SILICON AS A BIOMEDICAL MATERIAL

Mark Ming-Cheng Cheng^{1,2}, Ennio Tasciotti¹, and Mauro Ferrari^{1,2,3,4,5}

¹Center for Nanomedicine, Institute of Molecular Medicine, ²Biomedical Engineering, The University of Texas Health Science Center at Houston, ³Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, ⁴Bioengineering, Rice University, ⁵The Alliance for Nanohealth, Houston, Texas, USA

ABSTRACT

The cornerstone of microelectronics and MEMS, silicon-based manufacturing is taking over as a fundamental material in medicine. Research and laboratory diagnostic uses of biomolecular array technologies and microfluidics are well established. Novel sensor nanotechnologies are demonstrating features that make them an indispensable tool for the conquest of the post-genomic challenges, which present astronomical complexities in their requirement for multiplexed, real-time analyses. Controlled-release nanofluidic and microchip devices are under development as drug-delivery implants. Recently, nano-porous silicon has emerged as a material for medicine itself: it is biodegradable with tunable kinetics, eminently biocompatible, and provides suitable drug loading and release rates. Formed into micro- and nanoparticles of 'designer' size, shape and surface properties, nanoporous silicon is a particularly versatile material for the next generation of nanomedical therapeutic products.

INTRODUCTION

With apologies to the purists of nomenclature and scientific taxonomy, in this article we employ the word 'silicon' not only to refer to the celebrated material itself, but also to refer to the top-down fabrication techniques employed in clean room to manufacture electronic and micro-electro-mechanical artifacts, the common denominator of which is the employ of photolithographic techniques. Begging further compositional license, we encompass under the rubric 'silicon' also those techniques that derived from the bubbling cauldron of clean room innovation, though they do not directly employ lithography proper – thus, soft lithographies also pertain within the scope of this article. With a final expansive thrust, we will encompass also a broad set of materials, but only within the context of their manufacture by means of the aforementioned set of techniques – thus, silicon oxides and nitride, several metals, biological and synthetic polymers... a large family indeed, the operational descendants of silicon proper and its processing protocols in semiconductor manufacturing techniques.

The connection between these materials and protocols will probably be obvious to the expert, and impenetrable to the occasional frequenter; the objective of this paper is to connect the dots and articulate the family tree, maintaining a focus on applications to biology and most notably in medicine. What will hopefully emerge is a portrait of 'silicon' as a key enabler of progress in medical diagnostics and therapeutics, and – surprise? – an enthusiastic endorsement of silicon itself as a great material for in-vivo use in the clinic.

THE EMERGENCE OF SILICON-FAB AS AN ENABLING TECHNOLOGY IN BIOMEDICINE

The first thrust toward biomedical deployment of silicon fabrication technologies derived from microfluidics, which offered unprecedented performance in bioseparation and the controlled transport of reagents and analytes [1, 2]. Microfluidics has now become a household name in science worldwide, with academic journals, professional societies, and scientific congresses dedicated to the field. Many products have emerged for laboratory clinical and diagnostic uses, developed by companies ranging from the

start-up phase to publicly traded entities of the small- and mid-cap persuasion, to entire sector operations of large chip manufacturers. These products include separation technologies for blood into its components, the fractionation of complex biofluidic mixtures into, e.g., its protein and nucleic acid digest sub-populations, DNA amplification strategies via PCR-on-chip, precise fluidic dispensation technologies for automated high-throughput analyses, multiplexed analyte sensors for point-of-care diagnostics, and many more. A fundamental enabling breakthrough for the commercial and clinical transitions of microfluidics was the development of convenient methods of fabrication for polymers via soft-lithographic techniques, which do not require lithographic steps beyond the definition of an initial template or mold [3].

The transition from microfluidics to nanofluidics was enabled by the attainment of nanometer-scale resolution in UV photolithography, and the development of innovative, no-lithographic methods for nanometer-scale dimensional control. Our group has focused on nanofluidics for over a decade now, pioneering applications to cell transplantation [4], separation of biological molecules and nanoparticles [5], the encapsulation of implanted sensors to avoid biological fouling [6], and drug delivery from implants [7, 8]. The fundamental technique we have based our program on is the use of sacrificial oxide layers to define nanochannel dimensions [9, 10]. We have also explored the fundamental physical and chemical implications of nanochannel transport, developing and experimentally validating novel predictive laws for constrained non-Fickian diffusion [11, 12] and osmotic pressure [13].

Examples of microfluidic devices and approaches follow. The current standard for the separation of proteins or small nucleic acids (DNA, RNA, oligonucleotide), gel electrophoresis, suffers from labor-intensive processes as well as limited sensitivity and reproducibility. Microfluidics systems have been fabricated using photolithography and etching in glass to create microchannels with diameters between 10 -100 micrometers [14]. The devices utilize capillary electrophoresis and hydrodynamic pressure to implement the continuous-flow assays that automate and integrate sample injection, reaction, separation, staining and fluorescence detection steps. Different prototypes including binding assays, protein/enzyme assays, and cellular assays have been demonstrated [15]. Enzyme-linked Immunosorbent Assay (ELISA), commonly used in immunoassay to detect an antibody or an antigen in a sample involves a series of reaction/incubation and washing steps. It often takes many hours to two days because of the limited mass transport of antigen/antibody from the analytes to the surface. A polymer-based compact-disk (CD) like microfluidic platform has been developed to implement ELISA [16]. This device is fabricated using an UV-LIGA process. Different solutions involved in the ELISA process is stored in different reservoirs, followed by mixing and incubation that is controlled by centrifugal and capillary forces. Each step of the ELISA process is carried out by the rotation speed of the CD with no external power required. The advantages include reduction in reagent consumption and high-throughput; however, the non-specific adsorption on the polymer surface remains challenging.

The second avenue of import of UV- photolithography into medicine is in connection with microarrays for the sequencing of

nucleic acids, proteins, and an ever-growing taxonomy of biological molecules [17]. Photolithography was used to fabricate high density, spatially addressable DNA/RNA microarrays through and insight of Fodor, Stryer, and their coworkers [18, 19]. Contact lithography combined with the chemistry of light-sensitive removable protective groups allow for the synthesis of arbitrary polynucleotide bases at specified locations on the solid-state surface with a minimum of chemical steps. Current state-of-the-art microarrays have feature dimensions of 5 μ m and densities of about 4 $\times 10^6$ /cm². It is expected the size will continue to scale down. While such high-density microarrays are suitable for gene expression analysis, it takes a very long time for passive hybridization. Using lithography-patterned planar microelectrode arrays, electronic microarrays have been developed for addressable, and fast DNA or RNA hybridization [20]. The application of a positive electric current to the individual electrodes enables rapid movement and concentration of negatively charged molecules. Porous hydrogel is coated on the electrode to serve as a soft support for attaching DNA probes and to reduce effects of electrolysis. Novel fabrication technologies for arrays of bioanalytes are explored by many groups worldwide [17].

SILICON NANOTECHNOLOGY FOR PROTEIN SENSORS AND PROTEOMICS

An emerging application in biomedical research is the development of tools for reliable, rapid, quantitative, low-cost, and multiplexing identification of biomarkers using biological fluids (serum, urine, saliva) for screening, diagnostics, and staging of cancer and other pathologies. Early detection is particularly important in cancer, since the disease at its early stages, may be treated with the greatest probability of success.

However, the task is challenging because of the complexity and high dynamic range of protein and peptide biomarkers in the biofluids. It has been widely recognized that the number of different biomolecular species in the plasma proteome exceeds 300,000, and could be as high as 10⁶, with differences of as many as 12 orders of magnitude in relative abundances [21]. For quantitative detection of low-abundance biomarkers, new technologies are demanded including molecular recognition, bioconjugation protocols, and mechanisms for label-free, multiplexing detection. Three approaches are reviewed in what follows.

Cantilevers: Nanomechanical Detection of Biomolecules

Detecting biomolecular interactions by measuring nanomechanical forces offers an exciting means to develop highly sensitive, miniature, and label-free biological sensors [22, 23]. When molecular adsorption/binding occurs on one-side of a cantilever, the silicon cantilever beam is subjected to surface stresses and a change of mass. The cantilever beam manifests the forces involved in the adsorption process by nanometer scale displacement as well as by changes in resonant frequency. Such a direct transduction eliminates the need for the analytes to be labeled. The displacement of the cantilever can be quantified simultaneously by using a number of techniques such as measuring variations in optical beam deflection, piezoresistivity, piezoelectric, and capacitance. These forces can also be measured as a resonance frequency although the sensitivity is usually low because of the low quality factor of the resonator and the viscosity of the liquid. To improve this, microchannels suspended in the cantilevers have also been developed in which the cantilever is placed in vacuum and the solution is allowed to flow inside a hollow resonator [24].

Silicon offers no intrinsic chemical selectivity so that the chemical recognition on cantilevers is achieved by affinity binding reactions. Self-assembled monolayers, DNA probes, antibodies, or peptides can be coated on the cantilever by mechanism such as adsorption, covalent binding, or affinity binding. DNA hybridization on cantilevers has been extensively investigated by a number of groups [22]. Detection of proteins by cantilever nanomechanics is challenging, mainly due to the lack of reproducible and robust immobilization techniques for antibodies. However, prostate specific antigen (PSA) has been successfully detected on cantilevers using immobilized antibodies [23]. It has been shown the amplitude of the bending is proportional to the concentration of the antigen adsorbed and to the time of exposure.

Silicon Nanotextured Surfaces for Proteomic Analysis

It is hypothesized that the low molecular weight proteome (LMWP), comprising proteolytic fragments at extremely low concentration, contains a wealth of information of diagnostic and prognostic utility [25]. Because of the high range of variability of serum proteome, it is essential to develop fractionation technologies to deplete the most abundant interfering proteins. To overcome current limitations in sample processing and analysis, we developed nanoporous silicon chips (NSCs) to perform size exclusion, selective capture, and resultant enrichment of selected regions of the LMWP from body fluids and other biologic samples [26, 27].

NSCs incubated with human plasma mixtures spiked with peptide standards can be identified by Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF), one type of mass spectrometry analysis. The analytes can be detected with concentration as low as the ng/mL range, thus increasing the sensitivity of MS analysis by roughly 400-fold. In combination with the selective enrichment achieved by the use of NSCs, it represents a powerful tool to further improve the selectivity, sensitivity and reproducibility of peptide harvesting and identification in the search for disease-related biomarkers. They might provide clinical diagnostic tools for early detection of human diseases and for tracking individual patient's responses to treatment regimens.

Nanowires: Label-Free Electronic Biosensor

Nanowire sensors operate on the basis that the change in chemical potential accompanying a target binding event, such as DNA hybridization, can act as a field-effect gate upon the nanowire, thereby changing its conductance [28, 29]. The high-density and high sensitivity of nanowires holds promise for monitoring local chemical, electrical or physical property changes in cells or tissues.

The ideal nanowire sensor is a lightly doped, high-aspect ratio, single-crystal nanowire with a diameter between 10 and 20 nm. If the nanowire is much smaller, it couples noises easily, whereas if it is too large, it is not as sensitive. Other design considerations include the length, sensor geometry of nanowires, fluidic conditions and ion concentrations in the aqueous solution.

One great advantage of nanowire sensor is that they can be integrated with the circuit. Large scale circuits can, in principle, be constructed within very small (microfluidic) environments, creating very dense sensor libraries, thus enabling measurement of many different genes and proteins from very small tissue samples, or even single cells. However, functionalization of the individual nanowires with multiple ssDNA molecules or protein capture agents does represent a serious challenge.

SILICON-BASED THERAPEUTIC APPLICATIONS

Several implantable silicon-based microdevices have been explored in-vivo. For example, high-density silicon micromachined electrode arrays have been developed to interface with the central nervous system for the long-term monitoring of neural activity in vivo as well as the insertion of electronic signals into neural networks at the cellular level [30]. Another example is implantable silicon microchips for radio-frequency identification that have been approved by the FDA and commercialized.

Drug delivery is an extraordinarily exciting area of silicon-enabled nanomedicine [31]. For the chemotherapy of cancer, challenges remain to realize continuous local delivery of multiple drugs at a constant rate to the tumor microenvironment to overcome much of the systemic toxicity and to improve anti-tumour efficacy. The novel physical/chemical/biological properties of nanoscale materials offer new opportunities. For example, engineered particles for drug delivery can alter the pharmacokinetics and biodistribution of the drugs by immobilizing molecular recognition moieties (antibody, proteins, peptides, aptamers, etc) at the surface of particles. Another example is that we have shown when molecules transport through the nanochannels with a size approaching the hydrodynamic diameter of the molecules, the diffusion kinetics are linear, non-Fickian over a substantial period. This may be applied to drug delivery [32].

Silicon Microchips for Controlled-Drug Delivery System

An array of miniature reservoirs have been fabricated using semiconductor processing for drug delivery applications [33, 34]. Each reservoir is loaded with drugs and sealed with a gold membrane. The membrane ruptures in the presence of chloride ions when anodic voltage is applied to the membrane of interest, allowing the drug within the reservoir to dissolve and diffuse into the surrounding tissue. This device allows for the release of multiple, active substances in a pulsatile manner. The reservoirs can also be made of time-dependent biodegradable polymer without the need for a stimulus to trigger drug release [34].

We discovered non-Fickian diffusion phenomena using nanochannels designed for the controlled release of molecules [11, 32]. The nanochannels were generated by removing a sacrificial silicon oxide layer, which is sandwiched between two structural layers. Nanochannels of width 7-50nm can now be routinely manufactured without E-beam lithography. By tailoring the channel size and chemistry according to the molecules of interest, it is possible to attain the desired constant release profiles of drugs for a period of several months. Furthermore, applying a very small electrical field across the channel gives the ability to actively control the release rates. This offers the opportunity to develop smart pre-programmed, remotely activated, and self-regulated delivery implants. Nanochannels can also be used for immunoisolation of cell transplantation to perform size-exclusion [6]. The physical geometry affords sufficient permeability to small molecules such as oxygen, glucose and insulin, while impeding the passage of immune molecules and graft-borne viruses. Encapsulated rat neonatal pancreatic islets have been demonstrated to significantly outlive and outperform controls in terms of insulin-secretion capability over periods of several weeks.

Silicon Particles for Drug Delivery

Porous silicon particles can be formed using electrochemical etching in HF solution combined with lithography. These particles can carry drugs, proteins, peptides or small compounds. Large porous silicon particles of diameter 200µm have been investigated for oral delivery [35]. The particles carried FITC labeled insulin along with a permeation enhancer, sodium laurate. The release

profile was tested across differentiated human intestinal Caco-2 cell monolayers grown on Transwell® inserts. The flux of FITC-insulin from silicon particles across cell monolayers was nearly 10-fold higher as compared to liquid formulations.

Particles of diameter 30µm were used to carry radioisotope phosphorous 32 into close contact with the tumor [36]. The commercially available n-type silicon wafers contain dopant phosphorous 31 (0.001%), and it was irradiated to form 32P. These particles implanted intratumorally under local anesthesia directly into liver tumors under ultrasound or computed tomographic guidance showed a significant degree of antitumor efficiency at low doses.

Multi-Stage Nanoporous Silicon Drug Delivery Systems

It is the consensus that only between 1 and 10 parts per 100,000 of intravenously administered drug molecules reach their tumor targets in vivo [31]. The human body's natural defenses or biobarriers act as obstacles to foreign objects injected in the blood stream. These barriers include enzymatic degradation, phagocytosis in peripheral blood and in the reticulo-endothelial system (RES), and the crossing of the vascular endothelium. Margination and adhesion onto vascular endothelia are governed by haemodynamic forces that are effective barriers for injected agents.

To overcome this challenge, we have developed a multifunctional multistage delivery system (MDS) that is able to navigate in the blood stream and to efficiently dock to the abnormal vasculature associated with diseased tissues [37, 38]. The MDS comprises stage 1 mesoporous silicon particles (S1MPs) that can be loaded with one or more types of stage 2 nanoparticles (S2NPs), which can in turn carry either active agents or higher-stage particles. By loading S2NPs inside the pores of the S1MPs, the enzymatic degradation and RES uptake of the S2NPs would be prevented. Vascular penetration enhancers and other molecules can also be simultaneously delivered by the MDS to increase the crossing of the endothelial barrier. Future embodiments of the MDS are envisioned to integrate biological recognition moieties to allow for their selective targeting. In addition, the treatment can be optimized for the specific pathology treated by loading the particles with drugs such as chemotherapeutic agents, humanized antibodies, small interfering RNAs, and other agents. This novel, multi-stage strategy may eventually lead to significant improvements in therapy efficacy, and reduced systemic toxicity as well reduction in the total amount of injected drugs.

BIOCOMPATIBILITY AND BIODEGRADABILITY OF SILICON

Biocompatibility of medical device refers to its ability to perform its intended function, without eliciting any undesirable local or systemic effects in that host [39]. While silicon is a chemically-stable material, the oxidized silicon surface can show bioactivity, manifested in a layer of hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, which grows on the surface when exposed to a physiological solution. Recently, there have been reports on the testing of silicon and other MEMS materials as implantable candidates using ISO 10993 physicochemical and biocompatibility test [40, 41]. Silicon shows biocompatible but biofouling (the growth of adherent cells occurs)

Porous silicon has recently been discovered to have new biomedical properties [42, 43]. The bioactivity and biodegradation of porous silicon can be controlled by porosity and pore size. While high porosity silicon dissolves quickly in physiological buffers, biodegradation of medium porosity silicon is slower. Si dissolves mainly into monomeric silicic acid $\text{Si}(\text{OH})_4$, which is the

most natural form of Si in the environment and shows no toxicity. Silicon is an essential nutrient, and the average daily intake of silicon is about 20-50mg/day.

We showed in our pre-clinical studies that porous silicon is fully biodegradable and that the particles completely disappeared in 18-24 hours in physiologic solution, in 8-10 hours in serum and in less than 4 hours in whole blood. We cultured different cell types with porous silicon particles, and followed biocompatibility and cellular fate through proliferation assay, cytotoxicity assay, activation of the apoptotic program of the cells and cell cycle analysis. In all the experimental settings, the silicon particles proved to be completely harmless to cells.

CONCLUSION

Photolithographic methods applied to silicon and an ever-expanding spectrum of organic and inorganic materials have beneficially impacted biology and medicine, in the context of research and diagnostic laboratories. Silicon and silicon-based materials and methods first demonstrated their biomedical applicability in microfluidic systems, biomolecular sensors and microarrays. Many novel technologies are emerging that will be instrumental for the post-genomic era, and the advent of personalized medicine. Therapeutic uses of silicon include the controlled-release delivery of drugs and radiation from implants. Biodegradable and biocompatible nanoporous silicon-based multistage particulates open a new frontier for the intravenous, biologically targeted delivery of therapy across biological barriers. With these, itself silicon graduates from the role of the fabrication support enabler and gains citizenship in the domain of the true biomedical materials.

ACKNOWLEDGEMENT

These studies were supported by the following grants: DoDW81XWH-04-2-0035 Project 16, DoDW81XWH-07-2-0101, DoDW31P4Q-07-1-0008, NASA SA23-06-017, State of Texas, Emerging Technology Fund, NIH NCI 1R21CA122286-01, 1R01CA128797-01, and NSF ECCS-0725886. The authors would like to recognize the support from the Alliance for NanoHealth (ANH) and Center of Nanomedicine, The University of Texas Health Science Center at Houston.

REFERENCES

- [1] A. Manz, N. Graber, and H. M. Widmer, "Miniaturized Total Chemical Analysis System- A Novel Concept For Chemical Sensing," *Sensors and Actuators B-Chemical*, vol. 1, pp. 244-248, Jan 1990.
- [2] D. J. Beebe, G. A. Mensing, and G. M. Walker, "Physics and applications of microfluidics in biology," *Annual Review of Biomedical Engineering*, vol. 4, pp. 261-286, 2002.
- [3] Y. N. Xia and G. M. Whitesides, "Soft lithography," *Annual Review of Materials Science*, vol. 28, pp. 153-184, 1998.
- [4] T. A. Desai, W. H. Chu, J. K. Tu, G. M. Beattie, A. Hayek, and M. Ferrari, "Microfabricated immunoisolating biocapsules," *Biotechnology and Bioengineering*, vol. 57, pp. 118-120, Jan 1998.
- [5] J. K. Tu, T. Huen, R. Szema, and M. Ferrari, "Filtration of Sub-100nm Particles Using a Bulk-Micromachined, Direct-Bonded Silicon Filter," *Biomedical Microdevices*, vol. 1, p. 8, 1999.
- [6] T. A. Desai, D. J. Hansford, L. Leoni, M. Essenpreis, and M. Ferrari, "Nanoporous anti-fouling silicon membranes for biosensor applications," *Biosensors & Bioelectronics*, vol. 15, pp. 453-462, Nov 2000.
- [7] G. B. Lesinski, S. Sharma, K. A. Varker, P. Sinha, M. Ferrari, and W. E. Carson, "Release of biologically functional interferon-alpha from a nanochannel delivery system," *Biomedical Microdevices*, vol. 7, pp. 71-79, Mar 2005.
- [8] C. Cosentino, F. Amato, R. Walczak, A. Boiarski, and M. Ferrari, "Dynamic model of biomolecular diffusion through two-dimensional nanochannels," *Journal of Physical Chemistry B*, vol. 109, pp. 7358-7364, Apr 2005.
- [9] W. H. Chu and M. Ferrari, "Microfabricated Filter with Specially Constructed Channel Walls, and Containment Well and Capsule Constructed with Such Filters," *US Patent No. 5,798,042*, August 1998 1998.
- [10] T. A. Desai, D. J. Hansford, L. Kulinsky, A. H. Nashat, G. Rasi, J. Tu, Y. Wang, M. Zhang, and M. Ferrari, "Nanopore Technology for Biomedical Applications," *Biomedical Microdevices*, vol. 2, p. 20, August 1998 1999.
- [11] S. Pricl, M. Ferrone, M. Fermeglia, F. Amato, C. Cosentino, M. M. C. Cheng, R. Walczak, and M. Ferrari, "Multiscale modeling of protein transport in silicon membrane nanochannels. Part 1. Derivation of molecular parameters from computer simulations," *Biomedical Microdevices*, vol. 8, pp. 277-290, Dec 2006.
- [12] F. Amato, C. Cosentino, S. Pricl, M. Ferrone, M. Fermeglia, M. M. C. Cheng, R. Walczak, and M. Ferrari, "Multiscale modeling of protein transport in silicon membrane nanochannels. Part 2. From molecular parameters to a predictive continuum diffusion model," *Biomedical Microdevices*, vol. 8, pp. 291-298, Dec 2006.
- [13] A. Grattoni, M. Merlo, and M. Ferrari, "Osmotic pressure beyond concentration restrictions," *Journal of Physical Chemistry B*, vol. 111, pp. 11770-11775, Oct 2007.
- [14] L. Bousse, S. Mouradian, A. Minalla, H. Yee, K. Williams, and R. Dubrow, "Protein sizing on a microchip," *Analytical Chemistry*, vol. 73, pp. 1207-1212, Mar 2001.
- [15] S. A. Sundberg, "High-throughput and ultra-high-throughput screening: solution- and cell-based approaches," *Current Opinion in Biotechnology*, vol. 11, pp. 47-53, Feb 2000.
- [16] S. Lai, S. N. Wang, J. Luo, L. J. Lee, S. T. Yang, and M. J. Madou, "Design of a compact disk-like microfluidic platform for enzyme-linked immunosorbent assay," *Analytical Chemistry*, vol. 76, pp. 1832-1837, Apr 2004.
- [17] M. Ozkan, M. Heller, and M. Ferrari, *BioMEMS and Biomedical Nanotechnology, Vol. II: Micro/Nanotechnologies for Genomics and Proteomics*, 2006.
- [18] S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, and D. Solas, "LIGHT-DIRECTED, SPATIALLY ADDRESSABLE PARALLEL CHEMICAL SYNTHESIS," *Science*, vol. 251, pp. 767-773, Feb 1991.
- [19] R. J. Lipshutz, S. P. A. Fodor, T. R. Gingeras, and D. J. Lockhart, "High density synthetic oligonucleotide arrays," *Nature Genetics*, vol. 21, pp. 20-24, Jan 1999.
- [20] R. G. Sosnowski, E. Tu, W. F. Butler, J. P. Oconnell, and M. J. Heller, "Rapid determination of single base mismatch mutations in DNA hybrids by direct electric field control," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, pp. 1119-1123, Feb 1997.
- [21] M. M. C. Cheng, G. Cuda, Y. L. Bunimovich, M. Gaspari, J. R. Heath, H. D. Hill, C. A. Mirkin, A. J. Nijdam, R. Terracciano, T. Thundat, and M. Ferrari, "Nanotechnologies for biomolecular detection and medical diagnostics,"

- Current Opinion in Chemical Biology*, vol. 10, pp. 11-19, Feb 2006.
- [22] J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H. J. Guntherodt, C. Gerber, and J. K. Gimzewski, "Translating biomolecular recognition into nanomechanics," *Science*, vol. 288, pp. 316-318, Apr 2000.
- [23] G. H. Wu, R. H. Datar, K. M. Hansen, T. Thundat, R. J. Cote, and A. Majumdar, "Bioassay of prostate-specific antigen (PSA) using microcantilevers," *Nature Biotechnology*, vol. 19, pp. 856-860, Sep 2001.
- [24] T. P. Burg, M. Godin, S. M. Knudsen, W. Shen, G. Carlson, J. S. Foster, K. Babcock, and S. R. Manalis, "Weighing of biomolecules, single cells and single nanoparticles in fluid," *Nature*, vol. 446, pp. 1066-1069, Apr 2007.
- [25] L. A. Liotta, M. Ferrari, and E. Petricoin, "Clinical proteomics: Written in blood," *Nature*, vol. 425, p. 905, 2003.
- [26] D. Geho, M. M. C. Cheng, K. Killian, M. Lowenthal, S. Ross, K. Frogale, J. Nijdam, N. Lahar, D. Johann, P. Herrmann, G. Whiteley, M. Ferrari, E. Petricoin, and L. Liotta, "Fractionation of serum components using nanoporous substrates," *Bioconjugate Chemistry*, vol. 17, pp. 654-661, May 2006.
- [27] M. Gaspari, M. M. C. Cheng, R. Terracciano, X. W. Liu, A. J. Nijdam, L. Vaccari, E. di Fabrizio, E. F. Petricoin, L. A. Liotta, G. Cuda, S. Venuta, and M. Ferrari, "Nanoporous surfaces as harvesting agents for mass spectrometric analysis of peptides in human plasma," *Journal of Proteome Research*, vol. 5, pp. 1261-1266, May 2006.
- [28] G. F. Zheng, F. Patolsky, Y. Cui, W. U. Wang, and C. M. Lieber, "Multiplexed electrical detection of cancer markers with nanowire sensor arrays," *Nature Biotechnology*, vol. 23, pp. 1294-1301, Oct 2005.
- [29] Y. L. Bunimovich, G. L. Ge, K. C. Beverly, R. S. Ries, L. Hood, and J. R. Heath, "Electrochemically programmed, spatially selective biofunctionalization of silicon wires," *Langmuir*, vol. 20, pp. 10630-10638, Nov 2004.
- [30] K. D. Wise, D. J. Anderson, J. F. Hetke, D. R. Kipke, and K. Najafi, "Wireless implantable microsystems: High-density electronic interfaces to the nervous system," *Proceedings of the Ieee*, vol. 92, pp. 76-97, Jan 2004.
- [31] M. Ferrari, "Cancer nanotechnology: Opportunities and challenges," *Nature Reviews Cancer*, vol. 5, pp. 161-171, Mar 2005.
- [32] F. Martin, R. Walczak, A. Boiarski, M. Cohen, T. West, C. Cosentino, and M. Ferrari, "Tailoring width of microfabricated nanochannels to solute size can be used to control diffusion kinetics," *Journal of Controlled Release*, vol. 102, pp. 123-133, Jan 2005.
- [33] J. T. Santini, A. C. Richards, R. Scheidt, M. J. Cima, and R. Langer, "Microchips as controlled drug-delivery devices," *Angewandte Chemie-International Edition*, vol. 39, pp. 2397-2407, 2000.
- [34] A. C. R. Grayson, I. S. Choi, B. M. Tyler, P. P. Wang, H. Brem, M. J. Cima, and R. Langer, "Multi-pulse drug delivery from a resorbable polymeric microchip device," *Nature Materials*, vol. 2, pp. 767-772, Nov 2003.
- [35] A. B. Foraker, R. J. Walczak, M. H. Cohen, T. A. Boiarski, C. F. Grove, and P. W. Swaan, "Microfabricated porous silicon particles enhance paracellular delivery of insulin across intestinal Caco-2 cell monolayers," *Pharmaceutical Research*, vol. 20, pp. 110-116, Jan 2003.
- [36] A. S. W. Goh, A. Y. F. Chung, R. H. G. Lo, T. N. Lau, S. W. K. Yu, M. Chng, S. Satchithanatham, S. L. E. Loong, D. C. E. Ng, B. C. Lim, S. Connor, and P. K. H. Chow, "A novel approach to brachytherapy in hepatocellular carcinoma using a phosphorous(32) (P-32) brachytherapy delivery device - A first-in-man study," *International Journal of Radiation Oncology Biology Physics*, vol. 67, pp. 786-792, Mar 2007.
- [37] P. Decuzzi and M. Ferrari, "Design maps for nanoparticles targeting the diseased microvasculature," *Biomaterials*, vol. 29, pp. 377-384, Jan 2008.
- [38] E. Tasciotti, X. Liu, R. Bhavane, K. Plant, A. Leonard, B. Price, M. M. Cheng, P. Decuzzi, J. T. F. Robertson, and M. Ferrari, "Mesoporous silicon particles as a multistage delivery system for imaging and therapeutic applications," *Nature Nanotechnology*, March 2008.
- [39] A. Remes and D. F. Williams, "Immune Response in Biocompatibility," *Biomaterials*, vol. 13, pp. 731-743, 1992.
- [40] G. Kotzar, M. Freas, P. Abel, A. Fleischman, S. Roy, C. Zorman, J. M. Moran, and J. Melzak, "Evaluation of MEMS materials of construction for implantable medical devices," *Biomaterials*, vol. 23, pp. 2737-2750, Jul 2002.
- [41] G. Voskerician, M. S. Shive, R. S. Shawgo, H. von Recum, J. M. Anderson, M. J. Cima, and R. Langer, "Biocompatibility and biofouling of MEMS drug delivery devices," *Biomaterials*, vol. 24, pp. 1959-1967, May 2003.
- [42] L. T. Canham, "Bioactive silicon structure fabrication through nanoetching techniques," *Advanced Materials*, vol. 7, pp. 1033-&, Dec 1995.
- [43] L. T. Canham, C. L. Reeves, J. P. Newey, M. R. Houlton, T. I. Cox, J. M. Buriak, and M. P. Stewart, "Derivatized mesoporous silicon with dramatically improved stability in simulated human blood plasma," *Advanced Materials*, vol. 11, pp. 1505-+, Dec 1999.