Digital Printing of Bioinks

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Abstract

Recently piezo-based ink jet printers have proven to be reliable, productive manufacturing tools particularly where expensive materials are involved. As bioengineers, we are capitalizing on the ability of piezoelectric-based drop-on-demand ink jets printers to precisely deposit a wide variety of biologically active materials to create custom arrayed libraries and patterns. These applications and many others take advantage of ink jet strengths as an additive, non-contact process. We have developed disposable ink jet cartridges and a laboratory bench-top printing system. We have printed many biological materials, and optimization of ink compositions has been the focus of our research. At this conference, we will discuss both solvent-based and aqueous bioinks. We will show the spatial deposition of both nanoinks and microinks and show the resulting biomaterial designs and arrays including DNA, bacteria, proteins and carbon nanotubes. We have printed gold nanoparticles and protein-bound nanospheres to allow patterning of fluorescent proteins. We will discuss ink composition and printing characteristics of these materials. Finally we will discuss wave form parameters for printing a broad range of aqueous materials used in the biosciences and performance characteristics during printing.

Introduction

Recent advances in tissue engineering, biological sensing, and biotechnology have resulted from two complementary forces. First, there is a natural evolution towards microscale patterning and rapid prototyping of materials as novel technologies become available. Second, patterned materials provide the capability for specific interactions with cells, proteins, DNA, viruses, and other biological structures. Micropatterned biological materials are not only essential in medicine and biology but are also of increasing interest in microelectronics, microelectromechanical systems (MEMS), sensors, display units, and optoelectronic devices [1]. Rapid prototyping techniques like ink jet printing may allow for high-throughput production of patterned multiplexed biological materials without the use of masks, stamps, ribbons, or other costly and time-consuming conventional processing equipment. Piezoelectric-based ink jet printing is a thermally constant process with some important advantages over using bubble or thermal ink jets.

A digital materials printer manufactured by Dimatix, Inc. gives scientists and engineers a piezoelectric drop-on-demand ink jet printing tool for developing processes for depositing a wide variety of functional fluids. The Dimatix Materials Printer (DMP) is based on a cartridge printhead system: fluid is injected into the fluid module and then the fluid module is snapped to the jetting module to form a sealed cartridge. The ink jet printhead itself is a silicon die that consists of 16 individually addressable jets, an array of inline nozzles that are spaced 254 µm apart. The effective nozzle diameter is 21.5 µm, which provides droplets in the ~10 pl range. The printhead is powered by a thin piezoelectric unimorph, and actuation is in the plane of the wafer (bender mode).

Functional fluids are often developed in small quantities, and the fluid module's small volume of 1.5 mL and the negligible void volume in the printhead give researchers the chance to develop fluids for ink jet deposition processes without having to make large quantities. The DMP has a preloaded waveform that has been optimized for fluids that have a viscosity between 10 and 12 centipoise (cps) and a surface tension between 28 and 33 dynes/cm, and this waveform can be changed in pulse shape (amplitude, slew rate and duration), frequency and voltage as may be required for new fluids. The waveform has two segments, a jetting segment and a non-jetting segment, and the jetting segment is usually the segment that is manipulated the most to get non-optimized fluids (with viscosities above or below 10-12 cps and surface tensions above or below 28-33 dynes/cm) jetted. However, as in all ink jet printing systems, the physical characteristics of the fluid, details of printhead materials and geometry as well as the waveform that actuates the piezoelectric unimorph determine drop formation and overall fluid jettability. If waveform parameters are manipulated systematically, difficult-to-jet fluids may ultimately be deposited successfully.

Nanoinks and Microinks

Many functional inks are colloidal inks where a polymer or a metallic nanoparticle (one phase) is distributed in a non-polymer, non-nanoparticulate phase (second phase). These fluids can be stabilized by the addition of charged molecules such that the polymers or nanoparticles do not aggregate. We have expanded the particulate phase to include bacteria, yeast, nanospheres, and microspheres. We have produced many of these functional inks, and we will discuss a few of the inks developed.

Carbon Nanotube/DNA Composite Ink

Hybrid composites are a class of materials with several novel applications [2]. These materials are employed in the fabrication of sensors [3], restorative dental materials [4], and bone or heart valve tissue engineering scaffolds [5, 6]. One goal of ink jet printing is to generate thin films or three-dimensional structures of materials. Hybrid composites can be defined as inorganic nanoparticles mixed with organic polymers or organic polymers mixed with defined organic nanomaterials. In one example, a nucleic acid sensor has been generated by screen printing hybrid materials that contain multi-walled carbon nanotubes and nucleic acids [3]. Ye et al. characterized a multi-walled carbon nanotube modified screen-printed carbon electrode using alternating current impedance. They demonstrated that this structure was able to detect direct electrochemical oxidation of guanine or adenine residues of deoxyribonucleic acid (DNA) [3]. However, screen printing is inherently slow, wastes valuable materials, and is

plagued by low resolution. In addition, once a screen is produced, it has to be stored until it is used again, and if the pattern is changed, a new mask has to be produced for each change. Marquette et al. used screen printing to prepare an electrochemical biochip using a p53 target DNA sequence derived from exon 8 and graphite-containing ink [7]. A detection limit of 1 nM (150 pg) was observed, suggesting inadequate sensitivity for *in vivo* p53 detection. Their results suggest that increased feature resolution is correlated with increased sensor sensitivity. Increased feature resolution may be obtained using ink jet printing. For example, piezoelectric ink jet printing with a 21 µm nozzle may provide an individual drop resolution of 40 µm resolution. On the other hand, screen printing typically provides ~100 µm resolution. While it has been shown that carbon nanotubes can be easily deposited using vapor deposition [8], the ability to pattern using this form is a subtractive process that does not lend itself to high throughput or flexibility. In contrast, piezoelectric ink jet printing of multi-walled carbon nanotubes is an additive process that is inherently flexible with respect to formation of patterns. High resolution patterns of hybrid materials and other advanced sensor materials may be rapidly created using piezoelectric ink jet printing. In this example, ink jet printing of hybrid composite containing multi-walled carbon nanotubes and DNA was demonstrated. As shown in Figure 1, the solution was printed directly onto a gold sputter-coated silicon wafer (Figure 1a) or an aluminum scanning electron microscope puck (Figure 1b). These images demonstrate that high resolution microscale features may be obtained using piezoelectric ink jet digital fabrication technology. This printing process was greatly simplified by employing the Drop Manager software interface to manipulate the waveform and voltage to an individual nozzle.

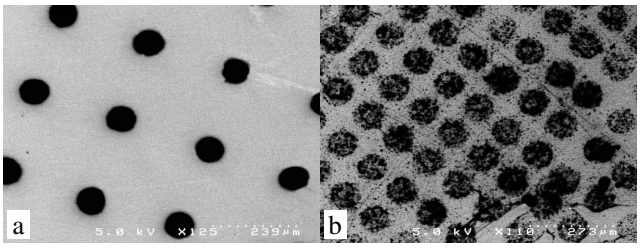

***Figure 1**. (a) Electron micrograph of ink jet-deposited hybrid multiwalled carbon nanotube/deoxyribonucleic acid pattern on gold-sputtered silicon wafer. (b) Electron micrograph of ink jet-deposited hybrid multiwalled carbon nanotube/deoxyribonucleic acid pattern on an aluminum substrate*

Ink Jet Processing of Streptavidin Patterns

Rhodamine-conjugated streptavidin (Pierce Chemicals, Rockford, IL, USA) was dissolved in phosphate buffer saline solution (Fisher Scientific, Fair Lawn, NJ, USA) to create a 1.6 mM solution. 1 % of polysorbate 20 surfactant (Fisher Scientific, Fair Lawn, NJ, USA) was added to isolate the water-soluble protein and ink jet printed. The streptavidin protein used in this study was labeled with rhodamine for imaging using the fluorescence microscopy. Figure 2 contains fluorescence micrographs of a rhodamine-labeled streptavidin microarray prepared using ink jet printing. Feature sizes of approximately 20 µm were observed. Unlike many other high throughput rapid prototyping techniques that require sample heating, piezoelectric ink jet deposition allows rapid deposition of heterogeneous microscale patterns of biomolecules. It is important to note that proteins were reproducibly printed using the Dimatix MEMS device piezoelectric ink jet printhead, and no obstruction of the printhead was observed.

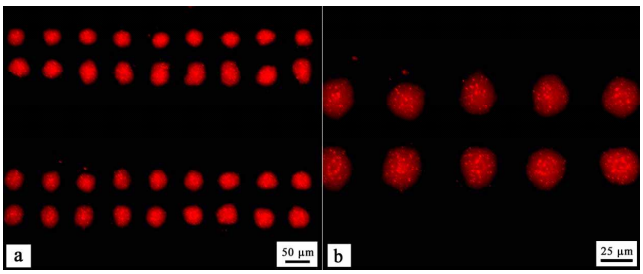

***Figure 2**. Optical fluorescent micrographs of rhodamine-labeled streptavidin deposited on a silicon substrate in a dot array pattern. Micrographs were obtained at both low (a) and high (b) resolutions*

Conclusion

Piezoelectric ink jet printing is a powerful non-contact, non-destructive rapid prototyping technique for biomedical applications. In contrast to thermal ink jet printing, MEMS-constructed piezoelectric ink jet printheads use a patterned lead zirconate titanate piezoelectric transducer bonded to a silicon diaphragm to generate acoustic energy that drives drop formation. This novel, non-contact, and nondestructive printing process may be used to immobilize a variety of biological materials while retaining their biological activity. We have demonstrated microscale patterning of several biological materials, including DNA scaffolds and proteins. Streptavidin protein microarrays with well-defined microscale features were obtained, and the functionality of the patterned strepavidin was demonstrated. In addition, ink jet printing of hybrid composites may be used to immobilize nucleic acids, proteins, cells, and other biological materials in advanced medical devices. Piezoelectric ink jet printing is a unique rapid prototyping technique that offers great potential for developing biosensors, tissue substitutes, cell-culture systems, and several other advanced biomedical devices.

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Author Biographies

Dr. Jan Sumerel is Manager for Biomedical Sciences for the Materials Deposition Division of Dimatix. She received her Ph.D. in Biochemistry and Biophysics under Professor Marzluff at UNC where she studied G1 cyclin/CDK complexes, and did her postdoctoral work with Professor Morse at UCSB where she studied biomimetics. As Director of Process Development at Invenios, Dr. Sumerel developed MEMS devices. She has a number of technical publications in the fields of biochemistry, molecular biology, and nanotechnology.

Anand Doraiswamy earned his B.S. degree in Chemical Engineering (2001), an M.S. in Materials Science and Engineering from the University of Arizona (2003), and is currently pursuing his Ph.D. in biomedical engineering at the University of North Carolina, Chapel Hill. His research interests include direct write microfabrication of medical devices, thin films and coatings for drug delivery applications, and laser processing of advanced materials.

Roger Narayan serves as Associate Professor of Biomaterials in the Department of Biomedical Engineering at the University of North Carolina. Dr. Narayan has published over fifty technical papers on pulsed laser deposition, laser direct writing, and two photon-induced polymerization of biomaterials. His current research on laser processing of nanostructured ceramics and laser direct writing of biomaterials is funded by the National Institutes of Health, the National Science Foundation, and the Office of Naval Research.