

Reactive Surface Micropatterning by Wet Stamping

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Hydrogel stamps are used to reactively micropattern various types of substrates. The method, called reactive wet stamping (r-WETS), is general in nature and overcomes several limitations of conventional soft-lithographic techniques. Illustrative applications of r-WETS in surface wettability modification, deposition of metallic microstructures, preparation of supports for electrostatic self-assembly, and multistep reactive patterning are discussed.

Micro contact printing (μ CP)^{1,2} and related methods have proven very successful in patterning self-assembled monolayers of alkanethiolates on metal surfaces,³ silanes on oxides and glass,⁴ and alkyl phosphonic acids on Al/Al₂O₃⁵ and have recently been extended to noncovalent patterning of proteins,⁶ DNA,⁷ as well as colloids⁸ and nanoparticles.⁹ At the same time, because these methods deliver chemicals onto surfaces in dry state, there are only a few examples of reactive μ CP, in which the molecules transferred from the stamp would covalently bind to the patterned support.^{10–13} Another limitation of techniques using elastomeric stamps is that they supply only miniscule quantities of chemicals from the tops of the microfeatures, and are thus unsuitable for deep patterning/etching of the supports. Recently, we^{14–18} and others¹⁹ have suggested that micropatterned hydrogel stamps can be used to deliver controllably large quantities of chemicals onto or into various kinds of supports. Here, we extend

this work to reactive surface micropatterning using polar inks (especially, water based). Our method, called reactive wet stamping (r-WETS), uses agarose or polyacrylamide stamps patterned in bas relief and soaked in a solution of a desired reagent to deliver this reagent onto a solid substrate. Because the chemicals at the patterned surface are solvated, they readily undergo chemical transformations that would be hard to achieve with other micropatterning methods. In addition, because the reagents are constantly resupplied from the stamp, they can penetrate into and modify the bulk of the substrate. We demonstrate how these capabilities can be used to tailor wetting properties of polymer surfaces, deposit metallic microwires, prepare supports for electrostatic self-assembly,²⁰ and perform sequential reactions at surfaces. These examples illustrate that reactive WETS is a simple and versatile alternative to μ CP and related techniques in applications in which inks incompatible with elastomeric stamps are to be used.

Typically,^{15,16} the stamps were made by pouring a hot, degassed 8–12% w/w solution of high strength agarose (OmniPur Agarose, Darmstadt, Germany) in deionized water against an oxidized poly(dimethylsiloxane) (PDMS) master having an array of microscopic features embossed on its surface (typical feature size, $W \sim 2 - 250 \mu\text{m}$, feature depth, $H \sim 2 - 100 \mu\text{m}$, $H/W 0.4 - 2$). After further degassing under vacuum and gelation, the agarose layer was gently peeled off, and cut into ca. 1–2 cm \times 1–2 cm \times 2–5 mm rectangular blocks (“stamps”) patterned with the negative of the array of features in the PDMS master. Next, the stamps were soaked for 10–120 min²¹ in an aqueous solution of a reagent(s) to be used in surface patterning. If the reagent(s) was not soluble or only sparingly soluble in water, it was first dissolved in a minimal amount of DMSO, dioxane, or ethanol and then diluted with deionized water up to $\sim 2\%$ v/v; small amounts of these organic solvents did not diminish the structural integrity of agarose stamps. Immediately prior to use, stamps were dried of excess water by placing on filter paper for 5 min and then on a glass slide (10 min, pattern side down) to equalize any hydration gradient that might have developed during drying. We note that agarose stamps could not be soaked for prolonged periods of time in strongly acidic media—in such instances, the stamps were made out of

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- (21) Soaking times for ionic solutions, such as K₂Cr₂O₇, were approximately 10 min; organic molecules (e.g., alkanethiols and silanes used here) required soaking for ca. 2 h.

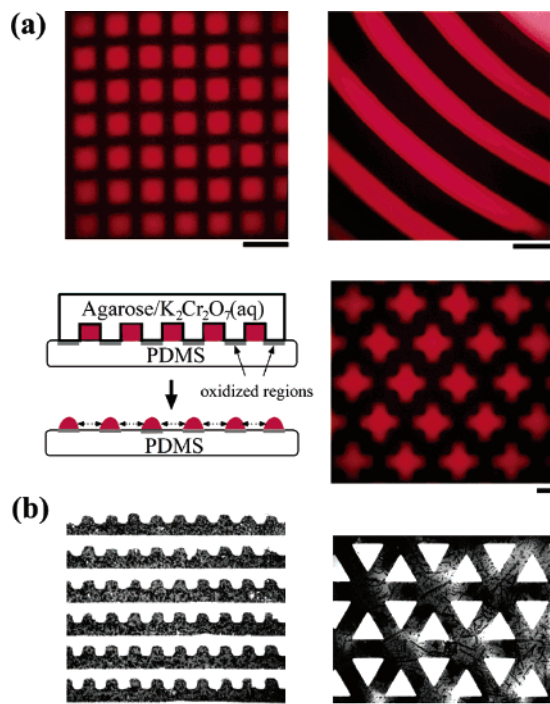


Figure 1. (a) Colored microdroplets forming on hydrophilic patches patterned onto a surface of PDMS via r-WETS with a solution of potassium dichromate. The scheme illustrates how the liquid introduced into the grooves of the stamp migrates onto the hydrophilic islands when the stamp is being removed. Scale bars correspond to $100\ \mu\text{m}$. (b) Two arrangements of planar metal “wires” on poly(styrene) prepared by first oxidizing the patterned regions with dichromate and then wetting with Tollen’s reagent and metallizing via exposure to the vapors of a 1:10 solution of formaldehyde (37% w/w formaldehyde in water, Sigma-Aldrich, St. Louis, MO) in water. Scale bars correspond to $250\ \mu\text{m}$.

a more durable material, poly(acrylamide), which, however, was mechanically less rugged (“brittle”) and slightly more difficult to handle than agarose.

Figure 1a illustrates application of r-WETS to modifying wetting properties of a polymer surface—here, hydrophobic poly(dimethylsiloxane), PDMS. Usually, such modification is carried out by a through-mask exposure to oxygen plasma²² or ozone.²³ In r-WETS, small (tens-of-micrometers in diameter) patches of arbitrary shapes were rendered hydrophilic by patterning the surface with an agarose stamp soaked (for 15 min) in a saturated solution of potassium dichromate, which oxidized the PDMS’s silyl groups to siloxane ones. Importantly, the degree of lateral (reactive) spreading was negligible, and the shapes of oxidized patches were faithful replicas of the shapes of the stamp’s features.

Differential wettability of the patterned surface was visualized by formation of rhodamine-colored microdroplets, which dewetted from the unmodified regions of the surface, and assumed the shapes of the hydrophilic islands. Interestingly, hydrogel stamps facilitated formation of drops of complex shapes (e.g., crosses), which could not be obtained reproducibly by simple dewetting²⁴ of a bulk

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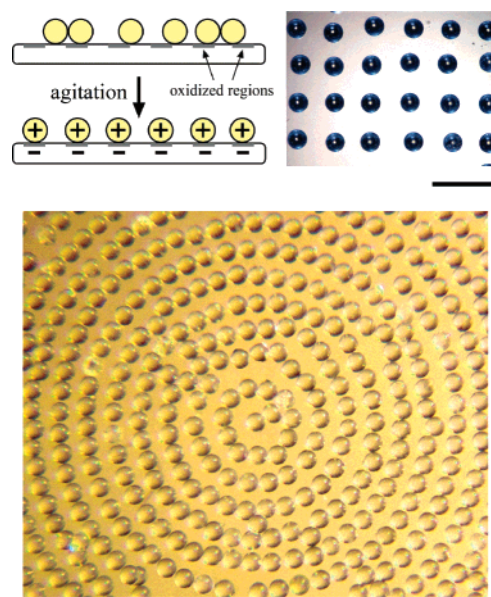


Figure 2. Scheme illustrating the process of electrostatic self-assembly of glass microspheres on a patterned polystyrene surface. The upper optical micrograph shows a square array of $50\ \mu\text{m}$ spheres organized by ESA on disjoint islands; the lower image has the spheres assembling on a pattern of concentric circles. Arrow bars in both pictures correspond to $200\ \mu\text{m}$.

liquid from the surface. Instead, with the stamp still on the surface, drops of a colored liquid were placed around the stamp’s edges and sucked into its grooves by capillarity²⁵ (wetting the stamp’s surface but not that of the unmodified PDMS support). When the stamp was being removed, liquid contained between the features minimized its surface free energy by transferring itself onto the hydrophilic patches that were now available on the surface.

The ability to conform the shapes of the liquid patches to arbitrary patterns allowed patterning surfaces with electrically conductive metal wires. This is illustrated in Figure 1b. After r-WETS with potassium dichromate, the patterned lines were covered with a solution of Tollen’s reagent and were then exposed to the vapors of 3% formaldehyde solution to give, through the silver mirror reaction, a continuous layer of silver. When dried at $50\ ^\circ\text{C}$ for 2 h, these lines were stable against washing with water and alcohol and were uniformly conductive, and their width could be as low as $50\ \mu\text{m}$, with a typical thickness of $1\ \mu\text{m}$.

Another application in which r-WETS has proven useful is the preparation of micropatterned substrates for electrostatic self-assembly (ESA, Figure 2). We have recently shown that ESA can be mediated and controlled by contact electrification between polymeric surfaces and small metal or polymer particles agitated on these surfaces.²⁰ We have also found that for certain polymers—notably, poly(styrene)—the degree and rate of contact charging depend on the degree of surface oxidation.²⁶ In this context, we used r-WETS to locally oxidize pendant groups of PS chains and thus to modify charging characteristics of the patterned regions on the polymer’s surface. Agarose stamps were soaked in a 30% w/w solution of hydrogen peroxide, applied onto surfaces of PS Petri

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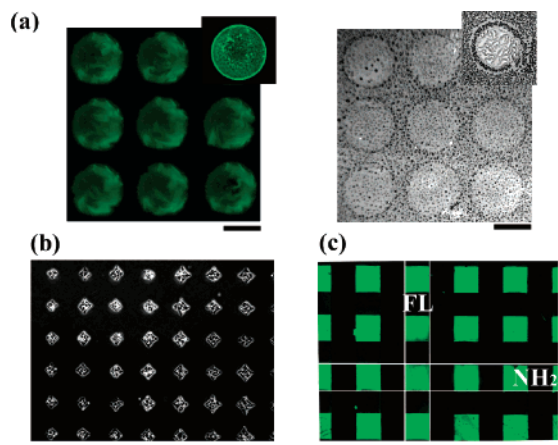


Figure 3. (a) Micropatterning of a mixed SAM on gold from a 2.5 mmol solution of disulfides (9:1 M/M of $\text{COOH}(\text{CH}_2)_{11}\text{SS}(\text{CH}_2)_{11}\text{COOH}:\text{COOH}(\text{CH}_2)_{11}\text{SS}(\text{CH}_2)_{11}\text{CON-Fluorescein}$) contained in an agarose stamp having an array of circles in bas relief. The background outside of the stamped circles was subsequently covered with a mixed SAM from a 9:1 M/M solution of methyl-terminated and carboxy-terminated thiols. The micrograph on the left is the fluorescence image; that on the right shows the corresponding condensation figure (smaller droplets on hydrophilic circles, larger droplets in the background). The insets show an island patterned with the fluorescein disulfide alone: notice that the condensation figure has much larger drops on this islands (thus providing evidence that r-WETS with a mixture of disulfides delivered *both* of them onto gold). (b) Array of Chinese hamster ovary (CHO) cells cultured on unmodified portions of gold; clear regions were wet stamped with an adsorption-resistant layer of SHC11EG6. In our procedure, the stamped coverslips did not require precoating with extracellular matrix protein (e.g., fibronectin) prior to cell plating. (c) An array of fluorescent squares prepared by two-step reactive patterning of glass. Scale bars in all pictures correspond to 150 μm .

dishes, and—to achieve deep patterning—left therein overnight; to avoid stamp drying, the dishes were kept covered during this time. When glass microspheres (Polysciences, Inc., glass beads 50–100 μm) were subsequently agitated mechanically on the patterned surface, they exchanged charge more readily with oxidized than with unoxidized regions of the PS surface—consequently, the spheres organized only over the oxidized islands (Figure 2). When the dimensions of these islands were commensurate with those of the spheres, it was possible to place one sphere onto each island. This is illustrated in the upper-right image in Figure 2, which shows an “open” square array of 50 μm spheres obtained by templated ESA.

Since agarose stamps do not lose their structural integrity when soaked in mixtures of water and alcohols or DMSO, they can be used to deliver onto surfaces chemicals that are not necessarily soluble in water. For example, r-WETS can be used to deliver various alkane thiols and/or disulfides from ethanolic solutions onto gold surfaces to form self-assembled monolayers therein. Figure 3b illustrates micropatterning—using an agarose stamp soaked in 2% SHC11EG6/DMSO solution—of protein-resistant self-assembled monolayers (SAMs) of thiols terminated in several units of ethylene oxide (EG). In conventional stamping methods, these polar mercaptans cannot be directly stamped onto a surface and form bioresistant monolayers²⁷ only from solution. WETS overcomes these limitations and gives a monolayer of

excellent quality on which cells do not grow even several weeks after stamping. Also, unlike PDMS (either hydrophobic or hydrophilized with PEO²⁸), hydrogel stamps provide a general method for the delivery of more than one thiol/disulfide at the same time, and thus for the formation of mixed SAMs under the stamped features.²⁹ When the unpatterned gold is then exposed to a mixture of thiols/disulfides of different concentrations and/or types, surfaces can be micropatterned with *two* mixed SAMs (Figure 3a)—such surfaces are interesting and potentially useful for the ability to tune the “contrast” between the properties (e.g., wettability, protein affinity³⁰/resistivity, etc.) of the regions of the two mixed SAMs continuously by simply adjusting the molar ratios of their components (and not by changing the constituent thiols themselves).

Our method is easily extended to multistep reactive micropatterning. In Figure 3c, r-WETS was first used to print parallel lines of an amino-terminated silane monolayer on glass (from stamps soaked in 2% v/v water solution of 3-aminopropyltriethoxy silane), and then—after dehydration at 150 °C for ~2 h—to apply a solution of fluorescein isothiocyanate (FTIC; 2% v/v solution in carbonate pH = 10 buffer) along lines running in the perpendicular direction. FTIC reacted with the terminal amino groups in the regions of overlap between the two patterns to give a pattern of fluorescent squares.

The major limitation of r-WETS is the possibility of lateral spreading³¹ of patterning inks from the stamped features. We have observed this happening when (i) the surface of the stamp was not sufficiently dried prior to patterning and (ii) if the patterned surface was very hydrophilic or became such as a result of patterning (for instance, formation of a SAM from EG-terminated thiols caused lateral spreading at an initial rate of ~10 $\mu\text{m}/\text{min}$). Even in such cases, however, spreading could be eliminated—especially in cases of polar reactants—by performing r-WETS under a layer of a liquid immiscible with the contents of the stamp. In a typical procedure, a stamp was placed upside down at the bottom of a dish filled with light mineral oil (Fisher Scientific, Fair Lawn, NJ). A substrate was then placed onto the stamp and came into conformal contact with the tops of the stamp’s features. At the same time, the grooves between the features remained filled with oil, thus preventing reactive spreading of the stamp’s contents onto a surface.

To summarize, we have described a new method of reactive micropatterning of surfaces using hydrogel stamps containing water-based “inks”. The method is simple, reliable, and versatile—the examples described here are but a few of its possible applications. We believe r-WETS will become a useful tool in reactive patterning of surfaces in applications in which reagents are to be delivered onto a surface in solution. In addition, r-WETS can be used as an easy method for multistep registration of compatible reagents on surfaces. Of course, gels other than agarose might be needed to affect other types of chemical transformations—in particular, water-sensitive reactions will likely require the use of stamps made of organogels and soaked in anhydrous organic solvents.

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