Surface Patterning with Fluorescent Molecules Using Click Chemistry Directed by Scanning Electrochemical Microscopy

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We introduce a new approach to pattern fluorescent molecules, which do not participate in an electrochemical reaction themselves, onto an azido functionalized glass substrate using "click" chemistry directed by scanning electrochemical microscopy (SECM). Our technique extends the surface patterning ability of SECM to the level at which any inert small molecule or macromolecule can be grown on an insulating substrate through a click chemistry reaction to form a covalent bond directly under a microelectrode.

SECM is a powerful tool for studying local electrochemical and chemical reactions and the kinetics of a fast reaction by scanning an ultramicroelectrode (UME) along a substrate while the currents produced by the oxidation or reduction of the electrochemical species at the electrodes are recorded.[1] When the UME is held about a radius distance from the substrate surface, electron transfer is confined to a small area and local chemical or electrochemical information can be obtained from the positive or negative feedback current, providing local topographic or kinetic information. By scanning the UME at a constant height, useful information about the spatial distribution of local surface conductivity and electrochemical activities of reactive sites can be obtained.

SECM-based surface patterning can be used to form a variety of μm-sized structures, for example, by electrochemical etching of a semiconductor,[2] metal deposition and etching,[3] deposition of conducting polymers,[4,5] and inducing biomacromolecules to immobilize on a self-assembled monolayer (SAM) which is patterned using a UME.[6] With SECM no preformed stamp or mask is needed prior to patterning the substrate, since the pattern is formed by scanning the UME. Surface patterning of different materials (metal, polymer, semiconductor) at high density onto various kinds of substrates at the μm, or even nm, scale is possible, and the surface chemistry is controlled to deposit precise amounts of materials by the UME at a given applied potential. These existing SECM patterning methods either depend on patterning a surface through destructive modification of a substrate (i.e., etching) or heavily rely on the electrochemical behavior of the target materials that will be deposited onto a substrate under an applied potential between the tip and the substrate. Recently Collman, Chidsey and co-workers[7] described the modification of a self-assembled monolayer coated Au microelectrode surface (ferrocene attachment) by "click" chemistry catalyzed by the electrochemical reduction of Cu(II) catalyst. Here we describe a different approach, where the Cu(II) reduction is carried out at a SECM tip to modify a glass surface beneath.

The "Sharpless click" reaction[8,9] refers to a Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition of azide and alkynes to form 1,2,3-triazoles with high efficiency.
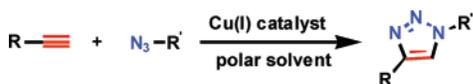
The reaction is useful in linking molecules onto a solid surface and can proceed in a variety of solvents, tolerate a wide range of pH values, and perform well over a broad temperature range. For example, azido-terminated SAMs can easily be connected with alkynes by 1,2,3-triazole formation in the presence of a Cu(I) active catalyst.[10] In this report, an acetylene-functionalized fluorophore was used in an electrochemical cell to react with an azido-terminated monolayer self-assembled onto a glass substrate to form triazole through a click reaction. As depicted in Scheme 1, a gold microelectrode was used to synthesize Cu(I) locally in the small gap between a tip and a substrate to catalyze the click reaction in a small volume. The small gap between a UME and a substrate can not only produce sufficient Cu(I) species to ensure immobilization of acetylene fluorophore derivatives onto a small area of the substrate, but can also maintain the stability of Cu(I), since it can be oxidized easily in solution. The amount of deposited molecules can be controlled by adjusting the tip–substrate distance, the amount of Cu(I), and the reaction time. The maximum amount of dye molecules attached to the SAM is determined by the total number of azido groups functionalized on the glass substrate.

Benzothiodiazole acetylene **1** was designed and synthesized as the fluorescent ink (see Supporting Information for the detailed synthesis and optical properties of **1**). The azido-terminated SAM on a glass substrate was prepared by treating a bromo-terminated monolayer with NaN$_3$. The bromo-terminated SAM was obtained by the immersion of a glass substrate in a solution of 11-bromoundecyltrichlorosilane in dry dichloromethane (1%, v/v) (see Supporting Information for detailed preparation conditions).

Since aquo-Cu(I) has poor stability and can disproportionate to Cu(0) and Cu(II), we selected Cu(II) salen as the Cu-source and DMF as the solvent to achieve stable Cu(I) species and solubility of the other reagents. Stable Cu(I) was generated at an UME in the SECM to catalyze the click reaction in the small gap between the gold UME and the glass substrate.[7] A typical cyclic voltammogram (CV) of Cu(II) salen[11] at a gold microelectrode in DMF, shows Nernstian behavior at -1.0 V vs Ag QRE (see Supporting Information). CVs at a larger electrode demonstrate that a stable Cu(I) intermediate can be obtained. This electrochemical behavior of Cu(II)/Cu(I) is attributed to stable tetrahedral intermediates of the Cu(I) species in the presence of the salen ligand in DMF.[11]

To pattern the azido-functionalized glass substrate with the fluorescent dye molecule, an approach curve to the azido-functionalized glass substrate with the gold microelectrode (100 μm diameter) at a potential of 0.8 V (vs silver quasi-reference electrode) (QRE) and a solution of ferrocene in DMF/n-Bu$_4$NBF$_4$ was obtained to allow the tip to be positioned at the desired height. The microelectrode was then held at a constant height of 28 μm, where the tip current decreased to 50% of the steady-state current at long distance (see the Supporting Information). This small distance above the glass substrate reduces the diffusion time of the Cu(I) species
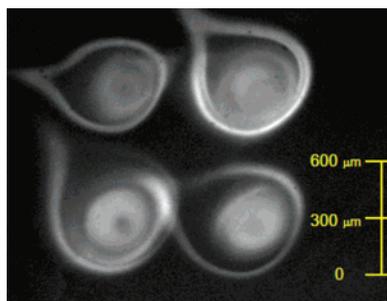
Figure 1. Typical fluorescence microscopy image of a patterned benzothiodiazole array on the glass substrate.

Scheme 1 Local Reduction of Cu(II) to Cu(I) at a Gold Microelectrode (Left) and Immobilization of Acetylene Fluorophore Derivatives onto a Glass Substrate through Click Chemistry.
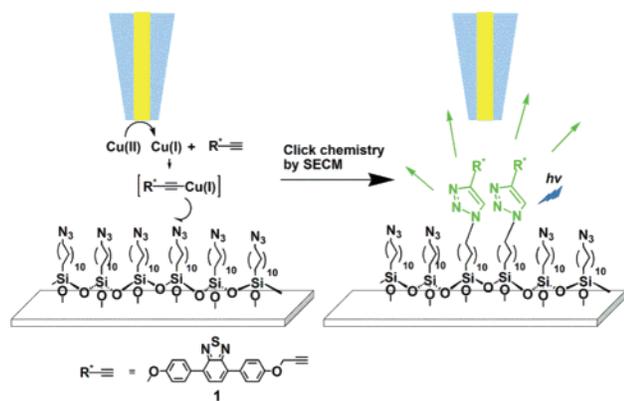
from tip to substrate and ensures a small reaction area. The ferrocene solution for tip approach and alignment was then replaced by the Cu(II) salen and **1** in DMF/n-Bu$_4$NBF$_4$ solution for surface patterning. The solution was deaerated with argon to stabilize the Cu(I) intermediate and prevent oxygen from participating in the electrochemical reaction. The Cu(I) species generated at the gold microelectrode at -1.08 V catalyzed the click reaction. After surface patterning, the glass substrate was washed well several times with acetone and EtOH to remove excess dye molecules nonspecifically anchored to the substrate. The pattern formed was measured by fluorescence microscopy. The fluorescence pattern on the glass substrate indicates that acetylene fluorophores were covalently linked to the substrate via 1,2,3-triazole formation.

Figure 1 shows a fluorescence microscopy image of a typical array. The array contains four spots, where each spot shows inner circular centers and outer rings. Since we used a 100 μm gold electrode for patterning, the 300 μm diameter inner centers correspond to the surface area of azido-functionalized glass directly below the gold microelectrode, where the produced Cu(I) has the highest concentration close to the glass substrate because of the short diffusion distance. The outer rings were about 500~600 μm in diameter and presumably result from a concentration gradient of Cu(I)−acetylene intermediates. The reaction rate of the click reaction and the thick insulating layer around the gold microelec-

trode may play a role in the formation of the interesting ring features. Such patterns could arise from the relative concentration gradients of the three components of the click reaction, e.g., Cu(I), azido monolayer and fluorescence dye derivative, and the spatial distribution of the reaction rates. The formed rings resemble "Liesegang rings",[12] which are formed in reaction-diffusion systems. Each of the rings also shows a pronounced "tail" which is probably formed because of convection caused by Ar gas bubbling. The distance between two ring centers was about 700 μm, which was controlled exactly by moving the microelectrode position with the SECM stepping motor. Different patterns of the dye molecule could be written on the azido-terminated SAMs in the same way by programming the SECM tip movement, as shown in previous SECM patterning.

This technique represents a novel method for attaching fluorescent molecules to a solid substrate by using SECM and click chemistry. This approach can probably also be used to transfer other molecules, and nanoparticles to a solid substrate. In addition, we have found the pattern generated by SECM-mediated click chemistry with a Liesegang ring-type behavior. On the basis of previous SECM work, the pattern feature size and shape should be controllable by selecting the tip size and the relative distance from tip to substrate. Larger scale and higher density patterning of protein arrays and other biologically interesting substances should also be possible.

Supporting Information Available: Additional experimental details of synthesis and photophysical properties of **1**, CV of Cu(II) salen, and procedures for the preparation of azido-functionalized SAM and SECM-aided patterning. This material is available free of charge via the Internet at http://pubs.acs.org.

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