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## Bioengineered Surfaces for Real-Time Label-Free Detection of Cancer Cells

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Nicola Massimiliano Martucci, Nunzia Migliaccio,  
Immacolata Ruggiero, Ilaria Rea,  
Monica Terracciano, Luca De Stefano, Paolo Arcari,  
Ivo Rendina and Annalisa Lamberti

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### Abstract

Biosensing technology is an advancing field that benefits from the properties of biological processes combined to functional materials. Recently, biosensors have emerged as essential tools in biomedical applications, offering advantages over conventional clinical techniques for diagnosis and therapy. Optical biosensors provide fast, selective, direct, and cost-effective analyses allowing label-free and real-time tests. They have also shown exceptional potential for integration in lab-on-a-chip (LOC) devices. The major challenge in the biosensor field is to achieve a fully operative LOC platform that can be used in any place at any time. The choice of an appropriate strategy to immobilize the biological element on the sensor surface becomes the key factor to obtain an applicable analytical tool. In this chapter, after a brief description of the main biofunctionalization procedures on silicon devices, two silicon-based chips that present an (i) IgG antibody or (ii) an Id-peptide as molecular probe, directed against the B-cell receptor of lymphoma cancer cells, will be presented. From a comparison in detecting cells, the Id-peptide device was able to detect lymphoma cells also at low cell concentrations ( $8.5 \times 10^{-3}$  cells/ $\mu\text{m}^2$ ) and in the presence of a large amount of non-specific cells. This recognition strategy could represent a proof-of-concept for an innovative tool for the targeting of patient-specific neoplastic B cells during the minimal residual disease; in addition, it represents an encouraging starting point for the construction of a lab-on-a-chip system for the specific recognition of neoplastic cells in biological fluids enabling the follow-up of the changes of cancer cells number in patients, highly demanded for therapy monitoring applications.

**Keywords:** biochip, surfaces, biosensing, optical, silicon, idiotypic peptide

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## 1. Introduction

Biosensing is a scientific and technological hot topic, given its potential in the field of medical diagnosis [1], healthcare [2], environment [3], defense [4], and food security [5].

Nowadays, the healthcare and pharmaceutical sectors continuously demand more powerful analytical and diagnostic tools for the identification of disease, the development of new medicines, and better diagnostic tests. In these fields, the specific and sensitive detection of targets in short-time analyses plays a key role. While a number of commercial bioassay kits are already on the market, there still remains a major challenge to develop novel biodetection methods to meet the ever-increasing request. High-capability optical biosensing systems are actually emerging as a way to reach this aim [6].

Optical biosensing is a powerful analytical tool used to detect optical changes upon the interaction between an analyte of interest and its ligand, previously immobilized on the sensing device as biological probe; the intensity of obtained signal can be measured, and its value is a function of the analyte concentration in the sample [7, 8]. Generally, optical biosensors use two different detection protocols: direct detection of the analyte (label-free detection) or indirect detection through optically labeled probes (label-based detection). In label-based detection, fluorescent, enzymatic, or radioactive tags, linked on target or on probe molecules, are used; the intensity of the signal indicates the presence of analyte and the interaction with the recognition molecule. This type of detection is extremely sensitive, since it is possible to detect up a single molecule [9, 10]; nevertheless, complex procedures are needed for labeling, and they may interfere with the functionality of the target molecule. In contrast, in label-free detection, target molecules are not labeled or altered and are free to interact in their natural forms: Recent progresses in this field are showing promising results [10]. This strategy is relatively easy and cheap to perform and allows for quantitative and kinetic measurement of molecular interactions. These advantages, with low detection limit, non-destructive approach, and with the ability to recognize a wide variety of analytes or multiple analytes at the same time with fast signal monitoring and analysis [11], make label-free optical detection one of the leading recognition methods in the biosensor field.

Label-free biosensors are very versatile platforms, since they can be developed in different materials, such as silicon or its compounds, glasses, metals, or polymers, and they offer different detection modes and configurations that can be combined [12]. In perspective, optical label-free biosensors are expected to replace fluorescent biosensors in DNA micro-arrays and lab-on-a-chip (LOC) applications [13–15].

The use of silicon-based technology—the same developed for microelectronics—could allow the integration of microfluidic circuits for analyte handling, sensing elements, and control/reading electronics into a single chip. This could pave the way to the mass production of micro total analysis systems and LOCs capable to provide rapid, sensitive, and multiplexed measurements in any place at any time.

The selection of the biological element to immobilize on the sensor surface is dictated by the application and must be chosen to be highly specific for the target molecule and stable enough

to be immobilized without losing functionality. Several types of routes can be used to biofunctionalize the sensor surface [16], and the choice of an appropriate immobilization procedure has become a key factor in the biosensor area. An ideal immobilization procedure should guarantee an efficient coverage by the molecular probes of the sensing layer without interfering with their properties (structure, biological activity, affinity, specificity). In addition, the possibility to preserve their stability in normal storage conditions and also for regeneration could be useful in the case of integration in portable LOC. Despite enormous research efforts have been made to find novel strategies according to the application, a universally valid procedure has not yet been developed and the realization of cheap handheld platforms is almost limited. For this reason, the choice of the most effective strategy of immobilization represents the critical step that turns a sensing device into an applicable analytical tool with the required quality standards. Currently, a lot of biomolecules can be used as bioreceptors (antibodies, nucleic acid, peptides, enzymes, cell receptors, and many others). Among these, artificial peptides provide an opportunity to develop the desired molecular biosensor due to their desirable properties such as diversified structure, high affinity to ligands, matured synthesis protocol, and modified approach [17].

## 2. Biosensing application in cancer

Cancer is one of the main causes of death worldwide. Early diagnosis is the key to enhance the success of medical treatment. In the last few years, in parallel with a growing interest in detecting cancer cells, a wide variety of techniques were developed for detection at the cellular level [18–21]; nevertheless, most of these modalities are expensive and time-consuming, and they are often associated to risks deriving from radioactive tracers.

At this point, despite some considerable achievements, the realization of simple, rapid, non-destructive, and low cost methods for early detection of cancer and minimal residual disease, important for diagnosis and reduction in mortality for certain cancers, still remains an unfulfilled goal [22, 23]. To meet these specific requirements, biosensors have attracted increasing attention since biosensing technology, taking advantage of the properties of biological systems combined to functional advanced materials, is providing rapid, reproducible, and highly sensitive cell detection.

Among the various types of cancer, lymphoma is the most common blood cancer, which incidence is recently increasing. This malignancy is a clonal expansion of neoplastic cells that may result in fatal outcomes [24]. Despite the great progress that has been made over the last several decades in the treatment of lymphoma, the prognosis for patients with particular subtypes of disease remains quite poor. Lymphomas are a heterogeneous group of tumors arising in the reticuloendothelial and lymphatic systems. The major types are Hodgkin lymphoma and non-Hodgkin lymphoma. Great progresses in the use of monoclonal antibodies, chemotherapy, radioimmunotherapy, and peripheral blood stem cell transplants have achieved significant responses in the treatment of these diseases and also markedly improved the outcome of the cure among elected sub-populations of patients, since not all the patients or

subtypes of lymphoma are responsive to these “conventional” approaches. For example, tumorigenic B-cell lymphomas are sensitive to anticancer treatments, including conventional chemotherapy, radiation therapy, and corticosteroids [25]. Nevertheless, the disease is associated with incomplete response to clinical treatments that result in a minimal residual disease in which a few neoplastic cells undetected *in vivo* replenish the cancer cell reservoir. This grim scenario calls for novel strategies to detect tumorigenic B cells. Random peptide libraries (RPLs) allow the selection of therapeutic peptides for tumor cell-surface receptors. Idiotypic determinants of the Ig-BCR, expressed by lymphoma cells, function as a specific tumor antigen that may be exploited for cell-specific detection or targeted therapy. Here, we present the complex different strategies that we adopted in order to construct a biosensor for the detection of tumorigenic lymphoma B cells and discuss all the difficulties that we encountered and the approaches we adopted for their overcoming.

### 3. The choice of appropriate support for biosensing

Our vision was to create a highly sensitive, label-free optical biosensing system for the targeting of patient-specific neoplastic B cells during the minimal residual disease. The material used to develop this unique tool for biosensing include mainly silicon, since it possesses great potential because of its many unique properties, including biocompatibility, which is an important precondition for biological and biomedical applications, abundance, tailorable surface chemistry, and unique electronic, optical, photonic, and mechanical properties, among others. In addition, high surface-to-volume ratio of silicon derivatives offers exciting opportunities to design high-performance silicon-based functional devices for biomedical applications. Moreover, silicon is very abundant on earth allowing inexpensive resources for large-scale and low-cost preparation for practical applications. Taking advantage of these attractive features, the interest in the use of silicon is widely grown leading to its applications not only in biology but also in a lot of other fields [26].

In our research activity, flat silicon devices of fixed thickness (400  $\mu\text{m}$ ) were obtained from highly doped p<sup>+</sup> type, 0.003  $\Omega\text{ cm}$  resistivity, (100)-oriented silicon wafers, cut into 10  $\times$  10 mm square pieces. The wafers were cleaned by a standard RCA process [27] and thermally oxidized at 1050°C for 5 h. An electrochemical etching process of planar silicon was used to pattern porous silicon with a high specific surface (up to 500  $\text{m}^2/\text{cm}^3$ ). An advantage in the use of porous silicon is that its morphology can be tuned by modification of process parameters [28] so that the resulting structures can be adapted to obtain the best performance for chemical and biological processes that happen on their surface. Moreover, the porosity of the material coupled to the low-cost production makes porous silicon an ideal bulky model system to follow each functionalization step: The concentration of reagents and molecules in the pores allow to quantify few nanometers thick films of passivating agents, exploiting signal enhancements, that cannot easily be measured on flat supports.



## 4. Chemical functionalization procedures

The selection of an appropriate procedure for the immobilization of a biological element on the sensor surface that interacts with the desired target for the specific recognition of an analyte has become a critical step in the biosensor area, and enormous efforts are continuously invested in order to optimize novel strategies according to the application. The biofunctionalization of chemical modified surfaces can be achieved in several manners that can be grouped in just two approaches: (i) direct adsorption and (ii) physical adsorption [29–33]: in both cases, each immobilization route presents advantages and drawbacks.

In the direct adsorption method, there is no bond formation between probe and device, reagents are not required so either structure or functionality of biomolecules is not affected. Nevertheless, the efficiency of this strategy is very low.

The bioreceptor physisorption is a quick and widely used approach to immobilize biomolecules on chip surfaces based on electrostatic, hydrophobic, and covalent interactions. Despite the efficiency and the simplicity, electrostatic, and hydrophobic approaches are direct fast methods, since no linker molecules are needed, but are limited to situations that require no directional orientation of the bioprobes. Moreover, both techniques request long incubation times and the risk of folding and desorption due to changes of parameters, such as pH, ionic strength, or temperature, cannot be excluded. The covalent attachment of probe is more efficient in terms of stability and binding strength. Generally, the binding occurs between a functional chemical group of the biomolecules, whose blocking does not affect the functionality, and one on the modified surface. For proteins covalent coupling, amino, carboxylic, or thiol groups are preferred, whereas in the case of nucleic acids, it is possible to take advantage of the versatility of their synthesis to insert reactive groups at the end of the sequence. More difficult is the immobilization of immunoglobulins in a correct orientation, which can be achieved by controlled linkage of carbohydrates groups in the constant region or using affinity proteins (such as A or G Protein) [31]. In all physical adsorption types, a chemical modification of the platform surface is required to the extent that the material properties are tuned to accomplish the best analytical characteristics.

The drawing up of an efficient and correct immobilization procedure is a crucial point to avoid a wide variety of factors that may negatively affect the biosensor functionality. The orientation of probe, the density of coating on the detection surface, pH, target concentration, operating conditions, and chemical environment provided by transducer must be closely explored. An efficient biofunctionalization process should take in account few important observances: The preservation of the molecular probe structure to guarantee subsequent binding of analyte; limited chemical steps and minimal consumption of reagents and samples to make the whole procedure lean and easily reproducible; low optical adsorption at the working wavelengths; homogeneously thin layer formation compatible with evanescent field sensing; uniform surface coating; saturation of eventually free binding sites to reduce the possibility of false-positive signals; biocompatible conditions; integrability with large-scale fabrication. The exploration of these traits offers the possibility to improve biosensors features increasing the power of detection.



**Figure 1.** Types of silicon surface chemical modifications for biosensors: (a) organosilane-based, (b) phosphonate-based, and (c) glutaraldehyde-based strategies.

**Figure 1** represents the main functionalization approaches employed to construct integrated optics (IO) biosensors. Before the biofunctionalization step, a previous chemical activation of the sensor surface is always needed. To this aim, our group employed the self-assembly of organofunctional alkoxy silanes (**Figure 1a**), an easy and versatile system for organic conjugating [34]. However, silicon-based surfaces require a prior activation step to oxidize the surface and to expose the silanol groups for cross-linking with the silane. The formation of a thin silane self-assembled film allows applying a great number of chemical reactions. Immediately before silanization, surfaces are cleaned with oxidant media to remove organic pollutants and to increase the hydroxyl moieties on the surface [35]. The used oxidant is piranha solution [36–39], consisting of a concentrated sulfuric acid mixed with hydrogen peroxide at 3:1 ratio. This treatment is performed by heating for 30 min only. Hundreds of different organosilanes with different structures and functionalities are nowadays commercially available, although the most commonly employed are those with short alkyl chain that present an amino, thiol, epoxy, or carboxylic group at the terminus. Among this vast variety of compounds, 3-aminopropyltriethoxysilane (APTES) was chosen for its reactivity to aldehyde, carboxylic acid, and epoxy functionalities.

The reaction between the oxidized surface and the organosilane is based on the condensation between the Si–O–Si of the silane and the OH present on the device; generally, besides the hydroxyl groups already present on the native silicon oxide layer, a thermal oxidation is a common procedure to form a new efficient oxide film [40–42] in order to assure a plenty of silanol groups for an efficient coverage of the organic layer.

Furthermore, after silanization, APTES layer was cured at high temperature [43]. The aminosilane is more reactive, and it can be applied on a surface using pure organic solvent. The advantage of the curing is that a more controlled deposition of the compound can be obtained to create a thinner film of the aminopropyl groups on the chip. The self-assembled monolayers generally present a thickness in the range of 1–3 nm and create a nanometer-scale organic thin coat [44, 45].

## 5. Choice of biomolecular probe

At this point, a wide variety of biomolecules (antibodies, nucleic acid sequences, peptides, enzymes, cell receptors) can be used as bioreceptors (**Figure 2**).

**Figure 2.** Types of bioconjugation methods on aminated surfaces: (a) *N*-hydroxysuccinimide-based, (b) succinic anhydride-based, (c) *p*-phenylenediisocyanate-based, and (d) glutaraldehyde-based strategies.

The choice of bioreceptor depends on the intended application of biosensor and it must meet two important requirements: high specificity for the target molecule and high stability to retain its biological activity when immobilized on the support.

A first biofunctionalization approach, based on the covalent bind of a biomolecule on the activated silicon sensor surface, included the use of an IgG antibody as molecular probe directed against B-cell receptor. The chip was treated with the homobifunctional cross-linker glutaraldehyde (GA): This molecule, besides to be employed to form an aldehyde-terminated surface, which allows the reaction of amine groups, by the formation of imines (Schiff bases), acts as spacer in order to keep away from the surface the immobilized bioprobe that can react freely with target molecules [46, 47]. By this strategy, the antibody has been immobilized on



the surface via protein A in an oriented fashion [48]. The whole process is checked monitoring surface changes by ellipsometric measurements and FTIR spectromicroscopy. As reported in Table 1, using a random sampling of four different wells, it was observed for all of them after each functionalization step the surface layer thickness.

Film	Thickness (nm)			
	Sample 1	Sample 2	Sample 3	Sample 4
Oxide	$75.8 \pm 0.4$	$72.4 \pm 0.2$	$75.0 \pm 0.3$	$73.4 \pm 0.2$
Aptes + GA	$3.0 \pm 0.4$	$3.1 \pm 0.3$	$3.1 \pm 0.3$	$2.1 \pm 0.2$
Protein A	$0.68 \pm 0.09$	$0.67 \pm 0.08$	$0.75 \pm 0.1$	$0.85 \pm 0.1$
$\chi^2$	0.54	0.43	0.47	0.45

**Table 1.** Surface layer thickness on four random samples after each step of functionalization measured by ellipsometry technique.

The analyses of the FT-IR spectra led to the identification of several characteristic vibration bands that were coherent with the various functionalization steps. Table 2 reports a list of the major bands identified together to peak assignment.

Predicted peak	Frequency $\text{cm}^{-1}$					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Si-O str	–	1127	–	–	–	–
Si-O-C <i>as</i> str	–	–	1250	1250	1258	1258
–(CH <sub>2</sub> )–str	–	–	1295–1305	–	–	–
–O–CH <sub>2</sub> –str	–	–	1445–1475	–	–	–
Amide II	–	–	–	–	–	1531
C=O str	–	–	–	–	1635	1642
Saturated primary ammine (–NH <sub>2</sub> def)	–	–	1650	–	–	–
Amide I C=O str	–	–	–	–	1650–1680	1638–1687
C=O str	–	–	–	–	1685–1705	1774
N–H str	–	–	–	–	–	3121
Primary ammine –NH <sub>2</sub> str	–	–	3250–3677	–	–	–

**Table 2.** Major bands identified by FTIR spectromicroscopy and corresponding assigned peaks.

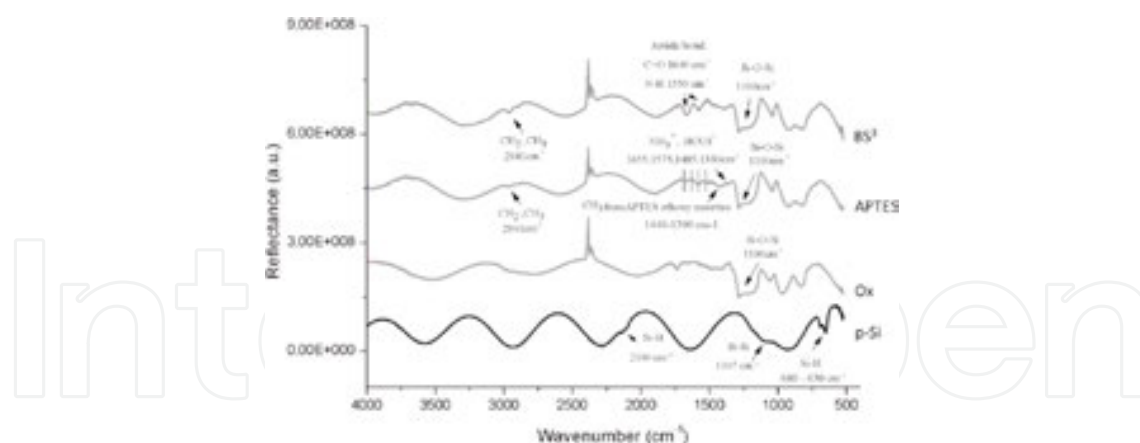
As experimental model, it was chosen a murine lymphoma cell line (A20) [49] that expresses high levels of membrane IgG. The most interesting point of this first approach is that the microfabricated biochip appears to be suitable to reveal specific bindings such as that between

cell-surface proteins (receptor) and corresponding specific antibody. In addition, the number of cells detected by the devices was  $2.0 \times 10^{-3}$  cells/ $\mu\text{m}^2$ .

Anyway, since this detection limit does not seem satisfactory and the idea that the contact probability between cells and antibodies on capture specific surface could be improved, we took advantage of a new functionalization strategy exploiting an Id-peptide as biomolecular probe. The choice of an Id-peptide was dictated by two main reasons: (i) Artificial peptides provide an opportunity to develop the desired molecular biosensor due to their desirable properties such as diversified structure and high affinity [50]. In addition, peptides with specific sequences can provide high affinity to particular ligands and be obtained by screening and optimization of artificial peptide libraries; (ii) the used idiotype peptide is a small peptide ligand able to be recognized with high affinity and specificity from the B-cell receptors present on the lymphoma B cells [51–53]. The use of a small ligand as biorecognition element endowed with great specificity could highly enhance affinity and selectivity of the detection layer. In addition, it simplifies the functionalization procedure with respect to that employed for antibodies in which controlling protein orientation is still very challenging [54]. The peptide was immobilized on the silicon surface following the functionalization strategy schematized in **Figure 3**.

**Figure 3.** Functionalization approach utilized on silicon surface to conjugate an Id-peptide to detect lymphoma cells. After each passivation step, the new synthesized layer is reported in the figure with the same color of the molecule used in the chemical reaction (APTES is blue, BS<sup>3</sup> is red, Id-peptide is green).

This chemical procedure was developed on both crystalline flat and porous silicon samples; the nanostructured porous was chosen because its peculiar morphology allows the immobilization of a greater number of molecules with respect to a planar substrate and a number of functionalization investigation methods could be more easily exploited [55]. The aminosilanized surface has been activated by the homobifunctional cross-linkers bis[sulfosuccinimidyl] suberate (BS<sup>3</sup>), which, acting as spacer, provide succinimidyl-activated carboxyl group that could react with amine-ended peptide to form an amide bond. Changes in chemical composition of PSi surface were monitored by FTIR spectroscopy after each functionalization step until BS<sup>3</sup> (**Figure 4**).



**Figure 4.** FTIR spectra of silicon surface after each chemical modification step.

The analysis of the FTIR spectra in the range from 2500 to 500  $\text{cm}^{-1}$  highlighted characteristic peaks of each molecule used in the different passivation steps, demonstrating the effectiveness of the functionalization procedure. Indeed, the characteristic peaks of Si-Hx bonds corresponding to the PSi after electrochemical etching (2100 and 680–630  $\text{cm}^{-1}$ ) are no longer visible when the devices were thermally oxidized, whereas the appearance of the Si-O-Si characteristic band at 1100  $\text{cm}^{-1}$  was detected.

The formation of the silane film was confirmed by the presence of peaks in the span 1440–1390  $\text{cm}^{-1}$ , relative to  $\text{CH}_3$  from APTES ethoxy moieties, and at 1655  $\text{cm}^{-1}$  relative to an imine group from oxidation of an amine bicarbonate salt [56]. Moreover, the appearance of the peaks at 1640 and 1550  $\text{cm}^{-1}$  that correspond to CO- and NH- groups of an amide bond, confirms the deposition of the BS<sup>3</sup>.

The functionalization of porous silicon surface was also confirmed by spectroscopic reflectometry.



**Figure 5.** Reflectivity spectra on porous silicon surface before (solid line) and after APTES silanization (dashed line), and after BS<sup>3</sup> functionalization (short dashed line).

Reflectivity spectra of porous silicon devices during functionalization steps are reported in **Figure 5**. The deposition on pores walls of a thin layer, constituted by the different organic chemical compounds, produces red shifts of spectra due to the increase of the average refractive index of porous silicon surfaces [57]. After silanization and cross-linker modification, a red shift of 21 and 15 nm was recorded, respectively. The same chemical modifications were performed also on flat silicon devices; in the latter, the whole functionalization procedure was followed by spectroscopy ellipsometry, in order to quantify layer thickness variations after thermal oxidation (SiO<sub>2</sub>), silanization (APTES), and cross-linker functionalization (BS<sup>3</sup>). As showed in Table 3, the thickness of oxidized silicon devices was 74 ± 1 nm; this value increased of 3 and about 2 nm after treatment with aminosilane and BS<sup>3</sup>, respectively.

Film	Thickness (nm)			
	Sample 1	Sample 2	Sample 3	Sample 4
Oxide	74.9 ± 0.3	74.9 ± 0.2	73.5 ± 0.2	74.7 ± 0.3
Aptes	3.5 ± 0.2	2.9 ± 0.3	3.1 ± 0.2	2.7 ± 0.4
BS <sup>3</sup>	1.72 ± 0.08	1.84 ± 0.03	1.82 ± 0.03	1.78 ± 0.02

The values reported are the average of five determinations on each sample.

**Table 3.** Surface layer thickness on four random samples after each step of functionalization measured by ellipsometry technique.

## 6. Biosensing

Once the chemical modified silicon chips have been obtained, a procedure to immobilize a small peptide for label-free detection of cancer cells was settled. The used experimental system takes advantage of the properties of an idiotypic peptide isolated from peptide libraries able to bind the variable region of the B-cell receptor on A20 lymphoma cells [51]. The selected peptide, named A20-36 (pA20-36), whose sequence is EYVNCDNLVGNCVI, was linked on silicon-modified surfaces and used as molecular probe. A random peptide (RND), SSAYGSCKGPCSSGVHSI, was used as negative control. To determine the optimal peptide concentration to obtain a uniform coverage of planar and porous surfaces, a titration was carried out. Based on the obtained results [58], 150 µM concentration was used for both peptides.

The detection of lymphoma cancer cells fulfilled on both planar and porous peptide-modified silicon surfaces is showed in **Figure 6**. The panels *a* and *b* report microscope light images of the planar device surfaces after incubation with a low (100 cells) or high number (50,000 cells) of A20 cells. The choice of the high number of cells was made in order to have saturation binding conditions. The same number of cells (50,000) was incubated on A20-36-peptide-modified porous silicon surface, but a lower number of detected cells were observed on light microscope (**Figure 6**, panel *c*). The chip was not able to bind lymphoma cells when function-

alized with RND peptide (**Figure 6**, panel *d*), whereas no myeloma cells (5T33MM), a surface IgG-positive B-cell line unable to bind to pA20-36 peptide [51], were detected when incubated on the device functionalized with pA20-36 (**Figure 6**, panel *e*).

The number of A20 cells detected on functionalized planar surface device was about 8500 and, taking in account an average area of  $80 \mu\text{m}^2$  for a single cell, filled  $\sim 680,000 \mu\text{m}^2$ , a value concordant with the available functionalized area ( $\sim 1.0 \times 10^6 \mu\text{m}^2$ ); when the detection was performed on porous silicon device, the number of cells that effectively bind the chip was lower (400), filling an area of about  $32,000 \mu\text{m}^2$ . The exiguous number of A20 cells on the porous silicon surface was probably caused by the peculiar morphology of the support; being highly porous, with pore diameter of about 50 nm, and pore upper edges lower than 1 nm in thickness, its inner surface is many order of magnitude greater than the top active one. Hence, just a very low number of peptides are really available on the pore upper edges to bind the cells (that cannot enter into the pores). Therefore, the consequence of this condition is the decrease in the number of cells detected on porous silicon biochip resulting lower respect to that on the planar surface.

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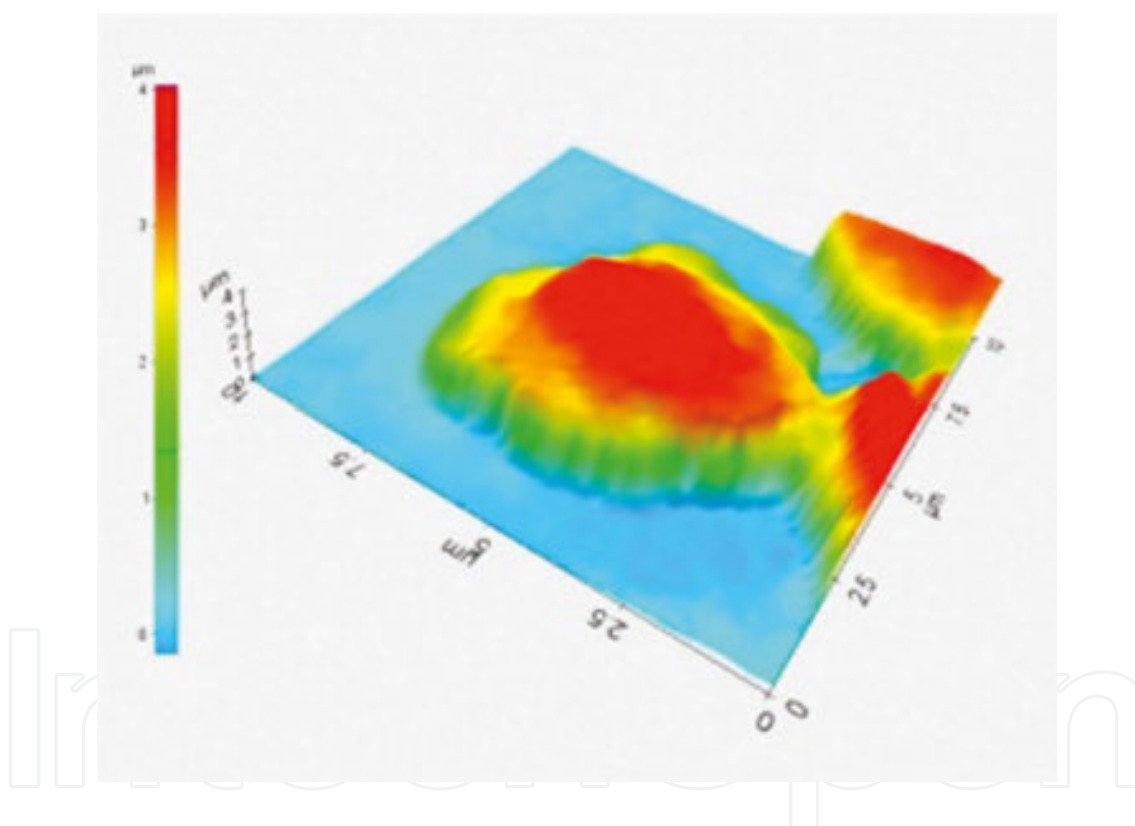
**Figure 6.** Optical images of A20 cell detection on both planar and porous silicon devices. Planar silicon pA20-36 modified sensor after incubation with  $1 \times 10^4$  A20 cells/mL (*a*) and  $5 \times 10^6$  A20 cells/mL (*b*). Porous silicon pA20-36 modified surface after incubation with  $5 \times 10^6$  A20 cells/mL (*c*). Planar silicon RND-modified-sensor after incubation with  $5 \times 10^6$  A20 cells/mL (*d*). Planar silicon pA20-36 modified sensor after incubation with  $5 \times 10^6$  5T33MM cells/mL (*e*).

The surface of each silicon chip presents a functionalized available area of about  $1.0 \times 10^6 \mu\text{m}^2$  so the maximum number of cells that can be bound on the device was 10,000 (covering an area of about  $800,000 \mu\text{m}^2$ ). Since the number of cells detected on planar and porous silicon surfaces was by count 8500 and 400 (evaluated by optical microscopy), the efficiency of detection is 85

and 4%, respectively. Moreover, comparing the efficiency of detection of the flat silicon device based on Id-peptide-BCR recognition with an analogous silicon-based bioanalytical system in which an anti-IgG-BCR was used as molecular probe [48], it is clear that the first biochip resulted more efficient in detecting A20 cells ( $8.5 \times 10^{-3}$  vs.  $2.0 \times 10^{-3}$  cells/ $\mu\text{m}^2$ , respectively).

This difference is likely due to the better accessibility of the A20-36 Id-peptide on the BCR with respect to the anti-IgG. In fact, the binding of the idiotype peptide should occur with the more exposed variable region of the receptor in contrast with the interaction between IgG and BCR in which the variable regions of the immunoglobulin bind the less exposed constant region of the receptor. Furthermore, also the difference in affinity constants between the two ligand-receptor systems coupled to diverse functionalization approaches might have had a decisive role in the detection efficiency.

Cell detection was also investigated by atomic force microscopy analysis (AFM) (**Figure 7**).



**Figure 7.** Representative AFM image in the trace direction of live A20 cells detected on silicon surface.

Both light microscopy and AFM analysis showed a good biocompatibility of substrate since viability and cell morphology were not affected.

At this point, since cancerous cells coexist with other cell types in the body and it is very important to selectively differentiate cancer cells from other ones, in order to assess the real performance of the biochip, lymphoma cells detection was carried out on devices incubated with mixed samples of A20 and 5T33MM cells ( $3.5 \times 10^5/\text{mL}$ ). The detection of lymphoma cells in system mixed is reported in **Figure 8**.





**Figure 8.** Detection of A20 cells (green) in system mixed with 5T33MM cells (red) by fluorescence macroscopy after incubation on planar silicon pA20-36 modified sensor.

The mixed system has been prepared in three different ratios (A20:5T33MM = 1:1, 1:10, 1:100) of the two labeled live cell lines [59]. The efficiency of detection also in a complex system demonstrated the high selectivity of the device, confirming that the use of an Id-peptide immobilized on a silicon-based chip could be a good proof-of-concept for future researches.

## 7. Conclusions

In this chapter, we focused on the functionalization and activation of crystalline and porous silicon surfaces to develop devices allowing the identification of specific ligand-receptor interactions.

As an example, we report new results about the realization of devices suitable to highlight the specific interaction between cell surface receptors and corresponding specific ligands. One of these devices was applied to detect the binding of extremely aggressive murine A20 lymphoma cells to a specific IgG antibody as molecular probe directed against B-cell receptor. The result was encouraging and prompted us to develop an improved device, more sensitive, for the specific recognition of different types of tumor cells. Another approach was based on the specificity of an idiotype peptide endowed with high-affinity toward A20 lymphoma cells. Particularly, the use of an Id-peptide as probe allowed to obtain a uniform sensor surface coating, thus enhancing capture ability also at low cell concentrations. Moreover, the biosensor was biocompatible and showed high repeatability as well as selectivity in label-free cell detection.

The improved device opens the way to the development of unique diagnostic tools in point-of-care testing for recognition and isolation of patient-specific neoplastic B cells during the

minimal residual disease. Any idiotype peptide is ideally endowed with a unique, clone-specific antigenic reactivity. Of course, this approach requires the selection of Id-peptides for each patient through laborious and costly procedures. This might be overcome focusing on a specific B-cell tumors, where a consistent number of patients share the same antigenic reactivity against a restrict pool of Id-peptides. Nevertheless, this strategy can be utilized for the characterization of other specific peptide–receptor interactions through the screening of a recombinant phage library.

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## Author details

Nicola Massimiliano Martucci<sup>1\*</sup>, Nunzia Migliaccio<sup>1</sup>, Immacolata Ruggiero<sup>1</sup>, Ilaria Rea<sup>2</sup>, Monica Terracciano<sup>2</sup>, Luca De Stefano<sup>2</sup>, Paolo Arcari<sup>1</sup>, Ivo Rendina<sup>2</sup> and Annalisa Lamberti<sup>1</sup>

\*Address all correspondence to: [nicolamassimiliano.martucci@unina.it](mailto:nicolamassimiliano.martucci@unina.it)

1 Department of Molecular Medicine and Medical Biotechnology, University of Naples, Federico II, Naples, Italy

2 Institute for Microelectronics and Microsystems of Naples, National Research Council, Naples, Italy

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