Application of optical sensing in POCT: near-continuous therapeutic drug monitoring in transplanted patients

S. Tombelli[1], S. Weber[2], C. Trono[1], A. Giannetti[1], M. O´Connell[3], M. Wen[2], H. Bittersohl[2], P.B. Luppa[2], F. Baldini[1]

[1]CNR-IFAC, Institute of Applied Physics "Nello Carrara", Sesto Fiorentino, Italy

[2]Institute for Clinical Chemistry and Pathobiochemistry, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

[3]Cornel Medical Limited, London, United Kingdom

***Abstract* — When treating transplanted patients, therapeutic drug monitoring (TDM) represents one of the most crucial aspects for the assessment of the correct dosage of immunosuppressants, in order to ensure an appropriate medical management avoiding the rejection of the transplanted organ. Optical sensing by Total Internal Reflection Fluorescence (TIRF) has been applied here to analyse immunosuppressants in samples from transplanted patients. The samples were collected by an intravenously applied microdialysis catheter, capable of extracting only the free drug fraction. Besides their analysis via optical sensing, the collected samples were additionally analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The results obtained with the two methods, optical sensing and LC-MS/MS, were then compared.**

Keywords — therapeutic drug monitoring, point-of-care-testing, immunosuppressants, optical biochip, fluorescence anisotropy

I. INTRODUCTION

Post-transplant care is one of the most challenging and complex area of modern medicine with the need for physicians to continuously determine the concentration of immunosuppressive drugs in patients in order to find the optimum balance between therapeutic efficacy and occurrence of adverse events. As these drugs generally show a narrow therapeutic range the difference in the drug concentrations exerting therapeutic benefits on the one hand and causing adverse events on the other hand is very small. Consequently, they can be easily under- or over-dosed [1]. It is evident that the fast and reliable verification of the correct dosage of these drugs is extremely important, especially during the first 48 to 72 hours after the transplantation. Consequently, therapeutic drug monitoring (TDM) plays an important role in the treatment of transplanted patients by enabling the assessment of the correct dosage of drugs characterized by narrow therapeutic windows. Typically, TDM of immunosuppressants involves blood withdrawal before their next administration to determine the minimal drug concentration, the so-called trough level. Standard analytical procedures are based on the measurement of the total immunosuppressive drug concentration in haemolysed whole blood samples. covering the sum of the drug concentration in blood cells, the concentration of the drug bound to proteins, and the free fraction. Since only protein-unbound (free) drugs can cross membranes and bind to receptors to produce the required pharmacological effect, this free fraction of the drug (2-8% for immunosuppressants) is more and more considered to be the parameter that best correlates with drug efficacy, but also with toxicity, and is capable of reflecting more accurately the clinical outcome when compared to the total concentration in plasma, serum, or whole blood [2]. Recent clinical studies have also demonstrated that a significant indication is given by the area under the concentration-time curve (AUC) of immunosuppressants, since this parameter is better correlated with efficiency and side effects than the trough level [3]. However, the accurate measurement of AUC requires multiple and repetitive blood samplings and analyses over 12 or 24 hours, which is both costly and time consuming for the clinical staff. A system that allows continuous/near-continuous monitoring of the free fraction of immunosuppressants instead of sparse sampling of whole blood samples within too long time windows, and that performs the monitoring close to the patient's bed without the need of delivering the biological sample to the central laboratories, may provide a much more accurate and faster AUC determination and would represent a significant progress in this area of research.

To this aim, a novel point of care testing (POCT) optical device, resulting from the EU project NANODEM (NANOphotonic DEvice for Multiple therapeutic drug monitoring; http://nanodem.ifac.cnr.it/), has been used for the determination of the free immunosuppressive drug fraction concentration in transplanted patients, using an intravenous microdialysis catheter as body interface, capable of providing microdialysates as clinical samples [4]. The benefit of this device can be an improved TDM and thus an optimized dosing of therapeutic drugs to support the patient management in a clinical environment.

II. ANALYSIS OF INTRAVENOUS MICRODIALYSATE SAMPLES

An optical platform for the monitoring of immunosuppressants in transplanted patients was developed within the European project NANODEM (http://nanodem.ifac.cnr.it/). The key elements are given by:

- the body interface constituted by an intravenous microdialysis catheter (MicroEye®, Probe Scientific Ltd.,

United Kingdom) which extracts continuously the free drug fraction from the blood to the microdialysate;

- a novel chip constituted by ten microchannels (produced by microfluidic ChipShop (Jena, Germany) under the authors design), where an inhibition binding immunoassay takes place for the possible simultaneous determination of up to three different immunosuppressants;
- a fluorescence-based optical platform for the chip interrogation.

The microfluidic optical chip based on total internal reflection fluorescence (TIRF) and fluorescence anisotropy consists of an array of microfluidic channels whose surface is chemically modified with the analyte derivative (Fig. 1). The excitation light coming from an external source is properly coupled and confined by TIRF into the optical waveguide constituting the chip and is guided towards the sensing area [5].

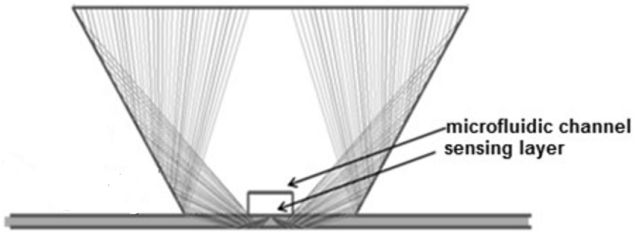

Fig. 1. Transversal cross section of the channel of the designed optical chip, including the ray tracing simulation of the fluorescence emitted by the sensing layer.

The potential of the established optical biochip device was tested within a small-scale clinical study addressing the analysis of total and free drug concentrations in patients under immunosuppressive therapy (cyclosporine A ± mycophenolic acid) after kidney transplantation, which was conducted at the Klinikum rechts der Isar hospital of the Technische Universität München (Munich, Germany). This study made use of the body interface module, based on intravenous microdialysis. By using the intravenously applied microdialysis catheter it was possible to take frequently patient samples (microdialysates) in a minimally invasive way and to measure the concentration of the exclusively free fraction of the immunosuppressant in the microdialysates, since only this component is able to diffuse through the microdialysis membrane. The concentration of immunosuppressants in these samples were determined by a highly sensitive LC-MS/MS method developed within this study.

The same samples were also tested with the optical platform after proper calibration and the results of the two methods were compared.

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