pb96@cornell.edu **Cell: (845) 401 3427**

Prior work: Through my experiences in academia and industry, I have learned to embrace *intellectual freedom* as well as *discipline* as cornerstones of my career. Freedom is necessary for innovation & creativity; and discipline is required to reduce ideas to practice and enable a return on investment. While I acquired discipline during my work at Intel and IBM, creativity is rather one of my assets. I became aware of my ability to innovate while obtaining my B.S. (1993-97) when I was awarded *1st prize at the national level* at the Indian Institute of Technology Kanpur (highest pedigree engineering college in India) on my proposal for "Generation of Electricity from Thunderstorms and Lightning" (1995). This independent self-initiated study began my interest in interdisciplinary projects. I was also recognized for my ability to "think outside the box" when I was awarded second-best "all-rounder" among 1,500 graduating students in the class of 1997. I have capitalized on my creativity and discipline to make the most of it in future.

My journey from heavy engineering industry to cancer research at MD Anderson Cancer Center (MDACC) and The Methodist Hospital Research Institute (TMHRI) evolved over 17 years. Each step was the combination of my *vision* to identify important issues in new fields, my *creativity* to address these issues, and my *passion* for innovation. My fascination with life-sciences started during my M.S. thesis in electrochemistry (1998-2000) when I realized the role that electrochemistry plays in neural signaling in particular and signaling events in general, and as a consequence I developed interest in pursuing graduate studies in biomedical engineering. My PhD thesis at Cornell University (2003-07) was on high resolution patterning of multiple proteins at hydrogel interface on silicon and bioMEMS (biological-micro-electro-mechanical systems) instrumentation. This has applications in regenerative medicine in formation of neuromuscular junctions and stem cell differentiation at osteochondoral junctions. After graduating with a PhD, I joined Intel Corporation (2007-2010) to understand challenges in industrial scale-up and statistical methods that I could apply to product development and biological experiments.

Current research: I am well positioned to accomplish research in the fields of materials science, and nanomedicine and immunology that capitalize on my interdisciplinary skills. In Dr. Laurence Cooper's laboratory at MDACC and in collaboration with Dr. King Li's laboratory, I am using my nano/microfabrication expertise to develop nanomedicine-based immunotherapies. I have initiated and developed collaboration with IBM Research to modify electronic nanomaterials for use as PET/MRI (positron emission tomography/magnetic resonance imaging) multi-modal imaging agents in cell therapy. With the guidance of my mentors, I have also gained experience in writing successful grants.

Long term goals: I look forward to a career in start-ups where I can bring together my interdisciplinary expertise to develop programs in BioMEMS Implants, Image Guided Cell Therapy and Drug Delivery, and collaborate for translation to clinic in oncology and regenerative medicine. I am further interested in business development and in writing collaborative grants to support development of scientific programs in biomedical applications of nano and microtechnologies. My experience in managing projects with teams of engineers, technicians, legal, and human resources has provided me with sensitivity towards diversity and maturity to handle complex interdisciplinary projects.

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SUMMARY

In my joint research position at MD Anderson Cancer Center and The Methodist Hospital Research Institute, my focus is on utilizing nanotechnology, microfluidics, positron emission tomography and magnetic resonance imaging with cell and gene therapy to develop solutions for imaging of therapeutic T-cells in human oncology trials. With my creativity and interdisciplinary expertise I feel passionate about developing the field of nanomedicine in translational research to directly impact patient care.

I bring a unique perspective and statistical knowledge from research and manufacturing in semiconductor industry, which makes me a strong candidate for translation of technologies from laboratories to clinical trials leading to commercialization. My experience in occupational relationships and building successful industry-academia partnership are recent evidence of my leadership qualities.

EDUCATION

Ph.D. Dept. of Biomedical Engineering Cornell University, Ithaca, NY 2007
- *Thesis*: Micropatterned multiplexed biofunctionalized surfaces by integrated microfabrication.
- *Minors*: Materials Science and Engineering; Physiology and Biophysics - Weill Medical College; Orthopedics - Hospital for Special Surgery; Business Studies, Johnson School of Management.
- *Courses*: Mammalian sensory systems, Molecular biology, Molecular/cellular neuroscience, Genetic engineering, Drug delivery.
- Teaching assistant for Spring 2003 and Fall 2004.
- Mentored one graduate and two undergraduate students on research projects.

M.S. Dept. of Metallurgical Engineering University of Utah, Salt Lake City, UT 2001
- *Thesis*: Electrodeposition of Zn-Ni alloy coatings outside conventional electroplating baths.
- *Courses*: Electrochemistry, Colloidal science, Surface analysis, Coating techniques, Alloys.
- Teaching assistant for Fall 1998 and Spring 1999.

B.S. Dept. of Metallurgical Engineering National Institute of Technology Warangal (NITW), India 1997
- *Industry Thesis*: Study of breakouts in continuous casting machines in Vishakapatnam Steel Plant.
- *Courses*: Physical metallurgy, Mechanical metallurgy, Process metallurgy.

WORK

MD Anderson Cancer Center **Dept of Pediatrics** **2010 - current**
The Methodist Hospital Research Institute **Dept of Radiology** **2010 - current**
Houston, TX
Research Engineer / Visiting Scientist *BioMEMS, Nanomedicine, Cell Therapy, Nuclear Imaging*

Image Guided Adoptive Cell Therapy – In this post-doctoral training position I am developing cell/gene therapy for conditional expression of immunoreceptors to manufacture tumor specific T cells and packaging nanoparticles inside these cells for PET/MRI imaging and to deliver a cytolytic payload to the tumor microenvironment. A major emphasis is on translation to clinical application and thus T cells are modified using approaches that can be undertaken in compliance with current good manufacturing practice (cGMP) for Phase I/II trials. I initiated and developed a partnership with IBM Research to modify electronic nanomaterials for use as PET/MRI multi-modal imaging agents in cell therapy and have significant experience in writing successful interdisciplinary grants geared for translational goals.
- *Magnetic and Plasmonic nanoparticles in T Cells:* Developing new methods (chemical and biochemical) to transfect T cells with nanoparticles to be used as contrast agents for *in vivo* imaging and tracking of transplanted therapeutic cells.
- *BioMEMS based microelectroporation device for increased cell transfection efficiency:* Microfluidics principles are utilized for development of disposable device to electro-transfer material into T cells with increased efficiency and low cell death.

Intel Corp., Hillsboro, OR **2007 - 2010**
Senior Engineer *Hard Mask Etch (HME) Group, Logic Technology Development*

Research Initiative (self-starter): Took initiative beyond my current responsibilities and conceptualized BioMEMS applications and consulted for Organic Photovoltaic Cells and Extreme Ultraviolet Lithography groups.

Back End (22 nm transistor node) – As part of the team I developed, qualified and owned RIE processes (6 trench layers), tools (8 RIE chambers: electron cyclotron resonance), and development module infrastructure for intermetallic mask and dielectric (HME) stack etch module. I made extensive use of Six-Sigma Methodology and Statistical Process Controls (SPC) for developing sampling plan in the white papers and implemented statistical methods for process and tool qualification. Final goal was to develop/transfer robust next-gen module to worldwide Intel factories in compliance with Moore's Law.
- *Etch Recipes (ER):* Developed/qualified RIE processes for resistance-capacitance targeting of Cu interconnects.
- *Plasma Clean (PC):* Led the continuous improvement project to develop and qualify PC process to reduce preventative maintenance and eliminate purchase of $5.0 million dollars RIE tool. Managed working group involving 4 process engineers; cross-disciplinary teams in industrial engineering and tool vendor; utilization of statistical tools for process qualification and matching; and determined the impact on production schedules.
- *Tool qualification:* Used statistical tools and trained new engineer with hands-on process and equipment modifications to qualify 4 new RIE chambers for high-volume manufacturing readiness.

- *HME Operations:* Developed WIP management systems for increasing equipment uptime and productivity. This entailed culture shift in work force of 29 operators and 4 shift managers that I accomplished by 'positive reinforcement'. My approach increased human sensitivity towards unprocessed WIP resulting in cycle time reduction from 3.5 to 1.2 hrs in 8 wks and was also adopted by other modules on the production floor.
- *Sustaining activities/floor operations:* Developed statistical sampling plans, qualification criteria, owned specification and created operational procedures/checklists/tool reports, investigated tool breakdowns and supervised repairs on RIE tools. Investigated process shifts and excursions using station/process monitors, inline and end-of-line monitors.

Cornell University, Ithaca, NY **Dept of Biomedical Engineering** **2003 - 2007**
Graduate Research Assistant *BioMEMS, Nanobiotechnology, Biomaterials & Bioinstrumentation*

Integrated BioMEMS – My thesis work involved extensive use of Cornell NanoScale Science & Technology Facility (CNF) & Nanobiotechnology Center (NBTC). Photolithography, electron-beam lithography and plasma etch was integrated with photo-labile protecting groups for bottom-up assembly for patterning DNA, multiple protein and cells. Procedures involved photo-generated acids/bases, nanomaterials, polymer brushes, hydrogel, dendrimer, self-assembled monolayer (SAM), surfactants, catalysts, buffers.
Multiplexed protein patterns (MPP) – Interaction of MPP was studied with mesenchymal stem cells (MSC) for *osteochondoral tissue junction engineering* (Prof. Alan Nixon, Large Animal Surgery, College of Veterinary Medicine). Collaborated with *organic electronics* (Prof. George Malliaras, Mat. Sc. & Eng.) and *nanophotonics* (Prof. Michal Lipson, Elec. & Comp. Eng.) to integrate MPP with microfluidics for label-free biosensing.
Electrospray Deposition (ESD) – ESD device was developed to dispense multiple proteins in non-contact mode for protein microarrays. Taylor-cone formation for liquid stream generation and hydrogel substrate for soft-landing was investigated.
On-chip DNA Sequencing – Miniaturized conventional gel electrophoresis system to microfluidic channels on the chip. This was conceptualized on the limitations of capillary electrophoresis and capitalized on micro-patterned hydrogel in the channels. Additives to reduce evaporation and improve electrophoretic mobility of DNA were investigated.
Anti-epileptic drug development (Univ. of Utah 2002) – Performed site directed mutagenesis to identify residues of UNC-49C in nematode *C. elegans* required for pregnonolone sulfate (PS) sensitivity to mammalian $GABA_A$ receptor.

IBM Microelectronics, Hopewell Junction, NY **2001 - 2002**
Staff Engineer *Reactive Ion Etch Development Group, Semiconductor Research & Development Center*

Logic Front End – Developed high aspect metal contact (MC) etch on LAM (Exelan-HPT) tool in BPSG eliminating SO_2 with in-situ strip. This eliminated downstream Cu corrosion increasing yield by 20%, reduced man-hours by 66% and process time by 25% thus saving $12.0 million per year per tool. In collaboration with Advanced Lithography Materials Group of IBM Research, 193 nm bilayer resist stack was integrated with MC etch process for 90 nm critical dimensions utilizing CH_3F as a component to reduce line-edge roughness. This process was transferred to similar tools in 300 mm fabrication line.
MRAM – Developed and sustained dielectric etch processes for trench and via (M1/V1, M2/V2). Lead process development working in alliance with production and maintenance groups beyond my job responsibilities. An out-of-commission AMAT ICP tool was qualified for MRAM dielectric etches increasing process yield from 10% to 95% saving $2.0 million per year.

IBM TJ Watson Research Center, Yorktown Heights, NY **2000**
Process Engineer (Co-Op) *Electrochemical Laboratory, Magnetoelectronics Group*

MRAM – Developed wet etch for 50 Å magnetic films selective to 10 Å tunneling barrier film and integrated with dry etch of various Ta and Ti based intermetallic masking layers. Integrated stack etch development involved studies on surfactants, buffers, inorganic & long chain carboxylic acids, and role of adsorbed plasma ions as catalyst. Team effort led to the transfer of MRAM technology from research in Yorktown to development in Hopewell Junction.

University of Utah, Salt Lake City, UT **Dept of Metallurgical Engineering** **1998 - 2000**
Graduate Research Assistant *Electrochemistry, Surfaces & Coatings*

Selective Electroplating – Designed portable electroplating equipment for in-situ repair of damaged corrosion protection coatings. Reduced environmental impact and operational cost was achieved by minimizing electrolyte consumption and restricting its flow. Formulation for fast zinc-nickel alloy electrodeposition under high electrical potential and suppressed hydrogen evolution reaction was developed. Ion-transport through membranes under the effect of electric field was investigated.

Other Experience **1995 - 1998**

Samtel Color Ltd., India (Color Picture Tube Industry): Revised/maintained module specifications in compliance with ISO 9001. Self-motivated for visual defect analysis of photolithographically patterned screen coating. Designed and developed single implosion protection device for four products, simplifying the handling process by 75% and saving $137,000 per year on material cost. 1997
Star Wire (India) Ltd. (Mini Steel Plant): Developed production planning and control systems for electric arc furnace, induction furnace, rolling mills, heat treatment, foundry, and quality control impacting the production of $2.0 million per year. 1998
Visakhapatnam Steel Plant, India (Integrated Steel Plant): Developed training guide for incoming engineers in continuous casting metal breakout causes. (6 months) 1996
Uttar Pradesh Steels, India (Mini Steel Plant): Electric arc furnace steel making (7 wks intern) 1996
ISGEC (Large Pressure Vessels): Welding (9 weeks); Foundry (4 wks intern) 1995

ADVISORY ROLES

- *Intel Research (2009 – 2010)*: Self-assembly processes for Extreme Ultraviolet Lithography & Organic Photovoltaics.
- *ScientiaAdvisors (May 2009)*: Processes and materials challenges for diagnostics/assay companies in bringing BioMEMS/Microfluidics based products from research to manufacturing.
- *Johnson Graduate School of Management, Cornell University (Jan 2006)*: Role of nanotechnology (nanostructured materials) to reduce automobile manufacturing cost. This was performed for Prof. Wesley Sine and was instrumental in attracting the interest of Mr. Ratan Tata (Chairman, Tata Sons Ltd., India) for producing $2500 automobile for Indian market.

PROFESSIONAL DEVELOPMENT TRAINING

- MD Anderson Cancer Center, 2011: Conflict theory & management
- Intel Corp., 2007-08: Statistical process control, Design of experiments
- Johnson Graduate School of Management, Cornell University, 2006: Innovation & technology
- IBM Microelectronics, 2001: Fundamentals and principles of plasma processes
- Samtel Color Ltd, 1997: Personal and inter-personal skills, Statistical process & quality control

AWARDS

2011 – NSF Fellowship – "Cancer Nanotechnology: analysis, imaging and treatment over multiple scales" (Jun 7-9), Houston, TX.
2003 - 2004 – Cornell NanoBiotechnology Center Fellow.
2002 – Achieved IBM First Invention Plateau.
1997 – Second Best All-rounder Student – among 1500 graduating students of RECW, India.
1995 – Most Innovative Idea – national level at Indian Institute of Technology (IIT), Kanpur, India.
1995 – Gold Medal – national level at Institution of Engineers (India).

RESEARCH GRANTS

Oct 2011 - Sep 2013 – Center for Transport OncoPhysics; Physical Science-Oncology Centers
- Title: Enhancing anti-cancer immunotherapy by combining Gene Research and Nano-Technology (Co-PI)

PRESENT & PAST AFFILIATIONS

American Association of Cancer Research, Biomedical Engineering Society, Materials Research Society, Electrochemical Society.

SKILLS

- ***BioMEMS/lab-on-a-chip*:** biomaterial/hydrogel, protein/DNA/nanostructure functionalization, surface polymerization/organic transformations, molecular self-assembly, microfluidics, surfactants/colloids, fluorescence microscopy
- ***Microfabrication*:** CAD (L-Edit), photolithography & photochemistry, deep reactive ion etching (RIE), thin-film deposition: plasma vapor deposition (PVD), chemical vapor deposition (CVD), electroplating & electrochemistry, vacuum & wet processes, SEM, AFM, statistical methods (JMP)
- ***Gene Therapy*:** mammalian cell culture, genetic engineering, polymerase chain reaction (PCR) amplification, restriction endonuclease, DNA ligations, DNA harvesting, bacterial cloning, site-directed mutagenesis, electroporation

PATENTS & DISCLOSURES

1) Bhatnagar, P.; Cooper, L.J.N.; Sly, J,; Miller, R. Image-Guided Adoptive T Cell Therapy Using Multi-Modal Contrast Agents. Disclosure# MDA11-134 (Aug 29, 2011).
2) Bhatnagar, P.; Cooper, L.J.N. (MDACC, USA). De novo magnetic iron nanoparticle synthesis in T Cells. Disclosure# MDA10-100 (Jun 7, 2010).
3) Bhatnagar, P.; Cooper, L.J.N. (MDACC, USA). BioMEMS based microelectroporation device for increased cell transfection efficiency. Disclosure# MDA10-093 (May 11, 2010).
4) Bhatnagar, P.; Craighead, H.G. (Cornell University, USA). Multiplexed electrospray deposition apparatus. U.S. Pat. Appl. Publ. (2009), US 20090317558.
5) Bhatnagar, P.; Limketkai, B (Intel Corp., USA). Conductive transparent electrode using molecular self-assembly for low-cost organic photovoltaic cells. Disclosure# 78772 (Dec 17, 2009).
6) Bhatnagar, P. (Intel Corp., USA). Modified resist stack and tool configuration for low photon count in EUV source. Disclosure# 72713 (complete title confidential). (Dec 1, 2008).
7) Bhatnagar, P. (Intel Corp., USA). Photolithographic patterning with increased resolution using self-assembled monolayer. Disclosure# 70879 (Aug 12, 2008).
8) Bhatnagar, P.; America, W.G.; O'Sullivan, E.J.; Wise, R.S. (IBM Corp., USA). Method for improving etch selectivity effects in dual damascene processing for semiconductor interconnect. U.S. Pat. Appl. Publ. (2004), US 2004175934.
9) Bhatnagar, P. (IBM Corp., USA). Apparatus for electroplating alloy films. U.S. Pat. Appl. Publ. (2002), US 6482298.
10) Bhatnagar, P. (IBM Corp., USA). Method and apparatus for electroplating alloy films. U.S. Pat. Appl. Publ. (2002), US 6344124.
11) Bhatnagar, P. (IBM Corp., USA). Method and apparatus for electroplating alloy films. U.S. Pat. Appl. Publ. (2002), US 6344123.

PUBLICATIONS

1) P. Bhatnagar, G.G. Malliaras, I. Kim, C.A. Batt. Multiplexed protein patterns on photosensitive hydrophilic polymer matrix. *Advanced Materials*, 22 (11), 1242-1246 (2010).
2) P. Bhatnagar. Multiplexed electrospray deposition for protein microarray with micromachined silicon device. *Applied Physics Letters*, 91, 014102 (2007).
3) P. Bhatnagar, S.S. Mark, I. Kim, H. Chen, B. Schmidt, M. Lipson, C.A. Batt. Dendrimer-scaffold-based electron-beam patterning of biomolecules. *Advanced Materials*, 18 (3), 315-319 (2006).
4) P. Bhatnagar, A. Nixon, I. Kim, J. Kameoka. Protein functionalized micro hydrogel features for cell-surface interaction. *Biomedical Microdevices*, 10, 567-571 (2008).
5) P. Bhatnagar, A.D. Strickland, I. Kim, G.G. Malliaras, C.A. Batt. Integrated reactive ion etching to pattern cross-linked hydrophilic polymer structures for protein immobilization. *Applied Physics Letters*, 90, 144107 (2007).
6) P. Bhatnagar, S. Panda, N. Edleman, S. Allen, A. Mahorowala, R. Wise. Controlling line-edge-roughness and reactive ion etch lag in sub-150 nm features in borophosphosilicate glass. *Journal of Applied Physics*, 101, 076102 (2007).
7) P. Bhatnagar, S. Panda, N. Edleman, S. Allen, A. Mahorowala, R. Wise. Integrated non-SO_2 underlayer and improved line-edge-roughness dielectric etch process using 193 nm bilayer resist. *Applied Physics Letters*, 88, 231501 (2006).
8) S.S. Mark, M. Bergkvist, X. Yang, L.M. Teixeira, P. Bhatnagar, E.R. Angert, C.A. Batt. Bionanofabrication of metallic and semiconductor nanoparticle arrays using S-layers protein lattices with different lateral spacings and geometries. *Langmuir*, 22 (8), 3763-3774 (2006).
9) S.S. Mark, M. Bergkvist, P. Bhatnagar, C. Welch, A.L. Goodyear, X. Yang, E.R. Angert and C.A. Batt. Thin film processing using S-layer proteins: biotemplated assembly of colloidal gold etch masks on chemically modified silicon surfaces for fabrication of semiconductor nanopillar arrays. *Colloids and Surfaces B: Biointerfaces*, 57 (2), 161-173 (2007).
10) P. Bhatnagar, M.L. Free. Selective electrodeposition of zinc-nickel alloy through porous medium. *Surface and Coatings Technology*, 200 (20-21), 6083-6087 (2006).

Conferences:

11) P. Bhatnagar et al. Image Guided T-Cell Therapy Using Nanoparticles Complexed With PET Imaging Probes. *Gordon Research Conference on Cancer Nanotechnology, Waterville, ME, USA, July 17-22, 2011*. [Poster]
12) P. Bhatnagar. Generation of electricity from thunderstorms and lightning. *Product of Future, Techkriti '95, IIT Kanpur, India, April 7-9, 1995*. [Poster + Report]
13) P. Bhatnagar et al. Immobilization of multiple proteins on photosensitive hydrogel surface. *Materials Research Society; MRS Spring 2007, San Francisco, CA, USA, April 9-13, 2007*. [Talk]
14) P. Bhatnagar et al. E-beam directed layer-by-layer assembly of dendrimer scaffold for biomolecule patterning. *Materials Research Society Proceedings Vol. 921; MRS Spring 2006, San Francisco, CA, USA, April 17-21, 2006*. [Talk]
15) P. Bhatnagar. Superconductors – design, present and future applications. *Composit '96, IIT Kharagpur, India. January 14, 1996*. [Talk]
16) P. Bhatnagar. Growth of powder metallurgy for economic development. *The Institution of Engineers (India), Students' Chapter Newsletter. July 1996, 1-4, 1996*. [Talk]

OTHER INTERESTS

Bonsai, Outdoors.

ADVANCED MATERIALS
www.advmat.de

www.MaterialsViews.com

Multiplexed Protein Patterns on a Photosensitive Hydrophilic Polymer Matrix

By *Parijat Bhatnagar,* George G. Malliaras, Il Kim,* and *Carl A. Batt**

Multiplexed functional proteins immobilized on microfabricated sensors and surfaces[1–3] have found applications in high-throughput screening[4] of drug molecules,[5] early disease detection,[6] organ printing, and complex tissue engineering.[7–10] Complex biological integrated patterns emulating physiological microenvironments have been used to engineer tissue junctions from stem cells by selective differentiation[2,11] and study interaction with the extracellular matrix (ECM).[11–13] Parallel developments in lab-on-a-chip (LOC) platform technologies have been identified for label-free biosensing[14] with faster analysis using less reagent and analyte volumes.[15–17] If LOC technology is to take advantage of the developments in the semiconductor industry,[16] efforts are needed to create biologically friendly microfabrication processes to allow integration of microelectronic circuitry with protein patterns.[17–19] Currently used methods for multiplexed protein patterns include soft-lithography,[1,20] inkjet printing,[21] and dip-pen nanolithography.[22] However, none of these have been integrated with complementary metal–oxide–semiconductor (CMOS) processing for high-volume manufacturing.[23] Soft-lithography and inkjet printing have proven to be versatile for protein patterning, however, resolution and hence alignment of the protein patterns with pre-existing features remains a challenge.[1,19] Dip-pen nanolithography, an analogue of scanning probe microscopy, can achieve high resolution but is extremely slow and has not been adopted by industry. Here we demonstrate a photolithographic process[24,25] on hydrogel-based biomaterial[26] for patterning three different types of proteins. The technique is scalable and capable of patterning a multitude of proteins aligned with respect to each other and surface microstructures. UV light (365 nm), benign to proteins and DNA,[27,28] was used. This strategy allowed us to integrate harsh upstream CMOS processing involving extreme pH, vacuum processes, and organic solvents, with downstream aqueous biomolecular processing at neutral pH.

We have earlier demonstrated methods to array single oligonucleotides or proteins.[29,30] Lift-off-based photolithography[2] and oxygen-plasma-etch-based patterning of two proteins[31] has also been demonstrated and is capable of scaling up to more proteins, but due to the subtractive nature of these processes none can be adopted with multiple layers in 3D.[8,9] Bochet et al. have described solution-based photochemistry of orthogonal photolysis of inter- and intramolecular acid groups using two different photolabile protecting groups (PLPGs) with differential sensitivity to 254-nm and 420-nm UV light.[25] This was further developed by Campo et al. who illustrated photopatterning to create chemically diverse areas for patterning colloidal particles and different biomolecules.[24] Photogenerated functional groups have also been used for solid-phase synthesis of multiplexed gene chips[32] and peptide chips,[33] which utilizes synthetic nucleotides or amino acid residues, respectively, protected by PLPGs.

Photochemical immobilization strategies can be categorized into two groups: photocatalyzed reactions and photodeprotection of reactive groups.[34] The former involves a single-step reaction by creating short-lived reactive groups on the surface by photoexposure. Although advantageous in facilitating a single-step reaction, this technique cannot be integrated with semiconductor industry equipment because it requires the substrate to be present in a liquid environment inside the photolithographic equipment. Due to the aforementioned limitations we resorted to a photodeprotection strategy to generate either an amine- (photogenerated base, PGB) or a carboxylic-acid- (photogenerated acid, PGA) functionalized surface[24,25] followed by subsequent immobilization of proteins.[35]

Cr microstructures, which serve as alignment marks in downstream protein patterning, were first patterned on a wafer using electron-beam evaporation of Cr, standard projection photolithography, and subtractive wet-etching of Cr. A self-assembled monolayer (SAM) of [3-(methacryloyloxy)propyl]-trimethoxysilane with a polymerizable terminal group was formed on the wafer surface from solution-phase (MOP-SAM).[30] A functional-group-containing monomer (FGM) (amine or protected carboxylic acid) was then polymerized with a thin film of acrylamide (AAm)–methylenebisacrylamide (Bis) copolymer [poly(AAm–Bis–FGM)][30] (Figure 1). 2-Nitrobenzyl succinimidyl carbonate (NBSC), a PLPG adduct prepared as described elsewhere,[36] was subsequently used to protect surface amine groups as 2-nitrobenzyl-derived carbamate (Scheme 1). In the case of 2-nitrobenzyl-derived ester groups (that yield surface

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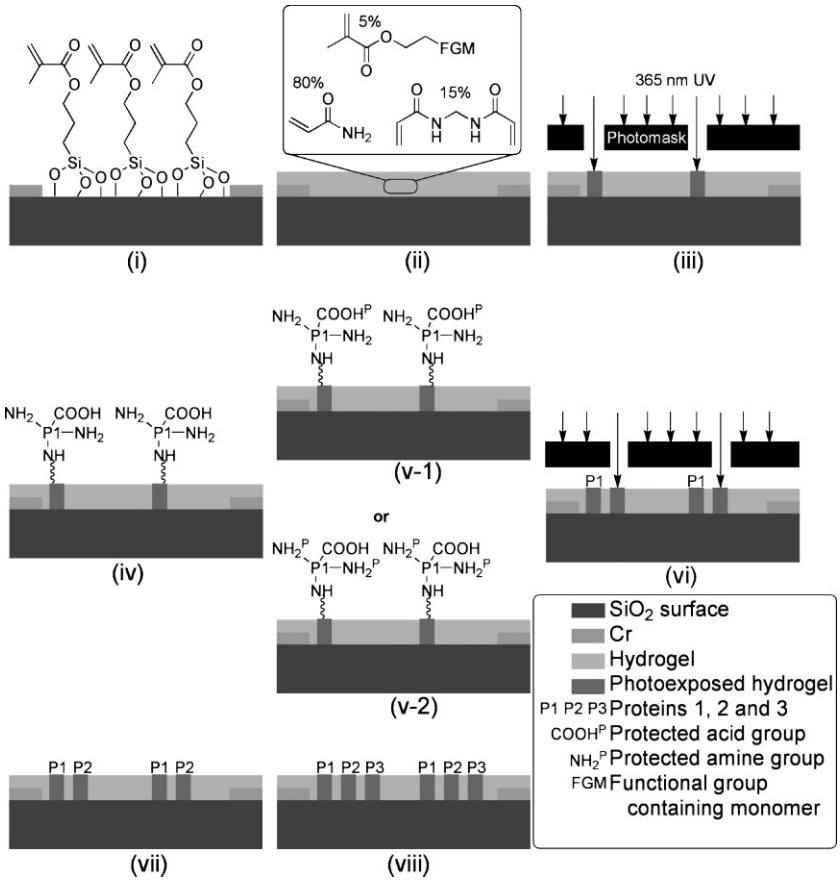


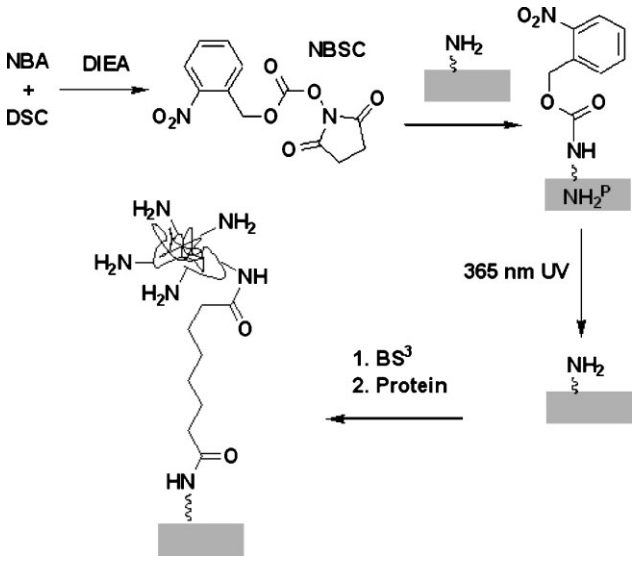
Figure 1. Fabrication of multiplexed protein patterns on a photosensitive surface. i) Electron-beam evaporation of Cr, projection photolithography (spin, 365-nm UV exposure, develop), subtractive wet etch of exposed Cr, MOP-SAM formation on SiO_2 surface; ii) formation of crosslinked polymer thin film containing FGM; iii) 365-nm UV exposure of selective areas, aligned to Cr patterns, on polymer thin film to cleave PLPG resulting into PGB or PGA; iv) use of Scheme 1 (for PGB) or Scheme 2 (for PGA) for covalent immobilization of proteins P1 on the exposed surface; v) chemical protection of functional groups on proteins P1 ((v-1) amine group protection for PGB based protocol and (v-2) acid group protection for PGA based protocol); vi) 365-nm UV exposure of selective areas on polymer thin film resulting into PGB or PGA; vii) immobilization of proteins P2 on newly exposed areas. Steps (vi–vii) are repetitions of steps (iii–v) for immobilization of second protein P2. viii) Steps (iii–v) are again repeated for immobilization of third protein P3. All three proteins are aligned to Cr patterns and hence also to each other.

carboxylic acid groups upon photoexposure), the monomer itself contained acid groups protected as 2-nitrobenzyl-derived esters.

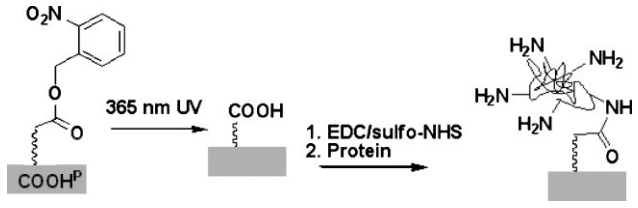
The photosensitive hydrogel surface was then exposed in selective regions to 365-nm UV light (730 mW cm^{-2} intensity) to create spatial patterns of PGB (Scheme 1) or PGA (Scheme 2) as a result of photochemical cleavage of 2-nitrobenzyl-derived carbamate or 2-nitrobenzyl-derived ester, respectively. Proteins were covalently immobilized on the PGB or PGA surface through stable amide linkages with primary amines available on lysine residues of protein molecules using bis(sulfosuccinimidyl)suberate (BS^3) (Scheme 1) or carbodiimide chemistry (Scheme 2), respectively.[35] After protecting the functional groups on the immobilized protein molecules (amine-group protection for PGB-based protocol and acid-group protection for PGA-based protocol), the process was sequentially repeated using different photomasks with a previously patterned Cr alignment mark to create a multiplexed protein surface. It was necessary to wash the surface with mildly acidic (pH 4) sodium citrate buffer for 3 h after every protein immobilization step to promote positive charges on nonspecifically adhered protein molecules, resulting in their electrostatic repulsion from the aminated surface.

Our initial efforts used photogenerated surface amine groups for protein immobilization (Scheme 1). Figure 2 shows an early effort to immobilize IgG antibodies from rat, rabbit, and mouse separately on individual chips with PGB, however, nonspecific adhesion was a challenge. It has been reported that photocleavage of 2-nitrobenzyl protecting groups to generate amine groups is accompanied by generation of an aldehyde-based side product (2-nitrosobenzaldehyde) that can react with primary amines to form imines,[24,27,37,38] thus rendering photogenerated primary amine groups unavailable for further conjugation. Semicarbazide hydrochloride has been used as an aldehyde scavenger in solution,[24,27,38] but due to CMOS equipment compatibility issues we were unable to use any solution-phase protocol during photoexposure. This led us to explore photogenerated surface acid groups[24,25] (Scheme 2). We believed that this approach would be a better choice since the photochemistry of this system is similar to the widely used *i*-line photoresists in the semiconductor industry, where acid functional groups are photogenerated by exposure to 365-nm UV light.

Carbodiimide chemistry was then utilized for surface immobilization of each protein on PGA regions (Scheme 2). Carboxylic groups on attached proteins were transformed to primary hydroxyl groups using carbodiimide chemistry.[35] This step prevented a chemical reaction between primary amines of subsequent proteins with carboxylic acid groups of proteins already immobilized on surface. Rat, rabbit and mouse IgG were immobilized sequentially as per Scheme 2. Proteins were detected using fluorescently labeled probe antibodies on a single chip and are shown in Figure 3a–c. Figure 3d shows an overlay image of the three images. Considerable nonspecific adhesion was still observed, which did not reduce after treatment with acidic or alkaline buffers. However, nonspecific physical adhesion is also an inherent property of IgG antibodies[39] and in fact this property of IgG molecules is utilized to block surfaces in biological assays (e.g., western blots) and eliminate nonspecific adhesion of protein-specific IgG molecules in subsequent steps. We argued that this property of IgG may have resulted in our observation of background fluorescence from nonspecific adhesion and may not interfere when we immobilize other proteins of interest. We might also be able to prevent nonspecific adhesion of labeled probe antibodies towards the protein of interest by blocking the



Scheme 1. Immobilization of proteins on surface using PGB.



Scheme 2. Immobilization of proteins on surface using PGA.

hydrogel surface using buffers containing unlabeled IgG molecules.

To validate if the photosensitive hydrogel upon selective exposure could be used as a generic platform to array non-IgG proteins without interference from nonspecific adhesion, we repeated the procedure using fibronectin protein (from bovine plasma) (Fig. 3e). Although we are unable to explain incomplete fluorescence in 100-μm patterns, we could faithfully reproduce 5-μm protein patterns over the entire 100-mm wafer from 25-μm projection mask patterns (5:1 pattern reduction) with minimal background noise due to nonspecific physical adhesion.

In summary, we have developed an additive method based on photolithography to pattern multiple proteins in alignment with each other and with surface microstructures at 5-μm resolution. This method can seamlessly incorporate biomolecular patterning with new advancements in high-resolution photolithography and materials processing for next generation integrated circuits. Further applications include creation of microenvironments with multiple differentiation signals including growth factors and ECM proteins confined to certain parts of the cell population,[40] and engineering vascularized tissues from undifferentiated stem cells.[10,11,13] Due to its additive nature, this methodology can also be integrated with rapid-prototyping methods[8,41] for 3D tissue or organ scaffolds.

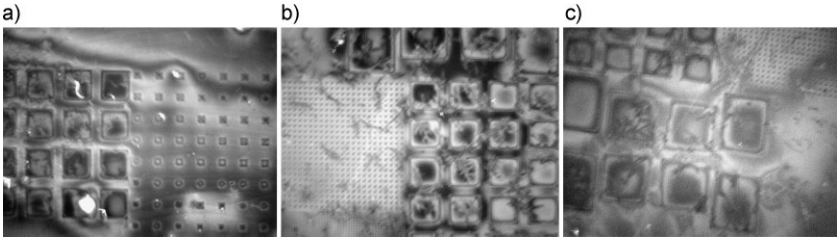


Figure 2. PGB groups (Scheme 1) were used to immobilize a) rat IgG, b) rabbit IgG, and c) mouse IgG.

Experimental

Surface Polymerization of the Poly(AAm–Bis–FGM) Hydrogel Film: MOP-SAM was formed on the silicon oxide surface followed by the poly(AAm–Bis–FGM) layer [30]. AAm (80%), Bis (15%), and FGM (5%) (w/w) were dissolved in a suitable solvent for the total monomer content to be 20% (w/v). The solution was then diluted 1:1 with 80% glycerol (v/v). Free radicals were generated by addition of 25% (w/v) ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) (Bio-Rad Laboratories, Hercules, CA). This solution (0.35 mL) was dispensed on 100-mm-diameter wafer, covered with a sacrificial 100-mm wafer, and polymerized for 4 h. It was then soaked in tris buffered saline (TBS) with 0.5% Triton X-100 (TBST) solution overnight. The sacrificial wafer was lifted-off mechanically by hand to obtain the thin hydrogel film attached to the MOP-SAM-functionalized wafer.

Surface Polymerization with Protected Amine Groups in the Hydrogel Matrix: 2-Aminoethyl methacrylate hydrochloride (Polysciences, Inc., Warrington, PA) was used as the FGM in water. APS and TEMED at 0.004 mL each per mL of gel precursor solution were used. The wafer containing surface amine groups was treated with NBSC [36] overnight in the dark.

Surface Polymerization with Protected Acid Groups in the Hydrogel Matrix: 2-Nitrobenzyl methacrylate (Polysciences, Inc., Warrington, PA) was used as the FGM in dimethyl sulfoxide (DMSO). APS and TEMED at 0.04 mL each per ml of gel precursor solution were employed. The higher initiator amount was required due to the free-radical scavenging activity of nitro-aromatic groups [42].

Protein Immobilization on Photogenerated Amines: Wafer with PGB was treated with BS^3 (30 mM) in phosphate buffered saline (PBS) for 30 min. After rinsing the surface with PBS, wafer was then treated for 3 h with protein solution (1 mg mL^{-1} in PBST (PBS + 0.5% Triton X-100), pH 7.3).

Protein Immobilization on Photogenerated Acids: Wafer with PGA was treated with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) (0.15 M) and *N*-hydroxysulfosuccinimide (sulfo-NHS) (0.03 M) dissolved in 2-[morpholino]ethanesulfonic acid (MES) buffer (pH 4.7) (0.1 M) for 30 min. The wafer was then treated for 3 h with protein solution (1 mg mL^{-1} in PBST).

Chemical Protection of Carboxylic Acid Groups on Surface-Immobilized Proteins: Wafer with surface- immobilized proteins was treated with EDC (0.3 M) and ethanolamine hydrochloride (0.5 M) in MES buffer (pH 4.7) (0.1 M) for 30 min.

Detection of Immobilized Proteins: Immobilized IgG antibodies were treated with ELISA grade bovine albumin serum (BSA) (Product #A7030, Sigma-Aldrich, St. Louis, MO) (40 mg mL^{-1} in TBST) and then with a cocktail solution of anti-rat, anti-rabbit, and anti-mouse IgG probe antibodies labeled with Alexa 647, Alexa 546, and Alexa 488, respectively (Invitrogen, Carlsbad, CA) (0.004 mg mL^{-1} each in TBST).

The surface with immobilized bovine fibronectin was treated with BSA as above and then treated with rabbit anti-fibronectin antibody (0.004 mg mL^{-1} in

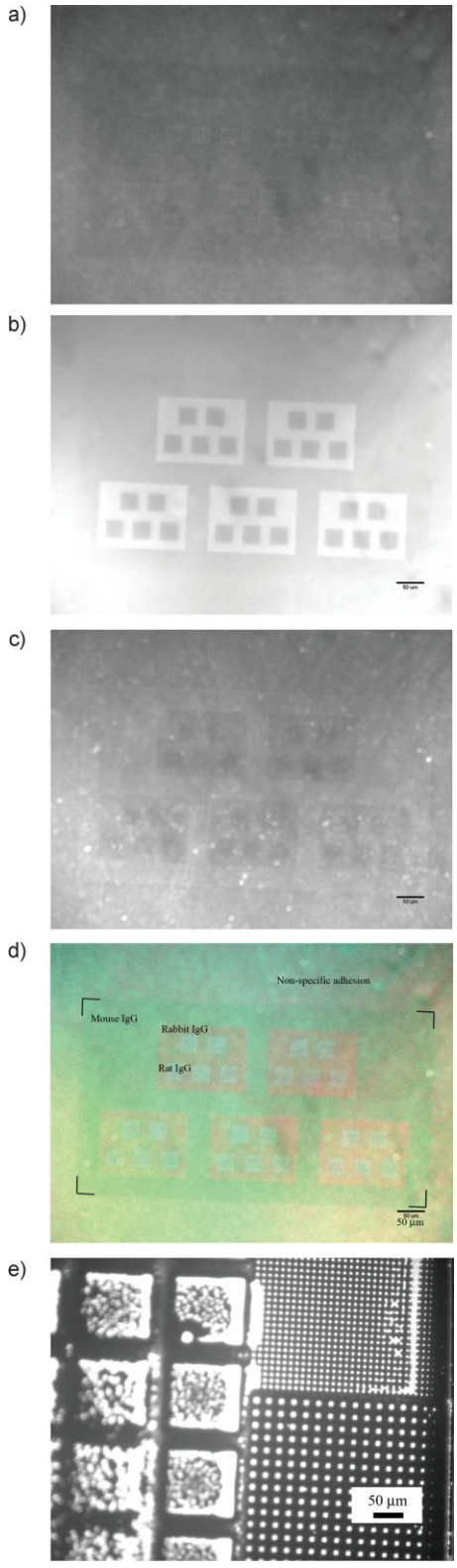


Figure 3. PGA groups (Scheme 2) were used to immobilize a) rat IgG, b) rabbit IgG, and c) mouse IgG. d) An overlay of (a–c) with a specific color attributed to each panel. Rat IgG: blue; rabbit IgG: red; mouse IgG: green. e) Bovine fibronectin immobilized on PGA groups (Scheme 2). Scale bar: 50 μm in all panels.

TBST). Finally, it was treated with anti-rabbit IgG probe antibodies labeled with Alexa 488 (0.004 mg mL^{-1} in TBST). Each treatment was 1 h.

An Olympus BX50 upright epifluorescence microscope (Olympus America, Inc., Center Valley, PA) with a Retiga Exi cooled monochrome CCD camera (QImaging, Surrey, BC, Canada) and a Metamorph V6.1 (Universal Imaging Corp., Molecular Devices Corp., Downington, PA) was used for imaging.

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Multiplexed electrospray deposition for protein microarray with micromachined silicon device

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Multiplexed electrospray deposition device capable of delivering picoliter volumes made by silicon micromachining technology has been developed as a deposition tool for making protein microarrays in a noncontact mode. Upon application of potential difference in the range of 7–9 kV, biomolecules dissolved in suitable buffer with nonionic surfactant and loaded on the electrospray tips were dispensed on the substrate with microfabricated hydrogel features (1–10 μm) in cone-jet mode. Schiff base chemistry followed by reductive amination was utilized for covalent immobilization. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2754642]

Lab-on-a-chip technologies have received considerable attention in the past few years where an integrated effort has been put forth by life science and physical science community to create functional platforms for cell-surface interactions,[1] early disease detection,[2] personalized medicine,[3] drug discovery,[4] and single molecule analysis.[5] One attractive aspect of this technology is in utilizing minimal volumes of the biological molecules and reagents which leads to higher throughput, sensitivity, and speed.[5,6] Silicon micromachining technology has been identified as one of the pillars[6] in the foundation of this technology that provides a capability to miniaturize through microfabrication of planar devices[5] with precision and reproducibility. Electrospray deposition (ESD) has lately emerged as one of the techniques to array proteins in noncontact mode[7–9] and allow generation of small volumes on the order of picoliters. Capillaries[7] and electrically collimating dielectric mask[8] have been used to make protein arrays using ESD process. However, these could not be integrated in planar microfabrication schemes of the lab-on-a-chip and introduces an added complexity of aligning the ESD tool to the collimating mask further aligned to the grounded surface features. Furthermore, to provide multiplexing capability to the ESD device,[10] it is important that multiple source tips of ESD be equidistant from the surface, a precision that can be achieved by micromachining. A slight misalignment of the different source tips in ESD device can cause the failure of all but one with the closest ground surface proximity. Here, we demonstrate such capability of micromachined silicon devices with four parallel ESD source tips. Multiple biological molecules were dispensed using multiplexed ESD device (MESDD) on a substrate with microhydrogel features (μhfs) to obtain micropatterns of immobilized biomolecules and the technique can be integrated into lab-on-a-chip technologies.

Standard integrated microfabrication was used for ESD device[11] and has been described in Fig. 1(a). Double sided polished silicon wafers with 2 μm thermal oxide (Silicon Quest International, Inc., Santa Clara, CA) were coated with 5 μm photoresist and exposed with a MESDD design mask using contact photolithography.[12] The resulting photoresist patterned wafer was subjected to CHF_3–O_2 based reactive ion etch process[12] to etch 2 μm silicon oxide, which was then used as a hard mask for deep silicon etching using the licensed Bosch process.[12] Finally, the silicon oxide hard mask was stripped using hydrofluoric acid[12] to obtain silicon MESDD. Figure 1(b) shows the design of the MESDD. Only one sharp edge was included for each tip in the MESDD design, as shown in the magnified view, to prevent charge accumulation at the corners and thus avoid multiple flows and corona discharge from single tip during MESDD operation.[13] The micromachined silicon MESDD was then claimed out of wafer by breaking the rest of the wafer and functionalized with self-assembled monolayer (SAM) of [methoxy(polyethyleneoxy)propyl] trimethoxysilane (Gelest, Inc., Morrisville, PA) (6–9 polyethylene glycol units).[14] This was done to prevent the nonspecific adhesion of proteins when loaded on the silicon surface of MESDD.

In a separate procedure, μhf was patterned on a silicon oxide surface and has been shown in Fig. 2. Briefly, a pho-

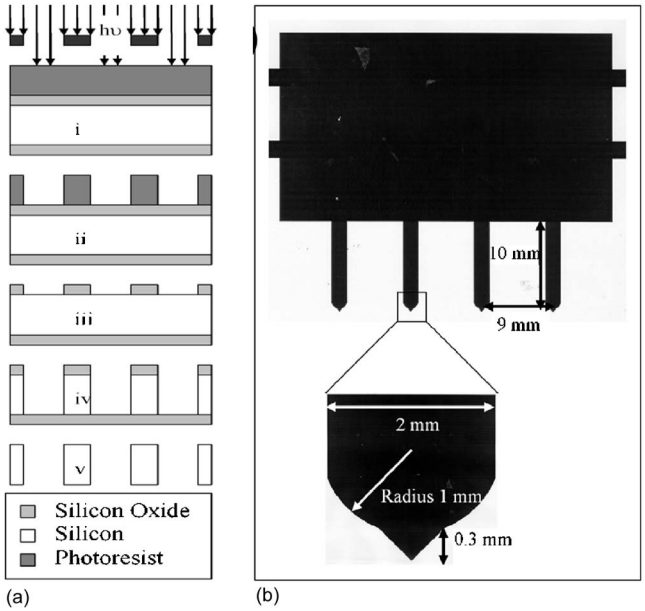

FIG. 1. (a) Microfabrication scheme for MESDD: (i) photoexposure (ii) photoresist development, (iii) CH_3F based reactive ion etching of SiO_2 hard mask, (iv) Bosch process for deep silicon etch, (v) hydrofluoric acid wet etch for removal of SiO_2 hard mask and back film; (b) Mask design for MESDD.

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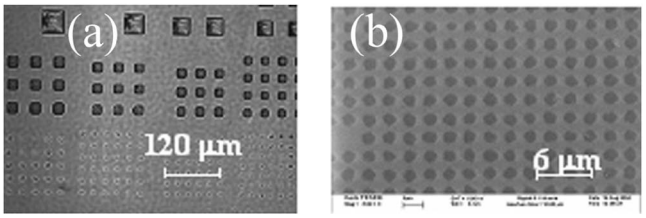


FIG. 2. (a) Microhydrogel features of different sizes; (b) SEM image of 1 μm hydrogel features.

tolithographically patterned parylene C polymer was etched in oxygen plasma.[9,15] The surface was functionalized with SAM of methacryloxypropyl(trimethoxysilane) (Gelest, Inc., Morrisville, PA) to covalently bind acrylamide [5% total monomer, 5% methylenebisacrylamide, in 0.01*M* phosphate buffer saline (PBS) and 40% glycerol] based hydrogel that was polymerized on this surface. Parylene C was then mechanically lifted to pattern μhf. The μhf was subsequently functionalized with aldehyde groups to covalently bind to the primary amine groups on the biomolecules.[16] Briefly, the μhf substrate was treated with aqueous $NaIO_4$ (20 min) and then with 5% glutaraldehyde in PBS (48 h).

Figure 3(a) shows MESDD mounted in operational configuration. Figure 3(b) shows the schematic of the circuit involved in the MESDD operation. Protein A (10 μl, 5 mg/ml) and biotin (10 μl, 2 mg/ml) solutions in PBS with 0.5% Triton X-100 were loaded by micropipette on the alternate tips of MESDD. Parallel streams of solutions with respective biological reagents were observed from all four tips in a cone-jet mode[7,13] when the potential difference at positive polarity in the range of +7 to +9 kV (Series EH, Glassman High Voltage, Inc., High Bridge, NJ) was applied across MESDD and grounded μhf substrate.[17] The addition of nonionic surfactant (Triton X-100) was essential in order to obtain the flow in a cone-jet mode.[7,13] This was also helpful in eliminating the nonspecific adhesion of the proteins on the substrate in the silicon oxide area available in between the μhf.[18] The surface with deposited proteins was incubated for 3 h at room temperature and treated with aqueous 0.1*M* $NaBH_4$ (20 min) for reductive amination of Schiff bases that formed between primary amine groups of protein A or biotin and aldehyde functionalized μhf.[16]

The μhf substrate with protein A and biotin immobilized through resulting amide bonds was then treated with Starting Block T20 (PBS) Blocking Buffer (1 h) (Pierce Biotechnology, Inc., Rockford, IL) and probed with a solution containing Alexa-488 labeled rabbit antimouse IgG (4 μg/ml) and Alexa-594 labeled streptavidin (4 μg/ml) (Molecular Probes, Invitrogen, Inc., Carlsbad, CA) in PBS with 0.5% Triton X-100 (1 h). Figure 4 show epifluorescence detection (water immersion objective, model AX70, Olympus America, Inc., Center Valley, PA) of immobilized protein A and biotin on different areas of the same chip. In the absence of blocking step [supplemental information, Scheme 1(a) and (b)], a nonspecific adhesion of probe molecules was observed on silicon oxide areas. As a control to determine that biomolecules only bind to the μhf through covalent amide bonds and not nonspecifically, protein A was also deposited on μhf substrate that was not functionalized with aldehyde groups [Scheme 1(a)]. The results indicate that protein A does not bind to nonfunctionalized μhf [Scheme 1(a)] but only to aldehyde functionalized μhf [Scheme 1(a)]. Scheme 1 also shows that when protein A is not present on the μhf substrate, the probe molecules do not bind to the hydrogel features.

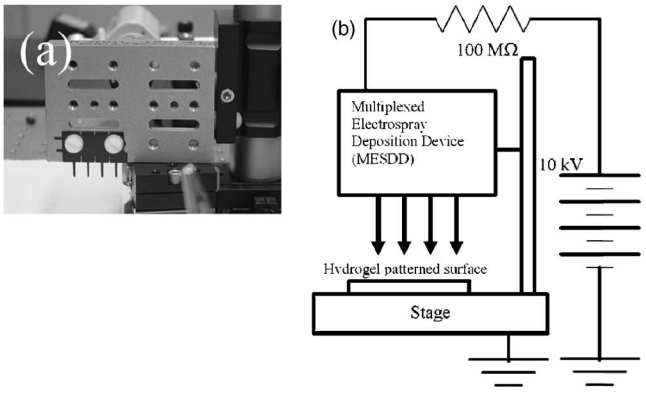


FIG. 3. (a) MESDD mounted in the operational configuration; (b) Circuit diagram of the electrospray protein dispensing unit.

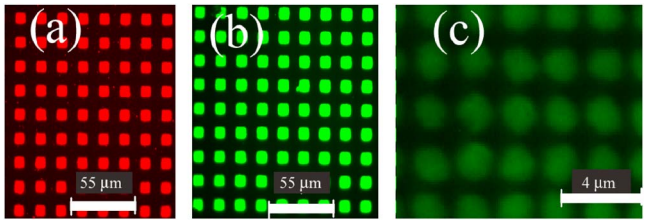
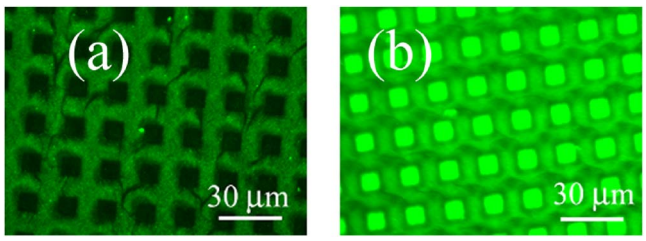


FIG. 4. Multiple biomolecules electrosprayed through alternate and parallel source tips of MESDD on a single μhf substrate. (a) Biotin on 8 μm hydrogel features; (b) Protein A on 8 μm hydrogel features; (c) Protein A on 1 μm hydrogel features. Biotin and Protein A were detected by a solution of streptavidin—Alexa 594 and rabbit antimouse IgG—Alexa 488 conjugates, respectively.

Lab-on-a-chip-type technologies rely heavily on micromachining techniques and miniaturization strategies.[6] While microfluidic approaches contribute toward planar devices designed for consuming lower analyte volumes, a complementary effort toward delivering smaller volumes would exponentially improve the capability of the integrated devices.[4–6] Providing a multiplexed capability using MESDD-type devices as demonstrated here would further enhance the throughput of lab-on-a-chip.[19] Interfacing the macroenvironment with on-chip microfluidic network has also been identified as a major challenge in the microfluidics.[1] Our approach may provide a solution for this by replacing pumps, which are still comparatively large, with on-chip reservoir



SCHEME 1. Fluorescently (Alexa 488) labeled rabbit anti-mouse IgG bound non-specifically to the silicon oxide background but not to the hydrogel. (a) Protein A did not bind non-specifically to hydrogel features that were not functionalized with aldehyde groups. (b) When Protein A was dispensed on the hydrogel features with aldehyde functionalization, the Protein A molecules bound through stable amide bonds as a result of Schiff base chemistry followed by reductive amination.

and multiplexed electrospray injection at the microfluidic interfaces. Due to the soft and semiwet nature of the hydrogel surface, which also make them more popular as protein microarray substrate,[20] it is not advisable to use contact mode deposition for making protein microarrays. Integrating ESD approach with μhf offers a tool for arraying of biological molecules in a noncontact mode.

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Dendrimer-Scaffold-Based Electron-Beam Patterning of Biomolecules**

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Microarrays have revolutionized the field of genomics and more recently proteomics and have proven to be an asset in high-throughput screening.[1–3] As the demand for improved sensitivity and throughput of biomolecular assays increases, considerable research effort has been put into developing microelectronic[4–9] and nanophotonic[10–12] biosensors, which are presumably more sensitive than conventional fluorescence-based assays and have a faster response time. The lithographic technology for making the densely packed microelectronic devices in a high-throughput manner is already quite advanced and may be integrated to form biosensors. This is expected to increase the pace of research in early detection of disease biomarkers,[5–7,12–17] discovering cell-signal transduction pathways,[15–22] and in drug discovery.[1,23,24] Denser arrays are also important for reducing reagent volume consumption and to improve sensitivity.[25] Successful biomolecule patterning on sensor chip circuitry requires a number of important steps. First, selective immobilization of the probe and reduction in non-specific binding should be achieved for higher signal-to-noise ratios. Second, reduction in the sensor size reduces the background, enhancing the signal-to-noise ratio, and therefore biomolecule patterns on the same order as the sensors are desirable. Third, the biomolecule pattern should be aligned with the sensor circuitry, which becomes more difficult as sensor size decreases. Finally, a fabrication process should be formulated to ensure that the biomolecules are intact and functional, which is a challenge given the harsh micro- or nanofabrication processing steps.[26] Here, we have demonstrated an electron-beam (e-beam)-based approach fulfilling the above requirements for patterning biological macromolecules that does not involve the use of resist, hence eliminating the exposure of these biomolecules to harsh resist-stripping processes that are normally employed to remove the resist. A non-biofouling poly(ethylene glycol) self-assembled monolayer (PEG-SAM) was selectively removed by e-beam and patterned with aldehyde-terminated polyamidoamine dendrimer (ald-PAMAM-SAM) in a layer-by-layer (LbL) manner to covalently immobilize the aminated oligonucleotide, which bind only to their complementary sequence targets and can be stripped and reprobed. The Generation-6 (G-6) PAMAM molecule, terminated with 256 primary amine groups and 6.7 nm in diameter,[27] was used to increase the surface density of aldehyde functional groups to increase the oligonucleotide-immobilization efficiency.[28–31]

Current techniques for patterning biomolecules involve the use of polymer-based templates,[32–35] which can be removed mechanically without the use of organic solvents after biomolecule immobilization. However, serious limitations exist in each case. Poly(dimethylsiloxane) (PDMS)-based soft-lithographic techniques[32–34] cannot be used to create high-resolution patterns,[36] as aligning the PDMS pattern with sub-micrometer features has been shown to work in a mix-and-match[37] manner with an accuracy of only 2 μm. Although alignment is not an issue for biomolecule patterning based on polymer lift-off,[35] as it is an integrated process, this method involves extra steps of deposition and etching a polymer film, which increases the complexity of the process. Challenges are also encountered as the size of the photolithographic patterns decrease due to the increase in line-edge roughness (LER)[38,39] and the isotropic nature of oxygen plasma etch.[39] Patterned gold has been used for creating protein patterns using thiol-based linkers,[40] but gold surfaces cannot be tolerated in some biosensors[4–6,10] as gold interferes with the optical signal or conductivity of the sensor. This technique also includes extra photolithographic and lift-off processing steps for patterning gold. Protein patterning using fluorescence-tagged proteins

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physically adhered to nanoparticles and assembled inside 60 μm etched features on a wafer[41] have been demonstrated; however, patterning in smaller dimensions with this technique is also limited by the LER obtained in the photolithographic dimensions of the etched features and, to some extent, nanoparticle size. E-beam patterning of octadecyltrimethoxysilane (ODS)-SAM was performed to create patterns of DNA;[42,43] however, ODS-SAM is not a preferred surface due to the non-specific adsorption of biomolecules on hydrophobic surfaces.[44] Dip-pen nanolithography[45–49] and nanografting[50,51] approaches provide impressive resolution but the technology is not mature enough for these to be used at the industrial scale, although some effort has been put in this direction.[52]

Figure 1 illustrates the LbL assembly technique we employed for preparing ald-PAMAM-SAM. First, a PEG-SAM layer was assembled in the vapor phase on a silicon wafer with 20 nm of thermal silicon oxide; this layer was then patterned

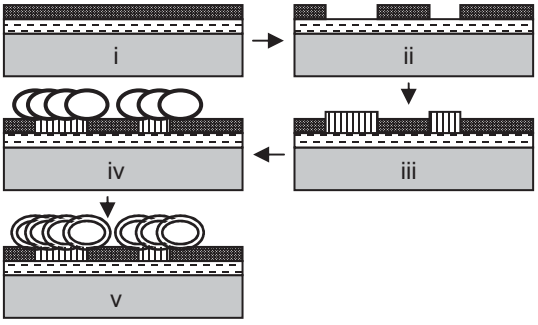

Figure 1. LbL method to pattern ald-PAMAM-SAM surrounded by PEG-SAM: i) Vapor-phase assembly of PEG-SAM, ii) e-beam patterning, iii) liquid-phase assembly of ald-SAM, iv) immobilization of G-6 PAMAM-SAM, v) modification with glutaraldehyde to create ald-PAMAM-SAM.

by an e-beam aligned to pre-existing features on the silicon oxide to regenerate the silanol groups. In general, the e-beam system can be used to achieve pattern alignment within 30 nm across the sample. Next, an aldehyde terminated SAM (ald-SAM) was assembled on the above regenerated silanol groups in the liquid phase. This was followed by the assembly of G-6 PAMAM on ald-SAM using Schiff base reaction between the aldehyde groups on ald-SAM and the primary amine groups on the PAMAM to create PAMAM-SAM. Schiff base reaction was again utilized to assemble glutaraldehyde on PAMAM-SAM to create aldehyde terminated PAMAM-SAM in the e-beam defined patterns (ald-PAMAM-SAM). Dendrimer-activated surfaces have been demonstrated for higher immobilization efficiencies and lower detection limits.[28–31] Here, PAMAM-SAM served to increase the surface density of the reactive aldehyde groups and also acted as a 6.7 nm spacer[27] for easier access of target DNA towards immobilized probe DNA. Figures S1,S2 (Supporting Information) show the preparation scheme of two control surfaces containing ald-SAM patterns surrounded by either a (1*H*,1*H*,2*H*,2*H*-perfluorooctyl) trichlorosilane (FOTS)-SAM background or a hexamethyldisilazane (HMDS)-SAM background. For both samples, vapor-phase assembly of the FOTS and HMDS was employed. These control samples were made to compare the non-specific adhesion of DNA on the FOTS-SAM or HMDS surface when compared with the PEG-SAM surface.

After exploring several aqueous and non-aqueous buffer conditions for optimizing the immobilization of the PAMAM dendrimers on the ald-SAM, we found that PAMAM dissolved in methyl alcohol with 0.08 % (v/v) acetic acid gave the most satisfactory results. This was done by checking the fluorescence emanating from probe DNA immobilized on the above-prepared ald-PAMAM-SAM.

Figure 2a shows a fluorescence image of the 1 μm patterns obtained after immobilizing carboxytetramethylrhodamine (TAMRA)-labeled probe DNA (pDNA) on the ald-PAMAM-SAM using Schiff base chemistry under aqueous conditions (pH 7.3) followed by reductive amination. Due to the resolution of fluorescence microscopy, we were not able to

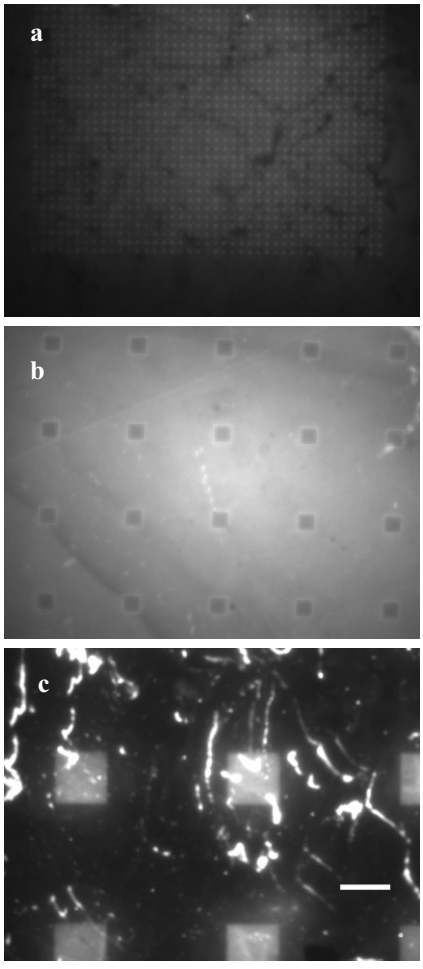

Figure 2. Aminated probe DNA immobilized on patterns of a) ald-PAMAM-SAM surrounded by PEG-SAM assembled in the vapor phase, b) ald-SAM surrounded by FOTS-SAM assembled in the vapor phase, and c) ald-SAM surrounded by PEG-SAM assembled in the liquid phase. Scale bar represents 30 μm in all three panels.

optically image the 30 and 300 nm patterns. The fluorescence image shows the expected uniform pattern of squares with minimal background noise. However, when substrates with pDNA patterns against a background of highly hydrophobic FOTS-SAM (or HMDS-SAM) (prepared as per Figs. S1,S2) were used, severe non-specific adhesion of pDNA was observed, as shown in Figure 2b. The high background noise observed for both FOTS and HMDS-SAM substrates is not completely unexpected, as hydrophobic surfaces are generally known to be susceptible to non-specific binding of biological molecules.[44] In an attempt to reduce the level of non-specific adhesion on the FOTS and HMDS surfaces, 0.5 % Triton X-100 (non-ionic surfactant)[44] (Sigma–Aldrich Corp., St. Louis, MO) was added to the pDNA solution during the probe immobilization step. However, the addition of Triton X-100 only resulted in the total elimination of any visible fluorescence patterns (not shown), indicating that the pDNA failed to immobilize on the patterned ald-SAM and ald-PAMAM-SAM areas. This result suggests that the aldehyde surfaces have become completely covered with surfactant, thus preventing covalent linkage with the pDNA. Subsequent washing steps with 0.5 % Triton X-100 in an ultrasonic bath to remove non-specifically adhered pDNA were also not successful. This is in confirmation with previous studies.[44] A significant amount of non-specific fluorescence was also observed when liquid-phase silanization was used to create PEG-SAM using long-chain (6–9 PEG units) 2-[methoxy(polyethyleneoxy)propyl] trimethoxysilane (MPEGTMS) (Gelest, Inc., Morrisville, PA) (Fig. 2c). This may be due to poor coverage of the surface by the PEG-SAM when using a liquid-phase versus a vapor-phase silanization protocol.[53,54] A larger pattern is shown in Figures 2b,c, as small patterns were not clearly discernible due to poor contrast from the high non-specific fluorescence background. Further control experiments were performed whereby the aldehyde functional groups on the ald-PAMAM-SAM were first quenched with a 0.05 M Tris/0.4 M glycine/0.05 M $NaCNBH_3$ buffer (Tris: tris(hydroxymethyl)aminomethane) before exposure to the pDNA solution. No fluorescence was observed in this case (not shown), demonstrating that the pDNA does not adhere non-specifically to the aldehyde surface.

Hybridization and selectivity assays were performed to test the functionality of the immobilized pDNA molecules by incubating the chips with either complementary target DNA (ctDNA) or non-complementary target DNA (ntDNA) tagged with Cy5 dye. Figure 3a shows a representative fluorescence image of ctDNA after hybridization to the pDNA immobilized on the dendrimer-activated patterns. No fluorescence was observed from pDNA pattern containing chips hybridized with ntDNA (negative control, not shown), proving that the immobilized pDNA was functional and retained its specificity towards its complementary target. In other negative control tests (not shown), a chip without any immobilized pDNA was hybridized with ctDNA; also, another chip with immobilized pDNA was exposed to a buffer solution without any ctDNA present. No fluorescence was observed in either case. These results show that ctDNA is not binding non-specifically to the ald-PAMAM-SAM, and that the pDNA does not give a false positive Cy5 signal due to the presence of the TAMRA fluorophore label.

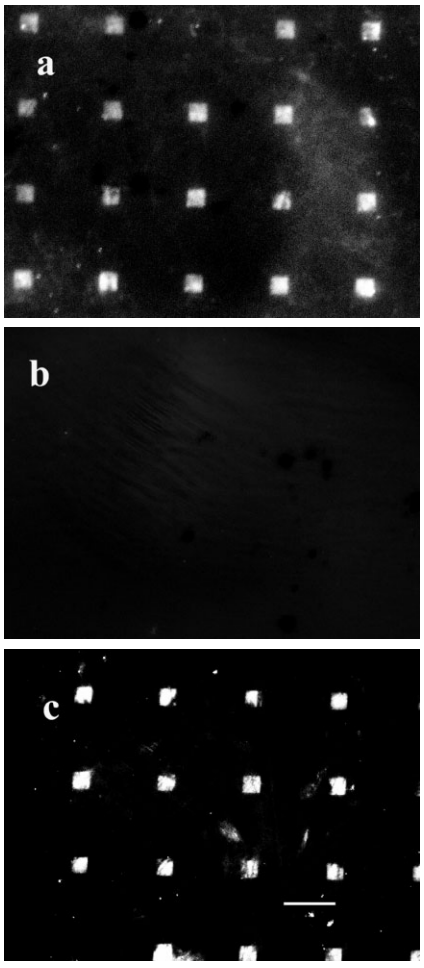

Figure 3. Hybridization assay of probe DNA with complementary target DNA. a) After hybridization with ctDNA. b) After treatment with 99.5 % formamide as a chaotropic medium. c) After rehybridization with ctDNA. Scale bar represents 30 μm for all three panels.

To test the reusability of the patterned surfaces with the pDNA, hybridized DNA complexes were first denatured by incubating in formamide solution for 10 min at room temperature and then rinsed in deionized water. This resulted in the removal of the bound ctDNA target and thus a complete loss of any visible Cy5 fluorescence (Fig. 3b). We found that exposure to the formamide for a period less than 5 min was insufficient for complete removal of the hybridized ctDNA. When the regenerated ("stripped") chip was again exposed to the ctDNA solution under the prescribed hybridization conditions, the Cy5 fluorescence patterns re-emerged (Fig. 3c) and appeared similar to those shown in Figure 3a. We also tried 8.3 M urea as the chaotropic medium to strip the hybridized ctDNA, but found that it required 30 min for complete stripping. The use of 8.3 M urea as the stripping reagent was also

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not advisable as it attacked the PEG-SAM, probably due to its basic nature, which resulted in specks of non-specific binding of ctDNA during the rehybridization step (not shown).

We have demonstrated here a biomolecular patterning technique capable of creating reusable DNA patterns using an e-beam process, without the use of a conventional e-beam resist, in 1 μm dimensions with respect to pre-existing micrometer-sized alignment features made by photolithography.[10] However, based on the accuracy of the e-beam tool, we suggest that pattern size and an alignment accuracy of 30 nm should be easily achievable. The process eliminates any requirement for a separate resist-removal step, and therefore exposure of the biomolecules to harsh chemical processing conditions during nanofabrication is avoided. This method uses the commercially available micro- and nanofabrication processes of vapor deposition[55] and e-beam lithography,[56,57] which can be easily integrated with fabrication steps for microelectronic or nanophotonic lab-on-a-chip type devices with biosensing capabilities.

Experimental

Preparation of PEG-SAM: A silicon wafer with 20 nm thermal SiO_2 was plasma cleaned, and PEG-SAM was assembled in the vapor phase at a chamber pressure of 0.5 torr using short-chain (single PEG unit) 2-[methoxy(polyethylenoxy)propyl] trichlorosilane (MPEGTCS) (MVD-100, Applied Microstructures, Inc., San Jose, CA) [55]. The process was repeated four times for 10 min each. The wafer was then rinsed in 2-propanol and cured overnight.

E-Beam Patterning of PEG-SAM: The PEG-SAM on the above wafer was then removed by e-beam lithography in patterns using a dose of 9 mC cm^{-2} using an accelerating voltage of 100 kV at a current of 20 nA with an approximate spot size of 20 nm (JBX-9300FS, JEOL USA, Inc., Peabody, MA) [56]. This was then rinsed in 2-propanol and blow dried in a N_2 jet.

Preparation of ald-SAM: The PEG-SAM on the wafer was removed by a 9 mC cm^{-2} e-beam and rinsed in 2-propanol. This was then treated ultrasonically for 2 h with a 3 % (v/v) solution of 11-triethoxysilylundecanalsilane (TESU) (Gelest, Inc., Morrisville, PA) dissolved in a stock solution of 95 % ethanol, 4.7 % water, and 0.3 % acetic acid and cured at 120 °C for 2 min.

Preparation of PAMAM-SAM: The wafer with ald-SAM was incubated for 2 h with a 0.05 % (w/v) solution of G-6 PAMAM dendrimer (Sigma–Aldrich Corp., St. Louis, MO, Product no. 536717) in methanol with 0.08 % (v/v) acetic acid.

Preparation of ald-PAMAM-SAM: The wafer with PAMAM-SAM was washed in methanol and incubated in a dilute solution of 7 % (w/v) glutaraldehyde (Sigma–Aldrich Corp., St. Louis, MO, Product no. G7776) in 97 % methanol and 3 % water for 2 h.

Probe DNA Immobilization: The above wafer was washed in methanol, and an aliquot of 5 μM pDNA (Integrated DNA Technologies, Coralville, IA) in phosphate-buffered saline (PBS) with 0.05 M $NaCNBH_3$ at pH 7.3 was incubated on ald-PAMAM-SAM for 5 min and rinsed with deionized water. The pDNA was functionalized with a free amino group (linked to a six-carbon chain spacer arm) at the 5′ end, tagged with a TAMRA fluorescent dye label on the 3′ end (5′-NH_2-C6-CAA GAT CGC ACT CCA GCC AG-TAMRA-3′), and bound to aldehyde groups with its 5′ end by Schiff base reaction followed by reductive amination. Any free reactive aldehyde groups remaining on the surface after probe immobilization were quenched with 0.05 M Tris/0.4 M glycine/0.05 M $NaCNBH_3$ buffer wash for 20 min.

Target DNA Hybridization: Silicon wafer pieces containing pDNA were incubated with a 2× SSPE (300 mM NaCl, 20 mM NaH_2PO_4, 2 mM EDTA, pH 7.4) buffer solution containing either 5 μM ctDNA (Integrated DNA Technologies, Coralville, IA) (5′-Cy5-TGT ACC GTA CCT GGC TGG AGT GCG ATC TTC-3′) or 5 μM ntDNA (5′-Cy5-GGG AAA AGG GAT CCG AAA AAA AGG GGT ACG-3′) for 30 min and subsequently washed with deionized water.

Denaturation of Hybridized DNA Complexes and Chip Regeneration: Chips containing hybridized ctDNA patterns were incubated in a 99.5 % solution of formamide (Sigma–Aldrich Corp., St. Louis, MO, Product no. F9037) for 10 min (25 °C) in order to denature double-stranded DNA complexes, and then rinsed with deionized water.

Fluorescence Imaging: An epifluorescence microscope (Labophot-2, Nikon, Inc., Melville, NY) fitted with a charge-coupled device (CCD) camera (Spot RT, Diagnostic Instruments, Inc., Sterling Heights, MI) was used for imaging of TAMRA- and Cy5-labeled oligonucleotides immobilized on the chip. All images were taken under the settings of a 20 s exposure time and a gain of two. No filter was used in the camera, and only the respective filter cube in the microscope for TAMRA (excitation: 541–551 nm, dichroic mirror: 575 nm, emission: 590 nm) or Cy5 (excitation: 590–650 nm, dichroic mirror: 660 nm, emission: 663–735 nm) was in place. For Cy5 imaging, a background image taken through a Cy5 filter on a separate chip without the ctDNA hybridization step was subtracted from the image of the chip containing hybridized Cy5-labeled ctDNA.

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Protein functionalized micro hydrogel features for cell–surface interaction

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Abstract Cross-linked hydrogel features have been patterned using subtractive lift-off of polymerized hydrogel film. Projection lithography and oxygen plasma etch was used to pattern parylene C polymer film. Molecular self-assembly of polymerizable monolayer was obtained in solution-phase and acrylamide based hydrogel was polymerized using free-radical polymerization on this substrate. Parylene C film was mechanically lifted-off to remove the blanket hydrogel film and micro hydrogel features (μhf) were obtained attached to the predefined patterns in the range from 1 to 60 μm. The μhf were functionalized with aldehyde functional groups, and proteins were coupled to them using Schiff base chemistry followed by reductive amination. Interaction of mesenchymal stem cells with transforming growth factor-beta 1 (TGF-β1) functionalized μhf was studied, and TGF-β1 was found to retain its tumor suppression activity.

Keywords Integrated microfabrication of hydrogels · Surface immobilization of growth factors · Cell–surface interaction · Biologically friendly microfabrication processes

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1 Introduction

Hydrogel based materials have proved to be an important biomaterial due to their ability to maintain physiological environment and have been recognized as a preferred interface for protein immobilization (Kiyonaka et al. 2004; Langer and Tirrell 2004). Patterning of proteins on micro hydrogel features (μhf) can be used for high-throughput applications in drug screening (Beske and Goldbard 2002; Bhadriraju and Chen 2002; Sidransky 2002; Koch 2004), early detection biosensors (Giaever and Keese 1993; Cornell et al. 1997; Sidransky 2002; Kaul et al. 2004), and fundamental cell biology studies (Chen et al. 1997; Alberts 1998; Takayama et al. 2001; Orth et al. 2003). Recent advances in the use of microfabrication processes for organ printing and complex tissue engineering motivated us to develop micron size growth factor environment (Hollister et al. 2000; Langer and Tirrell 2004; Hollister 2005; Arcaute et al. 2006; Cunningham et al. 2006; Khademhosseini et al. 2006; Mikos et al. 2006). Earlier, we suggested an e-beam directed patterning of oligonucleotides through dendrimeric interface (Bhatnagar et al. 2006). Here we describe patterning of proteins through hydrogel interface for cell–surface interaction studies. We suggest a

microfabrication method that produces patterns with 1 μm hydrogel feature aligned to the pre-existing surface topography. A photolithographically patterned polymer film was mechanically lifted-off (Ilic and Craighead 2000) to create cross-linked μhf attached to silicon oxide surface through self-assembled monolayer (SAM). The μhf were chemically modified (Arenkov et al. 2000) to covalently immobilize protein molecules using Schiff base chemistry followed by reductive amination. Further, the interaction of growth factor functionalized μhf with equine mesenchymal stem cells (eMSC) has been demonstrated that resulted into anoikis (apoptosis due to loss of anchorage) and immobilized transforming growth factor-beta 1 (TGF-β1) was found to retain its tumor-suppression activity (Lafon et al. 1996; Hartsough and Mulder 1997; Kim et al. 2003; Luginbuehl et al. 2004; Cao et al. 2006; Reynolds and Kyprianou 2006).

Current techniques for μhf patterning include photopolymerization (Arenkov et al. 2000; Rubina et al. 2004; Zourob et al. 2006), and soft lithography (Xia et al. 1999; Suh et al. 2004; Suh et al. 2004) and primarily suffer from alignment and resolution issues. Although e-beam based cross-linking (Hong et al. 2004) does not suffer from these issues, it is not a preferred method for high volume manufacturing. The method we report here produces an array of cross-linked μhf in sub-cellular dimensions capable of delivering localized stimulus to the cells (Chen et al. 1997; Takayama et al. 2001; Orth et al. 2003). Alignment to the pre-existing surface topography was achieved using standard microfabrication tool sets. This technique therefore can also be used to create an interface between microelectronic circuitry and proteins immobilized on μhf (Cornell et al. 1997; Kaul et al. 2004).

2 Experimental methods

Patterning of parylene C film Parylene (1.5 μm thick) C polymer film deposited (chemical vapor deposition) on 100 mm diameter thermally oxidized silicon wafer was patterned using standard projection photolithography. The patterns were transferred into the parylene C film using oxygen plasma in PlasmaTherm 72 reactive ion etcher (electrode area 585 cm^2). Microfabrication equipments at Cornell NanoScale Science and Technology Facility (CNF) were employed.

Polymerization of hydrogel thin film Parylene C patterns were treated for 2 min with 3% (*v*/*v*) solution of 3-methacryloxypropyl(trimethoxysilane) (Sigma-Aldrich Corp., St. Louis, MO, USA) in 95% ethanol, 4.7% water and 0.3% acetic acid. Five percent (*w*/*v*) monomer (95% acrylamide and 5% methylenebisacrylamide (*w*/*w*)) was dissolved in 0.01 M PBS with 40% glycerol (*v*/*v*). Free radicals were generated by addition of 4 μl of 25% (*w*/*v*) of ammonium persulfate and 4 μl of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (Bio-Rad Laboratories, Hercules, CA, USA) per milliliter of gel precursor solution. Pre-polymerized hydrogel solution was dispensed on the wafer and polymerized overnight.

Aldehyde functionalization of hydrogel The μhf substrate was treated with aqueous 0.1 M $NaIO_4$ for 20 min followed by 5% glutaraldehyde (in 0.1 M sodium phosphate buffer) for 48 h.

Immobilization of proteins on hydrogel TGF-β1 (R&D Systems, Minneapolis, MN, USA) was reconstituted as per manufacturer's instructions at 0.8 μg/ml in PBS without and with 0.25% Triton X-100 and dispensed on aldehyde functionalized μhf substrate. The surface was washed with PBS after 3 h and treated with 0.1 M $NaBH_4$ for 20 min. Detection protocol included the use of anti-TGF-β1 biotinylated affinity purified polyclonal antibody and streptavidin-Alexa 488 (Molecular Probes, Invitrogen Corp., Carlsbad, CA, USA) as the secondary probe.

Plating of eMSC cells on the μhf substrate The chips were incubated in 24-well plate in PBS with 0.1% NaN_3 for 30 min and copiously washed with PBS in cell culture hood. Equine mesenchymal stem cells (eMSC) were isolated as described elsewhere (Fortier et al. 1998) and passaged five times, washed with Hank's balanced salt solution, trypsinized, centrifuged at 300×*g* (4°C for 10 min) and plated at 50,000 cells/well. The cell culture media included low glucose Dulbecco's modified Eagle's medium, 1% ITS+ (Cat. # 354352, BD Bioscience, Bedford, MA, USA), 1% penicillin–streptomycin, 1% L-glutamine, and 0.2% amphotericin B (all constituents from Gibco, Invitrogen Corp., Carlsbad, CA, USA). The cells were fixed after 4 days with 3.7% paraformaldehyde solution and the nucleus was stained with 4′,6-diamidino-2-phenylindole (DAPI) for 10 min.

Fluorescence imaging Olympus BX51 upright epifluorescence microscope (Olympus America, Inc., Center Valley, PA, USA) with CoolSnapHQ monochrome CCD camera (Photometrics, Tuscon, AZ, USA) was used with Metamorph V6.1 image analysis software (Universal Imaging Corp., Molecular Devices Corp., Downington, PA, USA).

3 Results and discussion

Figure 1(a) illustrates the method for patterning μhf. A photolithographically patterned parylene C (sacrificial)

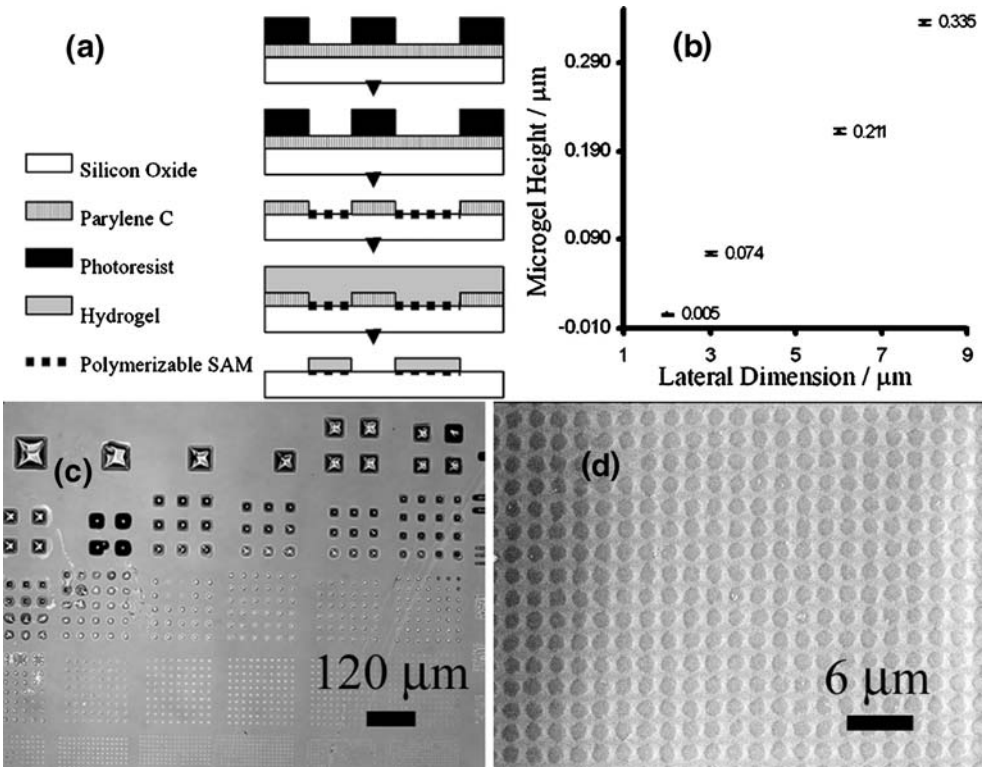

Fig. 1 (**a**) Fabrication scheme for μhf. (**b**) Relationship between the lateral dimension of μhf and their heights with *error bars*. Average standard deviation is ±0.002. (**c**) μhf of different sizes. Larger features are more prominent and higher. (**d**) SEM image of μhf of 1 μm diameter

polymer film on the silicon oxide surface was etched in oxygen plasma (Ilic, Craighead 2000; Orth et al. 2003). The surface was functionalized with polymerizable SAM and acrylamide based hydrogel was polymerized on the surface using free-radical polymerization. The parylene C film was then mechanically peeled (lift-off) from the surface (Ilic and Craighead 2000; Orth et al. 2003) releasing the μhf covalently attached to SAM on the silicon oxide. The μhf exhibited pyramidal shape with AFM and their heights varied with the planar dimensions (Fig. 1(b)). The observance of area effect is typical of microfabrication processes (Hedlund et al. 1994; Chang 2005). Different sizes of patterned hydrogel features were obtained based on the photolithographically defined patterns in parylene C (Fig. 1(c)). Figure 1(d) shows the SEM image of the μhf with 1 μm feature and space.

Through sequential surface treatment, (Arenkov et al. 2000), the μhf on silicon oxide surface was functionalized with aldehyde groups. TGF-β1 was immobilized through primary amines to form Schiff bases with the surface aldehyde groups followed by reductive amination resulting in secondary amide linkage that does not hydrolyze in aqueous environments (Hermanson 1996). Figure 2(a, b) shows the fluorescence detection of TGF-β1 on μhf and effect of non-ionic surfactant (Triton X-100), which was helpful in reducing the non-specific adhesion of the protein on silicon oxide surface (Marsh et al. 2002). The μhf without immobilized proteins did not exhibit any fluorescence when probed with labeled antibodies (negative control, Fig. S1). No non-specific adhesion of probe antibodies was observed on the μhf, but a blocking step with StartingBlock T20 (in phosphate buffered saline (PBS)) Blocking Buffer (Pierce Biotechnology, Inc., Rockland, IL, USA) for 1 h was introduced to prevent the non-specific adhesion of primary antibodies on the silicon oxide surface (Fig. S2). Similar results were obtained with interleukin-4 (IL-4) and interferon-gamma (IFN-γ; Fig. S3), biotin, and protein A.

Interaction of cells on functionalized surfaces and cell-adhesion studies can provide insight into the molecular basis of many diseases and is an area of active research (Lafon et al. 1996; Kim et al. 2003; Cao et al. 2006; Reynolds and Kyprianou 2006). Several applications for interaction of cells in lab-on-a-chip (LOC) systems have been reported (El-Ali et al. 2006), and the above protein functionalized μhf can further be added to the set of available LOC tool-set and utilized for studying cell-signal transduction events. The μhf with immobilized TGF-β1, IL-4 or IFN-γ were seeded with eMSCs and incubated for 4 days. It was observed that cells adhered to the substrate when μhf were functionalized with IL-4 (Fig. 2(c); or IFN-γ, not shown). However, when plated on the μhf with TGF-β1, the cells adhered to each other forming a sheet that was observed to barely attach on the corners of the chip but not

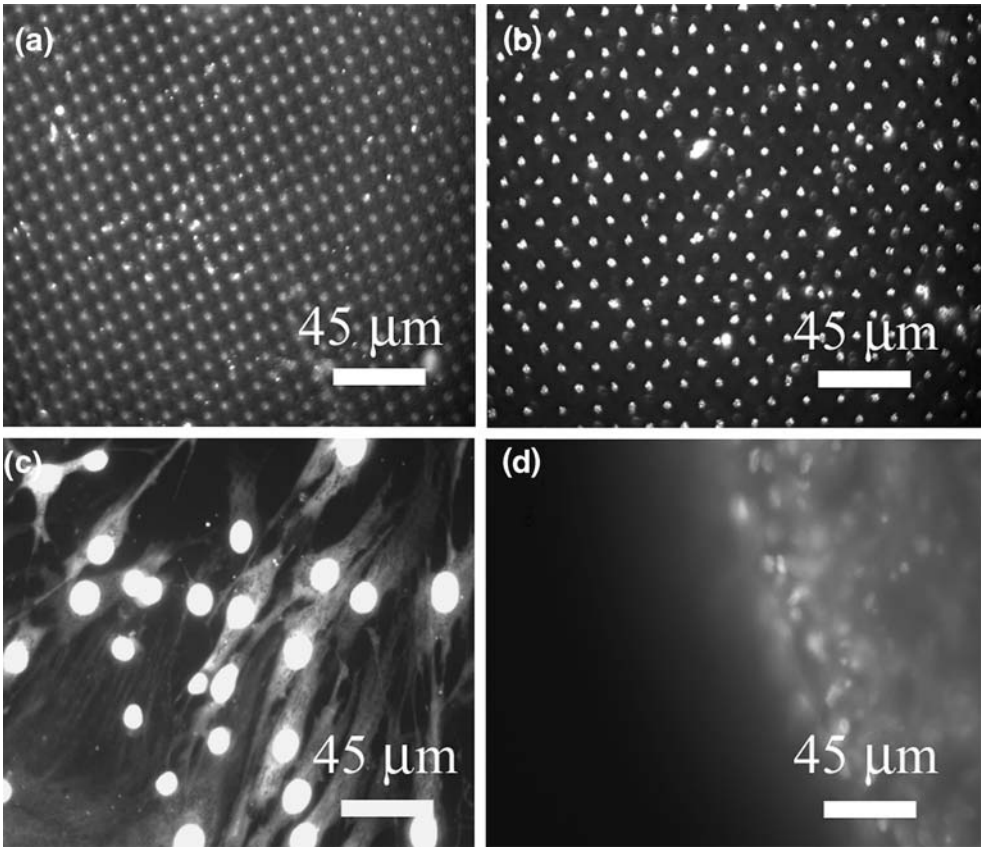

Fig. 2 (**a**, **b**) Fluorescent detection of TGF-β1 immobilized on the μhf. Protein was reconstituted in (**a**) PBS (**b**) PBS with 0.25% Triton X-100. (**c**, **d**) Plated eMSCs (**c**) cell-sheet adhered to the substrate when μhf were functionalized with IL-4 (**d**) cells adhered to each other to form a cell-sheet but did not adhere to the substrate when μhf were functionalized with TGF-β1

on its surface. Figure 2(d) is the image of the cell-sheet that consisted of cells adhered to each other but not to the TGF-β1 functionalized μhf substrate. We infer this non-adherent behavior of the cell-sheet was due to the tumor-suppression activity (Lafon et al. 1996; Hartsough and Mulder 1997; Kim et al. 2003; Luginbuehl et al. 2004; Cao et al. 2006; Reynolds and Kyprianou 2006) of TGF-β1, which at high dose has been found to induce anoikis in various cell types. TGF-β1 concentration in the range of 5 to 10 ng/ml has been accepted as normal dose for differentiating MSCs in chondrocytes (Cassiede et al. 1996; Johnstone et al. 1998; Gao et al. 2001; Worster et al. 2001; Luginbuehl et al. 2004; Li et al. 2005). However, we did not characterize the dose effect in this work since this was not our focus. As a control, the cells (Fig. S4) were plated on non-functionalized μhf, and cells were found to adhere similar to Fig. 2(c).

4 Conclusions

The intersection of biology and materials has been identified as one of the growing frontiers in materials science (Stupp et al. 2005; Whitesides and Wong 2006). Significant advancements have been made in the past decade in the field of proteomics. Interaction of cells with protein functionalized surfaces has been an area of considerable interest (Bhadriraju and Chen 2002; Raghavan and Chen 2004). Here we have demonstrated the use of standard microfabrication equipment for fabrication of μhf and its possible application in molecular and cell biology. Micro manipulating techniques such as microcontact printing (Xia and Whitesides 1998; Whitesides et al. 2001), inkjet printing (Lemmo et al. 1998; Heller 2002), dip-pen nanolithography (Piner et al. 1999; Ginger et al. 2004) or photolithography could be employed to immobilize different biomolecules in separate μhf and used for agonist-antagonist type of investigations in a single cell or to emulate extra-cellular matrix for tissue-engineering (Doh and Irvine 2006). Surface patterning of bio-functionalized nanoparticles through biomolecular interaction offers the possibility to construct integrated biosensors (Tokareva et al. 2004) and the use of standard microfabrication equipment allows integration of microelectronic or photonic sensors to be aligned (Tien et al. 2002; Verpoorte 2003) in high-resolution to the biomolecular patterns.

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