–29] These sequential processes lead to random polymeric chain scission, which produces hydrophilic groups such as -OH and -COOH. In this study, the FT-IR spectra revealed adsorption bands at 2970 cm^{-1} that were ascribed to the characteristic vibration of -CH- stretching from PU backbones. Additionally, broad 3200 and 3500 cm^{-1} bands were observed, which were attributed to R-OH and -COOH bands stretching from the PU chemical bond that was broken during chromic acid treatment. The functional groups provide binding sites for secondary chemical mod-

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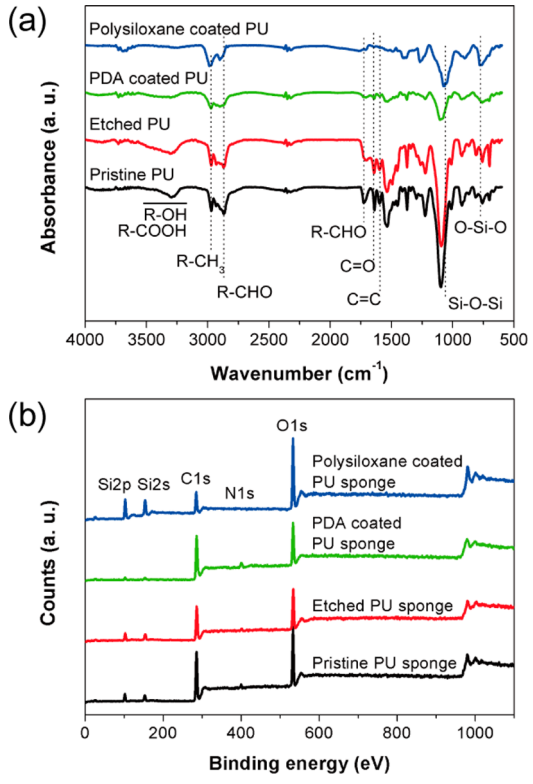

Figure 3. (a) FT-IR spectra of pristine PU (black), acid-treated PU (red), PDA-coated PU (green), and superhydrophobic PU (blue). (b) XPS survey spectrum of pristine PU (black), acid-treated PU (red), PDA-coated PU (green), and polysiloxane-coated PU (blue).

ification. The superhydrophilic PU sponge was fabricated through the employment of biocompatible and strong adherent PDA coating. After the etched PU sponge had been immersed in a DA solution, DA precursors were continuously attached and polymerized over the PU through the oxidation reaction of catechol groups to quinone. The reaction was started via oxidation, intramolecular cyclization, and rearrangement, followed by multistep reactions (Figure S4a of the Supporting Information). The conjugated aromatic structures exhibit a planar structure and further react with each other to form PDA over the PU frame.[30−32]

Finally, the etched PU surface was covered with PDA. The continuous chemical structural changes were evident from the FT-IR spectra as shown in Figure 3a. The broad absorbance 3293 cm^{-1} is ascribed to O−H stretching vibrations from the etched PU sponge. After adherent PDA coating, the broad bands of O−H stretching vibrations disappeared because of the condensation of PDA and formation of R−O−R structures (Figure S4a of the Supporting Information). The XPS survey scan and high resolution of N 1s XPS spectra for the N 1s peak as shown Figure 3b and Figure S5 of the Supporting Information also supported the formation of PDA over PU. From the pristine PU sponge, the main peak at 400.2 eV resulted from N−H backbone structures of PU. In contrast, the XPS spectrum of PDA-coated PU presented two peaks at 400.3 and 398.8 eV that are attributed to N−H and C−N═C structures. These peaks correspond to unique chemical features from both PU and PDA. Furthermore, the peak band of N 1s from the PDA-coated PU sponge becomes broader than that of pristine PU. These XPS results again confirm the successful incorporation of PDA coating onto the etched PU surface after DA self-polymerization.[33]

In the case of the superhydrophobic PU sponge, the polysiloxane coating was conducted over the surface of the etched PU sponge. The coating method is similar to the formation of silica layers. After acid treatment, the hierarchical roughness was formed on the surface of the PU sponge. For superhydrophobic surface modification, the surface of the PU sponge was modified by methyltrichlorosilane in hexane. The mechanism of the coating is initiated by the hydrolysis of methyltrichlorosilane.[34,35] Once the siloxane seed was attached over etched PU structures, the subsequent condensation of silane began to attach over the seed to form thin polysiloxane layers that finally covered the entire PU backbone (Figure S4b of the Supporting Information).

The existence of a polysiloxane coating over the sponge was identified by FT-IR. The adsorption band at 1068 cm^{-1} in the FT-IR spectra was ascribed to the unique characteristic vibrations of the Si−O−Si structure. The unique peak appearance from Si−C at 775 cm^{-1} also confirmed the formation of polysiloxane layers over the PU surface.[33] Furthermore, the appearance of new XPS peaks at 103.0 and 154.0 eV indicated the major coating product is composed of a silicone component over the PU surface (Figure 3b).

Overall, the peak at 1100 cm^{-1} attributed to C−O from PU was slightly shifted or diminished because of the secondary coating with either PDA or polysiloxane over the PU surface. Furthermore, the broad band at 3200−3500 cm^{-1} was mainly attributed to R-OH and -COOH, and the intensity of the band decreased because of the formation of chemical bonds with PDA and polysiloxane. These major peak changes from FT-IR spectra confirmed the proper surface modification with PDA and polysiloxane and promoted the synergetic effects on oil adsorption and tissue filtration.

3.3. Separation of Fish Tissue Fragment and Oils. In most cases, similar surface energies of the oil and material surface favor the adsorption of oil onto the surface as reported previously.[23,36,37] For this reason, the methyltrichlorosilane (i.e., superhydrophobic)-coated PU sponges presented a surface energy similar to that of the fish oil. Furthermore, the rough hierarchical structures were more favorable for the acquisition of superhydrophobic characteristics, similar to a lotus effect.[38−40] To confirm the capability of as-prepared sponges, the water/fish oil mixture was prepared as shown in Figure 4. The yellowish fish oil exhibited a density lower than that of water, and the boundary fish oil layer can be clearly observed through the container (Figure 4a). To demonstrate its oil adsorption capability, the superhydrophobic sponge was simply dipped into a mixture of water and oils. As soon as the oil touched the surface of a modified PU sponge, all of the fish oil was rapidly absorbed into the sponge, while the water remained in the container. As expected, the oils were completely removed from the mixture in a few seconds and no yellow solution (i.e., fish oil) was observed at the water interface (Figure 4a−d). Additionally, the absorbed oil could be recovered by squeezing the sponge (Figure 4e,f). Thus, the average oil adsorption capacity, k, of the as-prepared sponge was also measured and is presented in Figure 4g. The results indicate that the modified PU sponge could hold ~25 times more than its material weight.

From the results presented above, the superhydrophobic PU sponge demonstrated its performance by selectively absorbing fish oil from the mixtures. In addition, a soft and flexible polymer matrix could be a better candidate material for

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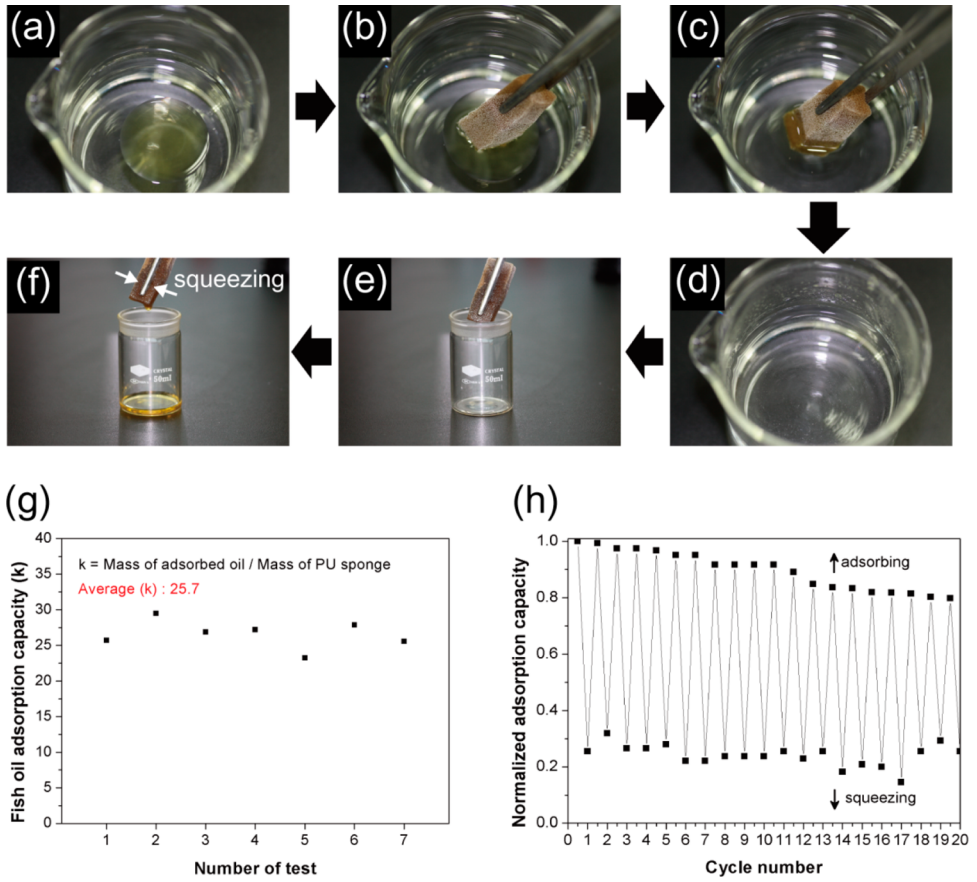

Figure 4. (a–d) Oil adsorption procedures for the superhydrophobic PU sponge and (e and f) quantification of the oil adsorption amount. (g) Fish oil adsorption capacity of several superhydrophobic PU sponges. (h) Adsorption capacity for repeated testing of absorbing and squeezing.

repelling water drops and be easily regenerated from the absorber by squeezing. The large surface area of the sponge allows an increased volume to weight adsorption capability compared with those of other materials, including textile, paper, and metal meshes.[41] The chemical and mechanical stability of as-prepared sponges is also important to their practical application. To check the properties, we tested an as-prepared sample by absorbing and desorbing oils and normalized the weight differences as shown in Figure 4h. As anticipated, the oils were removed and recovered fairly well, and good oil adsorption properties were maintained for more than 20 cycles. Therefore, these results indicate that the PU sponge is highly suitable for bio-oil recovery applications.

3.4. PCR Application. As-prepared superhydrophobic PU sponges and superhydrophilic PU sponges were introduced to demonstrate the oil absorbability and mechanical separability of tissue fragments. To isolate gDNA, a test tube containing a fish tissue chunk and 500 μL of deionized water was harshly shaken more than 20 times. Then, the superhydrophobic sponge was put inside the test tube for oil separation, and the remaining mixture was sequentially passed through the superhydrophilic PU sponge to isolate gDNA from tissue fragments. Finally, the superhydrophilic sponge was squeezed to extract gDNA. Once the tissues were mechanically broken into small pieces, fish oil was automatically separated and adsorbed onto the superhydrophobic PU sponge. In addition, small tissue fragments were also filtered through pores inside of superhydrophilic sponges, and the filtration performance of the tissue fragment was also confirmed by SEM (Figure 5a–d).

To verify the effectiveness of PU sponges in PCR amplification, the different portions of the PCR experiment were performed under those conditions as follows: (1) pristine PU sponge (i.e., partial removal of oil and tissue fragments), (2) only superhydrophobic PU sponge (i.e., only oil removal), (3) only superhydrophilic sponge (i.e., only tissue removal), and (4) both superhydrophilic and superhydrophobic sponges (i.e., both oil and tissue fragment removal). The amplified products under such conditions were identified by agarose gel images as shown in Figure 5e. Furthermore, the positive and negative control experiment was also performed as shown in Figure S6 of the Supporting Information. The agarose gel image of the PCR product from the oil and tissue removal gDNA exhibited a relatively strong and clear band compared with the bands of partially filtered oil or tissues as shown in Figure 5e and Figure S7 of the Supporting Information. The results indicate that oil and tissue fragments inhibited PCR amplification and the amplified product presented a smear band in the image. On the other hand, a strong band image was observed after removing the oil/tissue mixture with superhydrophilic and superhydrophobic PU sponges. The potential possibility of gDNA adsorption over a hydrophilic PU matrix was also investigated by adsorbing and desorbing through squeezing of the sponge and a known amount of gDNA. It was found that <6% of gDNA remained inside the PU matrix during the squeezing of the sponge (results listed in Table S1 of the Supporting Information).

To verify clear effects of those PU sponges and confirm that the tissue and oil affect PCR amplification as inhibitors, an

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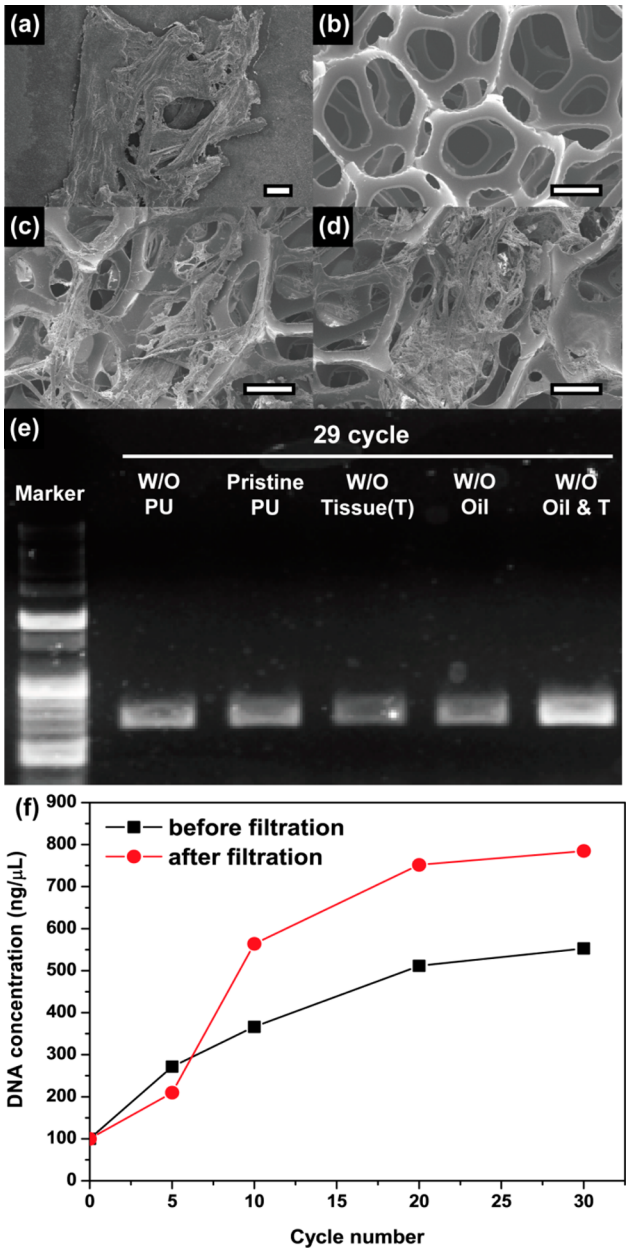

Figure 5. SEM images of (a) pristine fish tissue, (b) pristine PU sponge, and (c and d) tissue fragments filtered in PU sponge matrixes (scale bars of 200 nm). (e) Agarose gel images of marker and amplified DNA bands without the PU sponge and with the pristine PU sponge (partial tissue and oil removal), the superhydrophilic sponge (tissue removal), the superhydrophobic sponge (oil removal), and a combination of both superhydrophilic and superhydrophobic sponges (both tissue and oil removal). (f) Graphic image of amplified DNA changes over PCR cycles with and without filtering oil and tissue fragments.

additional experiment was performed with and without filtering of gDNA, tissue fragments, and fish oil. The amount of amplified PCR product was investigated every 5, 10, 20, and 30 cycles of PCR (Figure 5f). Until the fifth cycle, there was a relatively small amount of difference with amplified DNA between the PCR mixture before and after filtration. However, the final amount of PCR product amplified from the PCR mixture including filtered gDNA was gradually increased and more than doubled compared to that from the PCR mixture including nonfiltered gDNA. This means that treatment with superhydrophilic and superhydrophobic PU sponges enhances PCR amplification. Therefore, these multifunctional PU sponges are highly suitable for isolating gDNA from heterogeneous mixtures and lead to improvement of the performance of PCR amplification.

4. CONCLUSIONS

We developed a method for fabricating hierarchical structures with superhydrophilic and superhydrophobic properties using commercially available PU sponges and secondary PDA and polysiloxane coatings. These functionalized sponges can selectively remove oil and tissue fragments to enhance PCR amplification for powerful DNA amplification, while maintaining their structure, flexibility, porosity, and selective wettability and filtering. Moreover, PU sponges had the ability to extract target genes after tissue crushing and selective fish oil adsorption, which are critical steps for enhancing PCR. The PCR results using each conventional and sponge-based approach clearly show the improvement of PCR for DNA amplification, which will help identify fish species. Cost-effective and multifunctional PU could be a highly suitable platform material for identification of endangered animals, biosensors, biomedical pretreatment, point-of-care diagnostic systems, and filter industries.

■ ASSOCIATED CONTENT

Supporting Information

Contact angles of polysiloxane- and PDA-coated PU and proposed mechanism of polysiloxane and PDA coating. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

S. Seok and S. Shin contributed equally to this work.

Notes

The authors declare no competing financial interest.

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