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Application of inkjet printing technique for biological material delivery and antimicrobial assays

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ABSTRACT

A modified commercial inkjet printer was developed to deliver biological samples. The active *Escherichia coli* cells were directly printed at precisely targeted positions on agar-coated substrates via this technique to generate complex bacterial colony patterns. Viable cell arrays with a high density of 400 dots/cm^2 were obtained without the addition of any surfactants or other chemicals. Moreover, an applicable example of multiple-layer inkjet printing technique was adapted to deposit bacteria and antibiotics for antimicrobial potential assays. After fluorescent *E. coli* cells were printed, gradient concentrations of water-soluble antibiotics were ejected onto them to determine its minimum inhibitory concentration (MIC) to test the antimicrobial activities. This approach simplifies the experimental manipulation by replacing laborious manual loading processes with automatically controlled printing procedures, which makes it a versatile tool for high-throughput applications.

As a noncontact reprographic technique, inkjet printing reproduces digital images or characters on a substrate using ink drops according to a computer-aided design template [1]. As a versatile tool for microdeposition, commercial inkjet printers have been modified to print biomolecules onto target substrates with little or no reduction of their bioactivities. Currently, there are two main classes of inkjet printers including piezoelectric and thermal (also known as bubble-jets) in the market. In a piezoelectric printer, the ceramic piezoelectric layers were deformed when the computer control signals were sent to the piezoelectric actuators, which leads to shrinking of the capillary nozzle and causes the ejection of droplets through the orifices [2]. While in thermal inkjets, the resistive heater elements, which are connected to ink ejection orifices and placed in contact with an ink reservoir, were actuated according to the printing command. The evaporation of a small portion of ink located in the nozzles caused by rapid transfer of heat could create air bubbles, and thus force the ejection of droplets through the orifices [2]. Concerning the possible degradation of biomolecules caused by high temperatures (200–300 °C) of thermal inkjet technology [3], many reports indicated that there was no significant functional damage to the deposited biological samples [4–10]. Furthermore, the easier installation of a thermal print head and smaller load of samples make the thermal inkjet technology more applicable for the dispensing of biomolecules [2].

In this study, we have adapted a thermal inkjet printer to explore the potential of this technology. Different bacterial colony patterns and high-density cell arrays were generated via this printing method. Moreover, by applying inkjet printing to traditional antimicrobial experiments, we developed a novel prototype protocol for the MIC determination and susceptibility evaluation. Compared to traditional disk diffusion or agar dilution methods, this new approach has several advantages: (1) labor-intensive and time-consuming manual loading processes could be substituted entirely by automatically controlled printing procedures, which reduce the experimental error to a great extent and make the manipulation more reproducible; (2) with the precision and relatively high printing speed, inkjet printing technology has proven to be an extremely fast, cost-effective, and practical method for evaluating the antimicrobial activity of antibiotics or other medicinal materials; (3) using multicolor cartridges and multiple-layer printing techniques, it is possible for us to investigate different antibiotics against different microbes in parallel, which makes the results more comparable and convincible; (4) this approach is easier to be applied to industrial practices due to its automatic characteristic.

Abbreviations: MIC, minimum inhibitory concentration; IPTG, isopropyl β-d-1-thiogalactopyranoside; NCCLS, National Committee for Clinical Laboratory Standards.

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Materials and methods

Inkjet printer modification

A commercial four-color thermal inkjet printer (Canon PIXMA ip1880) with a black (PG-830) and a color cartridge (CL-831) was used to dispense biological materials. The color cartridge consists of three separate ink reservoirs connected by channels to three independent arrays of nozzles in the print head, and the black one only has one ink reservoir. The paper feeding mechanism was replaced with a fixed support to enable direct printing on solid substrates (e.g., microscope glass slides, microtiter plates). The distance between nozzles in the print head and the solid substrate is about 1 mm.

For the modification of the ink cartridge, the plastic upper cover of the cartridge was removed and the internal sponge soaked with ink was discarded. The thin metal sieve beneath the sponge was also removed carefully to expose the ink channel and thermal nozzles mounted directly on the bottom of channel. The cartridge was washed with 100% ethanol and distilled water repeatedly until all ink residual was removed. After being air-dried and sterilized under ultraviolet for 30 min, a low volume (about 50 μl) of biological samples could be loaded into the ink channel of the cartridge.

Sample preparation

For the construction of fluorescent cells, recombinant plasmid pET21–DsRed2 based on plasmid pET21a and pDsRed2-1 was constructed and transformed into *E. coli* BL21(DE3). The recombinant strain expresses red fluorescent protein after 0.2 mM IPTG induction. Cell pellets were collected by centrifugation (5000*g*, 10 min) after overnight cultivation, and then resuspended in a phosphate-buffered solution with a density of 10^7–10^8 cells/ml. The tubes containing bacterial cells were well suspended to break up clumps before printing and to ensure good distribution of the cells.

The stock solution of antibiotics was made in 1% phosphate buffer (pH 6) to a final concentration of 1000 μg/ml. The working solutions were diluted form the stock solution with the same phosphate buffer.

Bio-paper preparation

Microscope glass slides and metal plates were used as print substrates, respectively. Before use, these substrates were rinsed with ethanol and sterilized under ultraviolet. For the colony patterns and step-up antibacterial model, the substrates were coated with LB agar containing 0.2 mM IPTG for the induction of red fluorescent protein. For the high-density cell arrays, microscope glass slides without any coating were used. For the precision test and potency determination, the substrates were coated with a double-layer culture matrix, which was made up of an LB base support and a thin bacterial layer.

Fabrication of bacteria colony pattern

Several different bacteria colony patterns were edited by Microsoft PowerPoint software. These patterns included dots array (0.5 mm in diameter), gray strip gradient (0.5 cm wide and 2.5 cm long), and text (font, Arial; size, 16). Bacteria suspension was added into ink channel directly, and droplets of the cell suspension were ejected on the agar-coated substrates according to predesigned patterns. All printed slides were incubated at 37 °C for overnight and then held at 4 °C for 24 h for the visualization of red color. The whole process was conducted in a clean room to avoid any contamination.

Fabrication of high-density cell array

To evaluate the high-throughput potential of this inkjet printer, dot arrays with different diameters and distances were edited to match the layout of standard 96- or 384-well microtiter plates. To achieve higher throughput, the diameters of dots we reduced to only 100 μm, and the distances between dots were reduced as well. The bacteria cells were printed directly on microscope glass slides without agar coating. Fluorescent signals were monitored immediately using a laser scanner (GeneChip scanner 3000) with the excitation wavelength at 532 nm.

Precision test for inkjet printing of antibiotic solution

To evaluate the precision of inkjet printing technology, a traditional cup-plate diffusion assay was adapted for comparison. Cups with 6 mm inner diameter were placed on the surface of culture matrix containing a thin bacterial coating. Antibiotic solution was diluted to 4 μg/ml from stock solution, and 0.25 ml dilution was loaded into the cups carefully. For the inkjet printing method, solid dots with the same diameter were designed. According to the resolution of the printer and the volume of single droplet [11], the total volume of antibiotic solution printed for each dot was 0.5 μl. Antibiotic solutions with a concentration of 2000 μg/ml were loaded into cartridges and the amount of antibiotics printed in one dot was the same as that loaded in one cup. All plates were incubated for 16–18 h at 37 °C and the bacterial inhibition zones were measured using a multifunctional automatic analytical machine ZY-300IV (Xianqu Weifeng Technology Development Company, China).

Antibiotic potency measurement

Double dose assay was adapted for antibiotic potency measurement [12]. The oxford cups were replaced with 6-mm-diameter dots designed by software. Taking the advantage of color setup, two dots were edited to be magenta and another two were cyan. So the antibiotic standards and unknown samples could be loaded into magenta and cyan compartments, respectively, and controlled by printing designs. Furthermore, the gray value of these dots from both two colors was defined as 0% and 50%, respectively. Therefore, antibiotic standard and unknown sample could be printed synchronously onto culture matrix. One printed "dot" was of low dose and the other was of high dose. The ratio of the two doses for both the standard and the sample was 2:1. After incubation, the bacterial inhibition zones were measured by an automatic analytical machine, and the potency of sample was determined by its software according to its user manual.

Step-up antibacterial model

A novel step-up antibacterial prototype was developed by printing bacteria and antibiotics sequentially on agar-coated metal plates. *Escherichia coli* BL21(DE3) transformed with plasmid pET21–DsRed2 was used as indicatory bacteria. Bacterial suspension was added into black cartridge and antibiotic solution was added into color cartridges. Cell suspension was printed uniformly on the surface of culture matrix, forming a thin bacterial coating, on which the antibiotic solution was then printed. A rectangular (6×48 mm) gray strip gradient was edited for the step-up printing of antibiotic solution. All plates after printing were incubated for 16–18 h at 37 °C and then held at 4 °C for 24 h.