

Superhydrophobic substrates from off-the-shelf laboratory filter paper: simplified preparation, patterning, and assay application

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ABSTRACT: Off-the-shelf laboratory filter paper of different pore-sizes and thicknesses can be modified with fluorine-free organosilanes to be superhydrophobic, patternable, and ready for quantitative assay applications. In particular, we have demonstrated that cellulose filter paper treated with a binary hexane solution of short (methyltrichlorosilane, MTS) and long (octadecyltrichlorosilane, OTS) organosilanes, exhibits remarkably high water contact angles ($> 150^\circ$) and low wetting hysteresis ($\sim 10^\circ$). Beyond the optimized ratio between the two organosilanes, we have discovered that the thickness rather than the pore size dictates the resulting superhydrophobicity. Scanning electron microscope (SEM) images showed that silianization does not damage the cellulose microfibrils; instead they are coated with uniform, particulate nanostructures, which should contribute to the observed surface properties. The modified filter paper is chemically stable and mechanically durable; it can be readily patterned with UV/ozone treatment to create hydrophilic regions to prepare chemical assays for colorimetric pH and nitrite detections.

KEYWORDS: Filter paper; Silanization; Superhydrophobicity; Microfluidic paper-based analytical device (μ PAD), Nanostructure

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Introduction

The history of paper has been traditionally traced back to ancient China almost two millennia's ago.¹ Due to the excellent properties such as biodegradable, renewable, hygroscopic and economic, paper products have been widely used in our daily life (household, offices, and laboratories).² For a much shorter period (last decade also), paper has been adapted as a new substrate material to the development of analytical assays such as home pregnancy strips, blood glucose test strip, and pH test paper.^{3,4} In 2007, Martinez et al. reported the deposition and patterning of photoresist on chromatography paper to fabricate the first generation of paper-based microfluidic analytical devices (μ PADs).⁵ The basic idea was to create hydrophilic regions (confined by hydrophobic areas) for the selective spotting of assay-specific reagents, which allows small volumes of samples to be tested. Due to its ease-of-use, low cost and universal applicability, μ PADs have been witnessed overwhelmed development in recent years.⁵⁻¹⁸ Particularly, an assortment of analytes have been targeted, e.g., metals,¹¹ anions,^{12,13} proteins,^{5,10,14} with differently designed and fabricated μ PADs. It should be also noted that the flexibility in designing microfluidic patterns allowed for multiplex assays to be conducted on a single device.⁵⁻¹⁰

For the fabrication of μ PADs, the primary challenge is to modify the wettability of paper substrates (i.e., from hydrophilic to hydrophobic). In the past decade, several methods have been developed to fabricate hydrophobic paper substrates, which were based on either physical deposition^{5,19-21} or chemical modification approaches.²²⁻³⁴ Martinez et al. patterned filter paper by ink-jet printing a photoresist film followed by lithography;⁵ while Ogihara et al. sprayed dodecyltrichlorosilane-coated SiO₂ nanoparticles on paper, resulting in superhydrophobic surfaces (water contact angle, $\theta_{\text{water}} > 150^\circ$).¹⁹ Li et al.²⁹ and Delaney et al.³⁰ utilized the esterification reaction between alkylketene dimer (AKD) and -OH groups of cellulose to modify filter paper to be hydrophobic ($>110^\circ$). In comparison, silanization is a simpler alternative to above mentioned paper modification protocols; He et al. explored the OTS modification method to produce hydrophobic filter paper ($> 120^\circ$);²² Glavan et al. produced "omniphobic" paper by reacting with different fluoroalkyltrichlorosilanes in the vapor phase.²³ In most cases of silanization, the treated filter paper or cellulose did not show superhydrophobic properties, i.e., the water contact angles of modified filter paper surfaces are in a range of 100 to 130°.^{26, 27, 31-34} As a matter of fact, superhydrophobic paper was only achieved either via specialized reaction conditions (controlled vapor deposition)⁵ or with fluoroalkylsilanes.³⁵ Unfortunately, these organofluorine compounds can be accumulated in ecosystem and are acutely or chronically toxic to organisms.³⁶⁻³⁷

It should be emphasized that an improvement from hydrophobic to superhydrophobic is not simply the increase in water contact angles or the decrease in surface energy, but the switching in the contact mode between water droplet and solid substrates.³⁸ Particularly, for the superhydrophobic surface there are trapped air bubbles in between water drops and solid substrates, which facilitates the movement of reagents within designed hydrophilic “channels” on μ PADs. The fact that most μ PADs utilized hydrophobic filter paper with a water contact angle slightly above 90° as substrates, which can still lead to leakage of liquid into the hydrophobic regions. If paper is made to be superhydrophobic with water contact angles $> 150^\circ$,¹⁷ the hydrophilic channels and reaction regions of the μ PADs become completely defined. In addition, for the fabrication of μ PADs the patterning of the modified filter paper (either hydrophobic or superhydrophobic) is an essential step; therefore, the modification protocols should be carefully considered with the feasibility of further patterning.

Herein we report a facile and simple modification method to produce superhydrophobic assay substrates from off-the-shelf filter paper via controlled silanization reactions. As described below, the key advancement is to adapt a binary silane solution for the modification; upon optimizing the composition we were able to achieve superhydrophobicity on a number of WhatmanTM laboratory filter paper. In addition, we have shown that they can be patterned with conventional UV lithography, and therefore adapted for the fabrication of quantitative assays for various chemical analysis (pH test and nitrite detections as examples).

Experimental Methods

Materials and reagents

All filter paper samples (WhatmanTM Grade 1, 3, 4, 6, 597, 602h, 502b, 113) were purchased from GE Healthcare (Mississauga, ON). Methyltrichlorosilane (MTS, $\geq 99\%$), octadecyltrichlorosilane (OTS, $> 90\%$), citric acid, Na_2HPO_4 and NaH_2PO_4 were purchased from Sigma Aldrich (St. Louis, MO). Hexane was ordered from ACP Chemical Inc. (Montreal, Quebec). Thymol blue was purchased from Matheson Coleman & Bell (Cincinnati, OH), phenolphthalein from Baker & Adamson (Morristown, NJ). Methyl red and Bromophenol blue were purchased from Fisher Scientific (Pittsburgh, PA). Na_2CO_3 was purchased from Mallinckrodt (Paris, KE); NaHCO_3 and NaOH from Merck KGaA (Darmstadt, Germany). Ethanol (95%) was purchased from Commercial Alcohols (Brampton, Ontario). Deionized water ($> 18.3 \text{ M}\Omega \text{ cm}$) was produced with a Barnstead EasyPure UV/UF compact water system (Dubuque, IA). All reagents are of ACS reagent grade unless specified otherwise.

Filter paper modification and characterization

The modification reaction of small pieces of filter paper ($1 \times 3 \text{ cm}^2$) was performed in disposable glass scintillation vials (20 mL with a cap). At first, a binary solution of different ratios of MTS and OTS was prepared in 10 mL hexane, and de-oxygenated with argon gas (bubbling for 15 min). Then, the paper strip was immersed in the solution for different periods of time (from 1 s to 20 min). During the reaction, the vial was capped to ensure airtight; afterwards the filter paper was removed from the solution and gently dried under nitrogen stream.

To pattern the modified filter paper, the sample was irradiated in an UV/ozone cleaner (PSD-UV, Novascan Technologies Inc., IA) with a designed plastic mask on top. The water contact angles were measured using $1.0 \mu\text{L}$ droplets with a goniometer (AST VCA system, Billerica, MA). For each sample (at least 3 paper samples prepared under the same condition), 3 to 5 different regions were tested to verify the uniformity. The surface morphology was imaged with a scanning electron microscope (SEM) (FEI Nova NanoSEM 430, Hillsboro, OR).

Assay preparation and testing

A plastic mask with eight opening (holes of 0.8 cm in diameter) was used to pattern the superhydrophobic filter paper, i.e., create 8 hydrophilic reaction regions. For the pH test, $5.0 \mu\text{L}$ of universal pH indicator solution was pipetted onto each of the hydrophilic regions of the filter paper, following by adding $20 \mu\text{L}$ solutions of different pH. Different ratios of Citric acid and NaOH were used to make buffers with pH from 3 to 6; Na_2HPO_4 and NaH_2PO_4 pH = 7 and 8; Na_2CO_3 and NaHCO_3 for buffer with pH = 9 to 10). The universal pH indicator was prepared using Yamada Universal Indicator (YUI) recipe, for which 12.5 mg thymol blue, 31.0 mg Methyl red, 125.0 mg bromothymol blue, and 250.0 mg phenolphthalein were dissolved in 250 mL 95% ethanol; it was then neutralized with 1.0 M NaOH and diluted to 500.00 mL with water. Griess reagent for the detection of nitrite was prepared with 50 mM sulfanilamide, 330 mM citric acid, and 10 mM N-(1-Naphthyl) ethylenediamine in methanol solution. Initially, $5.0 \mu\text{L}$ of this indicator solution was pipetted onto the reaction zones; following by $20 \mu\text{L}$ different concentrations of nitrite solutions were then added. The results were analyzed by taking photos with an iPhone 5 and processed with the ImageJ program (ver. 1.48).

Results and discussion

Preparation of Superhydrophobic filter paper via binary silanization

Organosilanes have been commercially used to achieve hydrophobicity on various materials; in the field of surface chemistry silanization has been also extensively studied.³⁹⁻⁴⁵ It has been widely accepted that long chain alkylsilanes (e.g., OTS) can form ordered monolayers on surface,^{45, 46} while

short alkylsilane (e.g., MTS) produces rough surfaces with 3D nanostructures (as of the polymerization and condensation of the silanols) which end up with superhydrophobicity on the surface.⁴²⁻⁴⁴ Our initial tests to treat filter paper with MTS did not produce satisfactory results (not superhydrophobic, as discussed below), we have then explored the binary silanization approach, which was to treat the surface with a binary solution of long and short alkylsilanes (*vide infra*).

As commercial filter paper is available in different grades, with significant variations in pore size and thickness, therefore, experiments were performed on several types of filter paper that are popularly used in the laboratory. With a total concentration at 0.2% and 1:1 ratio of the two silanes (OTS and MTS), we have observed remarkable changes in the surface properties of filter paper samples (Figure 1); it is clear that all of them are no longer wicking to water and show contact angles above 125°. Notably three types of filter paper (Whatman™ Grade 3, Grade 113, and Grade 520B; Figure 1) became superhydrophobic upon treatment in the binary silane solution. Intriguingly, the water contact angle seems to be independent of the pore size (ranging from 2 μm to 30 μm) as we initially expected. Instead the water contact angle increases as the thickness increased from 160 μm to 550 μm; in fact, all three superhydrophobic samples have a thickness of >350 μm. It is known that besides low surface energies the superhydrophobicity relies on the creation of the so-called Cassie–Baxter state,⁴⁷ in which case microsize air bubbles are trapped in between water droplets and underneath solid substrate. As both are important parameters to define the porosity and flow rate, the pore size, precisely “particle retention”, is essentially the nominal dimension of the “pores” in the filter paper, which is determined by the size of particles that they can retain; the thickness dictates the “depth” of the micro/nanostructures (vacancies or gaps between the interconnected fibers). Despite different pore sizes (from 2 to 30 μm), all thicker filter paper samples produce superhydrophobicity, indicating that the depth of “vacancies” or “gap” is indeed the key structural parameter. Our SEM studies of the morphology of three representative types of filter paper confirmed above hypothesis. Grade 597 (Figure 2a) has a similar pore size as the Grade 3 (Figure 2b), but is much thinner. In contrast, Grade 3 and Grade 602 have similar thickness but different pore sizes. With thicker filter paper (the superhydrophobic samples, Figure 2b and 2c), the “vacancies/gaps” in between the fibers are much deeper (indicative of higher roughness factor) in comparison with the thinner sample (Figure 2a). The SEM images show slight differences in the packing densities of the cellulose fibers (Figure 2b vs. 2c); apparently, this is not the key factor for achieving superhydrophobicity.

With the above success, we further looked into if we can optimize the performance of binary silanization for modifying filter paper. Figure 3 shows the resulted contact angles with a solution of

different ratios of MTS and OTS to modify Whatman™ Grade 3, Grade113, and Grade 520B filter paper (the total silane concentration was kept as 0.2%). It is evident that neither pure OTS nor MTS creates superhydrophobic surface, and that the resulting water contact angle indeed depends on the ratio between the two organosilanes in all three cases. With increasing the amount of MTS in the binary solution, the water contact angle of the modified filter paper initially increases, up to a maximum (153° for Grade 3 filter paper) with the volume ratio around OTS: MTS = 3:7 (molar ratio of 1:3.4). The highest contact angles achieved for the other two samples are slightly lower (150° and 151°), and the optimal ratio was about 1:1 (OTS/MTS) in both cases.

Beyond the observed, remarkably high water contact angles, we estimated the surface tensions of the three superhydrophobic paper samples based on measurements with mixtures of water and ethanol (i.e., the construction of Zisman plots).⁴⁸ All three samples show similar wicking resistance at $33 \pm 1 \text{ mN m}^{-1}$ and the surface tension was determined to be as low as $27 \pm 1 \text{ mN m}^{-1}$. This value is comparable with that of the “fluoroalkylated paper” prepared by Glavan et al. (28 mN m^{-1}) via vapor-phase silanization,²³ slightly higher than those reported by Ly et al. (26.1 mN m^{-1})³² and Gaiolas et al. ($\sim 25 \text{ mN m}^{-1}$).³⁵ The other experimental observation is the low wetting hysteresis (i.e., the difference between advancing and receding contact angles); for all three superhydrophobic paper substrates, they are within a range of 8° to 14° , indicative of their uniform, nanoscale roughness (*vide infra*).

In the preparation of the above mentioned superhydrophobic filter paper samples, we have set the reaction time as 7 min, which was based on the reaction time reported by He et al. for the fabrication of hydrophobic filter paper with OTS silanization. To determine the optimal reaction time for the binary silanization, we have followed the reaction by measuring the water contact angle at different immersion time. As shown in Figure 4, the silanization reaction of Grade 3 filter paper is rather fast; the water contact angle rises exponentially and reaches 150° in less than a minute. The reaction reaches the “equilibrium” in about 5 to 8 min, indicates that we have indeed chosen the optimal reaction time. In comparison with the existing surface modification protocols, the solution-phase silanization is as efficient as plasma treatment and chemical vapor deposition.^{21, 23}

The remarkable wetting properties of laboratory filter paper upon binary silanization lead to our further investigation of the structural changes at the micro/nanoscale. As mentioned above, we have shown that the morphologies of filter paper of different grades are significantly different (Figure 2); the thicker filter paper would be more ideal to create “Cassie-Baxter state” upon in contact with water droplets. Taking Grade 3 filter paper as an example, we have obtained high resolution SEM images to

examine the morphological changes upon silanization (Figure 5). The silanization did not change the primary fiber structure, although the formation of nanoparticles and the creation of nanoscale porous structures are evident. For the sample treated with pure OTS solution, the surface of the fibers remained smooth (Figure 5a), which is similar to untreated filter paper (Figure 2b). We should note that the water contact angle on this surface is 140° to 145° , which is not in the superhydrophobic range. The MTS-modified samples show significant differences, i.e., the fiber is now coated with nanoparticulate structures (“islands”) in the range from 200 to 400 nm in diameter. They are rather uniformly distributed along the microfibers, with a minimal level of aggregation. As shown in Figure 5c, the morphology of the filter paper treated with the binary silane solution is rather unique, i.e., the microfiber becomes extremely porous and two types of nanoscale structures can be clearly identified. The dominant type consists of hollow particles with diameter of 200-300 nm (the inset of Figure 5c); the other type is porous “nanonetworks” (top right inset of Figure 5c).

Both OTS and MTS have been widely used for the modification of hydroxyl (-OH) terminated solid surfaces (silicon oxide, metal, glass, as examples),^{20, 41-49} but with a less extent for paper.²² It is also known that their reactivity towards hydroxylated surfaces are different, i.e., OTS tends to form ordered monolayers on SiO_2 or glass as of the strong inter-chain van der Waal forces, while MTS would form three-dimensional, aggregated nano/nanostructures, as discussed above. The hydrolysis of MTS occurs more rapidly and thus formed methylsilanols condense in an uncontrollable manner to form 3D interconnected nanofibers or nanospheres. We believe that the intriguing results obtained with the binary silanization should be attributed a combined effect of OTS and MTS modification; as depicted in Figure 5d, MTS molecules react with the cellulose fibers rapidly and primarily form nanospheres on the surface, while OTS would terminate the reaction and lower the surface energy. We note that in-depth morphological studies and their relationship with the silanization condition are warranted, but it is beyond the scope of this work.

Superhydrophobic paper patterning and assay development

As mentioned above, μ PADs have been developed for many different analytes under various measurement conditions, for example, they might be used in solutions of different pH and stored for prolonged period.^{20, 22, 50} It is important to examine the stability of the modified filter paper under different conditions; modified superhydrophobic filter paper substrates were soaked in aqueous solutions of different pH for one hour. We have shown that the water contact angles measured on these samples did not change significantly from those determined on freshly prepared samples for the pH range of 4 to 11, indicating their stability under mild acidic and basic conditions. It was observed that

the contact angles dropped slightly under strong acid conditions (i.e., $\text{pH} < 4$), which could be attributed to potential hydrolysis of Si-O bonds.⁵¹ More importantly, the modified filter paper remains superhydrophobic ($152 \pm 1^\circ$ for Grade 3) even after stored for 3 month under ambient conditions. Within the entire course of water droplets evaporated from the surface, there were no indications of either leakage or adsorption through the substrate.

The other important requirement of modified filter paper as bioassay substrates is the wetting property with respect biological samples; we have so far only demonstrated their superhydrophobicity with either water or pH buffers. We went ahead to test silanized three types of filter paper samples with a number of biological samples, including human saliva, human urine, serum-free media (SFM), 10% fetal bovine serum (FBS). In addition, tris buffer (100 mM, $\text{pH} = 7.42$) solution was also tested for comparison. The as-prepared superhydrophobic paper substrates were first soaked in biological samples for 30 min, before taking the contact angle measurements. As shown in Figure 6, the measured contact angles on the three types of silanized filter paper are all above 150° , indicating that the superhydrophobicity remains when contaminated with biological macromolecules. It is also noticeable that the variations of the repeated measurements are slightly higher than the data determined with pure water, particularly in the case of Grade 113 filter paper. The rather large pore size might be responsible for such a change, especially when the protein or salt concentration was high (sample 4 and 5).

The last not least essential requirement of new substrates for μPADs is the ability to be patterned into different sections of varied wetting properties; in retrospect UV-lithography has been the most popular method of degrading organosilanes in order to create hydrophilic reaction areas/channels on otherwise hydrophobic paper substrate.^{22, 52} We have shown previously that UV/ozone treatment (185 nm/245 nm) is a more efficient protocol than conventional UV irradiation (350 nm) in the activation of plastic substrates (polycarbonate in particular);⁵³ herein the superhydrophobic filter paper substrates were treated for different periods of time to explore the patterning feasibility. As shown in Figure 7, the water contact angles on modified Grade 3, Grade 113, Grade 520B filter paper decreases exponentially; the surface becomes hydrophilic ($\theta < 90^\circ$) in about 30 min and completed wetted by water droplets within 2.5 h. There are no significant differences in terms of the degradation rates among all three superhydrophobic paper substrates, though the Grade 3 filter paper (with the smallest thickness) seems to be a bit faster than the other two. Our SEM studies showed no significant change of the surface morphology to either the cellulose fibers or nanoparticulate structures upon UV/ozone treatment. The inset picture of Figure 7 is a demonstration of patterning 8 hydrophilic zones (0.7 mm in diameter) on such a superhydrophobic filter paper substrate. Not only the irradiated areas are totally wicked by the

ink solution, but also the borders are well defined and the area in between remained superhydrophobic. In comparison with the UV/O₃ patterning of hydrophobic filter paper reported by He et al. (1.5 h),²² the degradation time of the superhydrophobic paper is a somewhat longer. Beyond the difference in the water contact angles (150° vs 120°), the thickness of the filter paper (390-550 μm) herein is much thicker than that used in their studies (Grade 1, 180 μm).²² It should be noted that the activated filter paper substrates remain hydrophilic (wicked by water dropped on top) even after one week of aging under ambient conditions.

The patterned filter paper was then adapted for different assay applications; as pH test is the most widely performed analysis in labs, it was chosen as the first example. The Yamada Universal Indicator (YUI) was prepared according to the literature recipe,⁵⁴ and spotted on the 8 hydrophilic regions patterned above. As shown in Figure 8a, upon adding solutions of varied pH (as indicated in the picture) the assay develops expected color changes. To verify the accuracy of the expected color for particular pH values, the same test was performed on a microtiter plate (Figure 8a, bottom). For pH range from 3 to 10, the assay color changes from orange to violet, which is consistent with the results reported by Foster et al.⁵¹ and the paper assay (Figure 8a, top).

Figure 8b shows the results of a quantitative nitrite assay performed on the patterned superhydrophobic filter paper. The conventional protocol, Griess reaction was adapted: nitrite ion can react with sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (NED), following by the formation of a magenta azo compound.²² As shown in the inset picture, different magenta stains were generated, and the color becomes stronger upon increasing the nitrite concentration. With the JPG picture captured with an iPhone 5 camera, we were able to determine the color intensity with the ImageJ software program. Figure 8b shows that the color intensity increases initially as the concentration of nitrite increases, but approaches saturation above 1.5 mM. These results are comparable with the data reported He et al.;²² on the superhydrophobic filter paper substrates we were able to achieve a detection limit of 0.1 mM and a linear response range between 0.1 mM to 1.5 mM for the standard nitrite assay.

Conclusion

Superhydrophobicity can be achieved on a number of off-the-shelf filter paper via a simple and rapid binary silanization reaction. It has been shown that the remarkable wetting properties of modified filter paper are not depending on the pore size but the thickness. The high-resolution SEM images revealed distinct (hollow particle and porous network) structures when the filter paper was treated with a binary silane solutions. It was believed the different reactivities of short and long alkanesilanes are

responsible for the observed superhydrophobicity, i.e., MTS molecules rapidly aggregates into 3D nanostructures and OTS terminate the surface low surface energy long chain alkyl groups. Most importantly, the superhydrophobic filter paper can be readily patterned with UV/O₃ treatment, and adapted for both qualitative (pH test) and quantitative (nitrite detection) assays. Extended research with these novel superhydrophobic paper substrates for the creation of other more advanced μ PADs are currently in our laboratory.

Supporting information available

Additional experimental data including the determination of surface tensions and wetting hysteresis of superhydrophobic paper substrates; the stability tests in different pH solutions; aging effects and SEM morphology studies of as prepared and UV/ozone activated paper substrates. Tables summarize the characteristics (pore size, thickness, and application) of WhatmanTM filter paper and relevant references of paper silanization, respectively. This information is available free of charge at <http://pubs.acs.org>.

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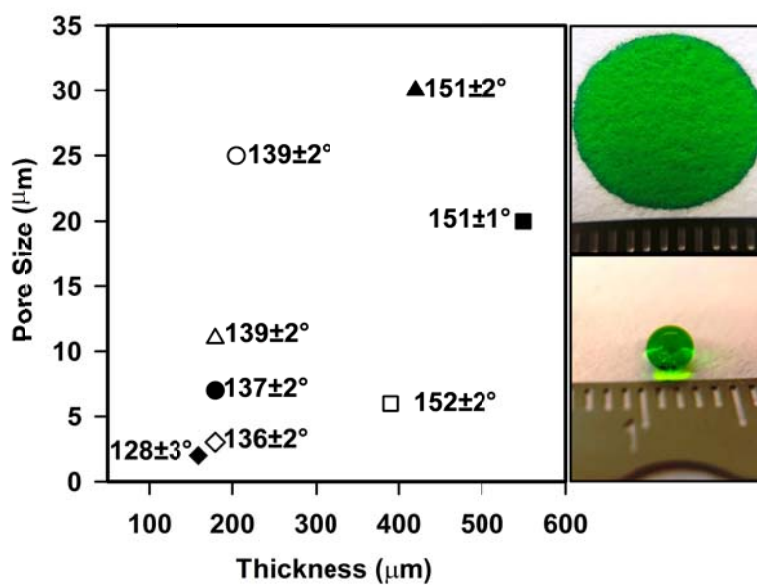


Figure 1. Water contact angles of modified filter paper strips with different pore size and thickness. All samples were treated with a binary solution of OTS and MTS with a volume ratio of 1:1 (0.2 % in total). Grade 1(△); Grade 3(□), Grade 4(o); Grade 6(◇), Grade 113(▲), Grade 520B (■), Grade 597(●) and Grade 602h(◆). The right insets show the pictures of water droplets (10 μL) on untreated (top) and silanized Grade 3 filter paper (bottom).

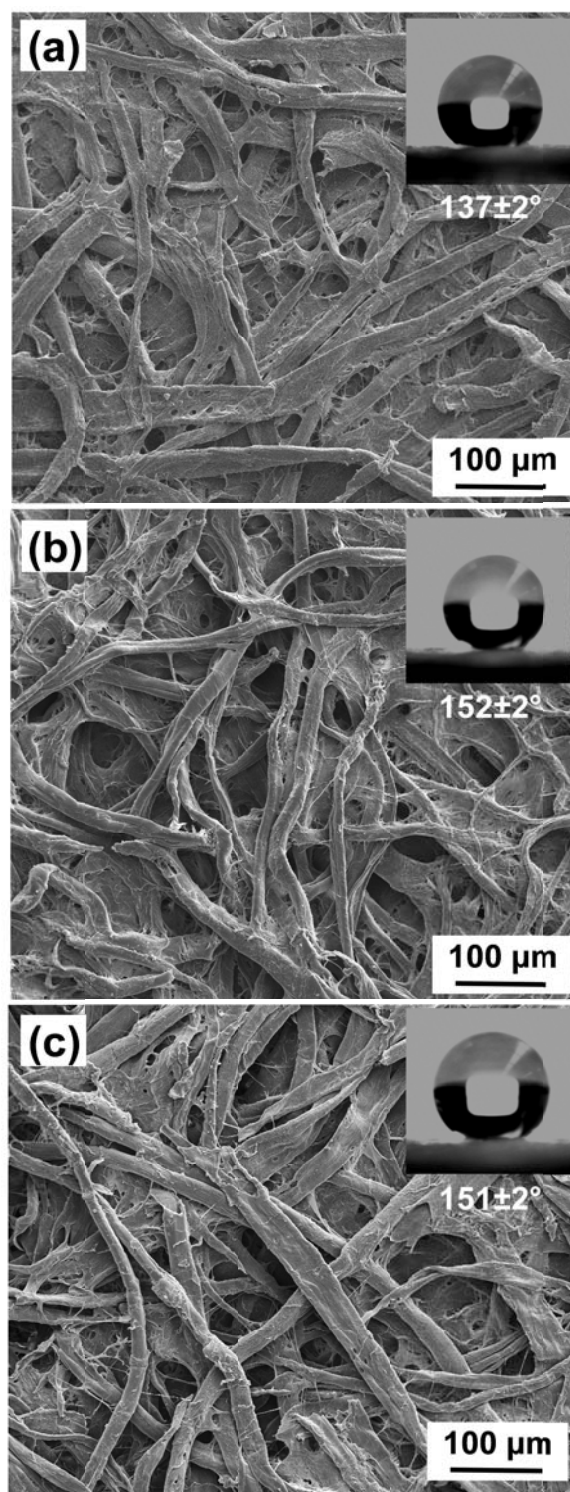


Figure 2. SEM images of WhatmanTM filter paper with different pore size and thickness: (a) Grade 597 (pore size: 4-7 μm , thickness: 180 μm); (b) Grade 3 (pore size: 6 μm , thickness: 390 μm); (c) Grade 113 (pore size: 30 μm , thickness: 420 μm). The inset images display water droplets and corresponding contact angles on the silanized filter paper, respectively.

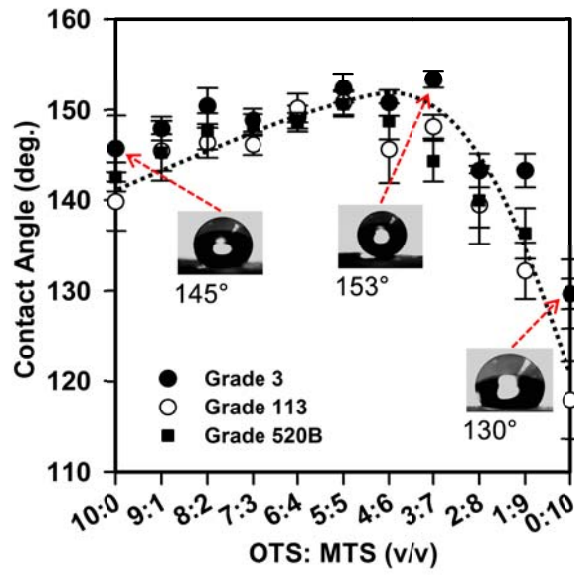


Figure 3. Water contact angle of modified filter paper as a function of OTS/ MTS ratio (v/v) in the binary organosilane solution. The insets show pictures of water droplets on Grade 3 filter paper modified with pure OTS, OTS/MTS (3:7), and pure MTS, respectively. The dash line was to guide the eyes only.

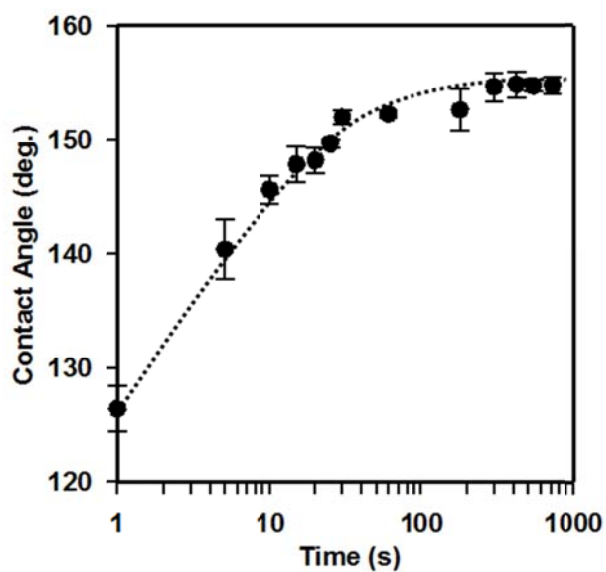


Figure 4. Water contact angle of modified Grade 3 filter paper as a function of the immersing time in a binary OTS/MTS (3:7) solution. The total concentration of the organosilanes was kept at 0.2%. The dash line is to guide the eyes only.

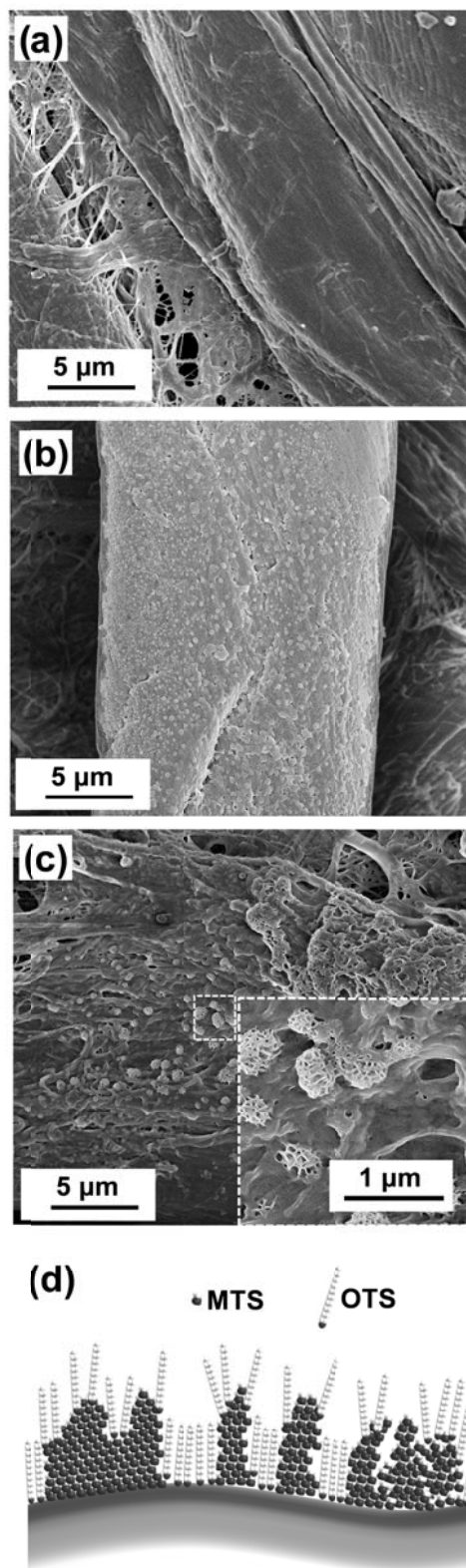


Figure 5. High resolution SEM images of Grade 3 filter paper after silanization with (a) OTS, (b) MTS, and (c) a binary solution of OTS and MTS ($v/v = 3:7$). (d) shows a schematic view of the nanostructured surfaces of modified paper fiber. The inset in (c) shows a zoom-in picture of the particulate nanostructures on modified Grade 3 filter paper.

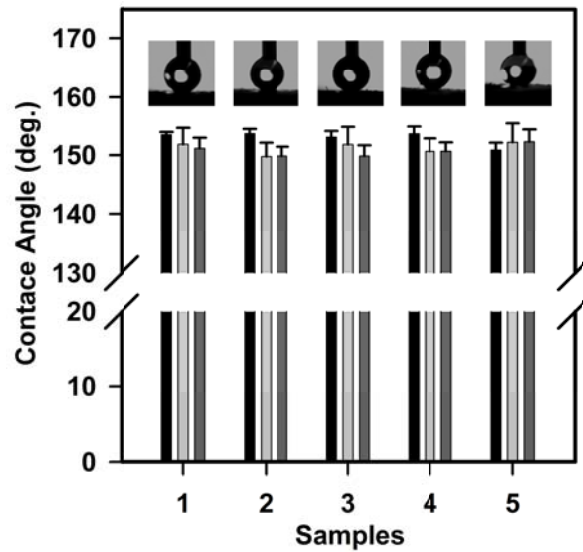


Figure 6. Contact angles of different biological samples on the superhydrophobic filter paper substrates. Samples 1 to 5 are: human saliva, human urine sample, SFM, 10% FBS, and Tris buffer, respectively. For each sample, three types of filter paper from left to right (Grade 3, Grade 520B and Grade 113) are tested for comparison. The top insets show biological sample droplets on superhydrophobic grade 3 filter paper, indicative their resistance to bio-sample contamination.

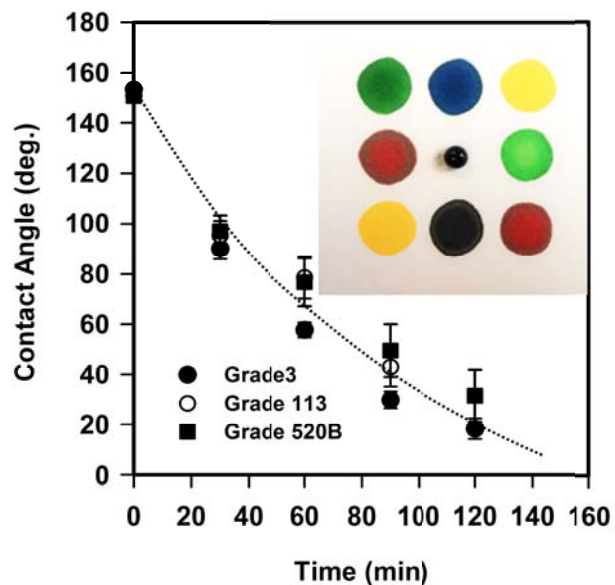


Figure 7. Water contact angles on silanized Grade 3, Grade 113, Grade 520B filter paper as a function of UV/ozone treatment time. The dashed line is to guide the eyes only. The top-right inset shows a picture of patterned paper substrates on silanized Grade 3 filter paper (with 8 hydrophilic reaction zones). The dyed water droplet at the center shows that the untreated sections of the substrate remains superhydrophobic.

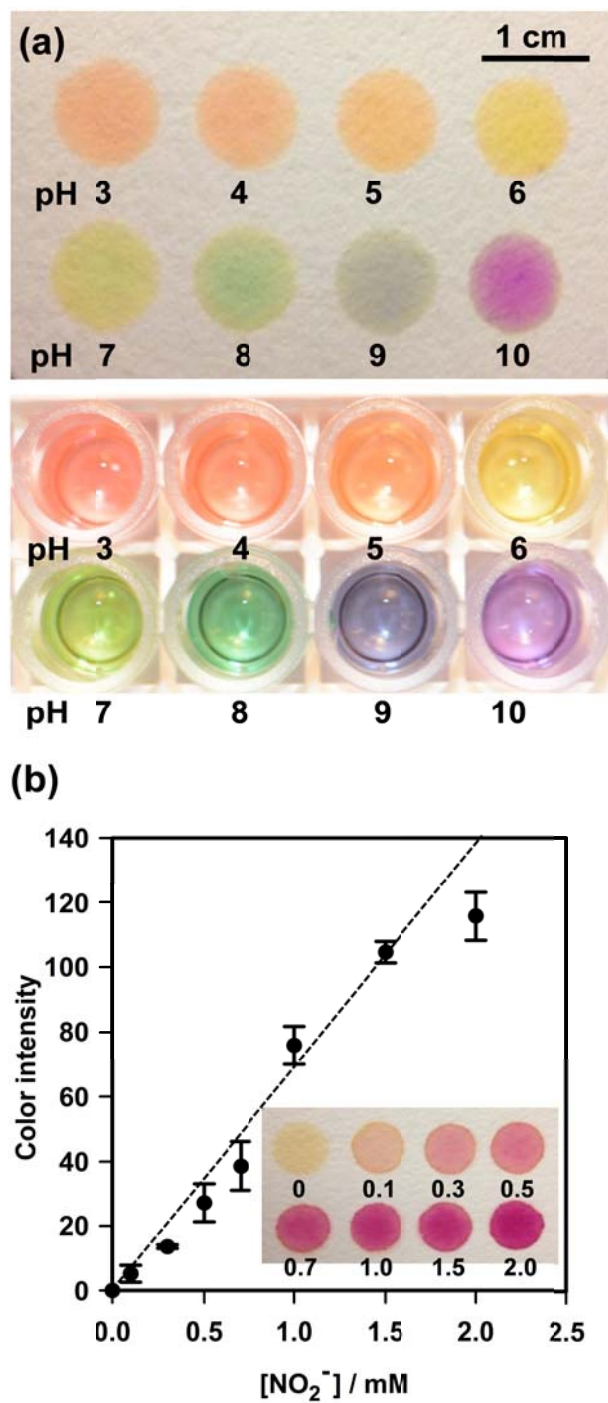
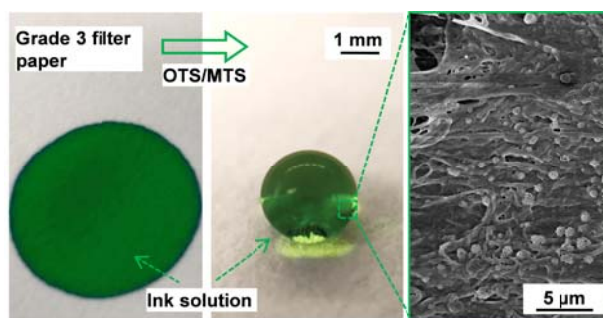


Figure 8. Colorimetric assays prepared on superhydrophobic filter paper substrates. (a) Comparative pH tests performed on paper and on microtiter plate; (b) Nitrite assay based on the Griess reaction; the inset shows the original picture of the developed assay (the number below is the analyte concentration in mM).

TOC image



Supporting Information

for

Superhydrophobic substrates from off-the-shelf laboratory filter paper: simplified preparation, patterning, and assay application

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Additional experimental data including the determination of surface tensions and wetting hysteresis of superhydrophobic paper substrates; the stability tests in different pH solutions; aging effects and SEM morphology studies of as-prepared and UV/ozone activated paper substrates. Tables summarize the characteristics (pore size, thickness, and application) of WhatmanTM filter paper and relevant references of paper silanization, respectively.

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Table S1. Characteristics of different types of Whatman™ filter paper tested in this work. Further information is available online at <http://www.gelifesciences.com>.

Whatman™ filter paper type	Pore size (μm)	Thickness (μm)	Application
Grade 1	11	180	Traditional qualitative analytical separations
Grade 3	6	390	Double the thickness of grade 1, for the retention of finer particles and allows for more precipitate to be held without clogging
Grade 4	20-25	205	Extremely fast filtering with excellent retention of coarse particles
Grade 6	3	180	Twice as fast as Grade 3 with similar fine particle retention
Grade 597	4-7	180	Medium fast filter paper with medium to fine particle retention
Grade 602h	< 2	160	A dense filter paper for collecting very small particles and removing fine precipitates. Used in sample preparation.
Grade 520B	20	550	Made from super-refined cellulose, designed for technical uses
Grade 113	30	420	High wet strength from the stable resin, for qualitative applications

Table S2. List of references for filter paper modification with different organosilane precursors and specified experimental conditions.

Corresponding author	Precursor (s)	Modification method	Water contact angles	Ref.*
K.-H. Paik	CF ₃ (CF ₂) ₇ (CH ₂) ₂ Si(OCH ₂ CH ₃) ₃ ; CH ₃ (CH ₂) ₁₆ CH ₂ SiCl ₂ CH ₃ ; (CH ₃) ₂ SiCl ₂ .	Vapor deposition	120°- 130°	31
M. N. Belgacem	CF ₃ (CH ₂) ₂ Si(OCH ₃) ₃ ; CF ₃ (CF ₂) ₅ (CH ₂) ₂ Si(OCH ₂ CH ₃) ₃ .	High tempt. Vacuum	140°	32
L. R. Arcot	CH ₃ (CH ₂) ₁₆ CH ₂ SiCl(CH ₃) ₂ ; CH ₃ (CH ₂) ₁₆ CH ₂ SiCl ₂ CH ₃ ; CH ₃ (CH ₂) ₁₆ CH ₂ SiCl ₃ .	Immersing	100°-120°	33
G. Guan	CF ₃ (CF ₂) ₆ (CH ₂) ₂ SiCl ₃ .	Vapor deposition	156°	34
N. Belgacem	CH ₂ =CH ₂ Si(OCH ₃) ₃ ; CH ₂ =CH ₂ COO(CH ₂) ₃ Si(OCH ₃) ₃	Plasma treatment	100°	35
G. M Whitesides	CH ₃ SiCl ₃ ; CH ₃ (CH ₂) ₉ SiCl ₃ ; CF ₃ (CF ₂) ₅ CH ₂ CH ₂ SiCl ₃ ; CF ₃ (CF ₂) ₇ CH ₂ CH ₂ SiCl ₃ ; CF ₃ (CF ₂) ₉ CH ₂ CH ₂ SiCl ₃ .	Vapor deposition	130°- 160°	23
L. Cai	CH ₃ (CH ₂) ₁₇ Si(OCH ₃) ₃	Immersing	123°	26
L. Cai	CH ₃ (CH ₂) ₁₇ Si(OCH ₃) ₃	Immersing	123°	27
Q. He	CH ₃ (CH ₂) ₁₇ SiCl ₃	Immersing	125°	22

* These numbers correspond to the reference list in the main text.

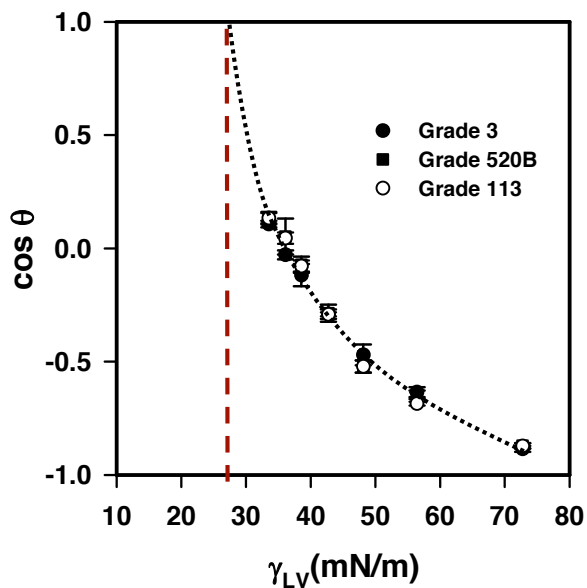


Figure S1. Zisman plots for three superhydrophobic filter paper substrates prepared by treating with binary silane solutions (with a total silane concentration of 0.2% and volume ratio of OTS: MTS = 1:1 for Grade 520B and Grade 113 filter paper, OTS:MTS = 3:7 for Grade 3 filter paper). Solutions of different surface tensions were prepared by mixing ethanol and ultrapure water following the procedure described in, Vazquez, G.; Alvarez, E.; Navaza, J. M. *J. Chem. Eng. Data* **1995**, *40*, 611–614. Particularly 1.0- μ l of each solution was dropped onto the paper substrate for measuring the contact angle. As mentioned in the main text, all three samples show similar wicking resistance at $33 \pm 1 \text{ mN m}^{-1}$ and the surface tension was determined to be as low as $27 \pm 1 \text{ mN m}^{-1}$.

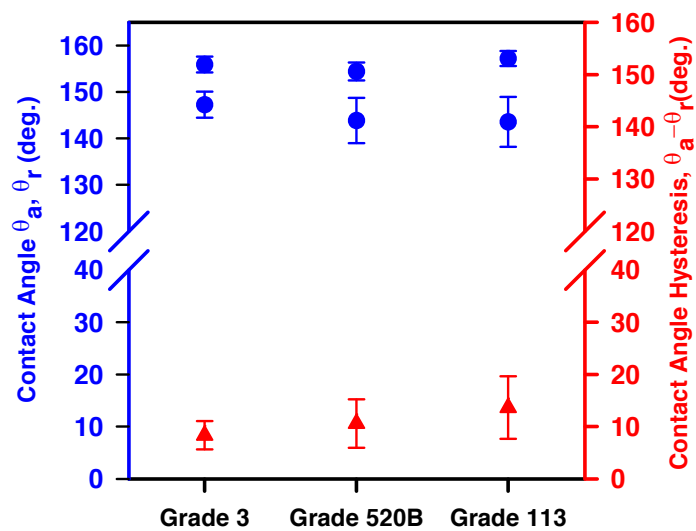


Figure S2. Advancing water contact angle (θ_a), receding contact angle (θ_r), and wetting hysteresis ($\theta_a - \theta_r$) of the three types of superhydrophobic filter paper. They were prepared in the same manner as described in Figure S1 and experimental section of the main text.

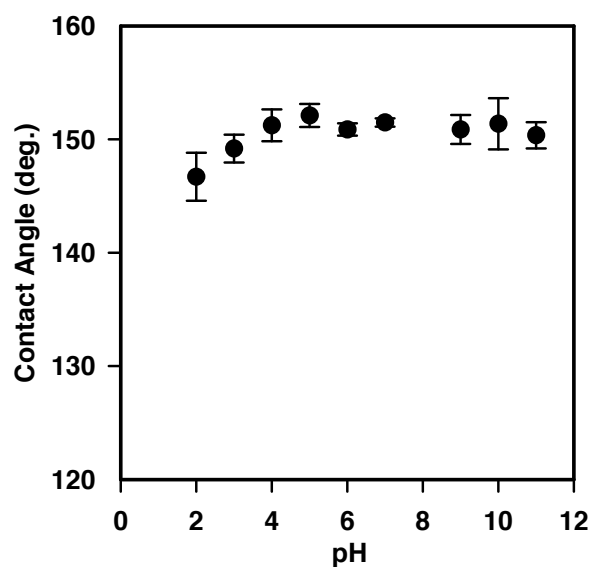


Figure S3. Stability tests of the superhydrophobic filter paper substrates (Grade 3). Water contact angles as function of the solution pH in which the paper substrates were immersed for an hour. The samples were prepared in the same manner as detailed in Figure S1. The preparation of the pH solutions ranging from 2 to 11 was described in the experimental section of the main text.

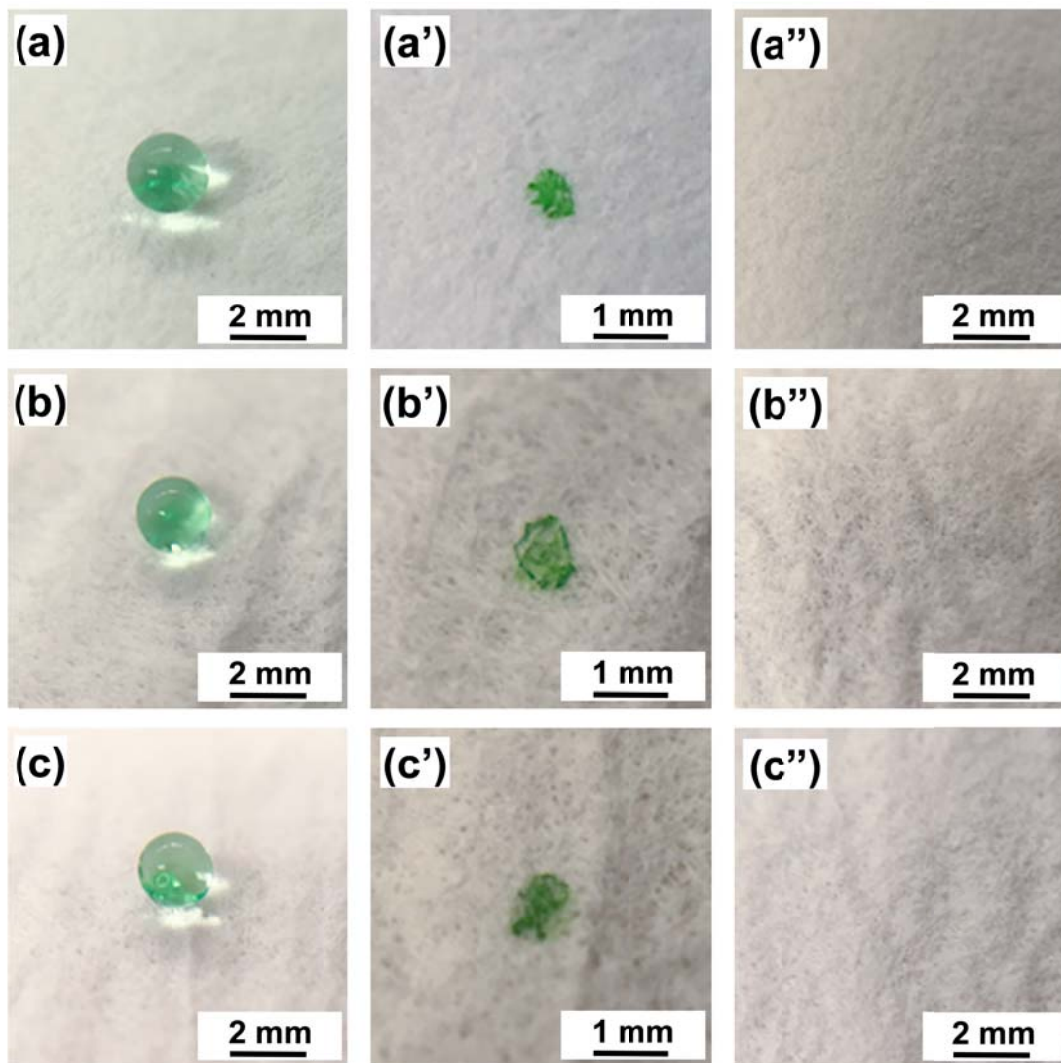


Figure S4. Leakage and adsorption tests of the three types of superhydrophobic filter paper with dyed water droplets: Grade 3 (a), Grade 11 (b) and Grade 520B (c). (a')-(c') show the pictures upon total evaporation of the dyed water droplets, and (a'')-(c'') show the back side of the substrates. It is clear that within the entire evaporation period (50 min) there is no leakage or adsorption of the dyed water droplets on or through the silanized filter paper, i.e., the dye stains stay on the surface and did not wick through.

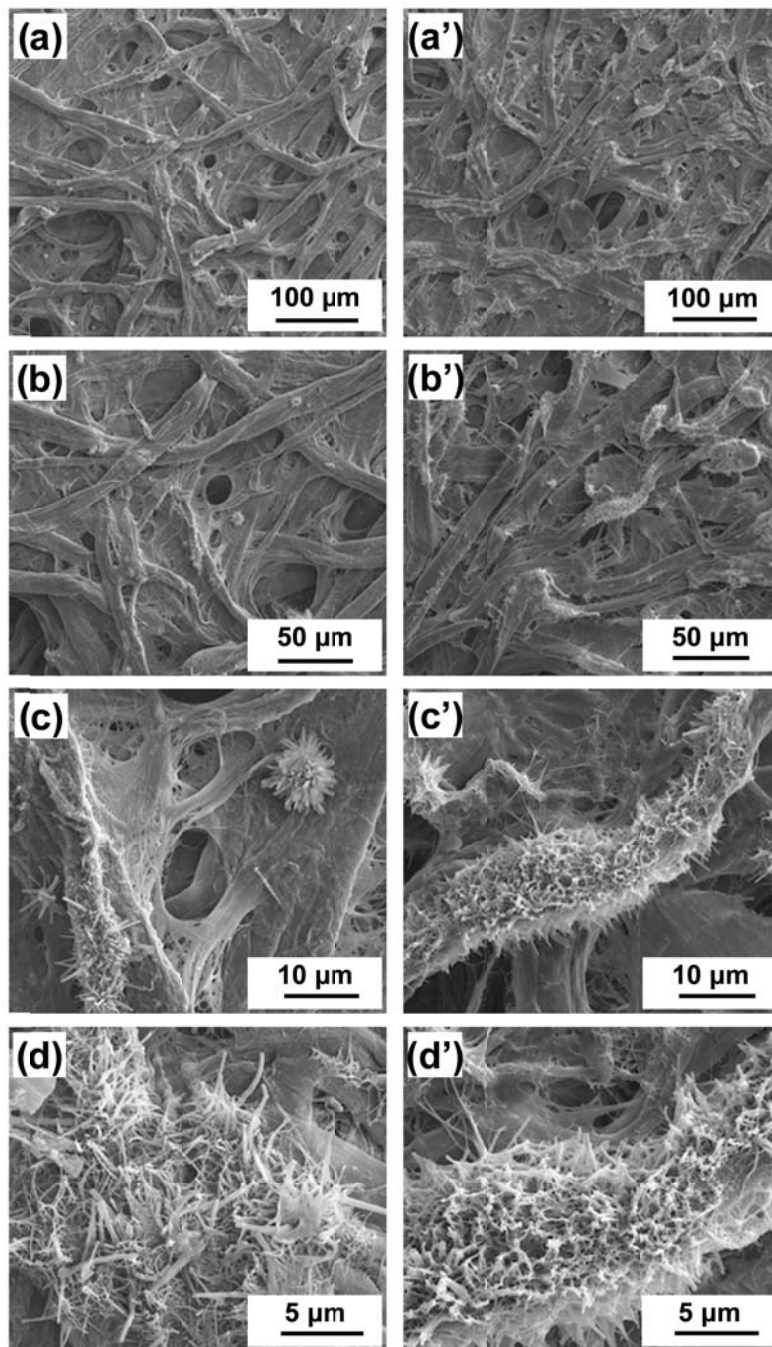


Figure S5. SEM images of superhydrophobic filter paper (Grade 3) as prepared (a-d) and after UV/ozone treatment for 3 h (a'-d'). There are no obvious changes on the surface morphology as illustrated with low to high magnifications. The samples were prepared in the same manner as described in Figure S1 and experimental section of the main text..

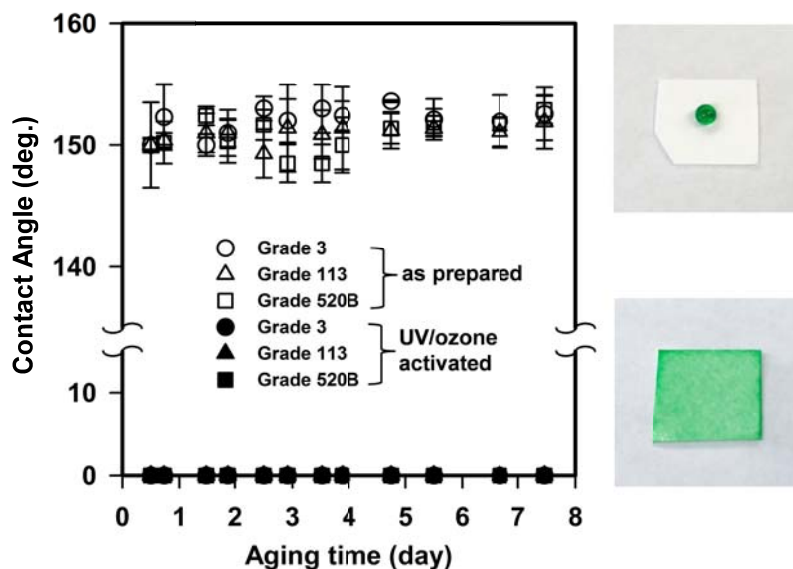


Figure S6. Aging effect of the as-prepared superhydrophobic filter paper and upon activation with UV/ozone treatment (3 h). Upon stored under ambient conditions for a week, there are no apparent changes in the water contact angles, i.e., the as-prepared samples remained superhydrophobic, while the activated samples are still hydrophilic (wickd by the water droplets). The right insets show the pictures of substrates stored for a week with a dyed water droplet on top. The samples were prepared in the same manner as described in Figure S1 and experimental section of the main text.