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Recent advancements in chemical luminescence-based lab-on-chip and microfluidic platforms for bioanalysis

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ABSTRACT

Miniaturization of analytical procedures through microchips, lab-on-a-chip or micro total analysis systems is one of the most recent trends in chemical and biological analysis. These systems are designed to perform all the steps in an analytical procedure, with the advantages of low sample and reagent consumption, fast analysis, reduced costs, possibility of extra-laboratory application. A range of detection technologies have been employed in miniaturized analytical systems, but most applications relied on fluorescence and electrochemical detection. Chemical luminescence (which includes chemiluminescence, bioluminescence, and electrogenerated chemiluminescence) represents an alternative detection principle that offered comparable (or better) analytical performance and easier implementation in miniaturized analytical devices. Nevertheless, chemical luminescence-based ones represents only a small fraction of the microfluidic devices reported in the literature, and until now no review has been focused on these devices.

Here we review the most relevant applications (since 2009) of miniaturized analytical devices based on chemical luminescence detection. After a brief overview of the main chemical luminescence systems and of the recent technological advancements regarding their implementation in miniaturized analytical devices, analytical applications are reviewed according to the nature of the device (microfluidic chips, microchip electrophoresis, lateral flow- and paper-based devices) and the type of application (micro-flow injection assays, enzyme assays, immunoassays, gene probe hybridization assays, cell assays, whole-cell biosensors).

1. Introduction

Over the past two decades, considerable research efforts have been dedicated to the development of miniaturized analytical devices, such as micro fluidic platforms, lab-on-chip, and micro total analysis systems (μTAS) [1]. Such analytical tools should be capable of performing all the steps of the analysis (sample pretreatment, reagents delivery, mixing, separation and detection) on a chip format and in an automated fashion. The possibility to scale down traditional bench-top analytical procedures and to transfer them in a portable miniaturized and self-operating system is paving the way for extra-laboratory analyses in clinical chemistry, environmental monitoring, food analysis, and bio-warfare protection.

With respect to their large-scale counterparts, miniaturized analytical platforms offer several advantages, such as system integration, portability, speed of analysis, low consumption of reagents and samples, enhanced ability to carry out parallel processing (multiplexing), and superior control of reaction conditions. Nevertheless, reaching low limits of detection might be challenging, since small sample volumes translate in few analyte molecules available for detection.

Biochips is a fast emerging field and it is expected that its market will increase up to nearly 9.6 billion dollars by 2016 [2]. Point-of-care (POC) clinical applications, i.e., diagnostic tests performed near the patient and in remote settings with minimal technological infrastructure, dominate the biochips market, especially with lateral-flow immunoassay (LFIA) dipsticks and electrochemical sensors (i.e., i-STAT from Abbott) [3]. Nevertheless, currently commercially available LFIA tests, although easy to use, inexpensive and robust, suffer from low detectability and lack of quantitative information. On the other hand, electrochemical biosensors offer high detectability and amenability to miniaturization, but they are prone to interferences by non-specific redox species and they are

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heavily influenced by temperature, pH and ionic strength variations, electrode fouling and redox by-products accumulation [4].

Optical detection, widely employed in microfluidics, provides instead high sensitivity, non-invasiveness, rapidity and easy implementation [4]. Among optical detection techniques, fluorescence is by far the most commonly employed one. On the other hand, chemical luminescence is particularly advantageous because it offers high detectability even in low volumes. Since the first reports about microfluidic devices exploiting chemiluminescence (CL) [5,6] or electrogenerated chemiluminescence (ECL) detection [7], the number of applications described in the literature have significantly increased, although never reaching the popularity of fluorescence- or electrochemical detection-based systems.

This review describes recent technical advancements and applications in microfluidic and lab-on-chip devices that exploit chemical luminescence detection principles, while non-fluidic microarrays will not be treated here. We emphasize those works which demonstrate on-chip integration of signal trigger and detection and multiplexed and/or integrated analyses. We focus primarily on the developments from 2009 to February 2013.

2. Chemical luminescence systems

Chemical luminescence, i.e., the production of light via a chemical reaction, can be distinguished in different subtypes depending on the type of stimulus able to trigger the reaction: CL and bioluminescence (BL) are referred to the chemical production of light started by mixing the reagents, the latter exploiting enzymes and photoproteins isolated from living organisms [8]; ECL is the luminescence generated by relaxation of exited state molecules produced during an electron-transfer reaction that occurs at the surface of an electrode [9]; thermochemiluminescence (TCL) is the emission of light produced by the thermally-induced decomposition of a molecule.

The analytical interest of chemical luminescence detection techniques mainly arises from their ability to produce photons with no need for photoexcitation, as it occurs in fluorescence detection, thereby avoiding problems arising from light scattering, background fluorescence or light source instability. Therefore, instrumentation for chemical luminescence measurements is in principle very simple, since no excitation source is required. In addition, when chemical luminescence detection is employed in conjunction with imaging detection systems, such as charge coupled device (CCD) or complementary metal-oxide semiconductor (CMOS) cameras, flexible configurations of the reading cell (e.g., the spatial distribution of microarray spots on a functionalized surface) are possible, provided that cross-talk phenomena are controlled. Finally, chemical luminescence detection showed wide dynamic ranges, thus facilitating analysis of samples with very different analyte concentrations. On the other hand, the main pitfall of these detection techniques is the potential effect of sample matrix constituents on the chemical reaction, which can either enhance or inhibit the light producing reaction. These effects are by definition unpredictable, and may lead to artefacts or spurious results. Furthermore, due to the high detectability of the chemical luminescence labels, non-specific binding must be carefully controlled to avoid high background signals and thus surfaces functionalization strategies are crucial for the success of the assay [10]. Another aspect that needs to be taken into account, especially when signal acquisition is performed in a flow regimen, is the a kinetics of photons emission that varies depending on the chemistry employed (ranging from flash- to glow-type). As the chemical luminescence signal is not stable over time, the light-emitting species are subjected to diffusion phenomena in solution, thus causing a loss in resolution [11]. Finally, integration issues are still to be solved, since luminescence reagents delivery and signal detection are most often performed with external systems, such as syringe pumps, cooled CCD cameras, or photomultiplier tubes.

The first analytical applications of CL date back to the late 1970s, with the advent of synthetic CL molecules, such as luminol and its derivatives, 1,2-dioxetanes, and acridinium esters. Nowadays such reactions are still widely employed, although enzymes have replaced CL molecules as labels in binding assays, for their signal amplification capability [8]. When enzyme labels are employed, care must be taken to avoid any situation in which the enzyme activity can be disrupted by experimental conditions and, as the enzyme activity depends on temperature, a thermostated readout cell should be employed. Horseradish peroxidase (HRP) is by far the most used enzyme label, and several efforts were dedicated to the improvement of the analytical performance of the HRP-catalyzed CL oxidation of luminol. For example the addition of enhancers, i.e., boronic acids, indophenols and N-alkyl phenothiazines, significantly increases the light output as well as the emission kinetics, reducing the HRP detection limit down to 8 amol [12]. Alkaline phosphatase is another frequently used enzyme label in CL bioanalyses, however its slow kinetics of light emission, that reaches a plateau several minutes upon addition of the substrate, make this label less suited for microfluidic applications.

As for BL, an amazing phenomenon observed in several terrestrial and marine species, researchers were able to steal the ability to luminescence from fireflies and other organisms (e.g., bacteria, copepodes, worms, click beetle) and to tune the BL emission according to bioanalytical needs [13]. Taking advantage of their high detectability, BL proteins (generally defined as luciferases) are widely used as reporter proteins for bioanalytical applications, such as the study of gene expression and gene regulation, the monitoring of protein–protein interactions and protein conformational changes [14].

ECL shares with CL its main advantages, while allowing a strict temporal and spatial control of the light emitting reaction. This peculiarity makes ECL species particularly appealing as probes for immuno- and geno sensors in a variety of analytical formats [9].

The implementation of TCL as a detection technique for immunoassays was first proposed in the 1980s, when the so-called fluorescence-amplified thermochemiluminescence immunoassay (FATIMA) was described. However this detection technique was soon abandoned mainly because of the high temperatures required to trigger the emission (typically 200–250 °C) and low detectability in comparison with enzyme labels. Nevertheless, being TCL a reagentless luminescence detection technique, it appears to be particularly suited for implementation in lab-on-chip devices. Indeed, recent reports might open new and unexplored perspectives of application of TCL in bioassays [15,16].

Chemical luminescence is also suitable for designing resonance energy transfer (RET)-based assays, involving non-radiative energy transfer between an excited donor and a fluorophore acceptor that are in close proximity [17]. These could represent convenient detection principles also in lab-on-chip devices since bioassays in homogeneous format can be developed. Fluorescence RET (FRET) is by far the most exploited RET technology in bioanalytical chemistry but it suffers from a high background signal caused by the direct excitation of the acceptor by the light source. This drawback is absent in chemical luminescence RET, in which donors are BL, CL, or ECL species. Despite potential advantages, chemical luminescence RET has not been extensively investigated for bioanalytical application. Few microfluidic devices based on chemiluminescence RET (CRET) [18] and ECL-RET [19] have been reported, providing superior detectability. However, in addition to problems inherent in all RET detection technologies, such as difficulties in obtaining correct orientation of the probes and in accurately measuring the