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RESEARCH PAPER

Prospects of low temperature co-fired ceramic (LTCC) based microfluidic systems for point-of-care biosensing and environmental sensing

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Abstract Low temperature co-fired ceramic (LTCC) based microfluidic devices are being developed for point-of-care biomedical and environmental sensing to enable personalized health care. This article reviews the prospects of LTCC technology for microfluidic device development and its advantages and limitations in processing capabilities compared to silicon, glass and polymer processing. The current state of the art in LTCC-based processing techniques for fabrication of microfluidic components such as microchannels, chambers, microelectrodes and valves is presented. LTCC-based biosensing applications are discussed under the classification of (a) microreactors, (b) whole cell-based and (c) protein biosensors. Biocompatibility of LTCC pertaining to the development of biosensors and whole cell sensors is also discussed. Other significant applications of LTCC microfluidic systems for detection of environmental contaminants and toxins are also presented. Technological constraints and advantages of LTCC-based microfluidic system are elucidated in the conclusion. The LTCC-based microfluidic devices provide a viable platform for the development of point-of-care diagnostic systems for biosensing and environmental sensing applications.

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1 Introduction

There is a pressing need today to develop microfluidic point-of-care systems for applications ranging from detection of clinically relevant biomarkers to environmental pollutants. Low temperature co-fired ceramic (LTCC) technology offers to fill this technological requirement. LTCC-based microfluidic systems have recently come into focus due to many potential advantages for microfluidic device prototyping. The last few years have seen an up-spring in the reporting of LTCC-based microfluidic systems. This paper presents a review of LTCC technology and its adaptability to microfluidics device development. An evaluation of recent development efforts of LTCC-based microfluidic systems for various applications is presented.

Microfluidics is an application of micro electromechanical systems (MEMS), dealing with the study and development of micro- and nano-liter fluid volume-based analytical devices (Whitesides 2006). Microfluidic systems offer many advantages over conventional bench top analytical systems such as small sample volumes, low energy consumption, utilization of micro- and nano-scale phenomenon, miniaturized form factors, portability, faster response time and disposability. These advantages of microfluidic devices make them the fundamental building block of point-of-care technologies. Microfluidics is a highly interdisciplinary field that brings together electronics (Cheng and Wu 2012), physics (Squires and Quake 2005), biotechnology (Barry and Ivanov 2004), optics (Psaltis et al. 2006), material science

and chemistry (Marre et al. 2012). Typical biomedical applications of microfluidics are focused on continuous flow devices for sampling (Browne and Ahn 2011), detection and counting (Zhe et al. 2007) sensing (Zaytseva et al. 2005) of protein biomarkers, microarray biochips for magnetophoretic DNA extraction (Karle et al. 2010) and electrophoretic polymerase chain reaction (PCR) amplification (Zhang et al. 2006). Other applications of microfluidics include optofluidic systems for tunable microlens arrays (Zappe and Shaik 2005), microfluidic fuel cells (Choban et al. 2004), etc.

The biggest application that drives the development of microfluidics technology is point-of-care diagnosis (Linder 2007; Sista et al. 2008). In this day and age, where early diagnosis can significantly bolster efforts to fight ailments ranging from lifestyle diseases (e.g., diabetes, stress), conditions arising from cancer, infectious diseases and bioterrorism threats, the need for continuous monitoring of target analytes at point-of-care is imperative. Ideal point-of-care systems need to be portable, of low form factor, be disposable, biocompatible, produce instantaneous results from raw samples and yet maintain a low cost per unit (Gervais et al. 2011). Microfluidics offers the potential to satisfy all these demands. The point-of-care system finds applications mainly in the biomedical and environmental sensing domain. For biomedical applications, point-of-care systems are best suited for personalized health diagnostics for day to day monitoring of physiological variables, prognostic kits for cancer, and applications in third world countries, where complete medical diagnosis is unavailable.

Food safety and environmental pollution monitoring are becoming new priority areas for human health care in today's day and age. Materials such as carcinogenic organic solvents, toxic materials, and even nuclear contamination (e.g., recent earthquake in Japan leading to nuclear contamination in water bodies), which are very hazardous for environment and human health are found in packaged foods, water bodies and air. Also, a wide range of environmental problems such as global warming, ozone layer depletion, acid rain, increase in harmful waste materials in the environment, dioxin, and pollution of air and water require solutions. Therefore, environmental monitoring is crucial to protect public from toxic contaminants and pathogens that can be released into air, soil, and water. Point-of-care detection systems, which provide real-time quantification of environmental and health hazards, are the need of the hour (Delattre et al. 2012).

2 Low temperature co-fired ceramic

To overcome the challenges in current processing technology and materials available, newer materials are constantly being investigated to add novel functionality and processing capability for microfluidic device fabrication. Ceramic-based microfluidics is one such approach that offers many novel structural and functional capabilities in microfluidic device design and fabrication. In recent time, low temperature co-fired ceramics (LTCC), a material used extensively in the integrated circuits (IC) industry as a packaging material over the last two decades, has gained attention as an alternative to glass, polymer and silicon for the fabrication of microfluidic systems (Gongora-Rubio et al. 2001; Peterson et al. 2005; Golonka et al. 2006, 2011; Ibáñez-García et al. 2008). LTCC offers a number of advantages over polymers, glass and silicon, making it an ideal material for developing microfluidic devices (Golonka 2006). Since the beginning of 1980s, LTCC has been extensively developed as a microelectronic packing material in multichip modules (MCM). A representative LTCC device consists of a multilayer stack of sintered ceramic tapes, each of which contains passive electronic elements such as resistors, capacitors and inductors buried in it. The various layers are interconnected through via filled with conducting materials such as gold (Au) and silver (Ag) paste.

In a generalized sense, LTCC has inherent properties such as chemical inertness, biocompatibility, high-temperature stability, excellent high frequency dielectric properties, mechanical strength, packaging capabilities and three dimensional structuring that lends itself well for the development of microfluidic devices (Jones et al. 2000; Gongora-Rubio et al. 2001). However, the wide variety of compositions available form different manufacturers of green tapes can significantly vary the above-mentioned properties. In particular, the chemical reactivity and biocompatibility have been seen to vary drastically with varying compositions of the green tape. A thorough study is, however, not available in literature currently and is a potential area of study. LTCC as a material for development of microfluidic-based systems has shown promise in overcoming the important drawbacks of polymer microfluidic systems. LTCC-based fabrication aids rapid prototyping with a significantly low turn-around time in a semi-clean room environment with minimal use of expensive tools compared to conventional clean room-based microfabrication techniques (Shafique and Robertson 2009). The parallel processing of the multiple layers and final integration into a multilayer stack easily accommodates design modification during initial device development. The maturation of the microelectronics packaging technology using LTCC allows for integration of the fluidic system with the support electronics needed for microfluidic operation and automation (Ibáñez-García et al. 2008). The sintering temperature of LTCC (850 °C) allows for integration of sensing and actuating electrodes using precious metals such as Au, Ag, Pd and Cu. This is a significant

advantage over high temperature co-fired ceramics (HTCC) which allows only the use of refractory metals such as W and Mo (Ramos and al 2009). At reasonable feature sizes, the integration of the working metals such as Au, Ag and Cu can be performed using a standard screen printing process. The relatively simple and inexpensive fabrication methods also lend itself to economical manufacturing in large scale. The multilayer approach accommodates the incorporation of three dimensional structures and high aspect ratio features such as overlapping microchannels, which is otherwise a tedious and complicated process in lithography-based microfabrication requiring repeating steps of sequential lithography. Leakage, one of the common challenges faced in microchannel bonding to substrates is a non-issue in LTCC microfluidics since all the layers are co-fired, resulting in a leak free, compact microfluidic manifold. LTCC, post fired, is also a mechanically stable, swell free, corrosion-resistant material.

2.1 LTCC—material review

Material selection is a crucial part of microfluidic system development as it impacts the processing, functionality, application and disposability of the sensor strips and the fluidic manifold. Processing-related details such as fabrication technique, integration with support structures and hermiticity are directly affected by the material used. Functional aspects of a microfluidic device such as capillary forces in microchannels, hydrophobicity and nonspecific adsorption of the sample analyte are determined by the material chosen. The choice of material also determines corrosiveness, temperature isolation and hermiticity for applications in implantable sensors and harsh environments.

Silicon, glass and polymers have long been the materials of choice for microfluidic device development. Silicon microfabrication using MEMS-based processing techniques is very well characterized for prototyping microfluidic devices. Silicon, however, has inherent drawbacks such as expensive cleanroom usage, opacity and impermeability to gases. After Whitesides et al. (Anderson et al. 2000) demonstrated the use of PDMS for the fabrication of microfluidic structures using soft lithography technique, significant efforts went into the adaptation of polymer processing techniques such as injection molding, nanoimprint lithography and hot embossing for microfluidic device processing. Polymers such as PDMS, PMMA, SU-8, Teflon etc., have characteristics such as transparency, ease of surface modifications, low electrical and thermal conductivity, biocompatibility, and flexible substrates, that has made them attractive materials for demonstration of lab-on-chip applications (Becker and Locascio 2002). Today, there is significant literature available on polymer material-based microfluidics; however, there remain many challenges in progress of the polymer-based microfluidic chips into the next stage of technology evolution, which is commercialization. Silicon and polymer materials essentially use a lithography-based fabrication technology, which involves thin film deposition and etching techniques combined with photolithography to define patterns that create microfluidic structures such as channels, reservoirs, vias and valves. Lithography-based approach confines the development of microfluidic structures to 2D, requiring multiple lithography steps to create 3D structures. Alignment errors introduced during lithography and leakage-free bonding of the multilayer microfluidic devices can cause device failure. Since the processing is sequential, failure during any one processing step leads to total process failure. While polymer materials are relatively inexpensive materials, only a handful of polymers are processable and cost of lithography-based fabrication is significantly high. LTCC technology has the capacity to overcome most of these challenges. In terms of the material properties, LTCC demonstrates an equal or better performance compared to silicon, polymers and glass. Table 1 provides a comparative summary of the relevant properties of LTCC, silicon, polymer and glass.

LTCC and HTCC are the two raw ceramic substrates used in the manufacture of multilayer ceramic substrates. The primary difference between the two comes from the material composition and firing temperature of the two materials. The main constituent of HTCC ceramics is Al_2O_3 (93 % to 99 %), which requires a high sintering temperature ranging from 1,500 to 1,700 °C. The high sintering temperature used for HTCC processing poses limitations which include incapability to integrate passive electronic components such as resistors and capacitors and use of refractory metals only. LTCC, adapted from the HTCC technology, is an amalgamation of Al_2O_3 (45 %) and glass (40 %) buried in an organic binder (15 %) (Ramos et al. 2009).

The constituent materials and their proportions are chosen to thermally match the sintering temperature of 850 °C, which is significantly lower than the HTCC sintering temperature. The lowered sintering temperature allows for the integration of resistors and capacitors and also the co-firing of screen printed metal on the ceramic substrates. A sintering model for the ceramic–glass composite system has been proposed by Gongora-Rubio et al. (2001). The unfired ceramic tapes consist of Al_2O_3 and glass granules dispersed in an organic binder. At sintering temperature, the glass granules melt and surface tension and capillary forces facilitates the encapsulation of the sintered Al_2O_3 granules. Annealing results in the formation of a dense ceramic structure. Nanomaterials incorporated into the green tapes have been reported (Zheng et al. 2007)

Table 1 Comparative table of the properties of LTCC with silicon, PDMS and glass

	LTCC DuPont 951	Silicon <100>	PDMS	Glass (borosilicate)
Mechanical				
Flexural strength (MPa)	320	700	2.24	69
Young's Modulus (GPa)	120	140	0.0018	64
Density (g/cm^3)	3.1	2.329	0.97	2.23
Shrinkage (X, Y, Z) (%)	13, 13, 15	–	–	–
Thermal				
Expansion coefficient (10–6/C)	5.8	2.7	310	3.2
Conductivity (W/mK) @ (25–300 °C)	3	120	0.15	1.1
Electrical				
Dielectric constant	7.8 (10 MHz)	2.32	2.3–2.8	4.6 (1 MHz)
Resistivity (Ω cm)	$>10^{12}$ (100 V DC)	$>10^5$	$>10^{14}$	$>10^{15}$

to enhance the processing compatibility of LTCC with other materials by controlling sintering kinetics. Nano-sized MgO was processed onto LTCC using electrophoretic deposition to vary the sintering temperature. Laser-induced surface modification on LTCC surface has been reported (Kordas et al. 2005) for chemical metallization to form electrodes. The laser-induced process activates the LTCC surface for an enhanced electroless chemical plating process with lateral resolution of few tens of micrometers.

2.2 LTCC processing

The unfired ceramic material is available in the form of sheets and is referred to as green tape, the color green representing the unfired state of the ceramic. Figure 1 presents a schematic of a standard LTCC fabrication process. The process starts by defening the substrate size and number of layers. In LTCC fabrication, all the layers that constitute the final device are processed parallely. After the substrate size is determined, the design features such as channels, grooves and vias are created using patterning tools such as laser cutting, punching heads, jet-vapor ethcing and CNC machines. Feature size is dependent on the patterning tool used with typical feature sizes in the 10–100 μm range. Conducting vias used as interconnects across layers are formed by filling the punched vias with conducting inks of Au and Ag. Electrical conduction lines and sensing/actuation electrodes are formed by screen printing. The individual layers are then stacked using an aligning chuck and a prefiring lamination step is performed at a pressure of 3,000 psi and temperature of 150 °C for 8–10 min. The lamination step is performed to temporarily bind all the layers while in the aligning chuck. The laminated LTCC device is then sintered in a programmable furnace. The temperature ramping profile is a critical parameter during sintering with slow ramping rates recommended to avoid thermal stresses and shrinkage mismatch between layers. At lower temperatures (200–400 °C), the organic materials burn out, and this step is critical for successful fabrication of microfluidic structures such as microchannels and vias and is discussed elaborately in the next section. At the sintering temperature of 850 °C, the glass granules melt and encapsulate the ceramic granules forming a homogeneous structure across all the individual layers. Figure 2 presents a typical firing profile for Dupont 651 green tape.

The burning out of the organic materials, which constitutes about 15 % of the total composition of the green tape results in a proportional shrinkage in the overall dimensions of the paternened structure in all the three dimensions. Hence, the shrinkage factor is always deliberated into during the initial design of all the critical features. Shrinkage-free LTCC tapes are also available from commercial vendors such as Heralock and ZST (Sea Ceramic Technologies).

2.3 Microfluidic components in LTCC

2.3.1 Microchannels

The fabrication of microchannels is one of the core processes in the development of microfluidic devices. Ideal design features of microchannels include vertical side walls, zero dead volumes, leak-free connection to inlet and outlet fluidic connectors, leak-free bonding to adjacent layers in multilayer architectures, low surface roughness and uniform cross section across the length of the channel to maintain the dynamics of the fluid flow (Smetana et al. 2009).

The most compelling problem faced during microchannel fabrication using LTCC technology is the occlusion of the microchannels due to collapsing of the LTCC layers during lamination (Fig. 3a, b). To overcome this challenge, many modifications to the standard LTCC

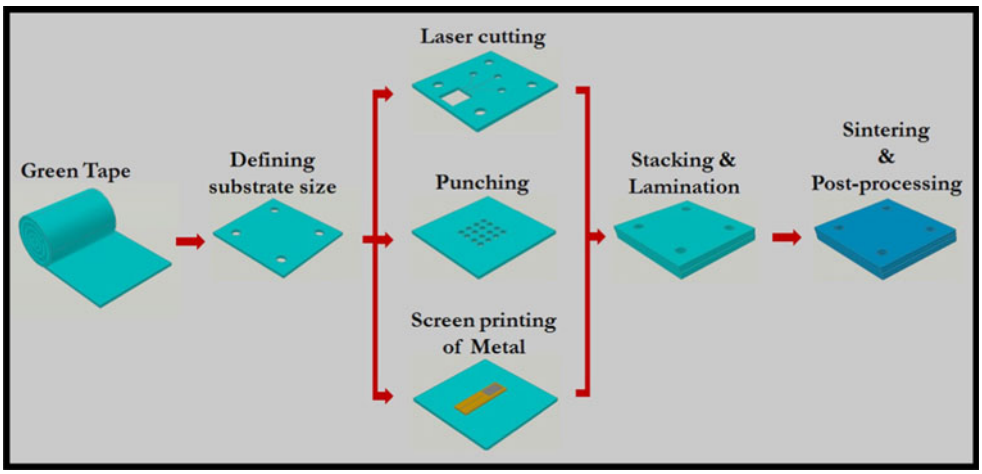

Fig. 1 Schematic of the LTCC fabrication process flow

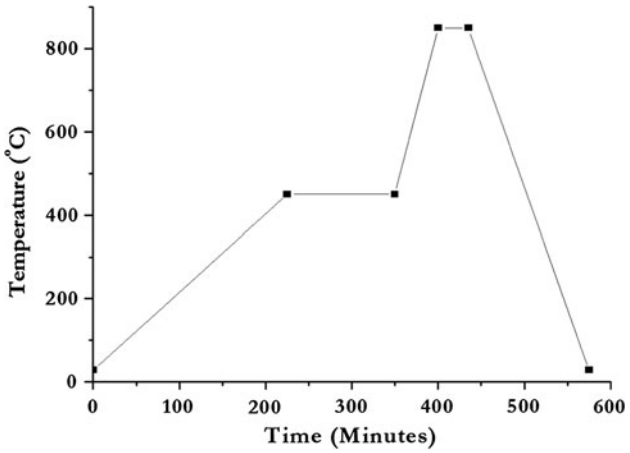

Fig. 2 A typical temperature profile for oxygen-rich sintering of LTCC materials

process flow have been reported. One of the popular approaches to overcome the channel sagging, or collapsing, has been to use a sacrificial material to fill the empty spaces in the microchannels prior to lamination (Birol et al. 2007). During the lamination process the sacrificial material provides the mechanical support required to maintain the structural integrity of the embedded microchannels and during the sintering process, the sacrificial materials burn out leaving a void that forms the empty microchannel (Fig. 3c). Golonoka et al (Malecha and Golonka 2008) have reported the characterization of an LTCC-based microchannel fabrication process using carbon-black paste and cetyl alcohol as the sacrificial materials with the DuPont DP 951 green tape. A similar characterization of the microchannel fabrication process has been reported using high purity carbon paste, carbon tape and cetyl alcohol with zero-shrinkage green tapes from Heraeus HL2000 (Malecha and Golonka 2009). Scanning electron microscopy (SEM) has been used to characterize the cross section of the microchannels fabricated using the different sacrificial materials as fillers. Significant improvements have been demonstrated leading to decreased sagging and channel constriction. The use of sacrificial materials requires a well-characterized temperature ramping profile during sintering to allow the complete burn-out of the sacrificial material to avoid residual retention in the microchannels after sintering. An alternate technique for overcoming the channel collapse and sagging issue, without the use of any sacrificial materials has been reported by Farhan Shafique et al. 2011). The technique uses the method of progressive lamination, where in, the lamination of the layers is performed multiple times individually at low pressures and also after aligning the layers in a progressive manner. This method, implemented successfully, provides a few notable advantages. Since the pre-lamination and post-lamination processes are identical, the overall process flow is not disturbed. The process is also considerably simplified by overcoming the use of sacrificial materials. The progressive method of lamination may, however, lead to a poor binding between the pre-laminated individual layers and can cause delamination of the layers during sintering. The delamination can lead to leaky channels and can cause bonding and peeling issues at integration and packaging stages. To overcome issue arising from channel collapse and delamination, more recently, Ulrike Deisinger and Roosen (2012) have reported the usage of low temperature, low pressure lamination along with double-sided adhesive tapes as the sandwich between the ceramic layers. This modification allows for the fabrication of cavities with very low aspect ratio (0.5:15 mm) with no observable sagging. μCT (Computer Tomography) has been utilized here for the non-destructive analysis of the internal features of the multilayer ceramic structure. Thomas Maeder et al. (2012) have reported the characterization of various compositions of hot melt adhesives as an alternative to adhesive tapes. The potential advantage of these hot melt adhesives is that the adhesives can be

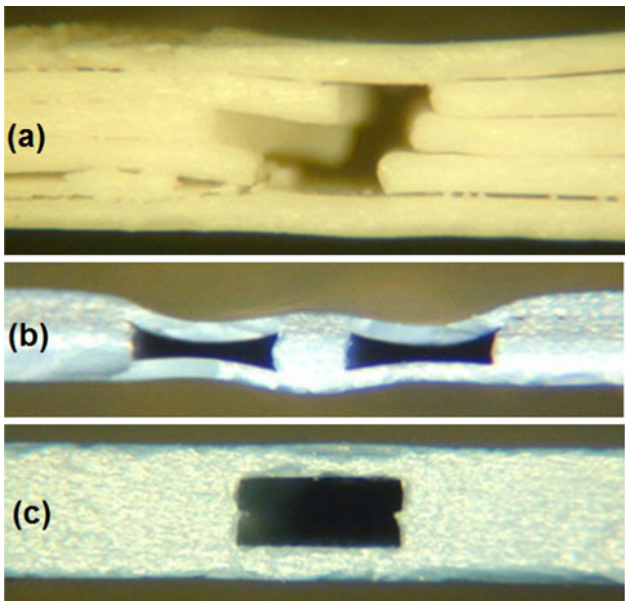

Fig. 3 Cross-sectional images of LTCC stack **a** delaminated and misaligned microchannel; **b** sagged channel structure and **c** defect-free channel structure

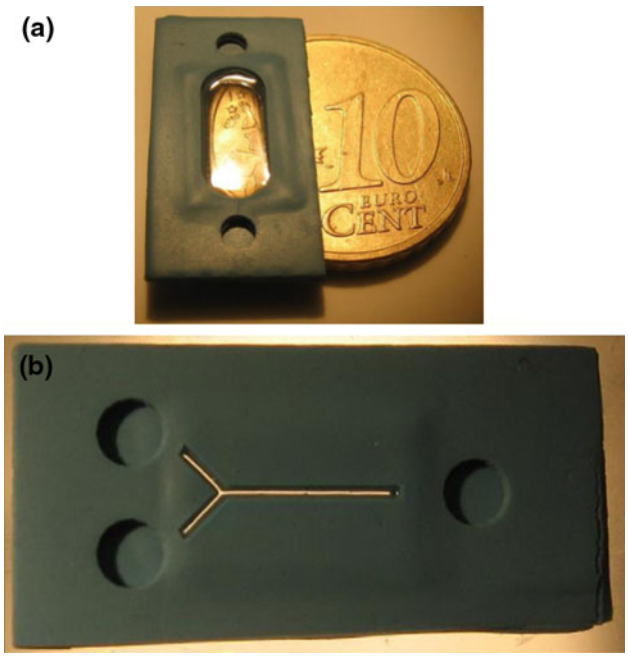

Fig. 4 **a** The LTCC-glass transparent microfluidic chamber; **b** picture of LTCC-glass mixer with a glass window incorporated

applied as thin film on the ceramic substrates in its green state and the subsequent processing steps for LTCC microfluidic device fabrication are not affected.

2.3.2 *Optical windows*

A perceivable drawback of using LTCC-based microfluidics is opacity of ceramic material. Transparency is a critical factor for applications involving optical detection such as fluorescence in μTAS systems. One of the approaches to overcome this challenge has been the use of a hybrid LTCC-polymer or LTCC-glass systems, in which, the microchannel and the underlying structure are made from LTCC and the top most layer that covers the microchannel and consists of the i/o fluidic ports, is made from a transparent substrate such as PDMS or glass. A technique for the bonding of LTCC substrates to PDMS has been reported by Malecha et al. (2009a, b, c). The laminated LTCC structure is first coated with a glaze layer using screen printing (Rusu et al. 2006). The glaze layer essentially forms a glass coating after the sintering process. Oxygen plasma exposure, that creates free radicals on the surface of PDMS and glass that assists in the formation of covalent bonds between the two materials, is a well-characterized process to obtain irreversible bonding for PDMS–PDMS, PDMS–silicon and PDMS–glass (Eddings et al. 2008). The same approach has been used to bond the PDMS cover to the glass-covered LTCC substrate. The bonding between the glass-covered LTCC substrate and PDMS provides a mechanically durable, leakage free, irreversible bonding. Pawel et al. (Bembnowicz and Golonka 2010) report the integration of transparent glass windows into the LTCC substrate to aid in optical applications of LTCC microfluidic devices. A thin glass wafer is used as one of the layers in the LTCC stack. During the sintering process, temperatures in the 670–720 °C range initiate the softening of glass, but the surface tension forces of the molten glass help in maintaining the structure of the glass sheet. At the LTCC sintering temperature, glass is conformal enough to accommodate the shrinkage of the LTCC substrate, while being strong enough to maintain its shape. Figure 4 presents the picture of a transparent glass window created in a LTCC device. This advancement lends itself well to the development of LTCC devices for optical sensing and actuation applications.

2.3.3 *Simulation of fluid behavior*

Fluid flow behavior in microchannels has been a key point of focus in the area of microfluidics research (Squires and Quake 2005). Microchannel geometry, surface roughness, hydrophobicity, surface tension and viscosity of the fluid are some of the factors that determine fluid dynamics in microchannels. Rapid and complete mixing of buffers, analytes and reagents is a critical requirement for chemical analysis and biosensors-based microfluidic systems. By understanding the fluid dynamics in microchannels and with careful design of the microchannel geometry, passive

mixing of fluids can be achieved. The opacity of LTCC material can cause issues while characterizing fluid flow in microchannels. Especially in micromixers and microreactors, where determining the mixing efficiency and the effect of microchannel geometry on mixing is important, flow characterization is not straight forward. Computational fluid dynamic (CFD) simulation can be a very useful tool in predicting fluid behavior and aid in design of efficient microchannel geometry. Malecha et al. (2009a, b, c) have reported the fabrication of microfluidic micromixer using LTCC technology. A detailed CFD modeling of fluid flow under (a) steady-state, laminar flow and mixing of incompressible fluids and (b) an application example of two chemicals, 1- and 2-naphthols has been reported. The findings from the CFD modeling, such as the effect of inertial and viscous forces on mixing efficiency, have been experimentally verified using a prototype I-shaped serpentine microchannel fabricated using standard LTCC processing. The aid of modeling and simulation has also been used to study the deformation and failure of LTCC layers to design and obtain optimal microchannel dimensions. The use of computer modeling to understand fluid flow behavior in LTCC microfluidic devices has also been presented by Schlottig et al. (2006). The network approach-based model which describes stationary flow in LTCC channels of non-circular cross sections, is essential due to the variance introduced in microchannel cross section and geometry fabrication using LTCC processing. Computational modeling has also been utilized to optimize application-specific microfluidic designs. In an effort to optimize the microfluidic design for an optic fiber-based optofluidic system, Malecha and Golonka (2006) have reported CFD simulations to understand the effect of fluid inlet and outlet spacing on the transmission of light in the microchannel. The set-up consists of three different microchannel designs (Π type, L type and I type) with varying inlet and outlet configurations (Fig. 5). The CFD simulation of fluid flow in the three different configurations indicates that the L type and I type structures showed decreased signal due to localized disturbances in fluid flow causing significant repeatability issues. An experimental approach for characterization of fluid flow in LTCC microchannels has been reported by Groß et al. (2008). Pressure drop method, which uses the pressure drop along the length of the microchannel and its corresponding friction factor to calculate flow rate as well as mixing efficiency. Another method described is the resistance time distribution measurement, which is based on the residence time of a liquid packet in a microchannel and its effect on mixing efficiency and yield. A pulse trace assembly is used to inject pulses of the experimental liquid and post processing on the recorded pulses and signal is used to understand the fluid flow behavior and mixing efficiency.

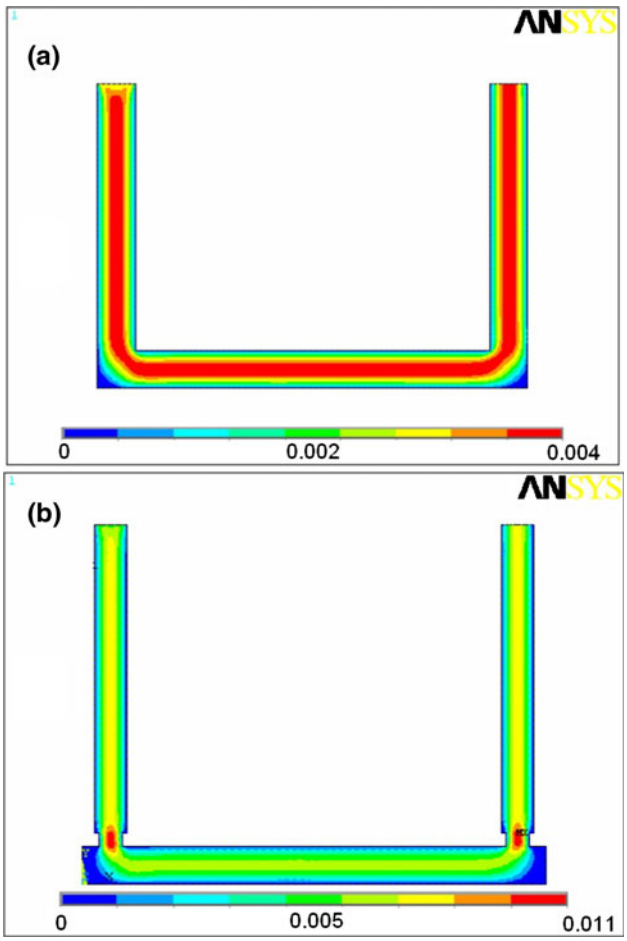

Fig. 5 Distribution of fluid velocity for **a** II type structure and **b** I type structure

2.3.4 *Valves*

Control of fluid flow using valves and pumps is an essential part of microfluidic operation. Directional flow control and regulation of fluid flow rate can be crucial for the successful operation of multi-analyte sensor and actuator systems. Satarkar et al. (2009) have reported the development of a magnetic hydrogel nanocomposite-based microfluidic valve integrated into an LTCC microfluidic channel. Magnetic nanoparticles were dispersed into hydrogel nanocomposites-based on *N*-isopropylacrylamide (NIPAAm). Application of an alternating magnetic field (AMF) produces localized heating of the hydrogel resulting in volumetric swelling. The hydrogel is incorporated into an LTCC microchannel system (Fig. 6) and the ON–OFF operation of microfluidic valve is demonstrated using the swelling and collapse of the nanocomposite hydrogel (Fig. 7).

Sobocinski et al. (2009) have reported a piezoelectric unimorph valve integrated into an LTCC microfluidic set-up (Fig. 8). A membrane-type piezoelectric disk is

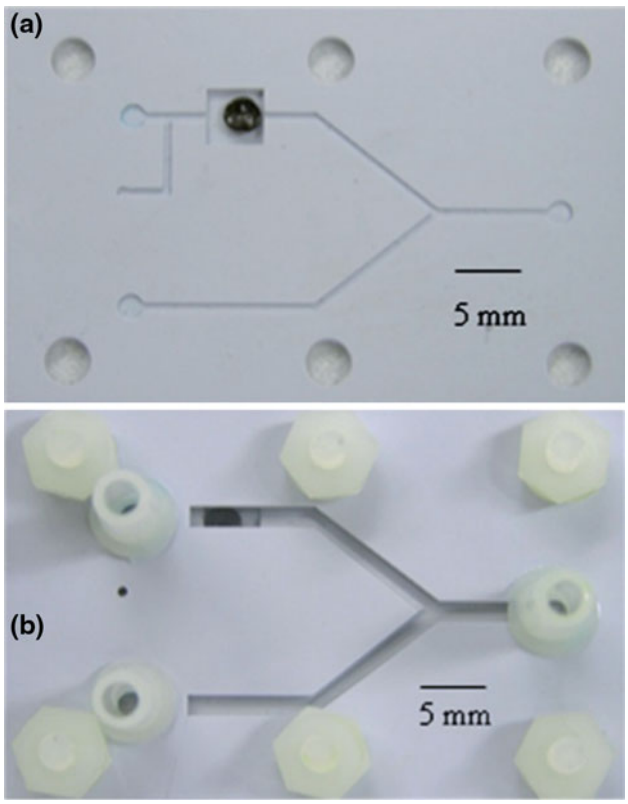

Fig. 6 Picture of microfluidic device with hydrogel composite as a valve in the upper channel: **a** bottom part of device and **b** assembled device

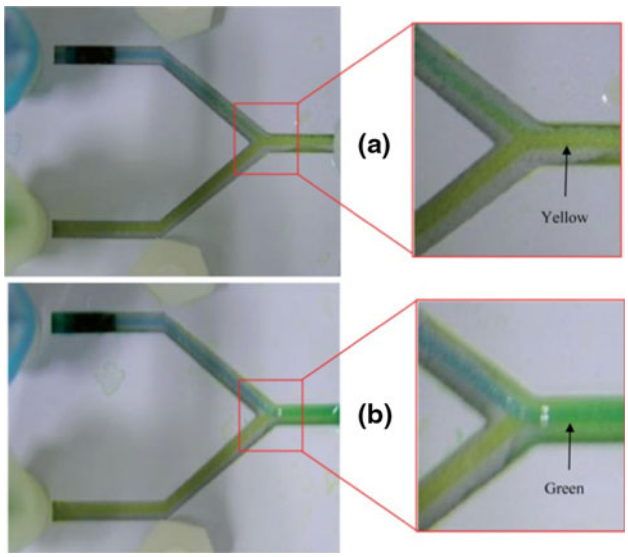

Fig. 7 Images showing ON–OFF control of flow with the hydrogel valve. In **a** the valve is OFF: swelling of the hydrogel blocks the upper channel preventing fluid flow. We see only yellow flowing. In **b** the valve ON: application of the AMF opens the upper channel allowing the blue stream to flow, leading to a *green color* as clearly visible in the *inset* at *right*

integrated into the LTCC stack. Application of an electric field across the piezoelectric disk resulted in a valve displacement of 1.3 μm at a response time under 30 ms.

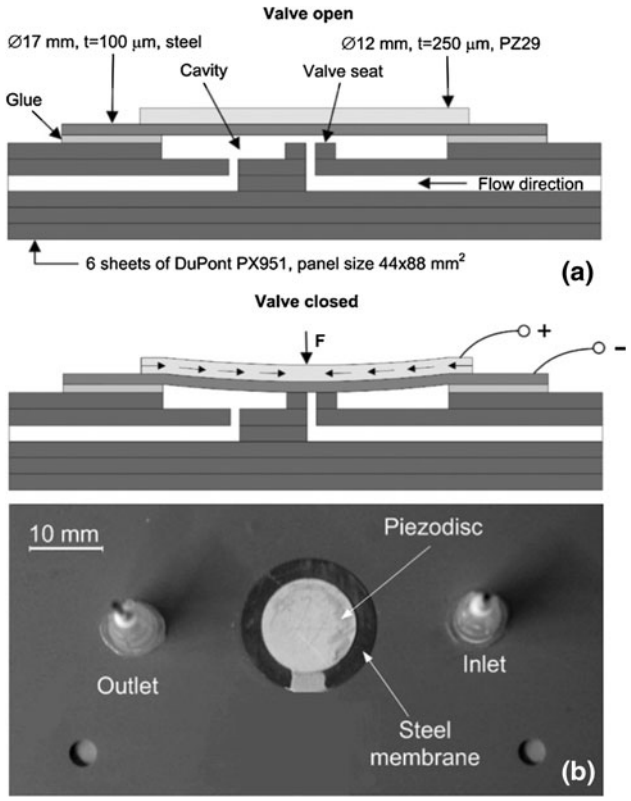

Fig. 8 **a** Schematic of cross-section of the piezoelectric LTCC valve; **b** integrated piezodisc into the LTCC microfluidic manifold

The valving action was characterized using gas flow at flow rates of 143 ml/min at 1 bar pressure and leakage levels of 4 %.

LTCC as a substrate material provides very little provision for movable parts; however, flexible membrane can be conveniently integrated into the LTCC stack either by co-firing or by post-fired integration. The development of microvalves and micropumps in LTCC microfluidic architectures offers a huge potential for innovation in LTCC process development. The advancement in LTCC processing through novel fabrication and bonding techniques will pave the way for more such demonstrations of microvalves and micropumps in microfluidic devices.

2.3.5 *Microelectrodes*

Fabrication of sensing and actuation electrodes is another important facet of microfluidic devices. The fabrication process and temperature profiles used in LTCC device fabrication allows for the integration of metals such Au, Ag, Pd, Cu etc. on LTCC substrates. The metal deposition process is commonly a screen printing or stencil printing process where in conductive pastes of the desired metals are patterned onto the LTCC substrate using a suitable screen printing mask. Factors such as surface energy and

viscosity of the paste are critical in determining the binding of the metal to the LTCC substrate and also the resolution of the metal conduction lines. In some cases, inorganic particles such as silica are added in the metallic paste to improve viscosity and surface binding (Wang et al. 2002). The metal deposition process is introduced in the unfired green tapes and is finally integrated into the multilayer stack which aids in parallel processing. A constraint of screen printing, however, is that the limitation on the smallest feature size is ~100 μm. Markowski et al. (2012) have reported the use of photoimagable metallic pastes for the fabrication of electrodes with feature size of only a few μm. This technique, however, requires the use of photolithography, an expensive fabrication technique, and hence may be an option where electrode dimensions are critical to the design (under 50 μm). The deposited metal can then be utilized for sensing purposes by functionalizing the electrode surface with application specific materials such as ion selective membranes (Ciosek et al. 2008), antibodies (Karlsson et al. 1991), DNA (Kerman et al. 2004) used in biosensors, or be used as actuators as utilized in electrowetting on dielectrics (Vasudev et al. 2009) devices and magnetohydrodynamic-driven flow devices (Aguilar et al. 2006). Fabrication of electrodes on LTCC substrate offers the potential to develop new sensing paradigms such as temperature pulse voltammetry (Voß et al. 1999), which requires high temperature tolerances from the underlying substrate.

2.4 Biocompatibility

An important material property that is taken into account during microfluidic device development is the biocompatibility of the structural and functional materials. While biocompatibility encompasses features such as immune and inflammatory response, they are more pertaining to implantable devices. The areas of interest for point-of-care sensor development in particular are facilitation of cell cultures on the material surface, material disintegration during material–fluid interaction, and the non-specific adsorption of bioreceptors (antibodies, enzymes and DNA) or the target analyte (antigens, hormones, etc.) onto the device surface. Smetana et al. (2007) have reported the characterization of cell proliferation of various cell lines (HeLa cells, BAC cells and L929 cells) on three commercially available LTCC materials in both the fired as well as green state. The results (Fig. 9) indicate that apart from the CeramTec tapes, all other LTCC tapes showed cell proliferation comparable to the control (glass).

Cell culture chambers in LTCC microfluidic devices are often integrated with electronic sensors and actuators, typically achieved through screen-printed metals. Hence, the understanding of the effect of metal compositions on

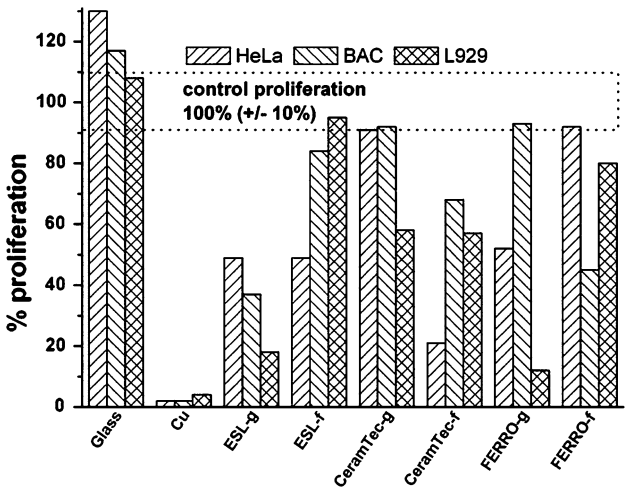

Fig. 9 Cell proliferation characterization of different LTCC tapes in the green state (-g) and the fired state (-f)

cellular responses is imperative for design considerations. During the process of development of an LTCC-based flow sensor for biological applications, Bartsch de Torres et al. (2010) have reported the investigation of cell culture growth (mammalian cell lines HEK 293 FT and CHO-k1) on LTCC substrates. Cellular response studies were carried out on both LTCC blank substrates (DuPont 951) as well as screen-printed metal compositions (DuPont Au, Pt, Ag). In addition, PCR experiments were also carried out in the presence of LTCC blanks and the screen-print metal compositions. The results indicate that the LTCC blanks provide a congenial surface for cellular activities; however, a negative cellular response was observed on the screen-printed metal compositions. Leachates from the metal have been singled out as the reason for the detrimental effects on cell cultures. Very recently, Mercke et al. (2012) have evaluated the viability of culturing of HUVEC (Human Umbilical Vein Endothelial Cells) Heralock® HL2000 tapes along with their recommended screen-printed Au and Ag paste. While their results indicate difficulty in initial attachment of the cells onto the LTCC substrate, no negative response was observed on cell cultured on the screen-printed films of Au and Ag. The cell cultures showed good viability up to 3 days.

Recently, Zhang and Eitel (2012) have reported a thorough characterization of the biostability of LTCC material for biomedical and microfluidic device applications. Leaching rate studies of three commercially available green tape materials have been studied during LTCC interaction with relevant fluids such as phosphate buffer saline (PBS), simulated body fluid (SBF), simulated gastric fluid (SGF), and a basic solution (1 M NaOH solution, no physiological analog). Leaching rate of LTCC in these fluids has been determined through weight loss and elemental analysis. The reported results suggest that

techniques such as surface passivation, flushing out of leached material and diffusion limited leaching may be required depending on the LTCC-fluid exposure time. Surface characteristics such as hydrophobicity, surface energy and available chemical groups determine the extent to which non-specific adsorption takes place. Various surface modifications such as increasing hydrophobicity and decreasing surface charge are well characterized to reduce non-specific adsorption on microfluidic materials such as silicon, glass, and polymers. A thorough investigation of the non-specific adsorption of bioreceptors such as antibodies, enzymes and DNA on LTCC material is currently not available in literature and is a potential topic of research in the near future. Information obtained from protein adsorption studies could lead to a thorough understanding of surface interactions and lead to development of novel surface modification techniques to optimize surface properties of LTCC for biosensing and implantable device applications.

3 Applications of LTCC-based microfluidic system

The favorable material properties and novel processing capabilities of LTCC to create active and passive microfluidic components such as microchannels, microelectrodes and valves have led to the successful development of LTCC-based microfluidic systems for a variety of applications. This section presents the detailed description of such successful applications in the field of biomedical microdevices and environmental monitoring systems.

3.1 Biomedical applications

3.1.1 Microreactors

Microfluidic microreactors are increasingly gaining importance in biosensors, chemical synthesis and pharmaceutical research applications due to many advantages such as excellent control over process parameters (temperature, pH, and humidity, etc.). Microreactors also aid in pilot small volume R&D activities, which is typical in chemical synthesis and drug discovery applications (Roberge et al. 2005). With the need for point-of-care systems gaining ground, microreactor technology has found good application in biosensing and PCR-based systems, which require highly controlled environments in a microchamber (Yang et al. 2002). Microreactors require the integration of multiple sensing and actuation components such as micromixers, microextractors, microheaters, microsensors for temperature, pH, humidity and redox measurements and many more depending on the demands of the application (DeWitt 1999). Polymerase chain reaction (PCR) is a powerful biotechnology tool finding many applications in the fields of science and medicine. PCR can be used to identify genetic diseases, detect infectious diseases, identify cancer types and also in forensic sciences for high accuracy matching and identification of human samples. PCR essentially creates multiple copies of a target DNA fragment through a sequential thermal cycling process. Traditionally, the PCR process is carried out in a laboratory using vials; however, advancement in microfluidics and microsystem fabrication technology has allowed the creation of portable and compact devices that can be used at point-of-care and can produce real time results known as real-time PCR (rtPCR). The success of microfluidic PCR systems is highly dependent on the creation of three temperature zones on a single chip with high stability, while being thermally isolated from each other. Typically, metals such as nichrome and scandium are microfabricated onto the substrate to form microheaters, and the samples are transported between the thermal zones using microchannels. Silicon, glass and polymers have been previously used as materials to create microfluidic channels in PCR systems. LTCC, due to its high thermal isolation, biocompatibility and microfluidic processing capabilities can prove to be a better candidate for PCR applications. Malodobra et al. (2011) have reported a study comparing the specificity, sensitivity and efficiency between traditional PCR systems and LTCC-based PCR systems. Bembnowicz et al. (2010) have reported the development of a LTCC-based microfluidic system for DNA amplification and measurement using PCR. The LTCC system consists of a microchamber which forms the integral part of the microreactor (Fig. 10). Temperature control is provided using a thermoelectric module and a micro-thermistor for measurement.

The detection system, optical based, consists of a fiber optic laser light source that is integrated into the LTCC chip and a CCD camera captures the fluorescent light emission using special filters. The developed system has been successfully tested for DNA measurement. An improvement to the above-mentioned LTCC PCR system has been reported (Bembnowicz et al. 2011). Here, the temperature control which consists of the microheater and the sensor is integrated into the LTCC substrate, thereby creating a more compact form factor for the envisioned system as shown in Fig. 11. A commercially available SMD (surface mount device) resistor is used as the microheater and a Pt100 element is used as the temperature sensor. Along with the heater and sensor, the support electronics are soldered onto the LTCC substrate. An additional metallic layer is screen printed to improve the thermal conductivity and achieve uniform temperature distribution in the microchamber.

In the above two reported developments, the sample is held stationary in the microchamber, while the temperature

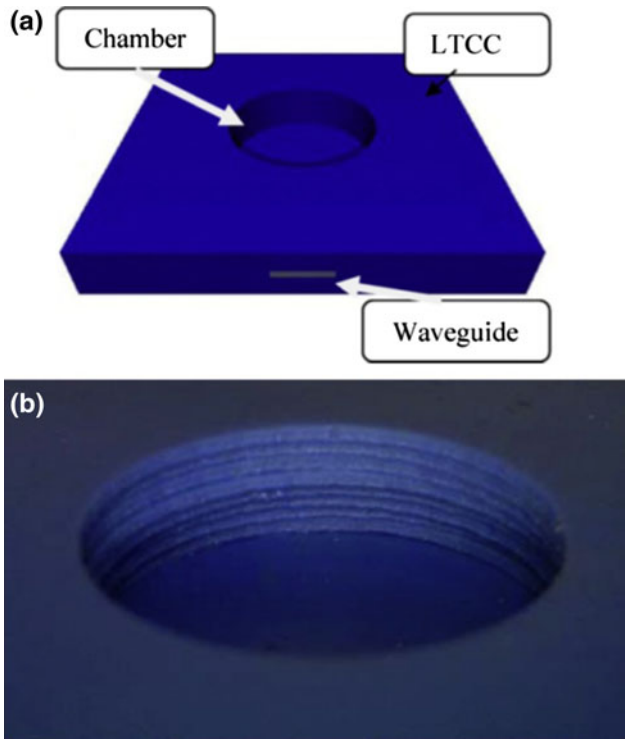

Fig. 10 **a** Schematic of LTCC-based PCR chamber with integrated waveguide detection system. **b** Fabricated multilayer LTCC PCR microreactor

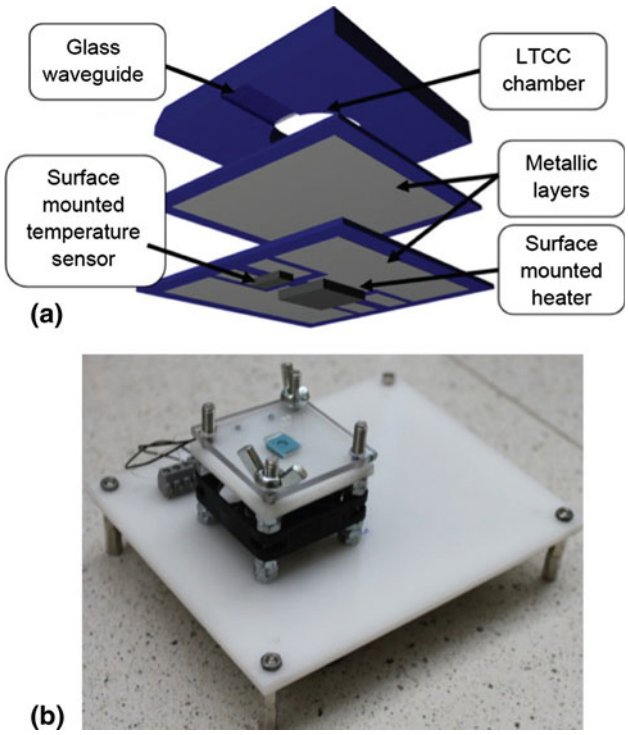

Fig. 11 **a** Scheme of LTCC microchamber connected with SMD resistors; **b** fabricated LTCC chip-based miniaturized PCR system

is cycled between three distinct temperatures using the embedded heaters. Another popular configuration for PCR microfluidic systems is to sequentially transport the sample between three chambers, each maintained at the required temperature. Sadler et al. (Sadler et al. 2003) have reported the development of an LTCC platform for PCR and DNA detection using a continuous flow microfluidic system (Fig. 12). Microchannels connect the three temperature zones which are realized using screen-printed Ag–Pd. Temperature sensors are surface mounted onto the LTCC substrate. One of the salient features of this design is the introduction of air-gaps between the parallel microchannels to provide thermal isolation during sample transport between the temperature zones. The functionality of the LTCC-based continuous flow microfluidic system is demonstrated by performing real-time PCR and also DNA detection.

Another fine example of the 3D architecture capability of LTCC processing has been demonstrated in an LTCC-based microfluidic microreactor developed by Smetana et al. (2007, 2010). The system consists of a spherical reactor cell for accumulation and mixing of reagents and other solutions (Fig. 13). The system also consists of sensor arrays for monitoring of pH-, oxygen-, temperature- and iodide-sensitive sensors. A surface mount optical glass fiber is used for reaction monitoring using absorption or fluorescence spectroscopy analyses. Temperature control is provided by pumping a thermal fluid through embedded ducts. A network

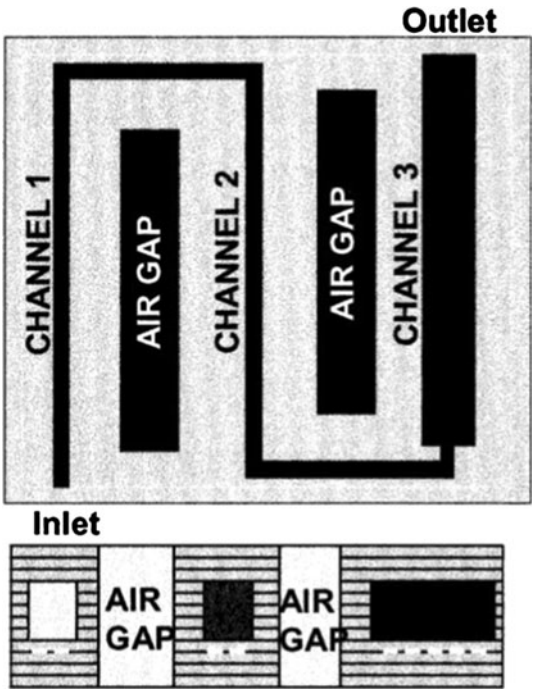

Fig. 12 Schematic of the top-view and cross-sectional view of the LTCC continuous flow PCR device

of micro-channels with cross-sectional dimensions varying from 200 × 200 mm up to 2 × 2 mm is connecting the different measuring sections within the ceramic module.

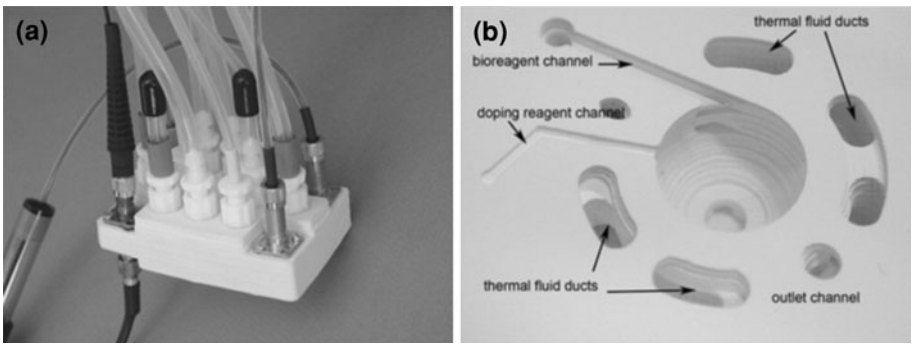

Fig. 13 **a** A fully integrated device with sensors, inlet/outlet ports and optical fibers; **b** microfabricated multilayer reactor cell with channels and fluidic i/o

3.1.2 Whole cell sensors

Cell cultures have been a great source of information for understanding the growth dynamics in the human body. Cell cultures are extremely sensitive to environmental variables such as temperature, pH, humidity, ion concentrations, nutrients and pathogens in the culture media. Monitoring, regulation and response of cell cultures to triggers is a huge area of research that acts as an enabling feature in drug discovery and toxicology. An LTCC-based microelectrode array for monitoring of cell cultures has been reported by Ciosek et al. (2009). The microelectrode array is realized by screen printing of Pd–Ag paste and electrochemical deposition of AgCl (Fig. 14). The microelectrode array is functionalized with ion selective membranes of high selectivity for monitoring and detection of various cell culture variables such as cell culture media change, cell growth rate and toxicity levels. Electromagnetic force (EMF) is utilized to detect the changes in electrode response due to the ion concentration variation. Principle component analysis (PCA) is used to segregate the multiplexed data obtained from the sensor array.

Bio-particle detection and counting has come into focus in the modern day due to the high potential of impact on daily life due to the presence of harmful chemicals and biological elements in the environment and other consumables. Bio-terror threats and spreading of allergens and bacteria in public places such as airports also require deployment of sensors that monitor and detect bioparticles. Flow cytometry is one of the most successful technologies used to detect particle concentration and size in a rapid manner. Malecha et al. (2011a, b) have reported the development of a LTCC-based micro cell analyzer for detection and counting of bioparticles (Fig. 15). The LTCC-based system works on fluorescence-activated detection in a flow cytometry set-up. The ceramic microfluidic system consists of a microfluidic chamber, microchannel, optic fibers, light emitting diode (LED) and photo detector embedded into the LTCC substrate. The integration of all the active and passive components of the system into a single substrate provides a form factor that is realistic for commercial applications. *Escherichia coli* and *Saccharomyces cervisiae* cells are used to demonstrate the functioning of the developed micro cell analyzer.

3.1.3 Biosensors

Biosensors are integrated small devices that employ a biological element such as an antibody, enzyme, receptor protein, nucleic acid, and cell or tissue section as the sensing element. Typically, a nanostructured matrix such as self-assembled monolayer, carbon nanotube, metal oxide or other nanocomposite material is used to immobilize the sensing element onto the detection surface. Various transduction techniques such as electrochemical, optical, acoustic, piezoelectric and gravimetry can be employed to quantify the concentration of the analyte that is sensed. Biosensors are a promising technology due to inherent advantages such as high selectivity, linearity, simple set-up, electrical output, high sensitivity and easy system integration. The sensing of various biomarkers using biosensors has been extensively researched. In recent years though, the focus has shifted toward integrating the developed sensors into a complete system that supports the sequencing of the sensing assays with the supporting electronics for data acquisition and analysis. LTCC technology, which facilitates the integration of microfluidic structures and electronic components such as resistors onto a single substrate, and the addition of integrated circuits by surface mounting provide the most suitable platform for developing miniaturized biosensing systems for a plethora of sensing applications.

Immunosensors, which use antibodies as the bioreceptor, are highly preferred due to the high degree of selectivity and sensitivity that is associated with antibody–antigen interactions. Antibody immobilization strategies are very well characterized and are highly suitable for various transduction mechanisms such as electrochemical and optical due to superior electron transfer and secondary fluorescence antibody tagging properties, respectively. For the fabrication of immunobiosensors, the availability of a surface that is conducive to antibody binding is a pre

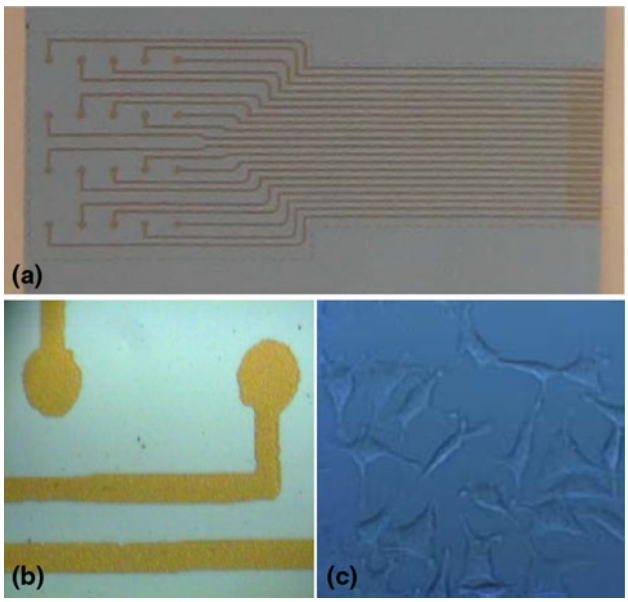

Fig. 14 Microelectrodes on LTCC substrate: **a** screen-printed electrodes on LTCC substrate using conductive paste; **b** microscopic image of the screen-printed electrode; **c** Vero green monkey kidney cells cultured on the LTCC microelectrode substrate

requisite. Typically, the antibody is immobilized at target areas such as sensor electrode surface, while attempting to keep the peripheral areas free of any non-specific binding. Nanomaterial-based immobilization matrices are used to improve surface to volume ratios, enhance the sensing response and also where the direct binding between the antibodies and the sensor surface is not feasible. With LTCC being investigated as a potential substrate material for the development of biosensing platforms, determination of optimum surface conditions is imperative. In this regards, Fakunle et al. (2006) have evaluated the feasibility of using LTCC as a substrate for immobilization of antibodies on screen-printed gold for electrochemical detection. Parameters such as pretreatment conditions (piranha cleaning v/s water), electrochemical response of the screen-printed gold electrodes and deposition of a nanomaterial immobilization of matrix such as self-assembled monolayer (SAM) on the electrodes were characterized and optimized. Electrochemical detection of Mouse IgG antigen using a sandwich-type immunoassay was used as a model to demonstrate the feasibility of using LTCC as a suitable substrate material for the development of immunosensors.

Fakunle and Fritsch (2010) have recently reported the development of a complete microfluidic system that adopts the screen-printed gold electrodes (previously discussed) into a multilayer LTCC microfluidic manifold containing microchannels (Fig. 16a). The sensing electrodes are incorporated onto all the four walls of microchannel (Fig. 16d).

Enzymatic biosensors are also a popular choice of bio-receptors due to their rapid, efficient and selective detection of important analytes such as glucose, cholesterol, urea and lactic acid. Enzymatic biosensors have found success in commercialization and have proven their potential for point-of-care diagnostics and personalize health care. Malecha et al. (2009a, b, c) have reported the use of an enzyme-based LTCC microfluidic biosensing platform for the detection of urea in biological fluids (Fig. 17). The system design consists of a micro-reactor chamber, an integrated micro-heater and temperature sensor. Micro-glass beads are used as the enzymatic carrier to determine the concentration of urea by measuring the pH at elevated temperatures. The enzymatic reactions are enhanced at elevated temperatures (37 °C) using the screen-printed Pt micro-heater electrodes. The temperature sensors are realized by screen printing of a resistance–temperature dependent ink (DP 3630). The developed sensor is successfully tested for urea concentration determination in biological fluids. The system also demonstrates impressive shelf-life and repeatability (18 days).

Another example of a LTCC-based enzymatic biosensor has been reported for the continuous monitoring of glucose (Malecha et al. 2011a, b) (Fig. 18). The detection system is based on the oxidation of glucose in the presence of the

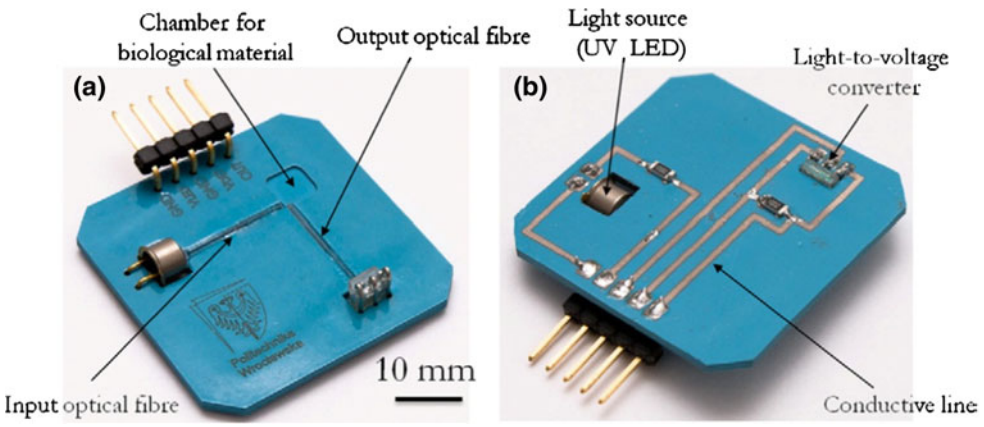

Fig. 15 Fabricated LTCC-based detection module for the micro ceramic cell analyzer containing the measurement chamber, i/o for the optic fiber and UV LED light source

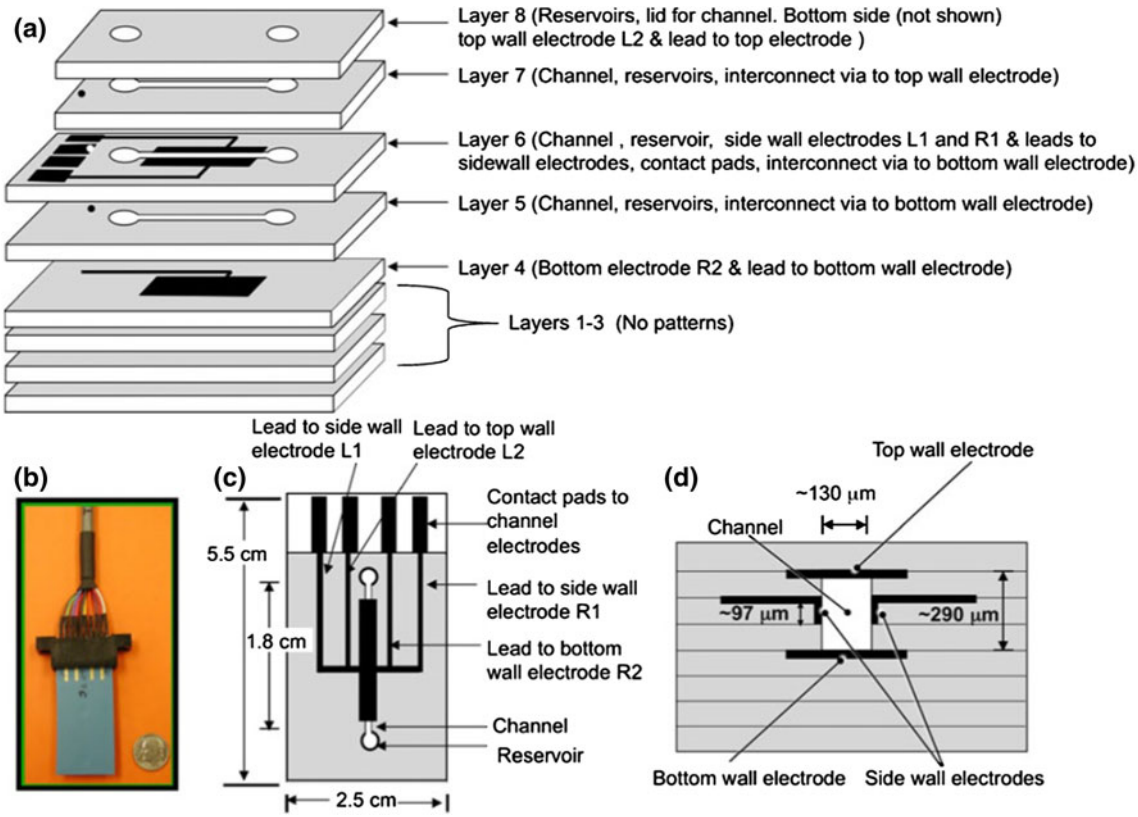

Fig. 16 **a** Multilayer stacking to create a screen-printed gold (SPG)/low-temperature co-fired ceramic (LTCC) microchannel device. **b** Photograph of a microchannel device with an edge connector. **c** A top view of the device. **d** Cross-sectional view of the microchannel showing the four SPG sites that can serve as electrodes

enzyme glucose oxidase, Gox. Hydrogen peroxide, a byproduct of the oxidation reaction is used to quantify the concentration of glucose. The sensing system, based on electrochemical (amperometric) measurements, consists of thick film working, counter and reference electrode, a microreaction chamber and a semi-permeable dialysis tube running along the length of the device. The system operates by the size-selective diffusion of the glucose molecule through the semi-permeable walls of the dialysis tube into the micro-reaction chamber. Glucose concentration up to 9 mM was successfully quantified at a high sensitivity of 147 nA/mM.

3.2 Environmental monitoring sensors

Owing to rapid globalization, a wide range of man-made chemicals and by-products formed in industrial or combustion processes have been, and still are, released in the environment. The toxic matter is known to adversely impact the ecosystem as well as human life. The increasing number of potentially harmful pollutants in the environment has resulted in stringent legislation toward environmental regulation. To implement these tight regulations, analytical instruments that can provide high sensitivity, accurate and instantaneous results in a form factor that can be deployed in remote locations are critical. There is a critical need to monitor contaminants and toxins in air (volatile compounds and gases), water (heavy metals, virus and bacteria), soil (chemicals, minerals) and food (bacteria, viruses, toxins, mycotoxins, pyrethroids, etc.). Where online and continuous monitoring of environmental variable is imperative, traditional laboratory-based analytical methods will not suffice. Point-of-care systems driven by microfluidic technology is a key player in the realization of such environmental sensors. Unlike biomedical applications where sterility and biocompatibility are critical issues considered during development, design criteria is mostly based on robustness and performance in harsh environments. Packaging of the sensors to withstand harsh environmental conditions is crucial, since the use of LTCC-based microfluidic systems can provide significant advantages over most conventional microfluidic systems, such as mature packaging techniques, inertness to harsh chemicals and gases and non-corrosiveness.

Achmann et al. (2008) have reported the development of an LTCC-based microfluidic gas sensing system for the detection of formaldehyde (Fig. 19). A biosensors approach, where formaldehyde dehydrogenase, an enzyme,

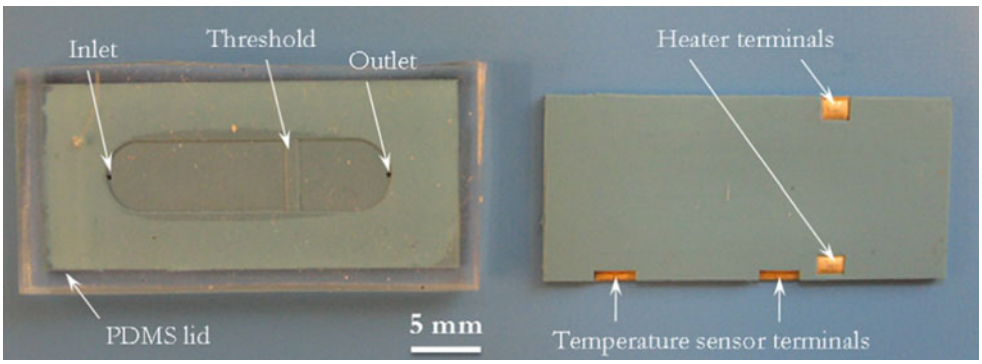

Fig. 17 The LTCC-based microreactor containing i/o microchannels, micro-heater and temperature sensor

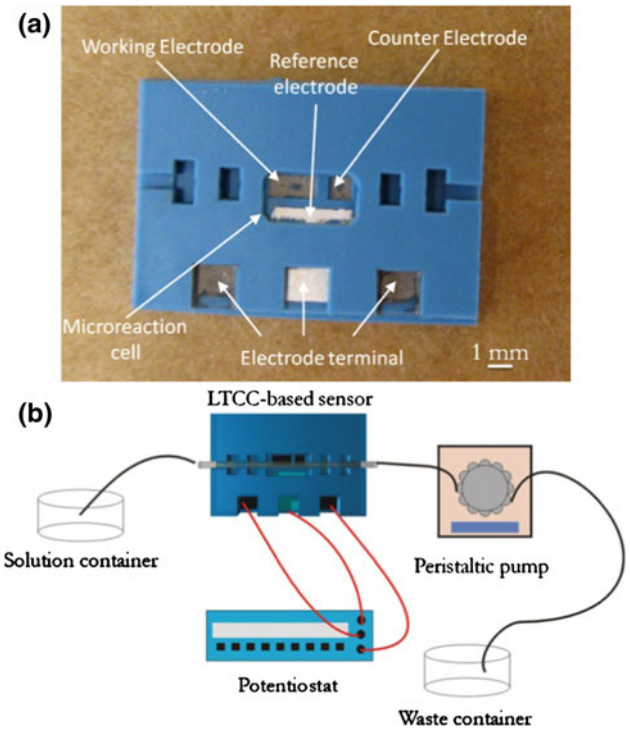

Fig. 18 **a** The LTCC-based manifold containing buried electrodes for amperometric glucose sensor; **b** schematic of the experimental set-up

is used as the sensing element in an amperometric sensing set-up. To support system integration, a ceramic diffusion membrane has been incorporated into the LTCC stack. The typical hydrophobicity required in the diffusion membrane is achieved by coating the ceramic membrane with an organochlorosilane in toluene. The sensing electrodes are realized by standard screen printing of Ag/AgCl. The sensor exhibits a low detection limit of 76 ppb with linear response in the range 500 ppb to 10 ppm. The sensor performance is comparable to a macro-sized counterpart while requiring half the enzymes and one tenth the sample size.

In an effort to develop a deployable on-site microanalyzer, Ibáñez-García et al. (2010) have developed an LTCC microfluidic system for the simultaneous detection of nitrate and chloride ions in water samples (Fig. 20). Two sets of screen-printed Ag/AgCl electrodes are used for potentiometric detection, and ion selective polymer membranes are used for selective detection of chlorine and nitrate ions. The integrated sensor performance demonstrated a linear working range of 10^{-3}–10^{-1} M for nitrate ions and 10^{-4}–10^{-1} M for chloride ion. Similarly, amperometric determination of free chlorine using carbon nanotube composite electrodes has been reported (Olivé-Monllau et al. 2011). The use of nanocomposites increases the system functionality and also enhances the surface renewability inherent in composite electrodes. A noticeably lower detection limit of 0.05 mg/L of chlorine ion was detected. Real samples collected from swimming pools were successfully tested for the determination of chlorine using the developed LTCC-based sensing system (Fig. 21).

Contamination of water bodies with metallic particulates originating as byproducts from industrial processes is a cause for serious concern. The presence of metallic contaminants in potable water has been correlated to many cases of carcinogenesis. An LTCC-based spectrophotometric microanalysis system (Alves-Segundo et al. 2010) has been developed for the determination of chromium (VI) in water (Fig. 22). The microfluidic system consists of inlet and outlet microchannels that transport the sample solution into and out of the measurement chamber. A glass window incorporated into the opaque LTCC structure allows the use of optical detection system such as colorimetry for determining the concentration of the target analyte. The developed system provides a linear sensing range of 0.1–20 mg/L at a detection limit of 50 μg/L.

An LTCC-based microfluidic system for detecting traces of heavy metals in biological and environmental fluids has been reported by Gongora-Rubio et al. (2004). The

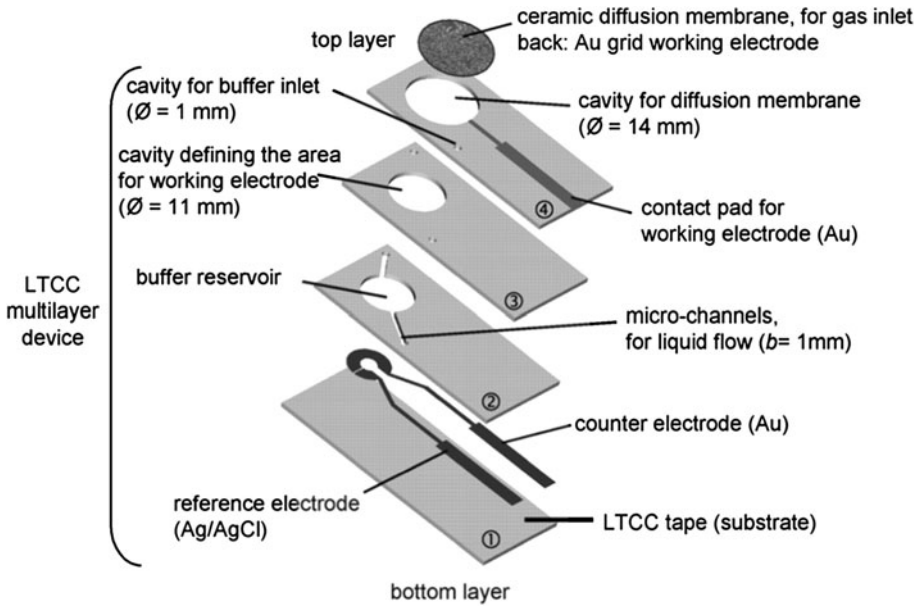

Fig. 19 Design of the LTCC multilayer sensor device. The device consists of four superimposed LTCC subunits (each comprising three layers). *1* Bottom layer with Ag- and Au thick-film tracks, *2* buffer reservoir with micro-channels and cavity for liquid storage, *3* protective layer for the channels and *4* top layer with a cavity clasping around the ceramic diffusion membrane and an Au thick-film track as contact pad for the working electrode. Gas inlet is provided by a porous ceramic membrane (modified with CTMS) with a Au grid working electrode screen-printed on the back

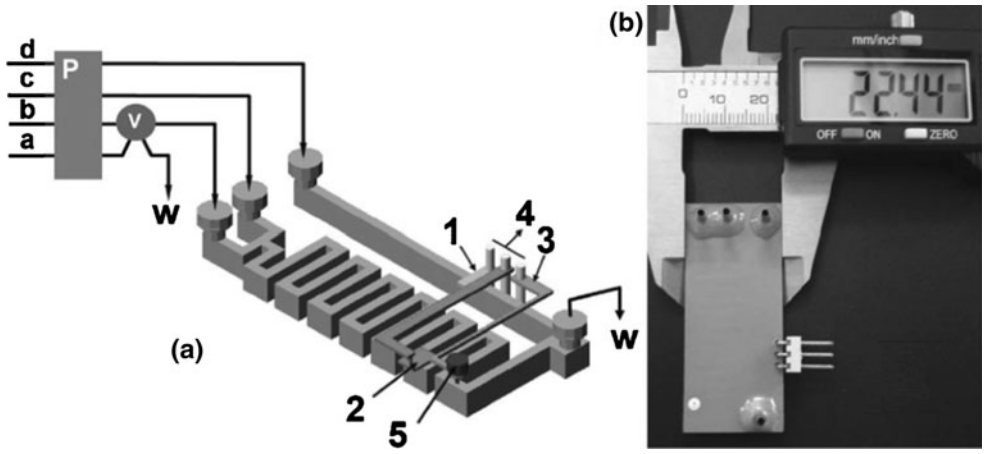

Fig. 20 a Schematic diagram of the constructed devices and experimental set-up. Fluidic elements: *a* sample solution; *b* carrier solution (H2O); *c* conditioning solution (K2SO4 0.1 M, pH 2.4); *d* auxiliary solution (KCl 0.1 M); *w* waste outlets. Electrical and detection elements: *1* reference electrode, *2* chloride selective electrode, *3* conductor path between the nitrate membrane and its corresponding via, *4* vias for the trough-hole connector, *5* deposited nitrate selective membrane. External actuators: *P* peristaltic pump, *V* six-port injection valve. **b** Picture of the constructed device and dimensions

design features of the LTCC microfluidic manifold include a spiral coil microchannel for passive mixing of two liquids required in most electrochemical sensing assays and a screen-printed microelectrode array that is required for the electrochemical detection technique (Fig. 23). An important part of the chip fabrication is the precise alignment of the electrode array with the microchannels. In this work, an innovative approach has been utilized; where in, a standard dental X-ray image is used to verify the alignment, since visual alignment is not possible in the opaque structure. The integrated system demonstrates detection of mercury and copper in low detection limits of 0.9 and 0.45 μg/L, respectively in urinary fluids. The same system can be employed for detection in environmental fluids such as river water and water bodies around industrial sites.

For the detection of organic compounds such as sulfamethoxazole (SMX) and trimethoprim (TMP), Almeida et al. (2011) have developed an LTCC-potentiometric microfluidic device. SMX, which has been used for many years as a human/veterinary antibiotic is now a potential threat as it is found in alarming quantities in water coming from municipal wastewaters and aqua farming water bodies. The developed sensing system uses synthesized antibodies as the sensing material. Molecular imprinting technique has been used to create ionophores of SMX on a plasticized PVC membrane. The membrane is then

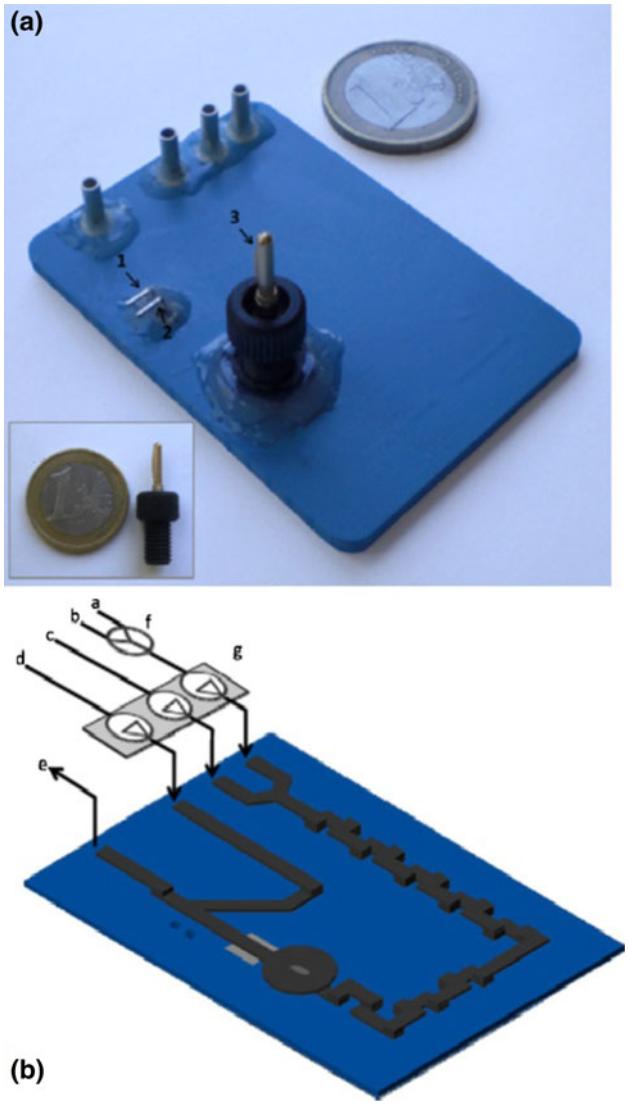

Fig. 21 **a** Amperometric microanalyzer integrating CNT composite electrode (*inset* CNT composite-based working electrode). External connection of: (1) reference electrode, (2) counter electrode and (3) working electrode. Experimental manifold used for chlorine determination in the ceramic microanalyzer based on CNT composite amperometric sensor. *a* Sample or stock solution, *b* deionized water, *c* carrier solution, *d* 0.2 M KCl, *e* waste, *f* solenoid valve and *g* pump

integrated into the LTCC microfluidic manifold for detection of SMX and similarly TMP. The sensor displays slopes of −58.7 mV/decade for a detection range from 12.7 to 250 μg/mL at a detection limit of 3.85 μg/mL at a high throughput of 36 samples/h. The sensor utilizes a sample volume of 3.3 mL per sample.

Various examples of systems application of LTCC-based microfluidic devices have been reviewed. However, there remain mainly opportunities for further development of the processing techniques of LTCC to realize novel functionality and applications. Realization of feature sizes in the nanoscale range in LTCC systems is currently limited by the fabrication techniques such as laser-assisted patterning, punching and screen printing. Novel fabrication techniques along with the use of new material compositions to create nanostructured green tapes, can in the future, enable the creation of nanoscale features such as nanochannels, nanowires and other nanotextures surfaces. While LTCC presents an attractive set of advantages, it does not necessarily overcome all the limitations of conventional microfluidic fabrication materials. Methods to integrate LTCC with silicon, glass, and polymers can lead to the creation of hybrid microfluidic devices that bring together the best from all the materials. LTCC microfluidic systems with actuators for microvalves and micropumps will be essential for realization of a stand-alone point-of-care system. Wearable sensors, which is gaining attention for personalized health care and environmental sensing applications could also benefit from the capabilities of LTCC-based microfluidic systems due to the applicability of LTCC in harsh environments (Benito-Lopez et al. 2009; Benito-Lopez et al. 2010).

4 Conclusion

A comprehensive review on LTCC-based microfluidic systems is presented. The application of LTCC technology for the development of point-of-care biomedical and environmental sensing systems has been reviewed. LTCC-based microfluidic systems provide many advantages that make LTCC a promising material for microfluidic device development. LTCC-based microfluidic system demonstrates faster processing time, parallel processing, easy creation of 3D architectures, biocompatibility, compatibility with ASIC and advanced packing technology. A comprehensive review of the material properties of LTCC and its comparison with its counterparts (Silicon, Glass and Polymers) is presented. General processing techniques used in LTCC fabrication and the specifics of the fabrication techniques for the creation of LTCC microfluidic components (microchannels, valves, microelectrodes, etc.) are discussed in detail. The constraints in LTCC-based microfluidic systems such as flexibility and opacity have also been discussed. The development of LTCC-based microfluidic systems is on the rise with new applications being reported in the recent past. With LTCC-based microfluidics expanding its applications and capabilities, several opportunities exist for innovation. The mechanically strong, chemically non-corrosive and thermally stable LTCC system finds good applications in point-of-care sensing both in biomedical (biocompatible) as well as environmental (harsh environments) sensing applications. Application example of LTCC-based microfluidic

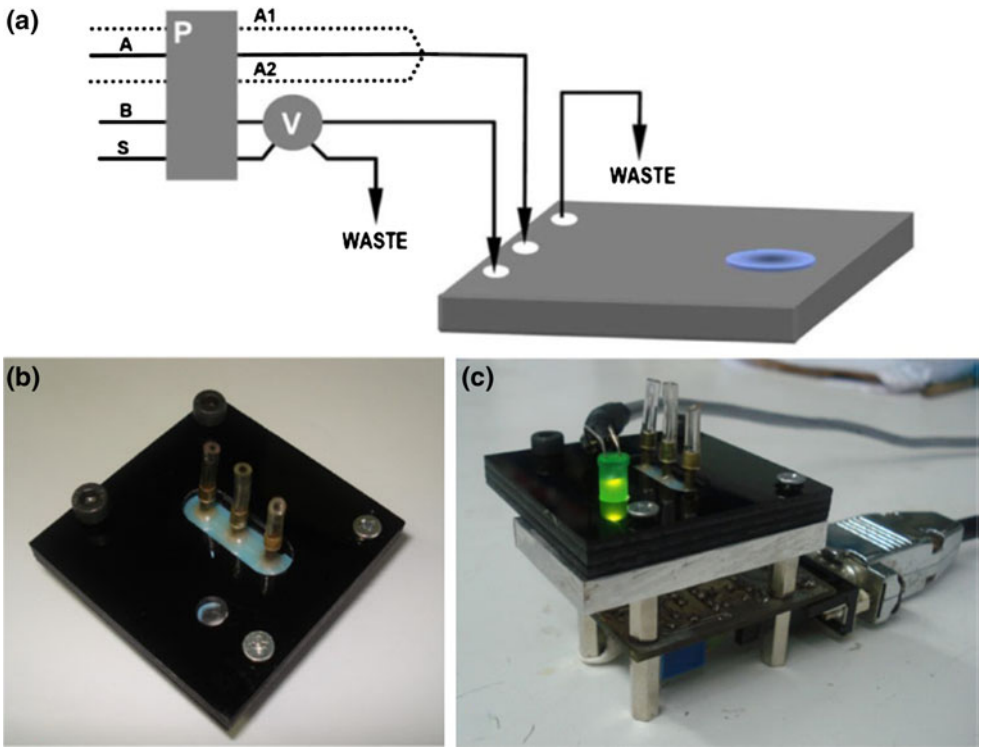

Fig. 22 Continuous flow microsystem set-up. **a** Experimental manifold: *A* acidified DPC reagent solution, *A1* H2SO4 solution, *A2* DPC reagent solution, *B* deionized water, *S* sample, *P* peristaltic pump, *V* six-port injection valve. **b** Protective PMMA black support for the external optical components. **c** Optical detection set-up: (1) LED; (2) PMMA support; (3) photodetector and associated electronics; (4) DB9 (RS232) computer connector

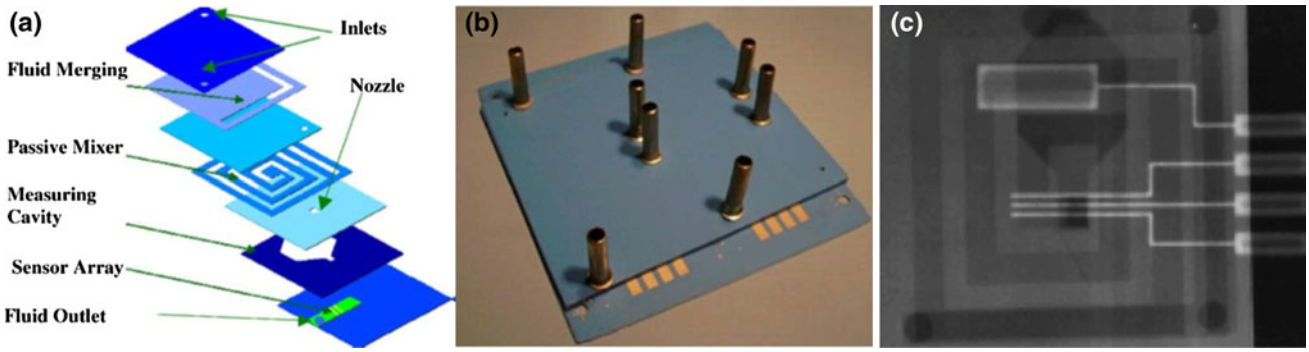

Fig. 23 **a** LTCC layers for manifold and sensor array. **b** Fabricated manifold with sensor electrodes. **c** Standard dental X-ray image for electrode alignment verification

systems for biomedical sensors (whole cell sensors, micro reactors, biosensors) and environmental sensors (gases, water pollutants) is also reviewed.

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