THE ADHESION AND GROWTH OF VASCULAR SMOOTH MUSCLE CELLS IN CULTURES ON CARBORANETHIOL-MODIFIED GOLD FILMS

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Abstract

Metal surfaces have become important over the last decade for potential surgical implants, and within this context we present here a study of the cell growth on modified gold surfaces. Gold films, deposited on glass plates and annealed with a hydrogen flame, were modified with four different carboranethiol derivatives: 1-(HS)-1,2-$C_2B_{10}H_{11}$ (A), 1,2-$(HS)_2$-1,2-$C_2B_{10}H_{10}$ (B), 9,12-$(HS)_2$-1,2-$C_2B_{10}H_{10}$ (C) and 1,12-$(HS)_2$-1,12-$C_2B_{10}H_{10}$ (D). The materials engendered from these modifications were used to investigate the adhesion and growth of rat aortic smooth muscle cells cultured on these surfaces in a DMEM medium with 10% of fetal bovine serum. One day after seeding, the highest number of initially adhered cells was found on the surface of a bare gold film. However, three days after seeding, the number of cells on carboranethiol-modified gold samples B, C and D was significantly higher than the number on a bare gold film. After seven days, the number of cells on a bare gold film and on gold films modified with derivatives A, B and D was very similar, but the surface of a gold film modified with derivative C exhibited a significantly smaller number of cells. This may be explained by the exposure of the CH vertices of the carborane cluster, which are more acidic than the BH vertices exposed toward the cells in either A or B.

***Keywords**: Metal coating, gold film, carboranethiol, cell adhesion, cell spreading, cell proliferation, biomaterials, tissue engineering, surgical implants.*

[Engineering of Biomaterials, 81-84, (2008), 117-119]

Introduction

Artificial and nature-derived materials, including metals, have been intensively studied in medicine and in various biotechnologies. Examples are bio-imaging, bio-sensing, drug delivery, cell cultivation, and the construction of replacements of irreversibly damaged tissues and organs. A largely accepted concept in recent tissue engineering is of surfaces supporting and controlling cell colonization associated with successful integration of an implant within the organism. This concept is used for the construction of durable bone prostheses persisting in the patient for many years, and is being developed to make bio-artificial replacements of blood vessels, liver, pancreas or even nervous tissue (for a review, see [1-3]).

Metallic materials have frequently been utilized in a hard tissue surgery, e.g., for constructing joint prostheses and other bone implants. However, these materials could be used in the form of thin nanocomposite films in soft tissue surgery, e.g. for coating artificial heart valves or vascular prostheses. Gold, one of the metallic materials that have been studied, has been used in medicine and in implantology because of its inert character and negligible toxicity. Gold reacts easily with thiol (-SH) groups [4], and this reaction can be used for modifying gold films to make surfaces suitable for the adhesion and growth of cells. In this study, we present the use of flat gold coatings modified with selected carboranethiol derivates as new substrates for cell colonization. Vascular smooth muscle cells were chosen as a model system because they are an important component of both hard and soft tissues and organs - including bone and blood vessels.

Experimental

Preparation of the gold samples

Gold films deposited on glass plates with dimensions 11×11mm were purchased from Arrandee (Germany). All films were freshly annealed with a hydrogen flame before use. These films were modified with carboranethiol derivatives in accordance with the literature [4]. The following carboranethiol species were used:1-(HS)-1,2-$C_2B_{10}H_{11}$ (A), 1,2-$(HS)_2$-1,2-$C_2B_{10}H_{10}$ (B), 9,12-$(HS)_2$-1,2-$C_2B_{10}H_{10}$ (C) and 1,12-$(HS)_2$-1,12-$C_2B_{10}H_{10}$ (D) (FIG. 1).

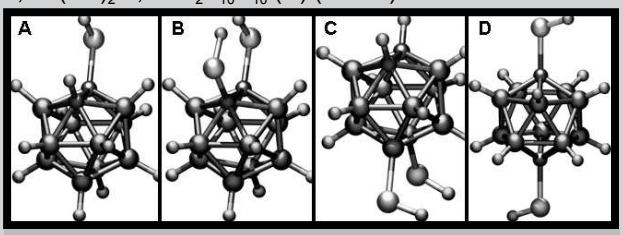

FIG. 1. Carboranethiol derivatives used for modification of gold films: 1-(HS)-1,2-$C_2B_{10}H_{11}$ (A), 1,2-$(HS)_2$-1,2-$C_2B_{10}H_{10}$ (B), 9,12-$(HS)_2$-1,2-$C_2B_{10}H_{10}$ (C) and 1,12-$(HS)_2$-1,12-$C_2B_{10}H_{10}$ (D).

Cells and culture conditions

For the cell culture experiments, the materials were sterilized with 70% ethanol for 1 hour, inserted into 24-well polystyrene plates (TPP, Switzerland; well diameter 1.5 cm), and seeded with smooth muscle cells derived from rat aorta by an explantation method [1,2]. The cells were used in passage 3 and seeded at a density of 17 000cm^2. The cells were cultivated for 1, 3 or 7 days in 1.5 ml Dulbecco's Modified Eagle Minimum Essential Medium (Sigma, U.S.A.), supplemented with 10% foetal bovine serum (Sebak GmbH, Aidenbach, Germany) and 40 μg/ml of gentamycin (LEK, Ljubljana, Slovenia), in a cell incubator with a humidified atmosphere of 5% of CO_2 in the air and at a temperature of 37°C. For each experimental group and time interval, three samples were used.

The cells on one sample for each experimental group were rinsed with phosphate-buffered saline (PBS), fixed by 70% cold ethanol (-20°C, 5 min) and stained with a combination of Texas Red C2-maleimide fluorescent membrane dye (Molecular Probes, Invitrogen, Cat. No. T6008; 20 ng/ml in PBS), and Hoechst # 33342 nuclear dye (Sigma, U.S.A.; 5μg/ml in PBS). The number and morphology of the cells on the sample surface were then evaluated from pictures (10 for each sample, size 0.136mm^2) taken under an Olympus IX 50 microscope using an Olympus DP 70 digital camera (FIG. 2).

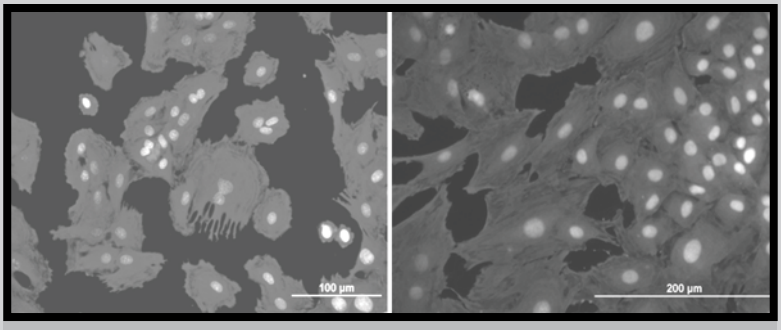

FIG. 2. Examples of the morphology of vascular smooth muscle cells on day one (left) and day 7 (right) after seeding on a gold film modified with the carboranethiol derivate 1-(HS)-1,2-$C_2B_{10}H_{11}$ (A). Stained with Texas Red C2-maleimide and Hoechst #33342. Olympus IX microscope 50, obj. 20, digital camera DP 70.

On the remaining two samples, the cells were rinsed with PBS, released using a trypsin-EDTA solution (Sigma, Cat. No. T4174) and counted in a Cell Viability Analyzer (VI-Cell XR, Beckman Coulter). As control materials, samples with a bare gold film, standard tissue culture polystyrene dishes (PS) and uncoated glass substrates were used.

The size of the cell spreading areas, i.e., cell areas projected on the material surface, was measured one day after seeding using Atlas software (Tescan Ltd., Brno, CR). Fifty seven to 170 cells from 10 pictures for each experimental group were evaluated.

Statistics

The results are presented as a mean value with a standard error (Mean ± SEM). Statistical significance was evaluated using the ANOVA, Student-Newman-Keuls method. Values of $p \leq 0.05$ were considered as significant.

Results and discussion

One day after seeding, the highest number of initially adhered cells was found on the surface of a bare gold film with 23000±1400cells/cm^2, compared to a range from 7900±800 to 16100±800cells/cm^2 on the modified gold surfaces (FIG. 3).

However, three days after seeding, the number of cells on the carboranethiol-modified gold samples B, C and D (24200±1500cells/cm^2 to 31400±1500cells/cm^2) was significantly higher than the number on the bare gold film (16900±900cells/cm^2, FIG. 3).

After seven days, the number of cells on the bare gold film and gold films modified with derivatives A, B and D was very similar, all in the range from 71800±2400cells/cm^2 to 72300±2200cells/cm^2 (FIG. 3). These cell population densities were significantly higher than the values obtained on standard polystyrene culture dishes. This result is favorable, especially in the case of derivative D. In this molecule, the SH groups are located in para-orientation (FIG. 1), which would facilitate the attachment of the carboranethiol to the gold surface and its simultaneous functionalization with other bioactive groups or molecules in order to further increase the attractiveness of gold surfaces for cell colonization [5-9].

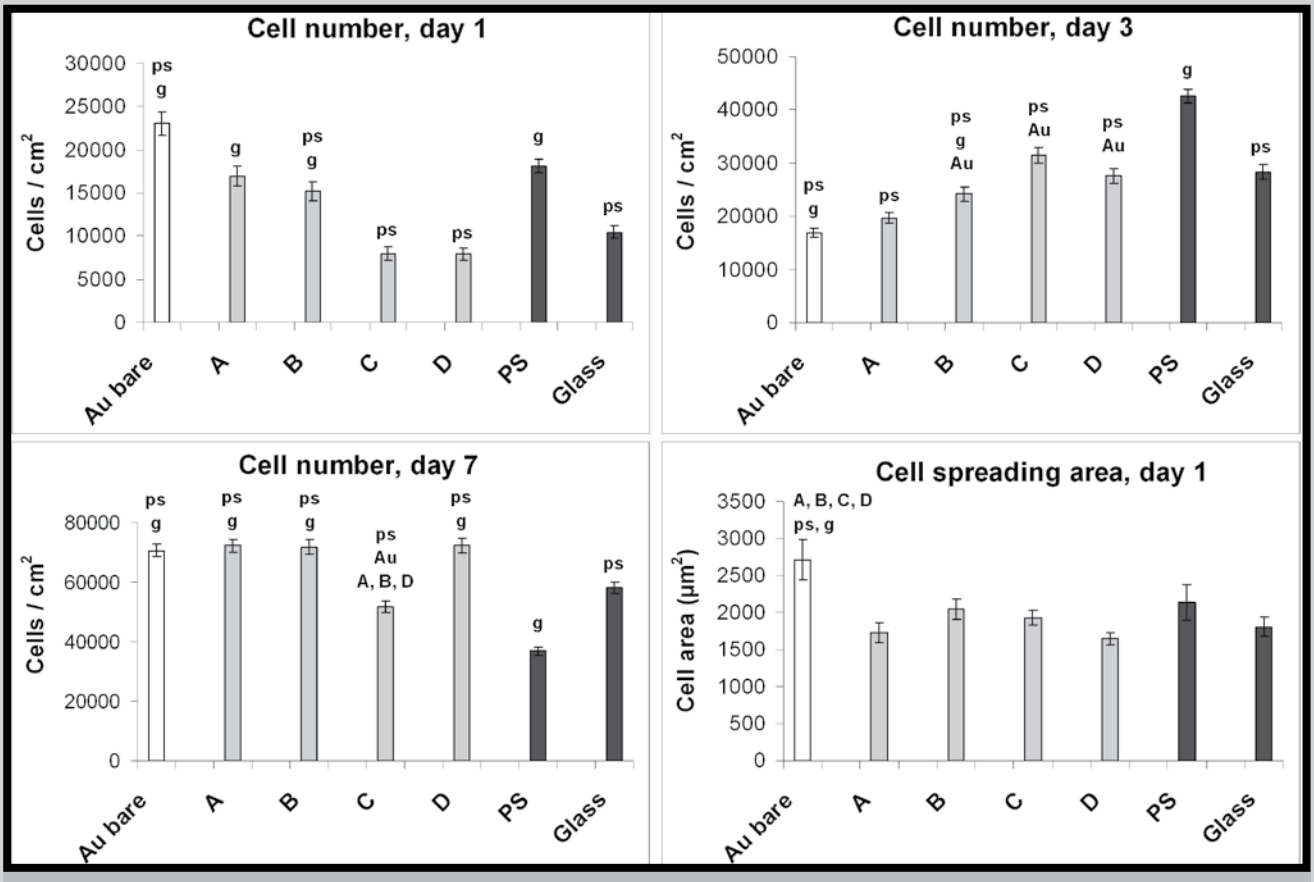

FIG. 3. Number and cell spreading area of rat aortic smooth muscle cells on day 1, 3 and 7 after seeding on gold modified by carboranethiol derivatives1-(HS)-1,2-$C_2B_{10}H_{11}$ (A), 1,2-$(HS)_2$-1,2-$C_2B_{10}H_{10}$ (B), 9,12-$(HS)_2$-1,2-$C_2B_{10}H_{10}$ (C) and 1,12-$(HS)_2$-1,12-$C_2B_{10}H_{10}$ (D), polystyrene culture dish (PS) and glass (G). Mean ± SEM from 100 measurements performed on 2 samples (cell number) or 57-170 cells from 10 pictures of each sample (spreading area). Statistical significance: ps, g, Au, B, A, D: $p \leq 0.05$ in comparison with polystyrene dish, glass substrate, bare Au film or carboranethiol derivatives A, B, D.

However, the surface of a gold film modified with carboranethiol C exhibited a significantly smaller number of cells, 51600±1900cells/cm^2 (FIG. 3). This may be explained by the exposure of the CH vertices of the carborane cluster, which are more acidic than the BH vertices exposed toward the cells in either A or B. Nevertheless, the cells on all tested surfaces were able to form a confluent layer.

The cell spreading areas on day 1 after seeding were significantly larger on the bare gold sample (2700±270μm^2) than in all remaining experimental groups. In these groups, the cell spreading areas were in the range from 1650±80μm^2 (on the samples modified with derivative D) to 2140±240μm^2 (on polystyrene dishes) but these differences were not statistically significant (FIG. 3).

Conclusion

Modification of a gold surface with carboranethiol derivatives A, B and D increased the population density of rat aortic smooth muscle cells after 7-day-cultivation on these surfaces in comparison with standard polystyrene cell culture dishes. However, on derivative C, the cell population density was significantly lower. This may be associated with the orientation of the carborane cluster, in which the acidic CH vertices face upward from the surface. Carboranethiol derivative D has thiol groups attached in the opposite (i.e., para-) positions, and can be considered as a promising linker for the attachment of various biological molecules to a gold surface.

Acknowledgements

This work was supported by the Grant Agency of the Acad. Sci. CR (Grant No. KAN400480701). Mr. Robin Healey (Czech Technical University, Prague) is gratefully acknowledged for his language revision of the manuscript.

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BIOMATERIALS WITH ANTIBACTERIAL ACTIVITY

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Abstract

The sol-gel derived silica spheres with surfaces modified by silver nanoparticles were used to enhance the photodynamic effect. The silica nanoparticles were prepared by modified Stöber synthesis. The diameter of obtained silica spheres was ca. 100 nm. These silica spheres we used as a carrier for noble nanoparticles. It was shown that Ag-Au nanoparticles have an antibacterial activity against Escherichia coli. This effect depends on the nanoparticles concentration and it is stronger for higher concentrations. Laser irradiation enhances this effect, and starting from certain concentration it is possible to kill Escherichia coli, totally, when using laser light.

[Engineering of Biomaterials, 81-84, (2008), 119-121]

Introduction

Recently, many new developments in nanomedicine are observed. One of the promising application of nanomaterials is in photodynamic medicine, where the nanoparticles may enhance the photodynamic activity [1-3]. Photodynamic therapy (PDT) over the past decades was mainly exploited for treatment of tumors. It can be also suitable for the inactivation of microbes by photodynamic activity. Antimicrobial photodynamic therapy (APDT) combines a nontoxic photoactive dye - photosensitizer to generate singlet oxygen and free radicals after light exposure that kill microbial cells [4-8]. In this paper we will demonstrate that silver-doped nanoparticles have antimicrobial activity and this effect may be enhanced by adding a photosensitive agent and exposing the bacteria culture to the light.

Materials

First, the silica nanoparticles were prepared by modified Stöber synthesis [9] from ethyl alcohol (95%, Polish Chemicals), ammonium water (25%, Polish Chemicals), hydrofluoric acid (35%, Polish Chemicals) and tetraethylortosilane (TEOS from Aldrich) mixed at room temperature prepared. Next, the Tollen's method for silica silver doped nanospheres production was exploited [10]. The preparation process used in this study is described in [11]. For reduction reactions glucose was used as the reducing agent. In second reduction reaction to the solution Ag-SiO_2 0,5M of $AgNO_3$ and 25% NH_4OH were added. For cementation process "gold liquid" (K_2CO_3+$HAuCl_4$+H_2O) was added.