Effect of microstructured and ta-C coated glass-surfaces on cell growth

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Abstract To investigate the effects of tetrahedral amorphous carbon (ta-C) coating and microstructuring of glass surfaces the growth of human osteosarcoma SAOS-2 osteoblasts and fibroblast-like mouse cells L-929 was analyzed. Microstructures were established by femtosecond laser with focused laser beam and the coating with tetrahedral amorphous carbon (ta-C) film was achieved by excimer laser ablation for film deposition. SAOS-2 and L-929 cells were cultured on these ta-C coated and/or microstructured glass-surfaces. The reaction of the cells to these modified surfaces was investigated by fluorescence staining. By application of the laser annealing process alternating to the deposition process, the preparation of several micrometer thick stress-free ta-C films with 80-85 % sp3 bonds and 60-65 GPa hardness was possible. Cultivation of SAOS-2 and L-929 cells on these modified glass surfaces showed no detrimental effects on cell viability, also if they grow slower and do not spread so well, like on control. By confocal laser scanning microscopy the cell morphology was analyzed and a positive influence of coating the microstructures was observed, in contrast to the non-coated microstructures, where cells avoid growing.

Keywords: microstructure, coating, ta-C, tetrahedral amorphous carbon, adhesion, cell growth

1 Motivation

The reaction and interaction of cells and surfaces are strongly affected by the properties and the topography of the surrounding surfaces. Several studies have shown that coating and structuring of surfaces can influence the adhesion, proliferation, and morphology of cells. Diamond-like carbon films can have a hardness and strength up to 90% of that of natural diamond. If they have a high fraction of sp^3 diamond bonds (80-85%) and do not contain hydrogen, they are called tetrahedral amorphous carbon (ta-C). Due to the high hardness, wear and corrosion resistance, chemical inertness, low frictional coefficient and excellent smoothness of ta-C, it fulfills the criteria of a good biomaterial.[1] Furthermore the structuring of surfaces can promote the adhesion of cells, influence the morphology and also induce aligned cell growth. [2, 3]
The goal of this study is to investigate the effects of tetrahedral amorphous carbon (ta-C) coatings and microstructured glass surfaces on the human osteosarcoma SAOS-2 osteoblasts and also on the fibroblast-like mouse cell line L-929.

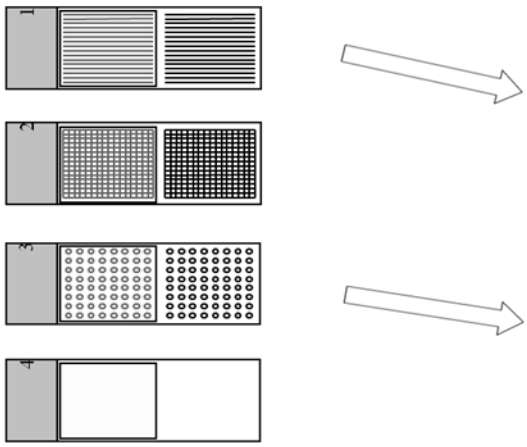
2 Materials and Methods

2.1 Coating Methods and Material Preparation

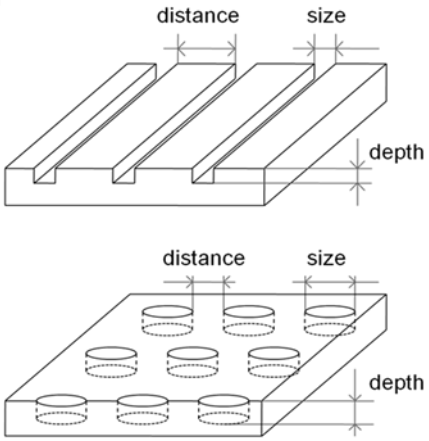
Tetrahedral amorphous carbon (ta-C) was deposited with excimer laser (LPX300 Coherent) ablation. A 100 nm thick ta-C film was achieved via pulsed laser deposition (PLD) on the samples with 80-85% sp^3 bonds and a hardness of 60-65 GPa (see also presentation number 89 – Katja Günther). The microstructures were created by femtosecond laser pulses of 775 nm mean wavelength and 130 fs pulse duration with focused laser beam.

The microstructures were done on 76 x 26 x 1 mm microscope glass slides and afterwards the ta-C film was deposited. The samples were separated in 2 half. Both of them were microstructured with the same pattern: lines, grids or points. One sample was left without pattern, but one half was coated (figure 1).

a)



b)



c)

sample	pattern	distance	size	deepness
1a	lines	100 µm	40 µm	5-10 µm
1b	lines	200 µm	50µm	15-25 µm
2a	crosses	100 µm	34-38 µm	5-10 µm
2b	crosses	200µm	45-50 µm	25-30 µm
3a	points	100µm	40-50 µm	5-10 µm
3b	points	120-150 µm	80-120 µm	15-20 µm
4	none (control)			

Figure 1. Schematic illustration of the structures and overview of all structures. a) Arrangement of the deepening, left half is coated, right half is non-coated. b) Magnification of the structures and definition of the measurements. c) Overview of different structures and their measurements.

2.2 Cell Lines, Growth Conditions, Staining and Microscopic Analysis

Two cell lines, the human osteosarcoma SAOS-2 osteoblasts and the fibroblast-like mouse cells L-929, were used for the experiments on the ta-C coated and / or micro-structured glass-slides. Both strains were cultured in Rosewell Park Memorial Institute RPMI-1640 medium (Biochrome AG) containing 10% fetal calf serum. Cells were harvested at 90% confluence, seeded on the samples and then cultured in 4-well dishes (Nunc) for 4 days. Growth was performed by 37°C in humidified 5% CO_2-in-air.

For fluorescence microscopy, the cells were fixed in 3.7 % formaldehyde, prepared with 0.1 % triton X-100 for staining and the actin-strains were labeled with BODIPY® Fl phallacidin (Invitrogen). The samples were visualized by fluorescence microscopy (Zeiss Axiovert 200 M). Microphotographs of non-overlapping areas and from separated growing zones were taken from all samples using 100x and 200x magnification.

3 Results

The investigation of the general growth properties shows that although the cells grow on the structured and coated surfaces, there are apparent differences in cell morphology in comparison to the control glass slide. The cells grow slower, they do not spread so well, the number of rounded cells is increased and the actin filaments do not show such a distinct shape. Additionally there are differences between the coated and the non-coated structures. On the coated structures the cells show no negative response to the deepening, into which they grow, as seen at the SAOS-2 and L-929 cells on sample 1b in figure 2.

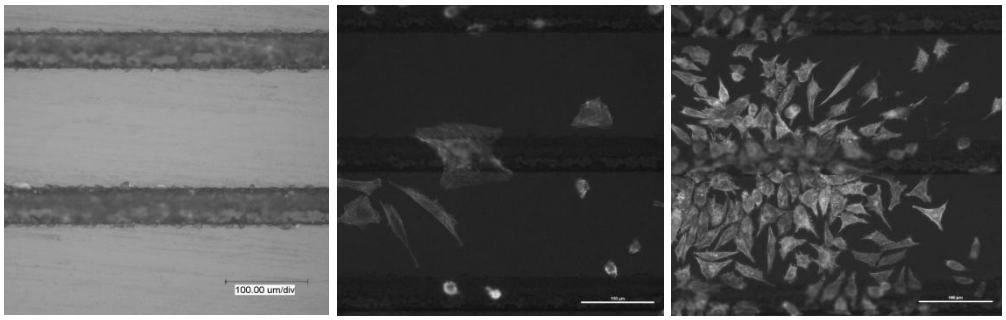


Figure 2: Microphotographs of cell growth on sample 1b (numbering same as in figure 1). Left to right: light microscopy photograph, fluorescence microscopic photograph of SAOS-2 cells on coated sample (BODIPY FL phallacidin green), fluorescence microscopic photograph of L-929 cells on coated sample (BODIPY FL phallacidin green).

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On the non-coated structures, the cells seem to avoid the deepening, for example L-929 cells on sample 1a and 2a. Even if they grow densely on the normal surface, nearly no cells can be observed in the deepening (figure 3).

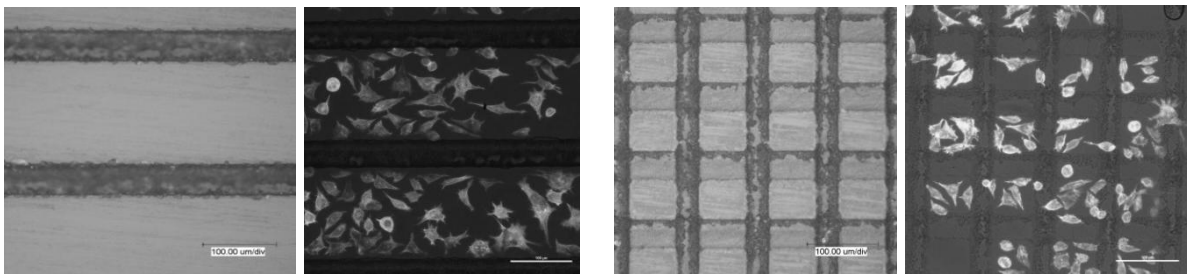

Figure 3: Microphotographs of cell growth (numbering same as in figure 1). Left to right: light microscopy photograph of sample 1b and fluorescence microscopic photograph of L-929 cells on non-coated sample (BODIPY FL phallacidin green), light microscopy photograph of sample 2a and fluorescence microscopic photograph of L-929 cells on non-coated sample (BODIPY FL phallacidin green).

In contrast to this, the cells that grow on coated, but non-structured surfaces, seem to grow worse than on non-coated-non-structured surfaces (figure 4).

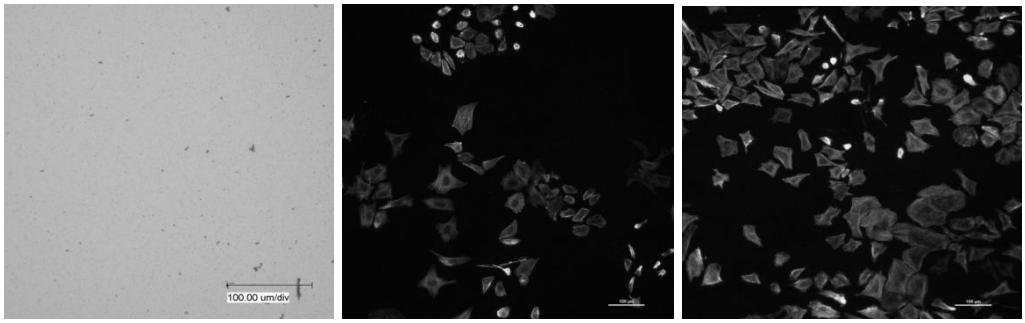

Figure 4: Microphotographs of cell growth on sample 4 (numbering same as in figure 1). Left to right: light microscopy photograph, fluorescence microscopic photograph of SAOS-2 cells on coated sample (BODIPY FL phallacidin green), fluorescence microscopic photograph of SAOS-2 cells on non-coated sample (BODIPY FL).

4 Conclusion

Based on these results, a correlation between cell morphology and the deepening is apparent. The structuring in combination with the coating of glass slides affects a homogenous cell growth. Although the cells grow moderately slower and fewer adhere in comparison on the control surface, there seems to be no sustainable indication that ta-C deposition is not suitable for cell culture applications.

In the future, further cell lines should be tested and additional experiments, like analyzing cell morphology with SEM (scanning electron microscopy) or measurement of adhesion force by AFM (atomic force microscopy), have to be done, to confirm these initial results.

5 References

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