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Donald J. McCallum

University of Wollongong, donmc@uow.edu.au

Cameron J. Ferris

University of Wollongong, cjf146@uowmail.edu.au

Paul D. Calvert

University of Massachusetts - Dartmouth, pcalvert@uow.edu.au

Gordon G. Wallace

University of Wollongong, gwallace@uow.edu.au

Marc in het Panhuis

University of Wollongong, panhuis@uow.edu.au

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Printed Hydrogel Materials

Don McCallum¹, Cameron Ferris¹, Paul Calvert², Gordon Wallace¹ and Marc in het Panhuis¹

¹ARC Centre of Excellence for Electromaterials Science, Intelligent Polymer Research Institute,
School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia.

²Department of Materials and Textiles, University of Massachusetts Dartmouth, Dartmouth, MA 02747, USA.

Abstract — An inkjet printer has been developed for printing hydrogel materials. The printer uses two piezo industrial printheads to print out combinations of cationic and anionic polymers, which then form gels on a substrate in a clear demonstration of reactive printing. The synthetic polymers polydiallyldimethyl ammonium chloride and polystyrene sulfonate, and the naturally occurring polymers, alginate, chitosan and gellan gum all formed gels via inkjet deposition.

Keywords—inkjet; printing; hydrogel; bionics; polyelectrolyte complexation.

I. INTRODUCTION

Digital fabrication of biological materials into useful structures is an emerging technology. Opportunities exist for digital deposition methods to assist in regenerative medicine, genetics, molecular, and other biological sciences. Biological systems exist on the cellular scale hence artificially fabricated devices require sub micron or nanometer dimension assembly.

Inkjet printing has evolved from its origins in graphical print to become a general method of materials deposition. Inkjet is now widely used in electronics, rapid prototyping, micro-dispensing, thin film production and protective coatings. Inkjet is essentially the delivery of low viscosity fluids in drop sizes of 1 to 100 picolitres (pL), making it inherently suited to the soft, wet processing required for bio-polymers. The reactive printing described herein, whereby two or more reactive materials are printed as fluids to form gel structures for bionics, is an innovative stream of work.

II. BACKGROUND

In recent years inkjet printing has been demonstrated as a method for cell deposition [1, 2], printing skin-like films [3], extra-cellular matrices or bio-scaffolds [4], and in many other biology related tasks. A culmination of these techniques is their potential use in tissue engineering, regenerative medicine, prosthetics, implants, and for procedures as ambitious as spinal cord repair or sight restoration [3, 5, 6]. Furthermore, there are commercially available inkjet machines for biological tasks. These are exemplified by those jetting organic micro arrays (<http://www.arrayjet.co.uk>) and rapid prototyping of complex three-dimensional structures that aid spatial understanding of biological forms [4]. These facts indicate inkjet printing has a role to play in the formation of advanced biomaterials.

A central requirement of bioengineering is the need to print a scaffold. The extra-cellular matrix supports cell life and provides the structural framework of the organ in multicellular animals. A number of synthetic and naturally derived biopolymers have been shown to form hydrogels suitable for artificial scaffolds due to their biocompatibility and similarity to the natural extra-cellular matrix. The polysaccharides chitosan and gellan gum have been identified as suitable scaffold materials [8, 9]. Aqueous solutions of these materials can be converted to gel or solid phase by ionic processes. Gellan gum has been shown to form hydrogels by cross-linking with CaCl_2 solution [10, 11]. When one solution is drop cast onto another, or when the liquids are layered, gellan gum (anionic polyelectrolyte) and chitosan (cationic polyelectrolyte) undergo complexation at their interface, forming a film. They can also be drawn into fibres by spinning methods [12, 13]. This is of particular interest to inkjet engineering as material delivery and assembly can be undertaken in the liquid phase and the final structure is a solid or gel phase.

Natural polymers are long chains [14], which gives rise to high solution viscosities with non-Newtonian characteristics. Synthetic polymers are also known to be useful as bio-scaffolds [15]. Some synthetic polymers have small and consistently sized molecular weights. This infers a simpler rheology, likely to be better suited to inkjet printing. Polydiallyldimethyl ammonium chloride (cationic) and polystyrene sulfonate (anionic) are two such polymers. Other polymers of interest and candidate materials for gel formation include polyethyleneimines, polylysine (cationic), poly(acrylic acid) and polyvinyl sulfonic acid (anionic). Complexes of many pairs of these polymers in very thin layers have been formed by the layer-by-layer self assembly process [16]. The inkjet printing approach allows us to make much thicker layers for bulk studies and to vary composition in a systematic fashion.

There are a large and complex number of material and operational parameters to be optimised when jetting a fluid using inkjet printing [17]. The two predominant methods of drop-on-demand inkjet printing are piezo inkjet printing (PIJ), where ink is jetted from a nozzle via deflection of piezo – electric materials, and thermal (TIJ), whereby ink is thrust out via a pressure bubble which forms in the fluid when a nearby resistor is charged. While PIJ has a perceived superiority as it is able to jet a wide range of materials on account of its mechanical action [18], it is noted that a large number of bio-materials are based around water or other solvents, and are thus inherently suited to deposition by TIJ. TIJ involves a localised heating of the inks and this may be a concern with some sensitive biopolymers. The fluid physics inside a nozzle

chamber is not well understood, therefore the exact effect on cells is also unknown, other than it has been numerously reported that cells have survived and reproduced after jetting via both methods [1,2]. In this work a purpose-built two-head piezoelectric printer is used to fabricate hydrogels from combinations of oppositely charged polymers.

III. EXPERIMENTAL

A. Apparatus

A bespoke printer was specially built for reactive printing hydrogels (Fig. 1). The printer consisted of two PIJ engineered Xaar 126 printheads [19]. This is an industrial printhead with 126 nozzles, each jetting approximately 80 pL drops at up to 5200 Hz. The printheads have a print width of 17.2mm, with nozzles spaced 137.1µm apart. Proprietary software and drive electronics connected to the printheads are driven by an ordinary PC [20]. The drive electronics could control 16 printheads all jetting a different fluid. In the work herein, only 2 printheads were used.

A servo actuated linear slide with 500mm travel was used to drive a substrate table back and forth, underneath the printheads. The slide operates up to 0.5ms^{-1} , meaning that each layer, 17 mm wide, only takes a few seconds to deposit. The linear slide was fitted with an optical encoder. The encoder delivers to the drive electronics a RS422 differential signal to indicate the position to start printing, and a further stream of signals every 1µm, indicating the position for each row of drops to be jetted. The substrate holder could support substrates up to 297 x 210mm in area, and manual adjustments could allow for variations in substrate thickness. The substrate holder had a pattern of grooves and clips to aid in positioning and holding the substrates. The optimal throw distance (distance between the printhead's nozzle plate and the substrate) is 1mm, with 3mm yielding acceptable print quality. The servo was programmed in a simple machine language to drive the linear slide and substrate table back and forth as required for layer by layer manufacturing.

Figure 1. The reactive printer. The substrate table supporting 6 microscope slides moves back and forth underneath the printheads.

Fluids (inks) were supplied to the printhead via a tube of 3mm internal diameter and 300mm length, which is connected to a 50ml syringe open to atmosphere (reservoir). Important for

good printing is the meniscus level of the ink at the nozzle orifice. The negative pressure at the nozzle plate is typically between -1 to -3.5 kPa. A negative pressure above -1kPa and inks seep out the nozzles covering the nozzle plate and inhibiting jetting. Below -3.5 kPa and ink is drawn back to the reservoir, starving the nozzle firing chambers of ink. Varying the height of the reservoir alters the negative pressure, compensating for variations in ink properties to achieve a good meniscus level.

B. Materials

Gellan gum (CP Kelco) was dissolved at 0.25% w/v in Milli-Q H₂O, alginate 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 added to water/acid at 50 °C, stirred at 500rpm for 2 hours. The synthetic polymer, polydiallyldimethyl ammonium chloride (PDDA), was received as a liquid at 35% w/v (Sigma, "very low molecular weight" 150,000) and was diluted with Milli-Q to 20% w/v. Polystyrene sulfonate (PSS, polystyrene sulfonate co-maleic acid, sodium salt Sigma, molecular weight 20,000) was prepared to 30% w/v. To all materials 1-2% w/v Triton X-405 (polyethylene glycol *p*-(1,1,3,3-tetramethylbutyl)-phenyl ether, Sigma) was added as a surfactant, and 1-2% w/v ethylene glycol (EG, Sigma) was added as a humectant. To allow easy monitoring of the deposition, the solutions were colored by adding dyes. PSS was colored with <1% polypyrrole (doped 5% wt. solution in water, Sigma) suspension, alginate with crystal violet, chitosan with bromocresol green (Sigma), and PDDA and gellan gum with food dyes. These compositions gave apparent viscosities in the range between 7-24 cPs, the recommended range for good jetting. A minimum of 5ml of each fluid is required to prime the heads. Inks were sonicated for 30 mins and filtered through a 3.2 µm glass mesh syringe filter immediately before printing. It was qualitatively observed that by adding the Triton and EG, jetting was more reliable, and quicker to achieve all nozzles firing and with less occurrence of nozzle drop out or misfire. The anionic polymer inks were supplied to one head and the cationic to the other. Processing was undertaken at 23 °C.

Substrates were typically a 75 x 25mm glass microscope slide, though polyvinylidene fluoride (PVDF) membrane, plain copier paper, and photopaper, were also tested. Typically a square of area 17 x 17mm was printed on each microscope slide, this size corresponding to the width of the printhead. In order to print the 17mm x 17mm squares, monochrome bitmaps were prepared in MS Paint at 126 x 126 pixels per bitmap. Bitmaps were opened with the drive electronics' software. Each pixel corresponds to a drop being ejected (black being *on* and white *off*). Manipulation of the bitmaps is a simple way of producing patterns, pictures and text or of controlling the ratios of each material deposited.

Samples were made with variations to the printed ratios of cationic to anionic ink, number of layers, and printed patterns. Characterisation methods included profilometry, weighing, optical microscopy, and scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS).

IV. RESULTS

17 x 17mm squares of PDDA were printed onto squares of PSS with 10mm overlap in a single print layer to leave a 7mm zone of pure PSS or pure PDDA on either side. This was repeated for 2, 4, 8, 16, 32, and 64 layers. The PSS: PDDA ink ratios as determined by the pixel density of the bitmap files were 50S:50D, 66S:33D, 33S:66D, 75S:25D, 25S:75D, 100S:100D (S denoting PSS and D, PDDA). The PDDA and PSS concentrations are 5.83% w/v and 23% w/v respectively. In all a gel formed where the two materials overlapped (see Fig. 2) whereas the areas of either polymer alone remained liquid and were subsequently washed away. This is a clear demonstration of the use of an inkjet printer to print two reagents in separate printheads to form a product.

Figure 2. (A) shows freshly printed 17mm square of PSS (blackish) and an offset 17mm square of PDDA (reddish) with overlap in the middle. (B) shows that after mild washing only the gel from the overlapped area remains

A profilometer was used to record heights of wet, freshly printed gels, and desiccated gels. The profilometer stylus was set to the lowest force settings in order to avoid indenting the gel surface. The elevation (thickness of gel above the substrate) and volume of the wet gels fluctuated greatly with variations in the ambient humidity. Desiccated samples gave reliable, repeatable elevation data. There was a linear increase in the dried gel elevation with increased number of printed layers with a thickness increase of about 1.7 microns for each cycle of printing the two polymers through all nozzles (Fig. 3). The samples with higher PSS to PDDA ratio showed higher elevation, and greater volume. The high PDDA samples give thinner layers, as would be expected from the lower concentration in solution and the similar densities of the polymers.

Gel elevation by polymer ratios and print layers

Figure 3. Plot of gel elevation by polymer ratio as a function of number of printed layers.

The 32 layer set of gels was then put through a series of wash cycles. The gel was annealed on a hot plate at 90°C for 15 minutes, soaked in water for 1 hour, then desiccated by drying in vacuum with silica. Fig. 4 shows that the gels are not losing significant amounts of material after the initial wash. The initial loss of thickness after washing, reflects the loss of salt (sodium chloride) that is released as the two polymers complex and loss of any excess PSS that does not become part of the gelled complex. Qualitative observations of the brightness and contrast of the dyes in the gels indicated that the soluble dyes were being lost with subsequent washing. The nanoparticulate polypyrrole grey coloring is not lost.

Gel elevation as function of wash cycles and polymer ratios

Figure 4. Plot of gel elevation after washing. There was an initial loss of material after the first wash, but with subsequent washing the gel degradation lessened.

In order to demonstrate the printer's capability to create larger hydrogel objects, not just thin films, a print of 256 layers was undertaken, with 5 minutes delay after every 64 layers, to allow evaporation of some excess water. Fig. 5 shows that after 1 week the gel has swelled to approximately 21 x 21 x 2mm, expanding out from the 17 x 17mm area that was originally printed.

Figure 5. A PDDA/PSS gel of 256 layers. The gel has continued to take up water. (A) 2 days after printing and (B) taken 1 week after printing. Scale is Australian 5 cent coin.

The bitmaps were altered to take into account the concentrations of the polymers to achieve a closer molar parity. Actual weight ratios of PSS:PDDA solids jetted were then 1:0.75, 1:1, 1:1.25, and 1:1.5. Based on the formulae of the polymer units, the weight ratio corresponds closely to the ratio of cationic and anionic units. 16 layer samples were prepared on PVDF substrate, annealed, washed and dried. EDS provided an elemental composition of the gels and the ratios of expected elements correlated with input ratios (e.g. percentage sulfur

content determined by the EDS was 4.17, 3.62, 3.19 and 1.44 with the decreasing PSS concentrations). The EDS confirmed that very low levels of sodium and chloride remained in the gels after washing.

V. DISCUSSION

The concept of reactive printing through poly-electrolyte complexation of ionic polymer fluids to form hydrogels has been clearly demonstrated. The use of readily available industrial inkjet printheads allows for an efficient trade off in micron scale details, but efficient build up to millimeter and centimeter scale devices.

The EDS analysis shows that the gels remaining after washing contain little sodium or chloride. The retention of thickness, shown in Fig. 4, peaks at a print ratio of 33S:66D which corresponds to a molar ratio of about 30% PDDA units. The reduced retention fractions for gels with very low PDDA contents does suggest that excess PSS is washed out.

The substrate table held 6 microscope slides, thus samples were typically produced in batches of 6. In the normal machine configuration the maximum sample size would be 17 x 297mm. By placing heads side by side or adding extra axes of motion the printed area could be increased to 297 x 210mm.

Gellan gum/PDDA and alginate/chitosan gels have also been formed by the reactive printer, Fig. 6. These demonstrate the versatility of the printer to be used for a range of polymers with different dyes. This is a possible precursor for showing drug and protein release through the hydrogels

Figure 6. (A) A gel formed from gellan gum and PDDA. (B) A lattice shape made from chitosan and alginate. 17 x 17mm squares.

In summary, reliable jetting of a number of bio-materials and their formation into hydrogel structures suitable for bio-scaffolds has been demonstrated. The concept of reactive printing by means of supplying two oppositely charged ionic materials to two separate printheads, jetting them sequentially and reacting on a substrate has been shown. The scaffolds have been shown as robust to washing cycles, and have been built up to volumes of magnitude relevant for tissue engineering.

This paper contributes towards the development of printing approaches for controlled fabrication of hydrogels.

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