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Electrophotography—An Efficient Technology for Biochip Fabrication

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Abstract. *At present, electrophotography has not drawn much attention as a digital manufacturing technique. But it has the potential to be an alternative to ink jet for some interesting applications. The authors introduce a novel and a highly efficient manufacturing process for biochips (peptide arrays) based on electrophotography. Peptide arrays are powerful tools for developing new medical agents for diagnosis and therapy techniques. They are predicted to become as important as DNA arrays provided it is possible to produce peptide arrays in sufficient complexity at moderate costs. After the major scientific goals of the manufacturing process were achieved, a step toward series production has now been made. © 2011 Society for Imaging Science and Technology.*
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INTRODUCTION

In the area of manufacturing, digital printing is generally used for the maskless application of highly resolved coatings. Functional substances are stabilized in a jettable ink or, in the case of electrophotography, they can be embedded into printable toner powders. Due to their different properties, both digital printing techniques complement each other not only in the graphics market but for manufacturing purposes as well. A fruitful application enabled by electrophotography is the efficient manufacturing of complex biochips, i.e., peptide arrays. Peptide arrays have a wide range of applications. In medical research, they are used for the development of new medical agents, vaccines, and methods for fast diagnosis of diseases. Other applications exist in fundamental research as, e.g., study of the immune system or characterization of enzymes and signal transduction pathways.

For (but not limited to) applications in biofabrication, electrophotography has proven to posses interesting advantages compared to ink jet or other dispensing techniques. First, it is a very stable and reliable process; no clogging of nozzles occurs; and no intermediate purging processes are needed. The printing resolution of electrophotography is comparable to ink jet, but while the ink jet process tends to become unstable with decreasing nozzle and drop size, the stability of the electrophotographic process is not affected by the printing resolution. The resolution is mainly limited by the mechanical accuracy of the printer and the quality of the functional toners, which is determined by the distribution of the particle size (that should be narrow) and the adhesion and tribo-electrical charging property of the toner particles.

An important property of electrophotography needed for biochip fabrication is that highly reactive biochemical agents can be deactivated by encapsulation into the toner particles. Uncontrolled chemical reactions, as easily happen on the intermingling of drops, are suppressed. Chemical reactions are started in a controlled manner by melting the toner powders; at this step, the toner matrix turns into a solvent. Additionally, the highly sensitive (and expensive) precursors used in peptide chemistry (Fmoc amino acid-OPfp esters) turn out to be surprisingly stable when encapsulated into toner particles. The decay rate of the most sensitive amino acid ester is about 5% per month while the same amino acid ester decays in a conventional solution (e.g., ink) within minutes. These properties are decisive for commercial fabrication of peptide arrays.

BIOCHIPS

Biochips promise to advance biotechnology and medicine by providing the opportunity for massive parallel screening for chemical binding partners. In these screenings, libraries of proteins or peptides (peptides are short protein fragments) are probed with molecules of interest, for example, important proteins in pathogens, cancer cells, or viruses, in order to identify binders of diagnostic or therapeutic capability; see Figure 1.

Proteins consist of 20 amino acids, which are linked to form long chains. A typical protein has a length of about 500 amino acids. However, protein fragments, so called peptides, with a length of 12–20 amino acids are sufficient for identifying characteristic binding reactions. These

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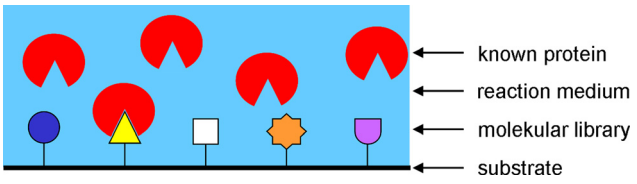


Figure 1. Characteristic chemical binding of a protein to a biochip.

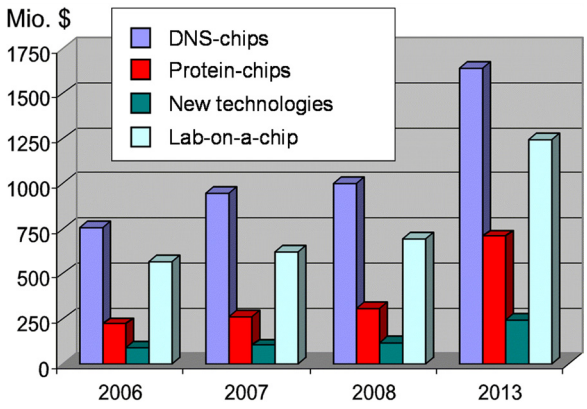


Figure 2. BBC Research: Global Biochip markets (see Ref. 1).

screenings can be regarded as a search for a biological key fitting to a lock. However, the problem is the enormous number of peptides needed. About 100,000 peptides are necessary in order to represent each of the approximately 1000 proteins in a typical bacterium (in the form of 100 overlapping peptides each), 500,000 for a malaria pathogen, and even many more peptides are needed when an array of stochastic peptides is desired (20^{10} peptides of length 10 are possible). Up to now, peptide arrays have been produced by a spotting technique. A maximum of 10,000 peptides fits onto a single letter-sized support; the main drawback is the manufacturing cost. An individual peptide spot amounts to about 6€, adding up to almost 60,000€ for a full array—too much for even the most promising applications.

But the market for biochips is predicted to grow rapidly with an emphasis on fundamental research, drug discovery, and molecular diagnosis;[1] see Figure 2. However, the spreading of this technology is impeded by the high production costs of biochips. Lithographic methods allow for the combinatorial synthesis of highly dense DNA chips, but peptide arrays that cannot be produced by this method lag behind. Now, the electrophotographic printing of peptide arrays provides a promising way forward.

PRINTED PEPTIDE ARRAYS

Peptides are synthesized layer by layer from amino acids on a glass slide where the printer is used as a highly efficient microdispensing tool. The process works as follows: Protected amino acids (Fmoc amino acid-OPfp esters) are embedded into toners (instead of colors) that have similar physical properties as commercial color toners. Since peptides and proteins consist of 20 amino acids, an according number of amino acid toners and printing units in a peptide printer are needed. In the first run, spots of up to 20 toners are printed in an array pattern onto a coated glass slide where the single spots do not overlap; see Figure 3. Then the slide is removed from the printer and heated up to about 90°C. The toner particles melt, turning the toner matrix into a solvent. At this step, the embedded amino acids are freed and couple to the coating of the carrier. Subsequently, the toner matrix and charge control agents are washed away and the coupled amino acids are deprotected. Now, a first layer of amino acids is bound on the glass slide. The complete synthesis cycle is depicted in Figure 4; for chemical details, see Ref. 2.

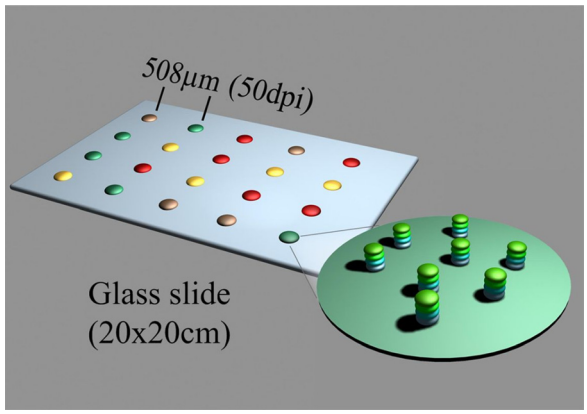


Figure 3. Layer by layer synthesis of a peptide array on a glass slide.

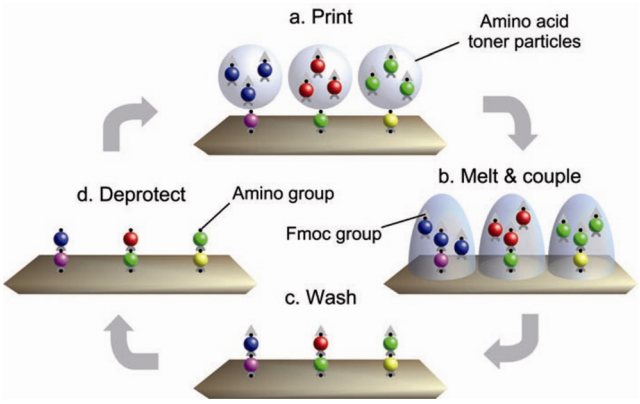


Figure 4. The synthesis cycle includes printing of the amino acid toners (a), coupling of the amino acids by melting (b), washing (c), and deprotecting the coupled amino acids (d).

During printing, the amino acids are processed in a dry state, preventing any undesired chemical reactions. As mentioned, the coupling reactions are started in a controlled manner by heating the carrier outside the printer. After completion of the first synthesis cycle, the probe carrier is put into the printer again, and a second layer of amino acid toners is printed precisely on top of the first as shown in Fig. 3. After finishing the second synthesis cycle, amino acid chains of length two are generated on the glass slide. When repeating this printing and synthesis cycle 12–20 times, several hundred thousand peptides of the

Figure 5. First prototype peptide printer, operated at DKFZ since 2005. Twenty printing units are arranged in a line.

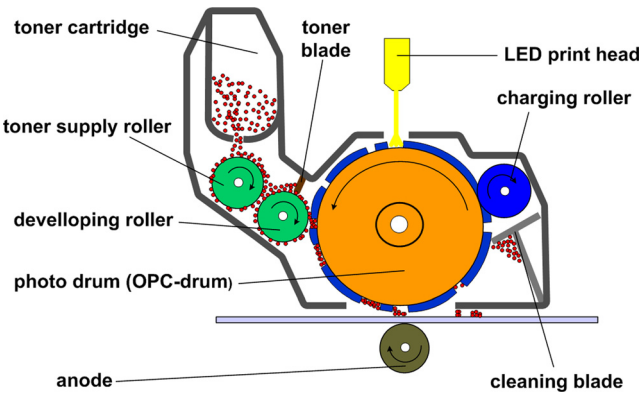


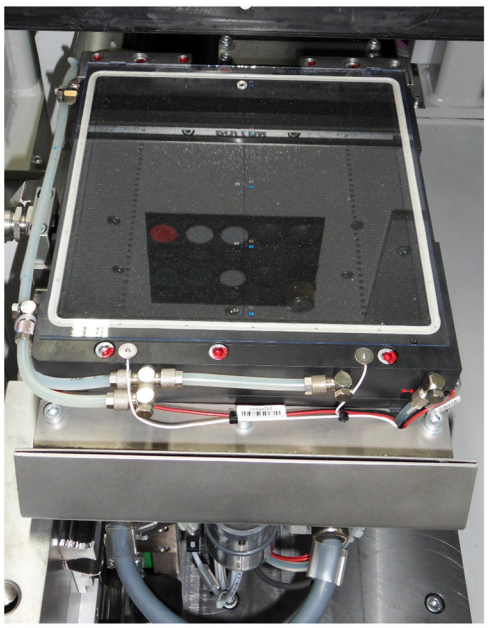
Figure 6. Printing unit: Single-component development system (nonmagnetic) with 1200 dpi LED-array (Type C2/C4 from Oki Company).

corresponding length are synthesized in parallel on a glass slide of size 20×20 cm^2.

Compared to the state of the art, printed peptides arrays are more complex, i.e., they contain many more different peptides. The first prototype peptide printer (Figure 5) is able to print over 150,000 microspots on a glass slide (20×20 cm^2). The second generation printer (Figure 6) designated for series production manages to print over 500,000 spots on a glass slide of the same size. This allows offering peptide arrays at a price 30- to 40-fold less in comparison to the state of the art (~0.13–0.20€ for a single peptide spot, PEPperPrint Company).

(a)

(b)

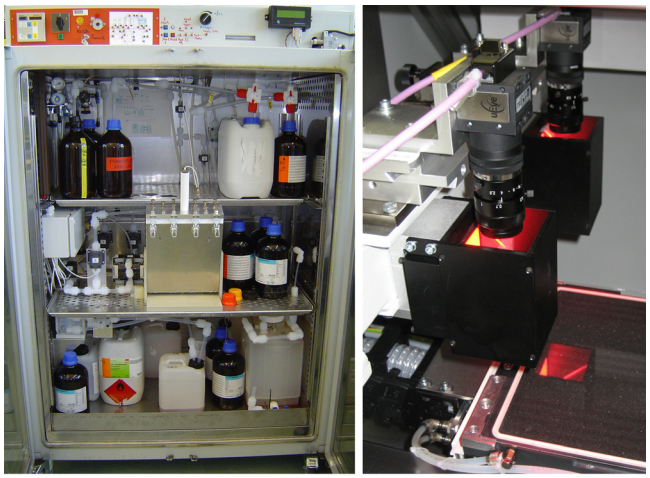


(c)

Figure 7. Peptide printer for series production. Designed and build by Fraunhofer-IPA and KMS Company, operated at PEPperPrint Company.

PRINTING TECHNOLOGY

The peptide printers deposit 20 amino acid toners onto glass slides; the first prototype is shown in Fig. 5. For each of the 20 amino acids, a developing unit (type C2, Oki Company, Figure 7) with a 1200 dpi light emitting diode (LED) array is provided where all developing units are arranged in a line. The glass slide is mounted on a linear slide, which moves below the synchronously rotating organic photoconductor (opc) drum of the developing units. The toner is transferred directly from the opc drum to the glass slide (single-pass printing). Toner transfer onto glass is more difficult than onto paper due to the stiffness and the smooth surface of the material. For successful toner



(a) (b)

Figure 8. Chemical reactor for processing peptide arrays (left). Vision system for calibration of the printing units, positioning of the glass slides, and reading of bar codes for identification (right).

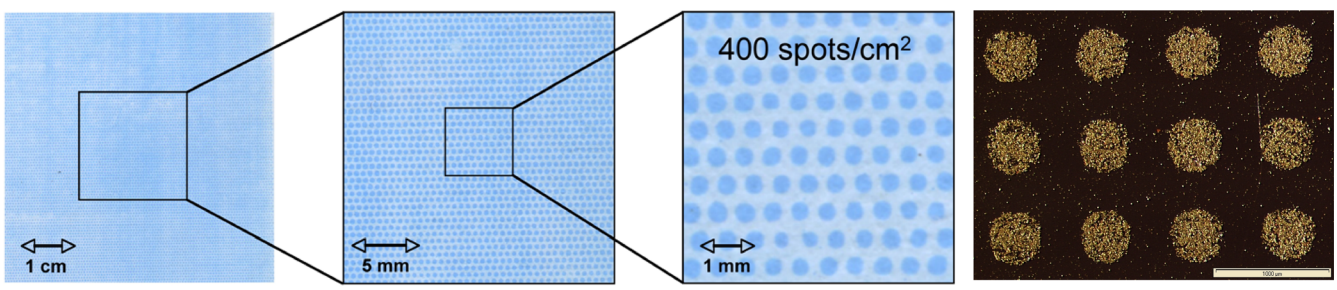
transfer, a conformal (uniform local) contact of the glass slide to the opc drum is required. Using the property that 1 mm thick glass slides are flexible within the range of about ± 0.2 mm, we achieve this by pressing the slide against the drum with an adjustable force. A transfer anode made from static dissipative foam is integrated below the glass slide. It enables toner transfer by an electrostatic field (anode voltage: ~2.5–3 kV). The maximum voltage of the anode is limited by gas discharge effects that lead to a breakdown of toner transfer due to discharge of the toner particles on the opc drum. The transfer efficiency is still significantly lower compared to commercial color printers, but the value creation of printing amino acids onto glass slides for peptide synthesis, on the other hand, is many thousand times higher than that of printing colors onto paper.

According to the principle of single-pass printing 19 opc drums roll over the toner spots deposited by the first printing unit. Considering a finite transfer rate, each drum removes a fraction of the previously deposited toner. But unlike in color laser printers, the toner spots deposited by different printing units in a single run never overlap, Fig. 3. Therefore, the regions of the opc drums, which contact the previously deposited toner, are still fully charged ($V_{Dark} \sim -600$ V). This property reduces the rate of removal of toner, thereby enabling single-pass printing with 20 printing units onto glass slides.

The mechanical accuracy of the peptide printers is much higher than commercial printers. For a peptide array of 12–20 layers with 20 amino acids, toners must be printed precisely on top of each other. Between the different runs, the probe carrier is removed from the printer for the chemical coupling, washing, and deprotection steps. The arrays printed by the first prototype (Fig. 5) contain 387 spots/cm^2; this corresponds to a point to point distance of 508 μm; see Figure 8. Its successor (Fig. 6) manages to print arrays with half the point to point distance, yielding four times as many peptides per unit area.

Besides an increase in resolution, improved reliability of the printing process and the chemical synthesis steps, convenient operation of the printer, and quality assurance of the arrays are major issues for the step from the laboratory to commercial production. To address these requirements, a major reengineering of the prototype peptide printer has been carried out.

The series production of peptide arrays is a batch process since the printing steps (~3 min) are fast compared to the chemical processing cycles in between: First, a layer of amino acid toners is printed on each of five or ten glass slides. During chemical processing of the slides in a chemical reactor (Figure 9), the second batch of glass slides can be printed on. For the identification of the individual slides, a bar code, which is read by a camera, is laser imaged at a corner of each slide (Fig. 9). A data management system then proposes the appropriate next data set to the operator. The vision system is also used for the calibration of the printing units and to measure the precise position of the glass slides on the mounting. The position of a slide is detected by laser imaged markers at its corners, and the printing data are electronically corrected accordingly to ensure that all layers are precisely printed above each other. Similarly, by measuring the position of printed calibration dots, the printing units are electronically calibrated. When the linear slide passes below a printing unit, it is pneumatically lowered onto the front rim of the glass slide and lifted back after finish of printing. Besides the 20 developing units needed for the amino acid toners, four additional ones are provided for test purposes and reserve (Fig. 6).



(a) (b)

Figure 9. Magnified amino acid spot and a coupled amino acid on a glass slide (stained).

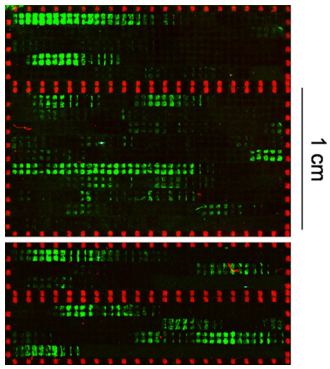


Figure 10. Fluorescent pattern of a printed peptide array incubated with antibodies.

AMINO ACID TONERS

The amino acids, which are embedded into the toner matrix, are protected against uncontrolled chain reactions (Fmoc-chemistry) but are chemically activated to enable the coupling reactions. To assure a defined tribo-electric charging of the toner particles, charge control agents and stabilizers are added to the matrix material. These ingredients need to be compatible with the chemistry of the synthesis cycle (Fig. 4), which excludes many substances. Compared to color toners, the melting point of the toner matrix must be lower (<90°C) to prevent undesired side reactions during peptide synthesis. To obtain a low melting point of the toner matrix and to optimize the coupling reactions, solid solvents are used, which are higher homologues of standard (liquid) solvents traditionally used for peptide synthesis, e.g., dimethylformamide (liquid) versus diphenylformamide (solid). Chemical coupling yields within this solid–liquid environment proved to be comparable to standard liquid processes.[2]

The physical properties (charge-to-mass ratio, size distribution, etc.) of the amino acid toners are close to those of the commercial color toners. As a result, the printing resolution and the toner transfer rates are comparable. In fact, at the beginning of the project, the physical properties of commercial color toners (from Oki Company) were defined as the interface for the parallel development of the peptide printer and the amino acid toners.

As mentioned, the highly sensitive amino acids prove to be surprisingly stable when encapsulated into toner particles. The decay rate of the most sensitive amino acid toner (Fmoc-Arg-OPfp-ester) is about 5% per month (stored at 25°C) while the corresponding amino acid ester decays in a conventional solution within minutes.

BIOLOGICAL FUNCTIONALITY

To prove the biochemical functionality of printed peptide arrays, 5500 different peptides were synthesized on microscope slides (387 peptides per cm^2) that display many permutations of two specific peptides, which are known to selectively couple to corresponding antibodies (so called Flag- and Myc-epitopes). When the arrays were incubated with these antibodies, all of the permutated peptide epitopes showed the expected staining pattern, which correlates in relative intensity with previously published results.[3,4] A more detailed description of the chemistry and biochemistry of the combinatorial synthesis of peptide arrays are beyond the scope of this article; it can be found in Ref. 2. In Figure 10, an example of the read out of a printed peptide array, which has been incubated with antibodies, is shown.

CONCLUSIONS

A novel application of electrophotography, the highly efficient manufacturing process of peptide arrays, has been introduced. Several hundred thousands of peptides are simultaneously synthesized layer by layer from amino acids on a glass slide. The 20 amino acids are embedded into 20 toners and printed onto the glass slide with high resolution and accuracy. This new production technique has the potential to advance research in life sciences by enabling many important projects, which are not possible today due to the lack of highly complex and affordable peptide arrays. Motivated by this result, other interesting applications of electrophotography in the area of manufacturing are currently under study.

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