THE GROWTH OF SAOS-2 CELLS ON DLC LAYERS DOPED WITH TITANIUM

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Introduction

Diamond-like carbon (DLC) coatings have been widely studied due to their potential usage as a coating of implants. DLC layers can be doped with various chemical elements, e.g. chromium, titanium, cobalt, and silver. Titanium(Ti)-doped or chromium(Cr)-doped DLC layers were observed to improve properties of the material internal stress, to reduce peeling from the material surface and risk of cracking [1]. Saos-2 cells are a human osteoblast-like cell line that has a potential to undergo osteogenic differentiation [2]. In this work, we evaluated the adhesion, growth, and osteogenic differentiation of Saos-2 cells on DLC layers enriched with different Ti content.

Materials and Methods

The Ti-DLC layers were prepared of a different Ti content on glass as follows: 0.0, 0.7, 3.3, 5.2, 10.0, 24.5 at% Ti. The glass coverslip (Gl) and tissue culture polystyrene (PS) were used as control materials. Prior cell seeding, the samples were sterilised in 70% ethanol for 2 hours. The Saos-2 cells were seeded in a density of 20,000 cells/cm^2 and cultured in McCoy's 5A medium with 15% foetal bovine serum for 7 days. On day 1, 3, and 7, the cells were fixed by 70% frozen ethanol and stained with Texas Red C_2-maleimide (day 1), stained for vinculin using primary mouse anti-vinculin antibody and secondary anti-mouse antibody, and for F-actin using phalloidin conjugated with TRIC (day 3), and for osteopontin (day 7) using primary mouse anti-osteopontin antibody and secondary anti-mouse antibody. The cell nuclei were counterstained with Hoechst 33258. The cell spreading area was measured from microphotographs of 289–399 cells stained with Texas Red C_2-maleimide (day 1) using Atlas Tescan software. The cell number was counted from microphotographs of immunofluorescence staining taken on day 1, 3, and 7. All microphotographs were taken under epifluorescence microscope Olympus IX 71 microscope, objective ×20 and ×10. The data is expressed as mean + SEM. ANOVA, Dunn´s method. The statistical significance ($p<0.05$) is specified above the columns (compared to every sample on the same day of the culture).

Results and Discussion

The number of initially adhered cells on day 1 was from 7,927 cells/cm^2 on DLC without Ti to 12,871 cells/cm^2 on glass sample. The cell number on day 3 ranged from 20,465 cells/cm^2 on DLC without Ti to 45,832 cells/cm^2 on PS sample. The cell number increased during the time period and on day 7, the cell number reached values from 98,887 cells/cm^2 on DLC with 5.2 at% of Ti to 176,815 cells/cm^2 on DLC without Ti. On day 1, the highest cell spreading area (FIG. 1), was observed on DLC with 5.2 at% of Ti and the lowest was on glass. On all DLC-based and PS samples, the cells were polygonal-shaped; however, on the control glass sample, the cells were rather round-shaped. On day 3, staining for vinculin showed a more apparent assembly of vinculin-containing focal adhesion plaques on samples with a higher Ti content (FIG. 2 A). On day 7, as the cells on all samples reached confluence, we found that the osteopontin, a marker of osteogenic differentiation, is present in Saos-2 cells on all Ti-DLC (FIG. 2 B) and control samples.

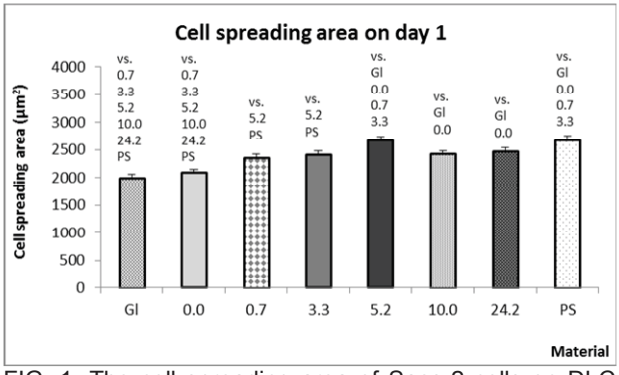

FIG. 1. The cell spreading area of Saos-2 cells on DLC doped with 0.0, 0.7, 3.3, 5.2, 10.0, 24.5 at% of Ti, on glass (Gl), and on polystyrene (PS) on day 1. The data is expressed as mean + SEM. ANOVA, Dunn´s method. The statistical significance ($p<0.05$) is specified above the columns (compared to every sample on the same day of the culture).

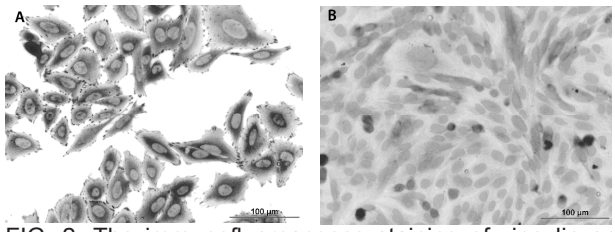

FIG. 2. The immunofluorescence staining of vinculin on day 3 (A) and of osteopontin on day 7 (B) in Saos-2 cells on DLC with 5.2 at% of Ti. The cell nuclei are counterstained with Hoechst 33258, Olympus IX 71 microscope, objective ×20.

Conclusions

We proved that Saos-2 cells adhered and proliferated during the followed time period of 7 days on Ti-DLC samples as well as on control samples. According to the cell spreading area and the presence of more apparent assembly of vinculin-containing focal adhesion plaques, the samples with higher Ti content seem to be more suitable for quick adhesion of the Saos-2 cells. The osteogenic differentiation was confirmed by the presence of osteopontin.

Acknowledgments

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