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Selected laser methods for surface structuring of biocompatible diamond-like carbon layers

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ABSTRACT

Laser based surface structuring methods are among the fastest growing techniques and displace other surface treatments for applications such as marking, change of topography and microstructure. Such changes may lead to controlled modification of material properties, including their biocompatibility. The paper presents two laser techniques — picosecond Direct Laser Writing (DLW) and multi-beam Direct Laser Interference Lithography (DLIL). The purpose of the research was to create different shapes and dimensions of 2D and 3D periodical microstructures on the surface of thin diamond like carbon (DLC) coatings on different substrates. Resulting structures were further tested to find the influence of laser processing on the interaction of created scaffolds with the living cells in the direction of improvement of their directional growth and adhesion. The use of two different patterning methods (DLW and DLIL) allowed creation of structures with periods ranging between sub-microns to tens of microns, with different pattern resolutions, shapes (linear, dotted, crossed, hierarchical) and depths. Preliminary analyses have shown the possibility of high degree of control and improvement in the directional growth of smooth muscle cells and the proliferation of the endothelial cells, cultivated on migration channels created at DLC layers.

1. Introduction

One of the main applications of laser radiation in materials engineering is the modification of surface properties [1]. Multiple surface topographies and microstructures are required in order to create specified properties in various scales. Moreover, material properties are strongly associated not only with their microstructure, but also its spatial distribution. In many cases it is actually the key to the surface functionality, often engineered by nature [2].

The description and broader impact of laser techniques for interfacing biological matter with the materials intended for bioengineering applications has been the topic of several papers and reviews [3–10]. Modulation of the surface topography and microstructure is mainly realised using the following methods:

- Laser direct writing (LDW) [11–13];
- Mask projection (MP) [14,15];
- Direct laser interference lithography (DLIL) [16–19];
- Laser induced, self-organised periodic surface structures — "ripples" (LIPSS) [20,21];
- Combined techniques.

The most popular two of them, namely LDW and DLIL were utilised in experiments described in this paper. These techniques have been previously successfully tested in the structuring of different engineering and biocompatible material surfaces [22]. In case of bioengineering, one of the main issues is topographical response of cells and tissues to the pattern and form of structuring [23–25]. If the response is different, then this dependence can be utilised in modulation of cell functions [26], design of implant materials [27], cell growth control as well as in spatial cell orientation [28–30].

Amorphous carbon known as, "Diamond-Like Carbon" (DLC), representing an almost perfect biocompatible material was selected for the laser patterning tests [31,32]. The DLC is characterised by high smoothness, low friction coefficient, is chemically inert and abrasion-resistant [33,34]. Moreover, the optical DLC properties, especially the refractive and extinction indexes, are particularly appealing due to the ability to process material by means of laser radiation, especially in the green and ultraviolet spectral ranges [35,36]. In the cardiovascular field, DLC is employed for blood-contacting implants and interventional devices, such as heart valves, vascular prostheses, dialysis membranes

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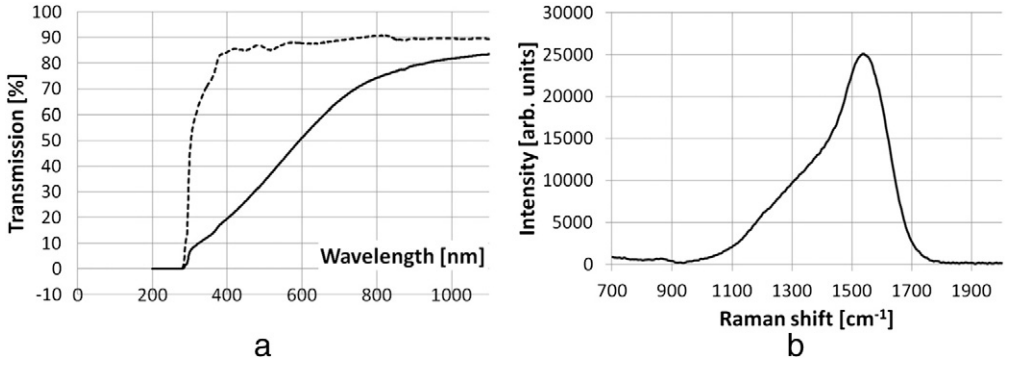

Fig. 1. Characteristic properties of the examined DLC layers (thickness 200 nm) on a polystyrene substrate: a) transmission as function of wavelength (solid line — DLC + polystyrene, dashed line — polystyrene); b) Raman spectrum registered during excitation via light with 514 nm wavelength.

and rotary pumps for ventricular assistance, stents and guidewires [37–40].

The surface modifications presented in the paper focused on controlled cell migration and proliferation. However, in the future, the performed analyses should lead to the general objective, which is the controlled integration of the implants with muscles. In the presented case, the integration should involve the heart. The work was conducted in response to the need of finding effective ways to properly prepare the external part of the inflow cannula of the heart support system. The rotor pump is introduced to the left chamber through the apex cordis. The external surface of the cannula should stimulate the tissue to integrate. It means that the muscle cells should partially grow on the surface, creating a smooth integration between the tissue and the metallic implant or the artificial device. It is crucial because otherwise, the light of the conduit will be closed by the uncontrolled proteinaceous and cellular growth. The heart is built of a special type of muscle cells. This is a striated muscle, but innervated with an autonomic nervous system. Because of the research nature of the work and the need for certain simplifications, we have decided at this stage to purchase and use the commercially available smooth muscle cells, also innervated with an autonomic nervous system.

With an artificially prepared tissue analogue, it is not possible to have control over one cell type. The necessary and the safest solution is to create a self-controlling cellular co-culture [41]. It was therefore decided to develop the muscle cell-material and the endothelium-material interactions separately. The preliminary tests of endothelium cell growth on modified DLC surfaces are shown in the last part of Section 4. Further results will be used in the subsequent stages of the work to develop a co-culture on artificial surfaces. This kind of interaction occurs in the blood vessel.

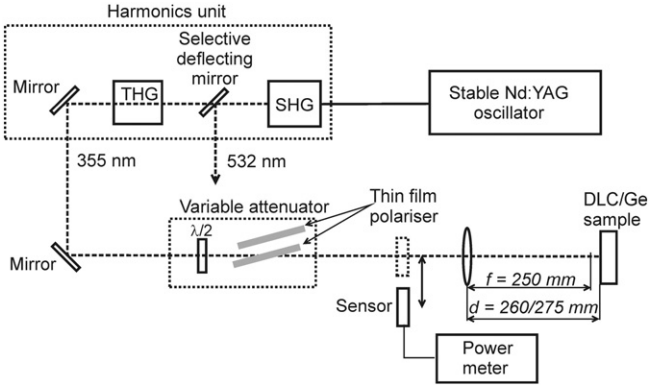

Fig. 2. Optical scheme of a measurement system determining ablation thresholds. SHG — second harmonics generator (532 nm); THG — third harmonics generator (355 nm).

2. Materials and methods

2.1. Materials and samples

The laser processing experiments were performed on DLC layers with thicknesses of approximately 200 nm, deposited onto glass, silicon and polystyrene (Petri dish), and with an approximate thickness of 1.2 μm on germanium. The coatings were all prepared on a Leybold Univac machine (Leybold Vacuum, Cologne, Germany), equipped with unbalanced AJA sputter cathodes and Advanced Converter power supplies.

The cell-material interactions were determined based on the comparisons of the morphology and proliferation of Human Umbilical Vein Endothelial Cells (HUVECs) and Smooth Muscle Cells (SMCs) for a 5-day culture. Cell lines were purchased from Lonza, Switzerland. The cells were seeded with the density of 4×10^3 cells/cm^2 and cultured on all the analysed variants of scaffolds. A detailed preparation of the suspensions can be found elsewhere [42]. The cell viability and proliferation was determined by the everyday MitoTracker Red CMXRos staining and counting of cells under the microscope for a 5-day culture. At appropriate points in time (after 4, 24, 48 h and 5 days) the samples were fixed (4% paraformaldehyde) and stained with Alexa Fluor 488 phalloidin in order to visualise the cytoskeletal structure, and with 4′,6-diamidino-2-phenylindole (DAPI) to show the cell nuclei. The images were acquired using an Exciter 5 AxioImager confocal laser scanning microscope (CLSM) with an incubation chamber (Carl Zeiss AG, Germany). The data was processed by means of the CLSM Zen 2008 software.

In order to quantify the similarities in morphology between the cells of all the investigated samples, the shape index (SI) was evaluated using image analyses. The SI is a dimensionless measure of cell roundness and it is defined as $SI = 4\pi A/p^2$, where A is the area of the cell, and p is the length of the cell perimeter. Thus, the SI values range from zero for a straight line to one for a perfect circle. The orientation of the elongated cells was evaluated by measuring the angles of individual cells by means of the AxioVision 4.8 image processing software package. The average angle was derived from finding the statistical average for all cell angles. The cells were then classified according to their angular deviation from the mean angle in each image. The standard deviations for the relative number of cells per class were obtained by the evaluation of $n = 6$ images from each sample series.

2.2. Microstructural analyses

A main research instrument in microstructural analyses was the KH 8700 model digital 3D microscope from Hirox, Japan. The microscope allowed observations and three-dimensional quantitative measurements of surface topography under varying illumination, from point to cylindrical, with a variable angle of incidence. The microscope