Hierarchical thermoplastic rippled nanostructures regulate Schwann Cell adhesion, morphology and spatial organization.

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Abstract

Periodic ripples are a variety of anisotropic nanostructures that can be realized by ion beam irradiation on a wide class of solid surfaces. Only few authors have investigated these surfaces for tuning the response of biological systems, probably because it is challenging to directly produce them in materials that well sustain long-term cellular cultures[1]. Here, hierarchical rippled nanotopographies with lateral periodicity of 300 nm are produced from a gold-irradiated germanium mold in polyethylene terephthalate (PET), a biocompatible polymer approved by the US Food and Drug Administration for clinical applications, by a novel three-steps embossing process. The effects of nano-ripples on Schwann Cells (SCs) are studied in view of their possible use for nerve-repair applications. Data demonstrate that nano-ripples can enhance short-term SC adhesion and proliferation (3-24h from seeding), drive their actin cytoskeleton spatial organization and sustain long-term cell growth. Notably, SCs orient perpendicularly with respect to the nanopattern lines. These results provide information about the possible use of hierarchical nano-rippled elements for nerve-regeneration protocols [2].

Biocompatible thermoplastic ripples: fabrication and characterization.

PerFluoroPolyEther intermediate mold

crosslinked with UV light (356 nm, 25 mWcm^{-2})

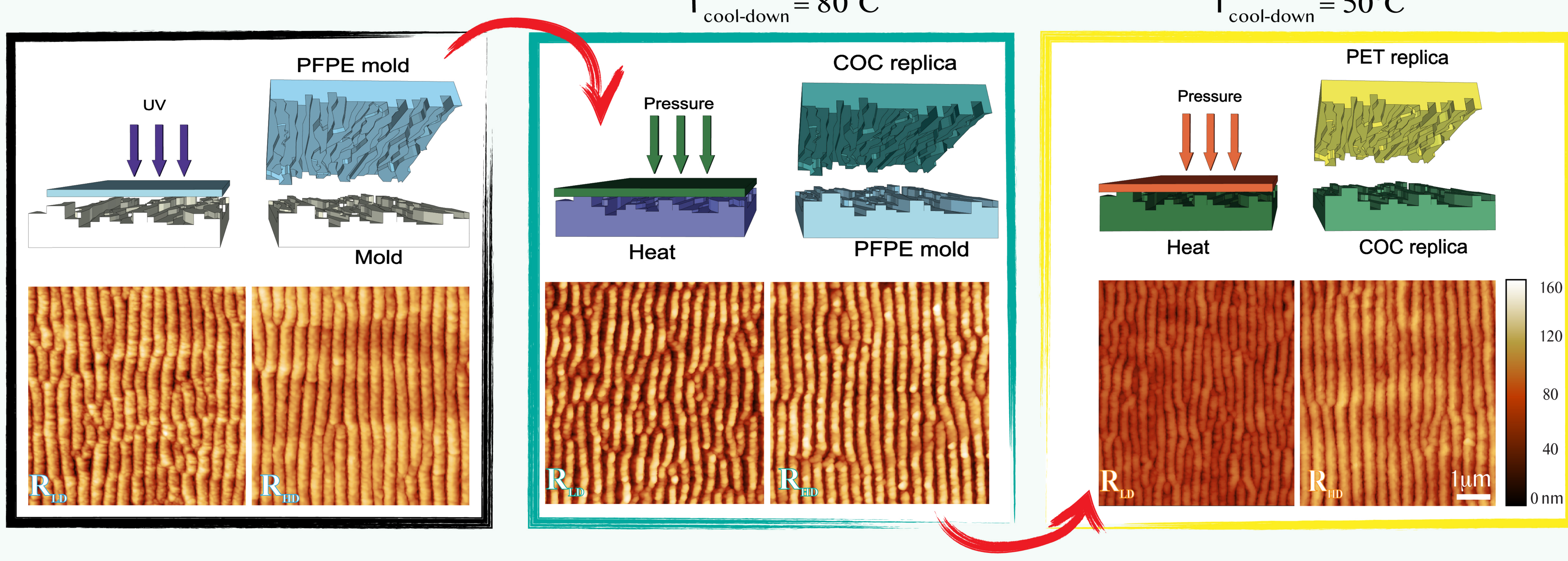
t= 180 s in nitrogen atmosphere [3]

Cyclic Olefin Copolimer replica

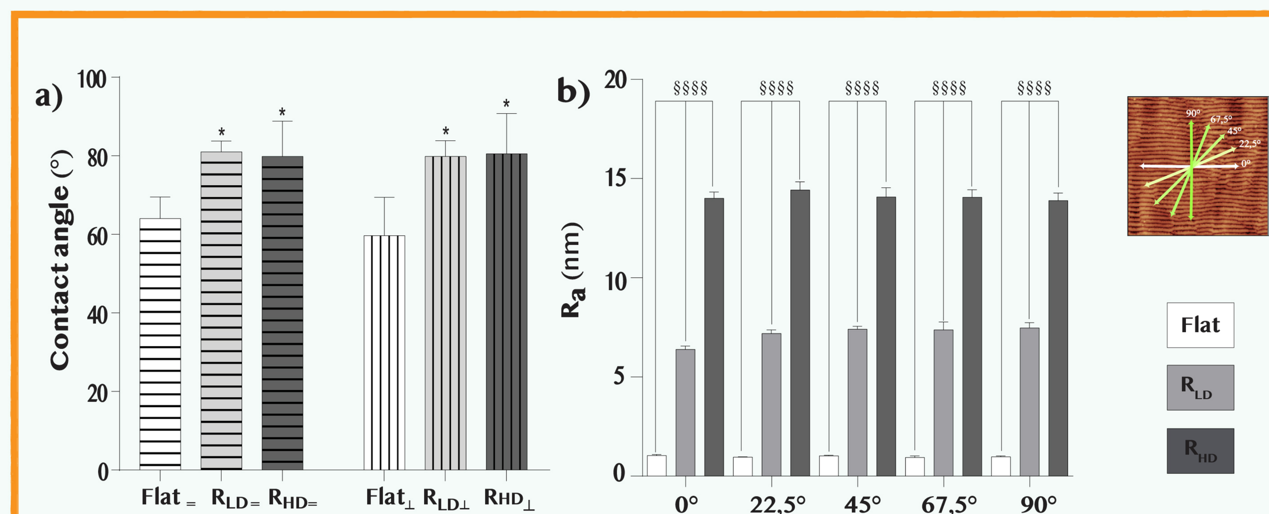
$T_{imprint}$=150 °C (Tg_{COC} = 134 °C), t = 300 s, P = 50 bar, $T_{cool-down}$ = 80°C

PolyEthylene Terephtalate replica

$T_{imprint}$=75°C (Tg_{PET} = 75°C), t = 300 s, P = 20 bar, $T_{cool-down}$ = 50°C



Contact angle and roughness measurements.

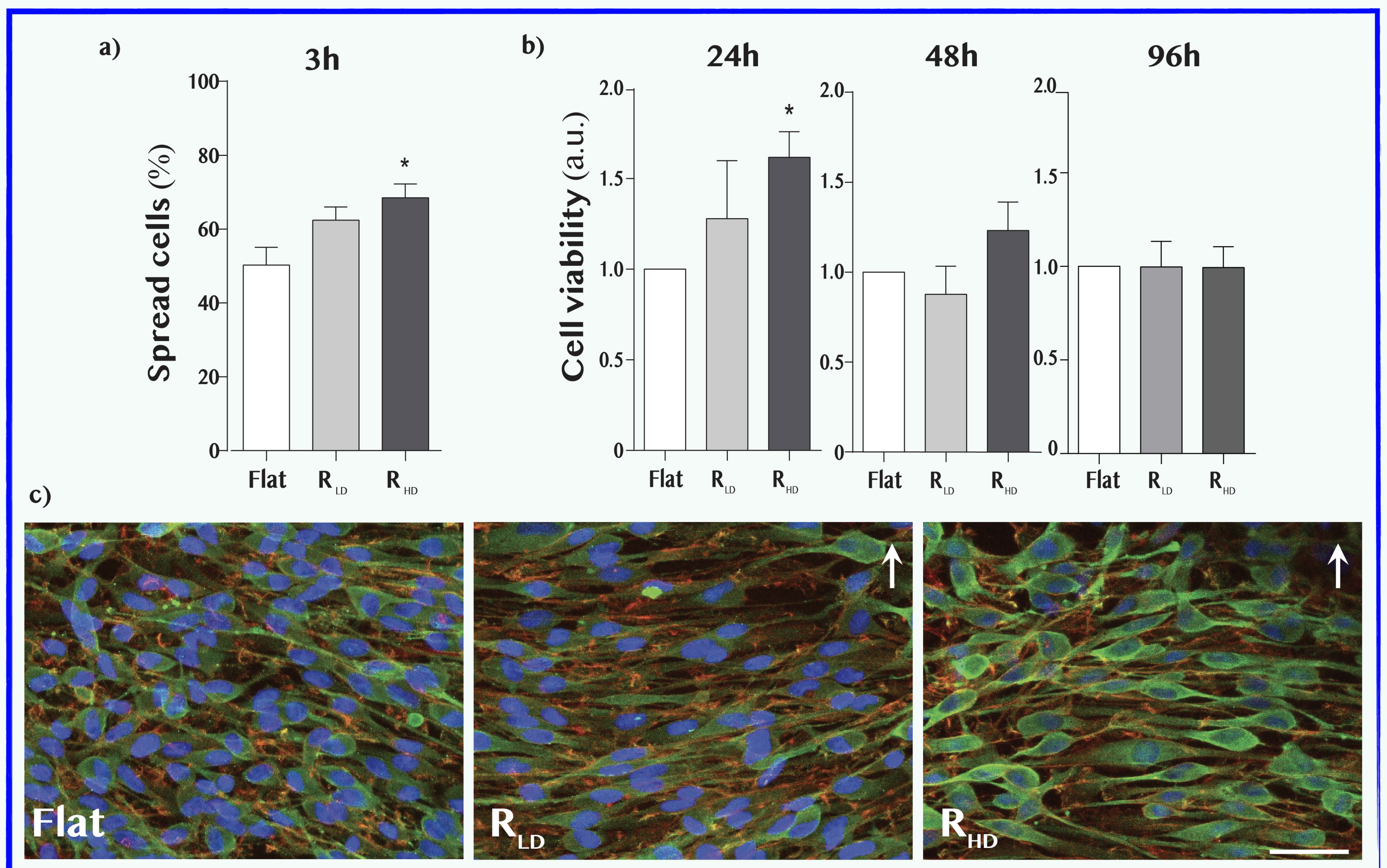


Surface characterization of the R_{LD} and R_{HD} PET periodic nano-rippled substrates. **a)** Contact angle measurement, along the parallel (=) and perpendicular (⊥) direction with respect to the main direction of the pattern; * P < 0.05, One-way ANOVA, Dunnett's test vs. Flat. **b)** Average roughness Ra (nm); §§§§ P < 0.0001, One-way ANOVA, Tukey's multiple comparisons test.

The three-step thermal embossing process to produce biocompatible and transparent thermoplastic substrates having directional, periodic nano-rippled structures on their surfaces. Nano-ripples topographies exhibited an increased hydrophobicity and roughness with respect to Flat controls.

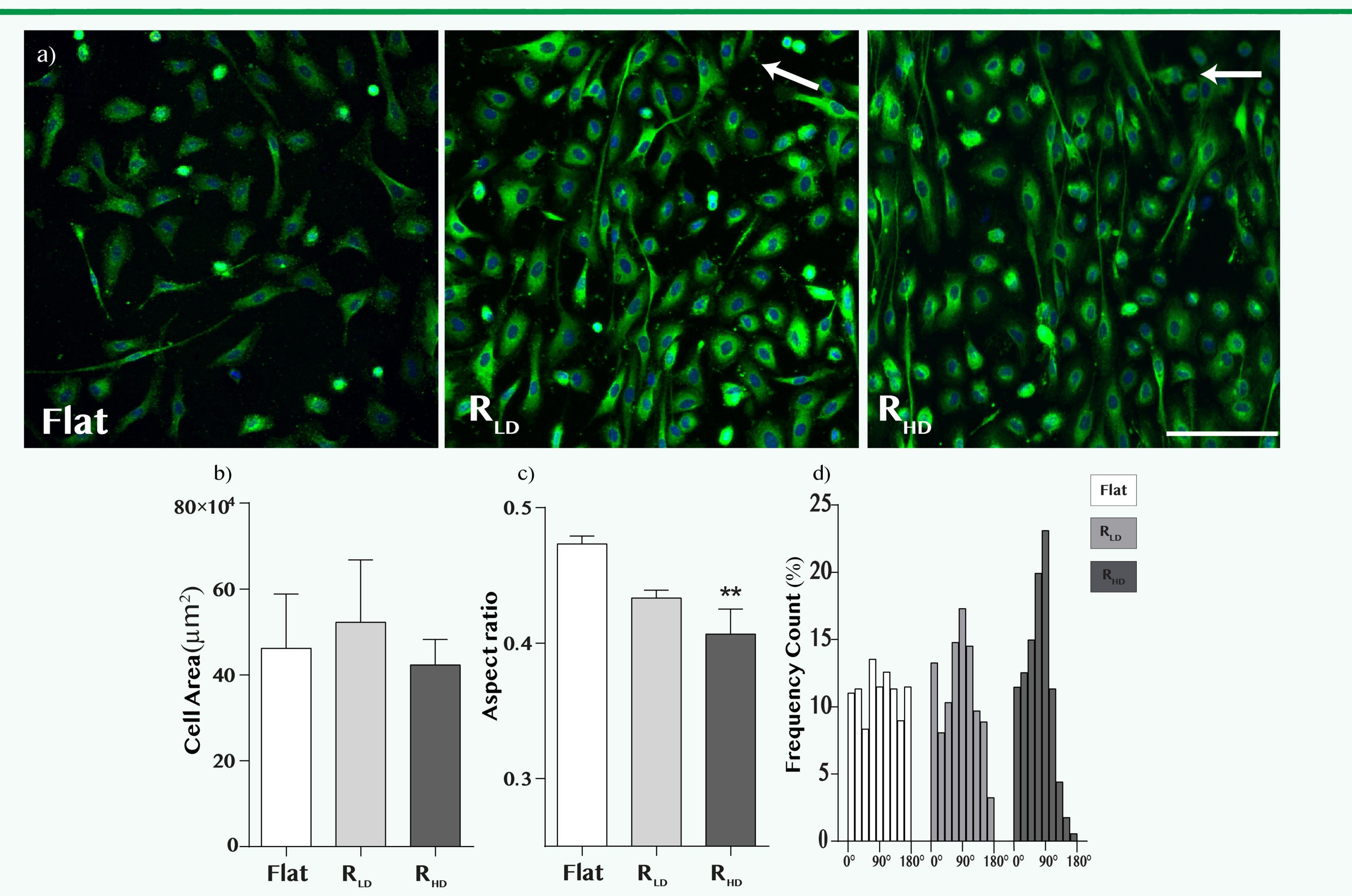
Schwann Cells behavior on nano-rippled surfaces: adhesion, viability, morphological parameters.

In order to study how SCs attach and spread on these substrates, we acquired bright field microscopy images and measured the percentage of cells that underwent full adhesion and spreading.



a) Percentage of spread cells at 3h. **b)** SC cell viability at 24h,48h and 96h after seeding. Data= mean ± SEM, n ≥ 3; * P < 0.05, One-way ANOVA, Dunnett's test vs. Flat. **c)** Representative confocal images of SCs cultured for 7 days on Flat, RLD, and RHD, and immuno-stained for S100 (green), actin (red) and nuclei (blue). SCs proliferated and reached confluence on nano-ripples and flat surfaces. Scale bar: 50 μm; white arrows: ripple direction.

After 48h of culture we immunostained the cells for S100 (SCs marker) and DAPI (nuclear marker) and evaluated a set of cellular morphological parameters (area, elongation and orientation).



a) Confocal microscopy images of Schwann cells on Flat, RLD and RHD nano-rippled PET stained for S100 (green) and nuclei (blue). Scale bar: 100μm. Arrows indicate the nano-ripple direction. b-c) SC morphological characterization: cell area **b)** and aspect ratio **c)** for the different substrates. **d)** Cell angular distribution (0° is the nano-ripple direction) for the different patterns. Data= mean ± SEM, n=3; ** P < 0.01, One-way ANOVA, Dunnett's test vs. Flat.

The nano-rippled PET substrates are suitable for SCs, determining an increased short-term cell adhesion (3h) and viability (24h); at longer term (48 and 96h and 1 week), SC maintain their typical spindle-shape, healthy morphology, and reach complete confluence.

SCs actively interact with nano-rippled topographies, and polarize in a perpendicular direction with respect to the pattern lines. This effect become more evident on RHD, the rougher substrate.

Conclusions

We have developed a new three-step fabrication process to replicate Ripple-low dose (RLD) and Ripple-high dose (RHD) hierarchical nano-rippled structures from germanium substrates onto PET surfaces. Overall, RHD nano-ripples emerged as the most effective scaffold in tuning SC adhesion and growth.

References
[1] Dell'Anna*, Masciullo* et al. 2017 RSC Advances "Multiscale structured germanium nanoripples as templates for bioactive surfaces."
[2] Masciullo et al. 2017 Nanoscale "Hierarchical thermoplastic rippled nanostructures regulate Schwann cell adhesion, morphology and spatial organization."
[3] Masciullo et al 2018 Nanomaterials "Perfluoropolyether (PFPE) Intermediate Molds for High-Resolution Thermal Nanoimprint Lithography."