ADHESION, DIFFERENTIATION AND IMMUNE ACTIVATION OF HUMAN OSTEOGENIC CELLS IN CULTURES ON CARBON-FIBRE REINFORCED CARBON COMPOSITES

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Carbon fibre-reinforced carbon composites (CFRC) have been considered as promising materials for use in orthopaedic and dental surgery, surgery of the spine as well as veterinary medicine [1-5]. For these applications, the CFRC needs surface modifications, e.g. coating and polishing, in order to optimize their roughness and resistance against the release of carbon microparticles [6-8]. As follows from our earlier studies, coating with pyrolytic graphite combined with grinding and polishing seem to represent a suitable surface treatment of these materials [7, 8]. These modifications improved spreading and subsequent proliferation of human osteoblast-like MG 63 cells on CFRC in vitro and significantly diminished the release of carbon particles from these materials [7,8]. In the present study, we concentrated on selected molecular markers of adhesion (β_1-integrins, i.e. receptors for collagen, focal adhesion protein vinculin [9]), differentiation (extracellular matrix protein osteocalcin [6, 8]) and potential immunoatractiveness (intercellular cell adhesion molecule-1, ICAM-1 [9]) of osteogenic MG-63 cells on pyrolytic graphite-coated CFRC with different surface roughness obtained by grinding and polishing. In addition, adsorption of collagen, which mediates cell adhesion, was investigated on these samples [10].

Two-dimensionally reinforced CFRC were manufactured in the Institute of Rock Structure and Mechanics, Acad. Sci. CR, Prague, as reported earlier [7, 8]. The following groups of samples (3x3 cm) with gradually decreasing surface roughness were prepared:

#1: control untreated
#2: ground by metallographic paper of 4000 grade
#3: coated with pyrolytic graphite (C_4H_{10}, 4 Torr, 1900°C, 325 min) in Tesla Vršovice Ltd., Prague, CR
#4: ground and coated with pyrolytic graphite
#5: ground, coated with pyrolytic graphite, then polished with metallographic paper of 4000 grade
#6: ground, coated with pyrolytic graphite, polished with metallographic paper of 4000 grade and diamond paste (PRAMET, Šumperk, CR) of 3/2 grade
#7: ground, coated with pyrolytic graphite, 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 [7, 8].

The CFRC samples were cleaned in distilled and deionized water, sterilized in an autoclave, placed in polystyrene Petri dishes (Gama, České Budějovice, CR, diameter 5 cm; sample "G"). Some of them were exposed for 24 hours at room temperature to 10 μg/cm^2 of collagen IV, conjugated with fluorescent label Oregon Green 488 (Molecular Probes, Eugene, OR, U.S.A.), and diluted in phosphate-buffered saline. Intensity and distribution of fluorescence on the CFRC surface was evaluated in confocal laser scanning microscope (Bio-Rad MRC600). The remaining samples were seeded with human osteosarcoma-derived MG63 cells (European Collection of Cell Cultures, Salisbury, UK) at the density of 25,000 cells/cm^2. The cells were cultured in 6 ml of Dulbecco-modified Eagle Minimum Essential Medium (Sigma, St. Louis, MO, U.S.A.) supplemented with 10% of fetal bovine serum and 40 mg/ml gentamicin. For immunofluorescence staining [9], cells in 3-days-old cultures were fixed with methanol for 5 min at -20°C. For enzyme-linked immunosorbent assay (ELISA) [9], cells on day 7 were harvested by trypsin-EDTA solution (Sigma) and homogenized in Ultrasonic Homogenizer (Cole-Parmer Instrument Co., Chicago, Illinois, U.S.A). As primary antibodies, rabbit polyclonal anti-human β_1-integrin chain (Chemicon Int. Inc., Temecula, CA, U.S.A.), mouse monoclonal anti-human vinculin (Sigma), anti-bovine Osteocalcin (Chemicon) and anti-human ICAM-1 (Exbio, Prague, CR) were used. The secondary antibodies were represented by goat anti-rabbit and goat anti-mouse IgGs conjugated with FITC or peroxidase (Sigma) [9].

Quantitative data are presented as mean values ± SEM from 3 experiments (each performed in triplicates). Statistical significance was evaluated by Student's test for unpaired data.

The MG-63 cells on modified CFRC (samples #6 and #7) contained a significantly higher concentration of β_1-integrins, i.e. a group of integrins involving receptors for collagen, than the cells on unmodified composites (FIG.1). As suggested by the intensity and distribution of fluores-

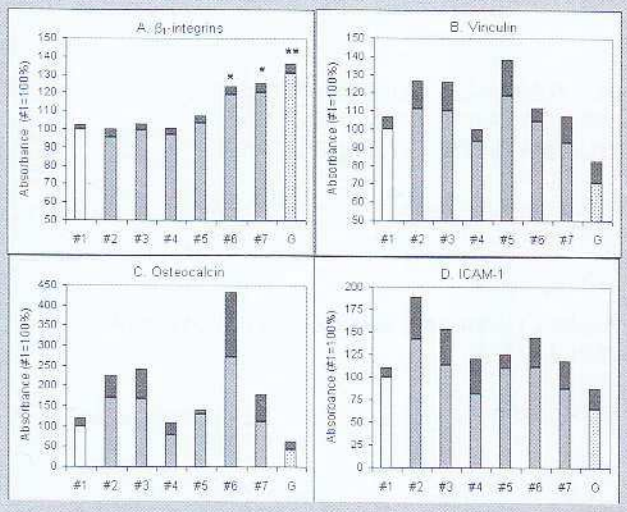

FIG. 1. Concentration of β_1-integrins (A), vinculin (B), osteocalcin (C) and ICAM-1 (D) in MG 63 cells growing on unmodified CFRC or CFRC of groups #1 to #7 and G (see Material and Methods; B). Measured by ELISA (per mg of protein) on day 7 after seeding. Absorbances of cell samples from the surface-modified CFRC are expressed in % of values obtained from control cells on untreated composites. Means ± S.E.M. from 3 experiments. Student's t-test for unpaired data, *p≤ 0.02 and **p≤ 0.01 compared to the control values in cells on unmodified CFRC.

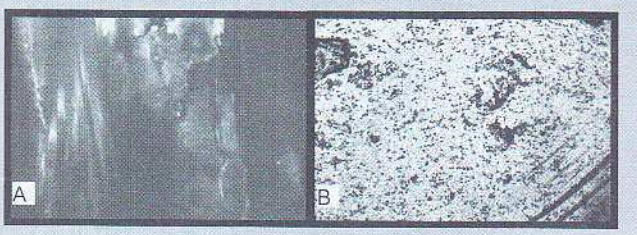

FIG. 2. Adsorption of collagen IV conjugated with Oregon Green 488 on unmodified CFRC (A) or CFRC of group #6 (B; see Material and methods). Confocal laser scanning microscope, obj. 40x.

cence of Oregon Green-conjugated collagen IV (FIG. 2), this finding could be explained by a higher and more homogeneous adsorption of collagen, which can be contained in the serum supplement of the culture media, or secreted by the cells themselves. Immunofluorescence staining of vinculin showed a relatively high formation of focal adhesion plaques in cells on samples #6 (FIG. 3). In these plaques, integrins communicate with a wide spectrum of signaling and cytoskeletal molecules, which control cell viability, growth and differentiation. On unmodified CFRC, a diffuse pattern of vinculin distribution (FIG. 3) indicated a low formation of focal adhesion plaques, which might explain a lower proliferation of MG-63 cells found on these materials in our earlier study [7]. In addition, the cells on modified CFRC, especially on samples #6, showed a tendency to contain more osteocalcin (FIG. 1), a non-collagenous calcium-binding extracellular matrix protein, considered as an important marker of osteoblastic differentiation and bone tissue formation [6, 7]. Concentration of ICAM-1, cell surface adhesion molecule of immunoglobulin type, which bind inflammatory cells [9], was similar in modified and unmodified CFRC; only in cell on samples #2, a slight but non significant tendency to increase was noted. These results suggest that coating the CFRC with pyrolytic graphite, as well as their grinding and polishing with metallographic paper and diamond paste, would not enhance the immunoattractiveness of the cell-material complex.

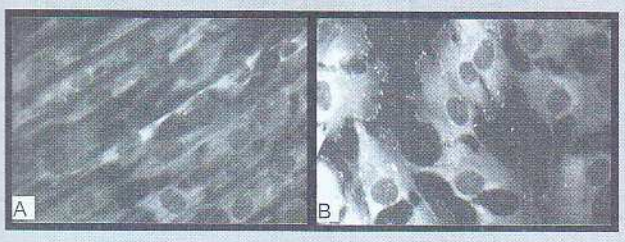

FIG. 3. Immunofluorescence staining of vinculin, a marker of focal adhesion plaques, in MG 63 cells cultured for 3 days on unmodified CFRC (A) or CFRC of group #6 (B; see Material and Methods). Confocal laser scanning microscope, obj. 60x.

Acknowledgements

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VASCULAR SMOOTH MUSCLE CELLS IN CULTURES ON LACTIDE BASED POLYMERS FOR POTENTIAL CONSTRUCTION OF ARTIFICIAL VESSEL WALL

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We focused on polymer-cell reaction, specifically on adhesion and spreading of vascular smooth muscle cells (VSMC) on lactide- and polyethylenoxide (PEO)-based polymers, potential materials for construction of vascular prostheses. On poly(DL-lactic acid), PDLLA, the number and spreading of initially attached rat aortic VSMC were similar as on standard cell culture plastics. However, the copolymer of PDLLA and PEO, MeO-PEO-b-PDLLA, almost disabled the cell adhesion and spreading. Grafting of GRGDSG peptide to the copolymer restored the cell adhesion and spreading almost to the values seen on PDLLA. Surprisingly, the concentration of 5% GRGDSG was more effective than a higher concentration of 20%.

Synthetic polymers can be used for construction of artificial vascular prostheses. Disadvantage of these devices is