A BIOLOGICAL MONITORING MODULE BASED ON A CERAMIC MICROFLUIDIC PLATFORM

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Abstract: A 3-dimensional mesofluidic biological monitoring module has been successfully designed and fabricated using a low-temperature co-fired ceramic (LTCC) technology. This mesofluidic device consists of a network of micro-channels, a spherical mixing cavity and measuring ports. A selection of appropriate commercially available ceramic tapes has been chosen with regard to their biocompatibility performance. Specific processing procedures required for the realization of such a complex structure are demonstrated. Three dimensional numerical flow simulations have been conducted to characterize the concentration profiles of liquids at a specific measuring port and verified by experiment.

1 INTRODUCTION

Microfluidic and mesofluidic analytical systems are becoming increasingly popular in chemical and biomedical applications due to the need of small volume reagents, small wastes and short reaction times. Microfluidic devices may be classified into functionally limited labs-on-chip (LOC) and micro total analysis systems (μTAS). Most LOCs are single function and single layer devices such as mixers, separation channels, etc. Micro total analysis systems, on the other hand, are more complicated and are capable of performing many functions such as mixing, reaction, separation, etc. on a single module. Generally these devices handle nanoliters of reaction volumes and often accomplish their specific tasks in milliseconds of reaction times. Most of these systems are based on silicon, glass, polymethylmethacrylate (PMMA) or polydimethylsiloxane (PDMS) substrates (Anderson, 2000). A range of rapid-prototyping methods using laser-techniques for the fabrication of microfluidic devices are reported in literature. Micro-stereolithography, selective laser sintering, laser writing method and microcladding techniques are applied for generating complex 3-dimensional (3D) microparts of polymer, metal and metal-matrix composite (Kathuria, 2001, Yu, 2006). Micropatterned ceramic components may be fabricated in a rapid prototying process combining stereolithography for the supply of master models with low pressure moulding (Knitter, 2003). But true 3D microfluidic structures cannot be easily implemented using these techniques due to process limitations or material properties. The use of LTCC-technology for microfluidic devices enables the realization of multiple 3D microchannels, a feature not easily attainable in other MEMS technologies (Gongora-Rubio, 2001; Golonka, 2006). Therefore, based on this experience LTCC technology has been considered as an adequate approach to realize a compact temperature controlled monitoring module for biological reactions with low sample consumption which may be considered as a μTAS device.

The process technique for making a 3D-architecture with LTCCs is rather simple and standardized. Device production in LTCC technology covers the machining, punching or laser drilling of vias and channels on individual layers. The individual layers are stacked and laminated in a heated platen press or in a heated isostatic press. Subsequently the laminated stack is exposed to the firing cycle which is a somewhat critical process where heating rate, dwell time at burnout

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temperature and total firing cycle time have to be matched to the thickness of the ceramic stack.

The field of nonlinear chemical kinetics has been investigated about half a century. Still only a few complex chemical reactions have been described by means of an experimentally backed system of coupled chemical equations. Moreover, in biological relevant nonlinear systems most of the reactants, e.g. proteins etc., are expensive to prepare and generally only available in limited quantities. Therefore, it is essential to use tiny reaction volumes for continuous flow experiments, as realized in this reaction cell. This hereby presented contribution outlines the procedures of fabrication and characterization of a reaction module designed to follow quantitatively complex biochemical regulatory reaction networks in vitro as a basis for advanced mathematical fitting. This device consists of a mixing module which provides fast mixing of reactants and four sensor ports for simultaneous measurements. The functionality was proved using the well known oscillatory behaviour of the chlorite-iodide reaction (Kügler, 2008).

2 DESIGN

Figure 1 shows the schematic of the monitoring module also used as model for establishing finite element analyses (FEA).

The module comprises a spherical reactor cell where continuous mixing of the reagent fluids is provided. Besides this mixing chamber the module is equipped with pH-, oxygen-, temperature- and iodide sensitive sensors for reaction monitoring as well as with SMA-connectors for glass fibres required for absorption or fluorescence spectroscopic analyses (figure 2). Pumping a thermal fluid through embedded ducts provides temperature control. A network of micro-channels with cross section dimensions varying from 200 µm x 200 µm up to 2 mm x 2 mm are connecting the different measuring sections within the ceramic module. Special attention has to be spent on the selection of appropriate ceramic tape material with regard to biocompatibility and suitability to build up a complex 3-dimensional structure which contains a large number of cavities and channels.

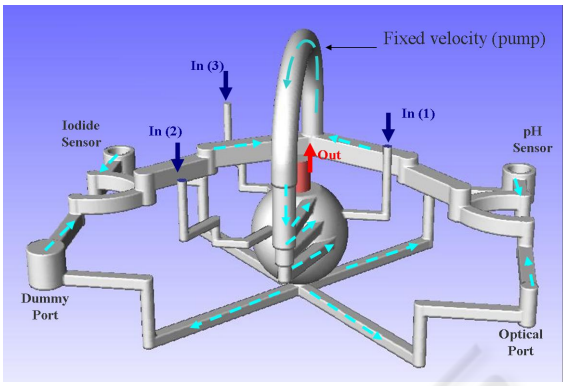

Figure 1: Scheme of the monitoring module with reactor cell and channel system.

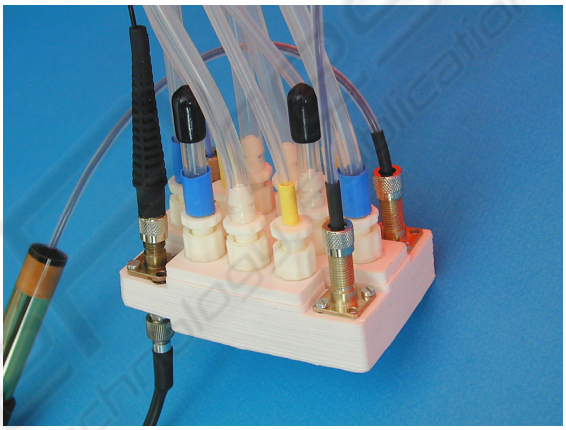

Figure 2: Biological monitoring module (completely assembled with sensor-, inlet-, outlet- ports and sockets for optical fibers).

3 DEVICE FABRICATION

3.1 Biocompatibility Testing

Three different lead-free tapes have been considered for this application like the ESL 41020, Ferro A6 and CeramTec GC-tape. The tapes should be biocompatible but nevertheless cells should not adhere and proliferate to a large extent on their surfaces. The latter requirements are critical with regard to clogging of channels by cell agglomerations. In order to compare the biocompatibility, proliferation, viability and adherence of HeLa (human, cervix epithelial) cells grown on sintered LTCC-tapes have been evaluated using standard test procedures.

3.1.1 Proliferation Test

As a first biocompatibility testing the ability of HeLa cells to proliferate on different LTCC tapes in contrast to glass and standard plastic surfaces was observed. The influence on cell proliferation was tested with the Bromodeoxyuridine (BrdU) assay (Gire, 1998) (Calbiochem, USA). BrdU incorporation is detected immunochemically with unlabelled primary antibodies and HRPO labeled secondary antibodies.

HeLa cells were exposed in a 96 well plate (1 x 10^5 cells/well) with the different test disks (4 mm x 4 mm) for 23 hours in DMEM (Dulbecco's mod. Eagle-Medium) containing 4.500 mg Glucose, 4.5 mM L-Glutamine, 44 mM Na-bicarbonate, 100 units/ml Penicillin, 100 µg/ml Streptomycin, 0.9 mM Na-pyruvate and 10 % fetal calf serum in a 6 volume % CO_2 humified atmosphere at 37 °C (standard conditions). During the final 3 hours of incubation BrdU is added. The BrdU concentration is measured using a multi-plate reader (BIORAD) at λ_{abs}= 455nm and $\lambda_{reference}$= 655 nm.

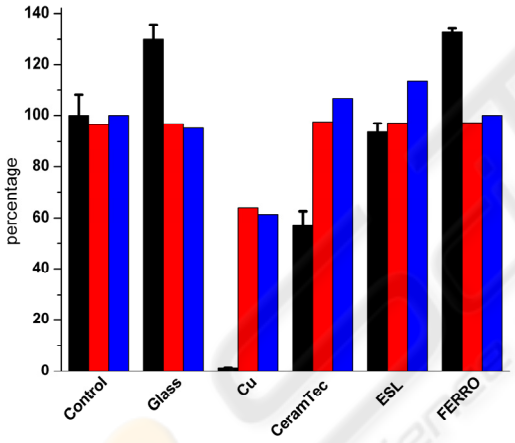

Figure 3: Biocompatibility testing for HeLa cells on fired LTCC- samples (CeramTec GC, ESL 41020, FERRO A6), glass-cover slides (Assistent, Germany), Copper (Cu) and standard plastic dishes (control-sample); Colour marking: black: proliferation rates, red: percentage of viable cells, blue: percentage of total cells compared to control group.

The results of the proliferation test are shown in figure 3 (black bars). With exception of the CeramTec GC-tape and the Cu- disks, the detected proliferation-rates of all other test samples are equal or even higher (glass support) compared to the growing rate of the control group on plastic support (100 ± 8.7 %). Also the deviation is comparable to the control group. In contrast CeramTec tapes show a 30-40 % reduction in proliferation. Nevertheless this reduced proliferation is low in comparison with cells incubated with Cu-disks, which under the same conditions show a proliferation rate of 2-3 % compared to control group.

3.1.2 Viability Testing

Next to the determination of proliferation rates it is necessary to analyze the ability of cells to adhere to surfaces and to determine the percentage of viable cells. For this test series HeLa cells were incubated in standard culture dishes with a diameter of 10 cm (about 5 x 10^5 cells/dish) for 23 hours in DMEM under standard conditions. The fired LTCC tapes to be tested, glass cover slides and Cu-plates were used with lateral dimensions of about 2.5 cm x 4 cm. Cells were incubated in the presence of these materials and on standard culture surfaces as control.

The number of cells and their viability was evaluated with propidium-iodide (Zarnai, 2001) using a Nucleocounter (Chemometec, Denmark). The results of the viability tests are presented in figure 3.

No significant reduction in number of total cells (blue bars) has been detected for all LTTC samples and glass as their percentage of total cells, in relation to the control group, are comparable within the error limits caused by inaccuracies of seeding the cells. Only samples grown on Cu show about 40 % less cells than the other samples.

Additionally not any significant reduction in percentage of viable cells (red bars) was shown for cells grown on LTCC tapes or glass support. Again, only samples grown on Cu-plates show a diminution of about 40 %.

3.1.3 Adhesion Testing

The side walls of channels and cavities are formed by the laser machined edges of tapes. To observe cell adhesion, which may rely on tape material but also on surface finishing a test method has been designed which enables to examine the potential adhesion of cells on the laser machined edges of the tapes. To evaluate the influence of the ceramic tapes on the adhesion of cells on glass surfaces, CeramTec and ESL ceramic tapes with laser-micro machined tapering channels with decreasing channel width (see figure 4-A) were mounted on standard glass cover slides (Assistant, Germany) with a super adhesive (Loctite). HeLa cells were seeded on the cover slides (10^5 cells) and incubated over night in DMEM medium under standard conditions. The growth behaviour of the cells was evaluated using a light transmission microscope.

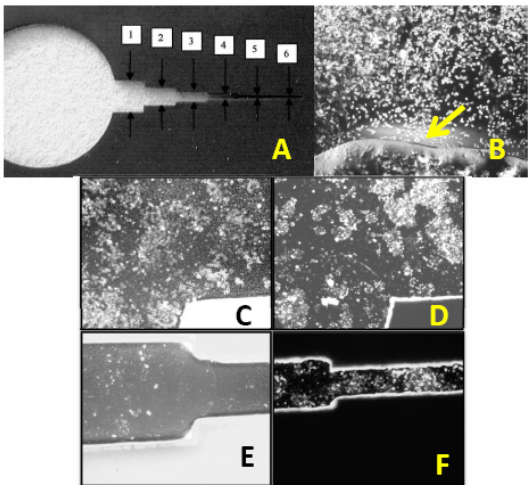

Figure 4: A: Test sample of a ceramic tape coupon carrying a continuous row of channel segments with decreasing width (central circular opening is 10 mm in diameter, the channel width is 2.122, 1.175, 0.672, 0.394, 0.228, 0.168 mm and 2.208, 1.224, 0.653, 0.396, 0.102, 0.054 mm for channels 1 to 6 of the ESL (D, F) and CeramTec (C, E) tape, respectively. C, E: HeLa cells grown on glass cover slides with a mounted fired CeramTec-tape at channels 0-1 (C) and 2-3 (E). D-F: HeLa cells grown on glass cover slides with a mounted fired ESL-tape at channels 0-1 (D) and 3-4 (F). B: HeLa cells grown on glass cover slides surrounded by mounting glue (arrow). Micrographs were performed with a Zeiss Axiovert S100TV at 10x magnification and a Digital Camera (Nikon DMX1200).

Exemplary the results for the CeramTec and the ESL tapes are shown in figure 4. It could be demonstrated that the initial circular opening and channel 1 machined in the CeramTec GC tape and the ESL tape show only a poor influence on the adherence of HeLa cells (figure 4-C, D; cell density in circular opening: 250 – 300 cells/mm^2, channel 1: 200 – 220 cells/mm^2) but already at channels 2 and 3 (figure 4-E) with a width of about 0.6 mm a significant decrease in adherence can be observed for CeramTec tapes (cell density in channel 2: 45 cells/mm^2, channel 3: 19 cells/mm^2) as no significant number of cells is observed at this area. In contrast the fired ESL-tape shows quite a different cell adherence performance. Also in channels 3-4 (figure 4-F) a rather dense cell population of cells (cell density in channel 3 and 4: ca. 100 cells/mm^2) and even aggregation near to the edges are observed. Only for smaller channels with width of about 0.2 mm a decrease of adherence can be observed (data not shown). The mounting glue has not any effect on the adherence behavior of HeLa cells to the glass surface (figure 4-B).

3.1.4 Summary of Biocompatibility Testing

It can be concluded that all three LTCC materials tested do not affect the viability of the cells, as the total number of cells counted after the same period was found identical within an error interval of ± 10 % for all LTCC-materials, standard plastic as well as glass. An increased cell death compared to the control group could not be found for any of the standard materials. Only copper showed a significant influence on viability, cell adherence and proliferation. If only the proliferation characteristic is considered, a distinction in tape performance can be made. Whereas the ESL-tape shows a similar behaviour with respect to the control assay of a standard plastic support, FERRO-tape reacts similar to a glass support and shows an increased proliferation. Only the fired CeramTec-tape exhibits a significant decrease in proliferation (figure 3). These results are also validated by the adherence test. When cell growth on a glass support was restricted by sidewalls of a narrow channel configuration, the cells reacted differently to ESL- and CeramTec-tapes: Whereas on ESL tapes the cells grow even in channels of very small width, it was not the case for channels machined into CeramTec-tapes. For these samples the area near to the glass – ceramic interface was widely free of cells and an efficient growth of cells was only detectable in rather wide channels. The reasons of the difference in cell adhesion on the channel walls are still under investigation. It might be related to the difference in surface energy as result of laser machining. The quality of the cutting edges and the surface of channel walls are defined by the absorption of laser energy in the tape material which may vary in dependence on the composition of the selected LTCC- material.

The results show, that on the one hand side fired ceramic tapes are generally biocompatible and do not restrict the viability of cells, and on the other hand side cells adherence can be specifically influenced by the material selected. CeramTec GC tape should be preferred, when adherence to the walls of the chamber or channels should be restricted as e.g. in microscopic observation chambers where single cells are preferentially observed in the center of an optical window and a cell agglomeration along the side walls or growth into micro fluidic flow channels should be avoided.

3.2 Processing Procedures

The CeramTec GC tape has been proved as a suitable candidate for the realization of the monitoring module not only with regard to the results of the biocompatibility tests but also due to its performance characteristic during processing. Since the module comprises a complex network of channels and cavities a tape material is required which does not tend to sag during firing. Beyond the green GC-tape with a thickness of 325 µm provides an adequate ruggedness for handling.

The three dimensional LTCC-structure is realized by forming a stack of adequately laser machined (diode pumped Nd:YAG-laser (Rofin Sinar, Germany) equipped with an acoustic optical switch, operating power: 12 W at TEM00-mode) single layers of tapes which are laminated and finally exposed to a firing process. For the realization of the complex module a single tape shows a rather delicate structure since it is penetrated by large-area laser machined perforations. A standard LTCC-processing technology cannot be easily applied for the realization of the module since it comprises 133 ceramic layers, which deviates from the number of tapes usually applied for conventional applications.

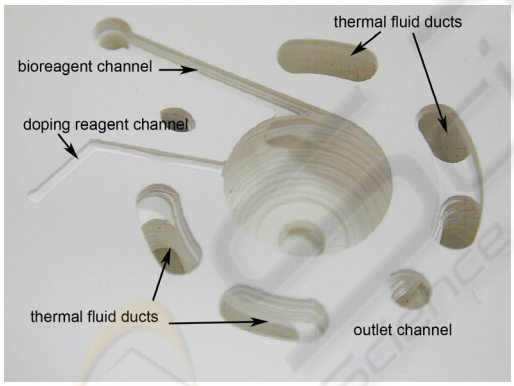

Figure 5: Inside view of reactor cell with various channels and ducts (collated stack of tapes before lamination).

Special attention has to be paid on the lamination of the ceramic tapes since the module contains a large number of cavities and channels (e.g. the reactor cell has a cavity volume of 1 cm^3). The finished sheets are collated in a mould (figure 5) and aligned by registration pins providing that successive layers are rotated by 90° to compensate for the texture (preferential orientation) induced by the fabrication of green tape. The lamination of the stack of tapes has been carried out in a heated platen press (Wabash).

A range of experimental work has been conducted (Wang, 2008) in order to optimize lamination parameters which should contribute to provide the shape integrity of channel and cavity structures. Sagging and delamination are typical for relatively wide channels (width equal to 500 µm or more) whilst contraction is characteristic for narrow channels (width equal to 200 µm and less). The application of sacrificial material for filling cavities and channel structures is a valuable approach to avoid sagging as well as the risk of delamination. The selection of an appropriate sacrificial material is absolutely essential for this complex microstructure device. It has been found out that the main task of the sacrificial material is to provide a uniform pressure distribution within the LTCC-tape stack during lamination. An additional supporting function of cavity structures during firing is not required since the considered LTCC material shows in all the phases of the firing process an adequate strength and stability. This substance should evaporate during the burnout phase of the sintering process without damaging the structure of the module. So the approach was to find a material, which is accommodated to the shrinkage performance of the tape. An obstruction of material's shrinkage should be avoided. Some authors recommend carbon black as sacrificial volume material (SVM) which may be applied as tape or paste (Birol, 2005). This material decomposes and exhausts at rather high temperature when sintering of tape already starts. Different polymer materials have been tested as potential candidates acting as SVM since they decompose and burn out at a temperature < 400 °C (before tape-shrinkage is starting).

Best results have been attained with PMMA chosen as SVM. It is also part of the organic constituents of the considered tape and exhausts free of residues at the burnout temperature of tape. The organic sacrificial material has been used in powder form. Channels and cavities are filled by a vacuum sucking technique. It has been found out that a uniform pressure of only 30 bar (sample temperature: 70 °C, lamination time: 3 minutes) has to be applied onto the ceramic layer stack which enables to maintain the rectangular cross-section of the buried channels while still avoiding delamination of the ceramic batch. The applied pressure was reasonably lower than recommended for conventional applications.

Thermal gravimetric analyses have been conducted to optimize the sintering profile (especially to provide a complete and slow burn out of the organics before sintering) in order to avoid

crack formation or delamination. The binder decomposition process was studied by means of Thermogravimetric Analysis (TGA) coupled with a Mass-Spectrometer (MS) to detect simultaneously the evolved gases (Balluch, 2008). Additionally, the binder burnout was analyzed by Dynamic Scanning Calorimetry (DSC). TGA revealed multistage decomposition behaviour of the binder system. The binder degradation is predominately governed by exothermic reactions in the temperature range up to 400 °C and the course of the heat flow may be correlated with the evolution of carbon dioxide. The heat flow of GC-tape shows two exothermic peaks: one at 250 °C and a major one at 364 °C. For the GC-tape the first exothermic peak corresponds to the maximum degradation rate in the TGA as can be seen in the insert of figure 6.

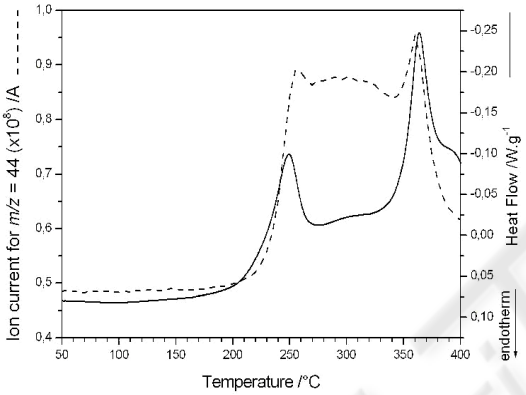

Figure 6: Comparison of the heat flow signal with the ion current for carbon dioxide for GC-tape.

The results of this study yield a useful approach to establish the firing profile of the considered LTCC with regard to heating rate and intermediate dwell time for the preheat phase of firing process. Based on the results of TGA it becomes evident that for the burnout stage of the GC-tape firing process an appropriate dwell time depending on the mass of LTCC-module has to be established. It has to be provided that the PMMA starts to pyrolyze slowly which enables to exhaust the gaseous decomposition products via the channels. In contrast a rapid decomposition of the organic filler induces a sudden intensified production of gaseous burnout products which cannot escape adequately via the channels and finally results in a destruction of the LTCC module. The burnout phase of the firing schedule has to be adapted to these requirements whereas an adequate dwell time (depending on the mass of the LTCC module) at the critical degradation temperature of the filler is of great importance. Practical experiments have shown that a dwell time at 250 °C is ignorable if an adequate slow heat rate is selected. A sufficient long dwell time at 350 °C for the total binder burnout is obligatory to be provided.

Firing of the samples has been conducted in a box furnace of Heraeus (heating rate for temperature range 25 °C – 350 °C: 0.8 °C/min, holding time at 350 °C: 6 h, heating rate for temperature range 350 °C – 500 °C: 0.8 °C, holding time at 500 °C: 4 h, heating rate for temperature range 500 °C – 920 °C: 1.8 °C/min, holding time at peak temperature of 920 °C: 1 h, cooling rate: 2 °C/min). Another parameter, which has to be considered during the firing cycle, was the temperature distribution within the sample. A temperature gradient > 6 °C within the ceramic module during the total firing cycle has to be avoided with regard to potential crack formation and fracture of the module (Kluge, 2006).

4 DEVICE CHARACTERIZATION

The influence of arrangement of inlet-channels along the perimeter of the reactor cell and the sink on the bottom of the cell on the flow performance of liquids within the reactor cell has been studied for different flow conditions by means of FEA using the FLUENT program package. The characterization of the device is based on time - dependent flow simulations. The numerical model describes the flow of dyed and pure water entering through different inlets at varying mass flow rates and passing into the spherical cavity of the reactor cell and the channel system. The local distribution of fluid at the spectroscopic port is predicted and contrasted with the results attained by spectroscopic analyses of light absorption at the considered port.

Exemplarily the flow characteristic for a representative assumption has been derived by numerical simulation and validated by experiment. The flow condition described starts with filling the cavity by injection of pure water at the radial inlets 1 and 2 (figure 1 and 7). The mixing initiated as a step inflow of dyed water (0.5 % trypan blue) is imposed on the radial inlet 2, which means that a stream of constant mass flow–rate and dye–concentration is applied at the respective inlet (figure 7). In the meantime the average residence time of dye concentration is measured at the optical port (figure 1).

The propagation of the streamlines depends strongly on the position of the inlet along the meridian of the spherical cavity as can be seen in figure 7 for the corresponding inlet arrangement.

The inlet 1 ("In 1") is placed 0.45 mm higher than inlet 2 ("In 2"). Within a period of 2.5 s the streamlines of the fluid entering inlet 1 are already diffusing into the interconnecting channels while those of the fluid starting from inlet 2 are still ending in the mixing chamber.

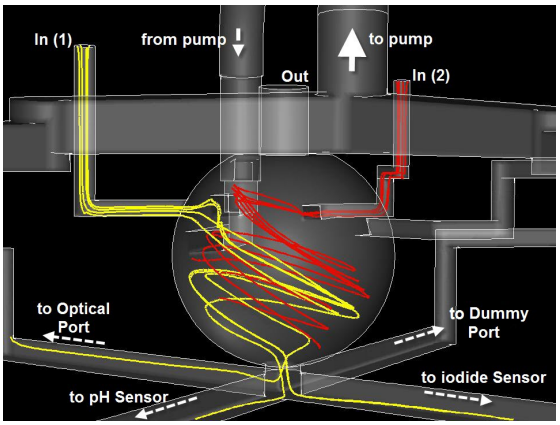

Figure 7: Propagation of streamlines of fluids entering inlet "In 1" (blue) and inlet "In 2" (red) with flow rates of 2 ml/min within a period of 2.5 s.

The model of the complete module was constructed and meshed with the program package GAMBIT (Fluent Inc.) Mesh elements were mainly hexahedral and only few tetrahedral elements were required for completely meshing the module. In all, the model (including the recirculation tube) has a volume of 3 cm³ and includes a total number of 292,194 mesh elements, of which 161,374 are hexahedral, 128,737 are tetrahedral and 2,083 are pyramidal.

The curves in figure 8 describe the increase of the dye volume in the optical port from initially pure water to the mixed state. For the supply of inlets with liquids two Flodos Stepdos 03 pumps were used and for providing the flow circulation a Flodos NF 5 diaphragm pump was applied. The flow rates considered in simulation and experiment are 2 ml/min at both inlets. At the optical port the absorbance was measured using a uv-vis array-spectrometer (EPP2000-50 µm Slit, StellarNet Inc.). The absorbance–characteristic in a scaled presentation is shown in figure 8. The steady state mixed flow condition at the optical port corresponds to a dye concentration of 47 %. It can be noticed that the ideal and the computed curves are almost identical. However, the experimentally derived characteristic shows a slightly less rate of rise as well as oscillating peaks. The oscillating peaks may be attributed to the pulsating characteristic of the pumps in use. Furthermore it must be noted that the equilibrated state concentration condition is attained also within the same period if the injection flow rate at the inlet ports has been varied synchronously while keeping the total sum of mass flow rates constant. If all three inlet ports are used simultaneously for the supply of the module different mass flow rates have to be selected to provide the desired mean concentration profile at the optical port.

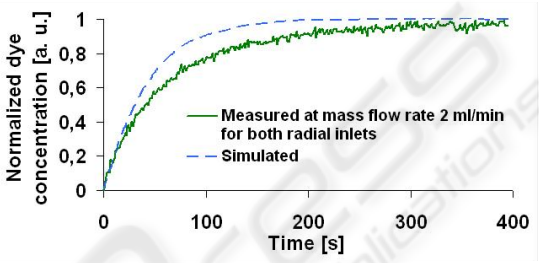

Figure 8: Dye concentration vs. time characteristic at the optical port.

Besides the performed dye distribution tests, the functionality of the microfluidic reaction module has been demonstrated by observing the time evolution of the iodine concentration due to the iodide-chlorite reaction, which involves only 2 inorganic ions and is characterized by an established set of reaction mechanisms according to the LLKE-model, which is named after its authors Lengyel, Li, Kustin and Epstein (Kügler, 2008). This complex reaction model involves an autocatalytic step and the coupling of different reactions can cause complex reaction patterns like oscillatory behaviour when measured under certain conditions in continuous flow. An intermediate of the chemical reaction leads also to production of the starting compound, which accelerates the reaction until the respective reaction partner is used up, but when the reaction is performed under continuous flow conditions oscillatory behaviour results which can be observed continuously. Figure 9 shows the oscillations observed for the iodine absorption measured around 470 nm (i.e. in a range between 456 to 484 nm). This means, that the optical density of the product is measured as a mean value over 60 pixels of the CCD-array (full black curve). The red curve shows the final result when the time dependence is additionally averaged over 20 seconds. The figure demonstrates that such an autocatalytic reaction can be measured in the microfluidic reaction module, although the total reaction volume and also the optical path of the optical detection port is very

small. Nevertheless oscillatory behaviour can also found under such conditions. The device is sensitive enough to record small concentration changes giving rise to changes in the optical density below 0.001. In future experiments the microfluidic module will be used for measuring complex kinetics in biochemical systems. In that case, limited amounts of reactive compounds, e.g. of enzymes, are available and the use of small reaction volumes is an essential prerequisite to allow quantitative measurements.

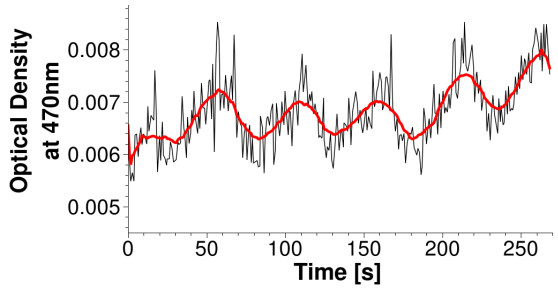

Figure 9: Oscillations of the I_2 absorption observed in the module produced by the reaction of I^- and ClO^{2-} in continuous flow.

5 CONCLUSIONS

The influence of different ceramic materials on the viability and proliferation of HeLa cells has been tested as a first basic characterization of the biocompatibility. In respect to their use in micro fluidic devices the growth of cells in channels of different width has been visualized. The LTCC-technology has been proved as a very versatile method to build up complex three-dimensional multilevel channel structures including even large-volume cavities, which are suitable for biological and diagnostic applications.

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REFERENCES

Kathuria, Y. P. (2001) "An overview of 3D structuring in microdomain", *J. Indian Inst. Sci.* vol. 81, pp. 659-664.

Anderson, J. R., Chiu, D. T., Jackman, J., Cherniavskaya, O., McDonald, J. C., Wu, H., Whitesides, S. H. and Whitesides, G. M. (2000) "Fabrication of topologically complex three-dimensional microfluidic systems in PDMS by rapid prototyping", *Anal. Chem.,* vol. 72, no. 14, pp. 3158-3164.

Yu, H., Balogun, O., Li, B., Murray, T. W. and Zhang, X. (2006) "Fabrication of three dimensional microstructures based on single-layered SU-8 for lab-on-chip applications", *Sensors Actuat. A Phys*., vol.127, pp. 228-234.

Knitter, R., Bauer, W. and Göhring D. (2003) "Microfabrication of ceramics by rapid prototyping proces chains", *J. Mechan. Eng. Sci.* vol. 217, pp. 41-51.

Gongora-Rubio, M. R., Espinoza-Vallejos, P., Sola-Laguna, L. and Santiago-Aviles, J. J. (2001) "Overview of low temperature co-fired ceramics tape technology for meso-system technology (MsST)", *Sensors Actuat. A Phys*., vol.89, pp. 222-224.

Golonka, L. J., Zawada, T., Radojewski, J., Roguszczak, H., and Stefanow, M. (2006) "LTCC Microfluidic System", *Int. J. Appl. Ceram. Technol.*, vol. 3, no. 2, pp. 150-156.

Kügler, Ph., Gaubitzer, E. and Müller, St. (2008) "Parameter identification for chemical reaction systems using the adjoint technique and sparsity enforcing stabilization - a case study for the Chloride Iodide reaction", submitted for publication to *J. Phys. Chem.*

Gire, V. and Wynfford-Thomas, D.W. (1998) "Reinitiation of DNA Synthesis and Cell Division in Senescent Human Fibroblasts by Microinjection of Anti-p53 Antibodies", *Mol. Cell. Biol.,* vol.18, no. 3, pp.1611-1621.

Zamai, L., Canonico, B., Luchetti,F., Ferri, P., Melloni, E., Guidotti, L., Cappellini, A., Cutroneo, G., Vitale, M. and Papa, S. (2001) "Supravial Exposure to Propidium Iodide Indentifies Apoptosies on Adherent Cells", *Cytometry* vol. 44, pp.57-64.

Kluge, C. P.(2006) *private communication*.

Birol, H., Maeder, T., Jacq, C., Straessler, S. and Ryser, P. (2005) "Fabrication of Low-Temperature Co-fired Ceramics micro-fluidic devices using sacrificial carbon layers", *Int. J. Appl. Ceram. Technol*., vol. 2, pp. 364-373.

Balluch, B., Lüftl, S., Seidler, S., Smetana, W. (2008) "Binder Burnout Studies of LTCC Tapes by Means of Thermal Analyses", *Proc. 32 nd Int. Conf. of IMAPS,* Pultusk, Poland: in print.

Wang, X. S., Balluch, B., Smetana, W., Stangl, G. (2008) "Optimization of LTCC-channel Fabrication for Micro-Systems", *Proc. of 30st ISSE 2008,* pp.420-425.