3D FEMTOSECOND LASER MICROPROCESSING OF BIOMATERIALS FOR APPLICATION IN MEDICINE

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Project goals

The principal goal of the regenerative medicine is to promote tissue regeneration and healing after injury or disease that can be achieved through a delivery of cells and/or factors in tissue engineered scaffolds designed to provide a biomimetic microenvironment conducive to cell adhesion, proliferation, differentiation, and host tissue integration. Scaffolds constructed from biocompatible polymers (gelatin, collagen and their blends) have been developed for the needs of skin tissue replacement. The main demands to engineered scaffolds are: biocompatibility, biodegradability, high surface area for cell attachment, and a good mechanical integrity suitable for treatment handling. The surface topography has been shown to be a key issue in cell proliferation. The scaffold design should mimic the *in vivo* tissue microarchitecture and the cellular microenvironment. The biomaterials' microstructure and mechanical properties influence the scaffold bioactivity.

The goal of the present project was to demonstrate the effectiveness of applying femtosecond laser pulses to the modification of surfaces of natural biopolymers – gelatin, collagen and collagen-elastin thin films – and the formation of micro and nanoscale structures, as well as to study the dependence of the thin-film structure evolution on the laser parameters.

Results

Limited information is available on the interaction of high-energy ultra-short laser pulses with thin collagen and gelatin films. The laser-induced formation of a nanometer-scale matrix of a biomaterial can be controlled by varying the number of applied pulses, the pulse duration, and the laser fluence, which can be powerful tool for controlling the cell proliferation. Our study showed that low and medium laser fluence levels with a small number of laser shots create the most suitable conditions for producing nanoporous surface modification (figure 1).

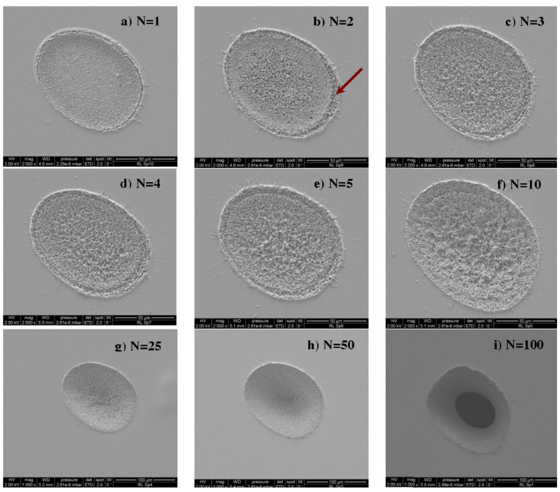

Figure 1. SEM images (2000×) of the surface modification of a thin gelatine film irradiated by 800-nm 30-fs laser pulses with laser fuence F = 2.5 J/cm^2: (a) N = 1, (b) N = 2, (c) N = 3, (d) N = 4, (e) N = 5, (f) N = 10, (g) N = 25, (h) N = 50, (i) N = 100.

Figure 2 shows the structure evolution as a function of the number of laser pulses applied. One can see that, as the number of pulses is raised to $N = 25$ (figure 2d), the structure of the porous matrix created loses its porosity, the pores become occluded and the modification is expressed mainly in melting of the inner part of the area irradiated. In this respect, it seems possible to tune the structure to a specific need.

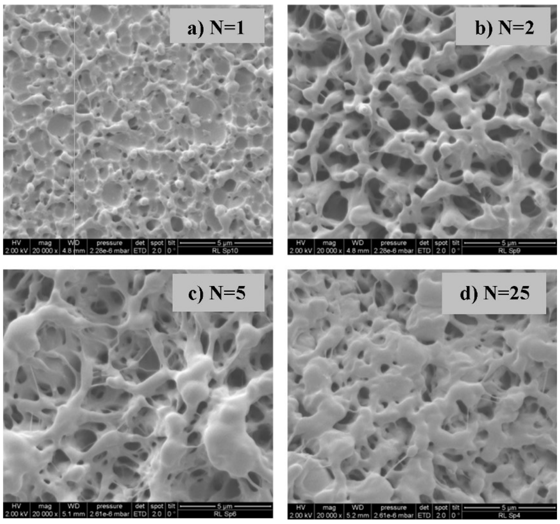

Figure 2. Higher SEM resolution images (20 000×) of the surface modification of a thin gelatin film irradiated by 800-nm 30-fs laser pulses with laser fuence F = 2.5 J/cm^2: (a) $N = 1$, (b) $N = 2$, (c) $N = 5$, (d) $N = 25$.

The interaction of the extremely high-intensity ultra-short pulses with transparent polymeric materials leads to multiphoton absorption in the material. This results in ionization of some atoms and molecules thereby providing initial carriers. The free electrons and ions absorb energy from the electromagnetic field of the laser radiation by inverse bremsstrahlung resulting in their acceleration. The subsequent avalanche-like multiplication of free carriers finally leads to a laser-induced optical breakdown (LIOB), rather than heat transfer, and to the generation of microplasma and voxels in the bulk of the transparent material. In addition, owing to the expansion of the heated plasma, a high-pressure transient wave propagates radially from the LIOB center into the surrounding environment.

The rapid energy deposition in a thin surface layer creates an extremely large energy density, leading to intense shock waves and a high pressure gradient in the interaction zone. For a laser fluence of ≈10^{14} W/cm^2 a few microseconds after irradiation, about 70 % of the absorbed energy is used by the expanding plasma to move the ambient gas, 20 % of the absorbed energy is lost as radiation to the environment, ultimately leaving less than about 3 % of the incident laser energy as heat inside the material. This thermal energy causes a thin layer of molten material to be formed immediately in the interaction zone. The lifetime of the molten layer (including both the melting and resolidification processes) depends on how quickly the energy is dissipated into the bulk. This in turn depends on the material's thermal properties (heat conductivity, specific heat, density, etc).

Controlling the morphology of the nanoporous matrix of biomaterial can be particularly powerful in controlling the cells' behavior (cell alignment, densities, orientation, migration, and localization). By increasing the number of laser shots at a constant laser fluence F = 6.4 J/cm^2 and a pulse duration of 30 fs the average size (diameter) of the surface nanopores created decreases from 400 nm to 100 nm. It was thus demonstrated that a control over the pores dimensions of the nanomatrix created, from 100 nm and up to 500 nm, can be achieved by varying the number of pulses.

Seeding of cells on functional biocompatible scaffolds is a crucial step in achieving the desired engineered tissue. In order to complete the cycle of experiments, human fibroblast cells were seeded on a laser modified collagen-elastin surface. Type I collagen is an extracellular matrix protein that is widely used as a scaffold material since it provides the necessary adhesive properties and tensile strength. The elastin provides elasticity to tissues/organs and is crucial component of blood vessels. We cultured human fibroblast cells in a collagen-elastin porous matrix to examine the effect of proliferation.

We created a series of rows on a thin film of 9:1 collagen-elastin spaced by 300 µm, the distance between each laser spot being 50 µm. The matrix created was irradiated by two pulses of 100 fs duration. Fluorescent microscope observations showed the relation between the surface topography and cells preferences during migration on the surface (figure 3).

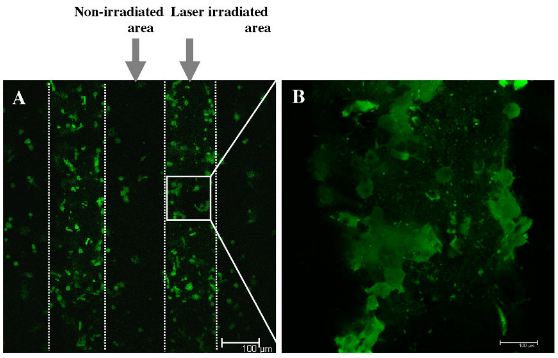

Figure 3. Fluorescence microscopy images of CFSE stained fibroblast cells cultured for 5 days on a 9:1 collagen-elastin porous matrix. Magnification: 10× (A) and 40× (B).

The cells in these images exhibited some migration after a 6-days incubation, but they were still confined within the patterns. The adhesion of the cells on the microstructured surfaces indicates that either 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 in their initial conformation; they also may be prone to undergoing structural changes. The flexibility of the cell's cytoskeleton depends on the rigidity of F-actin (major component of the cytoskeleton essential for such important cellular functions as the mobility), while their adaptation to the surface's topography determines the alignment of the cells.

In conclusion, we performed a systematic study of the influence of the laser pulse duration, pulse number, and fluence on the surface modification of thin film of biopolymers – gelatine and collagen. Irradiation in air of self-standing films of gelatin, collagen and collagen-elastin with single and multiple laser pulses at the wavelength of 800 nm produces a nanostructured layer with submicrometric dimensions on the film surface.

Femtosecond irradiation by different pulse temporal domains leads to the formation of the observed superficial fibrillar structures with different pores dimensions. Femtosecond laser modification is capable of producing a porous biopolymer matrix that mimics partially the structure and biological function of the extracellular matrix and can be potentially used for controlling cell behavior. It is tunable and can be used to design structures that affect cell proliferation, viability, and spreading.

Publications

1. Daskalova A, Nathala C, Bliznakova I, Stoyanova E, Zhelyazkova A, Ganz T, Lueftenegger S and Husinsky W 2014 Controlling the porosity of collagen, gelatin and elastin biomaterials by ultrashort laser pulses *Appl. Surf. Sci.* **292** 367-377
2. Chandra N, Daskalova A, Bliznakova I, Lueftenegger S, Zhelyazkova A, Enikoe S, Ganz T and Husinsky W 2013 Defined nano-structuring with ultrashort pulses in gelatine biopolymer films for tissue-engineering 2013 *MATEC Web of Conferences, EDP Sciences,* **8** DOI: 10.1051/matecconf/20130803009
3. Daskalova A, Selimis A, Manousaki A, Gray D, Ranella A and Fotakis C 2013 Surface modification of collagen-based biomaterial induced by pulse width variable femtosecond laser pulses *Proc. SPIE* **8770**

Conference participation

1. Nathala C, Daskalova A, Bliznakova I, Lueftenegger S, Zhelyazkova A, Enikoe S, Ganz T and Husinsky W 2013 Defined nano-structuring with ultrashort pulses in gelatin biopolymer films for tissue-engineering *Progress in Ultrafast Laser Modifications of Materials* (14-19 April 2013 Cargese France)
2. Daskalova A, Kasperski G, Rosseau P, Domaracka A and Lawicki A 2013 Interaction of slow highly charged ions with hard dental tissue: studies of fluoride uptake and remineralization efficacy *Eighteenth International Summer School on Vacuum, Electron and Ion Technologies* (7-11 October 2013 Sozopol Bulgaria)
3. Daskalova A 2013 Nano structuring by ultrashort laser pulses of biomimetic materials for application in tissue engineering *Second National Congress on Physics* (25-29 September 2013 Sofia Bulgaria)