Biological Damage Encountered through Inkjet Printing

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Statement of Purpose: Inkjet printing is an extremely useful tool for accurately placing small amounts of liquids onto a substrate. This is a promising tool that could be used within the medical field to deposit picolitre volumes of biological fluids onto a substrate. However, earlier work has reported some damage to biological materials after printing (Nishioka *et al*, 2004). This presentation demonstrates that there is some cellular damage present through inkjet printing, but will concentrate on assessing whether or not this damage occurs and is detectable in proteins. This will be done by using the percentage retained activity in an enzyme, glucose oxidase.

Methods: A standard glucose oxidase assay was used to detect the percentage retained activity of the enzyme after printing through a Microfab piezoelectric printhead (Microfab Technologies, Plano, TX, USA) and two commercial piezoelectric drop-on-demand printing systems supplied by Xaar (Xaar, Cambridge, UK) and Dimatix (Dimatix, Santa Clara, CA, USA). The assay used was an Amplex Red Glucose/ Glucose Oxidase Assay Kit (Product No: A22189, Invitrogen, Paisley, UK), which reacts glucose oxidase with D-glucose to produce D-gluconolactone and H_2O_2. In the presence of horseradish peroxidase, the H_2O_2 then reacts with the Amplex Red reagent in a 1:1 stoichiometry to generate the red-fluorescent oxidation product, resorufin (Mohanty *et al,*, 1997). The intensity of the fluorescence of the resorufin is then read in a microplate reader set at excitation in the range of 530-560nm and fluorescence emission detection at 590nm. The percentage retained activity can then be calculated in comparison to control curves carried out at the same time.

Results/Discussion: Work performed prior to this paper has demonstrated that cell death is encountered upon inkjet printing. The percentage cell death is variable with voltage and rise time and is not always statistically relevant. Here we present results that explore how the process parameters in each printer (excitation voltage, drive frequency, printer aperture) influence the extent of biological damage to macromolecules (fig 1 & 2).

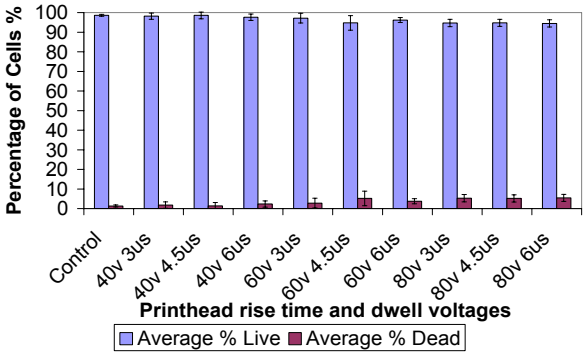

Figure 1. Percentage cell death of HT1080 Fibroblasts at different voltages and rise times.

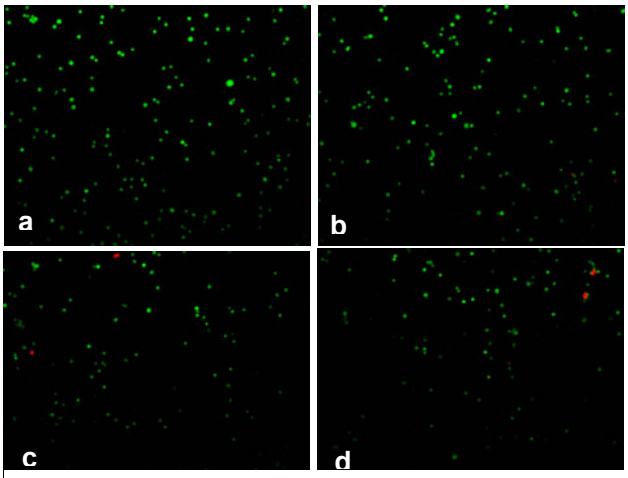

Figure 2. HT1080 fibrosarcoma stained with calcein AM and ethidium homodimer-1 a) control b) waveform amplitude 40V c) waveform amplitude 60 V and d) waveform amplitude 80V (All the above samples, excepting the control, were printed with a frequency of 10kHz and a constant rise and fall time of 3 us)

Conclusions: We demonstrate that it is the excitation voltage of the piezoelectric actuator which has the greatest influence on the extent of damage experienced by biological systems after inkjet printing. Statistical analysis of the data indicates that other parameters have no significant influence on behavior after printing. We also demonstrate that with some printing systems it is possible to identify regimes of printing process parameters that result in no significant difference between the behavior of the materials after printing when compared with an unprinted control.

References:

(Nishioka G.M., J. Am. Chem. Soc. 2004; 126, 16320-16321)
(Mohanty J.G., J. Immunol. Methods 1997; 202, 133-141.)

Sponsoring Organisation:
Xaar plc, 316 Science Park, Cambridge.