INVESTIGATION OF CELL INTERACTION WITH THE REGULAR PERIODIC STRUCTURE OF POLYETHYLENE NAPHTHALENE

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  - *Organised biopolymer nanopatterns prepared by replication*

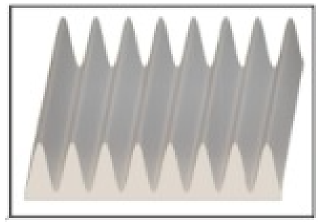
PUBLICATIONS:

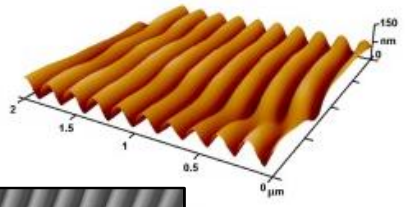
- Hurtuková, K.; Slepičková Kasálková, N.; Fajstavr, D.; Lapčák, L.; Švorčík, V.; Slepička, P. *High-Energy Excimer Annealingof Nanodiamond Layers, Nanomaterials* **2023,** *13* (3), 557-570, https://doi.org/10.3390/nano13030557.
- Slepička, P.; Rimpelová, S.; Svobodová Pavlíčková, V.; Slepičková Kasálková, N.; Hurtuková, K.; Fajstavr, D.; Švorčík, V. Mammalian Cell Interaction with Periodic Surface Nanostructures. *International Journal of Molecular Sciences* **2022**, *23* (9), 4676, https://doi.org/10.3390/ijms23094676.
- Hurtuková, K.; Juřicová, V.; Fajstavrová, K.; Fajstavr, D.; Slepičková Kasálková, N.; Rimpelová, S.; Švorčík, V.; Slepička, P. Cytocompatibility of Polymethyl Methacrylate Honeycomb-Like Pattern on Perfluorinated Polymer, *Polymers* **2021**, *13*(21), 3663-3678, https://doi.org/10.3390/polym13213663.

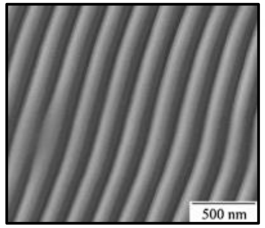
INTRODUCTION

- Periodically nanostructured surfaces have a wide range of uses and applications across various fields (optics and photonics, biomedical and healthcare, materials science, etc.) due to their unique properties and functionalities[1,2,3].
- Laser-Induce Periodic Surface Structure (**LIPSS**) is type of periodic surface pattern created on various materials using laser irradiation
- The formation of **LIPSS** involves a complex interplay of laser parameters (angel, wavelength), material properties, and the interaction between laser light and the material's surface[4].
- **LIPSS** structural size typically ranges from a few micrometers to less than 100 nanometers[5]
- **LIPSS** in biomedicine field has various properties[6,7,8]:
  - improved adhesion to biological structures
  - increased ability to absorb biomolecules
  - enhanced tribological property

LIPSS







EXPERIMENTAL

MATERIALS:

- polyethylene naphatele (PEN) (50 µm thick polymer foil)

MODIFICATION:

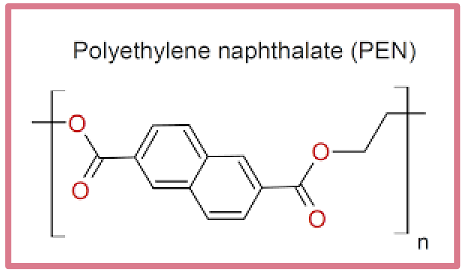
- KrF excimer laser influence: 10 mJ cm^{-2}, 6 000 pulses
- Angle of laser beam: 0° and 45°

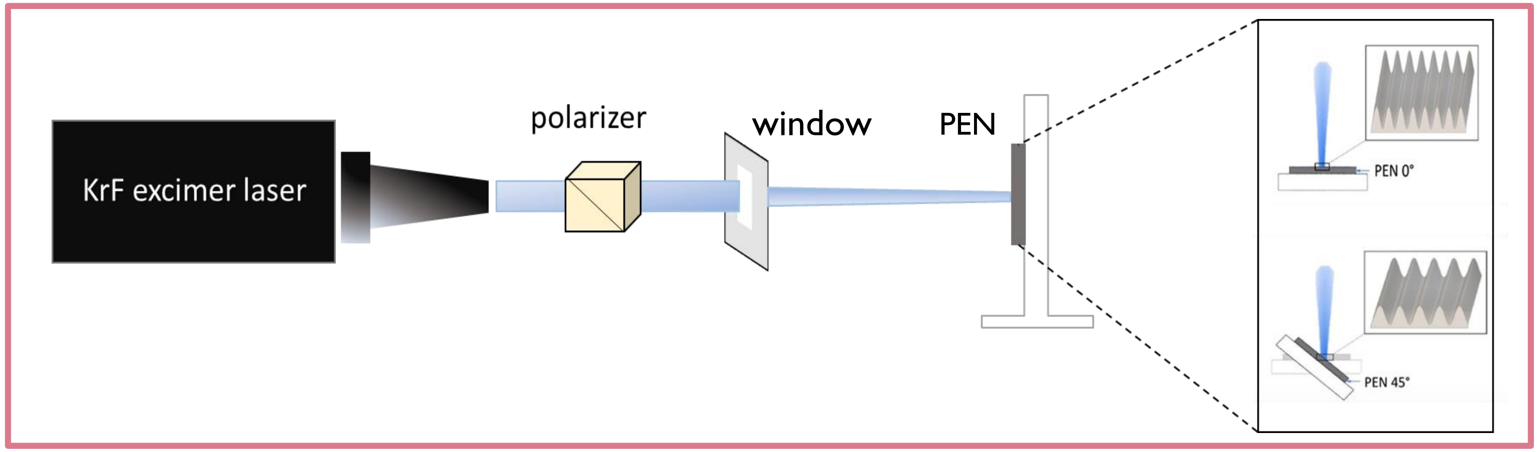
CYTOCOMPATIBILITY:

- C2C12 (mouse myoblasts)

ANALYSES:

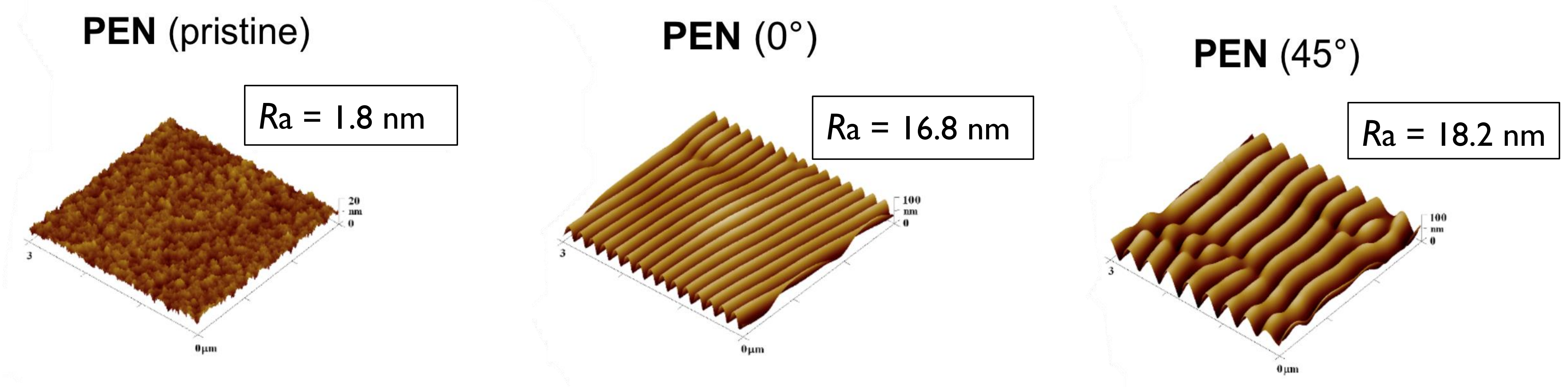
- Atomic Force Microscopy (AFM)
- Energy Dispersive Spectrocopy (EDS)
- Fourie-transform Infrared Spectroscopy (FTIR)
- Fluorescence microscopy





ATOMIC FORCE MISCOSCOPY

- Periodic nanostructures can take on different shapes and sizes depending on the wavelength of the primary laser radiation and the angle of incidence of the laser beam[9]
- With increasing of the laser beam angel the periodicity, roughness and height of the lamels also increase



Lamel height:
52.9 ± 3.7 nm

Lamel height:
67.9 ± 2.5 nm

Fig. 1 3D AFM images (3 × 3 μm^2) of PEN polymer samples unmodified (pristine) and modified (at an angle of 0° and 45°) with a laser energy density of 10 mJ cm^{-2} with the number of 6 000 pulses. *Ra* represents the average roughness.

FOURIER-TRANSFORM INFRARED SPECTROSCOPY

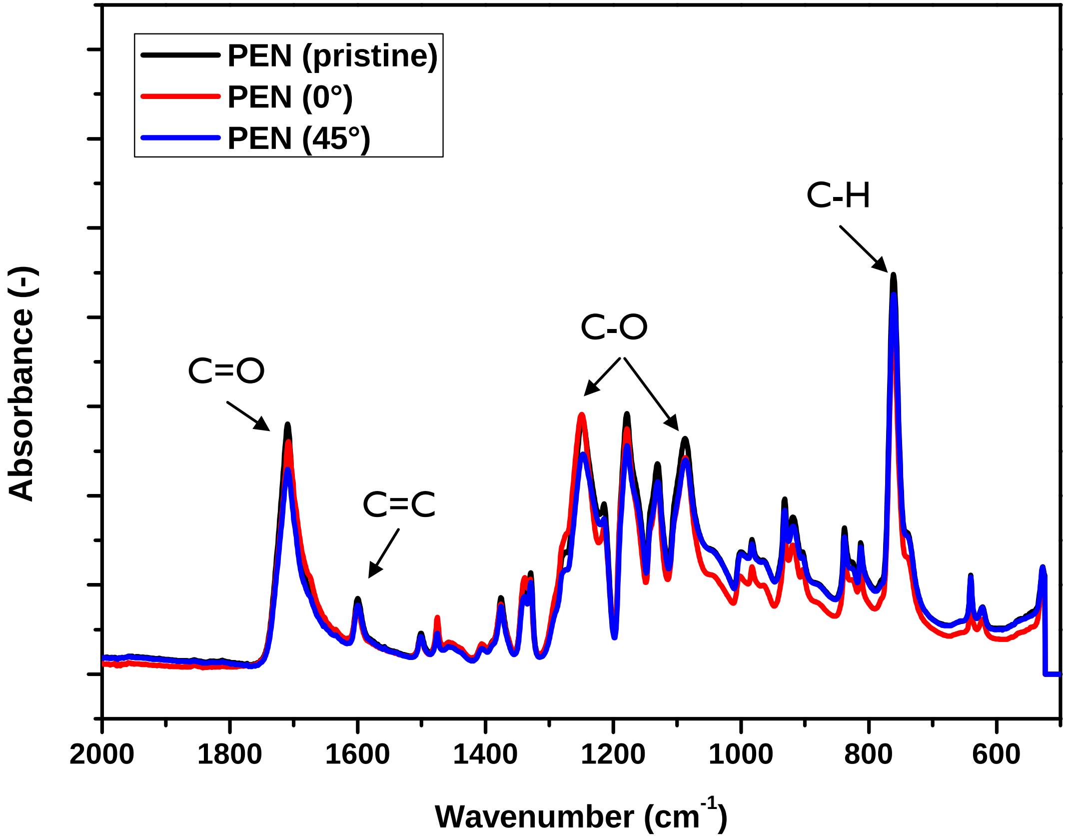
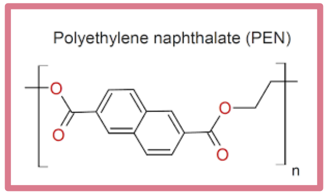


Fig. 2 FTIR sprectra of pristine PEN polymer and modified at 0° and 45° with a laser energy of 10 mJ cm^{-2} (6 000 pulses).

- The FTIR spectrum shows no major changes in the maxima of the absorption bands for modified PEN (0° and 45°) samples



FTIR spectrum[10]:

- 1725 cm^{-1} - carbonyl C=O functional groups from PEN ester group
- 1600 cm^{-1} - aromatic C=C bands from the naphthalene ring
- 1275 - 1000 cm^{-1} - C-O bands from the ester groups
- 750 cm^{-1} - aromatic out-of-plane vibrations of C-H bonds

ENERGY DISPERSIVE SPECTROSCOPY

- The increasing oxygen content in the modified sample is caused by the absorption of atmospheric oxygen after laser ablation

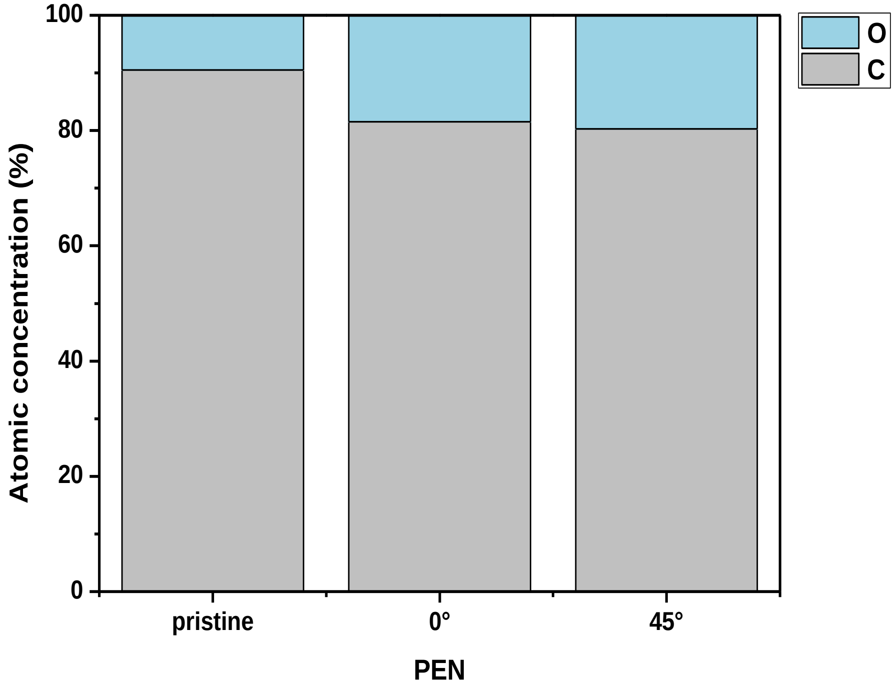


Fig. 3 Graph of the C and O elements distribution on pristine PEN polymer and modified at 0° and 45° with a laser energy of 10 mJ cm^{-2} (6 000 pulses).

- EDS mapping of homogenous distribution of the C and O elements on the modified PEN polymer

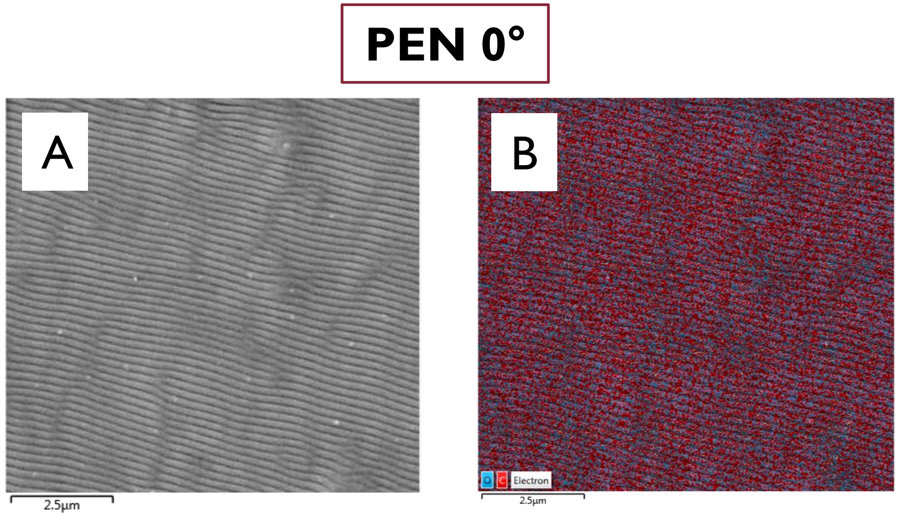


Fig. 4 SEM image (A) and corresponding EDS mapping (B) of the distribution of C and O elements on PEN polymer modified at 0° with a laser energy of 10 mJ cm^{-2} (6 000 pulses). Measured area 10 × 10 µm^2.

INTERACTION C2C12 CELL WITH PEN

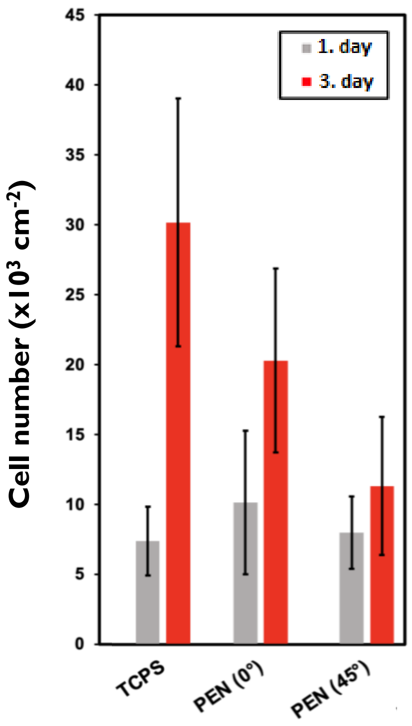


Fig. 5 Graph showing the number of adhered (day 1) and proliferated (day 3) C2C12 cells cultured on laser-modified PEN samples at a fluence of 10 mJ cm^{-2} compared to TCPS. The effect of modification at angles (0° and 45°) was also tested.

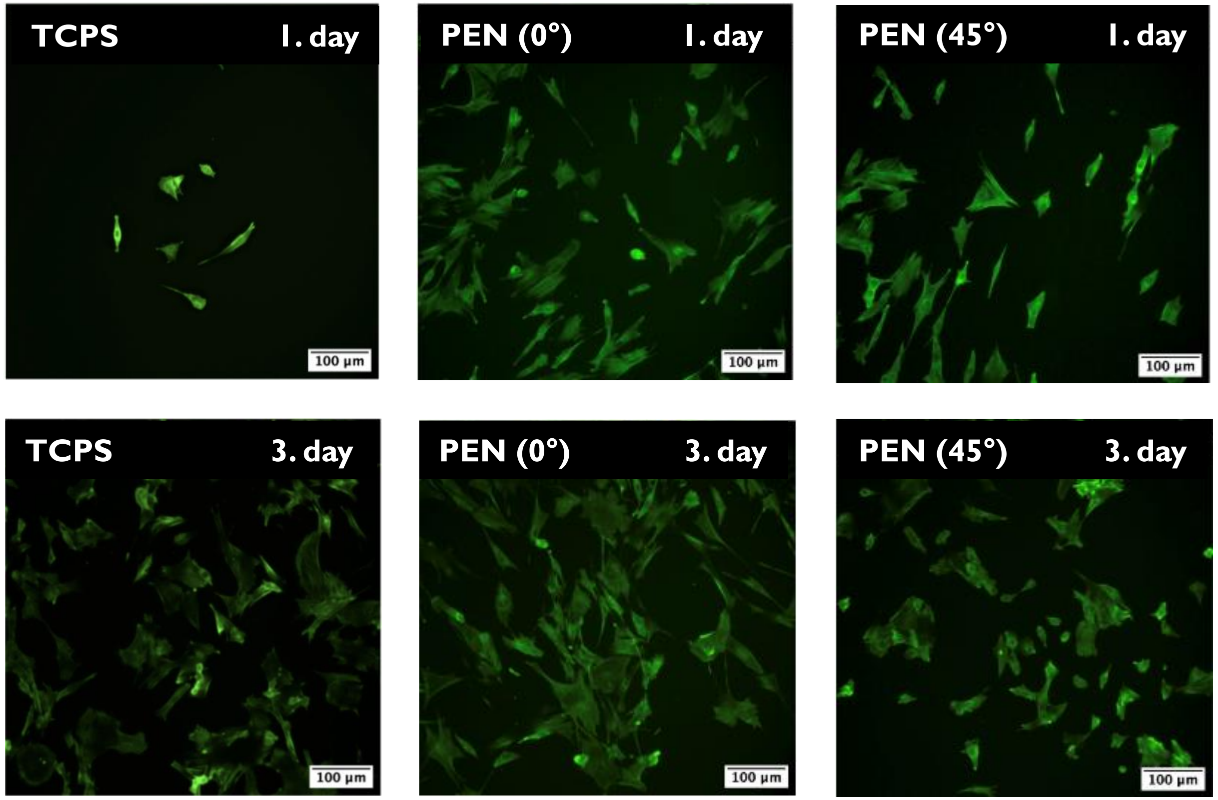


Fig. 6 Images of adhered (day 1) and proliferated (day 3) C2C12 cells cultured on laser-modified substrates and PEN (0°, 45°) compared to the TCPS sample.

- 1. day - cells were distributed homogeneously on the surfaces of all tested nanostructured PEN surfaces
- 3. day - the number of cells increased, but the cells were no longer homogeneously distributed and formed clusters
  - probably caused by the high surface roughness of the modified samples compared to TCPS (Ra = 5.4 nm)

CONCLUSION

- Succesfull preparation of a periodic structure (LIPSS) on the PEN polymer surface by the KrF excimer laser exposition at a different angle
- AFM results showed that the surface of the pristine samples was smoother (Ra≈2 nm) in comparison to samples with more significant surface pattern (Ra≈18 nm)
- FTIR method shows no major changes in chemical composition between pristine and modified samples
- EDS analytical method shows higher atomic concentration of O (reaction with atmospheric oxygen after laser ablation) and mapping of homogenous distribution of the C and O elements
- The results of the biological tests indicate excellent cell adhesion after 1. day in contrast to TCPS but worse proliferation after 3. day (forming cells clusters)

THANK YOU FOR YOUR ATTENTION!

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