Journal of Peptide Science
*J. Pept. Sci.* 2008; **14**: 217–223
Published online 26 November 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.963

JOURNAL OF PEPTIDE SCIENCE

# Controlled patterning of peptide nanotubes and nanospheres using inkjet printing technology‡

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*Received 6 July 2007; Revised 19 September 2007; Accepted 26 September 2007*

**Abstract:** Peptide nanostructures are expected to serve as a major tool in future nanotechnological applications owing to their excellent self-assembly properties, biological and chemical flexibility and structural simplicity. Yet one of the limiting factors for the integration of peptide assemblies into functional electro-organic hybrid devices is the controlled patterning of their assemblies. Here we report the use of inkjet technology for the application of peptide nanostructures on nonbiological surfaces. The aromatic dipeptides nanotubes (ADNT) which readily self-assemble in solution were used as an 'ink' and patterned on transparency foil and ITO plastic surfaces using a commercial inkjet printer. While inkjet technology was used in the past for the patterning of carbon nanotubes, it was not used for the deposition of biomolecular nanostructures. Furthermore, during the development of the application we were able to produce two types of nanostructures, i.e. nanotubes and nanospheres by the self-assembly of the same aromatic dipeptide, tertbutoxycarbonyl-Phe-Phe-OH (Boc-Phe-Phe-OH), under different conditions. Both spherical and tubular structures could be efficiently patterned on surfaces into predesigned patterns. The applications of such technology are discussed. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

**Keywords:** nanotechnology; bionanotechnology; peptide nanotubes; ADNT

## INTRODUCTION

Controlling the distribution, patterning and orientation of nanostructures is essential for many of the technological applications envisioned for supramolecular self-associated assemblies [1–3]. Most self-assembling materials are macroscopically disordered, a property which can limit their bulk properties and potential uses. In order to improve performances and enable new functions, patterning at the microscale is needed and will extend order in a predictable manner over large areas [4–9]. Several techniques for the growth of organized arrays of carbon and inorganic nanostructures were developed, including physical or chemical vapor deposition (CVD) and soft lithography techniques [1–3,10–13]. Recently, soft lithography was used to pattern and align PAs nanofibers [14].

Peptide building blocks demonstrate biocompatibility, chemical flexibility and versatility, biological recognition abilities and facile synthesis which make them a very attractive organic building block for bionanotechnology applications [15–21]. The recently identified ADNT represent a unique class of organic nanostructures. These bioinspired structures are formed by the self-assembly of the core recognition motif of the $\beta$-amyloid polypeptide into well-ordered hollow tubes [22]. The ADNT are readily formed under mild conditions from inexpensive building blocks. The dilution of a concentrated fluorinated alcoholic solution of the dipeptide into aqueous solution results in the efficient assembly of individual tubes with long persistence length. Furthermore, the ADNT have remarkable chemical and thermal stability [23,24] and extraordinary mechanical strength, which were directly measured through indentation-type experiments using atomic force microscopy. The averaged point stiffness of the nanotubes is 160 N/m, and they have a correspondingly high Young's modulus of ~19 GPa [25].

Methodologies were also developed for the horizontal and vertical alignment of the ADNT [26] and a controlled self-assembly of ADNT was obtained using enzymatic activation of self-immolative dendrimers [27].

ADNT can serve as a degradable mold for the fabrication of silver nanowires, as a scaffold for the organization of platinum nanoparticles and as a template for the formation of coaxial nanocables [22,28,29]. Furthermore, the tubes were used for the fabrication of sensitive electrochemical biosensors, [30,31] and a formation of ADNT biocompatible hydrogel has been demonstrated [32].

Moreover, tubular structures can assemble from noncharged aromatic dipeptide analog Ac-Phe-Phe-$NH_2$, in

Abbreviations: ADNT, aromatic dipeptide nanotubes; Boc, tert-butoxycarbonyl; Z, Benzyloxycarbonyl; CVD, chemical vapor deposition; $ddH_2O$, double distilled water; Fmoc, 9-fluorenylmethoxycarbonyl; HFP, hexafluoro-2-propanol; ITO, indium-tin oxide; MWCNT, multi-walled carbon nanotube; PA, peptide-amphiphile; Phe, phenylalanine; pLED, polymer light emitting diode; SEM, scanning electron microscopy.

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‡This article is part of the Special Issue of the Journal of Peptide Science entitled "Peptides in Nanotechnology".

which the *N*-terminal amine is acetylated and the *C*-terminal carboxyl is amidated, as well as the assembly of other amine-modified analogues: tertbutoxycarbonyl-Phe-Phe-OH (Boc-Phe-Phe-OH), Z-Phe-Phe-OH, and Fmoc-Phe-Phe-OH peptides into tubular and amyloid-like structures [33].

Inkjet printing, currently present in most households and offices, is a common method for transferring digital data to paper or transparencies. In recent years inkjet printing became a tool that can be used in various manufacturing processes to deposit minute quantities of materials, including lubricants for micromechanical parts, solder for microelectronics or UV-curable resins for the fabrication of micro-optical parts [34]. In the field of defined polymer deposition, inkjet printing is considered as one of the key technologies, in particular with regard to the fabrication of multicolor pLED displays and polymer electronic devices [35].

An inkjet printer was used to prepare nanometer-sized, unilamellar lipid and polymer vesicles with a controlled narrow-size distribution, this method also allows the efficient encapsulation of predefined drug mixtures [36]. Furthermore, conductive MWCNTs were modified to enable their dispersion in water and then dispensed on paper or polymer surfaces using an inkjet printer [37].

In the current work we present a cost-effective deposition method for generating patterns of peptide nanotubes on surfaces using a commercial desktop inkjet printer. The theoretical and practical considerations as well as the implications of the techniques for the field of bionanotechnology are being presented and discussed.

## EXPERIMENTAL SECTION

### Preparation of Initial Solutions of Peptides

The studied peptides, diphenylalanine (H-Phe-Phe-OH) and two *N*-terminal modified analogues: Boc-Phe-Phe-OH and Fmoc-Phe-Phe-OH, were purchased from Bachem (Bubendorf, Switzerland). Fresh stock solutions were prepared by dissolving lyophilized form of the peptides in 1,1,1,3,3,3-HFP (Sigma–Aldrich) at a concentration of 50 or 100 mg/ml. To avoid any preaggregation, fresh stock solutions were prepared for each experiment.

### Preparation of tertbutoxycarbonyl-Phe-Phe-OH (Boc-Phe-Phe-OH) Nanotubes and Nanospheres

For the preparation of nanospheres, a peptide stock solution was diluted in ethanol to a concentration of 10 mg/ml then immediately diluted in $ddH_2O$ to a final concentration of 5 mg/ml. For the preparation of nanotubes the 100 mg/ml peptide stock solutions in HFP was diluted into a final concentration of 2 mg/ml in $ddH_2O$. Immediately after dilution in $ddH_2O$, the peptide solution was allowed to dry at room temperature over glass cover slip for SEM analysis.

### Preparation of Solutions for Patterned Deposition

Diphenylalanine peptide, Fmoc-Phe-Phe-OH and Boc-Phe-Phe-OH stock solutions were diluted in ethanol to a concentration of 10 mg/ml and then immediately diluted in $ddH_2O$ to a final concentration of 5 mg/ml.

### Peptide Nanotubes Patterning using Inkjet Printer

HP Business Inkjet 2300 printer was used for 'printing' (i.e. patterned deposition) of the nanotube patterns on commercial transparency foil or on the conductive side of ITO plastic. An empty and cleaned cartridge was filled with one of the nanotubes or nanospheres solution, then 'printed' single or multiple times. Furthermore, different cartridges were filled with different solutions allowing the dispensation of different nanostructure on the same surface. All the surfaces were recorded using digital photography.

### Scanning Electron Microscopy (SEM)

The Boc-Phe-Phe-OH samples on glass cover slip and the samples deposited on transparency foil or ITO plastic were coated with gold. SEM images were made using a JSM JEOL 6300 SEM operating at 5 kV.

## RESULTS

### Preparation of ADNT Solutions and their Patterning using Ink Jet Technology

In order to enable accurate patterning of the ADNT, the printed solution should have appropriate evaporation ability; therefore we dissolved the dipeptide building blocks in a 50% ethanol solution. The diphenylalanine peptide (Figure 1(B)) was dissolved in HFP and then diluted into 50% ethanol to a final concentration of 5 mg/ml resulting in a peptide nanotube solution which evaporates rapidly. The ADNT solution was loaded into a clean printer cartridge and printed on transparency foil (Figure 2). The solution was dispersed on the transparency foil area once (Figure 3(A)) or several times (Figure 3(C)) in order to form condensed peptide nanotubes area. Following a single printing, as the dispensed solution dried on the surface, the printed area could easily be distinguished. Following multiple printing, minor inaccuracies due to the paper feeding mechanism could be observed. ADNT could be observed in the patterned area using SEM. The discrete, well-ordered, nanotubes were present only in the desirable areas. (Figure 3(B)). The use of multiple printing enables the formation of dense patterning of nanostructures. In addition, we were able to control the amount of 'ink' used in nanostructures solution, by simply changing the printer definitions and the quality of the printing. Moreover, we were able to control the density of the printed area by increasing or decreasing the concentration of nanostructure solutions (data not shown).



DOI: 10.1002/psc

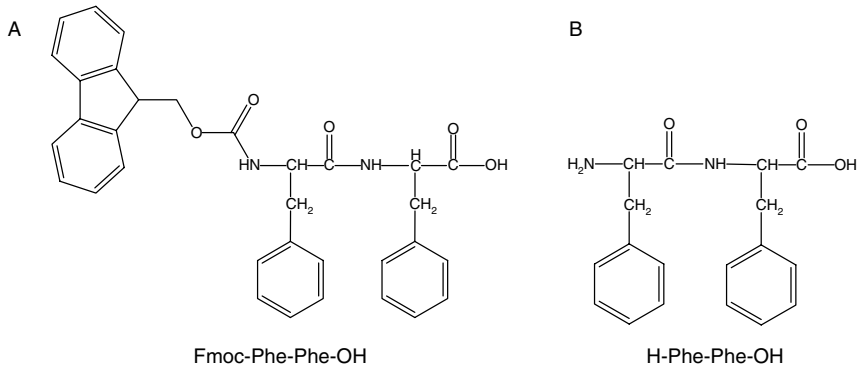


**Figure 1** The chemical structure of the studied dipeptides. (**A**) Fmoc-Phe-Phe-OH and (**B**) diphenylalanine peptides which self-assemble in solution to form ordered nanostructures.

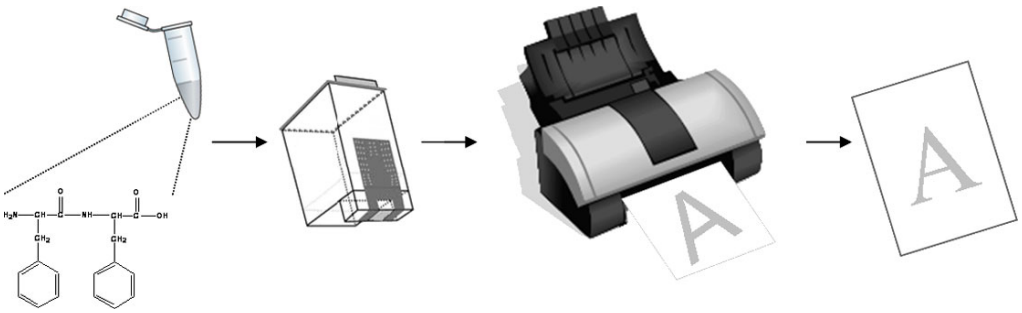

**Figure 2** Schematic presentation of the nanostructures patterning using inkjet printer. Dissolving the dipeptide monomer in HFP and 50% ethanol followed by their placing in an empty cartridge and 'printing' the solution on surface, which results in the patterning of peptide nanotubes on the surface. This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

Next we aimed at applying the inkjet printing on other self-assembling dipeptides. Similarly to the diphenylalanine peptide, its amine-modified analogs, the fluorenylmethoxycarbonyl-Phe-Phe-OH (Fmoc-Phe-Phe-OH) (Figure 1(A)), can self-assemble to form discrete ordered structures [33]. Here, Fmoc-Phe-Phe-OH was dissolved in HFP and then diluted into 50% ethanol at a final concentration of 5 mg/ml, then placed in the clean cartridge and dispensed on transparency foil-forming patterned nanotubes. The desirable pattern was observed on transparency foil surface and further analyzed using SEM which revealed the presence of nanostructures only in the printed area (Figure 3(F) and (G)).

## Self-Assembly of Boc-Phe-Phe-OH into Alternative Nanostructures

In addition, we were interested in the patterning of another self-assembling aromatic dipeptide, the tertbutoxycarbonyl-Phe-Phe-OH (Boc-Phe-Phe-OH) (Figure 4(A)). The peptide solution was prepared in the same manner as the previous dipeptides solutions. The solution was deposited on transparency foil, as the dispensed solution dried on the surface, the printed area was remarkably clearer and homogenous (Figure 5(C)) then the printed areas of diphenylalanine peptide or Fmoc-Phe-Phe-OH. Moreover, SEM analysis showed that the printed area is composed of spherical structures (Figure 5(D)) rather then tubular ones, which were observed in the other two dipeptides patterns. Interestingly, while dissolving the Boc-Phe-Phe-OH in HFP and water results in the formation of tubular structures, dissolving of Boc-Phe-Phe-OH in HFP and 50% ethanol results in the formation of spherical structures at a diameter of a few hundred nanometers. Hence, owing to different dissolution conditions, the same dipeptide, the Boc-Phe-Phe-OH, can alternately self-assemble into nanotubes (Figure 4(B)) or nanospheres (Figure 4(C)). Furthermore, we used several color cartridges each filled with different peptide solutions to form patterned surfaces composed of diverse structures and materials (data not shown).

## Nanostructure Patterning on Indium-tin Oxide (ITO) Surface

The application of ADNT for the fabrication of metallic nanowire has already been demonstrated [22,28,29]. In order to combine the ADNT in nanoelectrical devises there is a need of their patterning on conductive surfaces such as ITO. We used inkjet printing to pattern the



DOI: 10.1002/psc

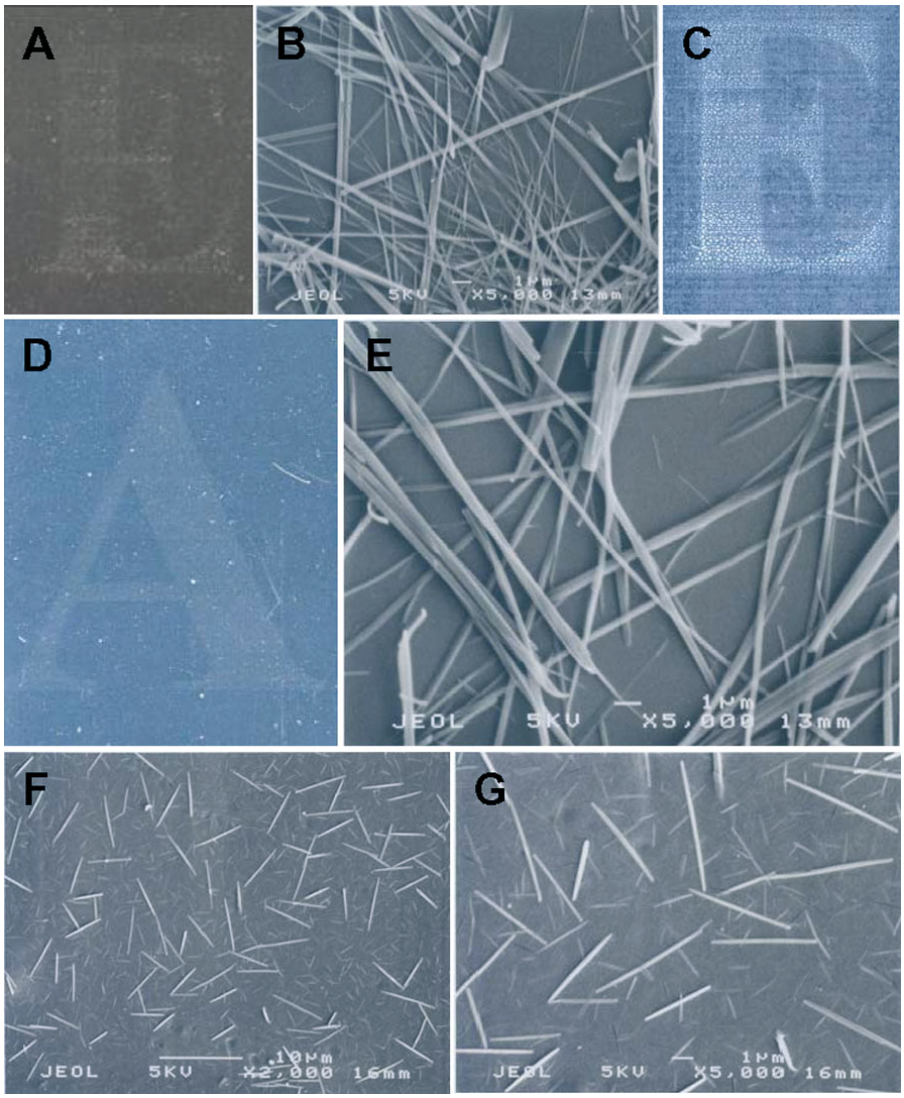


**Figure 3** Peptide nanostructure patterning using inkjet printer. Diphenylalanine peptide nanotube solutions deposited on transparency foil. (**A**) A single 'printing' of the letter 'E'. (**B**) SEM micrograph of the printed area in (A). (**C**) Multiple 'printing' (ten repeated 'prints') of the letter 'E'. Diphenylalanine peptide nanotubes solution deposit on ITO plastic. (**D**) A single 'printing' of the letter 'A'. (**E**) SEM micrograph of the nanotubes composed the printed area in (D). (**F**) and (**G**) SEM micrograph of a single printing of Fmoc-Phe-Phe-OH nanotubes solution deposited on transparency foil.

ADNT on the conducting side of ITO plastic. The nanotubes forming diphenylalanine and Fmoc-Phe-Phe-OH peptides solutions as well as the nanospheres forming Boc-Phe-Phe-OH peptide solution were dispensed on ITO plastic surface. The flexibility of the ITO plastic enables its easy transition in the printer. The patterned nanotubes and nanospheres could easily be observed on the transparent ITO plastic. Although the diphenylalanine peptide and Fmoc-Phe-Phe-OH nanotubes patterns on the ITO plastic were clear (Figure 3(D) and (E)), the Boc-Phe-Phe-OH nanospheres patterning on ITO (Figure 5(A) and (B)) appeared to be sharper and more homogenous as compared to the patterning of the nanotubes. These findings are similar to the findings of the patterning of the nanotubes compared to nanospheres on transparency foil (Figure 5(C) and (D)). Indeed, the ability to fill or coat the peptide nanotubes with metallic materials, together with their patterning on conductive surfaces may be highly useful in the application of nanoelectronics. Furthermore, both surfaces: transparency foil and ITO plastic patterned with each of the nanostructures, demonstrated durability. Eight months after the printing, the printed area is still visible and similar to a fresh pattern substrate (data not shown).

## DISCUSSION

The orientation and patterning of nanostructures is central to many nanotechnological applications. In the current study we present a novel approach for the patterning of ADNT on different surfaces using inkjet



DOI: 10.1002/psc

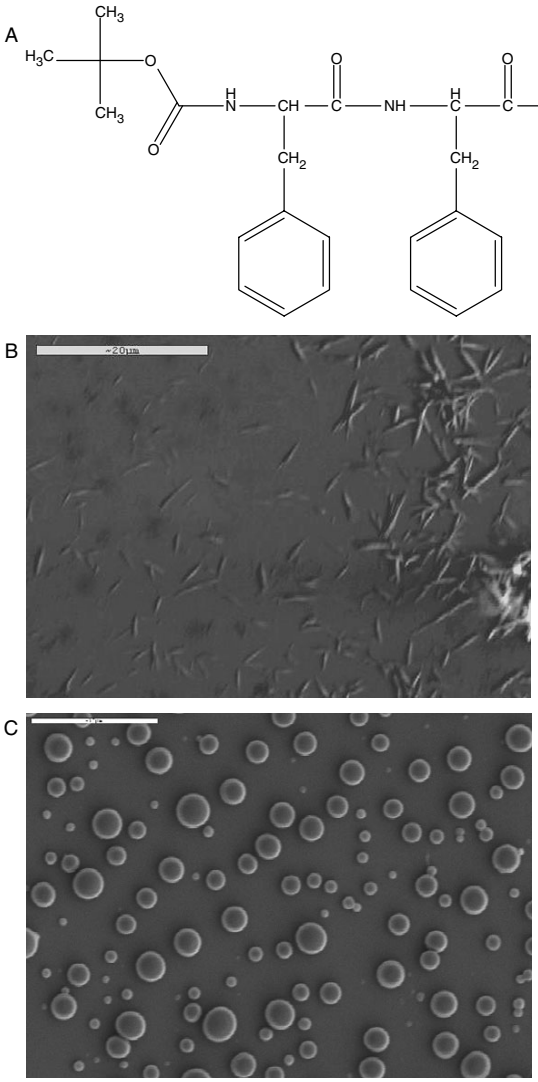


**Figure 4** Formation of nanotubes or nanospheres by the self-assembly of the tertbutoxycarbonyl-Phe-Phe-OH (Boc-Phe-Phe-OH) peptide. (**A**) The chemical structure of Boc-Phe-Phe-OH. (**B**) Dissolving the peptide in HFP and water results in the self-assembly of nanotubes. (**C**) Dissolving the peptide in HFP and 50% ethanol results in the self-assembly of nanospheres.

technology. One of the advantages of peptide nanotubes in general and ADNT in particular is their ability to disperse in aqueous solution, here we used this characteristic of the ADNT to form 'ink' solution for the patterning and deposition of nanotubes.

In the present study, aromatic dipeptides, diphenylalanine (H-Phe-Phe-OH) and two *N*-terminal modified analogs, Fmoc-Phe-Phe-OH and Boc-Phe-Phe-OH were studied. The diphenylalanine peptide and the Fmoc-Phe-Phe-OH, self-assembled in solution to form nanotubes. The other amine-modified analog, Boc-Phe-Phe-OH can self-assemble into nanospheres or nanotubes. All of the peptides were dissolved in HFP and 50% ethanol solution and then injected on the designated area using a simple computer-based ink-injection printer. The quick evaporation properties of the solutions ensured that the nanostructures will be placed accurately and rapidly at the desirable location. We demonstrate the deposition and patterning of the nanostructures on transparent foil and on conductive surface of ITO plastic.

The ability to fill or coat the peptide nanotubes with metallic materials was previously demonstrated, suggesting their use in nanoelectronic devices. The ADNT can serve as a degradable mold for the fabrication of silver nanowire [22] and allow the formation of platinum–nanoparticle composites [29]. Moreover, a coaxial metal nanocable formation using the ADNT scaffold was demonstrated [28]. The patterning of ADNT on conductive surface, as demonstrated in this work, is needed in order to combine the ADNT in nanoelectrical applications. We further demonstrated the ability to form multistructure patterns using a color inkjet printer with several cartridges each filled with different nanostructure solution. Moreover, in order to control the density of the nanostructure areas we used three approaches: (i) Multiple printing. (ii) Changing the printer definitions regarding the amount of 'ink', nanostructure solutions, used. (iii). Changing the concentrations of nanostructure solutions. We suggest that combining all of these manipulations to the nanostructure solutions and to the printing procedure will allow the formation of the desirable patterning of nanostructures.

One interesting aspect of the current work was the identification of the two alternating organization states of the Boc-Phe-Phe-OH peptide. We discovered that this peptide could form either tubular or spherical structures upon modification of solution composition. This is in line with other nanostructures such as carbon and layered inorganic structures that exhibit either tubular or spherical structures. This phenomenon is also observed for phospholipid structures that mainly exist in a spherical structure formed by the closure of two-dimensional lipid layer, but under certain conditions can form lipid nanotubes. Taken together, this is another support to our suggestion that the formation of peptide nanotubes and nanospheres by aromatic dipeptides is facilitated by the closure of a two-dimensional peptide layers [38].

In conclusion, the use of desktop inkjet printer for the deposition of ADNT opens a variety of possibilities for using peptide nanotubes in technological devices. Existing printing technology combined with novel nanomaterials can allow the creation of exciting new electronic and medical devices.

## Acknowledgements

We thank Asaf Tamir for technical assistance in this project, Dr Meital Reches for her help in

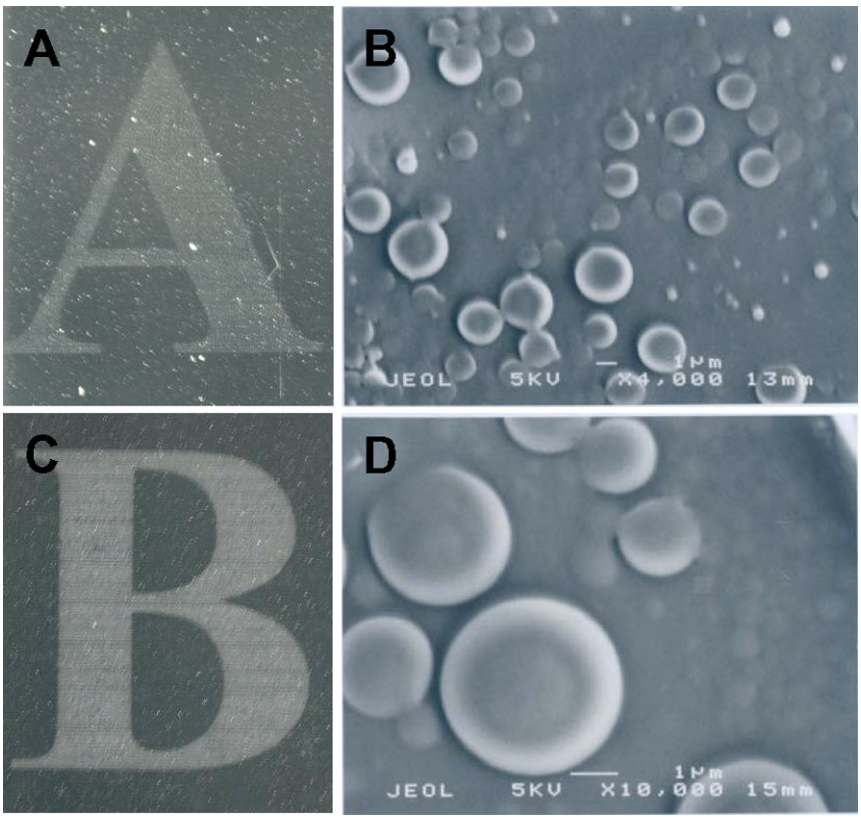


**Figure 5** Peptide nanospheres patterning using inkjet printer. A single printing of the letter 'A' using the Boc-Phe-Phe-OH nanospheres solution on ITO plastic. (**A**) The entire printed area. (**B**) SEM micrograph of the nanospheres composed the printed area in (A). A single printing of the letter 'B' using the Boc-Phe-Phe-OH nanospheres solution on a transparency foil, (**C**) The entire printed area. (**D**) SEM micrograph of the nanotubes composed the printed area in (C).

this project, Stephan Hauschild for his kind help with the printer and cartridge modifications, Moshe Alexandroni for help with the digital camera images, Dr Zahava Barkay for help with the SEM experiments and members of the Gazit laboratory for helpful discussions. We acknowledge the financial support of the European Commission 6FP BeNatural consortium. E.G. acknowledge the support of the Israel Science Foundation (ISF). L.A.A gratefully acknowledges the support of the Colton Foundation.

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