Innovative Bioanalytical Tools Combining Microfluidics and Plasmonics for Cell Screening Purposes

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In recent years, part of the scientific community has been moving towards the development of innovate bioanalyticaltools that allow to reach unprecedented resolution and to simplify the protocol of analysis. This is driven by the necessity to satisfy the need in the field of medicine of developing advanced screening and early diagnostic devices. In fact, in the majority of severe diseases, the detection of traces of biological species from the human body (blood and its components, saliva, biopsies, etc.) is of extreme importance for the possible impact on human health. The early detection of several illnesses (e.g. cancer or neurodegenerative diseases or others) could anticipate the therapy of the same disease and arrest its development and progression. This would improve the life quality of the involved persons and on the other hand reduce the health care costs. However, a great number of issues come into play before body fluids may be usedas routinely screening procedures and for early detection of severe illnesses [1].

The handling and analysis of complex biological samples, where individuating a single component among a cluster of molecules, is challenging and conventionally requires complicate protocols to pretreat complex samples [2]. Early diagnosis often is translated in the detection of few molecules in diluted solutions, which are invisible to the current sensors, due to a limit in the resolution of analysis. Moreover, for certain screening procedures is important to not affect the phenotype of the biological sample [3], especially for certain screening procedures which require analysis over long period of a specific sample (e.g. a specific cell populations) so that it becomes important to introduce new methodologies which are not invasive in respect to the analytes. Finally, the heterogeneity of behavior of the human body and its response to medical treatments requires the development of bioanalytical tools, which are compatible with the personalized medicine, and this is translated to the development of devices that are portable, fast, providing in parallel high-throughput and high content analysis with reduced costs.

One way to overcome these issues is the development of microfluidic devices and innovative label-free plasmonic nanosensors [4].

Microfluidics is an interdisciplinary discipline that focuses on the transport, manipulation and analysis of small amount of liquids, cells and particles [5]. Microfluidic devices have been developed and exploited from several research groups as bioanalytical tools [6]. This is due to several advantages over conventional bioanalytical technologies. In fact, these devices guarantee high portability, accurate control of for handling samples, simplified sample pretreatment protocols, low consumption of samples and reagents, high resolution of analysisand low costs of production and analysis[7].

There are many examples in literature of microfluidic devices used for analytical purposes. These have been developed for DNA detection [8], for protein analysis [9], and other biomarkers detection (e.g. discriminating healthy from tumor cells, for phenotypes cell screening, etc) [10-12].

A microfluidic device is capable to handle biological samples with very high accuracy and resolution simplifying the protocol of analysis [13]. A particular and interesting case is the possibility to screen cell populations with single cell resolutions [14].

It is well known that individual cells, even those identical in appearance, differ in numerous characteristics. Due to this heterogeneity, traditional biochemical assays, which analyze cells in bulk, do not allow to get rich information when single cells are studied. Single cell analysis allows investigating cell activities and potentially new biomarkers. It becomes then important for scientific research and clinical diagnostic applications, a sequential handling and manipulation of cells and cell suspensions. Usually, cells are cultured and analysed in large-scale environments such as Petri dishes. Therefore, it is difficult to analyse individual cells without the influence of other cells. These issues can be overcome by using microfluidics [15].

It would be also crucial to analyse the composition of a single cell at a sub cellular level or metabolites, this would bring a step forward the conventional analysis on cells. So that, another critical issue is to individuate a sensing methodology which allow to reach a resolution of analysis at single cell level [16]. Monitoring of cells to over a longer periodic most frequently performed by fluorescence microscopy. However, it is quite common that the fluorescent dyes used for specific subcellular staining interfere with the development of cells, changing their phenotype and metabolism. Another limitation is that fluorescent dyes bleach quite fast. Finally, this technique allows to investigate known molecules only. These aspects make fluorescence staining strategies less desirable in respect to label free sensing methodologies[17]. This can be done, for instance, integrating plasmonic nanodevices and Raman Spectroscopy.

Raman spectroscopy is an optic technique that allows obtaining the vibrational spectrum of a sample. The vibrational spectrum is determined by the chemical composition of the sample. In particular, the Raman effect describes the inelastic scattering between a laser and an emitter (e.g. a molecule) mediated by a vibrational or rotational mode of the emitter. Raman spectroscopy has been used to determine cellular status, such as living cells, dead cells, apoptotic cells, proliferating cells, differentiating cells, tumor or healthy cells. Raman spectroscopy can be integrated in microfluidic devices for having an accurate control over the biological sample (on single cells) allowing to leave them over long period in physiological or conditioned environments.

The main advantages of such a technique are the capability to perform analysis in a label free manner, reducing the steps to pretreat a biological

sample;the specificity of analysis due to a specific finger print for each biological substance the possibility to analyse biological substances in water solutions.

The main disadvantage is the probability that a Raman Effect can happen during the interaction between the sample and the excitation source and this is translated in very weak signals which are very difficult to detect. So that it is very important to integrate into microfluidic devices elements that allow enhancing the Raman signals, the plasmonic nanodevices.

These are metal nanostructures, in which surface plasmons are generated when excited by a laser source, and which, in resonance conditions, produce the enhancement of scattering of emitters placed close to them. An example of this is Surface Enhance Raman Scattering (SERS). SERS describes the enhancement of the Raman scattering by placing the molecule within the near-field of a metallic nanostructure or a roughened metal surface. The highest enhancements recorded to date are about 10^{14}, achieved on roughened silver surfaces which allowed to detect biological samples at femtomolar concentrations, few molecules in a very diluted sample[18].Finally the high spatial resolution of the nanostructures allow to resolve very complex mixtures simplifying the pretreatment protocol of a biological sample[19].

Acknowledgment

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