muscle cells in vitro [2, 17] and cells of the cartilage in situ [12]. It can be concluded that the bi-directional CFRCs infiltrated and coated with pyrolytic carbon exhibit a relatively good biocompatibility in vitro and they are promising materials for future biomedical applications.

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IMPROVED BIOCOMPATIBILITY OF CARBON-FIBRE-REINFORCED CARBON COMPOSITES IN VITRO AFTER THEIR POLISHING AND COATING WITH A CARBON-TITANIUM LAYER

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Abstract

The surface of unidirectionally reinforced carbon-carbon composites was modified either by polishing or coating with a carbon-titanium layer. In culture conditions, the composites were seeded with vascular smooth muscle cells derived from the rat aorta. On both types of modified samples, the number of initially adhered cells, degree of their spreading and their subsequent growth were significantly higher than on untreated samples, and in the case of carbon-titanium-covered composites, also higher than on standard plastic culture dishes Sterilin. These results obtained in vitro suggest possible good biointegration of the polished and carbon-titanium-covered carbon-carbon composites with the surrounding tissue in situ after their use in transplantation medicine for the construction of artificial implants.

Introduction

The carbon-fibre-reinforced carbon composites (CFRC) are promising materials for the construction of artificial implants, especially those of bones, joints and dental roots [1]. Their physical properties, namely density and porosity, are very close to those of bones, and their chemical composition is suitable for adhesion and growth of several kinds of cells in vitro as well as in situ [1,2]. However, the surface of unmodified newly fabricated composites usually seems to be too rough to ensure optimum degree of cell adhesion, which can be explained by heterogeneous composition of the CFRC, i.e. carbon fibres protruding from the carbon matrix. Moreover, the carbon matrix is brittle, which can lead to the release of carbon microparticles to the surrounding tissue, particularly on cyclic stretching [2]. These disadvantages of the CFRC could be minimised by polishing their surface and/or by covering it with a thin biocompatible layer. Thus, in this study we evaluate the initial adhesion and subsequent growth of rat aortic smooth muscle cells in cultures on CFRC either polished with SiO_2 or (in non-polished state) coated with a layer of carbon and titanium, a metal widely used in orthopaedic surgery and reconstructive dentistry [3,4,5,6,7].

Materials and methods

The composites were prepared from phenolic resin (i.e. matrix precursor) reinforced unidirectionally by Torayca carbon fibres T300. The prepregs were stacked, cured, carbonised at 1000°C in N_2 and graphitized at 2500°C in Ar. The material was then cut with a diamond knife into 8x10x3 mm samples, and either polished with colloidal SiO_2 (grain size 0.06 μm) or covered with a carbon-titanium layer approximately 3 μm thick. This layer was prepared by PECVD (plasma enhanced physical vapour deposition) method. Titanium was sputtered from the target by DC magnetron supported discharge in the environment of hydrocarbon gas. In this way, the layer consisting of a mixture of titanium and carbon atoms was created with a big number of free bonds. This caused strong adhesion of the layer to the substrate with chemically very reactive surface. Surface roughness was measured by Talysurf (Rank Taylor Hobson Ltd., England). For cell culture, the samples were washed in distilled and deionised water, autoclaved and placed on the bottom of plastic CellCult Multidishes (diameter 1.5 cm; Sterilin). Smooth muscle cells were obtained from the intima-media complex of the thoracic aorta of adult male Wistar rats by explantation method [1]. In passage 9, the cells were seeded on the composites at initial density of 17000 cells/cm^2 in 1 ml of Dulbecco Minimum Essential Medium supplemented with 10% of fetal calf serum and gentamicin (40 mg/ml). On days 1 and 4 after seeding, the cells were detached from the growth substrate by 0.2% trypsin in phosphate-buffered saline (PBS) and counted in the Bürker haemocytometer. The cell volume was calculated from the diameters measured by an ocular microscale in living cells resuspended in PBS after trypsinization. Morphology of cells growing on the composites was observed in fluorescence microscope after fixation in ethanol and staining with propidium iodide and also in scanning electron microscope.

Results and discussion

The surface roughness was significantly higher on untreated samples than on the polished samples. In the direction perpendicular to fibres, the departures of the rough ness profile from the mean line (Ra) were 3.55±0.2 μm on the untreated samples, 0.80±0.10 μm on the polished ones and 3.25±0.4 μm on the carbon-titanium-covered samples. Mean spacings of the adjacent local peaks (S) were 20, 37 and 24 μm on untreated, polished and carbon-titanium-coated samples, respectively. For the longitudinal direction, the Ra values were 1.25±0.15, 0.50±0.1 and 1.4±0.9 μm, and the S values were 85, 100 and 34 μm, respectively.

The number of initially adhered cells one day after seeding was significantly higher on the polished or carbon-titanium-covered composites than on the untreated samples. On the carbon-titanium-covered CFRC, it was even higher than on standard culture plastic dishes Sterilin (FIG. 1A). From day 1 to day 4, the cells on polished or carbon-titanium-covered surfaces proliferated more rapidly (doubling times 27.0±3.0 h and 21.7±1.5 h, respectively) than those on untreated surfaces (doubling time 36.0±6.0 h) and on day 4, they reached significantly higher population densities (FIG. 1). In the case of carbon-titanium-covered samples, the cell population doubling time was similar to that of cells growing on Sterilin dishes (22.4±1.6 h) and on day 4, the cell population density was significantly higher on these samples than on the Sterilin dishes (FIG. 1). The cell volume ranged from 2180 μm^3 to 3360 μm^3 and increased in the following order: untreated samples > Sterilin dish > polished samples > carbon-titanium-covered samples. The cells growing on untreated composites were mainly spindle-shaped with relatively small adhesion area and they were often oriented along the carbon fibres reinforcing the composite. On the polished composites, most cells were polygonal and well spread on the material surface, whereas on the carbon-titanium-covered samples, both elongated and polygonal cells were found (FIGS. 2, 3). The release of carbon microparticles was significantly lower in both groups of treated samples in comparison with the untreated composites (FIG. 4). These results are in accordance with our previous findings concerning slight improvement of cell adhesion and growth on uncoated and amorphous carbon-coated composites after their polishing [2] as well as with the findings of other authors concerning relatively good biocompatibility of titanium-containing materials in vitro as well as in situ [3,4,5,6,7]. It can be concluded that adhesion and growth of cells of mesenchymal origin on CFRC can be significantly improved by polishing and especially by coating these materials with a carbon-titanium layer.

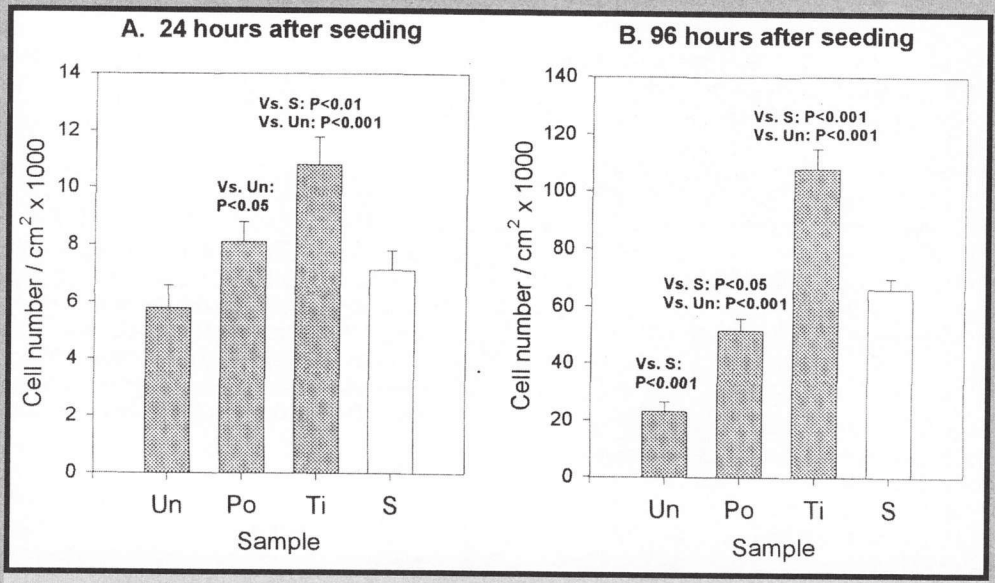

FIG. 1. Population density of rat vascular smooth muscle cells in cultures on CFRC with different surface treatment.
Un=untreated sample, Po=polished, Ti=coated with carbon-titanium layer, S=standard culture dish Sterilin.

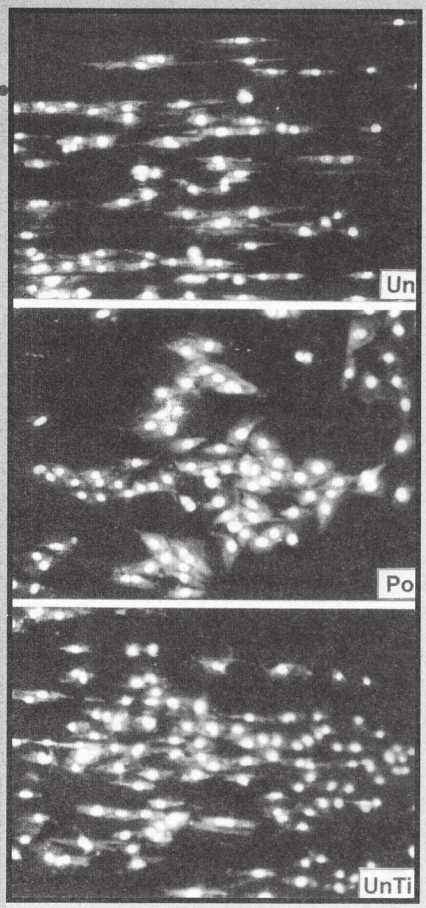

FIG. 2. Rat vascular smooth muscle in 3-day-old cultures on CFRC with different surface treatment. Un=untreated sample, Po=polished, Ti=coated with carbon-titanium layer. (Propidium iodide staining of ethanol-fixed cells. Axioplan fluorescence microscope, obj.20).

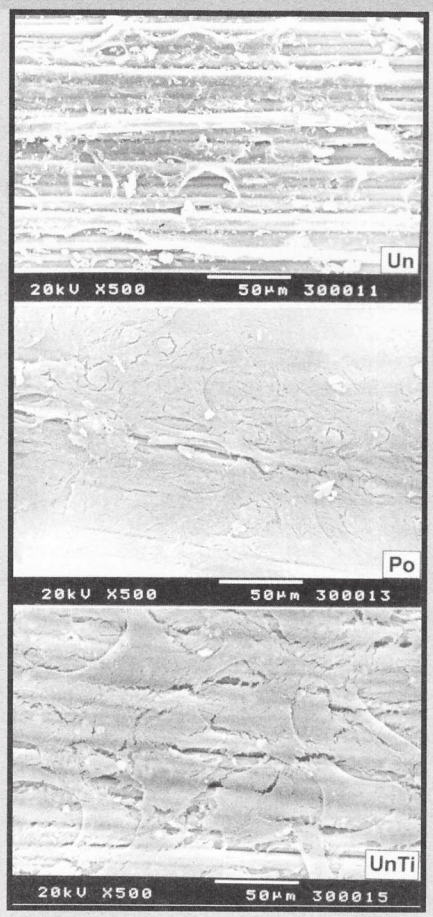

FIG. 3. Rat vascular smooth muscle in 4-day-old cultures on CFRC with different surface treatment. Un=untreated sample, Po=polished, Ti=coated with carbon-titanium layer. (SEM, 500x).

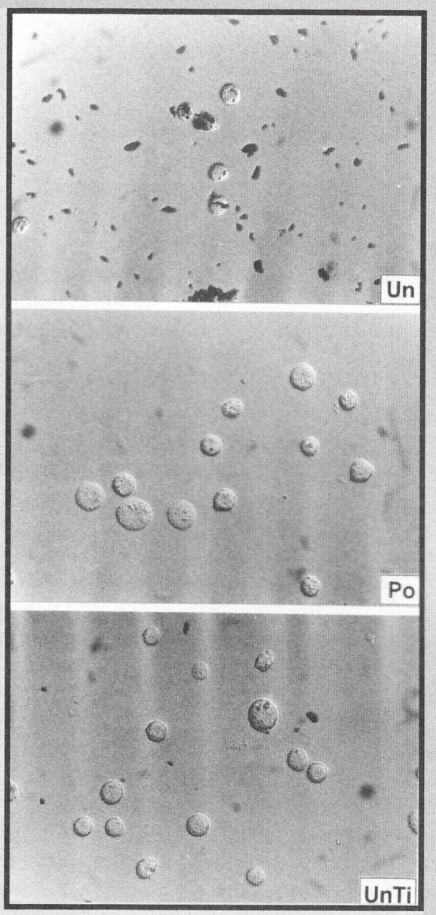

FIG. 4. Carbon microparticles in suspensions of rat vascular smooth muscle cells after detachment of the cells from CFRC by 0.2% trypsin in PBS on day 4 after seeding. Un=untreated sample, Po=polished, Ti=coated with carbon-titanium layer. (Interference contrast, obj.40).

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