GELIFICATION - A SIMPLE AND EFFICIENT METHOD FOR ON-CHIP STORAGE OF REAGENTS: TOWARDS LAB-ON-A-CHIP SYSTEMS FOR POINT-OF-CARE DIAGNOSTICS
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ABSTRACT
In this study, we describe a simple and efficient method for on-chip storage of reagents for point-of-care (POC) diagnostics. The method is based on gelification of all reagents required for on-chip PCR-based diagnostics as a ready-to-use product. The result reported here is a key step towards the development of a ready and easy to use fully integrated Lab-on-a-chip (LOC) system for fast, cost-effective and efficient POC diagnostics analysis.


INTRODUCTION
Current state-of-the-art of PCR-based molecular diagnostic techniques requires bulky equipment and laborious procedure. By integrating miniaturized components and automating functionalities, LOC technologies offer a valuable tool to perform fast, cost-effective and efficient DNA analysis [1]. In the LABONFOIL EU IP project (http://www.labonfoil.eu), a totally integrated microfluidic platform for PCR-based POC DNA analysis is being developed [2]. The newly developed LABONFOIL system (Fig. 1a and b) consisting of a two-chamber disposable polymer labcard made of cyclic olefin copolymer (COC) (Fig. 1a) and a control unit consists of an external fluorescence detector, two micro-pumps, two heaters and temperature sensors, two magnets, three bottles, a battery, 5 micro-motor pin actuators, and an automatic labcard insertion mechanism (Figure 1b).

All the steps from sample preparation, PCR-amplification and detection performing in a single chip have been reported [3, 4]. In order to fulfill the requirements of different biological applications, In the LABONFOIL system, the PCR amplification is carried out on the labcard in a separate chamber, where all the required PCR reagents are stored as ready to use gelified product.

EXPERIMENTAL
A stabilization method for complex enzymatic reaction known as “gelification” Developed and protected by Biotools (Patent US 7,919,294) was used for the stabilization and storage on chip of the PCR reagents. A gelification mixture developed by Biotools (Biotools A/S, Spain) was mixed with PCR reagent (1:7 v/v) according to the supplier’s instruction. The gelification process was performed at 30 ºC under a pressure of 30 mbar for 30 minutes in a vacuum drying oven (VO400, Memmert, Germany) or a freeze-dried machine (MartinChrist Alpha 1-2). Different volumes of the gelified PCR mixture ranging from 6-10 µl were tested on chip or on labcard in order to create a droplet shape in the middle of the chamber to avoid blocking the channels and the inlet and outlet of the chamber.

RESULTS AND DISCUSSION
Figure 2 shows photographs of a chamber with gelified PCR reagents from a chip (Fig. 2a) and from a labcard (Fig 2b). For large scale on-chip or on-labcard gelification, EVG 510 bonding system (Fig 3a, b) was modified (Fig 3c) and used as a tool where the gelification of 6-12 labcards can be performed at one time (Fig 3c) in the same condition mentioned above.

The stability of the on-chip or on-labcard gelified PCR reagents during storage is a key issue since we aim at development of a fully integrated LOC system for point of care. A multiplex PCR for rapid detection and identification of a food-born pathogen – Campylobacter spp., at sub-spices [5] was used to determine the activity of the gelified PCR reagents after a long period of storage at room temperature and at 4ºC using conventional real-time PCR (Fig. 4a and b) or on-chip PCR (Fig 4c). Full activity of the gelified PCR reagents was observed even after more than 3 months of storage at both room temperature and at 4ºC as no change in polymerase activity was observed. Figure 4 shows graphs of conventional multiplex real-time PCR using gelification product (Fig 4a) and melting curve analysis of such PCR (Fig 4b) and photos of gel electrophoresis end point analyses (Fig 4c).
Figure 1: LABONFOIL system. a) Photograph of a labcard. The disposable labcard comprises a luer syringe connector where the sample is loaded, five microvalves, and two chambers. Labcard is made of a COC substrate sealed by a polypropylene film coated with a pressure sensitive adhesive working with a COP disc as a valve. b) Photograph of the control unit and c) A schematic of the control unit.

Figure 2: Gelification for on-chip storage of reagents has been investigated in LBONFOIL European project. Pictures of gelified PCR reagents in (a) a 10 μL chamber of a COC injection molded chip (b) a 7 μL open chamber of a COC labcard. The gelification was carried out using EVG 510 Bonding system (see Fig 3)

Figure 3a and b: EVG 510 bonding chamber and c) modified EVG 510 with 6 labcards prepare for large scale on labcard gelification.

Figure 4: a) A graph of a conventional real-time PCR and (b) melting curve analysis to study the stability of large scale on chip and on labcard gelified. c) Photos of gel electrophoresis end point analysis of such PCR.
CONCLUSION
The results obtained in this study clearly demonstrate the feasibility of storing gelified reagents on-chip or labcard for POC diagnostics. We are now working on the integration and automation of all steps that includes on chip sample preparation, DNA isolation, PCR amplification and real-time detection on a single platform.

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