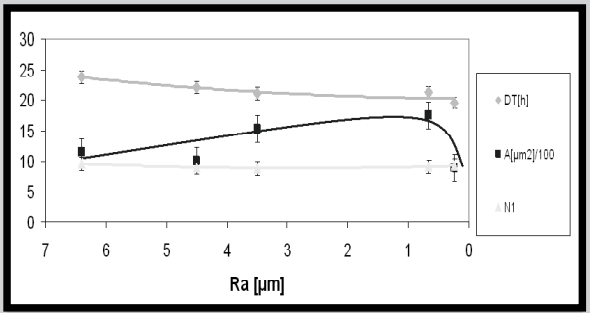

FIG.3. The dependence of biological parameters on the roughness parameter of the surface Ra for the pyrolytic carbon layer

Sample	#3	#4	#5	#6	#7
Cell density after 1 day [10^3/cm^2]	9,6 ±1,0	9,0 ±0,3	8,9 ±1,5	9,1 ±0,7	9,3 ±1,4
A [µm^2]	1155 ±222	1007 ±97	1527 ±425	1759 ±402	895 ±286
DT [h]	23,8 ±1,8	22,1 ±0,6	21,1 ±1,7	21,3 ±0,9	19,6 ±0,4

TABLE 1. Parameters of cell's features after one day cultivation: doubling time DT, number of cells N1 and cell areas A.

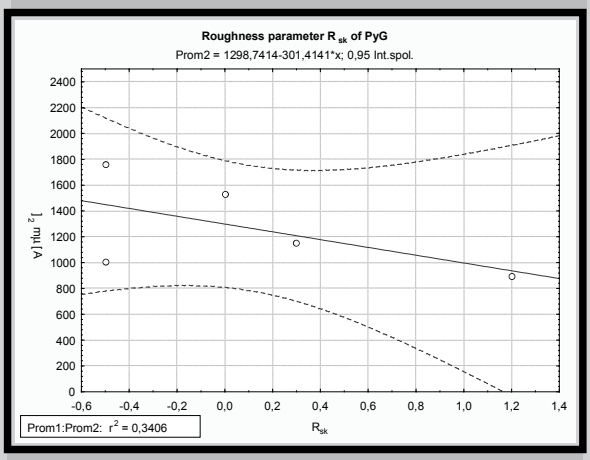

FIG. 4: Dependence of cell areas on Rsk.

profile, showed a tendency to be in negative values for best spread cells.

The next results for other types of surface films will be presented in the contribution.

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BIOLOGICAL EFFECTS OF POLYMERS MODIFIED WITH CARBON NANOTUBES ON HUMAN OSTEOBLAST-LIKE MG 63 CELLS

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Abstract

The tested materials were represented by a pure terpolymer of polytetrafluorethylene-polyvinyldifluoride-polypropylene (PTFE-PVDF-PP), pure polysulphone (PSU) and PSU modified with single- or multi-walled carbon nanotubes in concentrations of 0.5, 1 or 2 wt%. As control samples, a polystyrene cell culture dish and microscopic glass coverslips were used. The number and viability of human osteoblast-like MG 63 cells in cultures on these materials was detected with a Cell Viability Analyzer (Vi-CELL XR, Beckman Coulter) on 1, 3 and 7 days after seeding. On all tested samples, the cell number was similar or lower than that detected on the control polystyrene dishes. The cell viability on day 1 after seeding was relatively low on PTFE-PVDF-PP and some nanotube-containing samples, ranging from 10 to 100 % of living cells, but on day 7 after seeding, it reached at least 90% on all tested samples. The cell spreading area was detected in cells after immunocytochemical staining of β-actin on day 3 after seeding. In nanotube-containing samples, especially those with multi-walled nanotubes, this area was similar or even larger than that on the control materials. The beta-actin cytoskeleton was well developed in cells on all nanotube-containing materials and similar to that in cells on control surfaces. Thus, it can be concluded that nanotube-containing PSU supports the adhesion and growth of osteoblast-like cells and could be used for construction of bone implants in which the anchorage in the surrounding bone tissue is desirable.

Key Words**:** *carbon nanoparticles, carbon-polymer composites, bone cells, adhesion, proliferation, beta-actin*

[Engineering of Biomaterials, 58-60,(2006),14-16]

Introduction

In recent years, carbon materials, especially the carbon nanotubes, have been considered as very promising materials for biomedical applications including tissue engineering. Therefore, in our experiments, polysulphone was mixed with single- or multi-walled carbon nanotubes, because it is believed that the nanostructure of materials containing nanotubes can resemble the nanoarchitecture of the natural extracellular matrix (ECM). Nanostructured surfaces can

promote preferential adhesion and growth of osteoblasts over other "competitive" cell types, mainly fibroblasts, which can cause fibrous encapsulation and loosening of bone implants [1, 2]. Moreover, nanotubes can serve as a reinforcement of synthetic polymers, which are usually too soft and elastic for replacement of the bone tissue. When incorporated into a polymeric matrix, the nanotubes can resemble nanofibres of collagen and other extracellular matrix proteins in the bone, and together with other carbon nanoparticles (nanodiamonds, fullerenes), also hydroxyapatite and other inorganic crystals in the bone [1-3].

Material and methods

The tested materials were represented by pure terpolymer of polytetrafluorethylene-polyvinyldifluoride-polypropylene (PTFE-PVDF-PP), polysulphone (PSU; purchased from Aldrich Chemical Co., Inc. USA) and polysulfone modified with single- or multi-walled carbon nanotubes (NanoCraft Co. Inc., Renton, WA, U.S.A.) in concentrations of 0.5, 1 or 2 wt%. As control samples, a conventional polystyrene cell culture dish (TPP, Switzerland) and microscopic glass coverslips (Menzel GmbH, Germany) were used.

The polymer solution was obtained by dissolving 5 g of PSU in 50 ml of dichloromethane. This solution was mixed in a sonicator for 10 minutes with Single Walled Carbon Nanohorns (single-walled carbon nanotubes, SWNT); or High Crystalline Electric Arc Multi-wall Nanotubes (MWNT); in concentrations of 0.5, 1 or 2 wt%. The mixture was poured into a Petri dish and left freely to evaporate.

The materials in a form of circular foils (diameter 1.67 cm) were sterilized by H_2O_2 – plasma method (Sterrad 120, ASP, Johnson & Johnson), inserted in polystyrene 24-well-multidishes (TPP, Switzerland), seeded with human osteoblast-like cells of the line MG 63 (European Collection of Cell Cultures, Salisbury, UK) at the initial density of 7000 cells/cm$_c$, and incubated in 2 ml of Dulbecco-modified Eagle Minimum Essential Medium (DMEM, Sigma, U.S.A.) medium supplemented with 10% fetal bovine serum.

The cell number and viability was detected using a cell viability analyzer Vi-CELL XR (Beckman Coulter, U.S.A.) on the 1st, 3rd and 7th day after seeding.

The cell spreading and the size of cell-material contact area were evaluated in cells after immunofluorescence staining of β-actin on day 3 after seeding [4].

Results and discussion

The number of MG 63 cells on day 1, 3 and 7 after seeding on pure polymers (PSU, PTFE-PVDF-PP) as well as polymers mixed with single- or multi-walled carbon nanotubes was similar or lower than the values found on the control polystyrene dishes (FIG.1). Nevertheless, the cells on all nanotube-containing samples were relatively well spread. On the pure PSU and PSU with all concentrations of SWNT, the cell adhesion area was similar to that found on the control glass coverslips, and in samples with MWNT it was even significantly larger (FIG.2). On PTFE-PVDF-PP, the cell adhesion area was significantly smaller than on the glass coverslips, which was probably due to a relatively high surface hydrophobicity. Moreover, the cells on the polymer-nanotube composites, displayed well developed beta-actin cytoskeleton characterized by distinct beta-actin filament bundles (FIG.3). It was probably caused by the nanostructure of the surface that supported the spreading of osteoblast-like cells [1-3]. Similar results were obtained in MG 63 cells growing on carbon fibre-reinforced carbon composites covered with a nanostructured film of fullerene C_{60} (Bacakova *et al.* 2005).

The viability of MG 63 cells on day 1 after seeding was relatively low on PTFE-PVDF-PP (10% of living cells), PSU containing 0.5% SWNT (30% of living cells) and PSU with 1% MWNT (50% of living cells). On the other samples, the cell viability ranged from 63 to 100%, being 77% on the control polystyrene dishes. However, during 7-day-cultivation, the cell viability markedly improved. On day 3 and 7

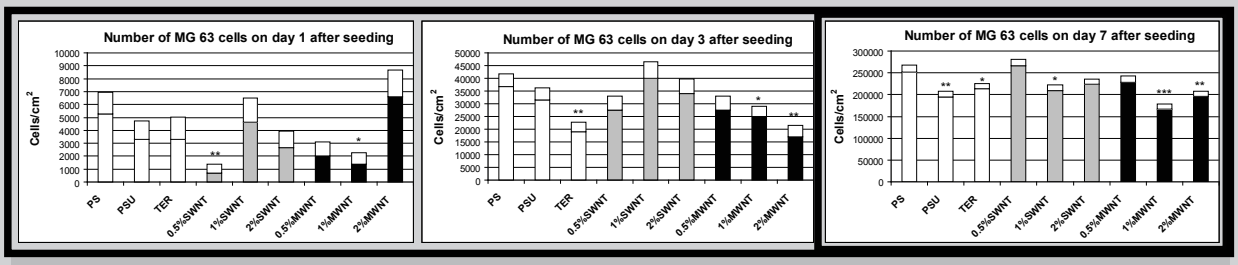

FIG.1. Number of MG 63 cells grown for 1, 3 or 7 days on tissue culture polystyrene (PS), polysulphone (PSU), terpolymer of polytetrafluorethylene-polyvinyldifluoride-polypropylene (TER), PSU mixed with 0.5, 1 or 2 wt% of single-walled carbon nanotubes (0.5%SWNT, 1%SWNT and 2%SWNT, respectively), and PSU mixed with 0.5, 1 or 2 wt% of multi-walled carbon nanotubes (0.5%MWNT, 1%MWNT and 2%MWNT, respectively). Mean ± SEM from 50 measurements, Student's t-test for unpaired data. Statistical significance: *p≤0.05, **p≤0.01 and ***p≤0.001 in comparison with the values on PS.

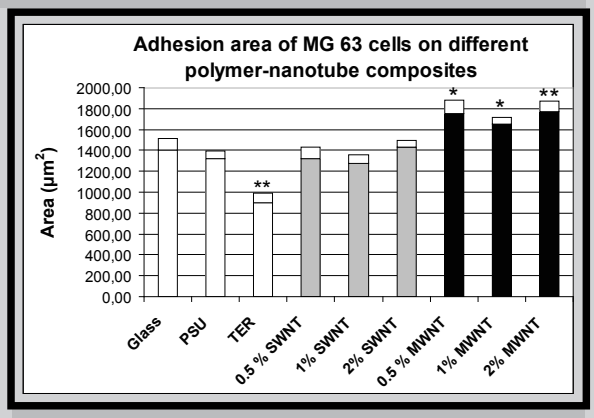

FIG.2. Size of the cell adhesion area of osteoblast-like MG 63 cells on grown for 3 days on various polymer-nanotube composites (see FIG.1). Mean ± SEM from 26-124 cells for each experimental group. Student's t-test for unpaired data. Statistical significance: *p≤0.05, **p≤0.01 and ***p≤0.001 in comparison with the control values obtained in cells on glass coverslips (Glass).

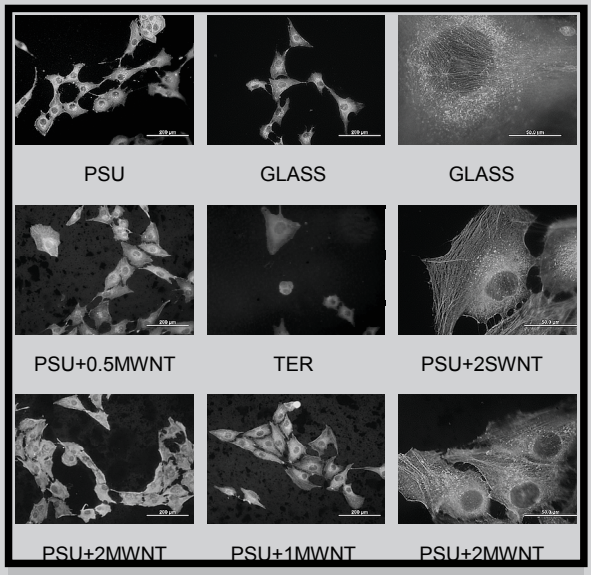

FIG.3. Immunoflurescence staining of β-actin in MG 63 cells grown for 3 days on microscopic glass coverslips (Glass), polysulphone (PSU), PSU mixed with 2 wt% of single-walled carbon nanotubes (PSU + 2 SWNT), terpolymer of polytetrafluorethylene-polyvinyldifluoride-polypropylene (TER), and PSU mixed with 0.5, 1 or 2 wt% of multi-walled carbon nanotubes (PSU + 0.5 MWNT, PSU + 1 MWNT and PSU + 2 MWNT, respectively). Fluorescence microscope Olympus IX 50, digital camera DP 70.

after seeding, it was at least 85% and 90%, respectively, in all tested samples.

Conclusion

Polysulfone supplemented with single-walled or multi-walled carbon nanotubes supported the adhesion, spreading and subsequent growth of human osteoblast-like MG 63 cells.

Acknowledgement

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THE STUDY OF THE SURFACE PROPERTIES OF C/C COMPOSITES

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Introduction

2D carbon-carbon composites (C-C) without or with the surface layer of pyrolytic carbon (graphite) is very prospective material due to its exclusive properties. This material system is often used in biomedical applications (bone and joint implants), machinery (friction-bearing parts) and in the aircraft industry (parts of the braking system). The aim of this work was to define the coefficient of friction and wear resistance of composite and of PyG layer.

Samples preparation

The investigated composites (IRSM AS CR, Prague, CR) were prepared from commercially available plain-weave carbon fabric made of the general purpose ex-PAN carbon fibre Toray T800. A stack of 8 layers of the fabric, soaked in an ethanol solution of phenol-formaldehyde resin Umaform LE (SYNPO Ltd., Pardubice, CR), was cured at 120°C. During its carbonization at 1000°C in nitrogen, conversion of the resin matrix to glass-like carbon took place; it was followed by high-temperature treatment of the samples at 2200°C in an argon environment. Pyrolytic carbon (graphite) (PyG) forms as result of pyrolysis, a chemical process where the bonds among the atoms of hydrocarbon molecules are broken due to high temperature. Partially crystalline pyrolytic carbon (pyrolytic graphite) layer of thickness about 0.5 mm was produced at process temperature 1900°C and partial pressure p=4Pa of butan+H_2 mixture. We studied the samples both with native ("as prepared") surfaces and also with surfaces prepared by grinding using metallographic paper of 4000 grade, to obtain samples with various roughnesses and chemistry. By this way, we have prepared 4 types of samples (I – IV) with 2 types of surface chemistry (C-C and PyG) and with various roughness – unground and ground.

Results and discussion

The measurements were realized using conventional tribometer in natural atmosphere (dry wear). The measurements demonstrate the excellent tribological properties of the surfaces, especially the very low friction coefficient and the very good wear resistance of the surface of the pyrolytic carbon layer on the polished 2D C-C composite.

To explain these measurements we also measured the surface roughness, microhardnes and surface energy of both the composite and the layer. The roughness and heterogeneity are common features of all real surfaces even if they were prepared very carefully. We have found, that both the type of surface and its roughness have the influence on the time dependence of friction and, moreover, the roughness has relatively low influence on the wear. Thus, the properties influencing the wear should be the hardness