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Dispensing an enzyme-conjugated solution into an ELISA plate by adapting ink-jet printers

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Abstract

The rapid and precise delivery of small volumes of bio-fluids (from picoliters to nanoliters) is a key feature of modern bioanalytical assays. Commercial ink-jet printers are low-cost systems which enable the dispensing of tiny droplets at a rate which may exceed 10^4 Hz per nozzle. Currently, the main ejection technologies are piezoelectric and bubble-jet. We adapted two commercial printers, respectively a piezoelectric and a bubble-jet one, for the deposition of immunoglobulins into an ELISA plate. The objective was to perform a comparative evaluation of the two classes of ink-jet technologies in terms of required hardware modifications and possible damage on the dispensed molecules. The hardware of the two printers was modified to dispense an enzyme conjugate solution, containing polyclonal rabbit anti-human IgG labelled with HRP in 7 wells of an ELISA plate. Moreover, the ELISA assay was used to assess the functional activity of the biomolecules after ejection. ELISA is a common and well-assessed technique to detect the presence of particular antigens or antibodies in a sample. We employed an ELISA diagnostic kit for the qualitative screening of anti-ENA antibodies to verify the ability of the dispensed immunoglobulins to bind the primary antibodies in the wells. Experimental tests showed that the dispensing of immunoglobulins using the piezoelectric printer does not cause any detectable difference on the outcome of the ELISA test if compared to manual dispensing using micropipettes. On the contrary, the thermal printhead was not able to reliably dispense the bio-fluid, which may mean that a surfactant is required to modify the wetting properties of the liquid.

Keywords: Microdispensing; Immunoglobulins; ELISA; Piezoelectric; Bubble-jet; Drop-on-demand

1. Introduction

Several types of recent bioanalytical systems require the use of dedicated means for delivering small volumes of bio-fluids (from picoliters to nanoliters) in order to speed up reactions and reduce the waste of reagents [1].

The Enzyme-Linked Immunosorbent Assay (ELISA) [2] is one of the most widely used analytical techniques for the detection of antigens or antibodies in a sample. The ELISA protocol uses two antibodies, one of which is antigen-specific, while the other is coupled to an enzyme. The latter one, referred to as conjugate, gives the assay its *enzyme-linked* name, and induces a chromogenic or fluorogenic substrate to produce a color change of the solution. Annual sales for immunoassay reagents and supplies are valued about 2 billion dollars in the US and 7 billion dollars worldwide. Developing microfluidic immunoassays is consequently a strongly pursued trend in bioanalytical research [3].

Commercial ink-jet printheads enable the rapid delivery of droplets (less than 20 pl) at high frequency (> 10 kHz per nozzle) ([4,5]). The dispensing of bio-fluids using such technologies requires some hardware modifications. Additionally, the shear and thermal (in case of bubble-jet printers) stresses occurring during the droplets ejection might be a source of molecular damaging. Therefore, experiments have been performed in the past to test the functional activity of the biomolecules after their dispensing. Several authors ([6–10]) adapted bubble-jet printers for the deposition of biomolecules, showing there was no significant functional damage to the deposited samples and proving the adaptability of ink-jet thermal technology for the dispensing of

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bio-fluids. Quite interestingly, they modified the wetting properties of the dispensed solution by adding a surfactant.

While thermal printers have been demonstrated to be adaptable to biomolecules dispensing, there is quite a lack of experimental information regarding the adaptability of piezoelectric heads. Currently, several microdispensing applications employ *ad hoc* developed piezoelectric actuation to generate droplets ([11–15]). As an example, Wallman et al. and Miliotis et al., both developed microfluidic platforms that include a piezoelectric microdispenser for the deposition of eluted samples on MALDI targets. The microdispenser has a single nozzle and can operate at a maximum frequency of 5 kHz with a minimum volume of the ejected droplets of about 60 pl.

Commercial ink-jet heads have the advantage of generating high flow-rates (of the order of 350 μl/min) as well as single droplets with volumes of the order of few picoliters. Therefore it is worth investigating whether commercial heads, relying on a well-assessed and widespread technology, could be adapted for the assembly of low-cost and high performance bio-dispensers.

The main goal of this work was to evaluate how prone the two main ink-jet technologies currently on the market are to be adapted for the dispensing of immunoglobulins to perform an ELISA test. To this aim, we modified the hardware of a thermal and a piezoelectric printer, while no alterations to the wetting properties of the bio-fluids were made.

This paper is structured as follows. The hardware modifications to both printers are briefly described in Section 2, as well as the protocol used for the dispensing. Section 3 summarizes the main results, which are discussed in Section 4. A short overview of the procedure and comments on its possible applications are reported in Section 5.

2. Materials and methods

Two commercial ink-jet printers were used: a HP Deskjet 5740, based on thermal bubble-jet technology, and an EPSON Stylus c46, based on piezoelectric technology. These two models were chosen because, according to the Manufacturers' specifications, they have similar flow-rates, which was confirmed by the experimental measurements reported in Section 2.3. The modifications to the printers and the dispensing procedure are briefly described in the next two paragraphs.

2.1. Thermal printer modifications

HP ink-jet printers employ thermal technology, also known as bubble-jet, to expel tiny droplets (less than 20 pl) of water-based ink onto the paper. The working principle of this technology is based on the generation of vapor bubbles by heating the liquid, which is confined in a series of micro-chambers. The heat is produced by resistors, having a typical size of few tens of microns, located beneath the floor of the chambers. As vapor bubbles expand, the liquid is pushed away and droplets are expelled from the nozzle [16].

The HP C8767EE black ink cartridge contains a polyurethane sponge soaked with ink. The sponge ensures that the liquid exerts a constant pressure on the nozzles, regardless of its hydrostatic level, thus preventing leakage. The sponge was removed and the cartridge was washed repeatedly with a 10% solution of distilled water and ethanol to remove ink residuals. A new cleaned sponge, filled with 1 ml of distilled water was used to test the dispensing procedure.

We then removed the paper feeding mechanism, including a tray, guide-rails and rolls, to place the ELISA plate under the printhead (Fig. 1). A rectangular base was fabricated with a 3D printer (Z Corp., Burlington, MA, USA) to support the plate at the right distance from the nozzles of the printhead (about 1 mm).

The paper feeding optical sensor was removed and a proper signal, produced by a MATLAB (The Mathworks Inc., Natick, MA, USA) routine, was sent from the DTR serial port pin to the printer motherboard using an USB to Serial converter (FT232BM, FTDI Ltd., Glasgow, UK). The signal was a square wave [0 V, 4 V] with period 51 s and duty cycle 5.9%. It emulates the signal produced by the paper detector during a regular printing session, thus allowing the continuous dispensing without further modification to the drivers.

2.2. Piezoelectric printer modifications

Epson ink-jet printheads expel ink droplets by means of piezoelectric crystals, whose deformation pressurizes the fluid contained in a series of chambers, thus expelling droplets through the nozzles [17]. The Epson Stylus c46 print cartridges (EPSON T036) contain a valve to prevent ink leakage [18]. We used an external feeding kit with four reservoirs and feeding tubes connected to initially clean cartridges. To test the dispensing procedure, the black ink reservoir was filled with 15 ml of distilled water. The paper feeding mechanism was removed and two supports were built and fixed to the structure of the printer to hold the ELISA plate under the printhead. The paper sensor could not be removed as it also sensed the home position of the printhead. As already described in par. 2.1, a proper square signal was produced by a MATLAB routine and sent via USB and a serial converter to the printer motherboard, thus allowing a continuous dispensing process (Fig. 2).

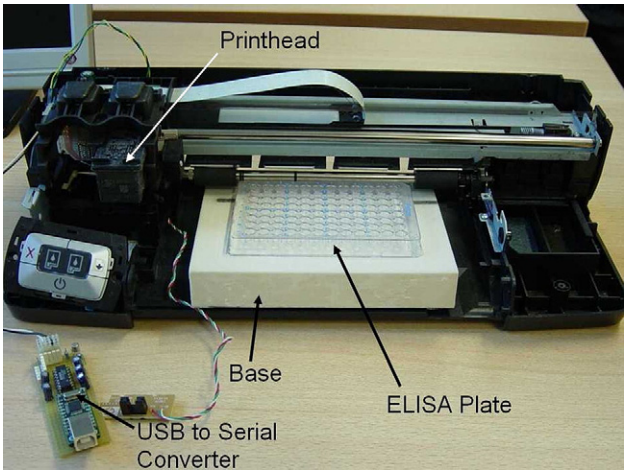

Fig. 1. The HP Deskjet 5740 printer modified for the dispensing in the ELISA plate. The USB to serial converter and the support for the ELISA plate are visible.