

Advanced Nanotechnological Approaches for Designing Protein-Based "Lab-on-Chips" Sensors on Porous Silicon Wafer

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Abstract: In this article, we will review the more recent patented approaches related to the design and development of micro- and nano-patterns of biomolecules on solid substrates for the realization of innovative biochips, including inkjet and spotting technology, and Scanning Probe Methods. In addition, we will report on some important patents based on the use of porous materials as substrates, exploiting the large specific surface for the design of highly sensitive biodevices. The main advantages and drawbacks related to each technological approach to the biochips fabrication will be pointed out, and future perspectives in the field will be discussed.

Keywords: Biosensors, porous silicon, Atomic force microscopy, nanolithography, biochip, bioarray, nanotubes, proteins.

INTRODUCTION

Nowadays, numerous interdisciplinary fields, such as biosensing and bioelectronics, require methods to immobilize biomolecules in specific portions on miniaturized solid substrates. In particular, the fabrication of advanced integrated "lab-on-chips", useful for the achievement of rapid and accurate analysis, is strictly connected with the progress in the micro- and nano-technology field. Indeed, the possibility of controlling the binding of biomolecules (proteins, antibodies, enzymes) on a chip with sub-micrometer resolution allows the fabrication of innovative high-performance biodevices, such as nanosensors and ultradense nanoarrays.

Among the main nanotechnological approaches addressing this issue, scanning probe methods appear very promising, due to their precise spatial control, which is missing in the so-called "bottom-up" approach, based on self-assembling processes. For instance, the Atomic Force Microscopy (AFM) tips can be used to construct nanoarrays of biomolecules, with the Dip Pen Nanolithography (DPN) technique [1] or the AFM nanografting method [2]. Recently, we have proposed a method based on Electron Beam irradiation which permits to easily create protein nanopatterns on a porous silicon wafer [3]. Proteins are very useful building blocks in the realization of a "Lab-on-Chip" due to their extreme specificity in binding analytes.

In this article, we will review some of the more recent patented approaches to the fabrication of micro- and nano-patterns of biomolecules on solid substrates, for the realization of innovative biochips.

SPOTTING AND INK-JET TECHNOLOGIES

The "dispensing" techniques are the backdate technologies for printing array of biological molecules, DNA and proteins, on solid substrate and the common approaches for

the array fabrication include the mechanical micro-spotting technique [4, 5] and the ink-jet ejection method [6,7]. The micro-spotting implies the contact with the substrate and it was developed first while the ink-jet is a non-contact printing technique and its use in the fabrication of "bioarray" is relatively recent. The ink-jet method can be further divided in two distinct types due to the ejecting actuators of the printing heads: thermal, also known as bubble-jet, and piezoelectric [8]. The most representative patents related to the upward technologies will be described starting from the 1997 patent of the Hewlett Packard (HP) company which manufactured a biological probe array using a vision-assisted micropipetting technique [9].

The mechanical spotting, in the HP patent, is achieved by pressurizing the micropipette to produce a droplet of the biological probe at the open tip of the micropipette and then by dispensing the droplet to a solid substrate following two different approaches: 1 - the droplet is brought in contact with the substrate, by lowering the micropipette or by rising the substrate; 2 - the micropipette is withdrawn, with a rapid acceleration, in a direction away from the substrate enabling the droplet to fall due to gravity force.

The droplet volume was estimated by visual monitoring its dimension with a video camera or a linear CCD array. The visual system is used also to estimate the droplet position on the array by monitoring the tip of the micropipette in relation to indices disposed on the substrate. Fig. 1 illustrates: a) a perspective view of the micropipette used to spot biological probes onto the array; b) the dispensing process; c) the vision apparatus to estimate the droplet volume and the droplet position on the array.

The patent by Brown and Shalon published in 1998 [5] used a similar method of spotting and specifically it involves the dispensing of a known volume of reagent by tapping a capillary dispenser on the target substrate. The capillary (Fig. 2) is formed by a pair of space-apart coextensive elongate elements adapted to hold a fixed amount of desired analytes

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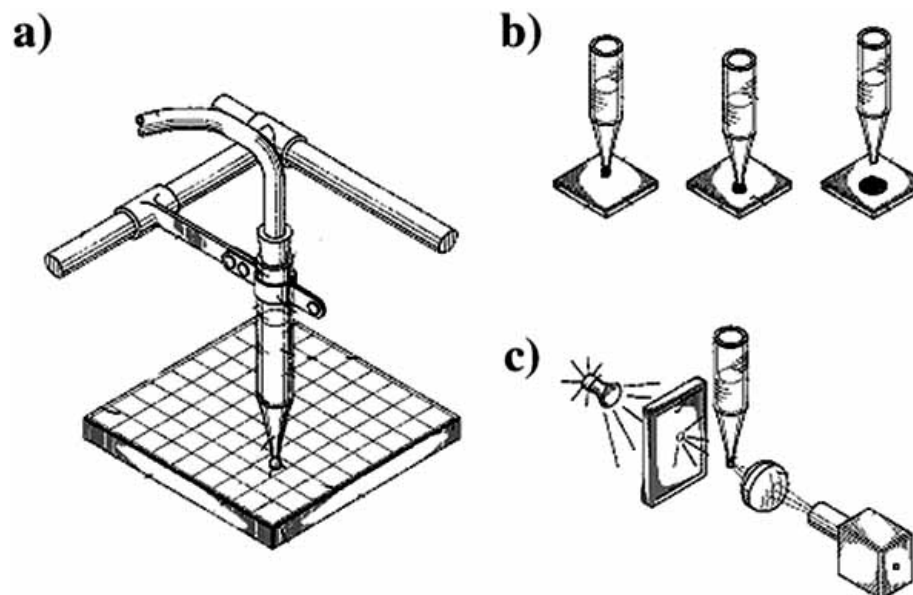


Fig. (1). Description of the invention reported in patent of ref. [9]: (a) view of the micropipette; (b) dispensing process; (c) vision apparatus.

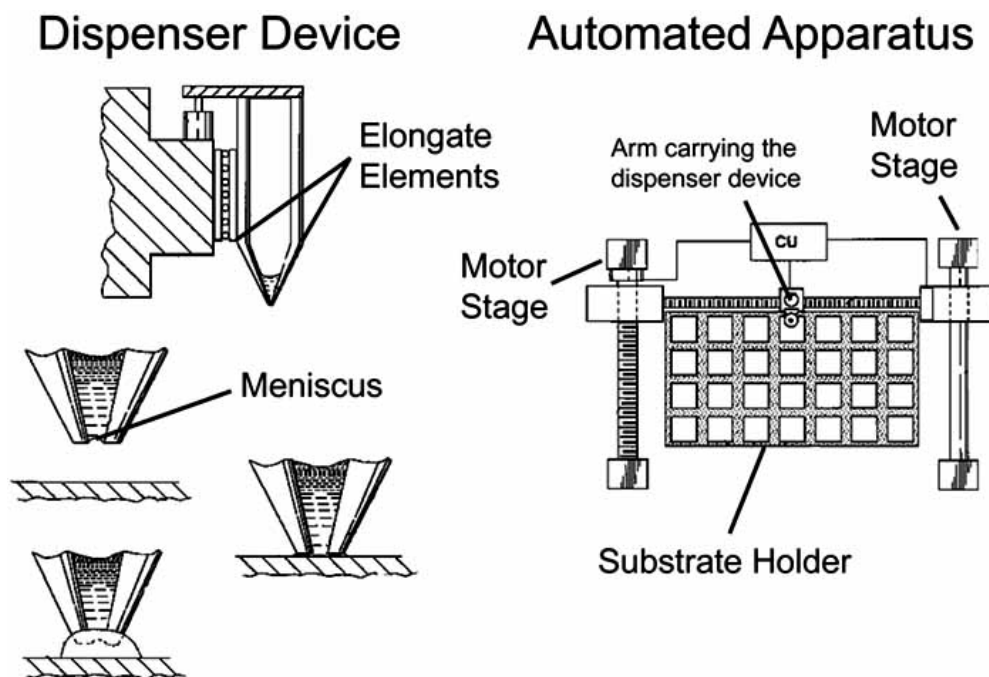


Fig. (2). Patent of ref. [5]: schematic of the dispenser device and of the automated apparatus used to form the array.

and having a tip region at which the analytes solution in the channel forms a meniscus.

To deliver the analyte, the tip of the dispenser is dipped into the reagent of interest and then tapped against the substrate at a defined position with an impulse effective to break the meniscus in the capillary channel and deposit a selected volume of solution (range 0.01 to 100 nl) on the surface. These two steps can be repeated several times giving rise to the desired array. The other new aspect of this patent is the automated apparatus (Fig. 2) proposed to form the microarray.

The dispensing methodologies based on mechanical spotting, here described, were overcome when in 2000 Okamoto *et al* found no damage, due to the high temperature, in the DNA strands (strands of DNA with 10 to 300 bases with concentrations ranging from 0.02 to 1.6 mg/ml have been tested) ejected on a solid substrate using a thermal ink-jet printer. This was the demonstration that ink-jet technologies can be used to prepared bio-array. Moreover in the same year Roda *et al* [10] used the same technique to print enzymes without detecting any loss of activity of the enzyme.

Some advantages of the ink-jet method are: a) non-contact spotting technique (also possible with the technique developed by HP); b) possibility to deliver droplet of small volume (picolitre range) insuring array with high density of biomolecules; c) good reproducibility of the droplet volume.

The ink-jet printing technology to prepare bio-arrays was patented by Okamoto and Yamamoto in 2002 using the bubble jet variation (Fig. 3) of ink-jet technology developed by Canon.

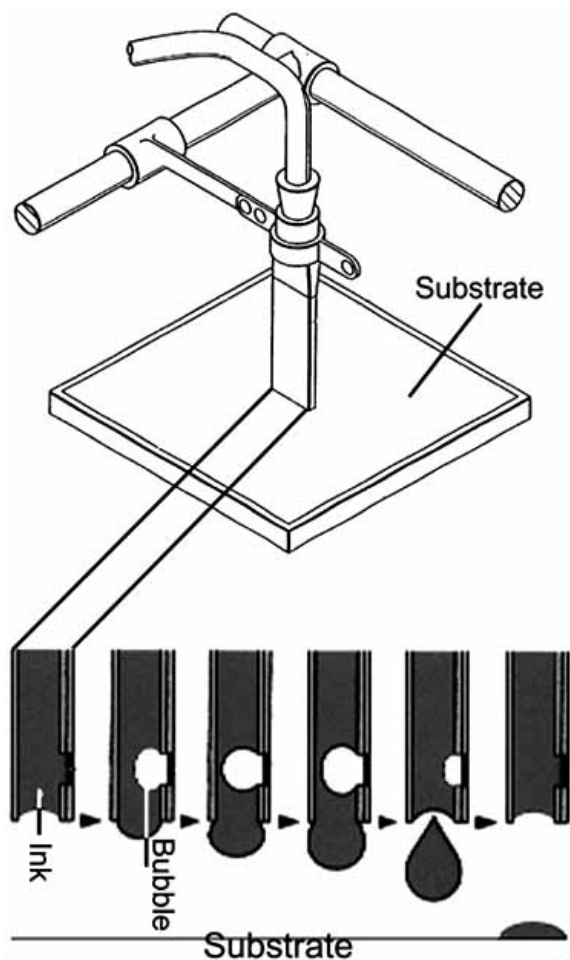


Fig. (3). Illustration of the bubble-jet technology.

Fig. 3 illustrates the liquid supply system (nozzle) which brings the liquid containing the probe such as DNA or enzymes to the printing head (a) and the bubble jet head (b) which consist of an ink reservoir and a resistive heater brought in contact with the ink. When the heater element is actuated a bubble is formed in the ink allowing droplet formation. Droplet size can be easily controlled by tuning the temperature, the frequency and the ink viscosity.

THE MULTI-ELECTRODE APPROACH

A microelectrode array (MEA) is an arrangement of several (typically more than 60) electrodes allowing the targeting of several sites for stimulation and extracellular recording at once. Typically, MEAs are used to examine the activities of whole cells and tissues rather than single

receptors (as in patch clamp experiments), studying the interaction of several cells in a culture or in their natural environment or even in whole organs. Scaling down to the sub-micrometer size, nanoelectrode arrays (NEA) have been fabricated employing nanotubes or nanowires. For instance, a nanoelectrode array based on vertically aligned multi-walled carbon nanotubes embedded in SiO_2 has been used for ultrasensitive DNA detection [11], and platinum nanowire arrays have been employed in the fabrication of a glucose sensor [12]. Thus, MEAs and NEAs can be considered as promising technological approaches for designing novel "Lab-on Chips". In the last years, some interesting inventions exploiting MEAs and NEAs for the fabrication of biodevices have been patented.

Voeroes and coworkers [13] patented a method for electrochemically patterning a MEA with at least two different kinds of macromolecules. This method is based on the finding that an adlayer of a polymer (e.g. protein-resistant polymer, in particular poly (L-lysine) -grafted- poly (ethylene glycol) (PLL-g-PEG)) can selectively be desorbed from a conductive microelectrode surface when it is subjected to a specific voltage. Subsequent to the desorption of the adlayer, probes of interest may be immobilized on the bare microelectrode surfaces. Suitable probes comprise proteins, DNA/RNA, carbohydrates, vesicles, but also cells, cell parts and combinations thereof. By providing a platform that is able to be selectively electrically polarized, and repeating the desorption/adsorption steps with a second or further kind of macromolecules, one can produce microchip arrays useful to study a large diversity of biological interactions, e.g. protein-protein interactions, protein-cell interactions, protein-nucleic acid interactions, etc.

Another patent [14] describes a similar method for the fabrication of multisensors chips for detecting analytes. In this case, a multielectrode chip, lithographed in a wafer, is put in contact with a solution of colloidal particles modified with an element of chemical or biochemical recognition. This element is selectively deposited over the electrode on which a potential has been applied. By serial repetition of the process, a biomolecular array with photolithographic resolution is obtained, and the fabrication of a chip for the simultaneous analysis of several analytes can be obtained.

C.-T. Lin and coauthors patented a microelectrode-based method to obtain a reconfigurable protein patterning process [15]. Operation of the electrode is based on a phenomenon called "electrowetting," where surface wettability can dynamically be controlled by varying the voltage across the device electrodes. When an electric field is applied across the electrode layers, the surface accumulates charge and becomes hydrophilic, binding the proteins to the surface via ionic bonding. Electrically controlling the amount of the surface charge permits controlled protein surface affinity. The device provides a means for reconfigurable protein patterning, which can be used for designing biodevices.

THE DIRECT WRITING APPROACH

The Scanning Probe Microscopy (SPM) techniques, such as AFM and Scanning Tunneling Microscopy (STM), are very useful and effective for nanoscale patterning and lithography, due to the inherent fine scale associated with

sharp SPM tips, and the ability of SPM machines to provide fine-scale imaging and spatial registration. Thus, SPM-based methods can be employed to fabricate innovative biodevices, using the SPM tips to directly write the biopatterns on solid substrates. For instance, some papers in literature report on employing AFM tips to create biomolecular nanopatterns, e.g. by local oxidation [16], nanografting [2], Dip Pen Nanolithography [1].

Among these methods, the latter (DPN) is one of the most interesting and promising. DPN is a scanning probe nanopatterning technique in which an AFM tip is used to deliver molecules to a surface via a solvent meniscus, which naturally forms in the ambient atmosphere (Fig. 4). This direct-write technique offers high-resolution patterning capabilities for a number of molecular and biomolecular 'inks' on a variety of substrates, such as metals, semiconductors, and monolayer functionalized surfaces.

Mirkin and coauthors have patented the technology [17] which exploits the ultrahigh resolution patterning carried out by DPN to construct peptide and protein nanoarrays with nanometer-level dimensions. The peptide and protein nanoarrays exhibit almost no detectable nonspecific binding of proteins to their passivated portions. This work demonstrates how dip pen nanolithographic printing can be used in a method to generate high density protein and peptide patterns, which exhibit bioactivity and virtually no non-specific adsorption. It also shows that one can use AFM-based screening procedures to study the reactivity of the features that comprise such nanoarrays. The method encompasses a wide range of protein and peptide structures including, for example, enzymes and antibodies. Features at or below 300 nm can be achieved.

Another similar patent involving AFM tips describes the use of a dedicated apparatus (nanoscale molecular arrayer)

for the formation of array that includes one or more deposition domains comprised of one or more deposition materials [18]. The invention may include an X,Y controller, an X, Y translation stage, a loading substrate, a deposition substrate, a Z controller, and a deposition probe. A computer controls all of the relative positions of each of the components. Furthermore, the method utilizes a humidity control system to create a capillary bridge between the probe and the substrate for transferring the deposition material between the loading substrate, the deposition probe, and the deposition substrate.

A technology based on the application of scanning probe methods in the multi-electrode approach has been patented by Protiveris Inc. [19]. The invention is based the fact that ultra small structures of a single or a few atomic layers can be built on a semiconductor surface by STM. Since these nanostructures can be built using different chemical elements (or the voltage applied to the structure can be selectively varied) and the spatial distribution, height width and shape of the structures can also be varied, these structures can be built in clusters to serve specifically as "molecular electrodes" whose electrochemical properties and spatial distribution can be made to correspond precisely with the external three dimensional shape and electrochemical properties of molecules, e.g. proteins. Therefore each of these clusters can serve as individual electronic protein "receptors" (or detectors). Since a very large number of these molecular electrodes can be placed on a single chip, the resulting nano-electrode arrays can be used to detect, characterize and quantify many different proteins on a single chip. In a variation of the technology, the chip can also be used to sequence DNA.

Another patent describes the integration of a direct writing method with the ink-jet approach [20]. This technology, named Biological Laser Printing (BioLP), is

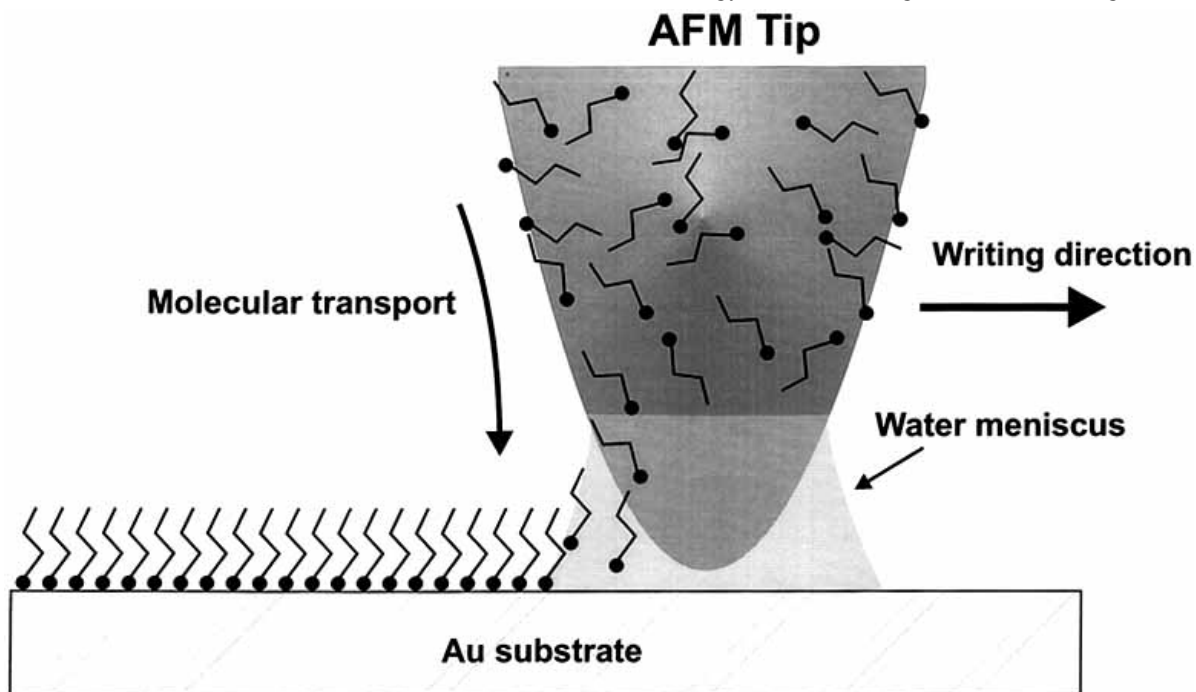


Fig. (4). Illustration of the Dip Pen Nanolithography technique.

based on a method of laser forward transfer. A focused laser pulse is utilized to transfer protein solutions, thereby eliminating the potential for orifice clogging, air bubbles, and unnecessary volume loss potentially encountered in commercially available spotting technologies. Photon energy is directed through a photon-transparent support and absorbed by an interlayer coated thereon. The energized interlayer causes the transfer of a biological material coated thereon across a gap and onto a receiving substrate (Fig. 5). BioLP has been used to print large-scale patterns of proteins, and the results have demonstrated reproducible protein printing with high resolution and efficiency [21].

BIOMOLECULES ON POROUS SUBSTRATES

It has been widely demonstrated that porous materials can be a very convenient choice as substrate for biochips and biodevices. Indeed, the inner surface of these materials can be exploited for the immobilization of large amount of biomolecules, allowing the fabrication of highly sensitive devices. Thereby, many inventions based on porous substrates have been patented in the field of biotechnology. For example, Nomoto and Tohda [22] have patented a method to provide a biochip substrate which is suitable for high density and high precision immobilization of DNA probes by stamping and for fabrication of a biochip which secures a high S/N ratio and high spot homogeneity at each spot without blurring or other problems when used. This method uses as biochip substrate a porous layer which contains inorganic particles such as silica particles or alumina particles and preferably has an average pore radius of from 1 to 100 nm and a pore volume of 0.1 to 5 cm³/g.

Among porous materials, Porous Silicon (PS) has been the object of the greatest attention from researchers, due to some important properties as photoluminescence, biocompatibility and it is easy to integrate it in microelectronic devices. Also, many patented biotechnologies involve the use of PS substrates.

Canham patented PS as a biomaterial [23], showing by *in vitro* experiments that certain types of PS cause the deposition of apatite deposits both on the PS and neighbouring areas of bulk silicon when immersed in a simulated body fluid solution. This deposition of apatite provides an indication that PS of appropriate form is bioactive, and therefore also biocompatible. A form of PS is dissolved in the simulated body fluid solution and this is an indication of a resorbable biomaterial characteristic. Thus, bioactive silicon may be used in the fabrication of biosensors for *in vitro* or *in vivo* applications.

Another patent describes a method for simultaneously detecting and separating a target analyte, such as a protein or other macromolecule, by means of PS [24]. This method includes providing a PS matrix on a silicon substrate, exposing the PS matrix to an environment suspect of containing the target analyte, observing optical reflectivity of PS, and correlating the changes in the silicon substrate to the target analyte. Thereby, the PS nanostructures are used to separate and, in preferred embodiments of the invention, simultaneously detect a variety of target analytes, such as a macromolecule, protein, protein fragment, polymer, biomolecule, biopolymer, biological cell, a small molecule or other molecular complex in a nanocrystalline matrix.

A PS-based method for the measurement of molecular binding interactions has been patented by Rauh-Adelmann and coworkers [25]. In this case, ligands are immobilized within pores of a porous silicon interaction region produced in a silicon substrate, after which analytes suspended in a fluid are flowed over the porous silicon region. Binding reactions occur when analyte molecules diffuse closely enough to the ligands to become bound. Preferably, the binding and subsequent disassociation reactions are observed utilizing a white light source and thin film interference techniques with spectrometers arranged to detect changes in indices of refraction in the region where the binding and disassociation reactions occur. In preferred embodiments

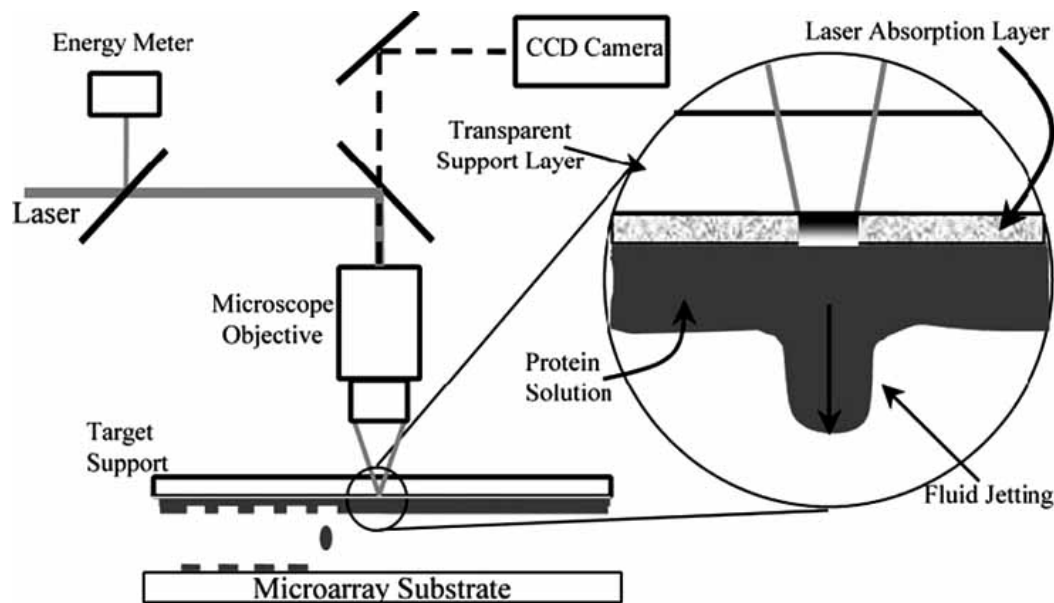


Fig. (5). Illustration of the Biological Laser Printing technology.

both ligands and analytes are delivered by computer controlled robotic fluid flow control techniques to the porous silicon interaction regions through microfluidic flow channels.

Another patented invention [26] relates to a device comprising a planar macroporous support material based on silicon, which has a plurality of pores with a diameter ranging from 500 nm to 100 m, distributed over at least one surface region, and extending in a continuous manner from one surface to the opposite surface of the support material. The inventive device is suitable for use as a base for a biochip base module in methods for detecting biochemical (bonding) reactions, in addition to analysing for this purpose enzymatic reactions, nucleic acid hybridizations, protein-protein interactions and other bonding reactions in the domain of genome, proteome or active substance research in the fields of biology and medicine.

EBAPS-BASED BIOCHIPS

As illustrated in previous sections, the SPM-based methods appear very promising due to the possibility of direct writing biomolecules with precise spatial control and high resolution. On the other hand, using porous substrates can be a key-factor to fabricate highly sensitive devices, thanks to the large specific surface of the substrate.

We have recently patented an innovative technology which implies the integration of an SPM technique with a porous substrate [27]. In this case, a standard Electron Beam Lithography (EBL) system permits a direct writing process free of organic contamination. As a substrate, we use a hydrogenated porous material, for example porous silicon (PS), easily obtained by electrochemical etching of silicon in hydrofluoric acid solution.

Our method is based on the electron irradiation of fresh PS in a Scanning Electron Microscope (SEM). This procedure leads to obtain Electron Beam Activated Porous Silicon (EBAPS) regions. The three steps involved in this nanopatterning process are summarized as follows:

- (i) anodization of a single crystal Si wafer in HF/EtOH solution, producing a PS layer;
- (ii) electron irradiation of PS in the SEM;
- (iii) addition of protein solution

After rinsing in deionized water and drying under nitrogen stream, we observed that proteins specifically bind to the EBAPS regions. It can be clearly appreciated the localization of proteins driven by the previous electron bombardment of the substrate. The same method has been successfully employed with different kinds of proteins (for example, the glucose-binding protein isolated from *Escherichia coli*, the glutamine-binding protein isolated from *E. coli* and an ultrastable sugar-binding protein isolated from the thermophilic organisms *Pyrococcus horikoshii*), and various biomolecules may be immobilized on a porous substrate by this technology. The EBAPS regions can be defined with sub-micrometer resolution, due to the high-resolution of the electron beam. Furthermore, by varying the electron beam energy, it is possible to control the dimension of the EBAPS patterns even in depth (in the z direction). Thus, 3D nano-bio-patterns can be fabricated by this

technique, exploiting the large inner surface of the porous substrate (see Fig. 6).

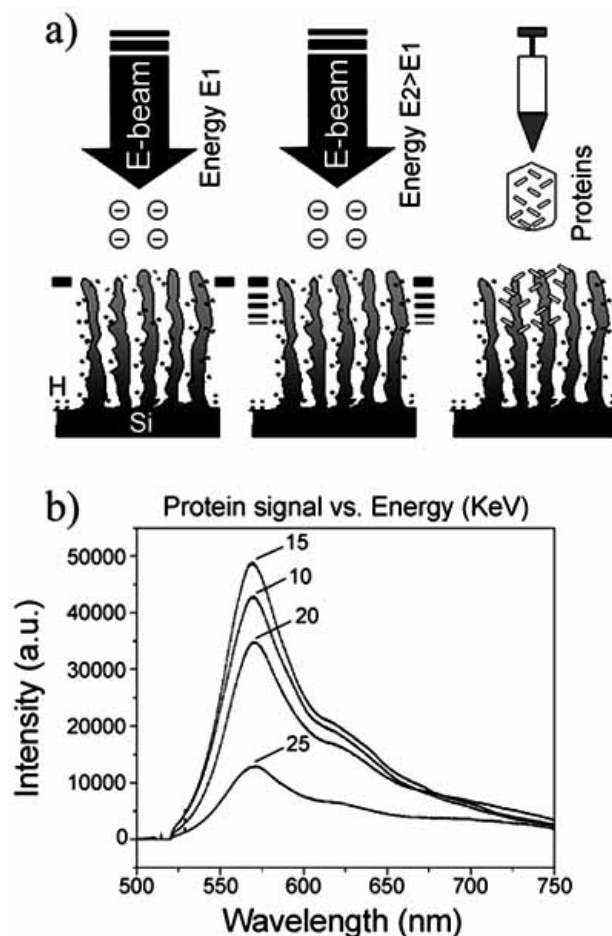


Fig. (6). (a) Schematic of the 3D nanopatterning process driven by the electrons hitting the silicon nanosponge at various energy values. In (b), fluorescence spectra acquired from different regions of the same sample are reported. The sample was irradiated with an electronic dose of 140 mC/cm², and exposed to a rhodamine-labelled glutamine-binding protein solution for 1 hour at 37 °C.

After the immobilization, proteins retain their functionality. Indeed, proteins bind a specific analyte, for example glucose in the case of the glucose-binding protein, even when attached on the porous substrate.

Importantly, the process can be serially repeated, so that different biomolecules can be patterned on the same chip, allowing the fabrication of protein-based Lab-on-Chips.

CURRENT & FUTURE DEVELOPMENTS

So far, several major nanotechnological approaches to the fabrication of biochips have been overviewed. To point out the main advantages and drawbacks related to each technique, we report a schematic comparison in Table I.

It can be clearly seen that "parallel" techniques (spotting and MEAs), although providing high fabrication throughput and good sensitivity of the obtained biodevices, are limited in

Table I.

TECHNIQUE	Speed / Throughput	Spatial resolution	Flexibility	Device sensitivity
Spotting / Inkjet	very high	medium	low	medium/high
MEA	high	medium	low	medium/high
Direct writing / SPM	very low	very high	very high	high
Porous substrates	high	low	low	very high
Spotting on porous substrates	very high	medium	low	very high
SPM on porous substrates (e.g. EBAPS)	very low	very high	very high	very high
Transfer method coupled to SPM on porous substrates	high	very high	high	very high

flexibility and spatial resolution. On the other hand, the "serial" SPM techniques give very good performance in nanofabrication but are very slow. Furthermore, the use of porous substrates, even though presents the advantage of producing very high sensitivity devices, cannot be *per se* a complete biochip fabrication technique. Therefore, an integration between various approaches appears highly desirable. For instance, spotting biomolecules or direct writing by SPM on porous substrates can result in a very fast building of highly sensitive devices, or in very high resolution and good flexibility coupled with high sensing performances, respectively. A further key ingredient to optimize the nanofabrication process may be a transfer methodology to get the replication of biopatterns, as pointed out in the last row of Table I. An example of such approach, called "supramolecular stamping" has been recently reported in literature [27].

The EBAPS technology described in our patent is an example of how advantageous can result the approach of mixing together different ingredients, such as the SPM high resolution and the large specific surface of a porous substrate, for succeeding in the invention of an effective nanofabrication method.

Generally speaking, we think that the integration of direct patterning methods with *bottom-up* approaches shall be a key-factor in the development of future methodologies for the fabrication of advanced nano-bio-devices. For instance, coupling a SPM technique with a peculiar self-organized substrate (e.g. self assembled monolayers, porous materials, block copolymers) may open new opportunities in the realization of Lab-on Chips.

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REFERENCES

- [1] Lee K-B, Park S-J, Mirkin CA, Smith JC, Mrksich M. Protein nanoarrays generated by dip-pen nanolithography. *Science* 2002; 295: 1702-1705.
- [2] Wadu-Mesthrige K, Amro NA, Garno JC, Xu S, Liu G-Y. Fabrication of nanometer-sized protein patterns using atomic force microscopy and selective immobilization. *Biophys J* 2001; 80: 1891-1899.
- [3] Borini S, D'Auria S, Rossi M, Rossi AM. Writing 3D protein nanopatterns onto a silicon nanosponge. *Lab Chip - Miniaturisation for Chem and Bio* 2005; 5: 1048-1052.
- [4] Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995; 270: 467-470.
- *[5] Brown, P.O., Shalon, T.D.: US5807522 (1998).
- [6] Okamoto T, Suzuki T, amamoto NY. Microarray fabrication with covalent attachment of DNA using Bubble Jet technology. *Nature Biotech* 2000; 18: 438-441.
- *[7] Okamoto, T., Yamamoto, N., Suzuki, T.: US20026476215 (2002).
- [8] Allain LR, Stratis-Cullum DN, Vo-Dinh T. Investigation of microfabrication of biological sample arrays using piezoelectric and bubble-jet printing technologies. *Anal Chim Acta* 2004; 518: 77-85.
- [9] Gordon, G.B., Conradson, S.A.: US5601980 (1997).
- [10] Roda A, Guardigli M, Russo C, Pasini P, Baraldini M. Protein microdeposition using a conventional ink-jet printer. *Biotechniques* 2000; 28: 492-496.
- [11] Li J, Ng HT, Cassell A., *et al.* *Nano Lett* 2003; 3: 597.
- [12] Yang M., Qu F, Lu Y, Hey, Shen G, Yu R. Platinum nanowire nanoelectrode array for the fabrication of biosensors *R. Biomaterials* 2006, 27, 5944-5950.
- *[13] Voeroes, J., Texto, M., Tang ,C., Keller, B.: WO2006032158 (2006).
- [14] Katakis, I., Campas, H. M.: WO03062456 (2003).
- [15] Lin, C.-T., Frost, A., Meyhofer, E., Kurabayashi, K., Fan C.Y.: US2006063207 (2006).
- [16] Yoshinobu T, Suzuk J, Kurooka H,,Moon W.C, Iwasaki H, *Electrochim Acta* 2003; 48: 3131.
- *[17] Mirkin, C.A, Della, C.G., Demers, L., Lee, K.B., Park, S.J.: WO03038033 (2003).
- [18] Henderson, E., Mosher, C.: WO02057200 (2002).
- [19] Peeters, J.P.: WO9924823 (1999).
- *[20] Barron, J., Ringeisen, B.R., Kim, H., Wu, P.: US2005018036 (2005).
- [21] Barron JA, Young HD, Dlott DD, Darfler MM, Krizman DB, Ringeisen BR. Printing of protein microarrays via a capillary-free fluid jetting mechanism. *Proteomics* 2005; 5: 4138.
- [22] Nomoto, H., Tohda H.: US2006078939 (2006).
- *[23] Canham, L.T.: WO9706101 (1997).
- *[24] Sailor, M.J.: US2006105043 (2006).
- [25] Rauh-Adelmann C, Patra S, Tigli, H., Martin, P.: US2006063178 (2006).
- [26] Dertinger, S., Fritz, M., Fuchs, K, Haneder, T., Lehmann, V., Martin, A., Maerz, R.: WO03089925 (2003).
- *[27] D'Auria, S., Borini, S.M., Rossi, A.M., Rossi, M.: WO06059356 (2006).