Sensors and Actuators B 171–172 (2012) 148–154

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Label-free, direct DNA detection by means of a standard CMOS electronic chip

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ARTICLE INFO

Article history:
Received 5 October 2011
Received in revised form 15 February 2012
Accepted 17 February 2012
Available online 3 March 2012

Keywords:
Biosensors
DNA sensor
CMOS device
Electronic DNA detection

ABSTRACT

An integrated chip for DNA hybridization detection was realized in a standard CMOS process: it hosts 80 biosensors subdivided in 2 channels, as well as D/A and A/D converters for electrical stimulation and readout. A microfluidic system, bonded on the surface of the chip, provides access to the sensing areas for test solutions and reagents. A simple, low-cost technique for the realization of a biocompatible interface on the aluminum sensing area was developed; it allows the immobilization of single-stranded DNA oligonucleotides on the sensing electrodes. The electrical response of the realized chip to the functionalization and hybridization processes was verified. A direct extrapolation of the density of the immobilized oligonucleotides was possible and this demonstrated the compatibility of the results with electrochemical data available in literature. Experimental results show sensitivity to target concentration down to 100 pM. Selectivity was tested by verifying that no response is generated by oligonucleotides either completely non-complementary or with 5 and 3 modifications.

1. Introduction

Integrated solid-state sensors represent a promising way to perform chemical and biological analysis with simple, reproducible and low-cost techniques. The very large scale of integration provided by the available commercial processes (mainly CMOS, Complementary Metal Oxide Semiconductor) permits the realization of self-consistent and complete systems, which integrate in the same device sensing units and transducing elements and that can be directly interfaced with processing platforms. Such systems could be portable, with low production costs and suitable for several kinds of analyses. In particular, a rising interest is directed to the realization of integrated sensors for DNA hybridization detection. The hybridization process, which determines the formation of a double-stranded DNA helix from two complementary single-stranded DNA, is widely used for genetic analysis and represents the common way for DNA detection. In the standard microarray technology, single-stranded oligonucleotides (probes) are anchored on a passive substrate; DNA sequences under analysis (targets) are labeled with fluorescent dyes and spotted on the same substrate. The surface is then rinsed in order to eliminate a-specific bounds; if hybridization occurs a fluorescent signal is observed in correspondence of the sites where the targets properly bound to the immobilized probes [1]. Performing DNA detection with CMOS devices means changing the substrate into an active component and facing the following challenges: (i) immobilization of chemical and biological species requires a well-defined knowledge of the chemistry of the involved surfaces, i.e. of the materials used in the realization process; (ii) biocompatibility of the materials is required; (iii) parallelization of the analyses is possible only by providing a direct access to the sensing areas with simple and low cost techniques, in contrast with the automated and complex ones used in bulky and sophisticated laboratory equipments (e.g., microarray technology for genetic analysis).

Gold is the most widely used material for the realization of active electrodes for integrated sensors which require the immobilization of biological species onto substrates. However, gold is not a standard material in the commercial realization processes of solid state chips. Lobert et al. [2] demonstrated the possibility of functionalizing CMOS- and bio-compatible materials, like silica and native alumina, with a proper coating of the surfaces with silane species; in particular, silica resulted more suitable for functionalization than native alumina, which is poorly uniform and robust to chemical treatments.

In literature, several examples of complete integrated analysis units can be found. For example, integrated chips for microarray fluorescent analyses have been proposed by Vo-Dinh et al. [3]. Schienle et al. [4] proposed a fully electrical approach for labeled analysis of oligonucleotides, where the hybridization process was detected by means of a redox current generated by a specific molecule used to modify the DNA strands. A similar approach was used by Stagni et al. [5] for a label-free detection based on capacitive measurements: the impedance between the electrodes and the electrolyte solution changes as a consequence of the

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doi:10.1016/j.snb.2012.02.052

immobilization of oligonucleotides on their surfaces. Both these chips are realized with non-standard CMOS process, in order to provide bio-compatible, gold electrodes; moreover, the sensing pads are accessible by means of direct spotting or contact printing of aqueous solutions of oligonucleotides. More recently, Levine et al. [6] proposed a fully integrated electrochemical system based on a CMOS chip, hosting sensor interface electrodes and active electrochemical detection circuitry; the chip is realized in standard 0.25 μm CMOS process, post-processing steps are required in order to realize gold, bio-compatible electrodes covering the standard aluminum pads. The electrochemical cell and the flow tubes are later mounted on top of the chip using epoxy and polydimethylsiloxane (PDMS). A fully CMOS-compatible chip was proposed by Cheng et al. [7], but the detection of oligonucleotides is indirect: probe and target strands are labeled with gold nanoparticles, and the system measures the current flowing between two electrodes when hybridization occurs and a low-impedance path is created by the gold particles.

In this paper we propose an integrated, fully CMOS-compatible chip for parallel and direct electronic readout of DNA hybridization. The chip, realized in standard 0.35 μm CMOS process, represents an innovative evolution of a previously developed chip [8]; it hosts 80 sensing units, temperature sensors and readout circuitry in order to provide a direct interface with processing instrumentation. The sensors are equally divided into two different arrays (channels), in order to allow differential measurements.

The complete parallelization of the operations is obtained thanks to a microfluidic system bound on the chip. It consists of a PDMS flow cell and silica capillary tubes, which permit the injection of the solutions on the sensing units. The microfluidic system is immobilized by means of conformal contact of the PDMS flow cell over the silicon nitride (Si_3N_4) passivation of the chip; plasma activation of the surfaces allows to realize a strong bonding between the structures without requiring glues which may contaminate the sensing areas.

Also, a novel, simple, low-cost and fully reversible process for the functionalization of active areas with single-stranded DNA has been developed. Such process consists of an ozone oxidation of the aluminum electrodes in order to realize a uniform and robust layer of alumina, on which molecules can be anchored by means of silanization processes. This interface is bio-compatible and suitable for biomolecular analysis in aqueous media.

2. Materials and methods

2.1. Oligonucleotides

Two different sequences of probe oligonucleotides have been provided by Sigma–Aldrich, later indicated as P0 (5′-$(T)_{13}$GGTTTCCGCCCCTTAGTG-3′) and P1 (5′-$(A)_{13}$GTCGGTGTAAAGGCTCT-3′). The sequences are modified with a thiol group (HS) in 5′ and with a fluorescent dye (Cy5) in 3′. The 13-bases thymine (adenine) sequence works as spacer, necessary to provide the correct distance from the surface. Similar sequences in terms of length and CG content obtained from 16S rRNA are commonly used for detection of different bacterial species in microarray analyses (Blaškovič et al., [9]).

Hybridization tests were carried out by using several sequences, complementary to the previously-described probes or with a few bases mismatch. T0 (5′-CACTAAGGGGCGGAAACC-3′) and T1 (5′-AGAGCCTTTACACCGACT-3′), provided by Sigma–Aldrich, represent the complementary sequences of P0 and P1 respectively; these strands are modified with fluorescent Cy3 dye in 3′. T0 sequences with five modifications (later referred as T0-5M: 5′-CACTAGATCTCGGAAACC-3′) and with 3 modifications (later referred ad T0-3M: 5′-CACTAAATCGCGGAAACC-3′) were used in selectivity tests; these sequences do not have fluorescent modifications.

2.2. Precursors of self-assembled monolayers

3-Mercaptopropyltrimethoxysylane (3-MPTS, Sigma–Aldrich) is well-known in literature for cross-linking of thiolated molecules and oxides (Alessandrini et al., [10]). After hydrolysis of the alkoxy groups, the central silicon atom can bind to hydroxyl groups normally present on the surface of the oxide. The thiol end group can form a covalent bond with the thiol head group of the oligonucleotides.

6-mercapto-1-hexanol (MCH, Sigma–Aldrich) is an alkyl chain, with a thiol head group and a hydroxyl end group that is used as a spacer in order to avoid the folding of the DNA strands (Herne et al., [11]).

3. Experimental

3.1. Detection mechanism

The sensing units are an improvement of the ones originally described in Barbaro et al. [12]: they consist of a pair of complementary floating-gate transistors, biased by a control capacitor. Part of the floating gate is exposed and works as the sensing area onto which DNA probes should be anchored. The simplified structure of a single sensing unit is shown in Fig. 1(a). The proposed device works as an electric charge sensor: a charge immobilized on the sensing

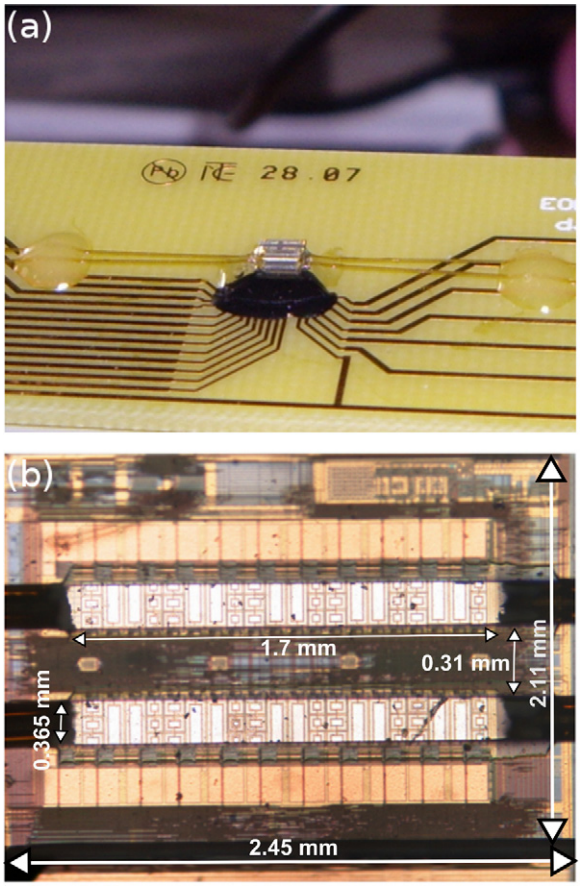

Fig. 1. (a) Structure of the sensing unit and basic working principle; (b) microphotography of the different structures implemented on the chip; (c) Scheme of principle for the readout and acquisition mechanism.