VASCULAR ENDOTHELIAL CELLS IN CULTURES ON METAL/ C:H COMPOSITE FILMS

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Abstract

Adhesion, proliferation and maturation of vascular endothelial cells in cultures derived from the bovine pulmonary artery (line CPAE) were studied on Ti/C:H or Ag/C:H films prepared on glass using dc magnetron plasma deposition. The concentration of Ti or Ag in the films ranged from 0% to 20%. On Ag/C:H layers, increasing concentration of silver markedly decreased cell viability. Only samples with very low silver content allowed cell spreading and formation of continuous endothelial layer. Although long-term presence of this material in patient's organism would not be advantageous, antimicrobial effect of silver may be favorable for short term applications, e.g. coating of catheters. On Ti/C:H layers, the cells were similarly or more active in adhesion, proliferation and maturation than those on glass or pure amorphous carbon, respectively. This material could be suitable for long-term use in both soft and hard tissue surgery, e.g. for inner coating of vascular prostheses or surface modification of artificial bone implants.

[Engineering of Biomaterials, 37, (2004), 18-20]

Introduction

Amorphous hydrogenated carbon (a-C:H, also called diamond-like carbon) has been used for surface modifications of various polymer- or metal-based biomaterials in order to increase their wear resistance, smoothness, hydrophobia and blood compatibility [1-5]. For example, it has been employed for coating of femoral heads of metallic hip prostheses in order to prevent the release of metal ions and wear particles [1] or deposited on various blood-contacting devices, e.g., blood pumps, in order to enhance their resistance to protein adsorption and thrombus formation [2-5].

The physicochemical properties of a-C:H films and their attractiveness for colonization with cells can be further modified by incorporation of metal ions into these layers [6, 7]. Thus, the aim of this study is to evaluate the adhesion, growth and maturation of vascular endothelial cells in cultures on Ti/C:H or Ag/C:H films deposited on glass. Titanium is widely used material for construction of bone, joint or dental implants, and it has been reported to support adhesion and growth of osteogenic and vascular smooth muscle cells [6, 7]. Silver is known by its strong antimicrobial activity [8, 9].

Material and methods

Ti/C:H or Ag/C:H films were deposited on glass using unbalanced magnetron with Ti or Ag target operated in the dc mode in a working gas mixture of n-hexane/Ar [10]. As estimated using transmission electron microscopy (TEM), atomic force microscopy (AFM), Rutherford backscattering technique (RBS) and elastic recoil detection analysis (ERDA), the concentration of Ti or Ag in the films increased proportionally to the amount of Ar in the working gas mixture, ranging from 0% to 20% [10].

The samples (size 8x8 mm) were sterilized by UV irradiation, inserted into Nunclon Multidishes (NUNC, Denmark; 24 wells, diameter 15 mm) and seeded with bovine pulmonary artery endothelial cells (line CPAE, ATCC CCL 209, 17 000 cells per cm^2). The cells were incubated in 1.5 ml of Dulbecco's Modified Eagle Minimum Essential Medium supplemented with 20% of fetal bovine serum for 1, 3 or 7 days at 37°C in air atmosphere with 5% CO_2.

Adhesion of endothelial cells on the metal/C:H films were evaluated by the number of initially adhering cells on day 1 after seeding, cell-material contact area (i.e., cell spreading area) and formation of focal adhesion plaques, detected by immunofluorescence staining of vinculin [11]. Cell proliferation was estimated by the percentage of cells newly synthesizing DNA. The nuclei of these cells were visualized by 30-min-incubation of cells with bromodeoxyuridine (BrdU), followed by anti-BrdU immunoperoxidase staining [11]. Markers of endothelial cell maturation, used in this study, were represented by formation of confluent cobblestone-like cell layer, content of von Willebrand factor and formation of distinct beta-actin cables. The latter two parameters were evaluated by immunofluorescence staining [11].

Results and discussion

On Ti/C:H layers, the endothelial cells adhered in higher initial numbers and by a large cell-material contact area than on pure amorphous carbon (FIGS. 1A, B). Similar results were obtained earlier on vascular smooth muscle cells, osteoblast-like MG-63 cells or bone marrow cells cultured on carbon-fibre reinforced carbon composites (CFRC) or titanium discs coated with Ti-containing a-C:H [6, 7]. The improved cell adhesion could be explained by formation of oxygen-containing groups in the Ti/C:H layers and their increased wettability [6, 7, 10]. The synthesis of DNA in cells on Ti/C:H samples, measured by BrdU incorporation, tended to be lower than on a-C:H, although these differences were not significant (FIG. 1C). The latter result is consistent with the findings that the proliferation activity is the highest at the intermediate strength of cell adhesion. When the adhesion is very high, the cells skip the proliferation phase and enter sooner the differentiation program [11]. Both a-C:H and Ti/C:H films allowed formation of continuous layers of cobblestone-like endothelial cells (FIG. 2A), although this formation was sooner on Ti/C:H. Thus, the Ti/C:H films showed a good compatibility with endothelial cells, so that these materials could be used for cardiovascular applications, such as coating of vascular prostheses or heart valves in order to improve their lining with endothelial cells. Another possible application is surface coating of bone implants.

On Ag/C:H layers, increasing concentration of silver

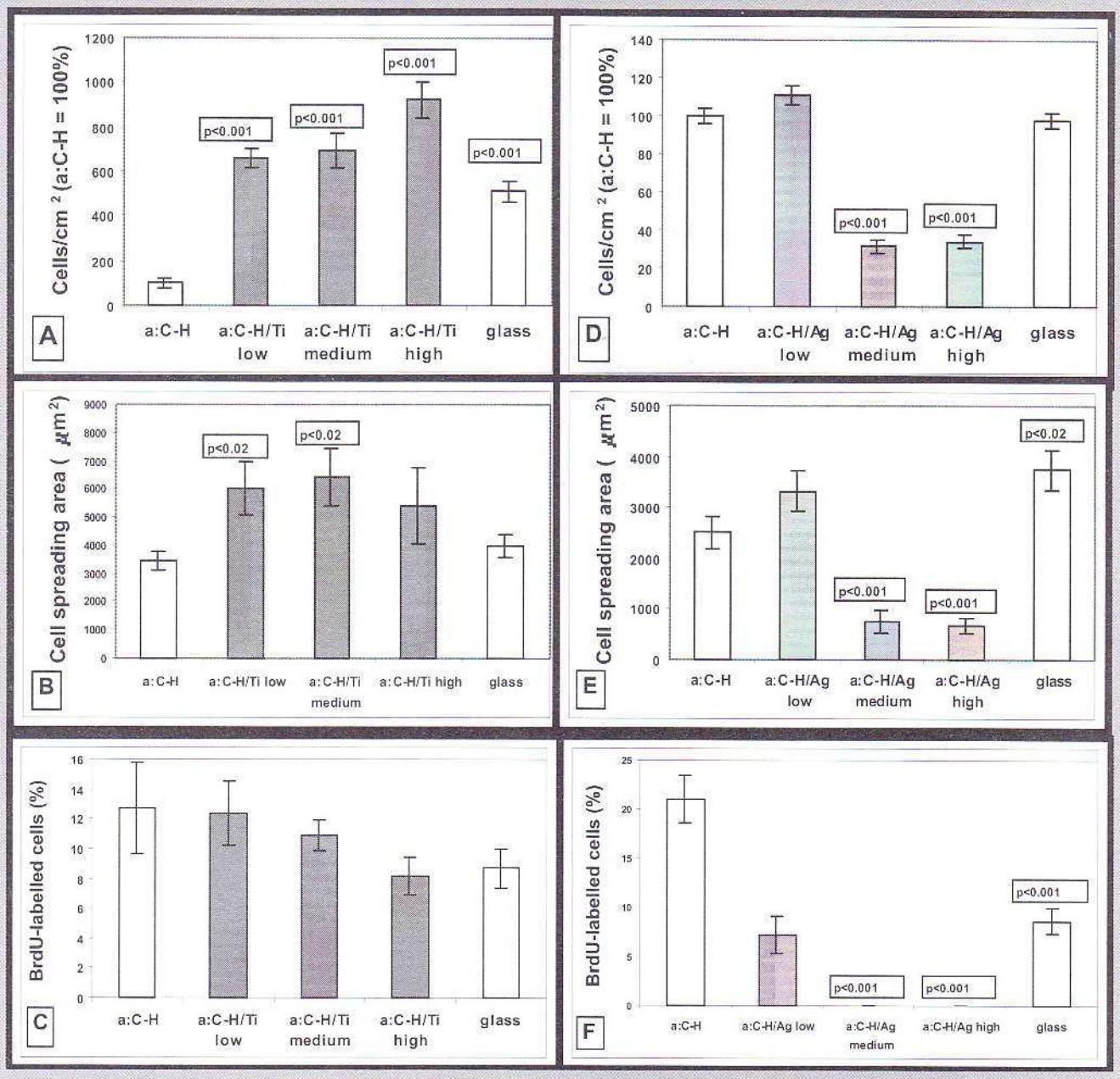

FIG. 1. Number (in % of values obtained on pure a:C-H; A, D), cell-material contact area (B, E) and percentage of DNA synthesizing endothelial cells (C, F) on a:C-H layers with low, medium and high concentrations (within the range approx. from 1% to 20%) of titanium (A-C) or silver (D-F) on day 1 (A, B, D, E) or day 3 (C, F) after seeding. Average ± SEM from 9-38 measurements, Student's t-test for unpaired data, statistical significance: p<0.02, p<0.001 in comparison with values obtained on pure a:C-H.

markedly decreased adhesion and proliferation of endothelial cells. Cells on the films with high and medium silver concentrations adhered at very low initial numbers and did not spread. They did not incorporate BrdU into DNA and usually died before day 7 of cultivation (FIGS. 1D-F, 2C). Only the layers with the lowest silver content allowed sufficient cell attachment and spreading, assembly of focal adhesion sites and actin cytoskeleton, synthesis of DNA and von Willebrand factor, and formation of continuous endothelial cell layer (FIG.1D-F, 2B, 2D-F). Similarly, polystyrene implanted with Ag- ions (energy from 5 to 30 keV, dose from 10^{14} to $6x10^{16}$ ions/cm^2) also supported growth of vascular endothelial cells [12]. Therefore, if the Ag/C:H layers with relatively low silver content ensure significant antimicrobial effects, which remains to be investigated, the use of these coatings for construction of durable implants into human body could be advantageous. The Ag/C:H layers with the higher and cytotoxic silver concentrations could be suitable only for short-term contact with patient's organism, e.g. as antimicrobial coating of intravascular or urinary catheters [8, 9].

Conclusions

Ti/C-H and Ag/C-H films with low Ag concentration supported adhesion, growth and maturation of vascular endothelial cells in cell culture conditions. These films could be used for construction of long-term artificial implants into human body, e.g. for coating of the inner surface of vascular prostheses or surface modification of bone implants.

Ag/C-H films with higher silver concentrations were cytotoxic and could be suitable for antimicrobial coating of intravascular and urinary catheters for short-term insertion.

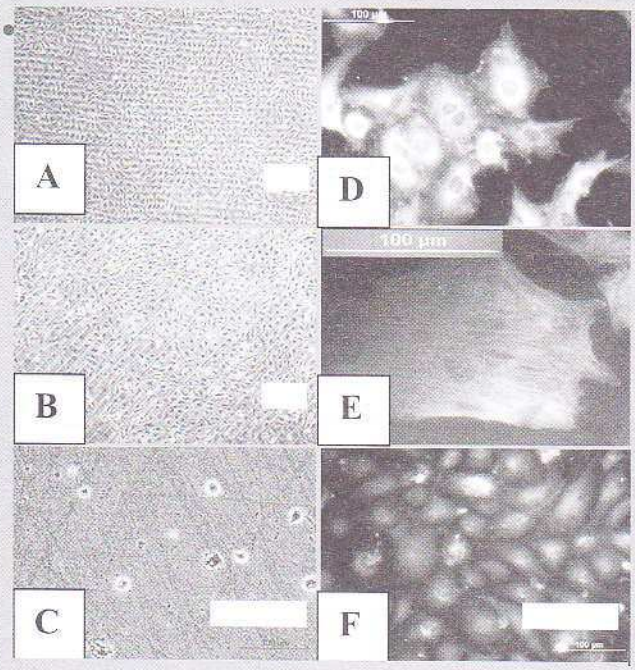

FIG.2. Morphology and molecular markers of adhesion and maturation of endothelial cells in cultures on Ti/C:H or Ag/C:H layers (concentration range of metals approx. from 1% to 20%). A, B: formation of a confluent cell layer on a-C:H with low concentration of Ti or Ag, respectively; C: dead cells on a-C:H with high concentration of Ag. D, E, F: immunofluorescence staining of vinculin-containing focal adhesion plaques, beta-actin

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EVALUATION OF THE EFFECT OF TRANSPHYSEAL BIOABSORBABLE SCREWS ON GROWTH OF RABBIT FEMUR

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Abstract

A self-reinforced bioabsorbable poly-L-lactide/polyglycolide (SR-PLGA) 80/20 screw 2.0 mm in diameter was implanted in a transphyseal location across the distal growth plate of the right femur in 24 immature rabbits. Radiological evaluation revealed a mean shortening of 3.1 mm at 3 weeks (p=0.050), 11.1 mm at 6 weeks (p=0.001), 9.3 mm at 24 weeks (p=0.011), 9.0 mm at 48 weeks (p=0.009) and 12.6 mm at 72 weeks (p=0.002) compared with the intact contralateral femur. Growth retardation continued for 6 weeks postoperatively (3 versus 6 weeks, p=0.003), after which the bones grew normally up to 72 weeks (p=0.6). The duration of temporary growth retardation correlated with that of strength retention of the SR-PLGA 80/20 copolymer. These findings suggest that SR-PLGA 80/20 screws can be applied in transphyseal bone fixation. The use of bioabsorbable screws for temporary epiphyseodesis seems attractive but requires further study.

Keywords: *Femur, growth, rabbit, SR-PLGA*

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