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PROOF-OF-CONCEPT EXPERIMENT REPORT

CHARACTERISATION OF NANOSTRUCTURED SURFACES TO DEVELOP A REPRODUCIBLE FABRICATION OF CELL CULTURE SUPPORTS

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Main aim of the proposal

Micro and nanopatterned substrates are of great interest in cellular biology and especially in the rapidly growing field of mechanobiology. The ability to realize nanostructured surfaces with high efficiency and productivity is therefore required for the production and commercialization of such supports. In detail, glass coverslips with nanostructures on the surface are widely used for the investigation of various cell processes such as migration, adhesion, differentiation. The nanostructured supports can be a strong instrument for tissue engineering, because these surfaces can be used to mimic the cellular microenvironment, and to reproduce tissue stimuli.

Before establishing a large production of nanostructured supports, it is fundamental therefore to evaluate the reproducibility of the imprinting technique Pulsed-NIL and the titanium deposition process on the substrate used by the company.

For this reason the characterization and the measure of the size of the imprinted structures (i.e. pillars diameter and distance, period length, label dimensions and grid lines distance) is needed.

Approach and Results

The sample platform developed by the company had the organization shown in Fig. 1.

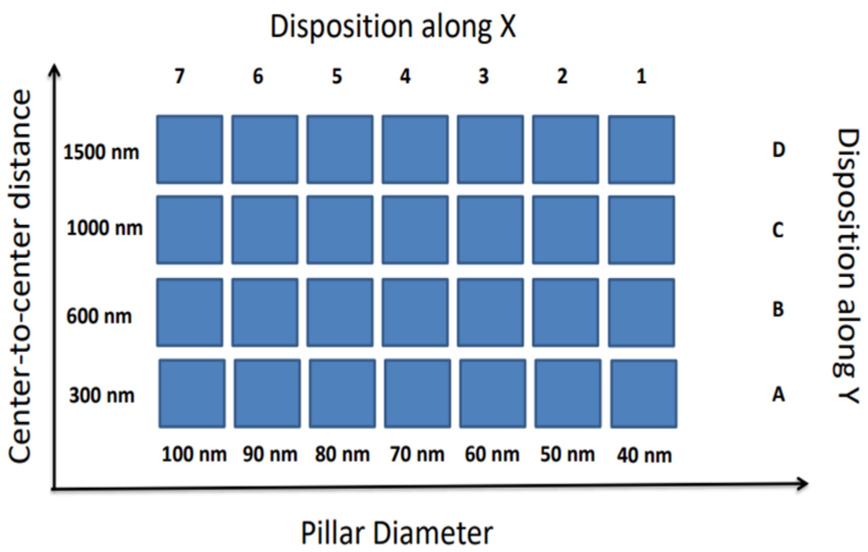

Figure 1: Organization of the printed field in the platform provided by the company.

The company was mainly interested in the evaluation of the pillar distance (center-to-center distance) and pillar diameter for the line A (A2-A7), while for the field B and C they were interested only in the field with larger pillar diameter (B7, C7).

To analyse these nanostructures we proposed Atomic Force Microscopy (AFM) measurements. This imaging technique can accurately measure the size of nanostructures on the surface and their position.

The measurements were performed with an Asylum Intrument in tapping mode by using cantilever with nominal spring constant 2.8 N/m (NanoWorld Arrow FM). Fig. 2 shows some representative images of the substrates with different pillar distance.

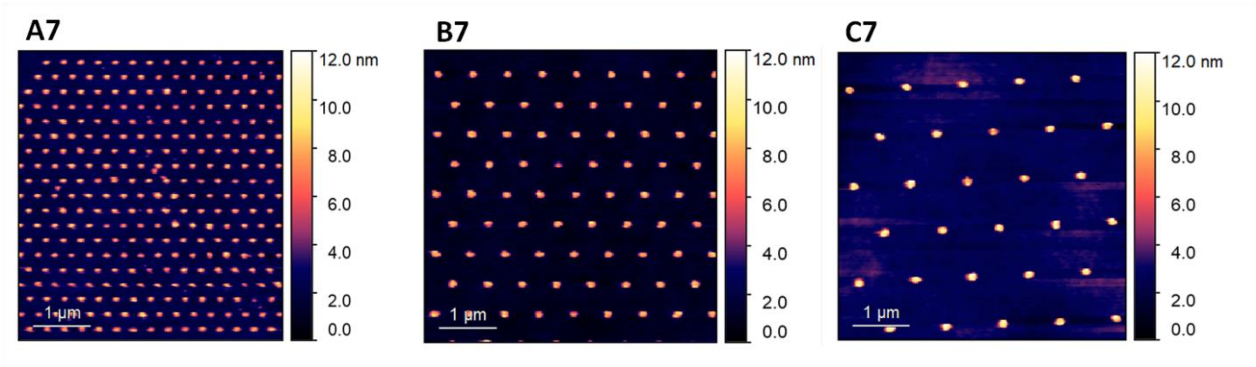

Figure 2: Examples of the AFM images obtained for three imprinted fields of the platform

On these images the size of pillar distances and pillar diameters were evaluated by profile analysis as shown in Fig. 3. The results are reported in Table I.

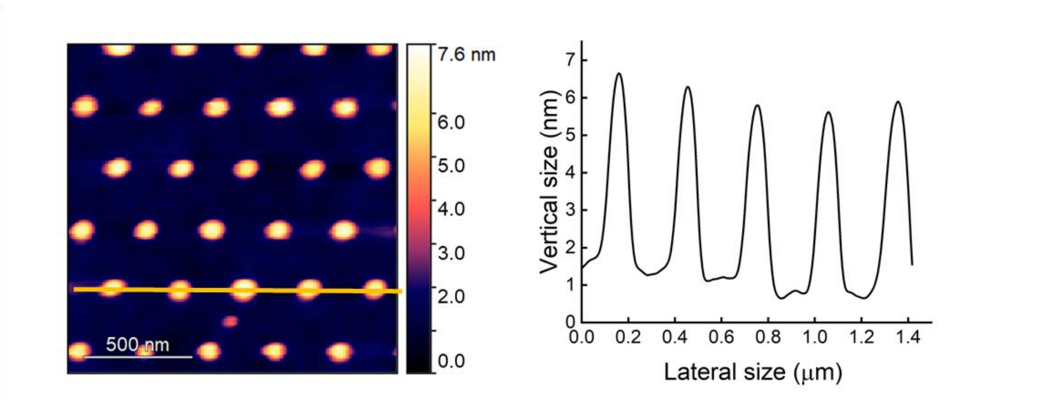

Figure 3: AFM image of a sample area and profile along the yellow line.

The pillar distances well match the expected size for A, B and C lines. For line A, the pillar diameters appear larger than the expected values, but this is not surprising in AFM images.

This is an inherent feature of AFM and it is very difficult to fully remove it. Any AFM image is a convolution of the shape of the probe, and the shape of the object imaged. This has the effect of making protruding features appear wide. However if we observe the diameter of pillars from A2 to A7, the evaluated diameters slightly increase in agreement with the expected increment of pillar diameter.

Table I : pillar distances and pillar diameters, these last ones evaluated as Full Width Half Maximum (FWHM).

	C1	C2	C4	C5	C6	C7
Pillar distance (nm)						1023 ± 29
FWHM (nm)						114 ± 16
	B1	**B2**	**B4**	**B5**	**B6**	**B7**
Pillar distance (nm)						594 ± 12
FWHM(nm)						112 ± 8
	A1	**A2**	**A4**	**A5**	**A6**	**A7**
Pillar distance (nm)		301 ± 11	297 ± 11	318 ± 13	303 ± 14	292 ± 22
FWHM(nm)		84 ± 8	100 ± 10	123 ± 20	111 ± 13	125 ± 15

Summary and final remarks about the effectiveness of AFM to evaluate the nanopatterning of the substrate.

The AFM provided a good and reliable method to analyze the nanostructures fabricated on surfaces. As respect to other high resolution imaging techniques (i.e. Scanning Electron Microscopy) the AFM does not require any metallization for the imaging and as result the sample can be imaged without any further coating, which could interfere with the real shape and size of the nanostructures. Moreover, AFM can provide the additional information about the height of the nano-pillars, which can be relevant in some cases.

Hence the AFM imaging is a valuable tool to examine the result of a nano-fabrication technique and to settle a reproducible standard methodology to generate large quantity of support with nanostructures.

This report has been written by Laura Andolfi

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