Highly sensitive detection of a neurodegenerative protein biomarker by using the pyro-electrohydrodynamic jet

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Abstract. A set of protein biomarkers are largely recognized as responsible of neurodegeneration mechanisms and hence as potential targets to be detected in low abundant concentrations in body fluids for performing early diagnosis. As an example, the Tau protein experiences a transition phase from a native disorder conformation into a preaggregation state, which leads to fibrillization processes. Here we show the possibility to detect Tau in urine samples at sub-picogram level, through the concentration effect of the pyro-electrohydrodynamic (p-jet) technique. An immunofluorescence protocol is applied to concentrated p-jet spots able to reduce drastically the diffusion effects in the antibody-antigen reaction. A set of diluted samples were prepared, and the fluorescence signal was detected by a confocal scanner. We achieved an excellent linear response with a significant signal-to-noise ratio down to 0.25 pg/mL. In perspective, the technique could be integrated into a compact device to be used for monitoring the early stage associated to neurodegenerative syndromes in different scenarios such as for example in long-term human space exploration missions.

1 Introduction

It is well known that nowadays the neurodegenerative diseases can be treated only in case of very early diagnosis. In the last years a great interest has been devoted to the long-term exploration missions of humans in space and microgravity has been demonstrated to have a crucial role in the production of excessive reactive oxygen species (ROS) and on mitochondrial dysfunctions leading to neurodegeneration [1,2]. The neurodegenerative diagnosis is based usually on brain imaging techniques and on dosage of specific protein biomarkers (e.g. Tau, amyloid) in cerebrospinal fluid thus requiring very invasive and expensive procedures that may require hospitalization with the additional issue of dealing also with fragile patients [3]. Therefore, clinicians still desire to detect the above-mentioned biomarkers in a peripheral body fluid, avoiding invasive procedure and thus allowing early diagnosis and reliable follow-up therapies. We propose here a fluorescence-based technique that makes use of a pyro-electrohydrodynamic jet for concentrating the biomolecules of interest through the overlapping of very tiny droplets of samples and hence reducing drastically the diffusion effects in the antibody-antigen reactions. We were able to detect Tau molecules in urine samples down to 0.25 pg/mL with high accuracy and specificity. This could open the route to a compact device for high sensitive detection of neurodegenerative disease in peripheral body fluids.

2 Experimental section

We prepared different solutions of Tau protein (Tebubio S.r.l.) in artificial urine (Chemazone Inc., Canada) in the range from 40 pg/mL down to 0.25 pg/mL, and we used the p-jet technique to print microspots of the solutions on a glass slide able to immobilize the target Tau molecules via a covalent bond. Figure 1 shows the schematic view of the p-jet procedure. A lithium niobate (LN) crystal 2×2 cm^2 sized (Crystal Technology Inc.) was used for the pyroelectric effect. A tungsten filament bent appropriately was positioned underneath to heat the crystal, thanks to the Joule effect induced by 3.5 s long current pulses. A glass slide with epoxy groups (PolyAn GmbH, Germany) was positioned on the top of the LN crystal and a commercial infrared camera was used to measure the temperature on such slide that did not exceed 35 °C, thus without detrimental effects for the deposited proteins.

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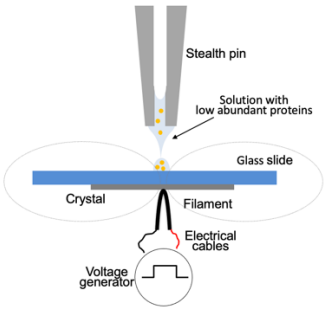

Fig. 1. Schematic view of the p-jet technique. The curved lines in grey indicate the electric field. The orange dots represent the target biomolecules in the solution. It is noteworthy that the scheme is not to scale.

A stealth printing pin (SMP3, Arrayit Corporation) with a capillary width of 20 µm was positioned on top of the slide and aligned with the tip of the tungsten filament. The pin was loaded in a well containing the Tau solution and, after switching on the current in the filament, tiny droplets (down to tens of pL) [4] were ejected from the apex of the printing pin and accumulated precisely on a single microspot on the slide. In fact, due to the pyroelectric effect, the spontaneous polarization P_i of LN changes according to $\Delta P_i \propto p_i \Delta T$, where p_i is the pyroelectric coefficient and ΔT the temperature variation. A surface net charge arises on the crystal because unbalanced by the polarization charge and, consequently, an electric field is generated with intensities that can reach values of tens of kV/mm [4]. Such electric field makes charges to accumulate on the meniscus and the Coulomb repulsion in the liquid elongates the droplet till the ejection of a tiny daugher droplet [5]. Ten replicates of the spots were realized for each sample. Negative control spots were obtained by printing the blank urine sample. Thanks to the small volume of the tiny droplets extracted from the pin and to the point-wise action of the electric field, we achieved the accumulation of the proteins on the microspots avoiding protein spreading. After printing, the slide was incubated at room temperature for 2 h for achieving the covalent bond on the surface and then incubated for 1 h with a blocking solution to saturate the free reactive areas. The slide was then rinsed with carbonate buffer, dried by nitrogen gas and incubated with a fluorescent Alexa 533-primary antibody against Tau at 4 °C overnight. At the end of the protocol new rinsing steps were performed to remove excess reagents. The fluorescence intensities of the spots were measured by the Innoscan 710 (Innopsys Inc) and then analyzed by the Mapix software.

3 Results

Figure 2 shows the behaviour of the fluorescence intensity recorded by the scanner for each Tau concentration, averaged over ten replicates of the p-jet spots and subtracted by the negative control. The error bars correspond to the standard deviations calculated over the ten replicates of the spots.

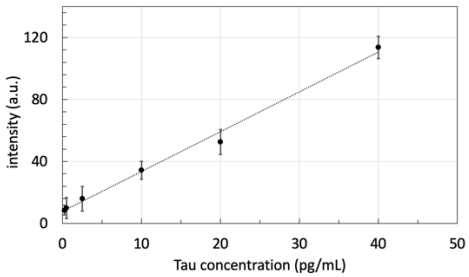

Fig. 2. Fluorescence intensity of the p-jet spots after the immunofluorescence protocol for each of the six solutions of Tau.

The signal-to-noise ratio was > 4 for all the data reported in Figure 2, thus demonstrating that a significant signal was obtained down to the challenging concentration of 0.25 pg/mL. The signal increased with the concentration according to an excellent linear behaviour with $r^2 > 0.99$. These results demonstrate the specificity of the technique and hence its reliability in a complex matrix such as that of human urine. In fact, the urine used in this work has a composition that resembles the real human urine (Chemazone Inc., Canada).

4 Conclusions

In conclusion, we demonstrated here the possibility to detect the neurodegenerative biomarker Tau in urine samples at very competitive concentrations, down to 0.25 pg/mL, thanks to the high-density fluorescence signal achieved by p-jet through the accumulation of tiny droplets at picolitre levels. An excellent linear calibration curve of the fluorescence intensity was achieved, opening the route to the development of an innovative fluorescence biosensor device based on p-jet. In perspective, this device would be used to monitor the level of Tau proteins in body fluids in follow-up therapies of neurodegenerative diseases but also in future scenarios of long-term human space explorations. In fact, the small volumes required by p-jet and the high sensitivity allow us to search for protein biomarkers in peripheral body fluids avoiding invasive withdrawals.

References

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