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Low temperature co-fired ceramic package for lab-on-CMOS applied in cell viability monitoring

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Abstract

Lab-on-CMOS chips (LOCMOS) are sophisticated miniaturized analysis tools based on integrated circuit (IC) microchips performing various laboratory functions. We have developed a low temperature co-fired ceramic (LTCC) package for a LOCMOS application regarding cytotoxicity assessment of nanomaterials. The LTCC packaged capacitance sensor chip is designed for long-term cell viability monitoring during nanoparticle exposure. The introduced LTCC package utilizes the flip chip bonding technique, and it is biocompatible as well as able to withstand the environmental conditions required to maintain mammalian cell culture directly on the surface of a complementary metal oxide semiconductor (CMOS) integrated circuit.

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Keywords: Lab-on-CMOS; low temperature co-fired ceramics; electronics packaging; cytotoxicity assesment; capacitance sensing

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1. Introduction

A lab-on-a-chip (LOC) is a miniaturized system based on microfluidics for applications such as chemical analysis, environmental monitoring, and medical diagnostics. LOCs perform rapid and cost effective reactions due to their small size: small sample volumes mean faster analysis times and less consumption of expensive reagents [1,2]. A subset of LOCs incorporate an analyzing IC microchip to integrate sophisticated laboratory functionality directly into the system; these are known as lab-on-CMOS (LOCMOS).

Here we report a LOCMOS system based on an IC chip designed for capacitance sensing [3] (Fig. 1 (a)) applied in cytotoxicity assessment during nanoparticle exposure. The IC microchip provides a mechanism for sensing capacitance at pre-defined locations, which reflects the surface attachment of adherent cells. Normally, healthy adherent cells spread out and attach to the surface on which they are cultivated, whereas dying cells ball-up and eventually detach. This morphology change of cells as an indication of viability can be monitored with capacitance sensing. An advantage of the method is that no cell stains or biomarkers are needed, and thus cell monitoring is fast and continuous in time.

However, a challenge with LOCMOS is to find a suitable electronics packaging method with material combinations that are not only biocompatible, but that also ensure electronics reliability in cell growth conditions, which include high humidity, elevated temperature, and corrosive electrolyte solutions. We have adapted the low temperature co-fired ceramic (LTCC) technology in combination with flip chip bonding to develop a biocompatible package for the capacitance sensor chip (Fig. 1 (b)-(c)).

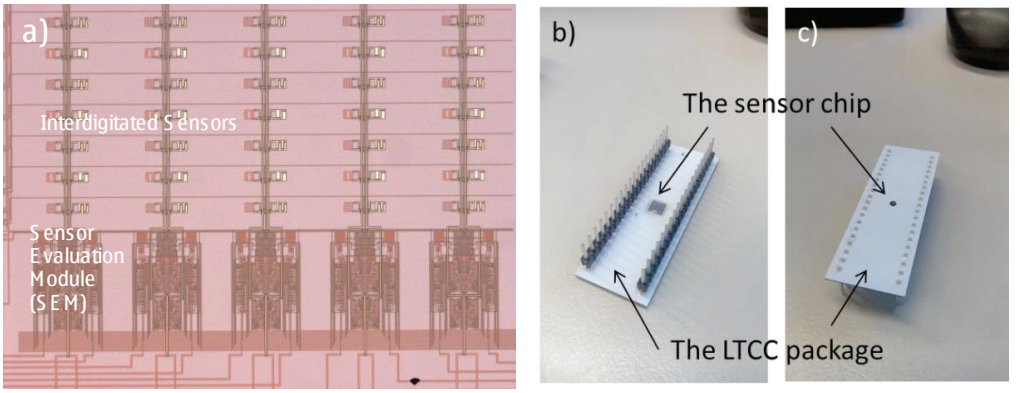

Fig. 1. (a) Microscopic image of the capacitance sensor chip with the interdigitated sensors and sensor evaluation modules (SEM); (b) The sensor chip "flip chip bonded" on the bottom of the LTCC substrate; (c) The top side of the LTCC package with sensing face of the sensor chip in the middle.

2. The LTCC packaging of the sensor chip

LTCC is a versatile technology due to the 3D processing of ceramic material in a constantly developing manufacturing process. During the past years it has developed from a simple substrate material technology to a complex microelectronic packaging system that involves buried passive components, heat sinks, sensors, actuators, micro-channels, and energy harvesters [4]. However, LTCC has not been utilized much in biological applications because it has not been considered as biocompatible due to toxic ingredients. Recently Luo & Eitel reported a biocompatible LTCC substrate material for biosensors [5], and versatile LTCC packaging has found its way also to biological applications [6,7].

In our case, the LTCC substrate for the sensor chip was made from Heraeus HeraLock® Tape HL2000, which is not considered bio-friendly. This was resolved by packaging the chip to avoid cells contacting the LTCC, as described below.

Silver conductor lines were screen printed on LTCC sheet using Heraeus Co-Firing Silver Conductor TC0307 paste recommended by the manufacturer due to low resistivity (≤ 0.003Ω sheet resistance) and good fine line printing characteristics (≥ 100 µm).

The sensor chip was glued on the back side of the LTCC sheet with Isotropic Conductive Adhesive (ICA) EPO-TEK H20E-PFC (Epoxy Technology), leaving the active area of the chip open through a pre-drilled hole on the top side of the LTCC. The two component silver epoxy was chosen due to its suitability for closely spaced contact pads of the sensor chip (pitch 160 µm and pad size 80 µm x 80 µm).

The ICA was applied on the contact pads of the LTCC as "bumps" by a stamping process. The stamp was made of alumina with laser processing. After stamping the bumps on the LTCC the sensor chip was mounted by using a flip chip bonder with alignment features, and the adhesive was cured with heat and pressure. An epoxy underfill was applied between the chip and the LTCC substrate to provide liquid sealing, to give additional attachment of the chip, and to protect the cells from the LTCC material. The stamp, stamping process, and chip bonding are shown in Fig. 2 (a). The underfill was also used to glue the cell well on top of the LTCC package (Fig. 2(b)).

The LTCC packaged sensor chip was inserted into a zero insertion force (ZIF) connector mounted on a printed circuit board and connected to a measurement set-up, including computer, controlling software, and data acquisition unit.

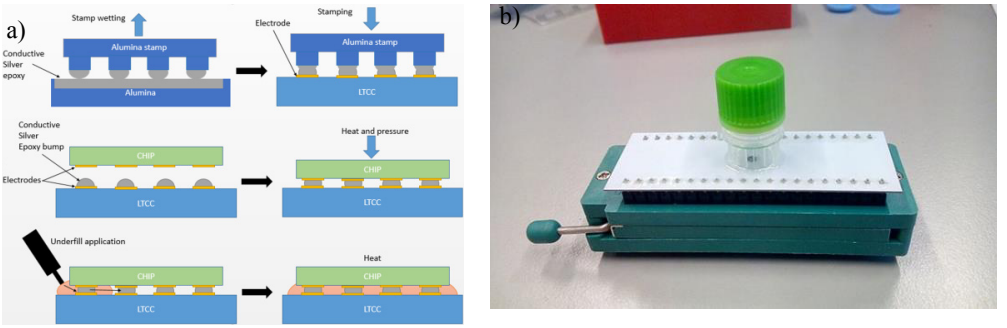

Fig. 2. (a) The ICA stamping process, sensor chip mounting, and underfill application; (b) The sensor chip inside the cell container and the LTCC substrate connected to zero insertion force (ZIF) connector.

3. The cell testing and reliability of the package

The biocompatibility and reliability of the electrical contacts of the LTCC module were tested first with dummy chips in standard cell culture conditions (5% CO_2, 37°C). The entire fixture was placed in an incubator for 3-7 days as human lung epithelial cells (BEAS2B) were cultivated on the passive chip. The total cumulative resistance between all contacts in the system, which were connected in a daisy chain pattern, was regularly observed. The cell proliferation was normal (Fig. 3) and the total resistance remained low (< 30 Ω) throughout the experiment.

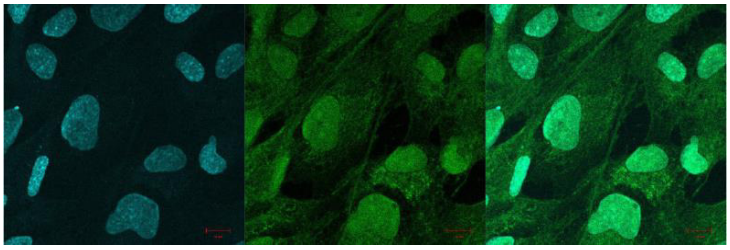

Fig. 3. BEAS2B cells on packaged dummy chip, size of the cells are 10 – 30 µm. Blue color in the left panel is the Hoechst stain indicating DNA; in the middle panel the green color distributed in the cells is COOH activated core-shell type CdSe/ZnS quantum dots; the right panel is the merged image of the two previous ones. The morphology of the BEAS2B cells on the dummy chip is normal.

4. Capacitance measurements with cells

After successful testing of the dummy chips, preliminary measurements were conducted with the sensor chip and the BEAS2B cells. Prior to the measurements, the sensor chip was calibrated using the controlling software until all the sensor evaluation modules reached their target values. A control test was done with plain cell growth media inside the incubator. The test was repeated with BEAS2B cells in growth media. Data collection ended 3 hours after cell deposition on the chip, corresponding to the sedimentation stage of the cells prior to attachment. There was a small but consistent 5 mV difference in the signals from pure growth media and growth media with cells (Fig. 4). Further conclusions require more data analysis.

The final intended application of the device is evaluation of the cytotoxicity of nanomaterials. We expect that the device will be able to detect an abnormal mortality rate of the cells after exposure to potential cytotoxins. This is an alternative method to traditional assay kits.

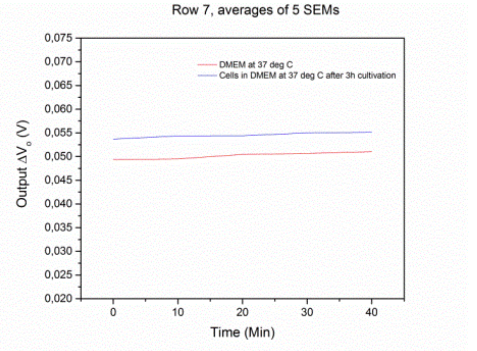

Fig. 4. Preliminary sensor measurements with the LTCC packaged sensor chip and cells. Blue curve: only cell growth media on top of the chip; red curve: cell growth media and BEAS2B cells on top of the chip 3 hours after deposition. Note that the cells are in the sedimentation stage, with attachment expected to occur after 24 hours. Data taken from sensor row 7 as the average of 5 sensor evaluation modules (SEMs). A data point was taken every 10 minutes.

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