PECTIN COATINGS ON TITANIUM ALLOY SAMPLES PRODUCED BY ADDITIVE MANUFACTURING: PROMOTION OF HUMAN BONE MARROW STROMAL CELL PROLIFERATION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 43]

Introduction

Titanium and its alloy, e.g. Ti6Al4V, are popular load-bearing biomaterials for bone contact. They can be fabricated by additive manufacturing technologies. The adhesion and proliferation of bone-forming cells is a pre-requisite for formation of new bone tissue on the implant surface, which in turn leads to implant stability and long-term success. Hence, surface coatings which promote cell adhesion and proliferation are desirable.

In this study, Ti6Al4V discs prepared by additive manufacturing were coated with layers of pectins, calcium-binding polysaccharides derived from citrus (C) and apple (A) containing alkaline phosphatase (ALP), the enzyme responsible for mineralization of bone tissue. Coatings were characterized biologically with human bone marrow stromal cells (hBMSC). Cell adhesion and proliferation were assessed.

Materials and Methods

Rough Ti6Al4V discs of diameter 2 cm were prepared as described previously [1]. Ti6Al4V discs and 0.8% (w/w) C (degree of esterification (DE) 34%, Galacturonic acid content (GalC) 74%) and A (DE 35%, GalC 75%) pectin solutions were autoclaved at 134ºC. ALP solution (1.6% (w/v)) was sterilized by filtration. ALP and pectin solutions were mixed 1:1 (v/v). 250 µl of this solution was spread on Ti6Al4V and allowed to air-dry in a laminar flow bench. The presence of a coating was confirmed by SEM after gold coating. hBMSC from two different donors were seeded at a density of 7,000 cells/cm². Cells were seeded onto the samples in 400 µl of cell culture medium (DMEM with 10% heat-inactivated fetal calf serum, and antibiotics (penicillin and streptomycin). After 2 h the medium was filled up to 4 ml and culture proceeded at 37°C in a humified CO_2 incubator. Proliferation was assessed by the MTS-Assay. Cells were treated with 10% dye solution in DMEM for 2 h. Analyses were performed 24 h and 7 days after seeding. Statistical significance was analyzed by one-way ANOVA and Bonferroni post-test (prism graph pad software). Cell morphology was assessed after 24 h. Cells were fixed with 4% paraformaldehyde and stained with Alexa488-phalloidine to visualize F-actin cytoskeleton (green fluorescence) and with DAPI to stain the nuclei (blue fluorescence). The images (three from each sample) were taken with Axiophot microscope (Zeiss) using a digital camera and Axiovision software. Focusing of cells of samples was complicated by the roughness of the sample surfaces.

Results and Discussion

A-ALP and C-ALP coatings formed on Ti6Al4V discs. Cells retained viability and proliferated over 24 h and 7 days (FIG. 1). Proliferation was significantly higher on C-ALP coatings than on A-ALP coatings after 1 day, and after 7 days, higher than on both uncoated samples and A-ALP coatings. A-ALP coatings were significantly superior to uncoated samples after 7 days. The reasons for this remain unclear. Cells on all substrates displayed a spread morphology and distinct, well organized F-actin fibers, characteristic for good adhesion (FIG. 2).

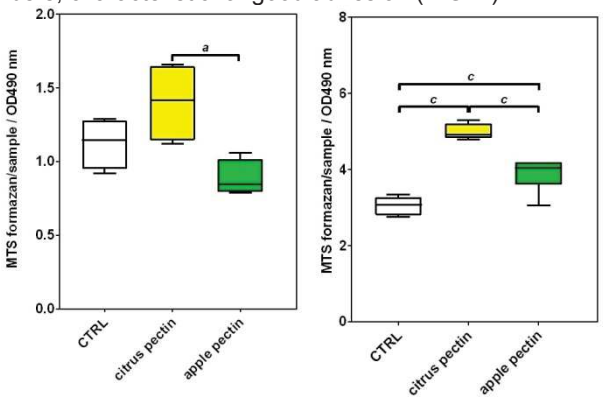

FIG. 1. MTS assay 24 h (left) and 7 days (right) after seeding of hBMSC on samples, a and c indicate significant differences (a: $p<0.05$, c: $p<0.001$).

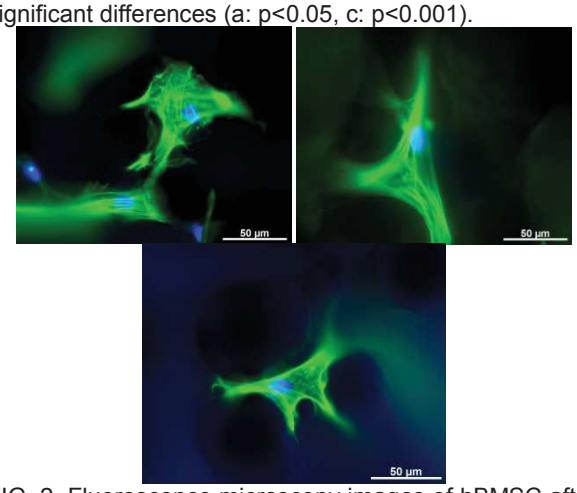

FIG. 2. Fluorescence microscopy images of hBMSC after 24 h. Top left: uncoated Ti6Al4V. Top right: C-ALP coating. Bottom: A-ALP coating. Blue: cell nucleus. Green: F-actin fibres. Scale bar: 50 µm.

Conclusions and Outlook

hBMSC proliferation after 7 days was increased by A-ALP coatings and, in particular, by C-ALP coatings. Cell morphology was similar on coated and uncoated samples. Future work should focus on differentiation.

Acknowledgment

Era-Net Rus Plus program for financial support in the framework of the project “Fabrication and investigation of new hybrid scaffolds with the controlled porous hierarchy for bone tissue engineering” (Intelbiocomp).

References

[1] Surmeneva MA et al, IOP Conf. Series: Materials Science and Engineering 98 (2015) 012025.