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Biological Microdevice with Fluidic Acoustic Streaming for Measuring Uric Acid in Human Saliva

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***Abstract*—The healthcare system requires new devices for a rapid monitoring of a patient in order to improve the diagnosis and treatment of various diseases. Accordingly, new biomedical devices are being developed. In this paper, a fully-integrated biological microdevice for uric acid analysis in human saliva is presented. It is based on optical spectrophotometric measurements and incorporates a mixture system based on acoustic streaming, that enhances the fluids reaction due to both heating and agitation generated by this effect. Acoustic streaming is provided by a piezoelectric β-PVDF film deposited underneath the microfluidic die of the device. Further, it incorporates the electronics for the detection, readout, data processing and signal actuation. Experimental results proved that acoustic streaming based on this piezoelectric polymer is advantageous and reduces in 55% the time required to obtain the analysis results.**

I. Introduction

The monitoring of uric acid levels in body fluids can provide important information for the diagnostic and therapy of several diseases [1, 2]. Uric acid analysis in saliva, instead of serum or urine, is a very attractive and advantageous method for a point-of-care device [3]. Usually, that kind of analysis is performed by spectrophotometry and carried out in clinical laboratories. As a consequence, the results become available after several hours, slowing patient's diagnostic and hindering quick monitoring and response. The development of test strips is an attractive method for saliva sample analysis [2, 3]. However, the result is based in a qualitative color readout, which can not lead to rigorous conclusions. Therefore, there is a large demand in the healthcare system to develop new microsystems for rapid and reliable point-of-care testing and monitoring that could be used to assess patient's health [4]. The use of such microfluidic devices allows the reduction of the response time and improves the analytical performance, laboratory safety and costs, due to the reduction of the sample size and chemicals storage [5]. For that, it is required that the involved fluids have a rapid mixing as well as an accurately controlled transport, which can be achieved by using passive or active mechanisms. Passive mixers use complex three-dimensional channels design and the mixing process rely only on diffusion, which can involve long transit and mixing times [6, 7]. The use of active mixers, like valves or magnetic/electrokinetical forces, is attractive for an effective transport and mixture due to the high chaos generated into the flows. However, most of these systems are difficult to miniaturize and to integrate in a single-chip, once they usually need complex control systems that increase the cost of the device and, more important, can damage sensitive fluids (e.g. fluids containing cells) [6, 8].

An interesting solution for the previously indicated limitations is the use of acoustic streaming achieved using a simple piezoelectric material. Applying an ac electrical voltage to its contacts, the piezoelectric film generates acoustic waves. These waves promote fluid motion in the direction of the acoustic attenuation and propagation. This phenomenon was demonstrated to pump fluids [9], to promote the mixture of fluids in microchannels [6, 9] and to enhance enzymatic microreactors [10].

II. Acoustic Streaming Phenomenon

Numerous efforts are being made to improve the fluids reaction at the microscale through the development of new and better mixing systems. An interesting mechanism that has received limited attention but has been able to improve the efficiency of the mixture in microfluidic devices is the acoustic streaming phenomenon. It can be described as the generation of a fluidic velocity due to the propagation and attenuation of the acoustic waves in a fluid [11].

This mechanism offers a variety of distinct advantages compared to other mixture techniques, like reliability (no moving parts), simple manufacturing and integration in a microfluidic system, less damage to sensitive fluids, scalable in very small channels and low-cost. On the other hand, acoustic streaming is a second order nonlinear mechanism; therefore, the majority of the electrical signal applied to the piezoelectric transducer is converted into heat, reducing significantly the efficiency of the agitation. Many studies of acoustic streaming consider this effect a disadvantage [9, 12]. However, in many cases, the fluids reaction is favored by a slight heating. Examples of the former are, among

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others, uric acid, urea, citric acid, potassium and sodium analysis in clinical diagnosis [13]. These heating can reach 37ºC or even higher temperatures. In these situations, both the agitation and the heating generated by the acoustic streaming effect is favorable, allowing a further reduction of the reaction time and obtaining, consequently, the analysis results in a shorter time. The heating can be also easily controlled by the amplitude and frequency of the electrical signal applied to the contacts of the piezoelectric film.

III. Biological Microdevice

A. Device Operation

The device described in this paper is developed for reaching the above objectives. The first prototype is illustrated in Fig. 1 and Fig. 2. It is composed by two dies: the fluidic die and the optical detection and electrical actuation die, all integrated into a single-chip.

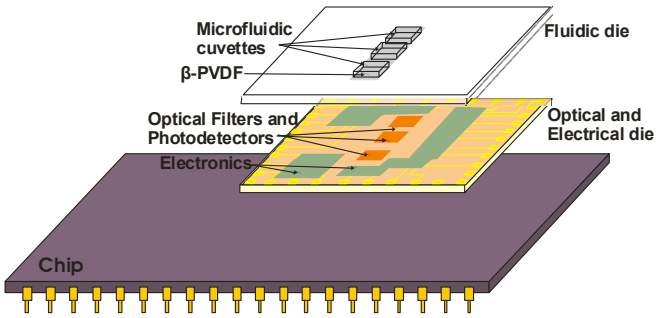

Fig. 1. Schematic representation of the several layers that constitute the microsystem.

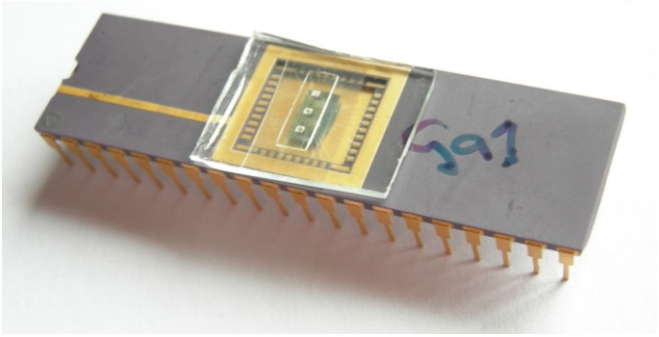

Fig. 2. Picture of the prototype of the biological microsystem. The fluidic die is glued above the CMOS die.

It is a portable, low-cost, plastic based and highly automated biological microsystem for testing and monitoring uric acid levels in saliva samples. Its operation is based on colorimetric detection by optical absorption of the saliva sample after mixing it with a proper reagent for uric acid analysis.

The fluids absorb the acoustic waves generated by the piezoelectric material, resulting in a radiation pressure and consequently in fluid microagitation. Therefore, the required time for the complete and homogeneous mixing is accelerated [11]. In this device, the acoustic streaming is implemented using an integrated piezoelectric polymer film, poly(vinylidene fluoride) in its beta phase (β-PVDF) with ITO (Indium Tin Oxide) electrodes [14]. This structure is deposited underneath the microfluidic die that is fabricated using the photoresist SU-8 (Fig. 3). The SU-8 epoxy provides low sidewall roughness and deep rectangular vertical structure of the fluidic die, which is suitable for the optical measurement. It also offers good properties like high-mechanical strength, biocompatibility, good adhesion on many different substrate materials and involves a low-cost fabrication process by UV lithography. Moreover, this die can be disposable to avoid contamination between analysis and complex cleansing that may involve hazardous reagents.

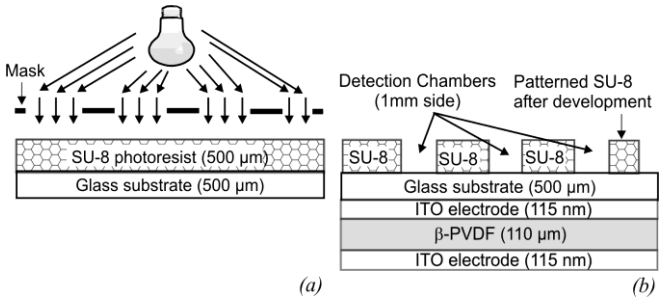

Fig. 3. Cross-section of the microfluidic die fabrication sequence and its dimensions: (a) deposition, spin coat, soft bake, UV exposure; (b) development of the SU-8.

The microfluidic die and consequently the detection chambers must have a minimum thickness of 500 µm, fundamental for the optical measurement technique, to provide a reasonable optical pathlength concerning the usual absorption coefficients of the biochemical parameters that are, generally, present in human´s physiological fluids [15]. Three detection chambers are required for a reliable analysis: one for the baseline reference given by the chemical reagent, a second for the calibration of the biochemical parameter concentration and for avoiding light fluctuations and a last one for the reaction of the sample with the reagent to perform the colorimetric analysis: the reaction chamber.

The detection die (Fig. 4) comprises the photodetectors, its readout electronics and the electronics for controlling the electrical signal applied to the β-PVDF film, all based on a standard CMOS technology. The photodetectors are pn-junction photodiodes designed to give the best quantum efficiency at the visible light [16]. The readout electronics is based on a Sigma-Delta converter, allowing the small data-acquisition and control system and providing a signal proportional to the light transmitted from the photodiode through the fluids. These data allow, after some processing based on the Lambert-Beer´s Law, to obtain the concentration of the molecule of interest [16]. Finally, the electrical actuation is based on a CMOS LC oscillator [17].

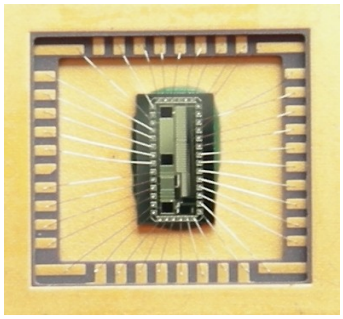

Fig. 4. CMOS optical detection die of the biological microsystem.

B. *Acoustic Transducer*

The acoustic streaming is generated by a piezoelectric transducer based on a 110 µm thick β-PVDF film with 115 nm thick ITO electrodes deposited on both side by magnetron sputtering. This polymer was chosen due to its excellent characteristics for the microfluidic device fabrication [18]. It is transparent, flexible, moldable and inexpensive. It has a high chemical agent resistance and it is easily produced into a thin-film. In addition, it has low acoustic and mechanical impedance, important for the propagation of the acoustic waves in the fluids. In opposition, ceramics piezoelectric materials have high-acoustic impedance, comparatively to fluids or plastics; therefore, the reflection coefficient on the boundary layer is above 90%. So, only a small fraction of the acoustic waves generated by the piezoelectric material is transferred to the medium, which drastically limit the efficiency of the acoustic streaming [19].

PVDF presents an unusual polymorphism constituted by four crystalline phases. For sensors, actuators and transducers applications, the β-phase is more adequate, due to its higher piezo-, pyro- and ferroelectric properties [20].

The electrodes of the piezoelectric film should be transparent due to the optical absorption measurements. Among the transparent conductive oxides, it was chosen ITO due to its suitable transmittance in the required spectral range (Fig. 5). In addition, it was also proved that this material does not degrade in contact with the used uric acid kit and, furthermore, the piezoelectric transducer can be deposited inside the microfluidic die, in direct contact with the fluids [21]. This study was based on High Performance Liquid Chromatography (HPLC) and proved that β-PVDF films with ITO electrodes do not degrade both with and without acoustic streaming (unlike AZO and Aluminum electrodes, for example).

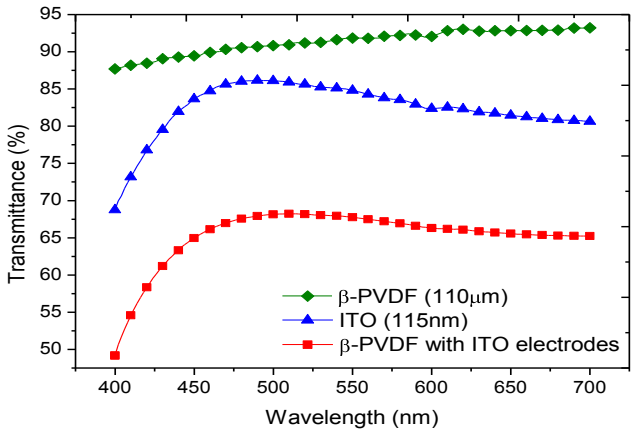

Fig. 5. Measured transmittance curve of the β-PVDF piezoelectric polymer (110 µm thick), the ITO electrodes (115 nm thick) and the β-PVDF with its ITO electrodes.

IV. Experimental Procedure and Results

The efficiency of the acoustic streaming generated by the β-PVDF film was proven experimentally. The evaluation of the mixing process was carried out using the Far Diagnostic kit with a 20 mg/dl standard of uric acid concentration [13]. The mixture produces a pink color and has a maximum absorption at 550 nm, monitoring by optical absorption spectrophotometry.

The measurement was performed in the cuvettes of the microfluidic die presented in Fig. 2. The cuvettes are 500 µm depth and 1 mm wide. In this study, the piezoelectric β-PVDF polymer was attached underneath the reaction chamber. The deposition of the ITO electrodes and the β-PVDF inside the reaction chamber is still being studied and optimized. However, it is expected that the enhancement obtained by the acoustic streaming effect will be slightly higher when the polymer is deposited inside the chambers due to the lack of reflection, absorption and dispersion of the acoustic waves in the glass substrate.

A 250 W quartz tungsten halogen lamp with a monochromator ORIEL Cornerstone 130™ was used as light source. An optical fiber is also used to direct the light into the measurement set-up. A Keithley 487 picoammeter (full-scale range from 10 fA to 2 mA and a resolution of 51/2 digit) is used for measuring the photodiodes current. These photodiodes were previously calibrated with a calibrated commercial photodiode as reference (Hamamatsu S1336-5BQ).

The acoustic streaming was set using a 5 V sinusoidal signal (supply of the CMOS chip) with 10 MHz (resonance frequency of the 110 µm thick β-PVDF film). The evolution of the reaction was studied to a maximum absorbance of 0.225 a. u., which is the absorbance value of the complete reaction. The results are shown in the Fig. 6.

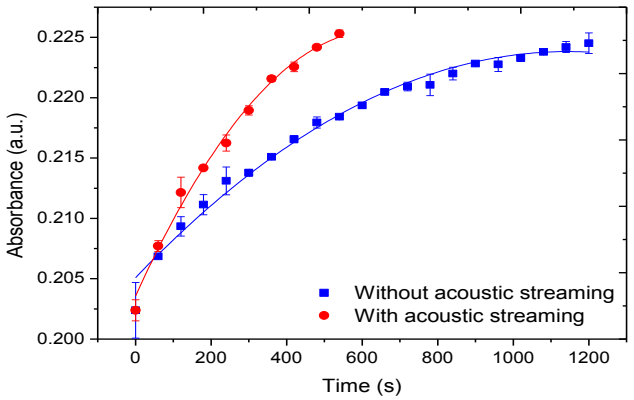

Fig. 6. Measured absorbance at 550 nm for 20 mg/dl of uric acid concentration, as function of time, without and with acoustic streaming (sinusoidal signal with 5 V amplitude and 10 MHz).

The complete mixture occurs in a faster way with the application of acoustic streaming, being the gain time approximately 55% of the reaction time without streaming. Moreover, the temperature of the fluids reached 32ºC at the end of the analysis time due to the heating generated by the acoustic streaming effect; in addition of the sound waves that leads the agitation. Consequently, the two separate contribution of this phenomenon (agitation and heating) was determined. First a new sample was heated with a temperature controller, setting the temperature gradient matching the one obtained with the acoustic streaming. Simultaneously, the absorbance curve was obtained and posteriorly was compared with the absorbance curve of the acoustic streaming (Fig. 6). It was demonstrated that 40% of the 55% improvement obtained by the acoustic streaming are due to the heating, and the remaining 15% are due to the acoustic waves. Therefore, it was proved that both the agitation and the heating generated by the acoustic streaming effect are favorable to enhance the reaction for uric acid detection.

V. CONCLUSIONS

A microsystem device for biological fluid analysis that incorporates an acoustic streaming system based on a piezoelectric β-PVDF film with ITO electrodes underneath the reaction chamber for improving the reaction between the fluids involved has been reported. It is a point-of-care device with all the microfluidic die, photodetectors, readout electronics and signal actuation integrated in a single-chip. It has been proved that both the agitation and the heating generated by the acoustic streaming phenomenon improve the fluids reaction with a gain of 55% for the uric acid analysis. Therefore, this solution becomes a suitable technique for an effective mixing and a reliable analysis. It avoids the limitations presented by other mixing systems, it does not interact or affect the fluids involved and it allows decreasing the complexity and the size of the microsystem. This study was conducted, particularly, for a possible use as a monitoring system of dialysis efficacy and level of renal function in patients with end-stage renal disease. It can be a simple device for instantaneous analyses that avoids the need to collect blood and urine samples (once it uses saliva), and, consequently, it avoids long response time as well as qualified professionals.

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