Electro Photography ("Laser Printing") an Efficient Technology for Biofabrication

***Stefan Güttler**, Oliver Refle, Simina Fulga, Andrzej Grzesiak, Christian Seifarth*
Fraunhofer Institute for Manufacturing Engineering and Automation, Nobelstr. 12, 70569 Stuttgart, Germany

Volker Stadler
PEPperPRINT and ***German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany***

Achim Weber, Christian Speyerer
Fraunhofer Institute for Interfacial Engineering and Biotechnology, Nobelstr. 12, 70569 Stuttgart, Germany

Abstract

Electro photography – the second wide spread digital printing technology beside ink jet – is on the way to prove its capabilities as a manufacturing technology, at least for applications in biofabrication. On DF2008 we first introduced a novel and highly efficient manufacturing process for biochips (peptide arrays) based on electro photography. After the major scientific goals were achieved the step towards a series production has been made in the last two years. We now present the biochip printing facility capable for a series production.

Next, we introduce the application of electro photography as a rapid prototyping technique for the (potential) production of artificial tissues. Similar to other rapid prototyping techniques 3D-structures are build layer by layer from toner powders. Since different approaches to manufacture scaffolds for tissue engineering have been made until today the comparison of the results is interesting.

Introduction

In manufacturing digital printing is generally used for the maskless application of highly resolved coatings. The substances to be deposited are stabilized in a jetable ink or alternatively, in the case of electro photography, they are embedded into a printable toner powder**.** Due to their different properties both digital printing techniques complement each other not only in the graphics market but as well for manufacturing purposes. An application in biofabrication enabled by electro photography is the efficient manufacturing of complex biochips – peptide arrays. This novel manufacturing process was first introduced on DF2008 [1,2]. After having established the major scientific results, i.e. proven the biological functionality of printed peptide arrays, the laboratory processes were transferred step by step into a series production. We now present the recent state of peptide printing capable for a commercial production.

But electro photography has even more potential in biofabrication. 3D-printing or rapid prototyping of scaffolds which are applicable for tissue engineering is a quite new and exiting field of research. Several rapid prototyping techniques as e.g. drop-on-demand printing of a binder into a powder bed, jetting of a hydrogel reactant into a hydrogel precursor, stereo-lithography, fused deposition modeling, and various dispensing strategies have been employed for the manufacturing of biocompatible and biodegradable scaffolds [3–5]. Our approach introduced here is to apply electro photography with customized bio-toners to build up 3D-scaffolds for tissue engineering.

Especially for (but not limited to) applications in biofabrication electro photography has interesting advantages compared to inkjet or other rapid prototyping techniques. First of all it is a very stable and reliable process; no clogging of nozzles occurs and no intermediate purging processes are needed. The resolution of electro photography is comparable to inkjet; but while the inkjet process tends to become more and more difficult (unstable) with decreasing drop size the stability of the electro photographic process is not affected by the resolution. The printing resolution mainly depends on the quality of the toner, i.e. the distribution of the particle's size (which should be narrow) and the tribo-electrical charge of the toner (which has to stay within a narrow window).

An important property of electro photography needed for biochip production is that highly reactive biochemical agents (Fmoc amino acid-OPfp esters, the amino acids) can be encapsulated and deactivated in the toner powders. Uncontrolled chemical reactions are suppressed and no interchange reactions are possible as it easily happens by the intermingling of drops. Chemical reactions are started in a controlled manner by melting the toner powders; at this step the toner matrix turns into a solvent. Additionally, highly sensitive agents are 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 months (stored at 25°C) while the same amino acid ester decays in a conventional solution (e.g. ink) within minutes.

Inkjet printing of aqueous or organic binder material into a powder bed or selectively sintering particles with a laser beam are well known rapid prototyping (or solid free form fabrication) techniques. But the spatial resolution of the obtained objects is moderate (in the range of ~250µm). More important, any 3D-object (e.g. a scaffold) can be build from only a *single* building material (powder), at least only a single one for each layer. Beside the superior resolution of electro photography compared to conventional rapid prototyping techniques it is an intrinsic feature that

each layer may be patterned from different toners. This provides the opportunity to introduce a more complicated structure into a scaffold.

We first present the series production of peptide arrays before we turn to the prospects of electro photography as a rapid prototyping technique for biocompatible scaffolds.

Biochips

Biochips promise to advance biotechnology and medicine by providing the opportunity of massive parallel screening for (chemical) binding partners. They are used for the development of new medical agents, vaccines, and methods for fast diagnosis of diseases. In these screenings libraries of proteins or peptides (short protein fragments) are probed with molecules of interest as for example important proteins in pathogens, cancer cells or viruses in order to identify binders of diagnostic or therapeutic capability.

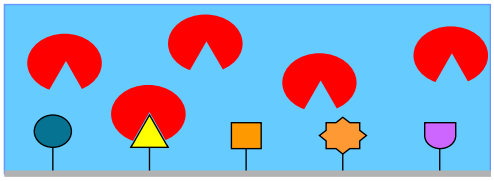

Fig. 1: *Characteristic chemical binding of a molecule to a biochip*

Proteins consist of 20 amino acids which are linked to long chains. A typical protein has a length of about 500 amino acids. However, protein fragments, so called peptides, with a length of 12 to 20 amino acids are sufficient for identifying characteristic binding reactions. These screenings can be regarded as search for a biological key fitting to a lock. But 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 bacterium (in the form of 100 overlapping peptides each) and even 500.000 for a malaria pathogen. Till today peptide arrays are produced by a spotting technique. A maximum of 10.000 peptides fit onto a glass slide where the main drawback is their price: An individual peptide spot costs around 6€, adding up to almost 60.000€ for a full array – too much for most promising applications.

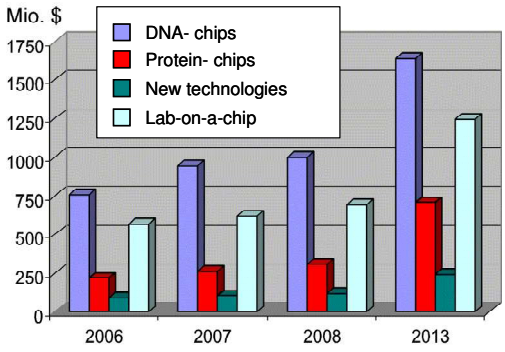

Fig. 2: *BBC Research: Global Biochip markets [6]*

The market of biochips is predicted to grow fast where the emphasis lies on fundamental research, drug discovery, and molecular diagnosis [6]. But the spreading of this technology is impeded by the high production costs of biochips. Meanwhile lithographic methods allow for the combinatorial synthesis of highly dense DNA- chips, but peptide arrays which can not be produced by this elegant method lag behind. Now printing peptide arrays by electro photography has shown the way out.

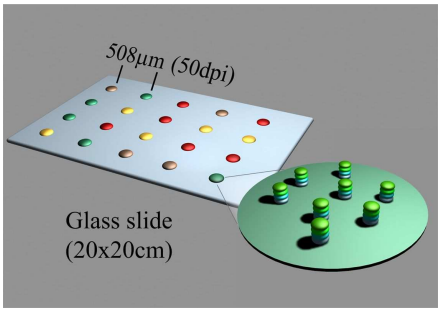

Fig. 3: *Layer by layer synthesis of a peptide array on a glass slide*

Printed Peptide Arrays

Peptides are synthesized layer by layer from amino acids on a glass slide where the laser printer is used as a highly efficient micro dispensing tool. The process works as follows [1,2]: Protected amino acids are embedded into toners (instead of colors) which 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 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, fig. 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 couple to the coating of the carrier. Subsequently the toner matrix and the charge control agents are washed away. Now a single layer of the amino acids is bound on the glass slide. The complete synthesis cycle is depicted in fig. 4.

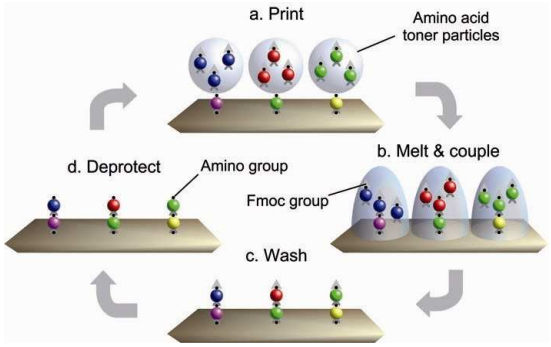

Fig. 4: *The synthesis cycle includes printing of the amino acid toners (a), coupling of the amino acids by melting (b), washing (c), and deprotecting the coupled amino acids (d).*

During printing, the amino acids are processed in a dry state preventing any undesired chemical reactions. 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 exactly on top of the first, figure 3. After 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 corresponding length are synthesized in parallel on a glass slide of size 20 by 20cm.

Printing Technology for a Series Production

Compared to the state of art printed peptides arrays are more complex, i.e. they contain much more peptides. The first prototype peptide printer (which is still under operation) is able to print over 155.000 micro spots on a glass slide (20 by 20cm). The next generation printer designated for a series production is now able to print more than 500.000 spots on a glass slide of the same size. This allows offering peptide arrays at a price which is at least ***30-times*** less compared to the state of art (~0.13 – 0.20€ for a single peptide spot, PEPperPRINT).

Beside an increase of resolution the reliability of the printing process and the chemical synthesis steps, a convenient operation of the printer, and the quality assurance of the arrays are major issues for a commercial production. To reach these requirements a major reengineering of the peptide printer and the chemical processing steps has been done.

Fig. 5: *Peptide printer for series production of peptide arrays. Designed and build in cooperation with KMS Automation GmbH.*

The series production of peptide arrays is a batch process since the printing steps are fast compared to the chemical processing cycles in between: A single layer of amino acid toners is printed on each of ten glass slides; then the slides are simultaneously processed in a chemical reactor, fig 7. The processing time allows to print the second batch of ten glass slides.

The printing process works as follows (fig. 6): The glass slide (220 x 210 x 1mm) is fixed on a vacuum table which is mounted onto a linear drive. Laser markers on the glass slide are used by a vision system to calculate its precise position on the holder in order to ensure that all layers will be printed exactly above each other. Each slide is identified by a lasered bar code; so a data management system can propose the next right data set to the operator. The printing process is single-pass; the probe carrier moves below the printing units which are arranged in a line. 20 – 24 printing units (OKI C7000 series with 1200dpi LED-arrays) are contained in the printer. At least 20 are needed for the 20 different amino acid toners, the additional ones are used for the testing of new toners. The printing units which are involved in the printing process (not all of them are needed for each print) are lowered onto the glass slide. The photo conductor drums are pressed onto the slide with an adjustable force such that a conformal contact of the OPC-drums to the surface of the glass is obtained which is required for a uniform toner transfer. As discussed in [1], the transfer of toner onto glass is more difficult than onto paper and requires a more sophisticated transfer process. After the print is finished the printing units are lifted back to their idle position. Finally the glass slide is removed from the printer and heated in an oven to start the coupling reactions before the chemical processing steps follow.

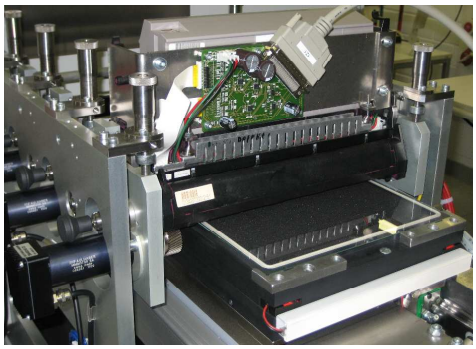

Fig. 6: *Printing unit and probe carrier holder.*

Fig. 7: *Chemical reactor for processing of peptide arrays.*

This new production technique is expected to advance research in life sciences by enabling many important research projects which are not possible on today due to the lack of complex and affordable peptide arrays.

3D- Printing of Functional Surfaces

The application of many rapid prototyping techniques for the manufacturing of biocompatible and biodegradable scaffolds for

tissue engineering has been studied since about 1996 [3-5]. Employed methods are drop-on-demand printing of aqueous or organic binder into a powder bed, jetting of a hydrogel reactant into a hydrogel precursor or vice versa, stereo-lithography, fused deposition modelling, selectively sintering of particles with a laser beam, and various dispensing strategies. Manufacturing of biocompatible scaffolds is a challenge since many biological and technical requirements must be met. First of all scaffolds must allow seeding of living cells, which requires biocompatible materials and a porous structure where the cells can grow in. The physical properties of biological materials are not always compatible to rapid prototyping processes.

A major obstacle of artificial tissues is the complexity of biological tissues, which have a complicated microstructure (with sizes down to 10µm) and may contain many different types of cells. A major disadvantage of many rapid prototyping techniques is that 3D-objects can only be build from a *single* material. In principle different layers of a layered 3D- structure could be made from different materials (e.g. powders), but this is of little help. A complex structure as depicted in figure 8 can not be build anyhow. 3D-printing of UV-curing inks or wax, on the other side, is not able to generate a porous structure which is important to guide the growing of cells. Another disadvantage of many conventional rapid prototyping techniques is their limited resolution which is in the range of about 250µm.

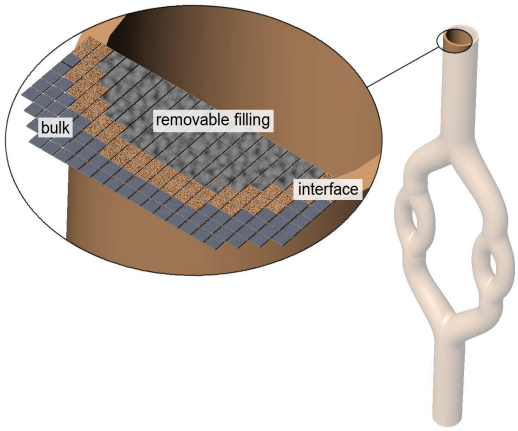

Fig.8: *Branched tube with bulk and surface made from different materials (bulk, surface, and support material)*

Beside the superior resolution of electro photography it is an intrinsic feature of this technology that each layer of a layered 3D-object may be arbitrarily patterned from different toners. These are the principle ideas which guide the application of electro photography as a rapid prototyping technique in biofabrication. In order to obtain a highly resolved 3D-object toner layers can not simply be printed one above the other and fixed by melting in between. The 3D-structure distorts by the frequent melting processes and its top surface fast corrugates. This prevents the uniform deposition of additional layers of toner. An alternative approach followed here is to chemically couple the consecutive added layers of toner. A moderate sintering of the toner after each printing step then suffices to provide the contact area between the particles needed for chemically coupling. A porous structure is obtained by the elaborate use of support material which can be chemically degraded after the printing process. Still a major problem is the highly uniform deposition of layers with different toners what is necessary to maintain a smooth surface. At the moment this process to generate biocompatible scaffolds from different materials is under development.

References

[1] S. Güttler, M. Gröning, P. Willems, B. Biesinger, F. Breitling, F. R. Bischoff, V. Stadler, Th. Felgenhauer, K. Leibe, S. Fernandez, "An efficient manufacturing process for highly complex biochips using laser printing technique", Proc. NIP24 and DF2008, p. 471-75 (2008).

[2] V. Stadler, T. Felgenhauer, M. Beyer, S. Fernandez, K. Leibe, S. Güttler, M. Gröning, K. König, G. Torralba, M. Hausmann, V. Lindenstruth, A. Nesterov, I. Block, R. Pipkorn, A. Poustka, F. R. Bischoff, and F. Breitling, "Combinatorial synthesis of peptide arrays with a laser printer", Angew. Chem. Int. Ed., vol. 47, p. 7132-35 (2008). http://dx.doi.org/10.1002/anie.200801616

[3] R. A. Giordano, B. M. Wu, S. W. Borland, L. G. Cima, E. M. Sachs, M. J. Cima, J. Biomater. Sci. Polym. Ed., vol. 8, p. 63-75 (1996).

[4] R. Landers, A. Pfister, U. Hübner, H. John, R. Schmelzeisen, R. Mülhaupt, J. Mater. Sci., vol. 37, p. 3107-3116 (2002).

[5] D. Hutmacher, M. Sittinger, M. Risbud, Trends Biotech., vol. 22, p. 354-62 (2004).

[6] Global Biochip Markets: Microarrays and Lab-on-a-Chip (BCC Research, BIO049B, 2007); Biomarkers: The expanding global market (BCC Research, BIO061A, 2007); Drug Discovery Technologies (BCC Research, BIO020C, 2007).

Author Biography

Stefan Güttler graduated in theoretical physics (diploma) form the University of Freiburg, Germany (1996). He obtained his doctoral degree (1999) at the Max-Planck-Institute for Physics of Complex Systems in Dresden with a thesis on signal processing in technical systems. In 2000 he joined the Fraunhofer Institute for Production Engineering and Automation in Stuttgart. His main working field is the development of digital printing technologies (electro photography and drop-on-demand printing) for manufacturing applications. Beside applications in biochemistry a focus is on printing of (ceramic) suspensions. For developing the efficient manufacturing process for highly complex biochips the Science Price of the German Stifterverband 2008 was awarded.