Cell contact guidance along topographical-directionality gradients

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Abstract

It is well established that the cell behavior is influenced by geometrical patterns in the micrometric and sub-micrometric range. In fact, the physical shape of anisotropic nanostructures induces alignment or directional growth of cells, the contact guidance phenomenon [1] . Ordered micro-/nano-structures coexist, *in vivo,* with some degree of topographical noise due to cellular debris, protein aggregates or scar-tissue invasion. Previous studies have enlightened the role of topographical noise on neurite guidance of single PC12 cells [2][3]. Contact guidance is also a strong regulator of collective directional migration, which could also be established either thorough intrinsic cellular mechanism or driven by single, or combination of external cues such as chemical gradients (chemotaxis) or gradients of substrate stiffness (durotaxis).
In particular, collective durotaxis has recently emerged as a far more efficient mechanism than single-cell durotaxis [4].
Here, we present a fibroblast and epithelial single cell motility study on topographical gradients of directionality. Starting from a perfect micro-grating of 2 µm in periodicity (50% duty cycle), the anisotropic signal is progressively lost in space by adding a increasing degree of nanotopographical noise. Moreover, in the second type of substrates, cell migration was followed on large areas (1cm x 454 µm) with a fixed degree of noise. Thanks to soft lithography techniques, we could replicate noisy gradients on biocompatible materials, such as polydimethylsiloxane (PDMS) and cyclic-olefin-copolymer (COC), and test them with Primary Human Dermal Fibroblasts (HDF) and MDCK-II cell line. Altogether, our results may provide a better understanding of tissue repair mechanisms, as well as information about cell migration during development or cancer invasion.

Biotopographical noise: a new method to fabricated and test topographical-directionality gradients.

- Our aim is an increased knowledge of contact guidance mechanisms by the study of the interaction of cells with a gradient of nanomodifications of perfect nanogratings (NGs).

- Nanomodifications were randomly added on a 2µm-periodical NGs, so that the directionality signal degrades adding a controlled percentage of bridges between two subsequent ridges, starting form a perfect grating down to a flat control surface.
- Each noise density (from 10% to 90%, density step of 10%) determines the actual single area directionality (**Figure 1**), as the average ratio between the substrate periodic signal component and the low-frequency noise in the Fourier Transform domain, defined in ref [5].

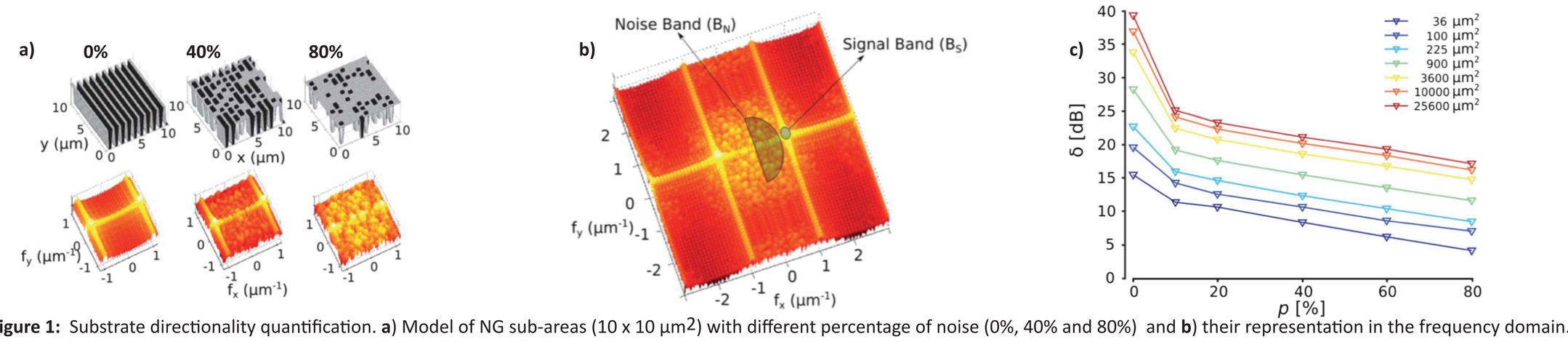

Figure 1: Substrate directionality quantification. **a**) Model of NG sub-areas (10 x 10 µm^2) with different percentage of noise (0%, 40% and 80%) and **b**) their representation in the frequency domain. **c**) Substrate directionality as a function of noise density, for several values of the size of the NG area[5].

- In order to evaluate how relevant could be the influence of bionoise on aligned cell motion, we have fabricated two types of artificial noise gradient: one for single-cell studies, where the single area of given noise density is comparable to the single-cell area (**Figure 2 a**), and 1cm^2 mirrored area, useful for next collective motion studies (**Figure 2 b**). Both types of noise gradients were replicated in two different biocompatible materials, COC and PDMS (**Figure 2 c**) thanks to standard soft lithography techniques.

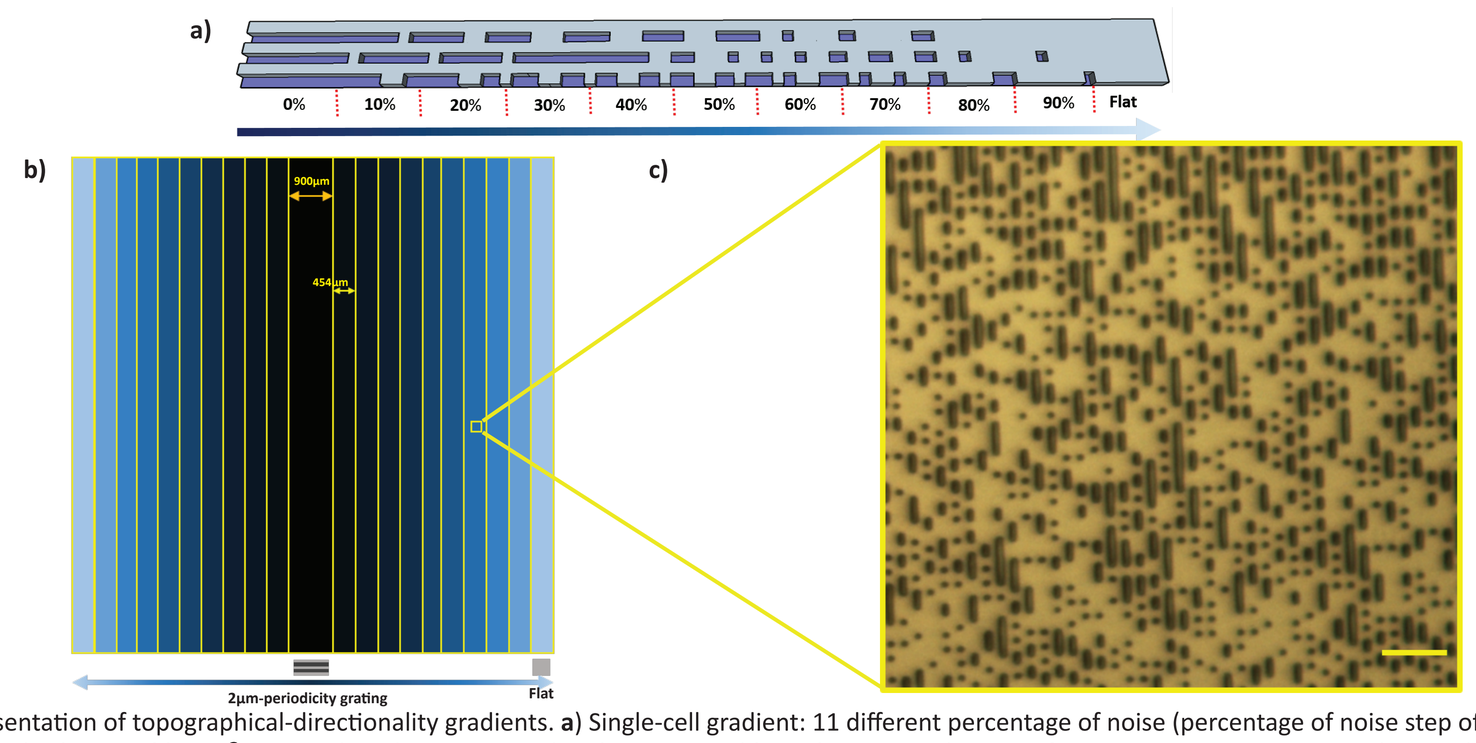

Figure 2: Schematic representation of topographical-directionality gradients. **a**) Single-cell gradient: 11 different percentage of noise (percentage of noise step of 10%) starting from a 2µm-periodical NGs down to the Flat control substrate. **b**) 1cm^2 mirrored gradient topography. **c**) Biocompatible replicas: optical image of 70% noise area on PDMS replica. Scale bar: 10µm.

Single-cell study *in vitro* Human Dermal Fibroblast and MDCK II cell lines

- Preliminary *in vitro* tests were made with two different cell lines seeded at low density (5*10^3 cell/cm^2): MDCKII (EGFP-nuclei stained) and HDF (far-red siRNA nuclei stained).

Those cell lines exhibit very different behavior: while MDCK II single-cell motility is influenced by neighboring cells, HDF cells behave more independently.

- Both types of cells were first seeded on Flat COC and Flat PDMS replicas, to evaluate cell speed and track straightness on both materials for 24h (**Figure 4**).

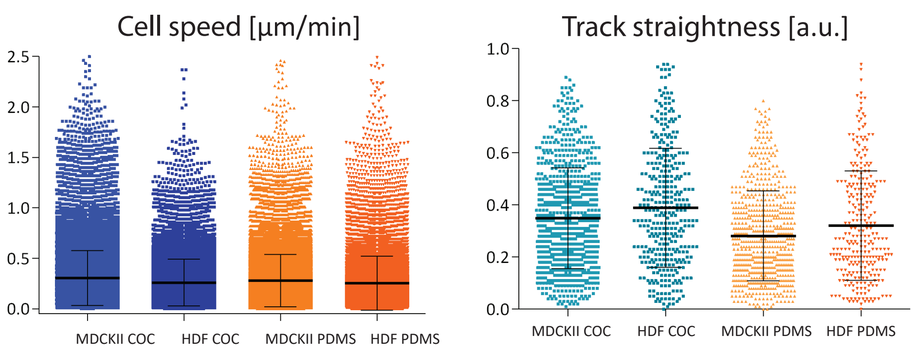

Figure 4: Cell speed and track straightness values of MDCK II and HDF seeded on Flat PDMS and COC replicas. Data sets are reported as mean±SD.

- We have tested topographical-directionality gradients for 24h in order to evaluate alignment angle of single cell (**Figure 5**) during their motion. MDCKII and HDF seeded on top of perfect grating seems to mantain the alignment in time, while cells seeded on the higher percentage of noise seem to have a randomic walk.

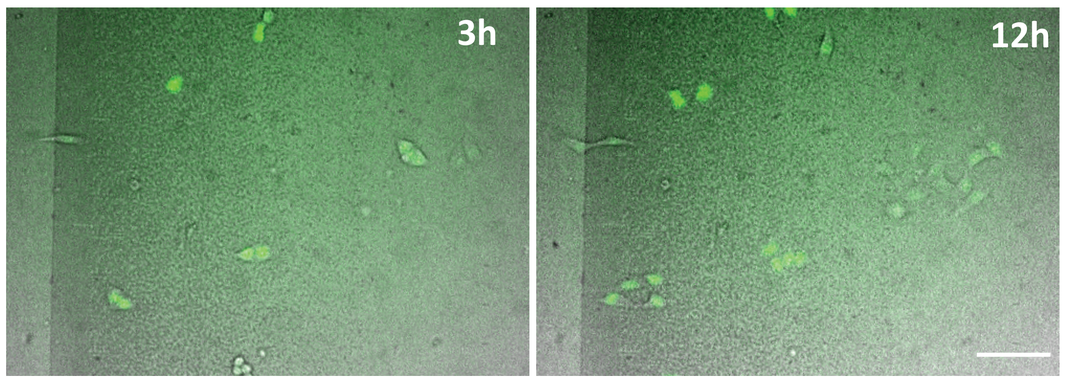

Figure 5: Representative images of MDCK II (EGFP-nuclei stained) seeded as single-cell on single-cell PDMS gradient. Scale bar: 100µm.

References:
[1] Ferrari et al., **Nanotopographic control of neuronal polarity,** 2011 Nano Lett. 11,2,505-511;
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[4] Sunyer et al., **Collective cell durotaxis emerges from long-range intercellular force transmission,** 2016 Science 353(6304):1157-61;
[5] Meucci et al., **Biocompatible noisy nanotopographies with specific directionality for controlled anisotropic cell cultures,** 2012, Soft Matter 8, 1109-1119.