the free polar energy and biological parameters are correlated. The question is now whether the different values of θ (and of the free po!ar surface energy) can be related with the real chemi-cal state given by FTIR and ESCA. The preliminary results of FTIR show that there is a different intensity of IR reflection indicating different occupation of the surface by the carbonyl and carboxyl group at CFRC in comparison with CFRC/PyG. No differences in FTIR spectra were found on surfaces with the same chemical nature but

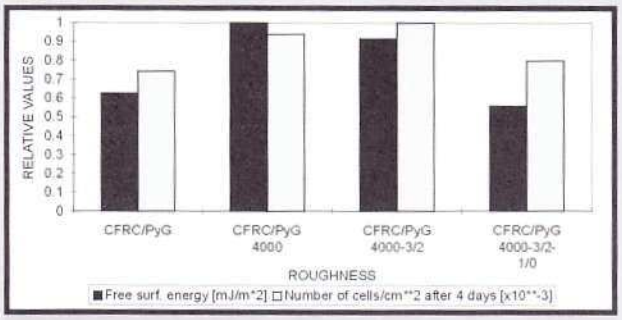

FIG.1. Dependence of free surface energy and cell density on surface roughness.

	CFRC	CFRC/PyG
Free surf. energy [mJ/m^2]	13.1	6.0
Cell density -1 day [1e3/cm^2]	5.8	9.3
Cell density - 4 day [1e3/cm^2]	77.8	130.6

TABLE 1. Comparison of the chemical and biological response of surfaces with the same roughness.

different roughness. One reason for the differences in surface free energy may be con-nected with the change in the ratio of the real area to the geometrical area of the surface in question.

Conclusion

The results indicate a different relation between biological response and free surface energy for roughness (with the same chemical state) and for chemical state (with the same roughness). The results of reflection goniometry, FTIR, and ESCA need to be compared.

Acknowledgements

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OSTEOBLAST-LIKE MG63 CELLS IN CULTURES ON CARBON FIBRE-REINFORCED CARBON COMPOSITES

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Carbon fibre-reinforced carbon composites (CFRC) have been widely used in technical and industrial applications because of their unique physical properties, such as specific strength, thermal resistance, and thermal and electrical conductivity [Fitzer *et al.* 1987, Bosdorf *et al.* 1995]. In addition, these materials seem to be promising for biomedical applications, particularly for hard tissue surgery. The mechanical properties of CFRC, namely the density, porosity and modulus of elasticity, can be tailored to be very close to those of the bone. The CFRC could be used, therefore, for bone and dental root replacements, external and intenal bone fixation, and the construction of artificial intervertebral plates [Blazewicz *et al.* 1997, Pesakova *et al.* 2000]. The chemical nature of CFRC is very close to that of pure carbon, so these materials can be well tolerated by the surrounding tissue. As follows from our earlier studies, the CFRC support adhesion and growth of osteogenic and vascular cells, both important components of the bone [Bacakova *et al.* 1998a,b,c; 2001). Colonization of an artificial bone implant with these cells types is desirable for its good integration with the surrounding bone tissue [for review see Bacakova *et al.* 2001]. However, the physicochemical surface properties of CFRC composites in their native, unmodified state usually are not ideal for optimal adhesion, subsequent growth and differentiation of bone-derived cells. As revealed by scanning electron microscopy, the surface roughness of CFRC seems to be too high, which is due to the prominence of carbon fibres over the carbon matrix [Bacakova *et al.* 1998c, 2001]. This study focuses on modifications of CFRC by grinding, polishing and coating with pyrolytic carbon in order to obtain a surface roughness optimal for interaction with osteogenic cells.

Two-dimensionally reinforced CFRC were prepared in the Institute of Rock Structure and Mechanics, Acad. Sci. CR, Prague. Commercially available woven fabric made of carbon fibres Toray T 800 was arranged in layers, infiltrated with a carbon matrix precursor (phenolic resin UMAFORM LE, Synpo Ltd, Pardubice, CR), pressed, cured, carbonised at 1000°C, and finally graphitised at 2200°C. The following groups of samples of various surface roughness were prepared:
#1: control untreated
#2: ground by metallographic paper of 4000 grade
#3: coated with pyrolytic carbon in Tesla Vrsovice Ltd., Prague, CR
#4: ground and coated with pyrolytic carbon

#5: ground, coated with pyrolytic carbon, then polished with metallographic paper of 4000 grade
#6: ground, coated with pyrolytic carbon, polished with metallographic paper of 4000 grade and diamond paste (PRAMET, Sumperk, CR) of 3/2 grade
#7: ground, coated with pyrolytic carbon, polished with metallographic paper of 4000 grade, diamond paste of 3/2 grade and finally of 1/0 grade

As measured by a Talysurf profilometer, the surface roughness decreased significantly from the group #4. For tissue culture experiments, the samples were cleaned in distilled and deionized water, sterilized in an autoclave, placed in 24-well CelCult plates (Sterilin, Feltham, UK) and seeded with human osteosarcoma-derived MG63 cells (European Collection of Cell Cultures, Salisbury, UK) at the density of 17,000 cells/cm^2. The cells were cultured in 1.5 ml of Dulbecco-modified Eagle Minimum Essential Medium (SEVAC, Prague, CR) supplemented with 10% of fetal calf serum and 40ug/ml gentamicin. Twenty-four hours after seeding, the medium was changed to remove non-adhered cells. Cells in 1- and 4-days-old cultures were harvested by trypsinization and counted in the Burker haemocytometer. The cell population doubling time (DT) was calculated as DT = (t-t_o) log 2/log Nt-log Nt_o, where Nt_o and Nt were the numbers of cells in 24- and 96-hour-old cultures, respectively. The data are presented as mean values ± S.E.M. from 4 samples for each experimental group. Statistical significance was evaluated by Student's test for unpaired data.

On day 1 after seeding, the numbers of initially adhered cells on all modified surfaces (groups #2 to #7) ranged from 5,750±380 to 10,520±600 cells/cm^2. These values usually did not differ significantly from those found on the control untreated samples (11,710±680 cells/cm^2), except of ground only samples (#2) where the cell number was lower. Nevertheless, the cell densities on modified CFRC (groups #3 to #7) were significantly higher in comparison with the values on CelCult dishes (4,550±610 cells/cm^2). In addition, on all modified samples, the cells proliferated significantly faster (doubling times from 18.8±1.2 h to 21.5±0.7 h in comparison with 77.1±9.0 h on control untreated CFRC), and on day 4 after seeding, they reached about 2.8 to 5.0 times higher population density (FIG.1). It seems that the growth of MG63 cells was improved mainly by coating CFRC with

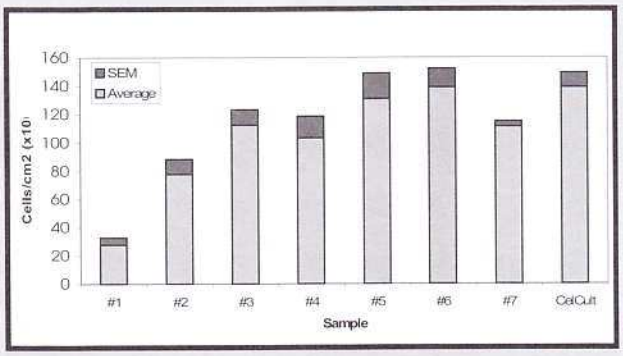

FIG.1. Number of MG63 cells on CFRC on day 4 after seeding.

pyrolytic carbon (group #3). Previous grinding or further polishing with metallographic paper and diamond had only a relatively little or even no additional effect on the improvement of cell growth, although this treatment markedly enhanced the cell spreading (FIG. 2A,B). Further experiments focused on beta-1 integrins, considered as the most important extracellular matrix receptors on osteogenic cells [Gronthos *et al.* 2001], and osteocalcin, a marker of osteoblastic differentiation [Josset *et al.* 1999], were carried out only on cells cultured on control unmodified and pyrolytic

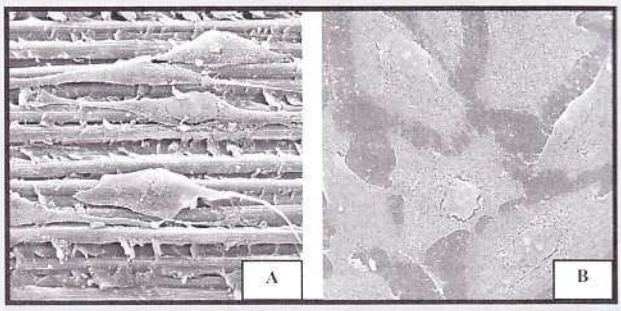

FIG.2. MG63 cells on unmodified CFRC (A) and a sample of group #5 (B). Scanning electron microscope, day 1 after seeding, original magnification 1000x.

carbon-coated CFRC (group #1 and #3). As revealed by the enzyme-linked immunosorbent assay (ELISA), the concentration of beta integrins and osteocalcin, measured in 6-day-old cultures per mg of total cell protein, was significantly higher (by 30±5% and 14±6%, respectively) in cells cultured on samples covered with pyrolytic carbon than in cells on unmodified composites. Moreover, the coating with pyrolytic carbon represented in our experiments the best protection against release of carbon microparticles from CFRC. It can be concluded that coating CFRC with pyrolytic carbon seems to be the most promising surface modification improving the compatibility of CFRC with bone-derived cells.

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