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Inkjet printing for high-throughput cell patterning

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Abstract

The adaptation of inkjet printing technology to the complex fields of tissue engineering and biomaterial development presents the potential to increase progress in these emerging technologies through the implementation of this high-throughput capability via automated processes to enable precise control and repeatability. In this paper, a method of applying high-throughput inkjet printing to control cellular attachment and proliferation by precise, automated deposition of collagen is presented. The results indicate that commercial inkjet printing technology can be used to create viable cellular patterns with a resolution of 350 μm through the deposition of biologically active proteins. This method demonstrates a combination of off-the-shelf inkjet printing and biomaterials and has potential to be adapted to tissue engineering and colony patterning applications. Adapting this method into the three-dimensional construction of cellular structures for eventual high-throughput tissue engineering using a bottom-up approach is possible.

Keywords: Tissue engineering; Micro patterning; Collagen; Hydrogel; Inkjet printing; Surface modification

1. Introduction

This work describes the application of commercial inkjet printing technology for the creation of viable cell patterns in a manner that is inexpensive, repeatable, flexible, and easy to use. Previously developed methods of constructing cellular patterns include soft lithography [1–3], laser-directed cell-writing [4], photolithographic techniques [5,6], and dip-pen nanolithography [7–9].

Soft lithographic techniques include microcontact printing and microfluidic channel flow patterning [3]. Of these, microcontact printing is more widely used. Microcontact printing has been used to create patterns of organic molecules such as alkanethiols, siloxanes, or proteins on solid surfaces using an elastomeric stamp primarily made from polydimethylsiloxane (PDMS) [1]. Due to the elastomeric nature of the PDMS, the stamp can be used to pattern nonplanar substrates. The resulting pattern is then used to control adsorption of biomolecules, such as proteins, to the surface, which subsequently interact with the cells to promote site-specific anchorage to the surface. The primary benefit of this method is the high-resolution capability to create features on the scale of 2–500 μm [2], allowing for the production of patterns on the scale of a single cell or cell process.

Laser-directed cell writing places single living cells onto a glass substrate in patterns by physically guiding the cells down hollow fibers with optical forces from a focused infrared diode laser [4]. It is an example of direct cell placement and requires no surface modification. This method, however precise, has limited potential application into product development or tissue construction due to its time requirements, limiting throughput, and its inability to control cell migration. However, it may prove to be exceptionally useful to study individual cell attachment and migration phenomena as well as cell–cell interactions and the behavior of cells in developing tissue.

Photolithographic protein patterning requires the linking of photosensitive groups on a substrate to proteins. Patterns can be made through selective activation of these groups by UV irradiation through a mask designed with the desired pattern. It has proven to be a useful technique to create micropatterned cultures [6]. In addition to protein patterning, photolithographic techniques have been used to pattern self-assembled monolayers (SAMs) on a surface to control

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cell adhesion for the creation of very-high-resolution cell patterns [10]. Kleinfeld et al. [11] pioneered the method of using lithographic masking with photochemical resist material to create patterns of silanes on N^1 ([3-(trimethoxysilyl)propyl]diethylenetriamine (DETA), an amine containing surface, to control neuron attachment and neurite outgrowth. Photolithographic patterning of SAMs has also been achieved using masked laser ablation techniques on DETA [12] and poly-D-lysine [13] for neuronal patterning.

Dip-pen nanolithography relies upon AFM techniques to create nanopatterns of proteins with a minimum feature dimension of 30 nm [7,9]. This method has been used to create high-resolution protein arrays to control cell attachment [8].

In contrast to these patterning methods, a procedure that applies inkjet printing of proteins to modulate cell attachment has been developed. Inkjet printing and computer-aided design (CAD) techniques have already made an impact in the field of biomaterials. Inkjet printing applications in biotechnology have been utilized previously including for biosensor development [14], biochips (i.e., immobilization of *E. coli* arrays) [15], DNA arrays [16,17], DNA synthesis [18], microdeposition of active proteins on cellulose [19], and free-form fabrication techniques to create acellular polymeric scaffolds [20,21].

For tissue engineering applications, inkjet printing processes have been pursued for the development of solid free-form fabrication (SFF) processes to construct three-dimensional tissue scaffolds. This method uses CAD images to create scaffolds one layer at a time, which allows for the integration of microbiological features, such as pores and channels, into the synthetic polymeric scaffolds. It takes advantage of the flexibility of inkjet printing to manipulate the designs for customized applications to create complex anatomical shapes. In conventional systems, a chemical binder is deposited with a drop-on-demand mechanism into a powder bed of polymeric material. Some drawbacks of this method include the need for specialized equipment and the requirement of harsh organic chemicals, a potential source of residual toxicity. Additionally, this process is not suitable for the printing of biological solutions such as polymer-encapsulated cells or cell suspensions. Park and coworkers [21] demonstrated the extension of SFF to the construction and selective modification of biodegradable polyester scaffolds to direct three-dimensional tissue development.

In this paper, we investigated the possibility of printing biological materials for cellular scaffolds using the conventional drop-on-demand mechanism demonstrated by inkjet technology. We demonstrate the feasibility of this concept by printing protein solutions via automated inkjet printing and their application to control cellular locations on a solid substrate.

2. Materials and methods

2.1. Substrate preparation and analysis

Glass microscope cover slips (Fisher Scientific, Pittsburgh, PA) were used as an optically transparent substrate material for this procedure. The slips, ranging in size from $22 \times 22\,mm^2$ to $24 \times 50\,mm^2$, were first cleaned in highly acidic piranha solution (1:3 hydrogen peroxide:sulfuric acid) over low heat for approximately 20 min. This resulted in a very clean hydrophilic substrate, which was very amenable to cell attachment and proliferation. To prevent the unhindered attachment of cells across the substrate, the cover slips were dip coated with an autoclaved 0.15% solution of type II agarose (Sigma Chemical Inc, St. Louis, MO) and dried prior to the automated patterning process. The coated substrates were then UV sterilized for at least 2 h in a laminar flow hood. The coated substrates can be stored for weeks prior to further usage.

A variable angle spectroscopic ellipsometer (Sopra, Inc., Westford, MA) was used to determine the thickness and uniformity of the agarose coating on the substrates. Reflective silica substrates were used for the ellipsometric characterization of the dried agarose coating as a model for the transparent glass surface. The silica substrates were cleaned, dried, and dip coated with agarose, similar to the glass substrates. Data were collected across wavelengths from 630 to 634 nm for five samples immediately before and after coating. Additional measurements were collected again after rinsing the substrates three times in triply distilled water to determine the resilience of the coating. In-house developed multilayer data reduction software was used to analyze the ellipsometry data [22].

A contact angle study was performed to compare the contact angle of water on clean glass, glass coated with agarose, glass coated with collagen, and glass coated with agarose and then collagen. Five $24 \times 50\,mm^2$ glass microscope cover slips were cleaned. Two-thirds of the slips were dip coated in autoclaved 0.15% agarose and dried. The bottom half of the coated area was subsequently coated with a 1.0 mg/ml collagen solution and dried. Each slip was then subjected to three water contact angle tests, one test in each third of the area, using a CAM 200 optical contact angle meter (KSV instruments, city, state). Additionally, five separate pieces of clean cover slips coated with only the collagen solution and also tested.

2.2. Print solution preparation and analysis

Collagen is the most common protein in the body and is readily recognized by cell receptors for attachment. Thus, by controllably coating a cell repellent surface with collagen, the attachment patterns of the cells can be

controlled. Two sources of type I collagen were used in the inkjet printing procedures including rat-tail (BD Biosceinces, Billerica, MA) and calf-skin collagen (Elastin Products Inc., Owensville, MO). The former was received at a concentration of 3.25 mg/ml in acid and diluted as necessary. The latter, a lyophilized, acid soluble, salt-free collagen was dissolved in dilute 0.0174 M acetic acid to create a stock solution with a concentration of 5 mg/ml. Working solutions were made by diluting the stock solution with sterile filtered water or Dulbecco's phosphate buffered saline (PBS) (Sigma Chemical Inc., St. Louis, MO), as necessary to a final concentration of 1.0 mg/ml. The gelation of the solutions of soluble collagen is dependent upon solution pH and temperature. Solutions were maintained at 4°C, above which the gelling occurs, until printing.

A rheometric study of the collagen solutions used for printing was conducted to compare the dynamic viscosities of the solutions to that of typical ink solutions and to determine how much the viscosity varied with the solution pH. From a stock solution of rat-tail collagen, with a concentration of 3.25 mg/ml, five solutions were made through dilution with a mixture of water and PBS, each with a resulting concentration of 1.0 mg/ml. The solutions tested were: (1) diluted with pure water (2) diluted with a 2:1 mix of water:PBS (3) diluted with 1:1 mix of water:PBS (4) diluted with a 1:2 mix of water:PBS (5) diluted with PBS only. The solutions were maintained at 4°C prior to testing. The viscosities were measured at room temperature (21°C) with varying shear rates from 0.1 to 1000 s^{-1} using an MCR 300 cone-on-plate rheometer (Paar Phyica, Ashland, VA).

2.3. Substrate patterning

Patterning of the substrates was achieved using the automated inkjet printing process. The patterns were designed using PowerPoint software (Microsoft, Redmond, WA) and printed using a modified Canon Bubble Jet (BJC-2100, Canon Inc. Tokyo, Japan). The printer modifications are described previously [23]. The cartridge was rinsed thoroughly with ethanol and sterile water prior to protein solution introduction. Slightly acidic collagen solutions were essentially the ink that was printed onto the substrates to form the cellular patterns which included lines, circles, dot arrays, and gradients. After printing, the patterns were dried in aseptic conditions and rehydrated prior to cell seeding.

2.4. Cell culture

A cell line of smooth muscle cells (SMC) (CRL-1476, American Type Culture Collection, Manassas, VA) derived from rat aorta, were plated on the collagen patterns. The cells were cultured in standard conditions (37°C, 5% CO_2) in Dulbecco's Modified Eagles Media comprised of 10% Fetal Bovine Serum and 1% antibiotic antimycotic, all obtained from Sigma Chemicals (St. Louis, MO). Each patterned cover slip $22 \times 22\,mm^2$ was placed in a 36 mm diameter well of a 6-well plate and seeded with 50,000 cells in 2 ml of media. Patterns were also made on $24 \times 50\,mm^2$ cover slips. These substrates were loaded into well plates and seeded with 130,000 cells in 3 ml of media. The media was changed every 1–2 days and the growth of the cells was monitored daily via light microscopy. In the event of visual interference due to cells attached to well surface under the cover slip, the cover slip was transferred into a clean, sterile well for microscopic inspection and additional culture.

Neuronal culture, using dorsal root ganglia (DRG) neurons, was also performed on the agarose and collagen modified substrates. The DRG neurons were isolated from day 14 rat embryonic tissue. The tissue was treated with trypsin-EDTA (0.25%) for 15 min in a 37°C water bath. The trypsin was removed, 100 μl BSA, 100 μl DNase from a 1 mg/ml stock solution and 2800 μl of L15 media (Gibco, Invitrogen, San Diego, CA) were added. Finally, the tissue was triturated manually, and the solution was spun down at 300*g* for 10 min on a BSA cushion. The resulting pellet was suspended at a density of 150,000 cells/ml and 400 μl of this solution was plated 50 min before media introduction. The DRG media consisted of 500 ml Neurobasal Media (Gibco), 10 ml Gibco B-27 Supplement 50×, 1.25 ml Glutamine (0.5 mM), 500 μl of 10 ng/ml Recombinant Human Cardiotrophin-1 (Cell Science), 75 μl of 1 ng/ml Brain Derived Neutrophic Factor (Invitrogen), and 75 μl Glial Derived Neutrophic Factor. The growth of the neurons was monitored daily via optical microscopy using phase contrast.

3. Results

3.1. Characterization of agarose-coated substrates

Ellipsometric measurements recorded at 632.80 nm ($n = 5$) indicated that the agarose film on the surface of the substrates was 583 ± 56 Å. After rinsing, the thickness of the coating measured 463 ± 57 Å. The results indicated that the agarose film was uniform and repeatable. Furthermore, the film remained stable and only a very slight decrease in thickness was observed after the surface is rinsed several times with water.

Water contact angle measurements of the substrates during stages of preparation were gathered. The results indicated that the agarose coating did not effectively change the hydrophilic character of the clean cover slip. The measured contact angles on the clean and agarose-coated substrates were $11.0 \pm 2.3°$ and

$8.8 \pm 3.7°$, respectively. In contrast, the presence of the collagen coating did significantly change this property, regardless of agarose presence. The collagen coating on the clean glass resulted in a contact angle of $51.0 \pm 1.3°$ while the agarose then collagen-coated cover slip displayed a similar contact angle of 49.0 ± 6.9.

3.1.1. Printing solution analysis

The resulting viscosity measurements of the five collagen solutions are shown, as compared to a reference value of commercial ink viscosity, in Fig. 1. Since the shear rate experienced by the collagen solutions at the nozzle is unknown, the measured viscosities of each solution were compared at a low shear rate of $11.3\,s^{-1}$. The fluids proved to be shear thinning, since the dynamic viscosity decreased as shear rate increased, so this low shear rate represented a conservative point of comparison. The measured viscosities of the collagen solutions with pH values up to 5.0 were statistically similar and averaged to 8.2 ± 0.3 cP, somewhat greater than the typical ink viscosity of 2 cP. At a pH of 5.5, however, the viscosity increased dramatically due to collagen fibril aggregation. Furthermore, it was observed that the collagen solutions at this pH became cloudy as partial solution gelation began. These observations correlated with previous printing experience, during which there was a high incidence of nozzle clogging when printing solutions of this pH. However, though the viscosity of the lower-pH solutions was more than 4 times that of the reference value for commercial ink, very infrequent clogging of the nozzle occurred when printing these solutions.

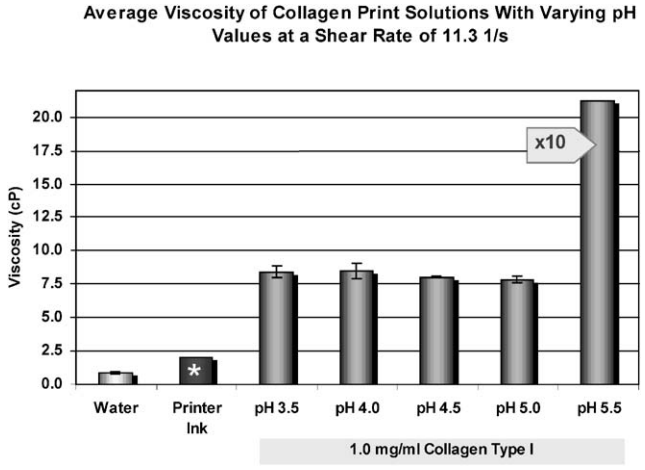

Fig. 1. Measured viscosity of 1.0 mg/ml type I collagen solutions at varying pHs compared at a low shear rate value to viscosities of commercial ink and pure water. (*) According to Calvert [28].

3.2. Protein pattern analysis

Soluble collagen solutions were printed onto the substrates to form the cellular patterns, which included lines, circles, dot arrays, and gradients. These designs, created using Microsoft PowerPoint, are shown in Fig. 2. The patterned substrates were analyzed with light microscopy after collagen deposition and again at daily intervals during cell culture using a Diaphot 300 (Nikon Inc., Melville, NY) inverted microscope. Fig. 3 shows ring patterns of printed 1.0 mg/ml collagen solutions prior to rehydration and cell seeding. The patterns seen in this figure showed visible indications of solution deposition and were used to verify that the patterns were intact after dehydration. These crystallized patterns are due to residual salt in the solvent. The substrates were subsequently rehydrated and seeded with cells.

3.3. Cell pattern analysis

After cell seeding on the protein patterns, the cultures were monitored daily via light microscopy. Prior to the first media exchange, it was common to have many rounded cells floating in the media due to the lack of locations to adhere, but these were easily rinsed away. The cells that settled on the collagen adhered and proliferated until confluent patterns were established. Figs. 4–8 indicate typical growth patterns of the cells to the deposited collagen patterns. A line pattern is shown in Fig. 4. The SMCs, seeded at a density of 30,000 cells per well, attached first to the boundary of the collagen pattern. Then, the cells began to fill in the pattern as they proliferated under static culture conditions until they reached confluency after 5 days. However, a confluent cellular pattern was achieved within 4 h by increasing the initial seeding density to 75,000 cells/well, as demonstrated in Fig. 4c. Patterns created using higher seeding densities had a shorter overall lifetime. In contrast, patterns seeded with lower densities took longer to reach confluency and, consequently, remain intact longer. Fig. 5 shows a ring pattern of cells. The

Fig. 2. Patterns designed using Microsoft PowerPoint software.

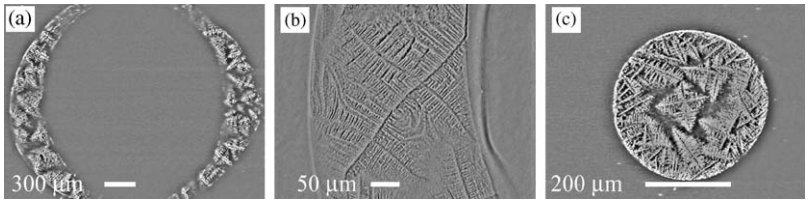

Fig. 3. Light microscopy images of printed collagen patterns, including a ring and a circle, prior to cell seeding at (a) 40×, (b) 100×, and (c) a circular pattern at 40× magnifications.

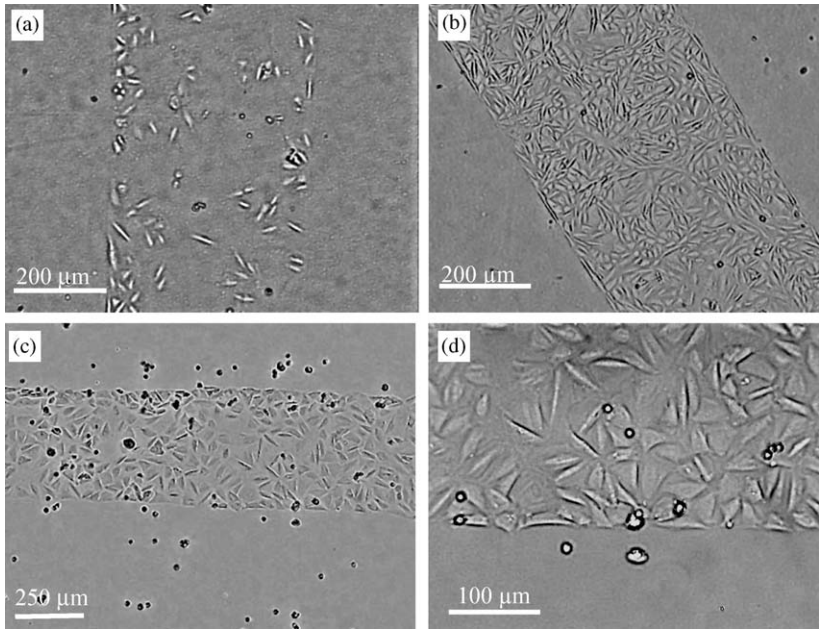

Fig. 4. 40× magnification light microscope images of a printed collagen line pattern seeded with SMCs after culture times of (a) 1 day, and (b) 4 days and (c) a 500 μm wide line 4 h after high-density seeding, but prior media rinse to remove unattached cells. The morphology of the patterned SMCs is demonstrated by (d) which is a 200× image of the line shown in (c).

tendency of the SMCs to align radially with the pattern along the boundary was observed and can be seen in Fig. 5a. Fig. 5b shows the overall shape of a printed ring. Cells were seen beyond the boundary of the printed collagen; however, the rounded morphology of these cells indicated they were nonviable, thus, it can be clearly seen that the 0.15% agarose coating worked well to hinder viable cell attachment and prevent cellular migration.

Fig. 6 indicates a cell colony array created by printing 8 pt font size periods in a 10×4 array. The figure shows four member colonies of this array, each with a diameter of approximately 350 μm. This array is typical of the highest resolution that has been achieved thus far with this method.

Cell gradient patterns were produced by printing collagen in a thin rectangle that fades in color continuously from pure black to pure white. The gradient was produced by a decrease in spatial density of ink droplets and was not dependent on changes of collagen concentration. On the low-density end of the gradient, the cell pattern greatly resembled the cell colony array constructed through the printing of individual dots. In contrast, the pattern at the high-density end of the pattern resembles the printed line patterns. Fig. 7 indicates the results after 4 h of seeding.

Finally, Fig. 8 shows patterned neuron cultures achieved by controlling cell attachment using inkjet printing of collagen. Fig. 8a shows a line pattern of DRG and glial cells. Fig. 8b shows an enlarged area of the patterned cells showing the alignment of the cells including their processes with the pattern. Healthy morphologically normal cultures have been maintained for at least 16 days. Long-term cultures are ongoing. These results indicate that this method can be used to pattern multiple cell types including neurons.

4. Discussion

These results represent a successful effort to develop viable cell patterns utilizing inkjet deposition of collagen onto a cell repellent surface. Typical attachment phenomena observed included the preference of the

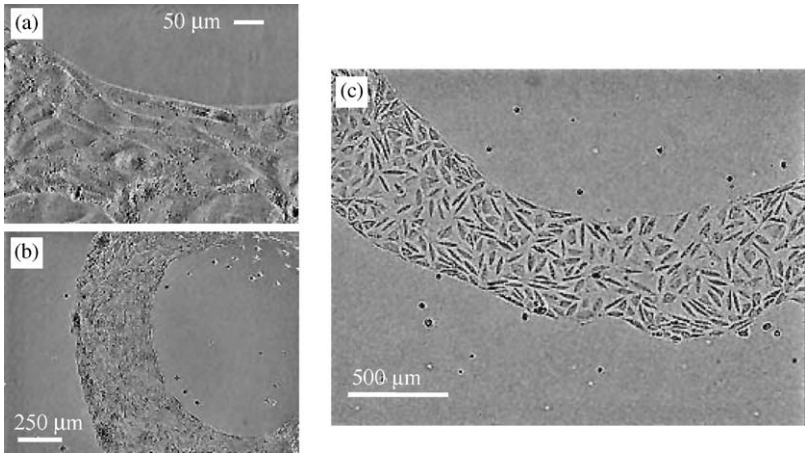

Fig. 5. Light microscopy images of a ring pattern after seeding with SMCs at a density of 30,000 cells 4 days into culture (a) 200× image, (b) 40× image, (c) printed ring pattern after 4 h of high-density seeding (75,000 cells) with SMCs.

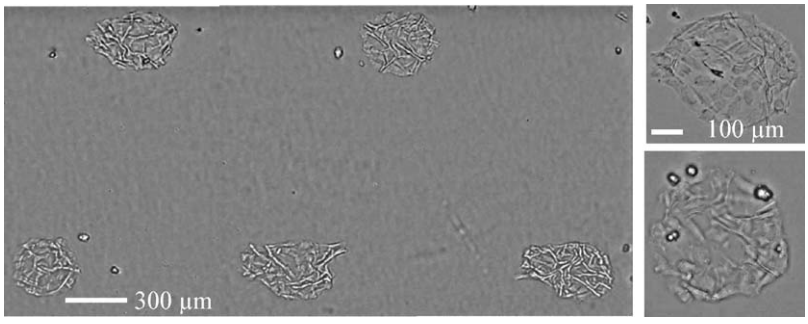

Fig. 6. Four members of an SMC colony array on the 4th day of culture shown at 40× magnification. The colonies each have a diameter of approximately 350 μm.

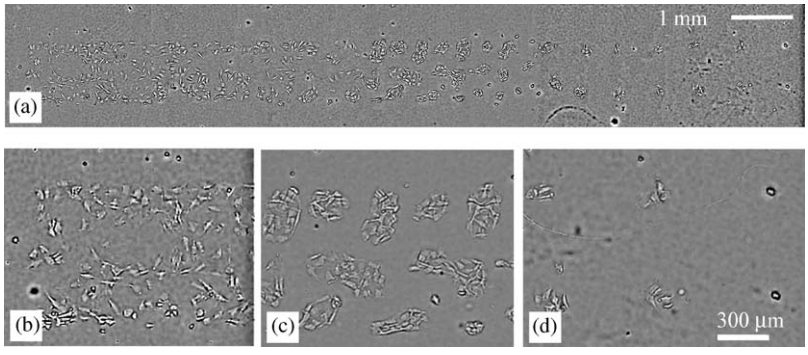

Fig. 7. SMC gradient pattern after 2 days of standard culture. (a) The overall gradient shown is a mosaic of small pictures. (b), (c), and (d) are enlarged views of the head, middle and tail of the gradient, respectively.

cells to attach to the boundary of the collagen pattern followed by other cells filling in the bulk of the patterns. This was probably due to higher surface energy at the interface sites. Presumably, the patterns seeded with the higher density also followed this preferential attachment. These patterns achieved confluency much faster than patterns seeded at a lower density, but in exchange for this benefit, a decrease in life span of the pattern was observed. This was probably due to cellular contraction causing disassociation of the collagen coating with the substrate. Independent of seeding density, the cells at the interface displayed tendencies to align with the pattern boundary. This phenomenon is demonstrated by the cell patterns shown in Figs. 4c and 5a.

The inkjet printing of proteins shows excellent potential in creating features in the size range 350 μm

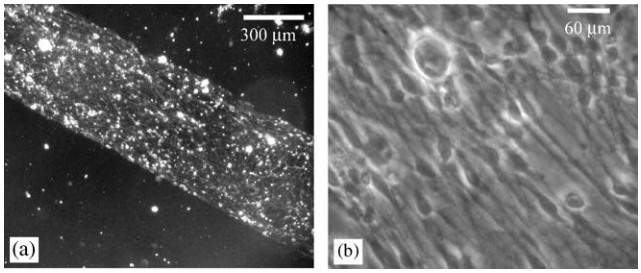

Fig. 8. Primary neuron growth was directed by inkjet printed collagen patterning shown with respect to the designed patterns. This figure shows DRG cells with larger round bodies and glial cells with smaller elongated cell bodies growing in a line (a) at 40× magnification obtained after 4 days of culture, and (b) at 400× magnification obtained after 7 days of culture. The cells are aligned predominantly with the pattern while the DRG cells exhibit their normal morphology with rounded cell bodies.

and above with the benefits of pattern flexibility, automation, and high-throughput capability. If a pattern change is required, a simple design modification can be done quickly on a software interface, without any additional materials. Additionally, since this process involves liquid deposition, it is possible to deposit hydrated particles such as fragile biomolecules or proteins. Thus, the potential for printing and maintaining viable biological solutions including cells, could be realized. In contrast, this feature is often not possible in microcontact printing processes during which chemicals are typically transferred in a completely dried state. Furthermore, inkjet printing of collagen has also proven to be a remarkably simple method to create viable cell gradient patterns. It is also an extraordinarily simple method to create gradients when compared to other chemical gradient production techniques such as scanning tunneling microscope for replacement lithography [24], photoimmobilization techniques [25], and cross diffusion of alkanethiols through a polysaccharide matrix [26]. Finally, since the protein solution is deposited without surface contact, as is the case in microcontact printing, surface contamination is more easily avoided [27].

Currently, microcontact printing is a more precise method for cell patterning than inkjet printing of proteins to control cell attachment, and is in common usage across many fields. In conventional inkjet printing applications, which deposit ink on paper, the resolution of the resulting pattern depends on a number of ink, printer, and substrate factors. These factors include ink viscosity, composition, and surface tension. Printer factors include nozzle size, droplet size, dot density, and lateral resolution of printer head, which determines the printed feature size. The corresponding specifications for the Canon BJC 2100 are approximately 20–30 μm, 10–20 pl, 360 Dpi, and 100 μm, respectively. However, since these printers were designed to function with ink and paper, the maximal resolution was probably compromised since the substrate characteristics, with respect to absorbency, porosity and hydrophilcity, differ from those of paper. Thus, an approximation of the resolution of this process is in the range of 300–400 μm. Recently, off-the-shelf printers with 1 μm resolution have become available suggesting a 10–20 μm resolution is possible for the cell patterns with the inkjet method. Moreover, resolution optimization may be less important since this technique can be expanded towards tissue engineering applications. For this application the goal would shift away from micron resolution towards three-dimensional bulk and integrity. Considering the dimensions of biologically relevant structures as well as cell motility and differentiation, the current resolution of the device may prove to be very applicable, particularly in the field of tissue engineering.

Solution deposition by the printer is driven by a thermal process which heats a small volume of "ink" to the temperature range of 200–300°C for a few microseconds to form a small bubble of vapor which forces a controlled volume of ink through an orifice [28]. The resulting pressure forces a small droplet through the nozzle onto the substrate. Concerns arise as to whether this elevated temperature will damage the function of the proteins. However, from the data presented here it is clear that the cell receptors can still identify the printed collagen, thus, it is logical to conclude that limited denaturization is occurring. This is furthermore strengthened by the fact that viable bacterial cells maintained viability when printed with this printer as detailed in a recent publication [15].

The collagen solutions at 1.0 mg/ml concentration showed very consistent results between subsequent printing processes. The patterning process has been repeated many times and no differences were observed between cultures patterned with rat-tail collagen and calf-skin-derived collagen. Additionally, it can be concluded from the rheometric study that a varying solution pH from 3.5 to 5.0 had no measurable effect on the viscosity of the solution, yet at pH = 5.5, a significant increase in viscosity was recorded. Although inkjet printers can be designed to handle liquids up to 100 cP [28], our experimental setup requires the collagen to be printed at a low pH due to this observation. Through the integration of a temperature control system and the implementation of a drive mechanism that does not heat the solution, solutions with neutral pH, which would be more amenable to cellular presence, could be printed.

Sterilization of this process can be achieved through ethylene oxide sterilization of the printer, proper cover slip cleaning and sterilization, the autoclaving of agarose solutions and manipulation equipment, filtration of collagen solutions, and finally, pattern construction and cell incubation under aseptic conditions. Furthermore, the technique can easily be amended to

high-throughput applications as over 100 samples could be produced on one printer in a single hour using the same or many different patterns. Although a cartridge can be reused over a number of experiments (>100), permanent blockage can occur, making it the limiting piece of equipment.

The potential applications of high-throughput cellular patterning include controlled construction of biologically active surfaces and devices and the study of cell attachment phenomena, including interactions to specific extracellular matrix components with cells. The latter application may lead to the ultimate goal of understanding tissue development, from the level of individual cells to the creation of functional customized organs built with automated processes. A more immediate application of this method, however, may include the application of small cellular colony arrays for rapid drug screening or cytotoxicity testing. The drugs or test chemicals could be printed simultaneously with the proteins to study the effects of the drugs on common extracellular matrix proteins and the subsequent cellular adhesion. This application could take advantage of the proven ability of inkjet printing to controllably blend and deposit small quantities of various solutions from multiple reservoirs. Alternately, the test substances could be deposited via the printer on top of the colonies after the desired culture time. Further benefits of inkjet printing include the potential to employ multiple nozzles to produce adjacent patterns consisting of multiple biological materials or polymers.

The authors do not currently advocate this method as a means for high-precision single cell patterning, since the resolution is currently hundreds of microns too large to be applied to individual cell patterning demonstrated by other patterning techniques. However, with further optimization and the development of specialized tools, this method may be able to perform with micron size precision with the additional benefits of flexibility and automation necessary for a high-throughput process for the rapid fabrication of patterns. In its present level of accuracy, this method demonstrates an exciting combination of off-the-shelf inkjet printing and biomaterials and has potential to be adapted to tissue engineering and colony patterning applications. This method is currently being adapted to the three-dimensional construction of cellular structures for eventual high-throughput tissue engineering using a bottom-up approach. A new printer is under development that will allow us to print solutions of higher viscosity, which will expand the capabilities of printing more mechanically stable and biologically active collagen solutions with or without a cellular component. Additionally, advancements in $x-y$ and z direction substrate manipulation could potentially take this high-throughput cell-patterning platform to the next level, 3D construction of soft tissues, and eventually organ printing [29].

5. Conclusions

The creation of cellular patterns of varying complexity and with multiple cell types using a simple automated printing process with a slightly modified commercial inkjet printer has been accomplished. In addition to the high-throughput capabilities, the benefits of this system include flexibility, the use of nontoxic materials, ease of use, and low cost. We have demonstrated that biologically active protein patterns as small as 350 μm can be created using a basic computer and common office software interfaced with a desktop inkjet printer.

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