"Functionalization of biopolymer thin films modified by femtosecond pulses for cell guidance improvement"

Albena Daskalova

Institute of Electronics
Bulgarian Academy of Science

1784 Sofia, 72 Tzarigradsko chaussee blvd.,
e-mail: a_daskalova@code.bg

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EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

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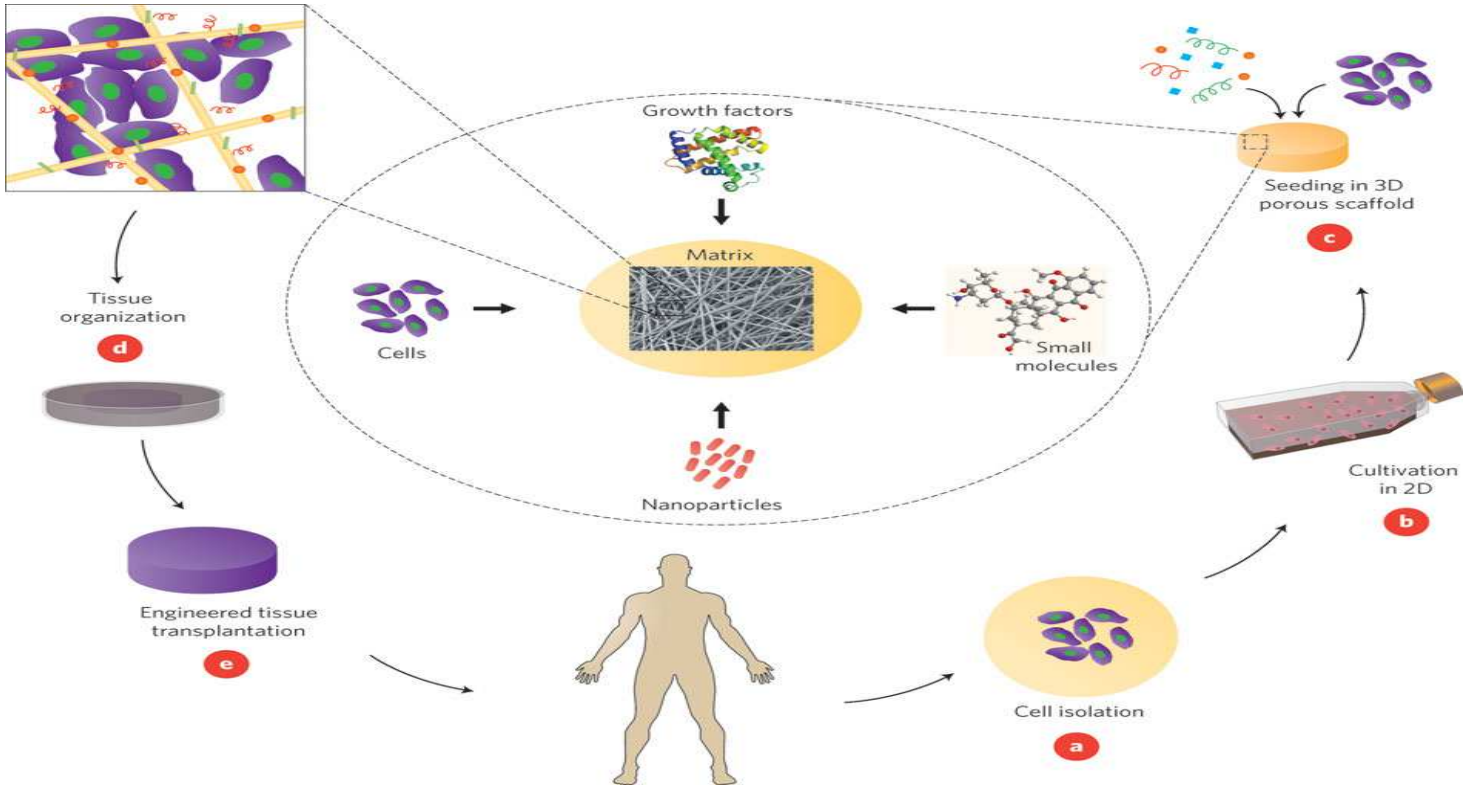
Tissue engineering

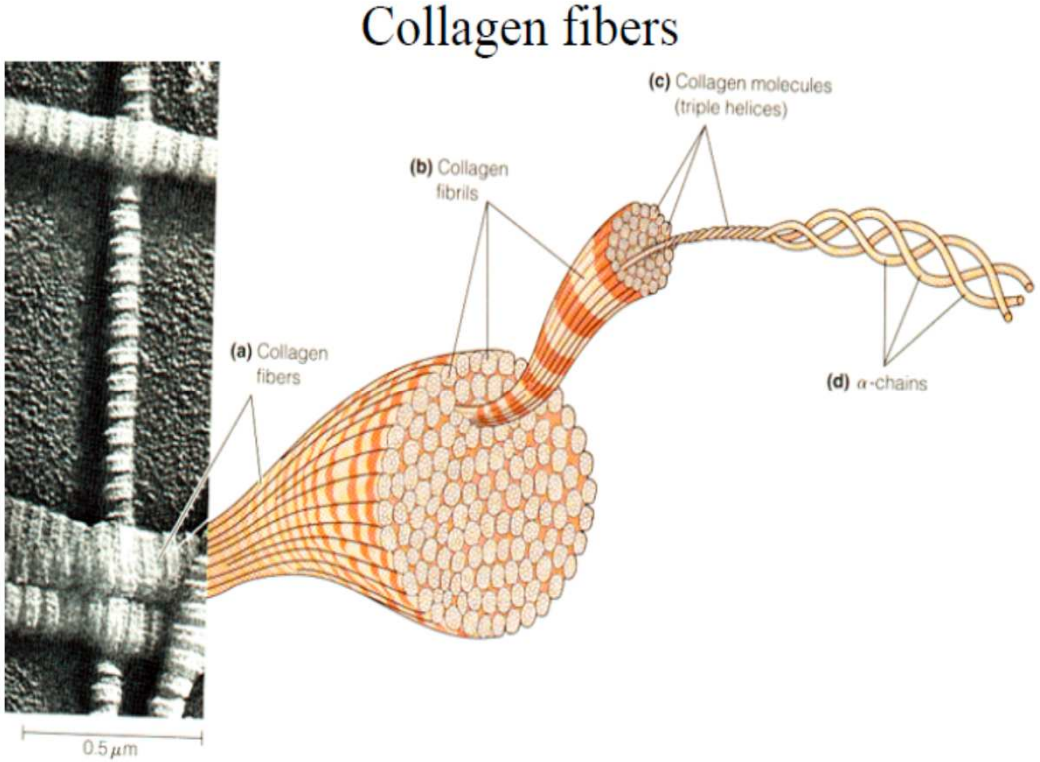


Biomaterials

- Main demands

to engineered scaffolds :

- biocompatible,
- biodegradable
- possess a high surface area for cell attachment
- have good mechanical integrity



- Collagen- key structural proteins found in ECM
- Gelatin - derivative of collagen and does not exhibit antigenicity
- Elastin - dominant part of the elastic fiber
- The microstructure and the mechanical properties of biomaterials influence the scaffold bioactivity

Methods	Porosity (%)	Advantages	Disadvantages	Pore size (µm)
Fiber Bonding (Unwoven mesh)	81	Highly porous scaffolds with interconnected pores	Use solvents which are poisonous to cells immersed in them for a long time	500
Solvent casting/Particulate leaching	87	Structure has high strength or electrical conductivity	Organic solvents used contaminate polymer Very long time required.	100
Gas foaming	93	biocompatible	Organic solvents used contaminate polymer	100
Phase separation/emuls-ification	95	Pore size and porosity easily changed	Solvents used are poisonous.	13 - 35

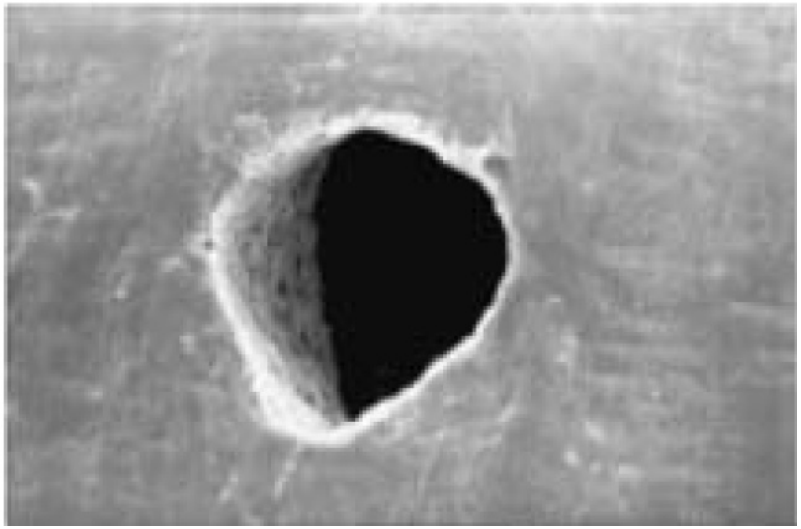
(L.Calvin, T. Wah, "Polymer Scaffold Structure for Tissue Engineering")

Short pulse laser-matter interactions

Ultra-short pulse durations:

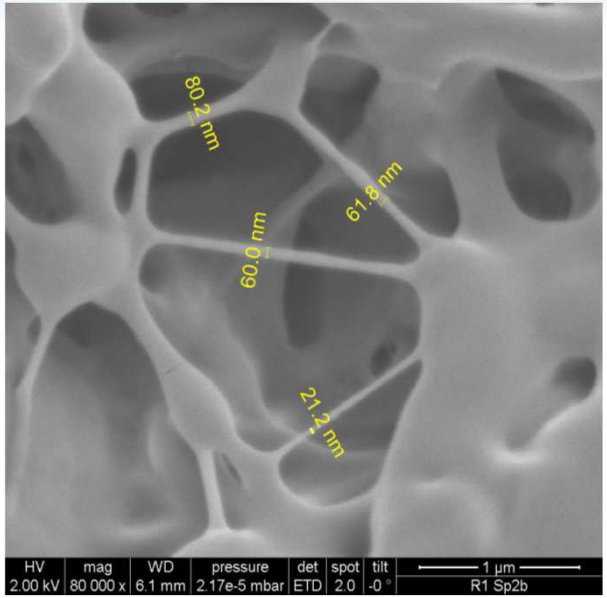
- Reduced ablation thresholds
- Negligible heat diffusion into the material
- Minimized energy loss
- Minimized heat affected zones
- Almost absent mechanical damage
- Absence of molten zones
- Absence of liquid phase
- Reduced molecular fragmentation

Ultra-short laser vascular wall engineering



An example of 20 μm holes drilled into vascular transplant-grafts by 30 fs pulses.

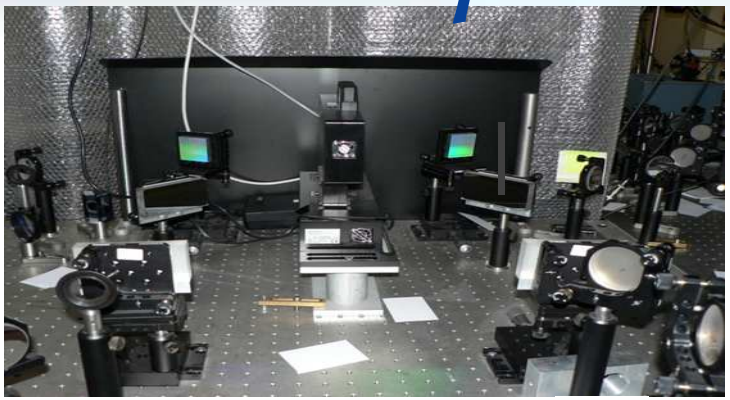
• Vascular grafts are structured by focusing a femtosecond laser beam down to a spot size of 20 μm.
• Sufficient endothelialization in the absence of any inflammation and fibrosis - the main requirement in the design of a stent surface.



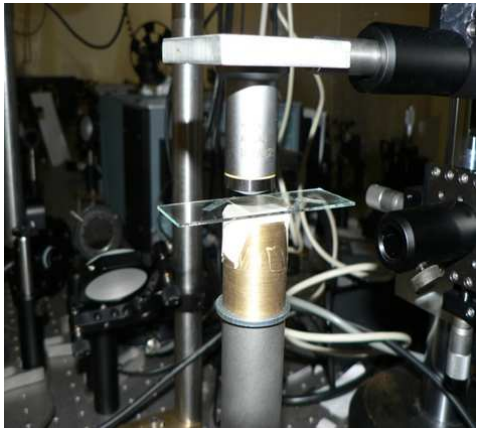
SEM micrographs of biopolymer nanofibers generated upon fs laser irradiation. The micrograph shows interconnectivity and the pore structure of gelatin laser induced foam.

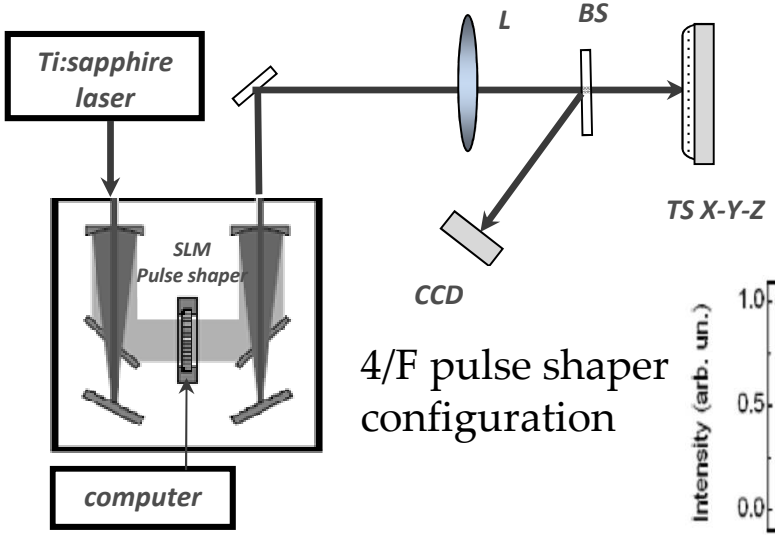
- Micro foam formation with enhanced porosity
- Interconnection fibers, in the order of several nanometers, between the separate pores

Experimental Setup

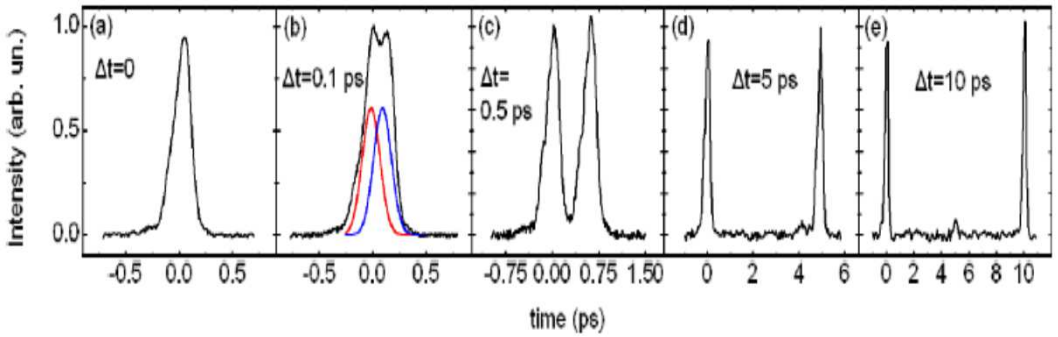


- CPA fs Ti: sapphire laser
- $\lambda = 800$ nm
- $\tau = 30$fs
- 1 KHz

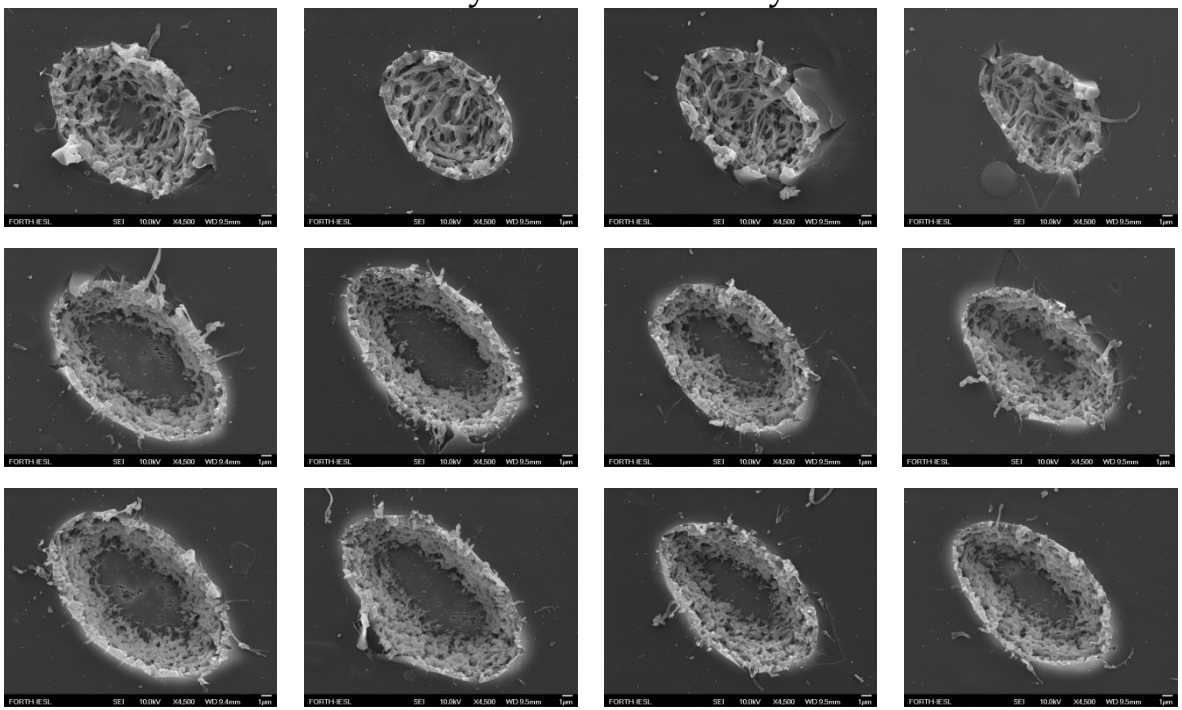




4/F pulse shaper configuration



FE-SEM images of laser irradiated spots using double pulses with different separation times



0 delay

0. 5ps delay

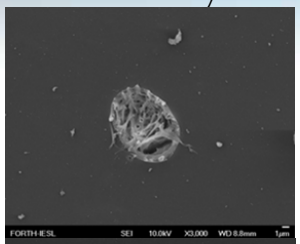
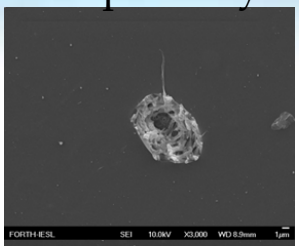
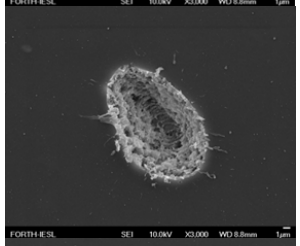
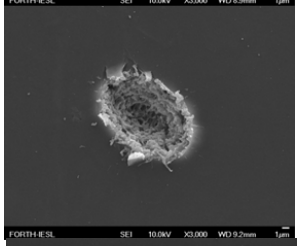
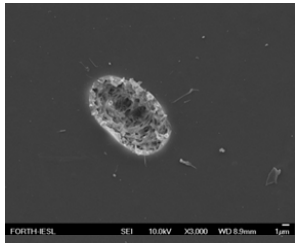
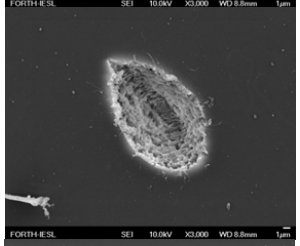
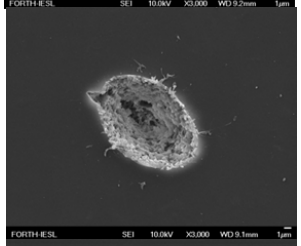
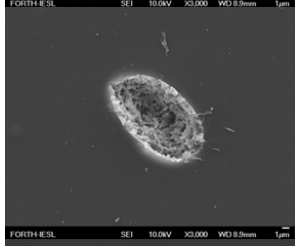
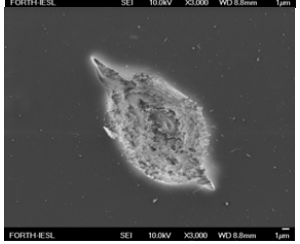
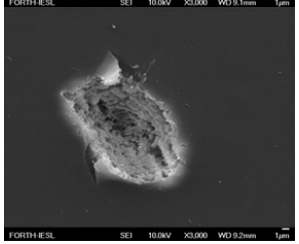
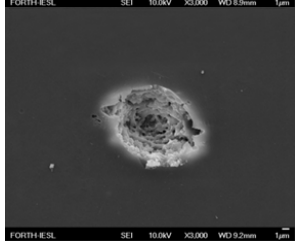
1ps delay

N of pulses =10

N of pulses =50

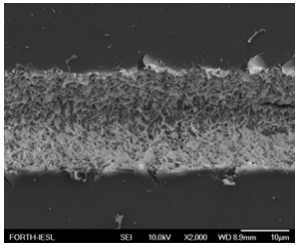
N of pulses =100

N of pulses =1000

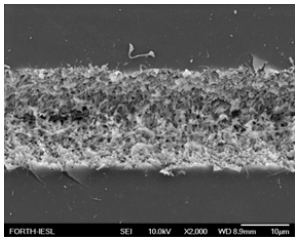












Continuous irradiation

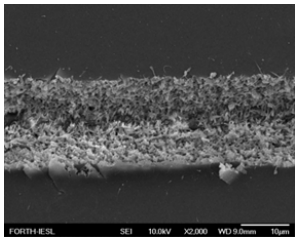
0 delay



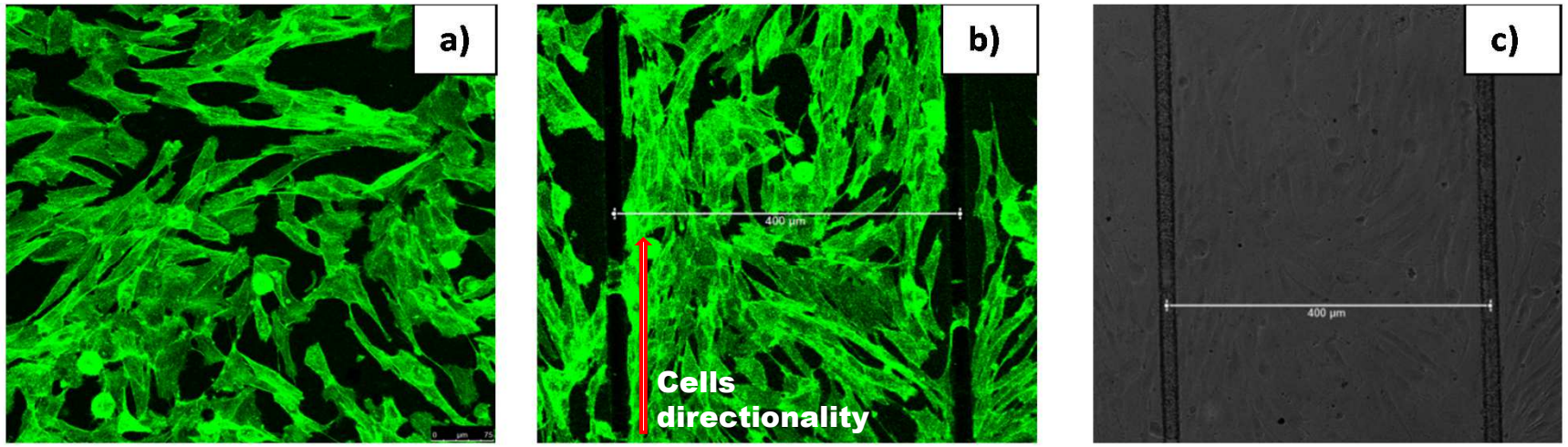
0.5ps delay



1ps delay



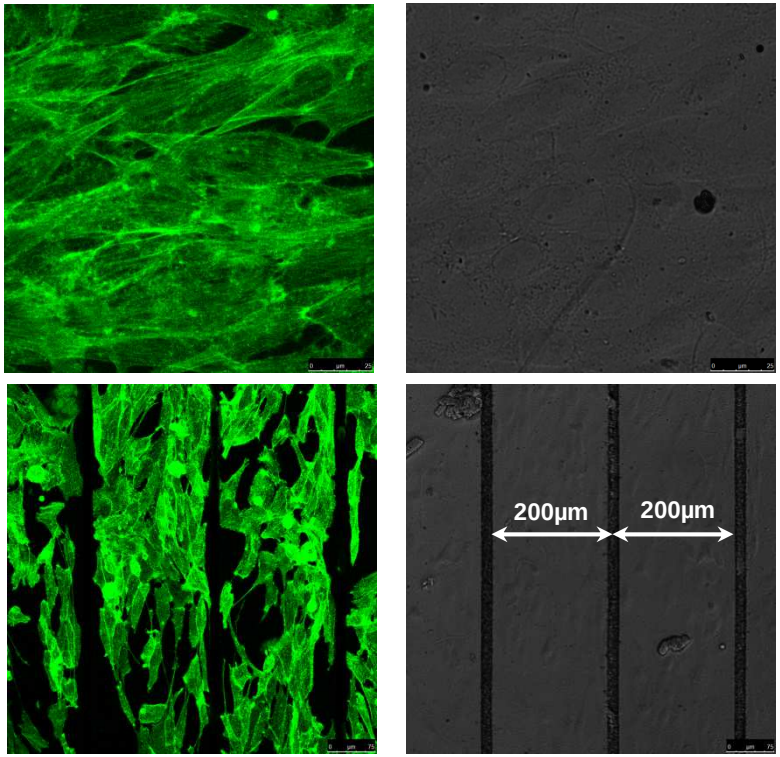
MG63 immobilization on topographically patterned collagen surfaces



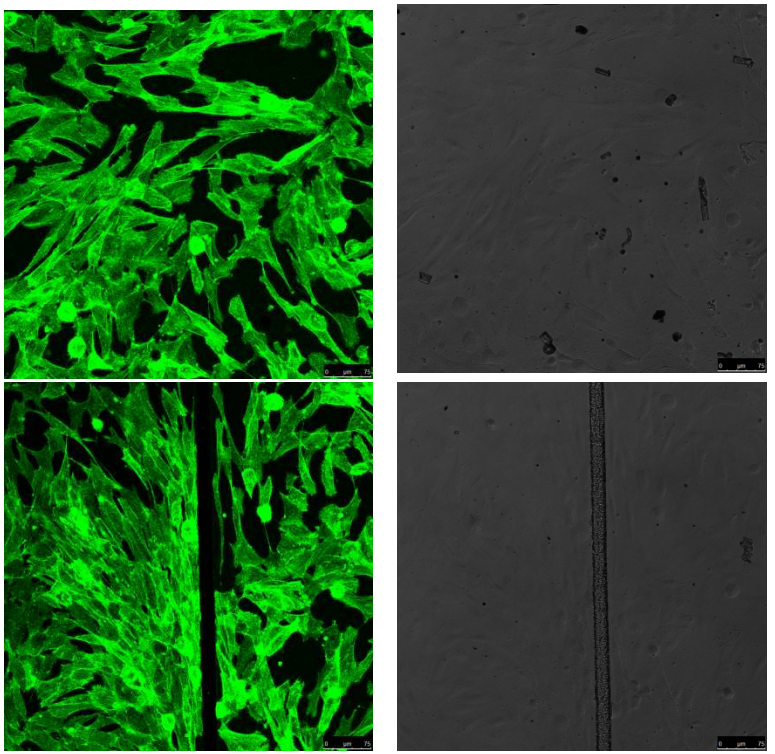
Confocal laser microscopy pictures of MG63 osteoblast cells on collagen films cultured for three days demonstrating the actin staining on the non-irradiated (a) and laser processed collagen thin film surfaces(b-c). (a)-(b) - the fibrillar network of actin cytoskeleton

- Laser-irradiated micro-grooves with different groove spacing
- Actin cytoskeleton staining - Rhodamine Palloidine conjugated
- Osteoblast alignment is crucial for the realization of anisotropic bone tissue microstructure.

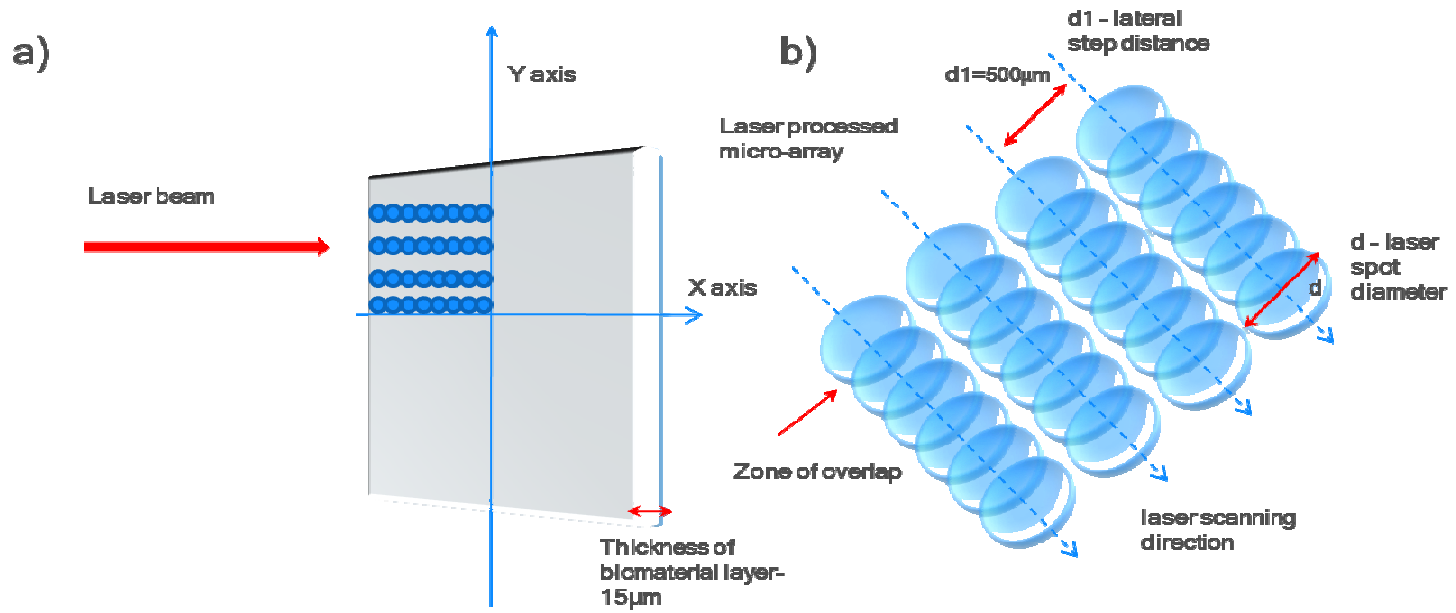
Collagen/elastin sample



Collagen sample

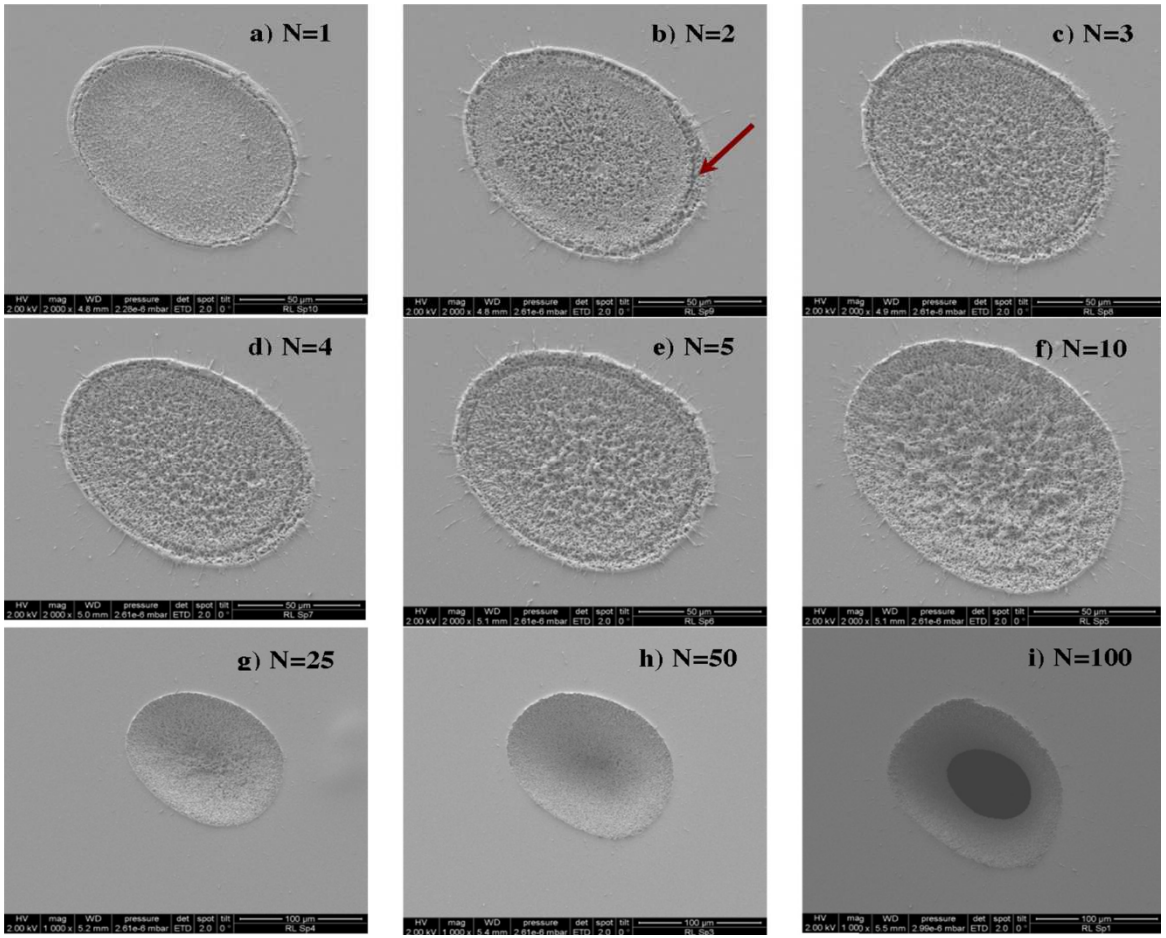


Scheme of the surface laser processing



a) parallel micro-grooves array on the surface of thin film of biomaterial produced by laser scanning along x-axis, b) close up of the processed array, distance of the scanning length is 5mm.

Surface modification of thin gelatin film



λ=800nm, τ=30fs , F=2.5J/cm², 2000x

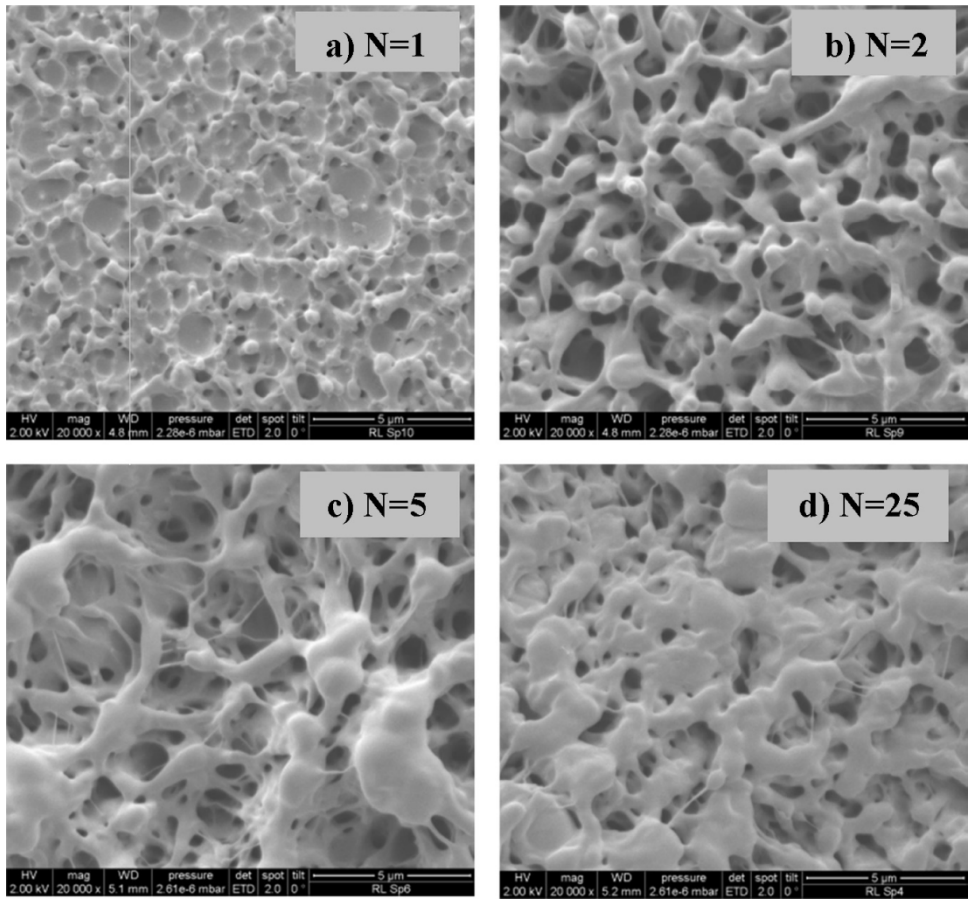
- First signs of surface modification, (N = 1), F = 2.5 J/cm^2

- As the number of pulses was increased further, we could observe the surface structure evolution, formation of porous matrix.

- In all cases larger micropores are dominant in the area irradiated by the higher energy part of the pulses, the smaller ones extend over the areas irradiated by the lower energy edges of the pulse.

- Formation of a rim

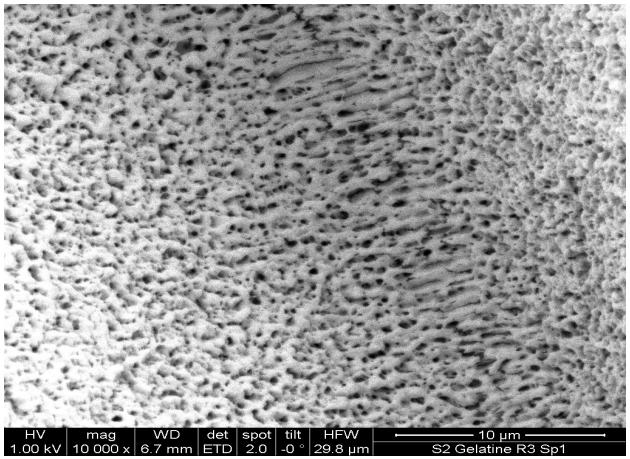
Surface modification of thin gelatin film

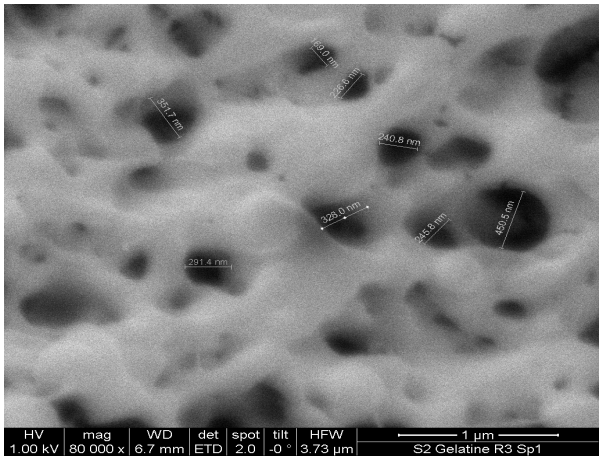


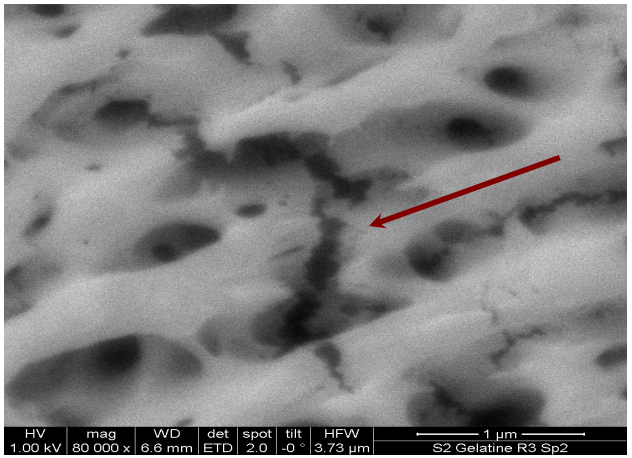
- Increasing number of Pulses ⇨ Lost of porosity
- Pores became occluded
- Signs of melting and sublimations occur by increasing the number of pulses

λ=800nm, τ=30fs , F=2.5J/cm^2, 20000x

Surface modification of thin gelatin film

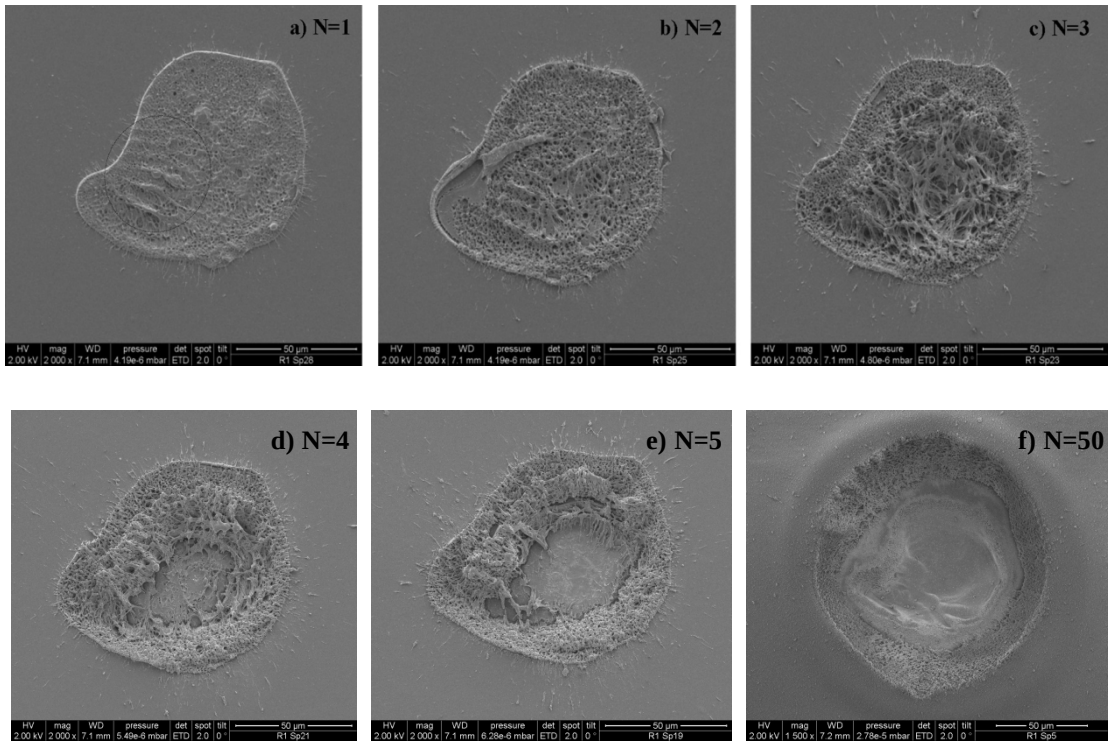






5 pulses, 30fs, F=6.4J/cm^2

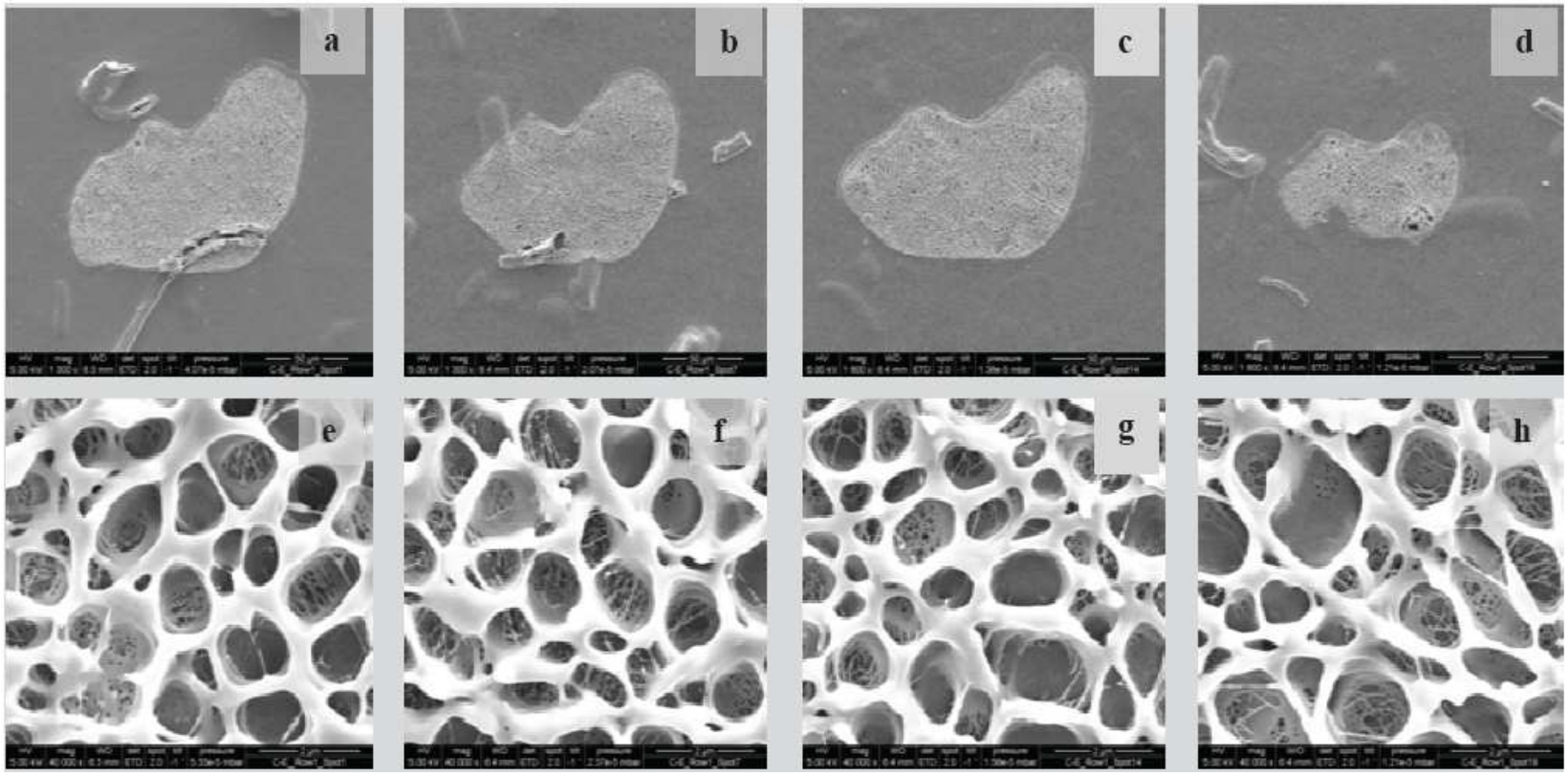
Surface topography of thin collagen film



- Presence of a liquid phase
- Presence of small interconnections
- Flow of molten material
- Covering by submicron sized redeposited grains from the laser plume

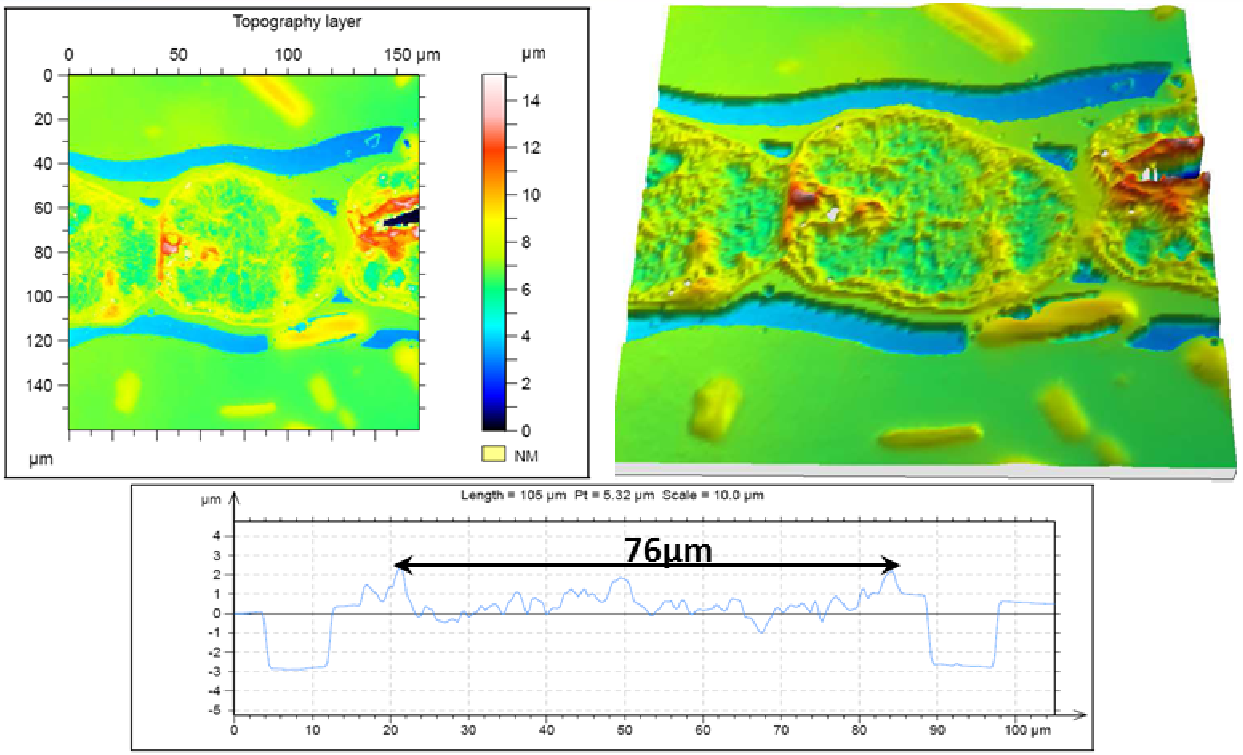
F = 5.7 J/cm², τ= 30fs, (a) N = 1, 2000×, (b) N = 2, 2000×,
(c) N = 3, 2000×, (d) N=4, 2000×, (e) N=5, 2000×, (f) N=50, 1500×

Surface topography of thin collagen/elastin film



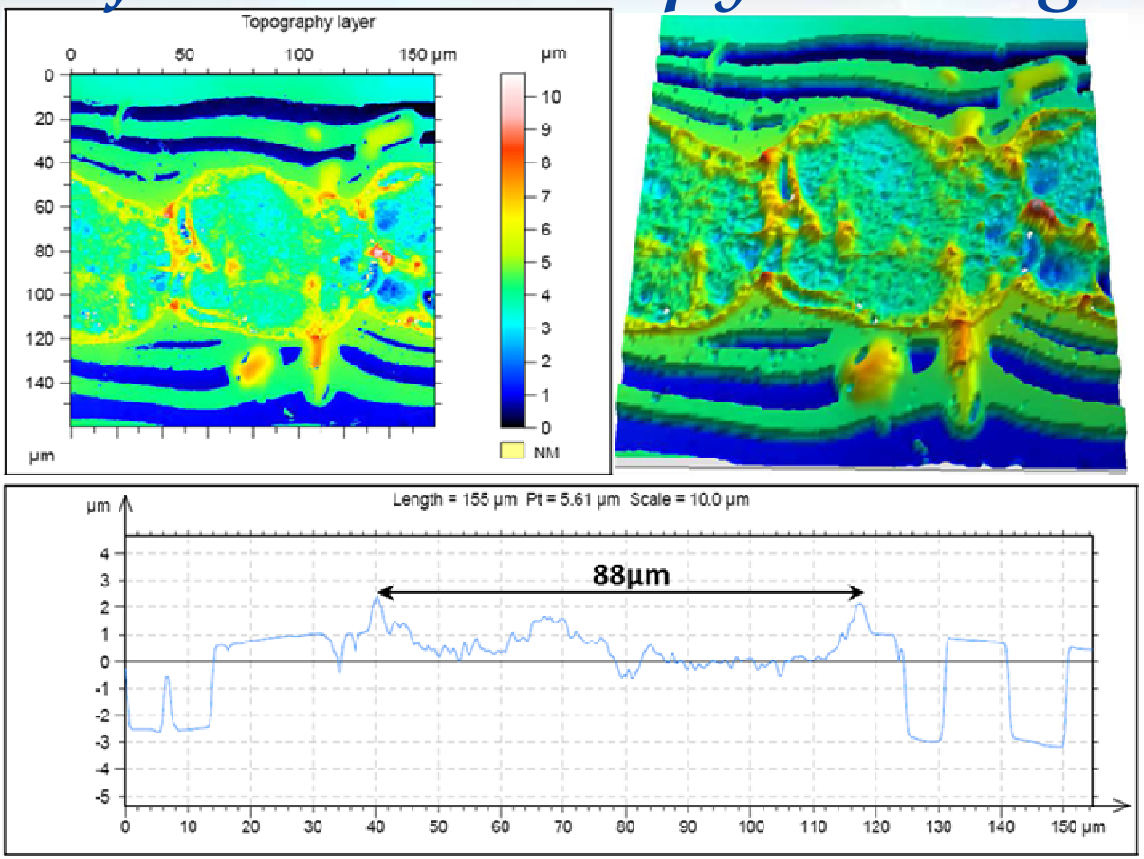
SEM images of single pulse irradiated collagen-elastin films at λ=800nm, τ=30fs: a) F=9.41J/cm^2, b) F=7.84J/cm^2, c) F=5.88J/cm^2, d) F=3.92J/cm^2

Confocal microscopy investigations



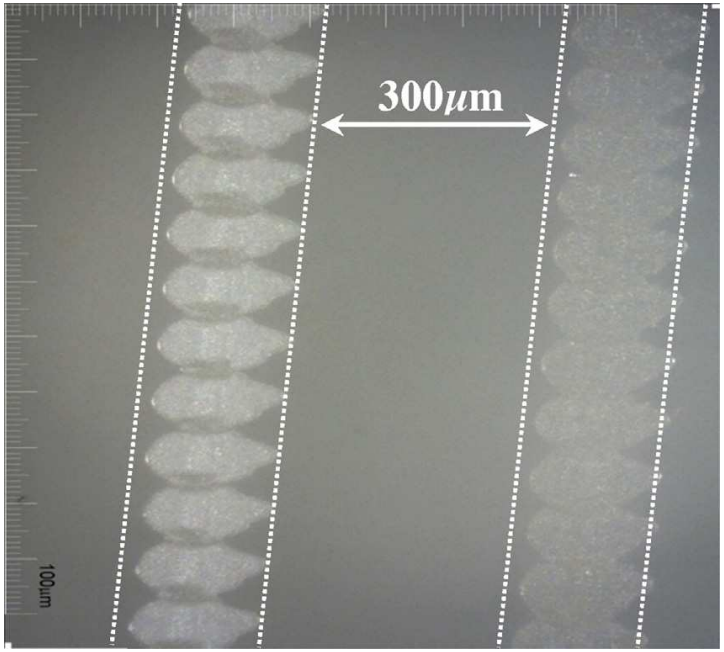
Confocal microscope image of the modified collagen/elastin region formed at F= 2.94J/cm^2, τ=30fs, N=2; a) topography image, b) 3-D topography image; c) cross section of the 3-D reconstructed image

Confocal microscopy investigations



Confocal microscope image of the laser induced modification formed at F= 5.88J/cm^2, τ=30fs, N=3; a) topography image, b) 3-D topography image; c) cross section of the 3-D reconstructed image,

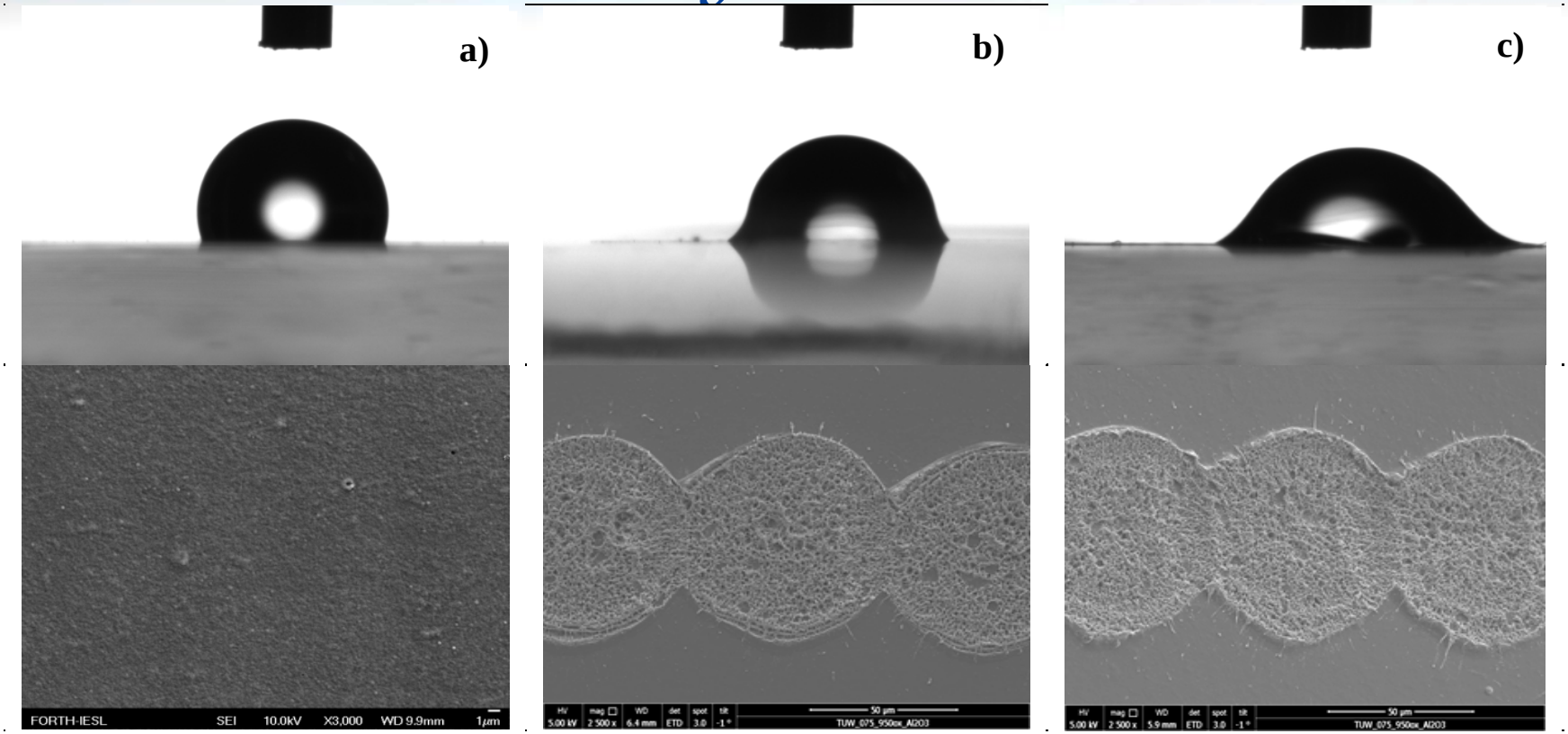
Cells adhesion on collagen–elastin laser induced porous matrix



Optical Microscopy image of examined surface with laser fabricated channels for fibroblast cells adhesion. Magnification power: 200×.

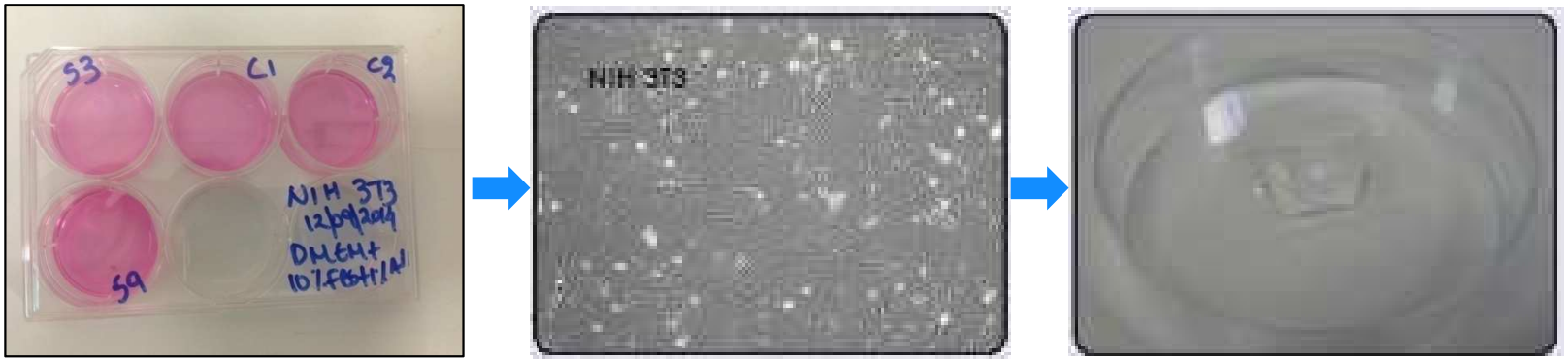
- Thin film of 9:1 collagen–elastin
- The created matrix was irradiated with N=2 pulses

Wettability measurements



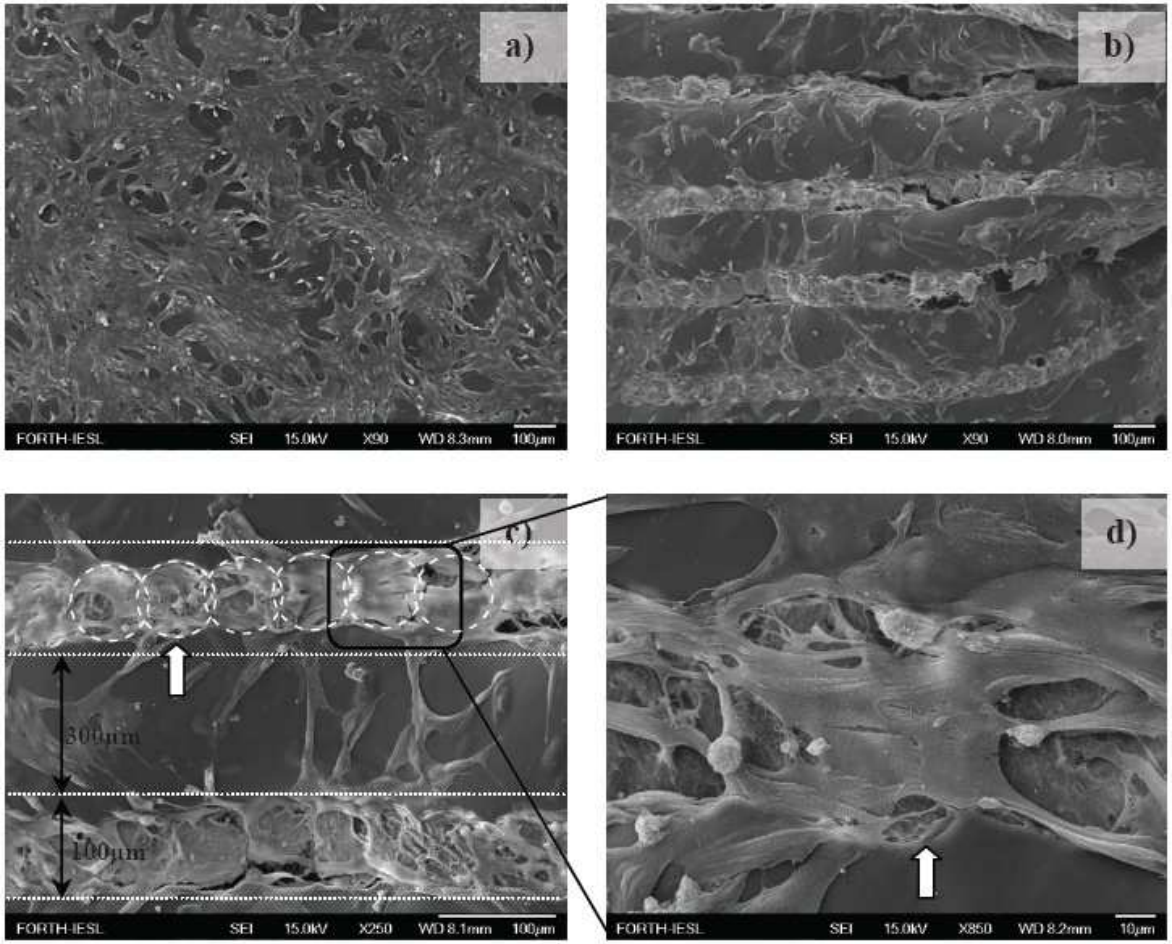
Images of contact angles of microscopic droplets of water on (a) flat, θ_w=109º and (b-c) patterned (N=2, 3 pulses), θ_w= 85.5º , 60.4º, collagen/elastin surfaces and corresponding SEM of non-modified and modified collagen/elastin surfaces.

Cells cultivation



- Density of 1920000 cells per cm^2.
- Culturing in (DMEM) medium supplemented with 10% (FBS) and 1% penicillin/streptomycin/amphotericin B.
- Dehydration procedure for examination with FESEM.

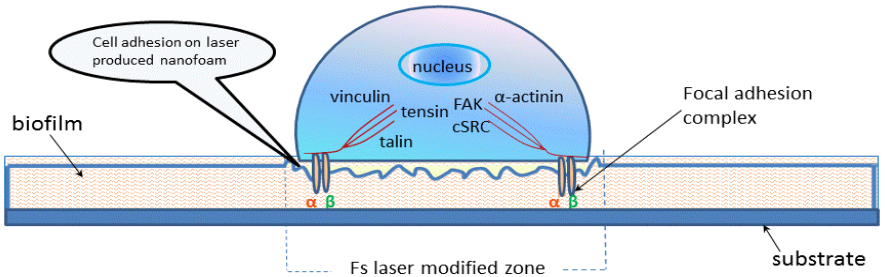
Field emission microscopy images of the cytoskeleton of NIH3T3 mouse fibroblasts migration



- Cells density raises towards the laser treated part of the surface.
- Enhanced adhesion of fibroblast cells on laser modified stripes.
- Mice fibroblasts remained viable for several days and showed little mobility outside of the micropattern.
- The actual mechanism of contact guidance and selective cell adhesion on the laser grooves is a complex process of chemical and topographical stimuli.
- The amount and/or orientation of the (initial) protein adsorption are different on the topographically structured samples than on the untreated ones.
- The hydrophobicity or hydrophilicity of the surfaces are also a factor which influences the proteins adsorption.

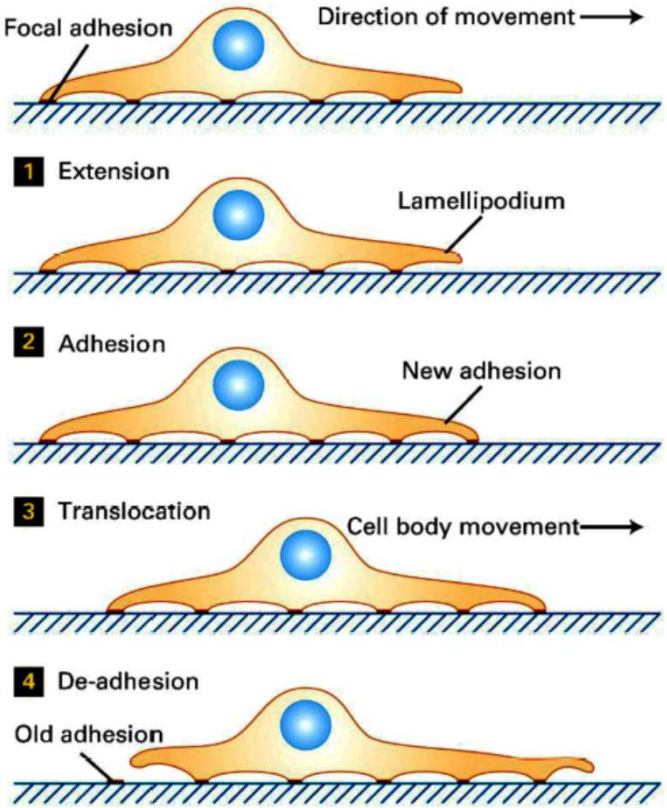
NIH3T3 attached on fs laser modified collagen/elastin layers following a 3 day culture period: (a) non-patterned surface, cell migration without a preferred direction (b-c) preferential cell migration on laser modified grooves.

Adhesion mechanism of cells on fs laser surface modified template.



- Cells mobility is governed by integrin-mediated focal adhesions .
- Cell locomotion occurs at the leading edge, when a membrane protrusion has emerged by small focal adhesions in order to make an initial contact with the ECM.
- Changes in local integrin adhesion force, influences cell membrane stretching and cell movement, thus affecting its response to surface roughness.

Steps in cells movement



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Conclusion

- Temporal tailoring of ultrashort pulses allows for the choice of the thermodynamic path the material undergoes during the excitation and its optimization with respect to different criteria. The results indicate the possibility of control of the generated microstructures .

- Longitudinal cell contact guidance (alignment along the groove directions) was observed on all of the types of laser-irradiated micro-grooved surfaces that were examined in this study.

- The material surface properties (chemical composition, wettability, surface roughness) can be modulated by ultra-short laser interaction in order to allow the adsorbed proteins to maintain their normal conformation and bioactivity.

- Femtosecond laser modification is able to produce a biopolymer porous matrix that mimic in part the structure and biological function of the extracellular matrix and can be potentially used for controlling cell behaviour.

- It was demonstrated that surface topography has an important effect on cells mobility and that cells are able to reorganize themselves in relation to surface nanofeatures.

Acknowledgment

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ФОНД НАУЧНИ ИЗСЛЕДВАНИЯ
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Thanks, for your attention!