Abstract—PCR is sensitive and specific tool for DNA fragments amplification. Nowadays PCR is used in a wide range of applications mainly in biotechnology, clinical and forensic medicine, microbiology, biochemistry etc. On the other hand, classical PCR requires sophisticated and expensive equipment. It makes this technique limited only to specific laboratories. In order to meet the demand the miniaturization of PCR platforms stands for excellent solution. Miniaturization is becoming very popular in a variety of scientific disciplines as well as in industry [2], [3], [4]. The interest in LTCC (Low Temperature Cofired Ceramics) technology has been growing recently. Thanks to its numerous advantages like low price, short time of a new structure development and compatibility with others materials, ceramics become widely used in military, data processing or medicine [5], [6]. Furthermore, ceramic is highly resistant to various chemical compounds and substances even to concentrated acids [7]. We have recently shown that ceramic is compatible with PCR components, especially with polymerase [8]. Thus LTCC might be also applied in biotechnology for PCR microreactor.

PCR is a basic tool for genetic typing in forensic purposes used for personal identification. Generally various panels of markers represented by several STRs (Short Tandem Repeats Polymorphisms) are used. Their analogs are miniSTRs that allow for generating even shorter amplicons (DNA fragments amplified during PCR). MiniSTRs are widely used for paternity testing, unknown persons identification and for investigation of forensic evidences.

In the paper we are presenting a device dedicated for miniSTRs amplification. MiniSTRs analysis is used worldwide for forensic purposes [9], [10]. We successfully performed multiplex PCR, where we were able to amplify 5 and 6 miniSTRs loci in one reaction. This resolution decreases the costs, accelerates the time and minimize the error of analysis [11], [12]. Adjustment the multiplex PCR with the miniaturized PCR system based on the LTCC technology might provides the possibility for faster and cheaper forensic analysis even in small laboratories or the one created in a place of catastrophes or scene of terror attacks [8], [13].

II. THE CERAMIC MICROCHIP AND TEMPERATURE CONTROL SYSTEM

The LTCC material is a complex chemical composition. The interaction with a PCR process is unknown. Thus, an experimental way of finding a proper material was performed.
Various commercially available LTCC products were tested. The DP951 ceramics was chosen as proper material for a PCR reactor [8]. The construction of chamber microreactor was proposed. The structure with buried heater and temperature sensor was designed (Fig. 1).

![Fig.1. The schema of ceramic microreactor with buried heater and temperature sensor. A - top view, B - bottom view.](image)

The LTCC technology was used for structure fabrication. The dimensions of the chip were 8 x 8 x 1.3 mm$^3$. The buried electronic components were defined in screen printing process. The pads, placed on bottom side of structure, enabled electrical contact with integrated passive components. A dedicated temperature management system was constructed. The structure was connected to the electronic device (Fig. 2). It was based on an ATmega168 microcontroller. The device enabled communication via USB (Universal Serial Bus) protocol with laptop computer. The thermal parameters of the PCR process were set in dedicated software and send to the device. The LTCC chip was placed in a handle. The construction enabled easy inserting of reaction mixture to the ceramic reactor. The reaction chamber was sealed by PDMS (Polydimethylsiloxane) lid. The computer application enabled constant monitoring of the thermal parameters.

![Fig. 2. Temperature control system with ceramic chip.](image)

### III. ANALYZED LOCI AND ALLELES

Ten various autosomal miniSTR loci and Amelogenin for sex determination were amplified with the use of the LTCC microchip. The alleles sizes were from 51 (TH01) up to 281 bp (base pairs) (FGA). Two multiplex PCRs were created. The first multiplex consisted of 6 loci and the second was composed of 5 loci. The alleles sizes for first multiplex PCR were from 58/63 bp (Amelogenin) up to 281 bp (FGA), the alleles sizes for second multiplex PCR were from 51 bp (TH01) up to 211 bp (D21S11). The names of miniSTRs, the type of fluorescence dyes used for labeling and expected sizes of analyzed loci are presented in Table 1. The examined loci are divided according to multiplex that their belong to.

### IV. DNA EXTRACTION

DNA was extracted manually from various forensic samples like blood stains, whole blood or others forensic evidences using Qiagen DNA Isolation Kit (Qiagen). The DNA isolation was carrying out according to manufactured protocol and differed depending on the type of material or tissue. Subsequently, DNA was either stored at 4°C or used for further analyses.

### V. PCR CONDITIONS AND PRODUCTS DETECTION

The PCR was performed with use of Qiagen Multiplex PCR Kit (Qiagen). 2µM primers were used. The temperature profile was modified as follow: 95°C, 7min, [(95°C , 30 s), (57°C , 45 s), (72°C, 45 s)]x30 for single locus PCR. The experiments in order to adjust temperature profile for multiplex PCR are still ongoing. 10 µl of reaction mixtures were applied on the chip. The whole reaction consumed 1 h, 45 min for single PCR. Next, PCR products were prepared for capillary electrophoresis by mixing 1 µl with 10 µl of Hi-Di Formamide containing GeneScan LIZ500 Size Standard (Applied
Biosystems) and denatured by heating at 95°C for 5 min and then snap-cooling on ice for 3 min. The PCR products were detected in capillary electrophoresis together with GeneScan LIZ500 Size Standard with the use of ABI PRISM® 310 Genetic Analyzer, Applied Biosystems and analyzed using GeneMapper ID v3.2.1 software. The results are presented as electropherographs with fluorescence intensity on Y axis and product size on X axis. The PCR product size was assessed based on size standard that contained known fragments from 50 bp up to 500 bp.

VI. RESULTS

A. Single PCR for each miniSTR locus

In the initial step of the experiments the separate PCR was set up for each miniSTR in order to optimize the PCR conditions. The PCR products for each miniSTR have been successfully obtained with the use of microreactor. Each locus gave the specific product that fit the expected alleles sizes (see table 1). Furthermore, the PCR process was much shorter with the use of microreactor than performed in commercial equipment and last 1 h 45 minutes. In some reaction we have observed the PCR products overload, what confirmed the high sensitivity of constructed microreactor. Heterozygotes were seen as two picks, homozygotes were seen as one pick. The examples of single locus PCR products are presented in Figure 3 A and B.

B. Multiplex PCR construction

The multiplex PCR was constructed depending on miniSTRs size and fluorescence dye used for labeling. The main limitation for multiplexing was to avoid the situation, where products of the different miniSTRs would overlap each other. This situation was strongly avoided. On the other hand, we allowed for overlapping in case where various fluorescence dyes with different extinction wavelengths were used.

Depending on the PCR product size and fluorescence dyes two multiplex PCR were made. The first hexaplex contained the Amelogenin for sex determination, D8S1179, D16S539, D19S433, D2S1338 and FGA. The second multiplex PCR was composed of five miniSTR loci and contained TH01, D3S1358, vWA, D21S11 and D18S51.

For labeling following fluorescence dyes were used: VIC, FAM, NED, PET with the extinction wavelength corresponding to green, blue, black and red wavelength, respectively.

C. Multiplex PCR performing

The PCR components and temperature conditions were optimized for each multiplex to obtain products of each analyzed loci. Initially we have focused on primer concentration adjustment. This experiment was made using commercial termocycler. At present further experiments in order to adjust the proper PCR conditions using microreactor are being performed.

Fig. 3. Electropherograms of single locus PCR products (A – classified for multiplex I, B – classified for multiplex II).
VII. DISCUSSION AND CONCLUSION

The multiplex PCR is a perfect technique useful in many applications. The possibilities to amplify numerous DNA fragments in one reaction enable for lowering costs of analysis, time and reagents consuming as well as flexibility in experiments design [11, 14]. This technique is widely used for diseases diagnosis [12], pathogen detection [15] or personal identification [9]. Applying multiplex PCR to a miniaturized PCR system may even more decrease the cost of analysis. Furthermore miniaturization gives further advantages like minimizing the space of equipment, accelerating the speed of analysis or mobility [16].

The lab-on-a-chip technology become more popular in a wide range of applications, especially in biotechnology. Integration of LTCC with electronic elements for heating and temperature measurements might provide sensitive and cheap device for PCR [8]. Furthermore, LTCC compatibility with others materials allows for multi step process integration.

Taken together, multiplexing with the use of microsystem technology provides fast, sensitive and cheap methodology useful in a wide range of analysis. The body of literature describes microsystems dedicated for various pathogens detection [17], [18]. This system seems to be perfect for forensic purposes [3], [19]. In present study we are presenting the utilization of the PCR microractor in forensic genetics. We have successfully amplified miniSTR loci used worldwide for personal identification. MiniSTRs markers allow generation of shorter amplicons than classical STR genetic markers, what influence the sensitivity and specificity of genetic analysis. Theses advantages are especially useful when working with degraded DNA, like numerous of forensic samples [9], [10]. In present study we have amplified in a single PCR 10 autosomal loci and amylogenin for sex determination. We have proved that DNA isolated from various forensic evidences might be successfully amplified using microreactor in the LTCC technology. Our experiments have proved as well that ceramic (DP951) is a good material dedicated to many biochemical processes. At present we are working on adjusting conditions for multiplex PCR.

Concluding, microsystem in LTCC technology might be successfully used for the PCR. As one of many instances we have performed multiplex PCR dedicated to forensic genetics.

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