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Effect on Thermal Ink-jet Printing on Bacterial Cells

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Abstract

Ink jetting is a technique with varied applications. The process of bubble/droplet formation of thermal ink jet printers is one that is known to involve a high temperature. This high temperature is however localized and short-lived. Investigations are hereby carried out to evaluate the effect of thermal droplet formation on bacterial cells. Bacterial viability tests were done by colony plate counting and measurement of bacterial absorbance after thermal ink jetting. Bacterial cells were also printed directly onto agar coated glass slides using different templates and incubated. The effect of thermal ink jetting on bacterial cell wall was also conducted by gram staining ink jetted cells. The results obtained indicated that the process of bubble formation did not have any significant effect on bacterial viability as compared to the pipetted versions. Also, bacteria printed directly onto agar gave colonies similar to templates used in printing. Gram staining experiments revealed that the bacterial cells still maintained ability to give a positive gram stain test.

Introduction

Ink jetting as a non-contact technique is capable of reproducing digital image data on a substrate. Applications of ink-jet printing have been varied and diverse in recent times ranging from electronics through polymer engineering to medicine [1]. Ink jetting is useful in procedures that require nano-manipulation but are limited by an inability to precisely position very small drops of liquid [2]. Most applications of ink jetting utilize piezoelectric rather than thermal inkjets, a major reason being the assumption that the high temperature involved in droplet formation with thermal ink-jet printers will result in damaging effect to materials [3]. This concern is further heightened when the work involves living cells. In this work, investigations are conducted to determine the effect of thermal ink jetting (TIJ) on bacterial cell viability and to evaluate any changes the process of droplet formation has on the bacterial cell wall. Studies to understand microorganisms and the microbiota (i.e., microorganisms in a particular environment) has triggered much interest recently partly due to the issue of antibiotic resistance. It is therefore imperative to come up innovative ways to study these. This is one of the reasons we explore the effect of TIJ of bacterial solutions as a foundation for other investigations. In May 2016, the United States Whitehouse launched the National Microbiome Initiative to advance the study of microbes in our bodies and environment with federal agencies investing more than $121 million towards the study [4]. Bacterial cells were chosen because of numerous potential applications thermal ink jetting of bacterial cells can be applied to. These include high throughput techniques like cell microarray technology, analytical concepts like evaluating antimicrobial efficacy of surfaces and devices, and determination of susceptibility of microorganisms to antibiotics [5-7]. With the combined increased interest in probiotic formulations (i.e., bacteria with health benefits to humans) and delivery challenges of probiotics, TIJ can offer an interesting alternative to on-demand formulation of probiotics both for advancing probiotic research and clinical purposes.

Method

A modified Hewlett Packard (HP) 5940 (USA) thermal ink-jet printer was used in this work. The modifications were done such that rather than substrate (i.e., paper) moving through the printer's rollers during operation, printing was to be done onto a stationary stage mounted underneath the print head when it's in motion. The printer's sensors were also activated manually to prevent the printer from detecting the absence of paper [8]. All printing experiments involved the use of only black cartridges (HP cartridge 337). The microorganism used in this work was *Lactobacillus acidophilus.* This organism was chosen because it is a generally regarded as safe (GRAS) organism to help in these preliminary determinations. The presence of a cell wall in this organism will also aid in evaluating any effect of droplet formation on the cell wall.

An evaluation of the viability of thermal ink jetted bacterial solution was performed initially. Bacterial viability was assessed by colony counting. A bacterial solution of known population was ink jetted into a sterile petri dish. Due to the picolitre droplet size of thermal inkjet printers, the procedure was repeated a number of times using a rectangular template (2 cm x 20 cm). When enough of the bacterial solution was in the petri dish, 50 uL was withdrawn and 1 in 10 serial dilutions made. The serially diluted solutions were then plated onto de man, Rogosa and Sharpe agar (MRS agar, Oxoid, UK) and incubated under anaerobic conditions for 48 hours prior to enumeration. A control test involved serially diluting and plating out a pipetted solution of similar bacterial concentration as the printed solution.

Bacterial viability was further evaluated by printing a variety of templates onto glass slide coated with MRS agar. The templates used included 3 sets of 1 cm x 1 cm squares, two rows of dots and text (figures 1–3). As a precaution, the distance between the print head and the agar coated glass slide was kept to an allowable minimum (2 mm) to reduce the effect of satellite drops that may fall outside the template design. The imprinted slides were then incubated for a period of 48 hours under anaerobic conditions.

Figure 1*- Template for three 1 cm x 1 cm squares*

Figure 2 *– Template for two rows of dots*

DODOO

Figure 3*- Template for text*

The ultraviolet absorbance by bacterial species was done by measuring the absorbance (at 600 nm) of ink jetted bacterial species in comparison to a pipetted equivalent over a period of 7 hours to observe any changes in the lag and early log phases of bacterial growth. This was done by repeatedly printing a bacterial solution of known concentration (Log 8 CFU/mL) into a petri dish using a rectangular template (2 cm x 20 cm) until there was enough bacterial solution in the petri dish to enable withdrawal of 50 uL. A 1 in 10 dilution was done (i. e 50 uL into 500 uL) and the resultant solution was then serially diluted further (300 uL into 3 mL). From the resultant 3 mL of ink jetted bacterial solution, 200 uL was transferred into 8 bijous and made up to 200 mL. The absorbance of 1 of the bijous was recorded immediately representing 0 hours, the remaining 7 bijous were then kept at 37 ^{0}C and absorbance of 1mL solution in clean cuvettes taken at hourly for 7 hours. This was repeated to obtain triplicate data. A control experiment was taken through above procedure with the only exception being pipetting of the initial 50 uL from Log 8 CFU/mL directly.

The effect of thermal inkjet printing on bacterial cell wall was also evaluated by gram staining. *L. acidophilus* being a gram positive organism was subjected to gram stain test after ink jetting onto a glass slide and compared with a pipetted version. The technique involves the use of four reagents (crystal violet, Gram's iodine, a decolorizing agent, and safranin). These were applied to bacterial cells in the order: crystal violet, Gram's iodine, decolorizing agent, and safranin [9,10]. Light microscope fitted with camera was then used for visualization.

Results and Discussion

Colony plate counting, a conventional bacterial enumeration technique was used to evaluate viability. Analysis of bacterial cells after thermal ink jetting showed the cells were intact and viability was comparable to the control. The impact of thermal ink jetting on bacterial viability showed that the printing procedure did not have any deleterious effect on bacterial viability. The bacterial population after ink jetting was very similar to that of control (pipetted); approximately 1 x 10^7 colony forming units (CFUs) per mL (figure 4). It is presumed that a temperature rise of about 200 ^{0}C is involved in bubble formation. It is this presumption that most scientists have about viability of cells being hampered when thermal ink jetted. This results further supports the findings by Xu and colleagues which show that the temperature rise is very short lived and the net effect across the medium is about 10 ^{0}C [11].

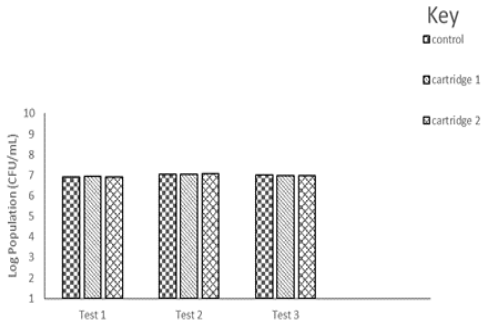

Figure 4*- Effect of TIJ on bacterial viability*

To investigate further viability of ink jetted bacteria, cells were printed using different templates directly onto MRS agar-coated glass slides and incubated. After incubation it was observed that bacteria had grown mimicking templates that was used in printing (figure 5 -7). The accuracy of the ink jetting procedure is even highlighted further with the printing of two rows of dots and the text (figures 6 ,7).

Figure 5 *-Bacterial growth post-incubation for three 1 cm x 1 cm squares*

Figure 6*- Bacterial growth post-incubation for two rows of dots*

Figure 7 *- Bacterial growth post-incubation for text*

U.V absorbance at 600nm is a technique used in bacterial enumeration. Bacterial absorbance at this wavelength is directly proportional to bacterial population. This was therefore used to observe the lag phase characteristics of thermal ink jetted bacterial growth. The absorbance of both the thermal ink jetted and control bacterial species were very similar in the lag and early log phases of bacterial growth (figure 8), indicating no significant change to growth properties.

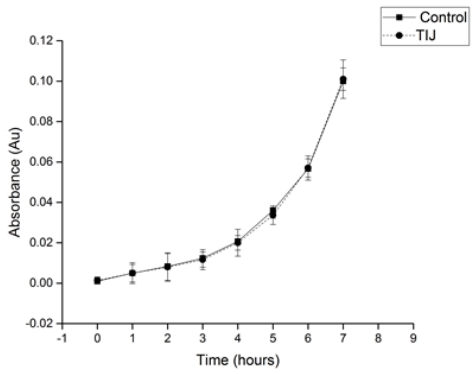

Figure 8- *Effect of thermal ink jetting on bacterial absorbance*

Gram staining is a simple useful technique to differentiate bacteria into two large groups, gram positive or gram negative. The basis for this differentiation is due to the physical and chemical composition of the cell walls of bacteria to retain and release stains. Gram staining is therefore key in evaluating the integrity of bacterial cell wall [9]. Visualization by light microscopy after gram staining revealed that both the ink jetted and pipetted bacteria gave positive gram staining results (figures 9–10). This implies the integrity of the thermal ink jetted bacterial species were still intact with no damage to the cell wall incurred as a result of bubble formation.

Figure 9 *- Light microscopy image of gram stained ink jetted bacteria*

Figure 10 *- Light microscopy image of gram stained pipetted bacteria*

Conclusion

It has been demonstrated here that the high temperature accompanying droplet formation in thermal ink jetting does not result in damaging effect to bacterial viability and cell wall properties.

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