Rapid Deposition of Hydrogel Layers by Inkjet Printing

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Abstract

A piezo inkjet printer was built for the rapid printing of hydrogels and other biological materials. Materials printed included the synthetic polymers; poly-diallyl-dimethyl ammonium chloride and polystyrene sulfonate, and the naturally occurring polymers chitosan and alginate.

Using these polymers, the printer was able to create robust hydrogels of size 17mm x 17mm x 2 mm high in under five minutes. This speed and size of production is of relevance in enabling tissue engineering, regenerative medicine and other technologies. Furthermore - live cells were successfully jet by the same printer, and could withstand the printing forces and remained viable in printed hydrogels for several days after printing.

Background

Three dimensional fabrication and printing of bio-materials such as hydrogels is a technology advancing on many fronts. Hydrogels have applications as matrices for cell growth, drug delivery devices and are expected to have a range of other applications in tissue engineering, bionics and soft materials engineering [1,2,3].

Calvert [4] has shown the ability to print viable cells using thermal inkjet printheads. Nakamura [5] has shown cell viability using peizo based inkjet systems. The Derby group has numerously shown that human cells can be printed [6].

It has been widely demonstrated that artificially fabricated biogels can be used to grow cells in-vitro [7]. However the ability to produce hydrogel devices of sizes several millimeters to several centimeters in three dimensions, is not as numerously reported in the literature. Where large (e.g. approaching human organ size) objects have been fabricated, it is often by techniques such as casting a mould or extrusion of bio materials through a syringe or other fine tip [8].

Inkjet printing is an established method of rapid prototyping. Many commercially available inkjet machines produce three dimensional objects. Despite differing underlying techniques (photo curing, thermal curing, jetting glue into a binder, etc.), they are all characterized by a layer by layer approach, considered slow in manufacturing terms. One way to help improve efficiency is maximizing the material used in the final structure, minimizing the material lost in evaporation or other processing waste. For a hydrogel, all of the inkjet drop maybe part of the final structure, especially if the solids loading in the inks are of the same range as that desired for the application, typically 10-50% for robust gels.

Natural and synthetic ionically charged polymer solutions are made to form gels by mixing anionic and cationic ions in a process called polyelectrolyte complexation [9]. There is a considerable literature on inkjet printing alginate gels for tissue engineering where calcium ions are printed into a bath of alginate solution, causing local gelation [10,11]. This has limitations as the gels are weak and revert to liquid if the calcium level drops. It would be beneficial to have a much wider palette of printable gels.

Printer for Hydrogels

A peizo inkjet printer has been constructed based around specially engineered Xaar printheads. Proprietary drive electronics were used (Xaar XUSB) to independently control up to 16 printheads at any one time, though typically two, and occasionally three or four heads loaded with different materials were used. Substrates were held in a clear Perspex plate, designed to hold in place 6 slides of 75 x 25 mm.

Motion between the printheads and the substrate was provided by a single linear axis (Reliance), with stroke length 400mm and 1 micron resolution via a linear encoder (Renishaw). In the first prototype of the machine a second horizontal axis was not included as the printhead's 17mm print width (126 nozzles at 137u spacing) was almost ideal for the types of studies envisioned, and they could easily be ganged side by side to increase this width. The printhead has an effective throw distance (gap between nozzle plate and substrate) of up to 3mm. This meant that three dimensional objects up to 2.5mm could be printed with little risk of interference and a vertical (Z) axis was not required. Conceptual schematic and photograph of the printer are shown in Figures 1 and 2.

The intention was to produce gel layers by alternately printing two components, a water soluble anionic and cationic polymer. Each head had an independent fluid path, without risk of cross-contamination in delivery, allowing reaction once the polymers were on the substrate.

The amount of each material delivered per print pass could be altered by simple changes to the bitmap patterns. This simple technique compensated for changes in fluid concentrations that were required to achieve the correct rheological properties required for jetting. Elevation of printed structures increased with number of print passes.

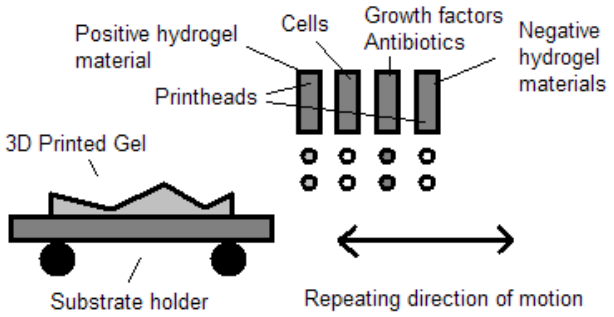

***Figure 1**. Schematic of the reactive hydrogel printer.*

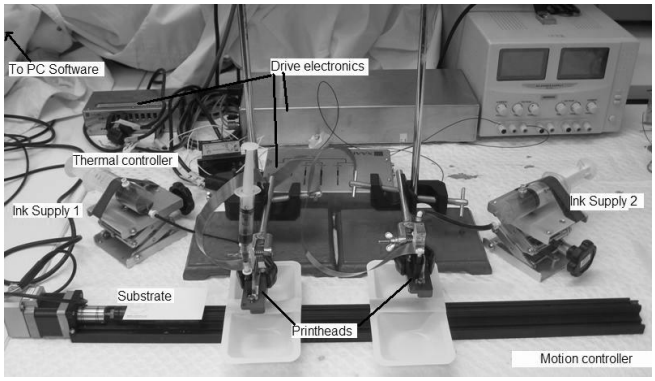

***Figure 2**. Photo of the reactive hydrogel printer.*

Materials for Hydrogels

Polyelectrolyte complexation: A central idea was the use of oppositely charged sets of fluid immersed polymers, which when in contact complex to form solids of water soaked gels [10,11]. Two main material systems were used.

One set of materials concerned the synthetic polymers PDDA (polydiallyldimethyl ammonium chloride, cationic) and PSS (polystyrene sulfonate, anionic). PDDA was received as a liquid at 35% w/v (Sigma, "very low molecular weight" 150,000 Da) and was diluted with Milli-Q water to 20% w/v. PSS (polystyrene sulfonate co-maleic acid, sodium salt Sigma, molecular weight 20,000 Da) was prepared to 30% w/v in Milli-Q. These polymers are characterized by short regular polymer chains.

The other set of materials consisted of the naturally occurring polymers alginate and chitosan. Inks were formulated using alginic acid sodium salt (Sigma) at 1% w/v in Milli-Q, and low molecular weight chitosan (Sigma) at 0.5% w/v in 1% acetic acid. Powders were weighed and dissolved in water/acid at 50 ^{o}C, stirred at 500rpm for 2 hours.

To all materials 1-2% w/v Triton X-405 (polyethylene glycol *p*-(1,1,3,3-tetramethylbutyl)-phenyl ether, Sigma) or Tween-20 (Polysorbate-20) were added as a surfactant, and 1-2% w/v ethylene glycol (EG, Sigma) was added as a humectant. Tween is regarded as being more bio-friendly. In concentrations of less than 5%, these materials were shown to have limited negative effect on cell growth (an indicator of bio-compatibility).

Mammalian cells (C2C12 mouse skeletal myoblasts, ATCC) were occasionally incorporated into the gels via a third printhead.

Substrates included printer paper, photo paper, polyvinylidene fluoride (PVDF) membrane and glass microscope slides depending on applications.

Printing algorithm

The gels were loaded into the printheads by means of standard laboratory syringes (50ml). About 3ml of each polymer solution was used in priming the printheads and connecting pipework, and 10-15mL of solution remained in each syringe as a reservoir. The syringes were laid flat on a laboratory jack, with the level of the ink held a few millimeters below the level of the nozzle plate. This allowed the correct meniscus to be set at the printhead by moving the lab jacks up and down. Occasional visual observation of the printheads to see if the nozzles were starved or flooding sufficed to maintain correct meniscus.

There is a saying about three dimensional printing using inkjet; that by joining drops we get lines, by joining lines we get films, and by layering films we can generate structures. Typically bitmap generated squares of 126 x 126 pixels (17mm x 17mm) were printed as layers which were then repeated. The bitmap image was sent to each of the printheads via the print electronics. If the cationic square was printed on the substrate first, the anionic material would immediately be jetted, initiating gellation of the materials. By altering the density of the bitmaps different polymer ratios could be obtained.

The concept was shown to be broadly successful. There was an expected correlation between increased print passes and increased gel height (see Fig. 3).

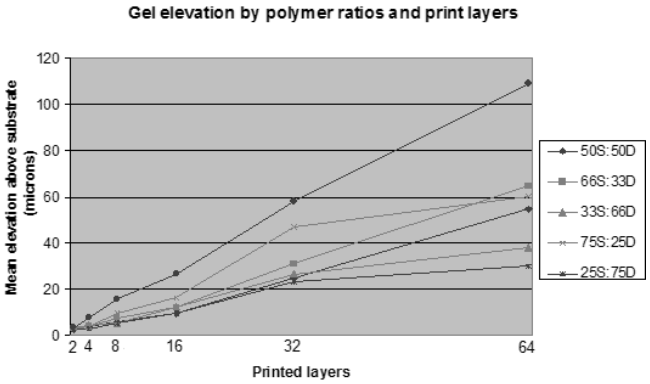

***Figure 3**. Relationship between print passes and dried gel heights. Legend indicates ratios of PSS (S) to PDDA (D).*

Excess water removal

The printer was able to deliver polymer containing inks at such a rate that there was often excess water present. Trials with the printer showed that the amount of water that was required to be delivered with the ink was excessive for the amount of gelling material. This was called 'puddling' and is shown in Figure 4. Ideally the gel could be built up on a table or platen and could then be picked up and handled without the need for any additional substrate. Glass slides are cheap and conveniently sized, while on non-porous, hydrophobic silca glass gels might simply slide or peel off.

Water removal was investigated by a number of methods. Printing onto a PVDF membrane on an absorbent filter paper showed to be an effective method for soaking away a part of the water while leaving the gel to form on the surface. Convection via fans and heating pads held below the slides were other effective methods of water removal. Interestingly, once gels had been formed they were then able to reabsorb approximately 50% more water than the excess water.

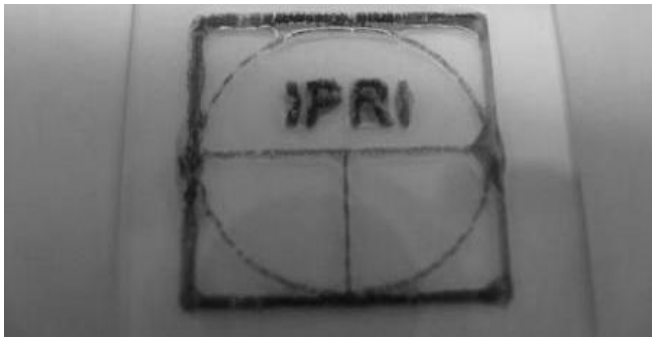

***Figure 4**. 'Puddling'of gels printed onto a microscope slide where there was excess water for the gelling materials to absorb.*

Cell inclusion

Cells could be added to the printed hydrogel by either seeding them onto the gel post printing or by including them via a third printhead. Cells were suspended at $1x10^6$ cells/mL in an ink consisting of Dulbecos Modified Eagles Medium (DMEM) with 10% fetal calf serum and penicillin/streptomycin. In a successful trial demonstrating the ability to print all components, cells were printed using one head, the hydrogel gellan gum (CP Kelco, dissolved in Milli-Q to 0.33% w/v) printed via a second head, and the gellan was crosslinked by jetting dissolved Ca^{2+} ions via a third head. This demonstrated the ability to produce a cell laden hydrogel, with all the component parts having been printed.

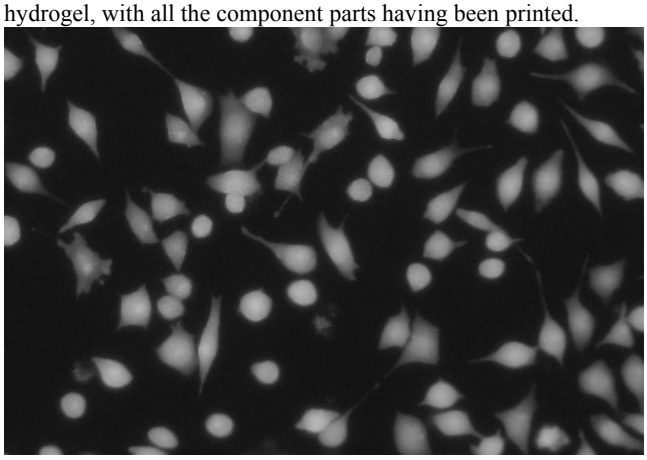

***Figure 5**. Printed structures seeded with C2C12 muscle cells.*

Drug and dye diffusion through hydrogels.

A topic of interest to biologists is the ability to have a secondary material release from a network of primary materials at a steady, even rate. Also if the release is slow e.g. over the order of days of weeks, then this is of special interest for the slow release of drugs.

The charged dyes bromocresol green ($C_{21}H_{13}Br_4O_5SNa$ Sodium Salt, Sigma), crystal violet ($C_{25}H_{30}N_5Cl$, Sigma) and indicator dye phenol red (ACS reagent, $C_{17}H_{14}O_5S$, Sigma), were added to the polymer ink solutions at less than 0.5% w/v of the host ink.

The bromocreosol green as added to the PDDA ink was measured for diffusion by taking micro-graphs at intervals over one week. The dye was still slowly releasing after one week (see Fig 6). The release was influenced by addition of salts, it was found that immersion in buffer solution slowed release, and polymer ratios closer to molecular parity also slowed the release.

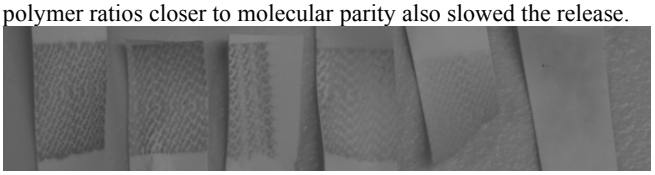

***Figure 6**. A gel formed of PDDA and PSS showing a slow release profile..The hatching is bromocreosol green dye diffusing over time. Images taken after immersion in phosphate buffer salt solution (pH 7.4) for 0.5,1, 2,4, 18 and 168 hours.*

Gels formed of chitosan dyed with crystal violet and alginate dyed with phenol red, showed repeatable, geometric diffusion patterns through printed three dimensional squares. The dyes were affected by position in the square, ratio of printed material and number of washing cycles.

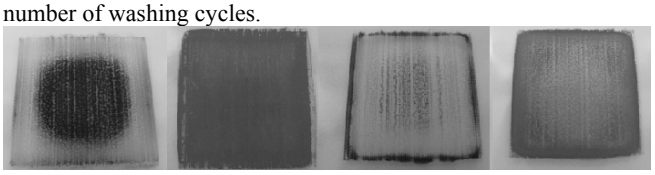

***Figure 7**. Images of a regular geometric dye diffusion through a printed gel of Chitosan and Alginate PDDS. Left to right. ratio Chitosan to Alginate 1:1 (Crystal Violet dye), ratio Chitosan to Alginate 1:1 (Phenol Red dye), ratio Chitosan to Alginate 1:3 (Crystal Violet dye), and ratio Chitosan to Alginate 1:3 (Phenol Red dye).*

Three dimensional bio-structures

The printer was readily able to produce structures that could be genuinely called three dimensional, not just films. The printer also over came limitations in having to print into pools or wells of solution.

Figure 8 showed a PSS/PDDA gel that has swelled beyond its original print size. Figure 9 shows a crosslinked chitosan/alginate, printed into a three dimensional lattice structure.

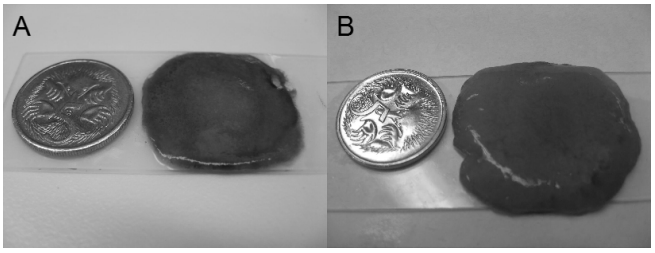

***Figure 8**. A PSS-PDDA gel that has reabsorbed water after being dried. The printed and dry gels were originally 17mm square. The gel after 1 day immersion in water (A), and after 1 week(B). (Coin diameter is 19mm).*

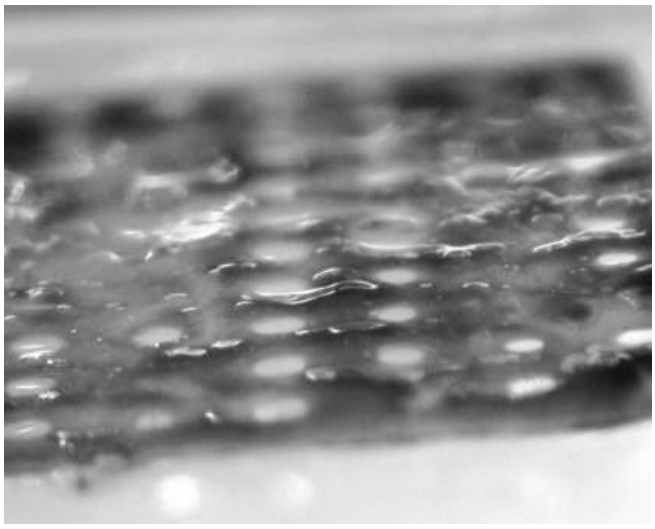

***Figure 9**. A Chitosan-Alginate gel lattice. The walls are about 600micron thick at 2.5mm spacing. Bulges in elevation go up to 1mm.*

Discussion

The development of printing hydrogels is likely to take much iteration and there is unlikely to be a fixed set of final materials. Inkjet systems are sensitive to even slight changes in ink formulation, and the understanding of the rheological properties is only just beginning to be understood [12]. The simple syringe supply systems together with easily flushed printheads help accommodate this sensitivity to the frequently required material changes.

The addition of surfactants and humectants was also qualitatively assessed to aid in print reliability (observed factors such as time and purge cycles for all nozzles to commence jetting, or the time all nozzles could remain jetting before dropping out.)

The synthetic materials (typically with shorter, more regular polymer chains) tended to jet more reliably, almost certainly due to simpler rheology in the peizo actuated firing chamber. In particular the naturally occurring polymer chitosan was found very difficult to jet reliably, even after the use of techniques such as sonication.

In many printing applications such as visual printer electronics, where a single wayward or missing inkjet inkjet nozzle can be noticed or lead to conductive tracks failing. However for many of the applications for hydrogels, the end product may be globular, swelling or flesh like. It may have cells growing on it and multiplying. In this case a number of faulty jets may be tolerated and have little or no effect on the final use of the hydrogel.

The issue of excess water removal can be addressed in a number of ways, with porous membrane substrates one demonstrated method. Increasing the solids content could also help this. This was as low as 0.5% for some of the natural polymers. The reaction kinetics of a multiplicity of drops is also an area for further consideration. In the reactive printing undertaken herein, the first layer of drops would typically join to form a uniform film on the substrate. The subsequent layer of oppositely charged polymer would contact the film as individual drops and partial or total cross-linking would occur. Thus heterogeneity was apparent in many of the films based around the drop spacing. This is evidenced in the hatch patterns of Figure 6 or the striped patters of Figure 7.

Conclusion

Is has been demonstrated that we can build a printer to produce hydrogels rapidly, up to 300 microns/min, with a fair degree of reliability and repeatability.

Experiments were undertaken that show practical use of such a printer to create devices for controlled drug delivery and as matrices for cell growth in a true three dimensional hydrogel structure.

Combined, these aspects of bio-printing contribute to goals such as tissue engineering where hydrogels are likely to find use.

References

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Author Biography

Don McCallum attained a Systems Engineering degree from Australian National University in 1996; Work on wood fibres, advanced ceramics and wear resistant metals for mining followed. From 1999 he investigated inkjet machinery for printing three dimensional Braille structures, this became the topic of his PhD (ARU, Cambridge).

2006-8 he worked as an applications engineer for the inkjet company Xaar. Don joined UoW in 2009 developing printing systems for biological, solar, and advanced electronic materials.