Biofunctional core-shell nanoparticle deposition for biochip creation by printing processes

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ABSTRACT

In order to set up an automated production-line for nanoparticle-based bio-chips, a piezo driven ink-jet printing process was developed which is biocompatible and allows for high flexibility as well as accuracy in nanoparticle deposition.
Highly dense and homogenous 3-dimensional amino-modified core-shell nanoparticle surfaces were achieved via inkjet printing with 2-methyl-1-pentanol based ink. Nanoparticles dispersed in a poly ethylene glycol (PEG) based ink with adjusted physical properties for jetting such as viscosity and surface tension were printed onto glass substrates which resulted in loosely packed nanoparticle monolayer coatings after washing procedures to remove low volatile PEG 200. 23-mer oligonucleotide spotted onto inkjet printed amino-modified particle layers showed homogenous and sharply defined DNA spots. Proteins (streptavidin) were suspended in glycerol-based ink and printed on epoxy-silanized glass and kept their bioactivity after this printing procedure.

Keywords: inkjet printing, bio-ink, piezo-driven, micro-array, ink formulation

1 INTRODUCTION

Nanostructured core-shell particles with tailor-made affinity surfaces are utilized to generate micro-structured affinity surfaces by micro-spotting the particles to form densely packed amorphous nanoparticle layers [1]. These layers provide an enormous surface enlargement and thus attract notice to chip-based analytical or diagnostic technologies [2]. Core-shell nanospheres, constituted from a silica core and an organic shell, can be used to couple specific capture-proteins or DNA-probes by application of state-of-the-art bioconjugate chemistry [3].
Further to a spotting of biofunctional nanoparticles in a micro arrayed format, the generation of a covering nanoparticle-coat in a user-defined shape [4] allows for flexible design of microstructures and for fabrication of large bioconjugative interfaces on various substrates, eg. glass and polymer.

In view of the advantages that have been experienced in the past decade using biochip-based approaches to screening type experiments, biochip-production should be made a cheap, flexible and reproducible process [5]. Ink-jet printing is a relatively straightforward fabrication process in order to assemble reproducible nanoparticle-coatings.
This process enables economical coating of substrates by integrating the molecular functionalization of substrates in a fully automated process. In contrast to classical microarray printing, many formats of 2-D drawings can be rasterized into X- and Y-coordinates to deposit materials in a corresponding printed pattern. Inkjet printing technology has recently been used to fabricate electronic, medical, optical and polymeric devices [6, 7].
Not only biomolecules but also viable mammalian cells can be printed for different applications such as tissue engineering [8]. Ink-jet printers can dispense fluid droplets with volumes in the picoliter to microliter range. In contrast to thermal ink-jet printing piezoelectric ink-jet printing is a thermally constant process that can be carried out at room temperature. The chemical properties of inks determine their jettability: surface tension and viscosity are two primary material properties that determine the success of a printing process.
To ensure simple adaptability to current bio-chip technology, we aim to develop ink formulations containing functional components, which can be used to feed ink-jet printers to generate automated production lines for nanoparticle-based bio-chips.
We will present results concerning activation of the substrates, preparation of PEG-based nanoparticle inks and 2-methyl-1-pentanol based inks. Viscosity and surface tensions are physical parameters affecting the characteristics of printability. An important criteria for not only wetting the printed surface properly but also for the formation of homogenous particle layers, is the adjustment of the surface energy of the ink to the needs of the substrates.
Ink formulation considering the maintenance of the biological function during the printing process was additionally observed both in form of micro-spotting Cy5 labeled oligonucleotide on the jetted organo functionalized core-shell particle layers as well as by jetting streptavidin and analyzing the bioactivity of the micro-structures in a fluorescent assay followed by an optical scan.

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2 MATERIAL AND METHODS

Chemicals

The NH_3 solution (p.a. 25 wt. %), H_2O_2 (30%), ethanol (HPLC grade), poly-(diallyldimethylammoniumchloride) (PDADMAC, MW ~100000, 20 wt. % in dH_2O) and poly-(sodium 4-styrenesulfonate) (SPS, MW~70000), 2-methy-l-1 pentanol (99%) were purchased from SAF (Taufkirchen, Germany). tetraethoxysilane and 3-aminopropyl-2-triethoxysilane were purchased from ABCR (Karlsruhe, Germany). Polyethylenglycol 200 (PEG 200) was purchased from Th. Geyer.

Functional nanoparticles

Monodisperse suspensions of spherical core-shell nanoparticles were generated via the method of Stoeber [9]. For detailed information concerning synthesis of the particles and their surface modification, see elsewhere [3].
Briefly: nanoparticulate silica spheres were synthesized from a mixture of 13.7 ml NH_3 solution (25 wt. %) 20 ml H_2O, and 5 g tetraethoxysilane in 400 ml ethanol in a sol-gel reaction. The average diameter of the particles used in this work was 117 ± 16 nm. The organic particle shell was prepared by employing 3-aminopropyltriethoxysilane, thus introducing amino-functions to the particle surface.

Pre-treatment of the substrates

Cleaning microscope glass slides (Menzel GmbH & Co KG, purchased via SAF, Taufkirchen, Germany) was carried out in 2% HELLMANEX solution (Hellma, Müllheim, Germany). The glass-surface was subsequently hydroxylated by incubation at 3:1 (v/v) NH_3 solution (25wt. %) and H_2O_2 (30%) for 40 min at 70°C and rinsed in ultrapure water. The surface activation was achieved by coating of glass surfaces with polyelectrolytes (PE) using layer-by-layer technique by Decher [10]. The coating of the substrate cyclic olefin copolymer (COC) (TOPAS 8007) was processed the same way.

Microspotting and Fluorescent read-out

DNA-micro-arrays were generated by using a GMS 417 pin-ring micro-arrayer. Fluorescence was detected using an Arrayworksx Biochip Reader (Applied Precision, Issaquah, WA, USA).

Organo-functional nanoparticle inks

The nanoparticles with a mean diameter of 117 ± 16 nm were dispersed in two different solvents. To obtain alcohol based inks the previously prepared nanoparticle suspensions were directly dispersed into 2-methyl-1-pentanol. Concerning polyethylenglycol (PEG) based ink the nanoparticle suspensions were dispersed in a premixed composition of poly ethylene glycol 200 (PEG 200) with solvent. The mixing-ratio of premixed solutions was adjusted in according to the jettability of ink-jet printer Dimatix Materials Printer 2800 (Dimatix Inc. Santa Clara, USA). Dimatix Materials Printer 2800 requires viscosities of $10 \leq \eta \leq 12$ mPa*s and surface tensions of $28 \leq \sigma \leq 33$ mN*m^{1}. Surface tensions were determined by using a SITA Dyno Tester tensiometer (SITA Messtechnik, Dresden, Germany) and viscosity by using a Brookfield rheometer LV DV – III (Brookfield E.L.V., Middleboro, Mass., USA) at contant temperature of 25°C. Printing was proceeded at room temperature with 40°C plate temperature The formulated ink was filled in a printing cartridge after filtering through 450 nm Filter.

3 RESULTS

Considering the needed ink properties from piezo-driven Dimatix Materials Printer, several mixing-ratios were established. To obtain a basic ink in which nanoparticle suspension can be dispersed, PEG 200 was mixed with the solvents. Viscosity was adjusted to approximately 12 mPa s after this. Table 1 shows the physical properties of PEG 200 basic ink. First printing experiments were done with PEG 200 with Solvent 1 to check how stable the process is.

Table 1: Ink properties such as viscosity and surface tension for selected basic ink measured at 25°C to achieve optimum printing performance.

Basic ink	Viscosity [mPa*s]	Surface tension [mN*m^{-1}]
PEG 200 + Solvent 1	12.16	31.95
PEG 200 + Solvent 2	11.93	29.6
2-Methyl-1-Pentanol	5.49	24.04
Glycerol + Additives	11.43	36.7

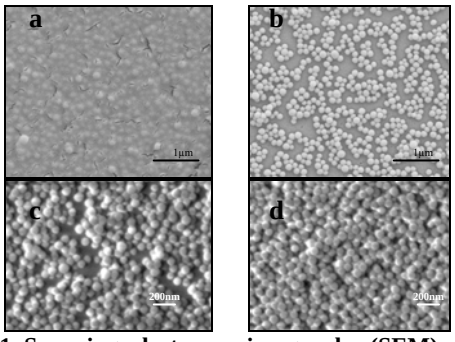

Figure 1 Scanning electron micrographs (SEM) of Amino-modified core-shell nanoparticle layers printed by a piezo-driven ink-jet printer. a) Printed particle monolayer covered by PEG, b) Loosely packed particle coating after washing step in ultrapure water to remove water soluble PEG, c) 3-fold particle layer printed onto pre-coated COC with 1-methyl-2-pentanol based ink, 20 µm drop-spacing led to loosely packed coating and d) 15 µm drop-spacing led to a highly densely packed 3-D particle layer.

Due to pre-processed glass substrates with a two-fold PDADMAC-SPS layer as described above, the amino-modified nanoparticle arranged after printing as a monolayer. PEG 200 is low-volatile and the deposited ink layer did not evaporate. Post processing, by washing the glass substrate in ultrapure water, removed PEG 200 but resulted in loosely packed monolayer particle coating as shown in Figures 1 a and b. Ultrasonication for 30 min prevented aggregation of particles at the nozzles

The printability of PEG 200 based ink on COC depends on its surface energy. With an 8-fold coating with PDADMAC-SPS which reduced the contact angle from 90° to approximately 60°, the programmed pattern could be printed (data not shown).

An alcohol-based ink is able to provide appropriate properties for printing without adding surfactants to reduce surface tension. Solvent-based nanoparticles were dispersed directly into 2-methyl-1-pentanol and physical properties were determined. Not only the printing process was stable without particle aggregation for at least 3 hours but also 3-dimensional patterning could be achieved. In addition to adjusting ink properties for an optimal printing process, printing parameters such as drop-spacing resulted in different particle coatings as shown in Figure 1 c and d. Drop-spacing is the distance in X and Y, center to center, of the drops that the in-jet printer will deposit to create the pattern. This parameter is most useful for altering the amount of jetted ink per unit area (fill density). The amount of jetted ink with 15 µm drop-spacing higher than that with 20 µm which resulted in loosely packed particle coatings with 20 µm.

Biofunctionality of the printed particles was tested by micro-spotting Cy5 labeled oligonucleotides (23-mer) followed by fluorescent scans. The oligonucleotides were spotted in 10 µM, 5 µM, 2.5 µM and 1.25 µM dilutions onto the COC surface which was coated with 4-fold PE and printed 3-fold with nanoparticles and resulted in homogenous and sharply defined DNA Spots (Figure 2).

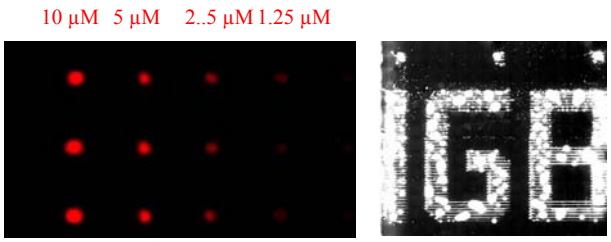

Figure 2 Bioactivity proven by fluorescent micrographs. Left hand: Cy5 labeled Oligonucleotide (23-mer) spotted onto ink-jet printed amino-modified core shell nanoparticle layer. Right hand: Streptavidin suspended in glycerol-based ink were printed onto epoxy-silanized glass substrate with a piezo-based ink-jet printer.

The protein streptavidin was dissolved in a glycerol-based ink and printed onto epoxy-silanized glass substrates. The microstructered patterns bioactivity was shown by fluorescence micrographs (Figure 2) thus showing that the bioactivity was maintained during the printing process.

4 CONCLUSIONS

Biochip fabrication needs processes for micro-structuring the surface which can easily be automated. This study shows that the process of nanoparticle printing to obtain bioactive 3-D surfaces which was previously established by contact printing techniques [1, 2, 4] can be successfully transferred to corresponding ink-jet printing processes. Creating homogenous and controllably packed particle surfaces is important for DNA micro-array technology as well as for protein micro-array technology to provide surface enlargement and hence maximize the observable flourescence signal intensities after biomolecular assay interactions [1]. The 2-methyl-1-pentanol-based ink showed optimal printing performance for bioactive core-shell nanoparticles. The alcohol enabled for a controlled printing process and evaporated excellently from the slightly heated substrates. The alcohol-based ink-jet printing process led to homogenous particle layers. For highest resolution of the printed patterns, surface energy of inks and substrates must be harmonized. The method shown here enables for micro-structure fabrication eg. for multiplex DNA hybridization experiments or protein-protein interaction screening.

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