

Cell and Organ Printing 1: Protein and Cell Printers

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ABSTRACT

We have developed several devices for positioning organic molecules, molecular aggregates, cells, and single-cell organisms onto solid supports. These printers can create stable, functional protein arrays using an inexpensive technology. The cell printer allows us to create cell libraries as well as cellular assemblies that mimic their respective position in organs. The printers are derived from commercially available ink-jet printers that are modified to dispense protein or cell solutions instead of ink. We describe here the modifications to the print heads, and the printer hardware and software that enabled us to adapt the ink-jet printers for the manufacture of cell and protein arrays. The printers have the advantage of being fully automated and computer controlled, and allow for the high-throughput manufacture of protein and cell arrays. *Anat Rec Part A 272A:491–496, 2003.* © 2003 Wiley-Liss, Inc.

Key words: organ printing; cell printing; tissue engineering; hardware

The promise of jet-based, computer-aided technology applied to biological problems includes the ability to detect disease early and inexpensively. The rationale for early disease detection is based on the immobilization of literally thousands of nanoscale markers for diseases (proteins) onto biochips, and the subsequent detection of signals stemming from these markers (Lockhart and Winzler, 2000). Microarray platform development has received wide attention recently because of its potential to be applied to massively parallel gene and protein expression profiling (Ramsay, 1998; Brown and Botstein, 1999; MacBeath and Schreiber, 2000). The current technology for making protein or DNA chips includes photolithography, which is mainly used for synthesis of oligo DNA chips (Southern, 1996; Gao et al., 2001), and pin arrayers (Shalton et al., 1996), which have been used as a platform for both cDNA and protein arrays (Wilson and Nock, 2002; Haab et al., 2001). Commercial ink-jet printers have been explored as economically feasible alternative platforms to photolithography or pin arrayers (Roda et al., 2000; Wilson and Boland, 2001).

Ink-jet-based printers may prove to be superior to pin arrayers, since there is less tendency to for cells to dry out using this technique. We describe here the hardware and software necessary to convert commercial ink-jet printers into cell printers. The novelty is that we use a jet-based, computer-aided device for cell printing. The printers can be used for a variety of applications and may be particularly useful when it is necessary to keep samples hy-

drated. We first show how to use the printers for protein printing, and then describe how they can be extended to print cells. The extension to cell-printing technology may have many applications, ranging from in vitro drug screening to organ printing.

MATERIALS AND METHODS

Printer Hardware With Adjustable Height

The printer is designed to print protein solutions to solid surfaces of any thickness. The body of the printer is made from PMMA with printing components (print head, logic board, encoder, etc.) from an HP 660C printer. Based upon the technical drawings for the original HP printer, the new printer was designed using a new gear mount pillar; closer tolerances were obtained by adding a horizontal support, changing the transistor in the circuit to one with

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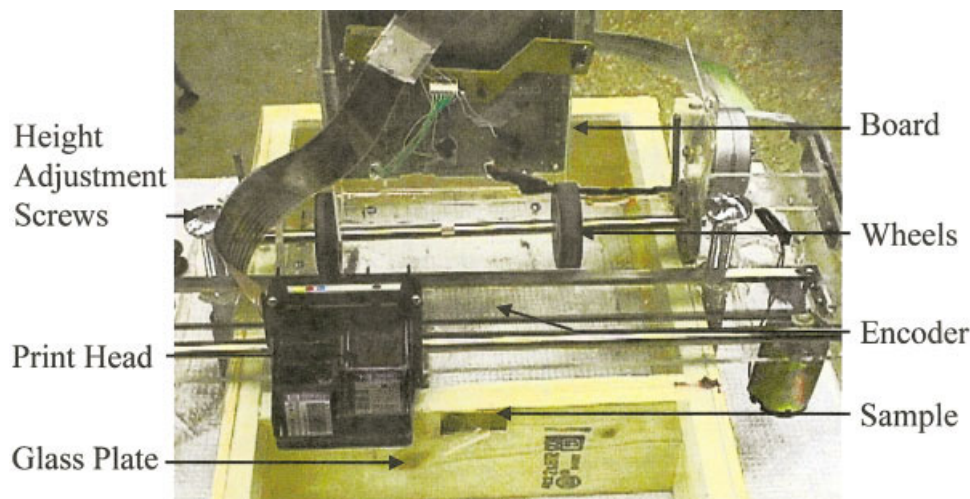


Fig. 1. Photograph of the inside of the protein printer, showing the print board, wheels, encoder, print head, and adjustable base. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

higher amplification, and reentering the horizontal position encoder.

The print head, horizontal bar, and encoder assembly are mounted on a height-adjustable pivoting support that is placed onto a 10-inch \times 13-inch glass plate. The rubber wheels that position the paper in the original printer are allowed to touch the glass, while the height of the print head is adjusted to match the sample thickness. The samples are held on paper or transparency sheets with double-sided tape, and the sheets are inserted between the glass plate and the rubber wheels. This allows the sample to be adjusted in the y-direction through the rotation of the wheels, while the print head sliding across the bar provides the movement in the x-direction.

A fixed base is used, as shown in Figure 1, and the print head, bar, and encoder assembly are mounted on this base. The base can be used with a variety of commercial printers (we used many different print heads from the HP 500–600 series).

Printer Hardware for Thin Samples

When thin samples, such as paper, transparencies, coverslips, or microscope slides, are employed, commercial printers may be used without much modification. We modified a Cannon BJ2200 for printing onto samples that are less than 1-inch wide and 1-mm thick by removing the base of the printer and replacing it with a PMMA support. The samples (e.g., microscope slides) are mounted onto transparencies and fed into the printer in the usual fashion. This has the advantage of increasing the printer throughput, since up to 50 transparencies with up to 10 slides each can be printed in one batch.

Printer Hardware and Software for Mammalian Cells

Large mammalian cells with diameters $>100 \mu$ will not fit through the nozzles of the ink-jet printers, and thus require specially designed print heads. The heads are designed to fit onto an HP 660C printer body. The print head consists of

nine independently operated piezo pumps, each of which possesses an inlet connected via flexible tubing to the cell suspension, and an outlet connected via a luer lock to a needle. The print head assembly may be used with the adjustable base described above, or mounted onto a conveyor belt as shown in Figure 2. The nine pumps are controlled by a Microchip programmable interface controller, using in-house-built software. The head position and the feeder are controlled by the HP driver software. The nine nozzles can, in principle, work simultaneously, but the current software instructs one nozzle at a time as a sequential code is read. However, during one print cycle, any or all of the nozzles can be addressed. The software for the controller lets the user enter the x and y positions of the desired print location and a jet identification value into a text data file. The software reads the text data file and assembles it into useful information for the printer, which then sends the print head to the position and activates the proper jet over that position. The values of the x position range from 0 to 57,150 μm in 50- μm increments. The values of the y position range from 0 to 25,400 μm in 50- μm increments. The jet identification is a nine-digit number of ones and zeroes; a one indicates that the jet is on at that position, and a zero indicates that the jet is off. For example, if one wanted to put a drop of fluid from jet 4 at a position of one-quarter of an inch (6,350 μm) from the left side of the slide and one-quarter of an inch from the top of the slide, the data file would have the following line: 6350 6350 000100000. The technique used to convert the data file into information useable by the printer cannot be shared at this time.

Newer software versions are using a graphical interface to select the printing positions and the jets to use. A grid is displayed on the user screen; the user clicks on grid points to select positions, and then clicks on the jets that are chosen to be active at that point. The software converts this information into data useable by the printer. A functional printing device has been built, but the hardware and software are undergoing constant revision to allow for more flexibility and user-friendliness.

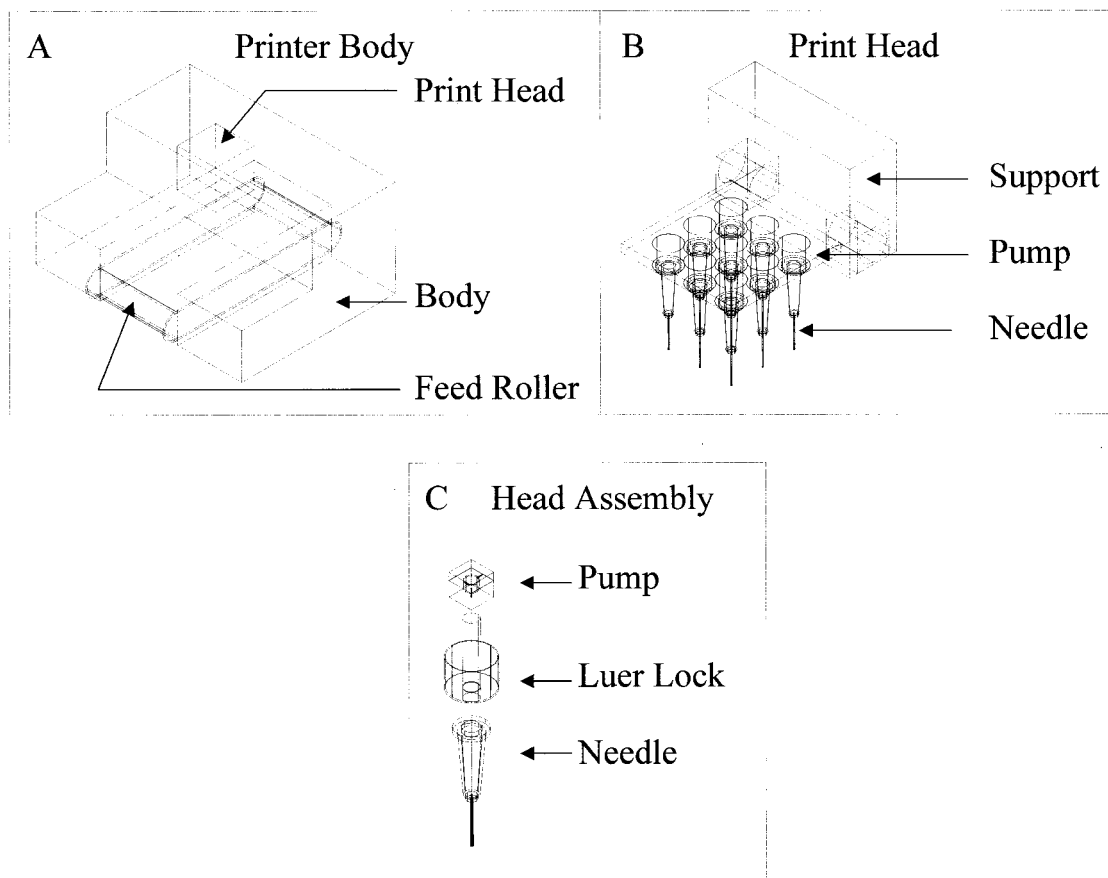


Fig. 2. Schematic drawings of the cell printer, showing the body, print head, and head assembly. The body was derived from a commercial HP 500 printer, and the print head was custom-built.

Printer Boards

In our design, we use original printer boards that are factory-matched to the print head. Therefore, when the print head is changed, the board is changed accordingly. This has proven to give the least amount of trouble with software.

Printer Software

The software drivers of the HP 550C are rewritten to allow for protein solutions of different viscosities and electrical charges to be printed. The source code for the HP550C printer was provided by the manufacturer at no cost. The new driver software constantly adjusts the voltages applied to the nozzle gate to account for different electrical resistance values in the solutions. This allows the appropriate amount to be dispensed, regardless of concentration, viscosity, or pH.

Other software modifications include repositioning the nozzle to the exact same location when addressing a different cartridge. In ink-jet printers the color effect is achieved by placing different amounts of primary colors close to each other; however, in biological assays the drops must be placed onto each other.

The drivers for the Cannon BJ-2200 printer were modified as described above, with the addition of a temperature control and settings for non-aqueous solvents. The

heat applied to the cartridges was adjusted so that the temperature of the ink bubbles did not exceed 100°F, to avoid possible denaturing of protein solutions. The nozzle gates were set to an empirically derived value that was optimized for ethanol. The use of solvents other than water or ethanol is discouraged. Although the drivers will most likely allow for many other solvents, the cartridges may partially dissolve and contaminate the samples. All of the software drivers can be downloaded from our website (<http://130.127.152.24/>).

Protein Printing

The four ink cartridges were emptied and thoroughly rinsed with deionized water until no ink was visible. The cartridges were then rinsed alternately with ethanol and water several times. Bovine serum albumin (BSA) (Sigma Chemical, St. Louis, MO) was dissolved in pH 7.4 phosphate-buffered saline (PBS) solution (Sigma) at 1 mg/ml. Biotinylated BSA (Biotin-BSA) (Sigma), streptavidin (Sigma), and Lucifer yellow-labeled biotin (Y-biotin) (Molecular Probes, Inc., Eugene, OR) were each dissolved at similar concentrations in pH 7.4 PBS. The four cartridges were filled with the following solutions: "yellow" was filled with BSA, "red" with Biotin-BSA, "blue" with streptavidin, and "black" with Y-biotin.

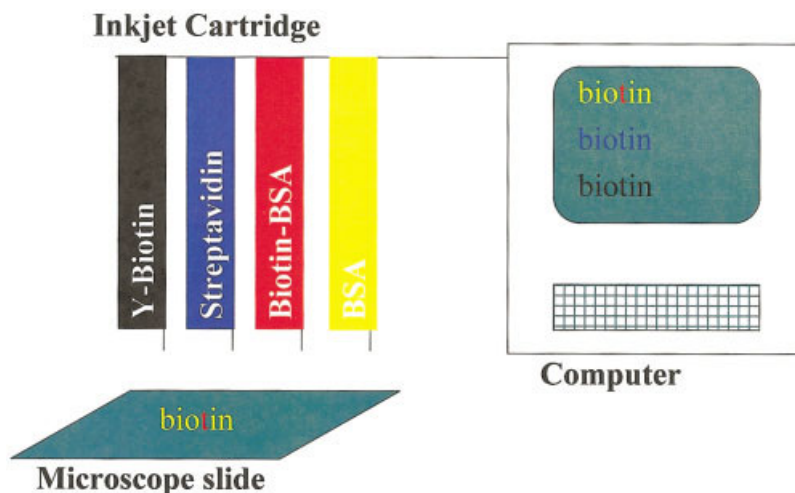


Fig. 3. Schematic of the protein printing experiment. Three successive passes first print BSA and biotin-BSA according to the yellow and red patterns, then streptavidin, and finally Y-biotin. Each passage spells out the word "biotin" on identical positions on the glass slide.

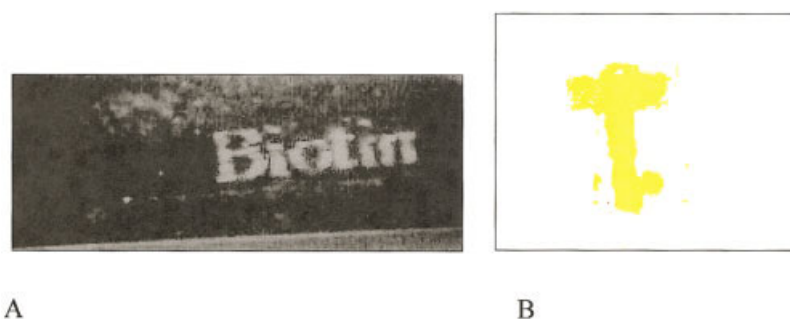


Fig. 4. Views of the protein pattern. **A:** Photograph of the pattern revealed after only BSA and bBSA are printed. **B:** Fluorescent image of the pattern after a second pass of streptavidin and a third pass of Lucifer yellow-labeled biotin.

A pattern was designed using Microsoft PowerPoint software. The color scheme was adjusted to print the desired compound. The pattern "Biotin" (font: Times New Roman, size 8) was selected for printing. The color cartridge was the one containing BSA; therefore, BSA was transferred onto the sample (with the exception of the "t," which was printed with the Biotin-BSA cartridge, and thus Biotin-BSA was transferred in the form of the symbol "t"). For subsequent passes the color cartridges addressed were changed to all blue, thus transferring streptavidin to the entire pattern, and black, thus transferring Y-biotin to the entire pattern. Between passes the slides were rinsed with PBS. A schematic of the experiment is shown in Figure 3.

Endothelial Cell Printing

Bovine aortal endothelial cells and a smooth muscle cell line (ATTC) passage 10 were trypsinized and resuspended in modified Eagle's medium (MEM) and 10% bovine fetal serum (FBS) with a concentration of 1×10^5 cells/ml, before they were printed onto a reconstituted basement membrane gel (MatrigelTM) and a 3 mg/ml collagen gel

(Elastin Products, Inc., Owensville, MO). The cell printer was placed in a sterile laminar flowhood. An individually wrapped, sterile 30-gauge needle (Becton Dickinson) was aseptically filled with the cell suspension, and screwed into the printhead. The drops produced by the needle have an average diameter of 0.006 inches, or a volume of 1.5×10^{-5} ml, resulting in an average of 1.5 cells per drop (either one or two cells per drop). The cell printing, which was carried out at room temperature, took less than 1 min per sample. The printed cells were then incubated in 5% CO₂ at 37°C for 30 min before the liquid medium was added. After 72-hr incubation, the cells were visualized with a live/dead assay (Molecular Probes, Inc.) under an epifluorescent microscope (Nikon, Inc., Melville, NY).

RESULTS

Protein printing results can readily be observed by inspecting the coverslips onto which the proteins are deposited. Figure 4A shows the pattern as seen immediately after printing. Rinsing removes about 90% of the protein, leaving about a monolayer of protein. This was confirmed by ellipsometry, by which we estimated the

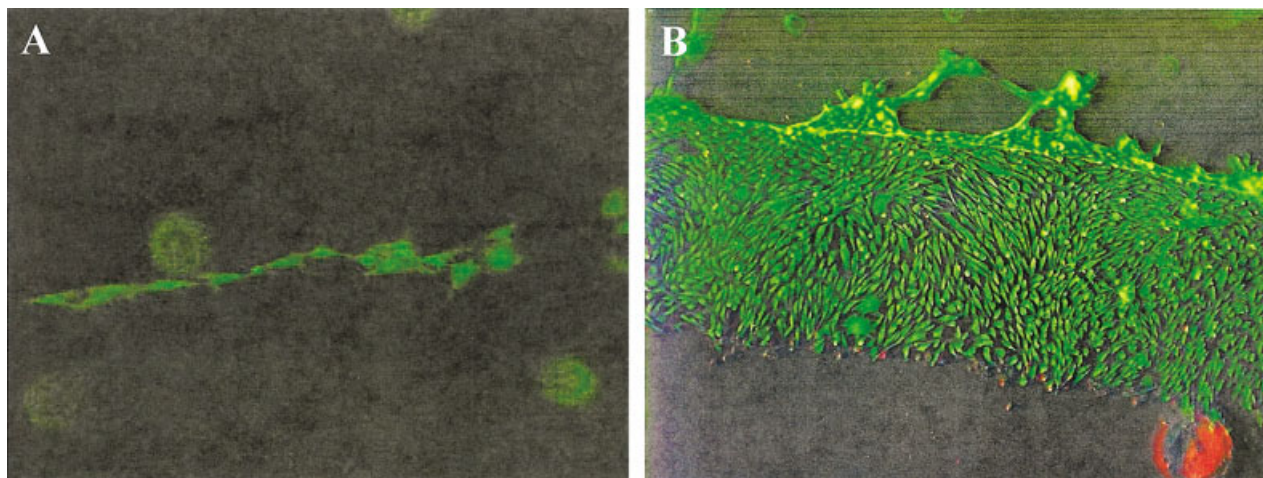


Fig. 5. **A:** Live/dead assay on printed BAECs. The image was taken after 3 days incubation (5% CO₂, 37°C), and shows that the endothelial cells attached to the Matrigel™. **B:** A strip of smooth muscle cells printed onto a collagen gel. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

amount of adsorbed protein to be 1,900 ng/cm² before and 190 ng/cm² after rinsing.

After the final printing, an examination of the glass slide under a fluorescent microscope revealed the letter “t” in yellow, indicating that the streptavidin bound only to the biotinylated BSA (Fig. 4B). The specific binding of the proteins indicates that the printing did not interfere significantly with the structures of the proteins or at least their active sites. These results suggest that the printer can be used for high-throughput colorimetric biochemical assays (Wilson and Boland, 2001).

Figure 5 shows the results of the cell printing. A line was printed by placing each cell in its position along a virtual line. The cells were incubated for 3 days and then imaged. The epifluorescence images depict the line of individual cells written with the printer. The results indicate that the cells stayed alive and attached to their respective positions after printing. Although the majority of the printed cells survived contact with the gel surface, we observed up to 25% cell death upon inspection of the printed patterns. The primary concern is the hydration of the cells, since the drop volumes are small and evaporate quickly under ambient conditions. Therefore, the water content of the gel support must be as high as possible to compensate for the evaporation rate. In the current study we observed no cell death caused by passage through the needles; however, we are planning a more thorough investigation of the cell damage that occurs during printing.

The most common application of protein microarrays is the microimmunoassay, whereby different antibodies are arrayed on a surface and subsequently exposed to an unknown sample. Binding of proteins to the immobilized antibodies is then detected by a variety of optical techniques, such as fluorescence and luminescence. Other protein-binding compounds, such as synthetic proteins (Nygren and Uhlen, 1997), RNA and DNA (Brody et al., 1999), allosteric ribozymes (Seetharaman et al., 2001), peptides, and small molecules (Kodadek, 2001) have been used.

The ink-jet-based delivery mechanism has several advantages over spotting techniques. These include the ability to work with hydrated samples, the simplicity of the

technique, the possibility of using many types of proteins and cells, and the high-throughput nature of the mechanism. The ability to work with hydrated samples is important when working with proteins that are highly susceptible to denaturation when they are dried out. Furthermore, by using liposomes in the cartridges the technique can be used for membrane-bound proteins. Thus, the method can be used to array membrane proteins that are otherwise difficult to immobilize in patterns.

The ability to print cells onto hydrated samples raises the possibility of generating inexpensive, dense cell-based arrays such as phage libraries, bacterial artificial chromosome libraries, and cell-based sensors. Cell printing is strikingly different from the approach of engineering surfaces for cell patterning, for two main reasons. First, cell printing is automated and fully computer-controlled, which enables high-throughput manufacturing of cell arrays. Second, cell printing is not limited to two-dimensional patterns because it does not depend on surface modifications. Systematic three-dimensional cellular assemblies may become possible with the use of the ink-jet approach.

CONCLUSIONS

We have introduced new hardware, derived from commercial ink-jet devices, for the printing of proteins and cells. These devices may have many potential applications, ranging from drug screening to tissue engineering.

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