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Measuring of calcification risk with polymer microchips

Abstract: It has been shown that the formation of calcium phosphate crystals can be detected in patient blood using a polymer microchip manufactured by ultrasonic processing. Ultrasonic processing is recently evolving for the fabrication of low-cost microfluidic devices from thermoplastic polymers. The formation of calcium phosphate crystals can be measured in the blood serum of a patient both optically from a change in turbidity or in electrical resistance.

Keywords: calcification risk, polymer microchips, ultrasonic processing.

https://doi.org/10.1515/cdbme-2018-0088

1 Introduction

It has been shown that the delayed formation of calcium phosphate nanocrystals is associated with cardiovascular events and mortality. These can have pathological effects on the body by inducing calcification. Calcification is a pathological accumulation of calcium phosphate at undesired locations in the human body such as organs and blood vessels. In particular, dialysis patients have a high risk of calcification [1]. For these patients, it would be important to provide a calcification risk test enabling discovering and counteracting the calcification with medication.

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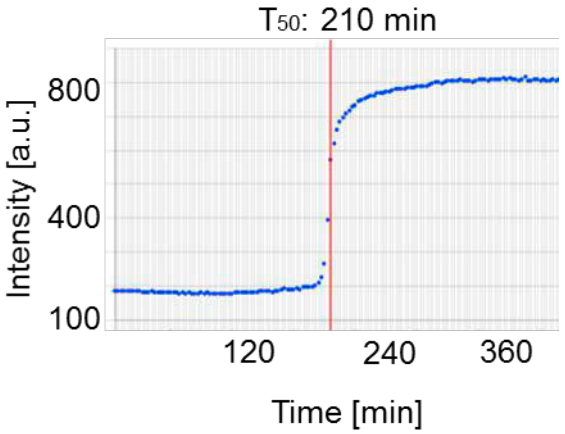

Figure 1: Intensity of the light scattered in a blood serum as measured in a nephelometer. © Calciscon

Calcification risk of patients has been determined in comparatively large and expensive nephelometers in a temperature controlled room at 37°C [2]. In these machines, the situation in human blood vessels is simulated in a microtiter plate. The blood serum of patients is mixed with calcium and phosphate and dosed into the wells of the titer plate. Virtually instantaneously so-called primary calcium protein particles (primary CPPs) are formed when the protein fetuin A is encapsulating calcium phosphate particles, and this way, preventing precipitation as hydroxylapatite [Ca10(PO4)6(OH)2]. At 37°C, after approximately 200 minutes the primary CPPs undergo a transformation into secondary CPPs which are larger increasing light scatter of the blood serum. The change in light scattering is measured in the nephelometer, and thus the time T50 of the transformation of the CPPs is determined. Figure 1 shows the change in light scattering as a function of time. The transformation of CPPs occurs earlier in the blood serum of patients who tend to calcification, and therefore, T50 is a measure of the calcification risk of patients.

The aim of this work is to develop a microfluidic chip in which the crystal formation time in patient samples can be measured fast, reliable and inexpensive. To achieve reliable results, the chip needs to be disposable avoiding contamination from a former measurement. Therefore, it has been decided to employ ultrasonic processing to fabricate a chip from polymer. Ultrasonic processing has been developed by

several groups in recent years [3 - 11]. Cycle times of a few seconds, investment costs of a few 10,000 € and realization of new designs in one working day make it a promising new way both for development and fabrication. However, up to now there are only few examples of working micro systems fabricated by these processes [3].

2 Ultrasonic processing

The microfluidic polymer chip is fabricated in two steps. First, cavities are generated in the surface of a polymer plate from polycarbonate (PC) by ultrasonic hot embossing, and then, these cavities are closed with a lid foil from the same polymer by ultrasonic welding.

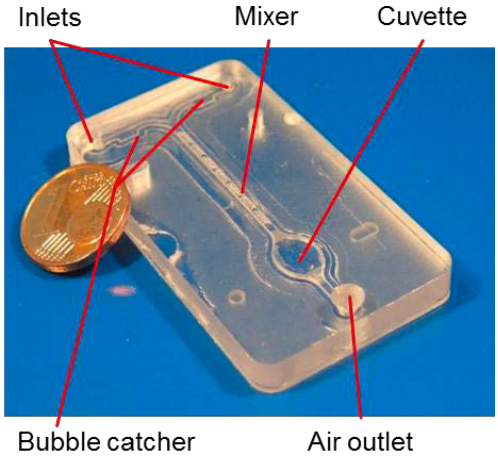

Figure 2: Microchip fabricated by ultrasonic processing for measuring the calcification risk.

For ultrasonic hot embossing, a tool with the inverse of the cavities to be generated was placed onto an anvil. The tool had been milled into an aluminium plate, 40, 60 and 4 mm in width, length and thickness, respectively. There are a lot of other possibilities to manufacture a tool, but milling in many cases is the easiest one [12].

A foil, 125 µm in thickness, and a plate, 30, 55 and 5 mm in width, length and thickness, respectively, both from PC, were placed on top of the tool (see Figure 3a). Then, the sonotrode of the ultrasonic welding machine was pressing plate and foil down onto the tool. After exceeding a predefined force threshold, ultrasonic vibrations started for the so-called ultrasound time. The vibrations generated friction heat between tool and foil, and between foil and plate. The polymer was molten by the heat and adapted to the microstructures on the tool (see Figure 3b). The polymer was further

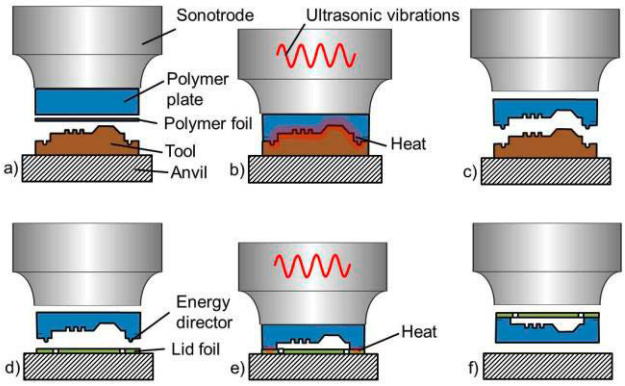

Figure 3: Schematic drawing of the fabrication of a microchip by ultrasonic hot embossing and welding.

pressed down onto the tool for the so-called holding time after the ultrasonic vibrations had stopped allowing the polymer to cool down. The sonotrode was driven up again and the polymer chip was released from the tool (see Figure 3c). This way, cavities were generated in the surface of the plate within 3.3 s by ultrasonic hot embossing. Foil and plate were joined to a single piece of polymer where there had been micro structures on the tool. In a second step, a lid foil from PC, 250 µm in thickness (see Figure 3d), was ultrasonically welded sealing the cavities and, this way, generating a closed microfluidic system. Protruding walls with a half-dome shaped cross-section, so-called energy directors, surrounding the micro cavities were molten by the friction heat and served as a kind of glue joining lid foil and plate (see Figure 3e). The entire time of ultrasonic welding was 1.8 s. The detailed process parameters are shown in Table **1**.

Table 1: Parameters of ultrasonic processing.

Process	Hot embossing	Welding
Force threshold	290 N	290 N
Amplitude of vibration	27.2 µm	24 µm
Force during vibrations	912 N	1370 N
Duration of vibrations	1.3 s	0.2 s
Holding time	2 s	1.6 s
Force during holding	912 N	1370 N

3 Optical measurement

In the microchip, calcium and phosphate were mixed in a micro mixer of herringbone type [13, 14]. 0.4 mL blood serum was mixed outside of the polymer chip with 0.1 mL NaCl solution (140 mmol) and 0.25 mL Na2HPO4 solution

(24 mmol). This sample was injected into one of the inlets of the chip while 0.25 mL CaCl solution (40 mmol) were injected into the other inlet.

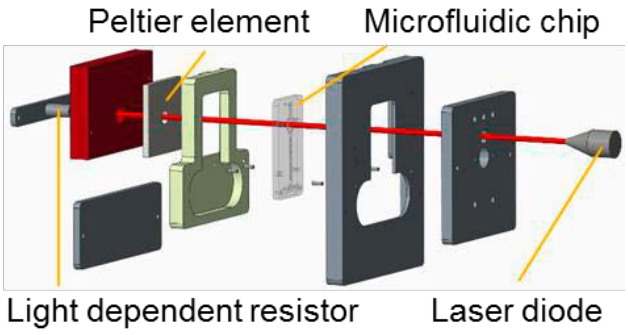

Figure 4: Schematic drawing of the measurement set-up with the microfluidic chip inside.

The transformation of CPPs was observed by the intensity of the light of a laser LED transmitting the sample in a cuvette in the chip (see Figure 4). A Peltier element was employed to elevate the temperature inside of the polymer chip to more than 50°C. This way, the transformation of the CPPs was accelerated and observed earlier enabling a quicker measurement of the calcification risk in the chip (see Figure 5). The measurement time was shortened by factor 17 and makes the device suitable for point of care diagnosis.

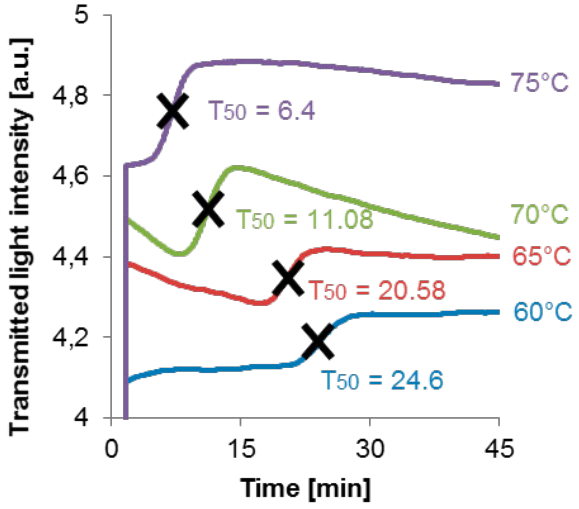

Figure 5: Measured light intensity transmitted through the polymer chip at 60, 65, 70 and 75°C.

4 Electrical measurement

The calcification risk can also be measured by a change of the electrical AC resistance of the blood sample. Such a chip is shown in Figure 6 and in Figure 7 there is shown the comparison of the optical measurement of turbidity and the resistance measured at 50 °C. The electrical measurement was performed with an AC voltage of 65 mV and 30 kHz. The obtained T50 were 81.1 min and 81.7 min for the optical and electrical measurement, respectively. The electrical signal shows more noise which maybe can be reduced with an improved design of the electrodes, but the measurements of T50 are very close to each other. Further experiments will show whether the electrical measurements can also be performed in blood directly taken from a patient without any processing to generate blood serum.

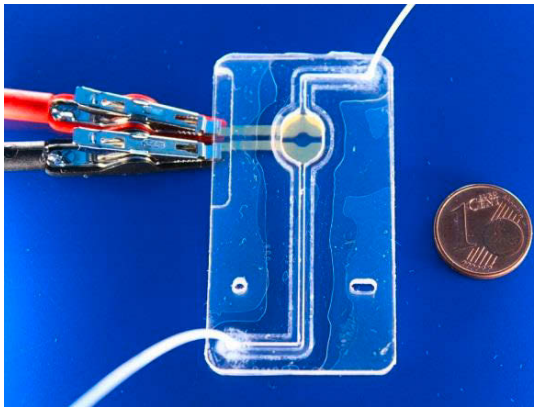

Figure 6: Polymer chip for electrical measurement.

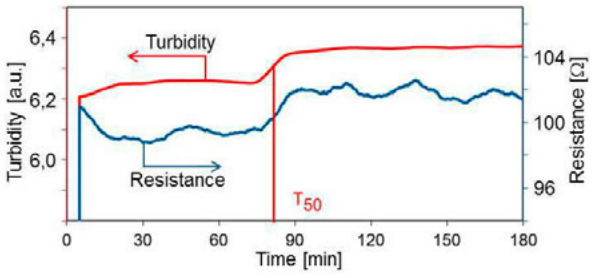

Figure 7: Turbidity and resistance measured in a single chip.

5 Conclusion

A measure of the calcification risk of patients, the so-called T50, can be determined in a micro fluidic chip manufactured by ultrasonic fabrication. This process enables the cheap production of polymer chips which are expected to allow affordable point of care measurements.

The investigations described here, show that both optical and electrical measurements can be employed to measure T50. Optical measurements up to now show less noisy results but electrical measurements could open up the way to measurements in blood without any more preprocessing.

Future investigations need to show below which T50 a therapeutic invention is necessary. Then the development of a diagnostic device suitable for point of care application is desirable.

Disclosures: AP and WJD are inventors of the T50-Test and co-founders and CEO, respectively (AP) of Calciscon AG which holds patents in the T50-test and commercializes the T50-test.

Author Statement

Research funding: The author state no funding involved. Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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