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Published on Web 02/04/2010

Tailored Electroactive and Quantitative Ligand Density Microarrays Applied to Stem Cell Differentiation

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Abstract: The ability to precisely control the interactions between materials and mammalian cells at the molecular level is crucial to understanding the fundamental chemical nature of how the local environment influences cellular behavior as well as for developing new biomaterials for a range of biotechnological and tissue engineering applications. In this report, we develop and apply for the first time a quantitative electroactive microarray strategy that can present a variety of ligands with precise control over ligand density to study human mesenchymal stem cell (hMSC) differentiation on transparent surfaces with a new method to quantitate adipogenic differentiation. We found that both the ligand composition and ligand density influence the rate of adipogenic differentiation from hMSC's. Furthermore, this new analytical biotechnology method is compatible with other biointerfacial characterization technologies (surface plasmon resonance, mass spectrometry) and can also be applied to investigate a range of protein−ligand or cell−material interactions for a variety of systems biology studies or cell behavior based assays.

Introduction

Stem cells possess the ability to self-replicate to give rise to identical daughter cells and they can also undergo a complex differentiation process to generate new cell lineages.[1,2] While stem cells hold much promise as an unlimited source of cells for transplantation therapies, and for treating numerous cancers and diseases, the precise control of the differentiation process is challenging and little is known about the complex interplay of the multitude of crucial factors ranging from signaling molecules to the cell microenvironment that influences stem cell differentiation.[3] For example, stem cells use cell surface receptors to receive important signals from the extracellular environment in order to initiate differentiation.[4] A major issue in using stem cells as therapies is the ability to control their interactions with man made materials. Modulating their growth and differentiation behavior on or within these materials, which are used as scaffolds for implant devices and as delivery vectors, will be important for a range of biotechnologies and therapeutics.[5,6]

While the chemical nature of the interaction between the cell surface receptors and the extracellular environment is complex and unclear, it is highly possible that the surface conditions and properties of the material to which the stem cells adhere would have an influence on the stem cell differentiation process. Therefore, investigation of the material-stem cell interaction and how this association can be manipulated is essential to discover new features of stem cell differentiation that can ultimately be utilized to build therapeutic devices or delivery systems. The ability to simultaneously survey different surface conditions in a high-throughput way to assess the factors that influence stem cell differentiation would facilitate our understanding of the material−stem cell interaction and potentially generate new biomaterials. In a landmark study, Langer and co-workers showed that the modulation of stem cell differentiation could be accomplished by modifying the *macroscopic* properties of the supporting materials. In their study, they used microarray technology to determine the most suitable polymer blends that cause stem cell differentiation for tissue engineering applications.[7−9]

We believe, to elucidate and further investigate the chemical nature of the surface effects on stem cell differentiation, a general high-throughput, multiplex, and quantitative model

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surface system that meets the following criteria is required. (1) The surface composition can be defined at the molecular level, thus analytical techniques can be used to tune and characterize the surface properties. (2) A general surface immobilization strategy to install a library of ligands/molecules in arrays where the amount and therefore surface density is measurable and controllable. (3) The surface must be biocompatible and inert to nonspecific protein and cell adhesion. This allows for the correct interpretation of ligand−receptor mediated interactions, that is, biospecific associations where the only interaction between cell and material is a receptor−ligand mediated interaction (no nonspecific surface interactions).

To achieve a molecular level investigation of surface properties that might influence stem cell differentiation, we have developed a multiplex and quantitative microarray strategy to study a range of surface effects on stem cell differentiation. This novel surface strategy is compatible with a powerful synthetic immobilization technique, which results in the capability of immobilizing a variety of molecules onto the surface quantitatively to create various surface properties in a high-throughput microarray format. The surface is also conductive and therefore electrochemistry can be performed on the substrate to precisely characterize the ligand density presented on the surface. This provides an opportunity for quantitatively determining the interplay of several surface properties such as surface roughness, hydrophobicity, chemical functionality, and specific ligand−receptor interactions as potential factors in stimulating stem cell differentiation.[6] Based on this model substrate, the role of specific surface properties on stem cell differentiation can be studied at the molecular level.

Our surface chemistry methodology is based on self-assembled monolayers (SAMs) of alkanethiolates on gold. SAMs of alkanethiolates on gold are molecularly well-defined and synthetically flexible and therefore can be modified with a variety of functional groups, which determine the characteristics of the substrate.[10] Importantly, SAMs are compatible with tissue culture conditions and optical and fluorescence microscopy.[11] Furthermore, the conductive feature of gold substrates enables the use of several analytical surface spectroscopy techniques including mass spectrometry, SPR (surface plasmon resonance), XPS (X-ray photoelectron spectroscopy), STM (scanning tunneling microscopy), and cyclic voltammetry (CV) to characterize interfacial associations.[10,12,13]

Microarray technology has revolutionized basic science and biotechnology, is recognized as a very powerful high-throughput experimental tool for screening a variety of conditions, and has been applied to material science, tissue engineering, proteomics, and drug discovery.[14] By using standard microarray technology many microliter-scale and even nanoliter-scale chemical reactions can be performed rapidly with spatial control on a single substrate. For cell-based assays, each of the spotted regions can be used for an independent analysis of a certain material−cell interaction. To create a variety of surface chemistries on one substrate and to precisely quantify the amount of ligands on each spot and then determine their influence on the rate of stem cell differentiation, we developed a multiplex analytical biotechnology that combines electroactive SAMs and microarray technology.

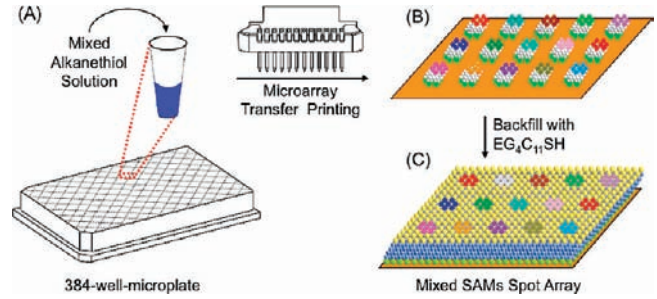

Figure 1. Strategy to transfer a range of mixed alkanethiol solutions (each solution may contain more than one alkanethiol) from a microplate to a bare gold substrate via microarray printing to generate a corresponding array of self-assembled monolayers. (A) A 384-well-microplate contains a number of different ratios of mixed alkanethiols. (B) The pins of a spotbot microarrayer will pick-up a specific solution from the microplate and transfer print the solution onto the bare gold substrate at programmed positions, allowing the alkanethiols to form ordered SAMs. Each color represents a unique ratio or combination of SAM composition. (C) A solution of tetra(ethylene glycol) terminated alkanethiol ($EG_4C_{11}SH$) was then used to backfill the remaining regions to render the surface inert to nonspecific protein adsorption and cell attachment. Based on this strategy, the gold substrate is composed of regions that present varying surface chemistries to study cell−material interactions (arrayed regions) and regions that do not allow cell adhesion.

Results and Discussion

Using a spotbot2 microarrayer, we printed mixed alkanethiol solutions in various ratios directly onto a bare gold substrate. By using a 384-well microplate with different ratios of mixed alkanethiolates in each well, a range of surface chemistries can be installed onto one gold surface (Figure 1). Upon spotting, the alkanethiols immediately adsorb to gold and efficiently self-assemble on the surface. Since small volumes are printed (less than 1 μL), the solvent (ethanol) rapidly evaporates resulting in further concentrating the alkanethiol solutions. The substrate is then washed thoroughly with ethanol and backfilled by immersion into an ethanolic solution of tetra(ethylene glycol) terminated alkanethiol ($EG_4C_{11}SH$) for 12 h. The ethylene glycol group is known to resist nonspecific protein adsorption and cell attachment.[15] Upon cell seeding, the cells only attach to the spotted regions if the underlying surface chemistry supports adhesion. In this way, the ability to test many different combinations of surface chemistries on cellular behaviors such as cell attachment and differentiation can be performed.

To precisely control and characterize the surface composition, we printed, via a microarrayer, different ratios of mixed alkanethiol solutions containing 2-(11-mercaptoundecyl) hydroquinone (H_2Q) and 11-mercapto-1-undecanol ($HOC_{11}SH$) onto bare gold surfaces. Because the H_2Q molecule is electroactive, the absolute surface density of the H_2Q within the SAM spots can be quantitatively determined by using cyclic voltammetry (CV) and compared directly to the concentration of H_2Q solution transferred from the original microwell.[16] Mixing H_2Q with 11-mercapto-1-undecanol ($HOC_{11}SH$) in different ratios creates a series of mixed alkanethiol concentrations that can be transferred

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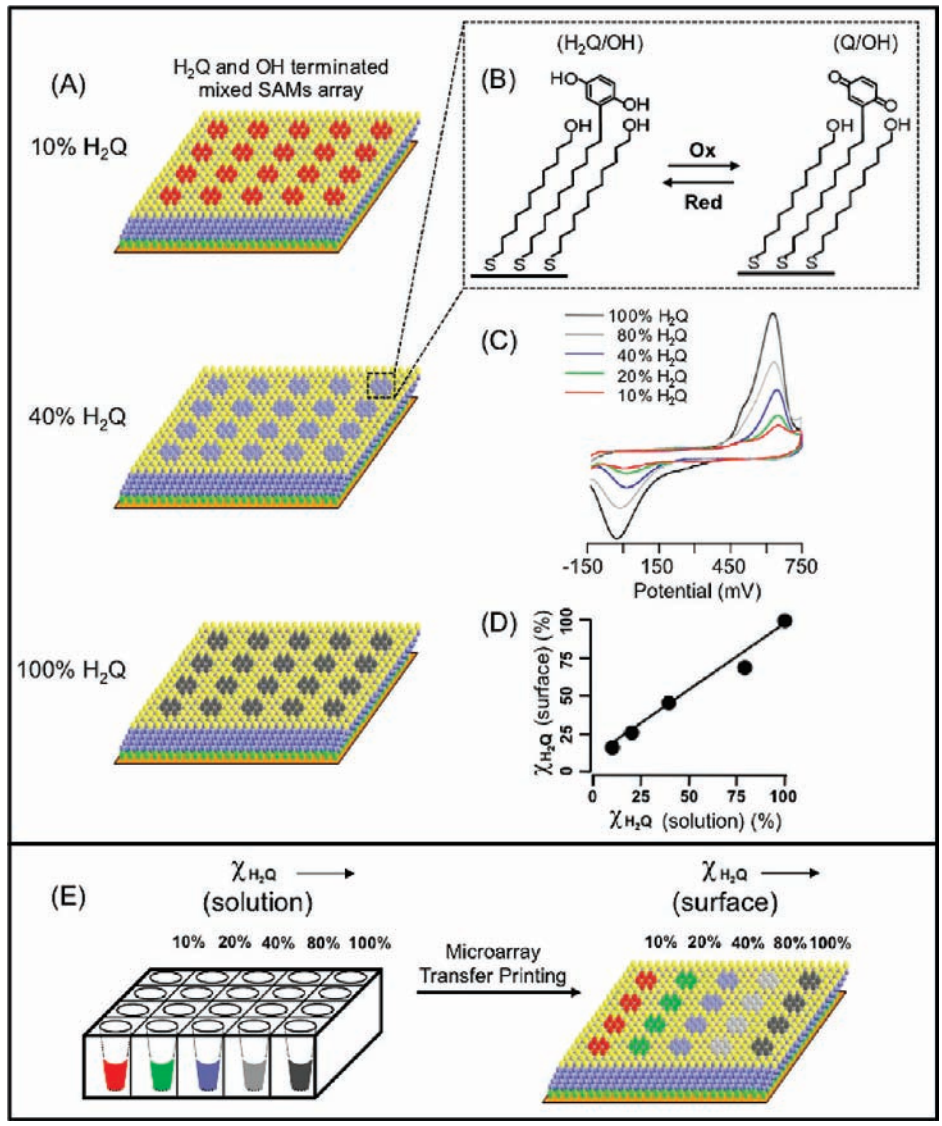

Figure 2. Strategy to develop quantitative and electroactive ligand density spot arrays. (A) Examples of SAM spot arrays of mixed $H_2QC_{11}SH$ and $HOC_{11}SH$. By printing mixed ratios of solutions of $H_2QC_{11}SH$ and $HOC_{11}SH$, different electroactive SAM compositions can be generated (examples show 10%, 40%, and 100% of H_2Q) and characterized. (B) The H_2Q group is redox active and can be reversibly oxidized and reduced to the quinone (Q) form. (C) The density of the H_2Q on the surface in each array can be determined by integrating the area underneath each peak in the cyclic voltammagram for each H_2Q density array generated. (D) A plot of the integrated peaks for each H_2Q surface density was used to determine the relationship between the solution composition of H_2Q (χ_{H_2Q} solution) and the surface density of H_2Q (χ_{H_2Q} surface). The straight line shows the solution concentration of H_2Q matches the surface density of H_2Q with an r value of 0.96. (E) Strategy showing the transfer of mixed solution of H_2Q- and −OH-terminated alkanethiols to form the corresponding H_2Q- and −OH-terminated SAMs.

onto the gold substrate to generate SAMs of the same spot size but with different amounts of H_2Q on the surface (Figure 2).

We used electrochemistry to quantitatively correlate the relationship between the concentration of H_2Q in the mixed alkanethiol solutions from the 384-well microplate with the surface density of H_2Q generated on gold via microarray transfer printing. SAMs presenting hydroquinone groups can undergo a reversible 2 electron, 2 proton, oxidation and reduction process in aqueous conditions, as shown in the cyclic voltammogram (CV) in Figure 2c.[17] The surface density of the redox active H_2Q molecule (Γ_{H_2Q}) can be precisely determined by integration of the CV peaks to determine the total charge Q, and using the simple equation $Q = nFA\Gamma$ (where Q = total charge, n = number of electrons, F = Faraday constant (96500 C), A = surface reaction area [(spot size) × (number of spots)] and Γ = H_2Q density on surface (molecules/μm^2)).[18] The surface density of H_2Q can then be directly correlated with the spotting solution concentration of H_2Q by plotting χ_{H_2Q} (surface) versus χ_{H_2Q} (solution) (Figure 2(d) (χ_{H_2Q} surface represents the ratio of the redox active H_2Q molecule on the mixed SAMs surface, and χ_{H_2Q} solution represents the ratio of H_2Q in the microwell solution. The slope is linear, indicating that the H_2Q density within the spots on the SAM surface is in accord with the spotting solution H_2Q concentration. This unique feature allows for the transferring of a mixed alkanethiol solution from a microplate (via a microarrayer) to generate a mixed SAM

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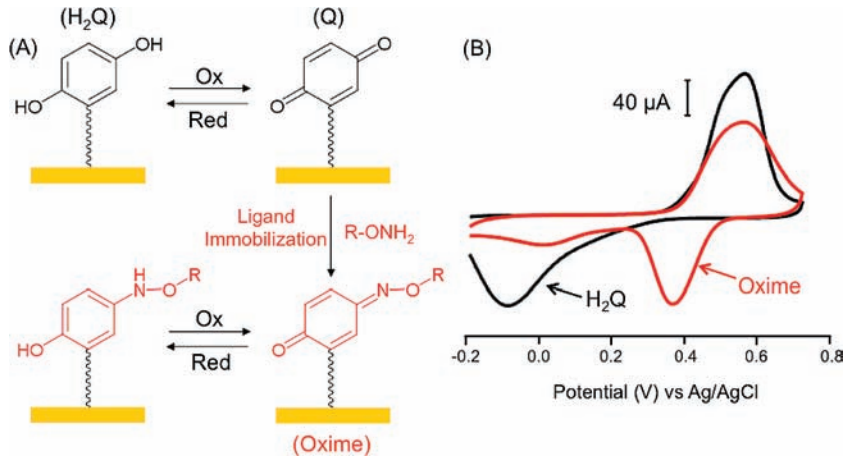

Figure 3. Strategy to generate quantitative, chemoselective, and electroactive ligand density spot microarrays. (A) The H_2Q group can be oxidized to the quinone group which can chemoselectively react with oxyamine tethered ligands ($RONH_2$, where R can be any ligand, small molecule or biomolecule) to generate an interfacial oxime conjugate. The oxime is also redox active but with a distinct cyclic voltammogram that allows for the precise monitoring and quantification of ligand immobilization. (B) Cyclic voltammograms showing the diagnostic peaks that characterize the hydroquinone to quinone redox couple and the oxime product. By integrating the peak area of the oxime conjugate from the cyclic voltammogram the yield of interfacial reaction and therefore surface density of ligand can be determined.

surface spot that can be characterized (via electrochemistry) precisely because the H_2Q is electroactive (Figure 2e).[19]

To prepare surface microarrays presenting a range of ligands with different densities for stem cell differentiation studies, we used the electroactive H_2Q molecule not only as a quantitative read-out of surface density but also as a quantitative chemoselective immobilization strategy (Figure 3). We have shown previously that the H_2Q group can be oxidized to the quinone (Q) group, which can selectively react in high yield with a number of functional groups (cyclopentadiene, hydrazide, and hydroxylamine).[16,20,21] This important feature permits chemoselective immobilization of a library of molecules with differing functional groups and provides a powerful method for tailoring surfaces for a range of applications. In this study, we incorporated an immobilization strategy based on the reaction between Q and oxyamine-tethered ligands ($R\text{-}ONH_2$) to form an interfacial oxime conjugate. This reaction is rapid and stable under physiological conditions.[22−30] Introducing the oxyamine ($\text{-}ONH_2$) group into a range of molecules is straightforward and allows for the generation of libraries of compounds that can be precisely arrayed on these electroactive surfaces.[18,23] A unique feature of this system is that the oxime conjugate is also redox active with diagnostic peaks in the cyclic voltammogram allowing for a sensitive probe to monitor the extent of the interfacial reaction in situ and as a quantitative determination of amount of immobilized ligand to the surface. By integrating the oxime peaks and comparing it with the original H_2Q peaks, the extent of the immobilization can be quantitatively monitored and the surface density of the immobilized ligand can be precisely controlled.[18] This quantitative electroactive immobilization strategy provides a general strategy to immobilize a range of ligands with precise control of density of each ligand to study a range of cell behaviors.[23]

To study the role of ligand density on stem cell differentiation, we synthesized a small library of oxyamine tethered ligands to generate an electroactive microarray presenting a range of molecules with different surface densities (Table 1). We first created a substrate presenting various H_2Q densities in many spots (Figure 4). We then oxidized the surface to generate the Q and then arrayed different oxyamine tethered ligands ($R\text{-}ONH_2$) to each spot. Cyclic voltammetry was used to show the quinones reacted completely to provide the corresponding oxime, indicating the ligands are immobilized at the same density as the original hydroquinone (For example, a 10% H_2Q spot density is oxidized to generate the Q at 10%; when an oxyamine tethered ligand ($R\text{-}ONH_2$) is reacted to completion as indicated by the shift in the cyclic voltammetry signal to the oxime product, 10% of the ligand is now presented on the spot). Stem cells were then seeded onto the ligand density microarray substrate and only adhered to the spot regions that supported adhesion. Once the cell array is formed, induction medium was added to the entire array to induce stem cell differentiation. Therefore, the rate of stem cell differentiation can be monitored over time as a function of the underlying ligand composition and ligand density (Figure 5).

In one application, we applied the electroactive ligand density microarray strategy to human mesenchymal stem cells (hMSCs) to study the role of ligand composition and ligand density on rate of differentiation. Human mesenchymal stem cells, as multipotent stem cells, have the ability to differentiate into several lineages including adipocyte, osteocyte and chondrocyte cells.[31] hMSCs are increasingly being used in therapeutic applications for bone, cartilage and adipose transplantation and repair. Unlike embryonic stem cells, hMSCs are more amenable

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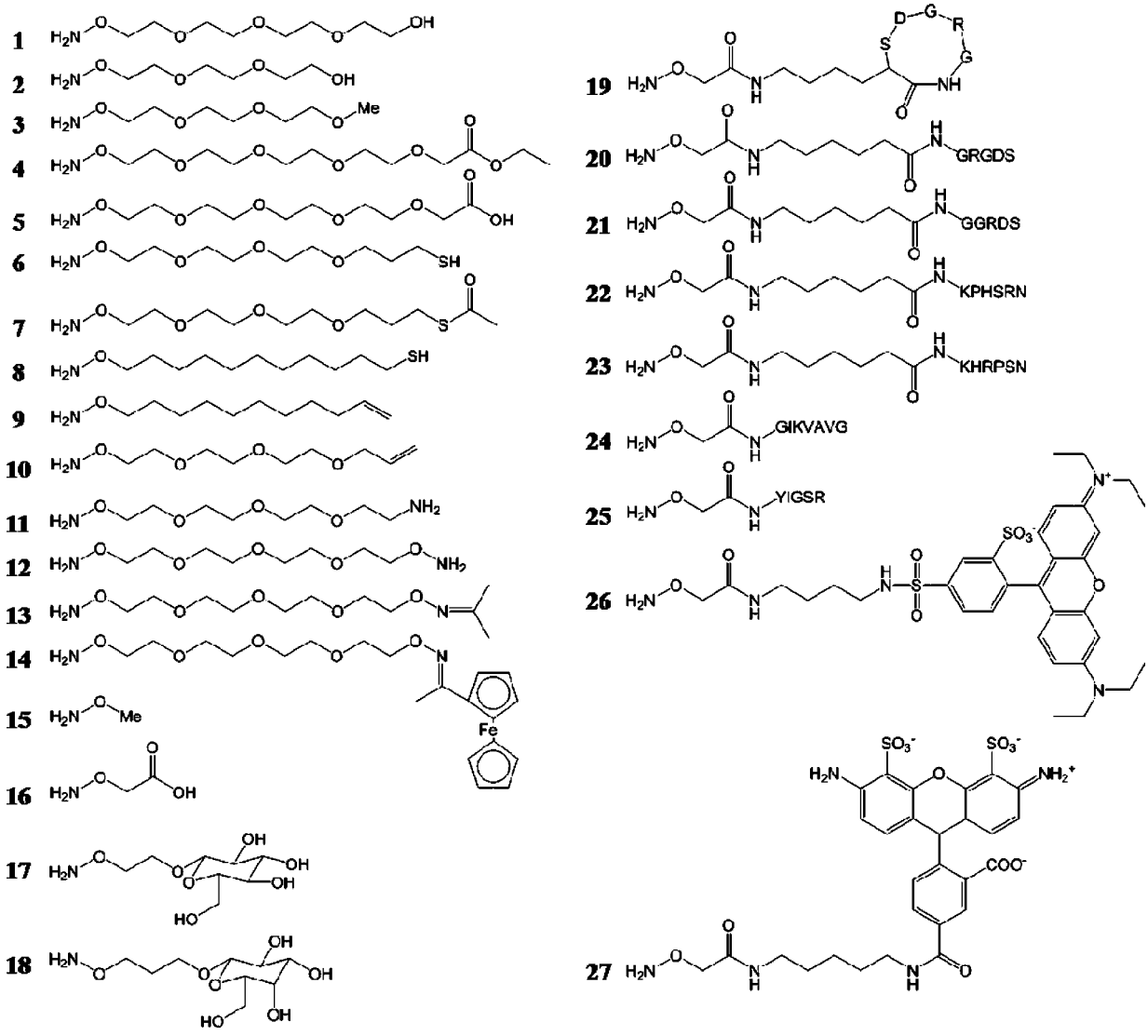
Table 1. List of Oxyamine-Tethered Small Molecules Used To Generate Quantitative Ligand Density Microarrays for Studying hMSC Differentiation



to controlled differentiation and can be readily induced to produce relatively pure differentiated cells. Because of ethical concerns regarding embryonic stem cell research and the ease of manipulation of hMSCs, many studies concerning hMSCs have been performed in recent years. The ability to precisely control stem cell differentiation into the corresponding lineage is crucial for developing new biomaterials for a range of therapies.

To study the effect of ligand composition and ligand density on hMSC differentiation, we used a visible dye to analyze specific differentiated lineage. To distinguish which cell lineage is derived from mesenchymal stem cell differentiation, specific marker dyes have been developed. For adipogenic differentiation, Oil Red O and Harris Hematoxylin are used as the specific marker stains. The generation of many lipid vacuoles is characteristic of adipocytes and can be specifically targeted and therefore visualized by Oil Red O staining. Nuclei are stained blue by Harris Hematoxylin (Figure 4).[32,33] A sample of micrographs showing adipocyte generation versus ligand composition and ligand density and time is shown in Figure 5.

To determine the differences in stem cell differentiation rate on the varying ligand density microarray surfaces, we developed a new quantification method that measures differentiation rate versus ligand density. This strategy is based on measuring the ratio of red pixels to the total number of pixels within the cell patterns and can precisely determine the subtle differentiation differences at any time point on any transparent surface without damaging the sample.[34] Conventional quantification strategies rely on manually counting the number of differentiated cells after weeks or measuring fluorescence absorbance of cell elution or measuring gene expression. Using this quantification method, even subtle differences in stem cell differentiation can be measured on the gold substrate. On the basis of this analysis, a 3D plot of stem cell differentiation versus time versus ligand density was generated and averaged for over 15 experiments (Figure 6, Table 1). The plot clearly shows that the ligand

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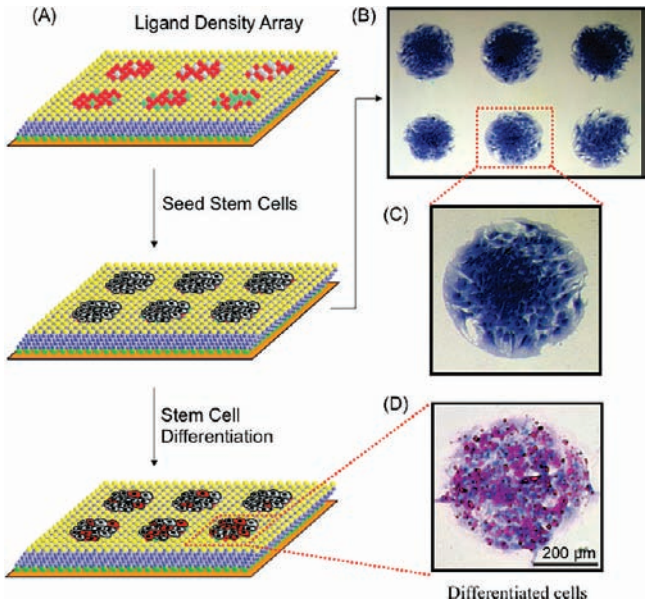

Figure 4. Application of the ligand density microarray to study the rate of hMSCs differentiation. (A) Schematic describing the generation of stem cell arrays to study differentiation as a function of ligand composition and ligand density. (b) A 4× micrograph of hMSCs patterned on a ligand density array. To determine differentiation the cells are stained with Oil Red O, which selectively targets lipid vacuoles to indicate adipocyte cells and Harris Hematoxylin which targets the nucleus and shows blue; (c) 20× micrograph of control hMSCs patterned with no differentiation; (d) 20× micrograph of a pattern of fully differentiated adipocyte cells. The rate of differentiation is influenced by the ligand properties and ligand density. All images were taken by phase contrast microscopy.

composition is important but the ligand density also has a dramatic influence on the rate of differentiation. For example, carboxylic acid terminated oxyamine ligand (**5**) differentiates to a greater extent at higher ligand density than at low ligand density. For thiol terminated ligand (**6**) the stem cells differentiate at lower ligand density compared to higher ligand density. We only show a sample of the differentiation data in Figure 6 (5 day time point). At various durations the differentiation profile changes based on ligand composition and ligand density. At 10 days, approximately all cells on the ligand density spot arrays are fully differentiated (normalized to 1.0, see methods section). As a comparison to the quantitation method used to generate the 3D plot, we also examined the gene expression profiles of the adipocyte markers lipoprotein lipase (Lpl) and peroxisome proliferators-activated receptor gamma 2 (PPARγ2) (Figure 7).[34] We observed that the marker genes were turned on at high levels at approximately 5 days and stayed at a nearly constant level afterward during the duration of the differentiation study. However, some expression was observed at earlier time points, indicating the gene expression profile is complementary but not as sensitive as the oil red O staining analysis.

Conclusion

This report shows for the first time the development of a quantitative ligand density microarray that can immobilize a variety of molecules for a range of cell biological and biochemical studies. This analytical biotechnology strategy is based on the transfer printing of an electroactive hydroquinone alkanethiol that can be oxidized to a quinone for subsequent ligand conjugation. All surface bound molecules are redox active and therefore provide a sensistive in situ probe to monitor and characterize the interfacial reaction. We applied this method to develop a unique microarray to study the effect of ligand composition and ligand density on stem cell differentiation. We observed that the density of ligands influences the rate of hMSC

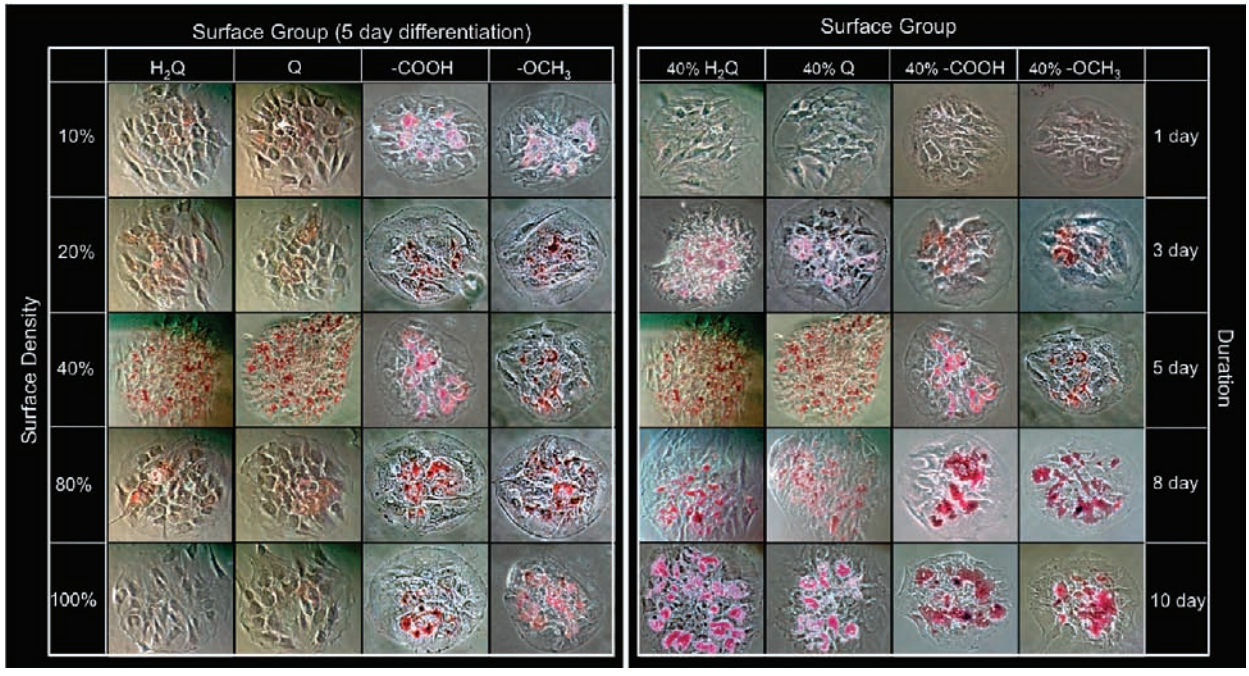

Figure 5. (Left) Representative micrographs showing adipogenic differentiation of hMSCs on different ligand density surfaces after 5 days. Each column shows micrographs of hMSCs differentiating to adipocytes on a particular surface ligand at varying ligand densities. Each row shows the same ligand density but varying ligand composition on hMSCs differentiation. The first and second columns show hMSCs on hydroquinone (H_2Q) and quinone (Q) surfaces, respectively. Shown in the third and fourth columns are hMSCs on immobilized carboxylic acid (−COOH (**5**)) and methoxy (−OCH_3 (**3**)) presenting surfaces. (Right) Representative micrographs showing adipogenic differentiation of hMSCs on surfaces presenting the same density (40%) at varying durations. Each column shows hMSCs on surfaces with 40% ligand density, including hydroquinone, quinone, (−COOH), and (−OCH_3) group, respectively. Cells were stained with Oil Red O and Harris Hemotoxylin. Images were taken by phase contrast microscopy.

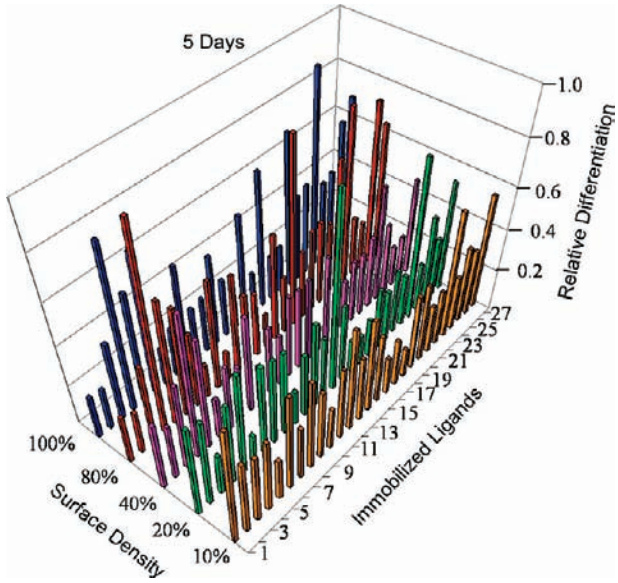

Figure 6. A three-dimensional plot comparing differentiation rate versus ligand composition versus ligand density. The data were normalized to 1.0 for fully differentiated cells after 10 days. There is a clear dependence on differentiation rate and ligand composition as well as ligand density. The representative plot shows data for 5 days differentiation.

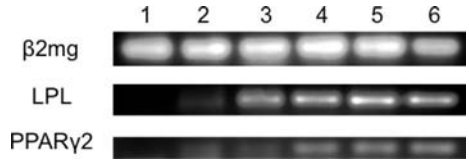

Figure 7. Representative gene expression comparison of adipogenic differentiation rate. An adipose-specific gene, liproprotein lipase (Lpl) and peroxisome proliferators-activated receptor gamma 2 (PPARγ2), and a control gene, $-$2-microglobulin (2 mg), were used to ensure equal loading of the DNA. hMSC's were cultured on varying surface ligand compositions and ligand densities and then induced to adipose and monitored. Total RNA was extracted and analyzed by reverse transcription PCR. Lane 1, control cells; lanes 2$-$6, days of induced differentiation.

differentiation to adipocytes. This feature of ligand density is often overlooked when developing biomaterials for stem cell therapies due to the difficulty in preparing materials where the relationship between ligand density and cellular behavior is molecularly controlled. The electroactive microarray strategy is general and can be used to prepare a wide range of microarrays for a variety of biointerfacial studies including cell-based assays to enzymology platforms. Since the substrates are conductive they may be used in conjunction with surface plasmon resonance technology to measure small molecule or protein binding and mass spectrometry to identify protein partners to the presented ligands. Furthermore, as an additional feature, since the ligand bound molecule is also electroactive the reaction can be reversed to selectively release the immobilized ligands to regenerate the H_2Q, which allows for a renewable microarray platform.[17,35] Finally, interfacing with microfluidic technology will allow for the discrete delivery of reagents to select regions of the surface for a range of systems biology approaches to study a variety of signaling pathways.[36–38]

Experimental Section

Microarray Printing. Microscope glass slides were cleaned by 1:1 mixed solution of hydrogen peroxide and sulfuric acid for 4 h (Caution! Piranha solutions react explosively with trace quantities of organics), followed by washing with distilled water and 200 proof ethanol. After drying with a stream of N_2, a 5 nm adhesion layer of titanium followed by 20 nm of gold was evaporated onto the glass slides. Different alkanethiol solutions (total 1 mM in ethanol) were mixed in varying ratios, filled in designated positions of the 384-well microplate, and then printed in a programmed array format on the gold-coated microscope glass substrate by a spotbot2 microarrayer, which allows for transference of the alkanethiols to programmed positions on the gold substrate. The substrate is then thoroughly washed with ethanol and immersed into a 1 mM ethanol solution of tetra(ethylene glycol) terminated alkanethiol for 12 h, rendering the remaining surface inert to nonspecific protein adsorption and cell attachment.

Electrochemical Characterization. Alkanethiols terminated with the hydroquinone group ($H_2QC_{11}SH$) and the tetra(ethylene glycol) group ($EG_4C_{11}SH$) were prepared as previously described.[19] By microarray printing mixed ratios of solutions of $H_2QC_{11}SH$ (H_2Q) and $HOC_{11}SH$ ($-$OH), varying electroactive SAM compositions can be generated. Cyclic voltammetry (CV) was used to quantitatively follow the oxidation and reduction process on the surface. All electrochemical experiments were performed using a Bioanalytical Systems CV-100 W potentiostat. Cyclic voltammetry (CV) on SAMs was performed in PBS (pH 7.4), using a platinum wire as the counterelectrode, Ag/AgCl as the reference electrode, and the gold/SAM substrate as the working electrode. All cyclic voltammograms were scanned at 50 mV/s.

Cell Culture. Human mesenchymal stem cells (hMSCs), basic medium, growth medium, and differentiation medium were obtained from Lonza. hMSCs were cultured as instructed by the vendor. After cells were washed with PBS and trypsinized for 3$-$5 min, they were centrifuged in serum containing medium and followed with gentle resuspending in serum-free medium. The cells were then seeded onto the substrates containing a ligand density array and then incubated at 37 °C in a humidified atmosphere of 5% CO_2 overnight. Adipogenic differentiation was induced by induction medium and kept by induction/maintenance cycles as described in the Lonza protocol.

Immunohistochemistry. The substrates were washed by PBS and fixed in 4% formaldehyde for 30 min, followed with sterile water and 60% isopropyl alcohol for 2$-$5 min. Samples were then stained by Oil Red O for 5 min followed by Harris Hematoxylin for 1 min.

RT-PCR Analysis. Human mesenchymal stem cells (hMSCs) were induced to adipogenic differentiation for varying durations. Total RNA was then extracted by RNA isolation kits (Qiagen). A 1 μg portion of total RNA was converted to cDNA using AMV reverse transcriptase and random hexamer primers (Promega). The resulting cDNA was used in PCR with the following primer, Lpl (sense 5′-GAGATTTCTCTGTATGGCACC-3′, antisense 5′-CTGCAAATGAGACACTTTCTC-3′), PPARγ2 (sense 5′-GCTGTTATGGGTGAAACTCTG-3′, antisense 5′-ATAAGGTGGAGATGCAGGCTC-3′), β2 mg (sense 5′-ACCCCCACTGAAAAAGATGA-3′, antisense 5′-GCATCTTCAAACCTCCATGAT-3′), at annealing temperatures of 52, 55, and 53 °C, respectively. Amplification

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reactions were carried out for 1 min through 30 cycles, and the reaction products were subjected to 1% agarose gel electrophoresis. The reaction products are 276bp (Lpl), 351bp (PPARγ2), and 116bp (β2 mg), respectively.

Quantification of Adipogenic Differentiation. Quantification by Matlab program is based on measuring the ratio of red pixels to the total number of pixels within the cell pattern. Data from 10 days were used as a frame of reference and normalized to 1.0, which indicates complete differentiation. Data were obtained by this method for various durations (1−10 days) of differentiation to determine the differentiation rate on the ligand density microarray.

Acknowledgment. This work was supported by the Carolina Center for Cancer Nanotechnology Excellence (NCI) and grants from the Burroughs Wellcome Foundation (Interface Career Award) and National Science Foundation (Career Award) to M.N.Y. We also thank Professors Sergei Sheiko, Wenbin Lin, and Rudy Juliano for their comments and suggestions.

Supporting Information Available: Experimental details and data. This material is available free of charge via the Internet at http://pubs.acs.org.

JA907187F