Applications for Ink Jet Printing in Biology and Medicine

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Abstract

Ink jet printing has attracted increasing interest in recent years as a tool for medical research. The ability to precisely position pl volumes of liquid has a number of potential applications in tissue engineering. The dispensation of bioactive chemicals, e.g. adhesion and growth factors, can be used for the directed growth of nerve cells. Whole cells have been dispensed without excessive damage to their function. This leads to the possible construction of biologically active structures from cells and other materials with applications in: fundamental cell biology, cell arrays for toxicity testing, bioreactors and cell factories for pharmaceutical and tissue (cartilage) manufacture, external assist devices (e.g. next generation dialysis, and even complete organs for implantation.)

There are a number of applications in the life and medical sciences that require precise manipulation of very small volumes of liquid: e.g. microdosing, surface patterning, tissue engineering, and the direct placement of living cells. Ink jet printing, with its potential for accurate deposition of volumes in the pl range may satisfy this need, either by the direct positioning of cells, or by directing spatially resolved cell behaviour through patterning chemicals such as adhesion or growth promoters.[1] Potential applications for inkjet printing include: cell based biosensors, tissue engineering for reproducing complex organ function, implanted cell-factory devices, or external assist devices for organs.[2]

Inkjet printers deliver picolitre droplets of fluid with positional accuracy < 30 μm. They can be used to position cells in an environment allowing their viability. They can also deposit, in parallel, other materials such that a complete device including: cell substrate (or scaffold), nutrient delivery mechanism, chemical and other stimuli, can be incorporated in a single manufacturing operation. Inkjet printing equipment is attractive to researchers in biology and medicine because the technology that enables colour graphics reproduction can be easily modified to allow the printing of different functional fluids – "bioinks".

Inkjet printing is of great interest to the enzyme-based biosensor market (e.g. glucose sensors based on electrochemical cells containg glucose oxidase). Current manufacturing practice is based on screen printing with consequent wastage of expensive enzyme suspensions. Inkjet printing dispenses fluid solely where it is needed, eliminating waste and also allowing finer feature resolution and hence smaller and more compact sensor designs.

There is a possibility that enzyme activity is influenced by the fluid stresses generated through inkjet printing. Nishioka *et al* studied the activity of horseradish peroxidase after piezoelectric actuated DOD printing and reported a decrease in activity decreased with increasing actuation. [3] However, a study of the activity of glucose oxidase after printing with a thermal printer found no reduction in activity after printing.[4] Recent work by Di Risio and Yan also studied the effect of inkjet printing on the activity of horseradish peroxidise.[5] They also investigated the effect of second phase additives on the activity of the enzyme after printing. It is well known that inkjet printing is only possible within a relatively narrow range of physical properties (viscosity, surface tension and density)[6-9] thus it may be necessary to modify biological fluids to enable them for printing. Di Risio and yan found that the greatest influence on activity of the enzyme was the concentration of the fluid property modifiers, once a fluid had been optimised for printing there was not significant further denaturing after printing.[5]

Another application for printing enzymes and proteins is to use inkjet delivery to pattern cell culture plates with factors to engineer cell development and differentiation. There have been recent developments in the field of stem cell biology that have used this methodology. Ilkhanizadeh *et al* investigated the influence of three biological molecules or fluids, fibroblast growth factor-2 (FGF2), ciliary neurotrophic factor (CNTF) and foetal bovine serum (FBS), on the differentiation of rat embryo neural stem cells (NSC). They modified a desktop thermal DOD inkjet printer and commercial graphics software was used to define surface patterns and concentration gradients through using greyscale printing. By patterning adjacent regions on the substrate with FGF2 and CNTF stem cell differentiation was controlled.[10]

Phillippi *et al* studied the differentiation of mouse muscle derived stem cells (MDSC) in the presence of printed patterns and concentration gradients of bone morphogenic protein-2 (BMP2).[11] Phillippi *et al* used a more sophisticated method for the deposition of protein suspensions than reported by Ilkhanizadeh *et al*.[10] Phillippi used an inkjet printing system specifically developed for protein and biochemical deposition rather than adapting a graphics system. As with the NSC studies, MSDC differentiation could be controlled by printing appropriate patterns and gradients of protein.

The ability to pattern surfaces with proteins and the retention of protein activity after printing has now been demonstrated. It is also clear that strategies are available to immobilise proteins under cell culture conditions for sufficient time to carry out cell biology experiments and that useful work on cell behaviour and stem cell differentiation has been reported. In order to fully exploit the capability of inkjet printing to engineer biomimetic tissue analogue constructs, cell deposition is necessary.

Boland used thermal DOD inkjet for cell deposition and found that fewer than 3% of the cell population died during printing.[12] However, preparation of a cell-containing bio-ink led to much greater death rates during ink preparation. Thus indicating, as with proteins, there may be difficulties in achieving a printable fluid while retaining biocompatibility. However, in a further study Boland was able to print neuronal cells and show that those that survived printing retained neuronal electrical activity.[13]

Nakamura *et al* succeeded in printing bovine vascular endothelial cells using a DOD printer with an electrostatic actuation mechanism.[14] Recently a thorough investigation of the influence of piezoelectric DOD printing on the survival and viability of a human fibroblast cell line has been reported by Saunders *et al*.[15] This found that at the lowest values of the

actuating pulse capable of producing drops, cell death rates were statistically indistinguishable from unprinted control cell populations.

A major objective of the research that has used printing technology is to develop engineered human tissue or tissue-analogue constructs for applications in Regenerative Medicine. To achieve this we need to be able to fabricate 3-D cell containing structures. This is still at a relatively early stage of development and needs significant advances in developing new cell-compatible materials that can be delivered by inkjet printing. To date most workers have used sodium alginate solutions gelled in the presence of Ca^{2+} ions, normally from $CaCl_2$ solutions.

Most work in this area has been carried out by the groups of Boland and Nakamura. In each case one component of the alginate gelling system is printed into a bath of the other. Boland group has taken the approach of printing the less viscous $CaCl_2$ solution into a tank of Na-alginate solution.[16] In order to generate 3-D structures, the tank containing the Na-alginate contains a moving table that is initially positioned so that a thin film of < 100 μm thickness of the liquid is exposed to the printer. By selectively patterning this film with $CaCl_2$ solution, a defined region of the liquid film is gelled. After this, the platform is lowered a defined distance and a second sequence of printing is used to gel a second layer. This process is repeated until the final desired structure is achieved. Boland reported the attachment and growth of bovine endothelial cells onto these printed structures but did not print the cells. Nakamura *et al* have succeeded in printing cells in a gelling medium.[17] In this case the Na-alginate solution, containing cells in suspension, is printed into the $CaCl_2$ medium to form beads or rings of gelled material containing cells. A similar approach was taken by Xu to form alginate beads containing cells.[18]

To conclude, there has been considerable pioneering work into the use of inkjet printing as a delivery mechanism for proteins, cells and biomaterials. This technology has enabled new experiments in cell development and has an exciting potential for applications in tissue engineering and regenerative medicine. However, it is likely that the application nearest to market is the use of inkjet printing in the fabrication of biosensors.

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Biography

Brian Derby is a professor of Materials Science at the School of Materials, University of Manchester, UK, where he leads a research group of 12 students and postdoctoral workers. Prior to that he was on the faculty of the University of Oxford, and director of the Oxford Centre for Advanced Materials and Composites. For more than 10 years he has been one of the pioneers of the application of ink jet printing as a general manufacturing tool and has carried out pioneering work in the 3-D printing of ceramics, polymers and biomaterials. He has been active in the field of Bioprinting: the use of printing technology (particularly ink jet printing) to deposit biomaterials along with proteins and cells for applications as sensors and tissue engineering scaffolds. Brian Derby's research has been funded by a number of sources including: Engineering and Physical Sciences Research Council (UK), Biotechnology and Biological Sciences Research Council (UK), the Technology Strategy Board (UK), the Office of Naval Research (USA), the European Commission, and directly by industry.