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A New Porous Reaction Layer for Developing Addressable Molecular Libraries

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Microelectrode arrays hold great promise as platforms for monitoring ligand−receptor binding events in "real-time".[1−4] Because each microelectrode in an array is individually addressable, each can be used to monitor a unique member of a molecular library that is associated with its surface. For this reason, we have been developing the synthetic tools necessary for site-selectively building and placing molecules by the Pt-microelectrodes in an active-semiconductor array.[3] Key to this work is coating the arrays with a porous reaction layer that allows for the attachment of substrates or completed library members to the surface of the arrays proximal to the microelectrodes.

To date, both agarose[5] and sucrose[6] have been used for this purpose. Both approaches have significant weaknesses. In the case of agarose, the polymer is not stable. It delaminates from the surface of the array with time, dissolves in a variety of solvents, and reacts with a number of the reagents used to perform site-selective syntheses.[7] For this reason, agarose is mainly used as a "practice-polymer" for studying new reactions on the arrays. The use of a sucrose-based coating solves these problems by providing a stable surface for generating functionalized arrays. However, like agarose the sucrose-coating provides a polyhydroxylated surface on the array. This surface limits the use of

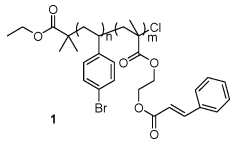


Figure 1

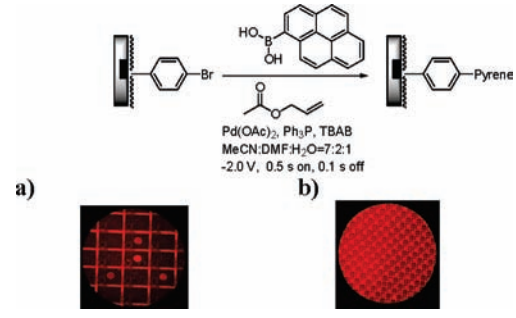
the microelectrode arrays for monitoring the behavior of small molecules that are synthesized by constructing core scaffolds and then diversifying the scaffolds through the use of protected amine and alcohol functional groups. In addition, preparing a stable sucrose surface requires special cleaning and handling of the microelectrode array.

With these things in mind, we sought to develop a new approach to coating the arrays that would allow for customization of the surface. Any porous reaction layer developed needs to be chemically inert, stable to multiple reaction steps and washings, functionalized in a manner that allows for site-selective modification proximal to the microelectrodes in the array, and porous enough to allow for both electrochemically mediated synthetic reactions[5,6] and electrochemical impedance experiments.[4] In addition, preparation of the coating needs to be general so that it can be tailored for specific uses in the future. To this end, it appeared that a diblock copolymer like **1** might be ideal (Figure 1).[8] One block in the polymer could be used to fix the polymer to the surface of the array, and the second used to provide attachment points for substrates to the resulting surface. To fix the polymer to the surface of the array, the first bock of the polymer was designed to take advantage of the cinnamoyl-substituted polymethacrylate strategy developed by Guojun Liu and co-workers.[9] This chemistry takes advantage of the polymethacrylate backbone to coat surfaces and then cross-links the polymer on the surface by photochemically dimerizing the cinnamoyl groups to provide stability to the coating. The key question for this strategy was whether the resulting nonconducting, cross-linked copolymer would be porous enough to allow for both the electrochemically mediated reactions needed for placing molecules on the surface proximal to the microelectrodes and the electrochemical impedance experiments needed for monitoring ligand−receptor interactions on the arrays.[10] To test this question, the second block of the copolymer was constructed from 4-bromostyrene. In this way, the compatibility of the cross-linked polymer with mediated electrolyses could be probed using microelectrode array-based Suzuki,[11] Heck,[12] and Cu(I) coupling reactions.[13]

The synthesis of polymer **1** was accomplished using atom transfer radical polymerization.[14] It was then coated onto the surface of an array by taking advantage of the suggestion by Liu and co-workers that polymer brushes from block copolymers like **1** are best formed using a solvent system comprised of a solvent that solubilizes both blocks of the copolymer and a solvent that solubilizes only the polystyrene block of the copolymer. Hence, **1** was taken up in a 1:1 mixture of THF and xylene, and then the resulting solution was spin-coated onto the arrays with a speed up to 800−1000 rpm for 30 to 40 s. The coating was allowed to dry, and the microelectrode array was subjected to irradiation using a 100 W Hg lamp for 20 min giving rise to a porous cross-linked polymer with pore sizes on the order of 19 ± 3 nm.[14]

The first reaction attempted on the new surface was the Suzuki reaction outlined in Scheme 1.[11] In this reaction, the Pd(0)-catalyst

Scheme 1[a]



[a] For (a) lower left = 300 cycles, lower right = 600 cycles, upper = 900 cycles, and center = 1800 cycles.

needed for the transformation is generated at selected electrodes by using them as cathodes (−2.0 V relative to a remote Pt counter electrode) to reduce $Pd(OAc)_2$. The conditions selected were identical to those reported previously for the reaction on an agarose surface.[11] The reaction proceeded nicely in a site-selective fashion on both an array having 1024 microelectrodes cm^{-2} (1K-array, picture a) and an array having 12 544 microelectrodes cm^{-2} (12K-array, picture b). The

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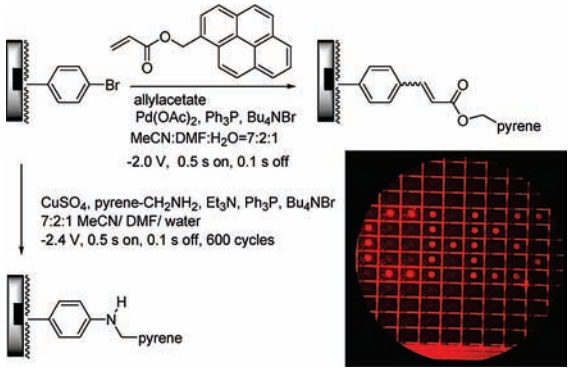
reaction conditions given in Scheme 1 were those used for the 1K-array. On a 1K-array, the reaction was conducted for four different time periods between 300 and 1800 cycles. The diblock copolymer was stable for each spot, a change from the previously used agarose polymer that decomposed after only 600 cycles. Arrays employing the block copolymer could be used for the Suzuki reaction 2 months after being coated, while arrays coated with agarose typically remained viable for less than 3 days. The polymer was stable to 15 consecutive experiments each using 300 cycles without any sign of delamination from the surface. For comparison, the agarose polymer begins to peel off of the array after as few as three consecutive experiments. The reaction on the copolymer coated 12-K array was conducted in a similar manner.[14]

To show the generality of the polymer support to site-selective chemistry on the arrays, three reactions were run side by side on a 1-K array. One reaction was the Suzuki reaction described above, and the other two were the Heck and Cu (I)-catalyzed reactions shown in Scheme 2. The Cu(I) reaction was used to place a "C-pattern" on the array, the Heck reaction an "H-pattern", and the Suzuki reaction an "S-pattern". The resulting "CHS"-patterned array is shown in the scheme.

Scheme 2



With the cross-linked methacrylate-based copolymer proving compatible with site-selective preparative electrolysis experiments, its compatibility with the desired electrochemical signaling experiments was probed. The signaling experiments are conducted by taking advantage of an electrochemical impedance-based approach.[4] This is done by first measuring the current associated with an iron species in the solution above the array and then adding a protein to the solution and monitoring drops in the current associated with the iron at the microelectrodes in the array. A drop in current at an electrode indicates binding of the added protein to the surface of the array proximal to the electrode. To test the compatibility of the copolymer with this approach, the nonspecific binding of Bovine Serum Albumin (BSA) to the unfunctionalized copolymer was examined (Figure 2).[14] The figure shows the cyclic voltammetry results of four experiments, each of which was run using a block of 10 microelectrodes in a coated, 12-K array. The first was recorded for a solution without the BSA protein, and then second through fourth were recorded after varying amounts of BSA were added to the first experiment. The initial addition of 2.5% by wt. BSA to the solution led to a slight increase in current observed. This increase indicated the presence of the BSA in solution (the increase is caused by a catalytic current associated with iron(III) oxidation of the C-terminus of the protein) with limited if any binding to the surface. The addition of 5.0% by wt. BSA to the solution showed a small amount of impedance indicating the start of surface binding, while the addition of 7.5% BSA showed maximum impedance. Such impedance experiments do not give linear responses because protein binding to the surface impedes both the iron from reaching the surface and the catalytic current. The result is a very sensitive test for the

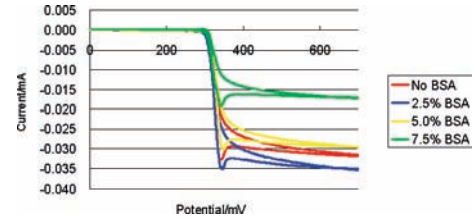


Figure 2. Conditions: For the solution above the array, 8 mM/L of both potassium ferrocyanide and potassium ferricyanide were dissolved in a 5X PBS solution.[14] Varying amounts of BSA were then added to the solution. For all the experiments, a scan rate of 200 mV/s was used.

binding to the surface of the electrode. In this case, the strong impedance observed indicated that the diblock copolymer was compatible with the signaling experiment. Similar results were obtained using an antibody in place of the BSA.

In conclusion, a diblock copolymer has been synthesized, coated onto microelectrode arrays, and cross-linked to provide additional stability. The resulting polymer coating is stable with respect to time and multiple electrolysis reactions, compatible with site-selective synthetic reactions on the arrays, and compatible with electrochemical signaling experiments. The overall approach opens the door for developing customized porous reaction layers for the microelectrode arrays.

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Supporting Information Available: Experimental details for the synthesis of polymer **1**, details for coating the arrays with polymer **1**, AFM images of the polymer on the surface, and details concerning both preparative and analytical experiments on the arrays are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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