Vertically Aligned Patterned Peptide Nanowires for Cellular Studies

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ABSTRACT

In this study, we present the influence of vertically aligned diphenylalanine peptide nanowires (PNWs) on cell growth and adherence using PC12 cells. Following the cell growth study, we simply concluded that the PNWs promoted PC12 cells' growth compared to bare gold surfaces. Moreover, we show a technique for the patterning of PNWs down to 10 micron wide strips to further investigate cell behavior and functionalization possibilities. We also investigated how the structural properties of patterned nanowire strips could be used as a cell scaffold in combination with adherence enhancers such as laminin (LAM) .

Keywords: self assembly peptide nanostructures, cell culture, patterning, PC12, diphenylalanine

1 INTRODUCTION

Over the years, scientific studies have shown that one crucial point when designing biological platforms is the strict environmental conditions required for cell and tissue culturing, such as pH, temperature, medium content and other parameters which affect the system's biocompatibility.

Diphenylalanine is reported to be the core recognition peptide in amyloid fibrils that are responsible for Alzheimer's disease [1]. Studies have revealed that diphenylalanine peptides with different functional groups and fabrication conditions can self-assemble into nanostructures such as nanowires, nanotubes, nanoparticles, and hydrogels. Self-assembled peptide nanostructures make an excellent candidate as a material for biological applications due to the inherent properties they hold, such as mechanical and chemical stability, various functionalization options, and mild, fast and inexpensive synthesis conditions [2].

Vertically aligned PNWs are grown by high temperature aniline vapor aging from thin amorphous diphenylalanine films [3]. Since their first report, PNWs aroused great attention among scientist. While some of the researches were focusing on discrete functionalization of PNWs to alter properties like hydrophobicity [4] and conductivity [5], others tooled with the patterning of PNWs and building up functional micro devices [6, 7]. Moreover it is strongly highlighted that we should expect a vast number of studies regarding 3D cell culture, drug delivery, bioimaging and biosensor development involving these biological nanostructures in the very near future [8].

Recently, our group has demonstrated that PNWs are a useful tool for cellular studies and sensor applications [9]. In this paper, we expand this study with different approaches. Firstly we cultured PC12 cells, which are neuronal stem cell models [10], onto different substrates (bare gold, laminin coated gold, PNWs on gold and laminin coated PNW on gold) to obtain an investigation of cell growth onto the mentioned surfaces. Secondly we patterned PNWs into strips of various widths onto a gold electrode surface by soft lithographic methods to evaluate the patterned structures' effects on cell growth and adherence using PC12 cells.

Combining this work with other approaches like discrete functionalization of PNW will reveal possible future platforms for cellular studies and biosensing.

2 EXPERIMENTAL

2.1 PNW Growth

PNWs were fabricated prior to experiments from freshly prepared 50 mg/ml diphenlyalanine solution in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) as described [3]. Gold surfaces were fabricated in cleanroom by e-beam evaporation on 10 nm titanium coated silicon wafers and were diced into 0.5 x 0.5 cm^2 squares. After placing a drop of peptide solution on the desired surfaces, samples were dried in a vacuum desiccator to obtain an amorphous film. Then the samples were located in a sealed chamber in the presence of aniline vapor, by including a separate container of aniline reagent in the chamber. The chamber was left for aniline aging in an oven for 14 hours at 120 °C.

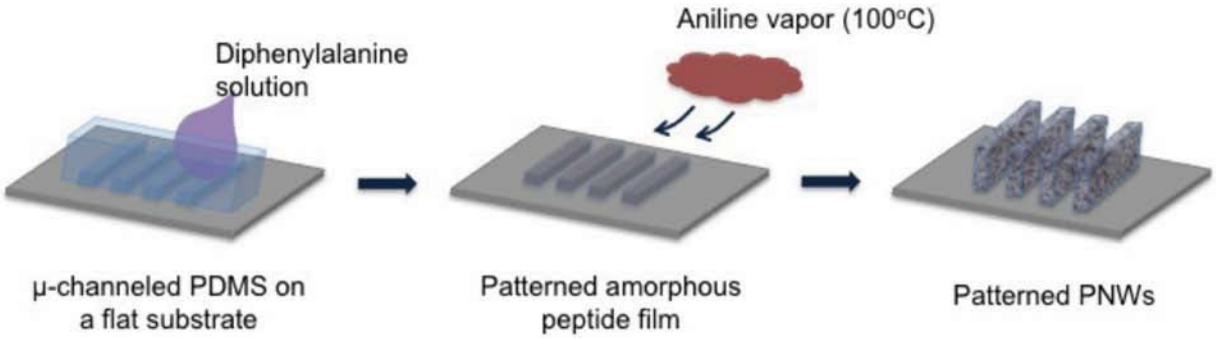

Figure 1 An illustration of PNW patterning using soft-lithorgapic methods.

2.2 PNW Patterning

PNWs were patterned onto substrates using a soft-lithographic method (see Fig. 1). PDMS stamps were fabricated in cleanroom using a patterned mask to etch channels with different dimensions on a silicon wafer. Etched silicon PDMS stamps with 10-20 μm wide micro channels used to fabricate PDMS molds. After locating the PDMS mold on a gold surface, a drop of peptide solution was introduced to the micro channels' openings and the channels were filled by capillary force. This process was followed by conventional PNW fabrication.

2.3 Cell Culturing

A thin laminin layer was introduced to the gold surfaces by 2 hours of direct contact with a 20 ug/mL laminin solution in cell culture tested water. The coated surfaces were washed two times with phosphate buffered saline solution (PBS).

PC12 cells in concentration of 10000 cells/ml were seeded onto the following surfaces: bare gold, LAM coated gold, PNWs on gold and LAM coated PNWs on gold. Cells were cultured in Dulbecco's Modified Eagle medium/Ham's Nutrient Mixture F12 supplemented with 10% fetal bovine serum, 10% horse serum, 100 Units/mL penicillin, 100 μg/mL streptomycin and 25 mM HEPES medium for one day before replacing the cell culture rmedium with a differentiating medium containing DMEM/F12 supplemented with 0.5% HS, 0.5% FBS, 100 μg/mL penicillin/streptomycin, 25 mM HEPES and 0.1 μg/mL Nerve Growth Factor (NGF). The samples were investigated after 48-96 by Optical and Scanning Electron Microscopy (SEM).

PC12 cells, in concentration of 330000 cell/ml were seeded on patterned PNWs on gold surfaces with and without LAM. The same cell culturing protocols and materials mentioned above were used for these experiments. Samples were investigated after 4 days of culturing.

2.4 Microscopy Imaging

Cell cultures were fixed prior to inspection with SEM. Direct contact of 2 wt/vol % gulataraldehyde solution in 0.1 M PBS was employed for fixation. After that, samples were rinsed twice with PBS for 5 minutes and washed with sterile water for 10 minutes. This step was followed by dehydration step using ethanol in concentration of 60-70-80-90-100 vol/vol % respectively and the samples were air dried.

A FEI Nova 600 NanoSEM system (FEI Company, Oregon, USA) was used to obtain Low Vacuum SEM images of Patterned PNW cell cultures.

For the cell growth study, an optical microscope was used to count cells on 48^{th}-96^{th} hours of culture. Several areas of samples were scanned and an average number of cells that were observed was used to plot cell growth bars including standard deviations.

3 RESULTS AND DISCUSSION

The cell growth study has shown that PNWs were suitable scaffolds for PC12 cells. PNWs clearly promoted cell adherence compared to bare gold surfaces (see Fig. 2). More over results indicated that introducing a standard protein adherence enhancer, laminin, increased cell growth as expected. It is also important to note that PNWs coated by LAM yielded a more favorable environment than LAM coated gold surfaces.

The patterning of PNW strips with widths of 10-20 μm were successfully done using the techniques mentioned above. The structures and morphology of the patterned nanostructures were found to be exactly the same as in the case of unpatterned PNWs.

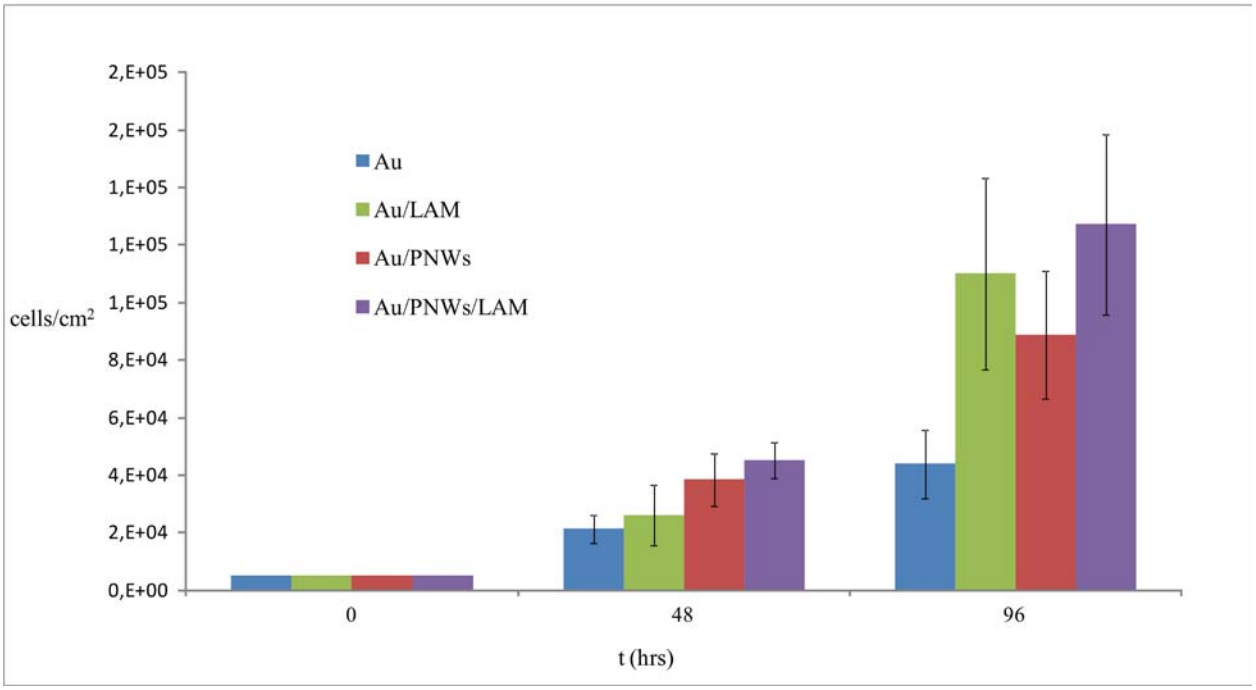

Figure 1 PC12 Cell Growth Study for various surface conditions, showing cells present on samples with surfaces of: i) bare gold (Au), ii) laminin coated gold (Au/LAM), iii) PNWs on gold (Au/PNW), and iv) laminin coated PNWs on gold (Au/PNWs/LAM).

However, patterned PNWs did not prove to be a convenient scaffold for PC12 growth without the LAM coating. No significant cell adherence and growth was observed on patterned PNW surfaces without the thin LAM coating (see Fig. 3). The LAM coating to patterned PNW surfaces drastically improved the PC12 cell growth (see Fig. 4). From these results, it can be concluded that although there is a significant interaction between PNWs and PC12 cells that results in this surface being a suitable scaffold, this interaction is not powerful enough to offer a relevant cell growth in the case of patterned PNW surfaces without the LAM. These results could be related to the narrowness of PNW strips being in the same range as the dimensions of a PC12 cell, which is around 5-10 µm. Moreover, the packing of PNWs, related to the diphenylaniline solution concentration used in the PNW fabrication, could interfere with cell growth. Further studies regarding wider PNW strips and PNWs with lower density will give deeper insight to these issues.

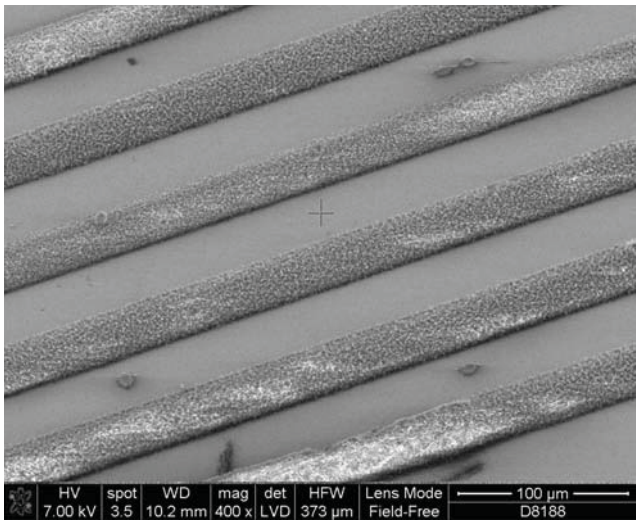

Figure 3 SEM image of PC12 cells on 20um wide PNW strips on gold chips.

OUTLOOK

We showed how PC12 cells' growth was affected by different surface modifications based on PNWs, showing how the presence of PNWs increased cell growth and adhesion as compared to plain gold surfaces. A method for the patterning of PNWs based on soft lithography was described. The growth conditions investigated in this study yield a useful technique for substrate modification that can be useful for discrete cellular studies. This study can be expanded with PNWs of lower packing densities which may create a more favorable environment for PC12 cells while preserving the functionalization possibilities offered by the PNWs. Furthermore wider PNW strips may create a more stabile cell culturing environment. Additional biocompatibility and cell viability studies can be carried out to incorporate PNWs in cellular studies with the novel approaches mentioned above.

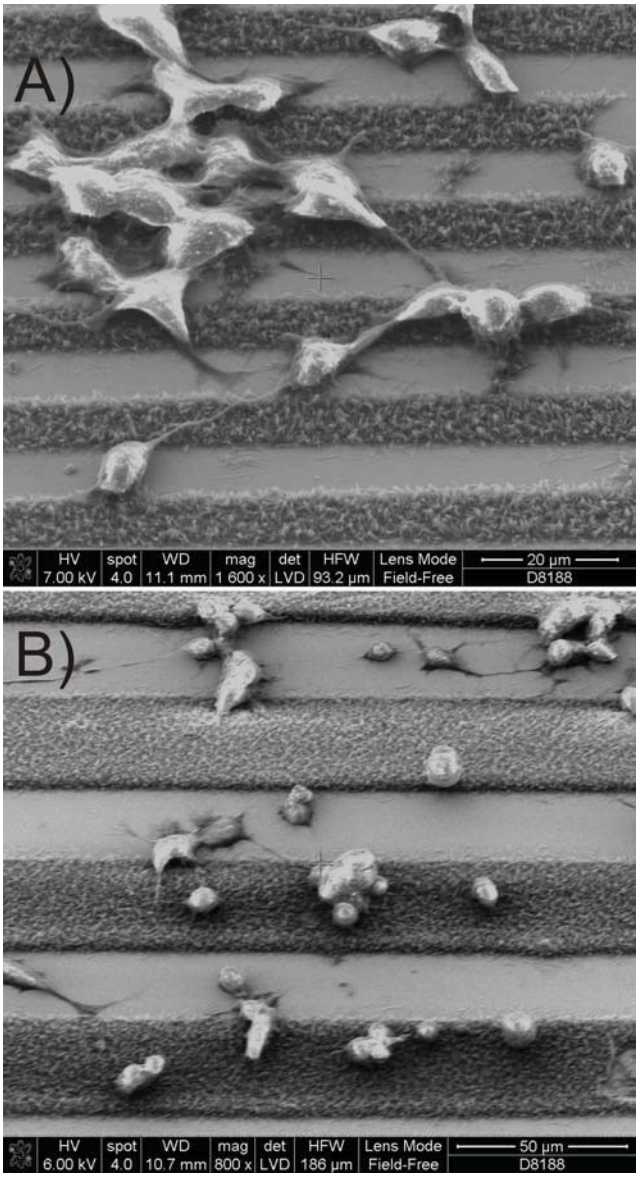

Figure 4 SEM images of PC12 Cells cultured on top of LAM coated PNW surfaces of A) 10 µm wide strips B) 20 µm wide strips.

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