Biosensors and Bioelectronics 54 (2014) 499–505

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A simple and portable device for the quantification of TNF-α in human plasma by means of on-chip magnetic bead-based proximity ligation assay

Vanessa Castro-López [a,*], Jorge Elizalde [a,b], Marcin Pacek [c], Elizabeth Hijona [d], Luis Bujanda [d]

[a] CIC microGUNE, Goiru kalea 9, 20500 Arrasate-Mondragón, Gipuzkoa, Spain
[b] IK4-Ikerlan, Pº JMª Arizmendiarrieta 2, 20500 Arrasate-Mondragón, Gipuzkoa, Spain
[c] POC Microsolutions, Goiru kalea 9, 20500 Arrasate-Mondragón, Gipuzkoa, Spain
[d] Department of Gastroenterology, Hospital Donostia/Instituto Biodonostia, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Universidad del País Vasco UPV/EHU, San Sebastián, Spain

ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form
11 October 2013
Accepted 22 October 2013
Available online 6 November 2013

Keywords:
TNF-α
Solid-phase proximity ligation assay
qPCR
Microfluidic chip
COP
Point-of-care

ABSTRACT

There is a general need in healthcare systems all around the world to reduce costs in terms of time and money without compromising patients outcome. Point-of-Care Testing (POCT) is currently being used in some applications (e.g. POC coagulation devices) as an alternative to already established standard central laboratory tests to overcome sample transportation and long turnaround times. The main objective of this investigation was to quantify Tumour Necrosis Factor-alpha (TNF-α) on-chip within the clinical relevant range of 5–100 pg/mL in human pooled plasma. The novel solid-phase assay developed in this study was a magnetic bead-based proximity ligation assay (PLA) in which one of the assay proximity probes was directly immobilised onto streptavidin-coated magnetic beads. The portable device was based on a disposable and single-use cyclo-olefin polymer (COP) microfluidic chip interfaced with a quantitative real-time polymerase chain reaction (qPCR) device previously developed in-house. Sample volume was 10 μL and total assay time under 3 h. The POC device and assay developed offer portability, smaller reagent and sample consumption, and faster time-to-results compared with standard ELISAs. Determination and monitoring of TNF-α therapy at the point-of-care will help to improve clinical and/or economical outcome in governmental healthcare budgets.

1. Introduction

Autoimmune diseases are inflammatory processes occurring in various organs of unknown aetiology (Kunz and Ibrahim, 2009). Their incidence has increased in recent years with an estimated prevalence of above 2% in the general population (Cooper and Stroehla, 2003; Eaton et al., 2007). One of the key multifunctional polypeptidic cytokines present in these inflammatory processes is Tumour Necrosis Factor-α (TNF-α), which has been reported to occupy a central role in the initiation and/or perpetuation of inflammatory processes such as rheumatoid arthritis (Lorenz and Kalden, 2002), inflammatory bowel disease (Kraus and Arber, 2009), psoriasis (Sharma and Sharma, 2007) and cancer (Huang et al., 2005).

Several TNF-α blockers (e.g. infliximab, adalimumab, and etanercept) have been developed over the past few years to control and improve the prognosis of patients (Jin et al., 2010). However, occurrence of adverse effects (De Greef et al., 2012), the high treatment costs (Hallinen et al., 2010) (i.e. it is estimated more than 20,000 euros per adult person per year), variable anti-TNF-α therapy effectiveness between patients (Bongartz et al., 2006), and loss of drug response throughout disease evolution (McDermott et al., 2013; Seminerio et al., 2013), makes monitoring levels of TNF-α in patients serum or plasma a must to assess both treatment efficacy and disease prognosis.

The most widely used technique to quantify and monitor TNF-α in biological samples is Enzyme-Linked ImmunoSorbent Assay (ELISA) (Ahrén-Moonga et al., 2011). There are several commercially available ELISA kits with various sensitivities and broad linear dynamic ranges in different biological matrices (Ledur et al., 1995). Despite TNF-α ELISA kits being able to detect TNF-α from a few pg/mL up to ng/mL in serum, plasma and cell culture supernatants, it has been reported that serum TNF-α levels in

* Corresponding author. CIC microGUNE, Goiru kalea 9, 20500 Arrasate-Mondragón, Gipuzkoa, Spain. Tel.: +34 943 73 98 05.
E-mail address: vcastro@cicmicrogune.es (V. Castro-López).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.10.039

healthy individuals are usually below 10 pg/mL (Scully et al.., 2010), while in more than 80% of patients with severe autoimmune diseases may range from 10 to 100 pg/mL (Arican et al., 2005; Katz et al., 1994; Martínez-Borra et al., 2002; Namas et al., 2009). Hence a very narrow and sensitive therapeutic window from 10 to 100 pg/mL needs to be addressed and well characterised. Additionally, ELISA is expensive, not very reproducible, lacks in standardisation, it is time-consuming as it requires multiple assay steps, large sample volumes are needed, and since it is not available in most hospitals for routine use, transportation to a reference laboratory is usually required (Huang et al., 2012; Liu et al., 2012).

An alternative to the traditional immunoassays is the detection and quantification of proteins based on the Proximity Ligation Assay (PLA) technology, which has been established over a decade ago (Fredriksson et al., 2002; Gullberg et al., 2004). The technique consists of conjugating polyclonal antibodies or matched pairs of monoclonal antibodies to oligonucleotides by which each oligonucleotide in the probe pair presents a 5′ or 3′ end. When the antibodies bind to the protein of interest, the oligonucleotides are brought into proximity in such a way that ligation of the two oligonucleotides by DNA ligase occurs. The ligation product constitutes the DNA template for the amplification in the quantitative real-time Polymerase Chain Reaction (qPCR) assay (Gullberg et al., 2004; Swartzman et al., 2010). Although PLA is more sensitive (due to the proximity-dependent signal), faster (as it does not require washing steps), simpler, it allows for multiplexed detection and requires less sample volume than traditional protein detection methods, it has not been widely used to date.

Most of the PLA assays developed up to now make use of the homogenous assay format whereby binding, ligation and amplification occur in solution without washing steps (Fredriksson et al., 2002; Gullberg et al., 2004). However, just a few solid-phase PLA experiments have been reported over the last few years whereby the target protein is first incubated with a capture antibody immobilised on a solid support and then the target protein is detected with a proximity ligation assay. One of the advantages of solid-phase PLA assays compared to homogeneous-phase PLA assays is that the former allows for removal of potentially interfering substances and excess reagents reducing background signal, thus rendering very sensitive assays with broader dynamic ranges (Fredriksson et al., 2007). The first solid-phase platform used streptavidin-coated PCR tubes (Gustafsdottir et al., 2006) but very recently streptavidin-coated magnetic beads have been introduced for different biologic al applications where limits of detection of 10 pg/mL (Jiang et al., 2013), 250 pg/mL (Nan et al., 2012), and in the fM range (Darmanis et al., 2010) have been reported. The solid-phase assay investigated in this study differs with respect to those previously mentioned in the sense that direct immobilisation of one of the proximity ligation probes onto streptavidin-coated magnetic beads occurs as opposed to immobilising a capture antibody first and then run the PLA assay. Since one of the assay proximity probes is already immobilised on the magnetic particle, a cheaper and faster assay can be developed.

The first microfluidic devices were made out of silicon and pyrex, but since the discovery of polymeric materials, fast and low-cost processes have been developed opening the door for mass production of single-use disposable microfluidic devices (de Mello, 2002). Moreover, polymeric materials offer versatility and a wide range of physical and chemical properties to match the requirements in an application-dependent manner. For instance, microfluidic chips made out of SU-8 were successfully fabricated to carry out PCR reactions (Calvo et al., 2011; Verdoy et al., 2009) as well as injection moulded COC (Cyclic Olefin Copolymer) and COP (Cyclic Olefin Polymer) cartridges (Ruano-Lopez et al., 2011) reducing the amount of sample and reagents required, as well as the heating up and cooling down process times in each cycle due to different scaling laws concerning the heat transfer. This single-use assay device approach is valid for portable systems and applications where a fast result is needed such as in critical care units or at the point-of-care.

Here we report a novel solid-phase and sensitive on-chip method for the quantification and monitoring of TNF-α in both buffer and human plasma within the range of 5–100 pg/mL in a COP single-use disposable microfluidic device that is easy and cheap to manufacture. The development of a more accurate anti-inflammatory test to detect, quantify and monitor TNF-α in human serum and/or plasma will help to improve the prognosis and treatment efficacy of autoimmune inflammatory diseases. In addition, rapid time-to-results at or near the site of patient care will allow for reducing time and cost on health service budgets.

2. Material and methods

2.1. Polymeric substrates

The polymeric material used to fabricate the microfluidic chips by injection moulding was COP (1420R grade) from Zeon Chemicals as it is a specific grade for diagnostics (e.g. ultra low fluorescence, sterilisation compatibility, and high Tg for PCR thermo-cycling conditions). The abovementioned microfluidic device was sealed with ZF14-100 COP thin film purchased from Zeon Chemicals. COP chips were firstly washed with nuclease-free water and a DNase- and RNase-free solution to remove any unwanted qPCR inhibitors. Consequently, COP chips were either used directly or treated for one hour with a commercial blocking assay buffer from Invitrogen (CA) to reduce non-specific binding and to improve signal-to-noise ratio. After the blocking step, three washing steps of 10 μL each with nuclease-free water were carried out to ensure complete removal of the blocking assay buffer, which contains bovine protein as carrier and 0.5% ProClin™ as a preservative. COP chips were left to dry at RT for one hour.

2.2. On-chip experiments: qPCR setup

The custom-made qPCR setup is based on a previously developed portable integrated POC device (Verdoy et al., 2012). Briefly, it is composed of the following components (Fig. 1(a)):

- Computer-based control program: The computer program has the following features: (i) it defines the protocol to be used in the reaction, (ii) it runs the protocol controlling the time and temperature of each cycle, and (iii) it displays the parameters in the current step including cycle number, time, temperature, and fluorescence values.
- Controller (USB connected to the computer): According to the parameters sent by the control program, the controller drives the heater and measures the temperature and the fluorescence signal sending these measurements to the computer.
- Chip holder: A custom-designed capsule was made in aluminium painted in black. This capsule allows the sealing of the fluidic inlet and outlet in the plastic chip, integrates the fluorescence detector, the heater and the temperature sensor. It also isolates optically the system preventing from external light interference.
- Polymeric chip: Injection moulded 10 × 10 mm^2 COP chips with an inlet, an outlet, and a 10 μL rhombus-shaped reaction chamber of 450 μm height (Laouenan et al., 2012; Fig. 1(b)).