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GROWTH OF CELLS ON CARBON COATINGS MANUFACTURED IN NEW MW/RF REACTOR

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Abstract

Due to unique physico-chemical properties and good biocompatibility are carbon layers considered to be promising material for wide field of biomedical applications. In this study, carbon films were manufaktured in microwave and radio frequency plasma reactor using dual frequency method (MW/RF PCVD - microwave and radio frequency plasma chemical deposition). Four various processes of deposition were used for preparation layers on substrates of medical stainless steel AISI 316 L. On samples of all four processes, growth and adhesion of cells were observed.
[Engineering of Biomaterials, 56-57,(2006),19-21]

Experimental details

Materials and deposition procedure

In the present study we have studied carbon coatings (NCD) prepared in microwave and radio frequency plasma reactor using dual frequency method MW/RF PCVD - microwave and radio frequency plasma chemical vapour deposition. Parameters of the deposition were optimised to get uniform films on the stainless steel AISI 316L. Stainless steel substrates used in the present study were shaped as 2,5 mm thick circular discs with diameter of 8 mm. Substrates were machined, electropolished, ultrasonically cleaned in methanol and dried. Prior to NCD deposition, the surface were cleaned in an argon inert plasma in the vacuum chamber for 10 minutes. [1] Four various processes of deposition were used TAB. 1. In present paper, coated samples were named after number of deposition process, uncoated were just AISI 316L.

Cells and cultures conditions

Coated and uncoated samples were sterilized in steam autoclave by temperature 120°C for 20 minutes, immersed in tissue water. All samples were placed into polystyrene multidishes (Costar, 24 wells, diameter 15 mm) and seeded with human osteosarcoma cell line MG 63 and Dulbecco medium at the initial density 30 000 cells/well. Glass coverslips and the polystyrene dish as control materials were used. Cells were cultured for one, three and seven days in the temperature of 37°C in atmosphere containing 5% of CO_2. On day one and three after seeding, the cells were rinsed in phosphate-buffered saline (PBS), fixed with 70% cold ethanol (-20°C, 5 min), vizualized by propidium iodide (5µg/ml, 5min). Their morphology was evaluated and documented using epifluorescence microscope IX 50 equipped with a digital camera DP 70 (Olympus, Japan). Fluorescent cell linker kit PHK 26 RED and propidium iodide for better vizual representation, were used. The number of adhering MG 63 cells was counted in 10-16 randomly selected mi-

PROCESS	Methane [%]	Bias [V]	Power [W]	Pressure [Pa]	Time [min]
1.	30	400	150	10-60	6
2.	30	500	250	10-30	3
3.	20	600	250	10-25	4
4.	15	700	250	10-30	5

TABLE 1. Parameters of deposition processes.

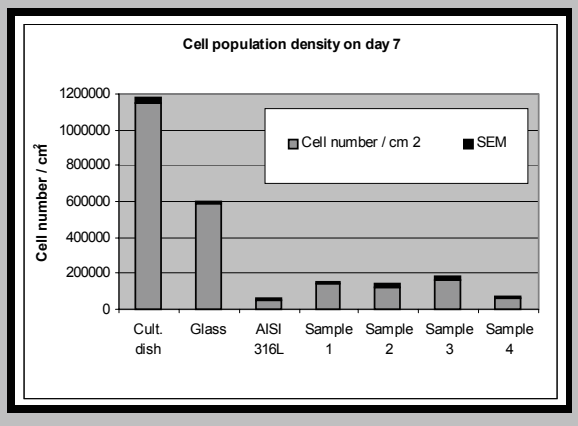

FIG. 1. Number of initially adhered osteoblast-like MG 63 cells on tissue culture polystyrene (Cult.dish), on glass coverslips (Glass), uncoated sample AISI 316 L, Sample 1, 2 , 3, 4 on day 7 after seeding. Average ±SEM from 10-16 measurements, statistical significance in comparison with values obtained on culture dish.

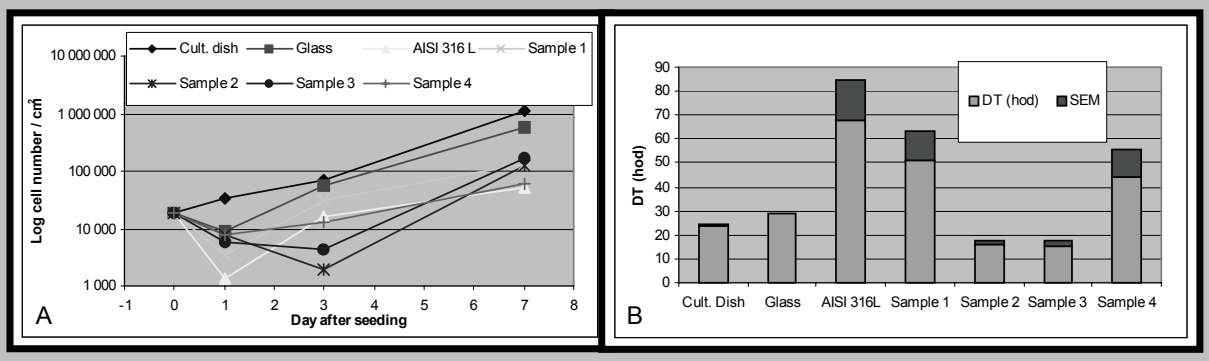

FIG. 2. Number of initial adhered osteoblast-like MG 63 cells (A) growth curves of these cells from day 0-7 (B) the population doubling time DT between days 3 and 7 after seeding on tissue culture polystyrene (Cult.dish), on glass coverslips (Glass), uncoated sample AISI 316L and coated Sample 1, 2 , 3, 4.

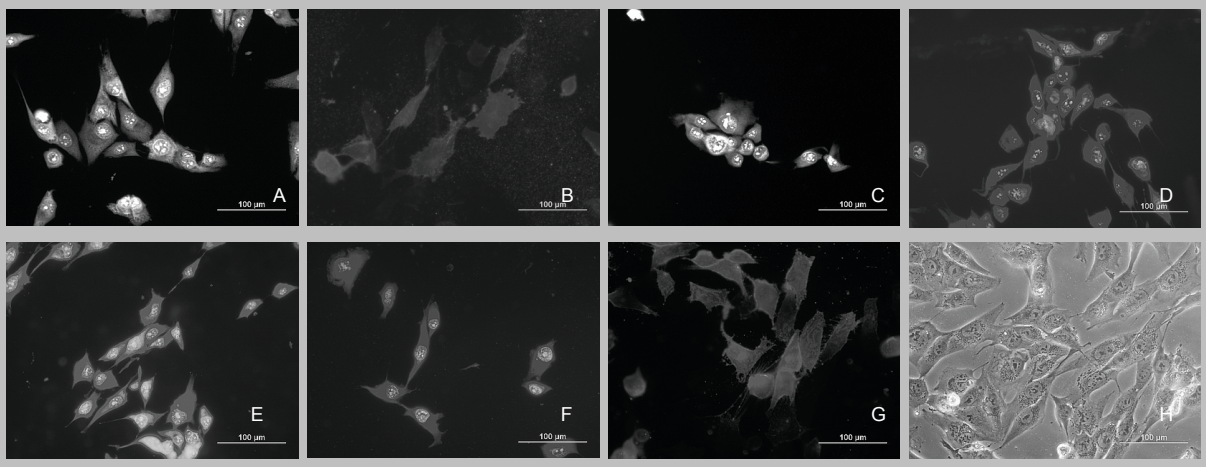

FIG. 3. Morphology of osteoblast-like MG 63 cells on day 1 after seeding, stained with propidium iodide or red fluorescent cell linker, epifluorescence (A) culture dish polystyrene (B) glass coverslips (C) sample 1 (D) sample 2 (E) sample 3 (F) sample 4, (G) AISI 316 L, (H) native cells in medium on culture dish.

croscopic fields (20x objective, 1 and 3 day after seeding). On day 7 after seeding, when the cell counting in microscopic field was disabled by a high cell population density and formation of multi-layered areas, the cells were detached by trypsin-EGTA for 5 min at 37°C and counted in a Bürker homeocytometer (18 measurements for each sample). [2,3]

Results

On day 1 after seeding, the number of cells initialy adhered on all samples was lower than found on conventional tissue culture polystyrene dishes and than on glass coverslips. In comparison with uncoated sample AISI 316 L was the number of cells on coated samples significantly higher. Morphology of osteoblast-like MG 63 cells on day 1 after seeding is represented in FIG.3. From day 1 to 3 after seeding, the cell number on all samples incresased in the following order: Polystyren > Glass > Sample 1 >AISI 316 L > Sample 4 > Sample 3 > Sample 2. On day 1 and 3 after seeding, clear preference of cell growth on edges of samples was observed. Clusters of cells on edge areas of samples were detected, but in the middles of samples only isolated cells were found. On day seven the higher proliferation activity of cells on the all coated samples in comparison to uncoated sample was observed. The most number of adhering cell were on polystyrene culture dish and on the glass coverslips. Non of our samples has better cell adhesion. In comparison with coated samples has higher number of cells the Sample 3 than Sample 2, Sample 1 and AISI 316L (FIG.1). Average cell numbers were compiled as growth curvers (FIG. 2A). The population doubling time DT of MG 63 cells between day 3-7 was shortest for Sample 3 (FIG. 2B), followed with Sample 2, Culture dish, Glass, Sample 4, Sample 1 and AISI 316L. Statistic data were presented as means ± S.E.M. from 10-16 measurements obtained from 1 sample for each experimental group. Statistical significance was evaluated by the Student's t-test in comparison with values obtained on polystyren culture dish.

SEM observation were performed in VEGA TS 5130 scanning electron microscope. The results of observation

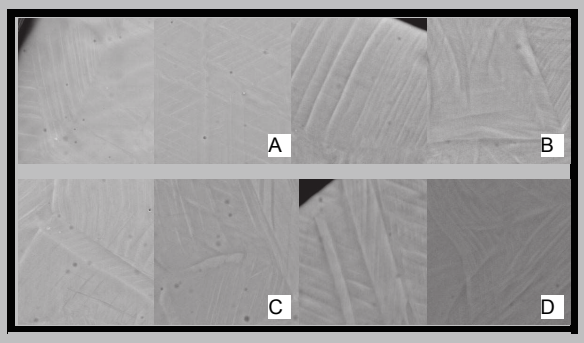

FIG. 4. SEM micrographs of surfaces of coated samples; photo on left side presents edge of sample, right is the middle of the sample (A) sample 1 (B) sample 2 (C) sample3 (D) sample 4

were presented in the FIG.4. Different surface morphology of layers on samples could be one of the reasons to growth preference of cells on individual samples. It may be the cause of the growth preference of cells on the edge areas of samples with comparison to the middle areas of samples, where on day 1 and 3 after seeding rather isolated cells were found.

Conclusion

In this study growth and proliferation of osteoblast-like cells MG 63 on four carbon films deposited under different process conditions in MW/RF reactor were investigated. In this paper the comparison of cell growth on coated, uncoated, glass and tissue culture polystyren was performed. We have noticed potential dependence of cell adhesion on surface morphology on edges and in the middle of sample. This study may be helpful in a selection of coating conditions in MW/RF reactor to create suitable carbon films for biological application.

Acknowledgements

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ELECTRIC POTENTIAL OF BIOMATERIALS COATED WITH DIELECTRIC CARBON LAYER AND NON-COATED IN WATER AND SERUM

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Abstract

Electric potentials, established in water and human blood serum, of the electrodes made of platinum, graphite, Ti6Al4V alloy, TiN, AISi316L steel, oxidized steel and the materials coated with diamond like carbon layer (DLC) and a nanocrystalline-diamond layer (NCD) were determined. The values of the potentials of non-coated materials increased with increasing electron work function (Φ) of investigated materials in reasonable accordance. The difference between the potentials measured in serum and in water was ascribed to the differing molecular electron structure of the proteins in contact with the electrodes. Electrode potentials of different materials coated with the same thin dielectric carbon layer varied significantly depending on the Φ value of the substrate. In this way the selection of a substrate material permits influencing on the interaction between NCD (DLC) coating and serum compounds. The potential of an electrode has appeared to be a simple but sensitive indicator of the phenomena that take place on the biomaterial surface.

Keywords: *electrode potential, electron work function, Pt, TiN, carbon, Ti6Al4V, AISI316L, DLC, NCD.*
[Engineering of Biomaterials, 56-57,(2006),21-24]

Introduction

The bulk biocompatibility of implant materials (i.e., the similarity of their mechanical, magnetic etc. properties to the properties of the human body) can be evaluated in the macro-scale in a quite simple way by in vitro examinations. The interaction between the surface of the implant material and the tissues, blood and cells is usually examined in vitro by observing the phenomena in the microscopic scale using biological methods. The specific drawback of the biological methods carried out in vitro lies in the necessity of using certain additional substances, absent in the living organism, that ensure the stability of the specimen. Moreover, the results of biological examinations obtained by various investigators are difficult to compare. Simple physicochemical methods suitable for estimating macroscopically the biocompatibility of the implant surface are scarce. They primarily include corrosion examinations and tracing the presence of the markers of selected bio-chemical processes that proceed in blood.
M.J. Jones et al. [2], who examined the haemocompatibility