Immobilization Strategies to Functionalize Tantalum Surfaces with Cell-Adhesive Peptides: Physicochemical and Biological Characterization

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INTRODUCTION

Tantalum (Ta)-based implants are commonly used in orthopedics to replace trabecular bone tissues. This is due to the combination of optimal mechanical properties and excellent biocompatibility displayed by this material[1]. Moreover, biomimetic approaches aiming at conferring a superior bioactivity on Ta surfaces could increase osteointegrative rates and improve the efficacy of such materials[2]. However, to date only a few studies have attempted the biofunctionalization of this material with cell-adhesive molecules[3,4]. The objective of this work was thus to investigate immobilization strategies to functionalize Ta surfaces with an RGD peptide. The process of biofunctionalization was characterized by means of physicochemical methods and cell adhesion assays.

EXPERIMENTAL METHODS

General: Smooth (Ra ≈ 40 nm) Ta disks were used as model material. A linear RGD peptide containing a bioactive sequence, a spacer unit and an anchoring group (Figure 1) was synthesized in solid-phase and used as cell adhesive molecule. **Biofunctionalization:** Ta samples were activated (passivation with HNO_3 or treatment by UV/ozone) and the RGD peptide immobilized either by physical adsorption or silanization (APTES). **Surface characterization**: the physicochemical properties of the samples were analyzed by means of contact angle measurements and surface energy calculations, white light interferometry, scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS). **Biological characterization:** Cell adhesion studies were conducted using human osteogenic sarcoma (Saos-2) cells. The number of cells attached on the functionalized Ta samples was measured by LDH assays, and their spreading by immunofluorescent methods. Proliferation of adherent cells was also evaluated by LDH activity. **Statistics:** Significant differences were analyzed either by ANOVA or by non-parametric Mann-Whitney test. Confidence levels were set at 95 %.

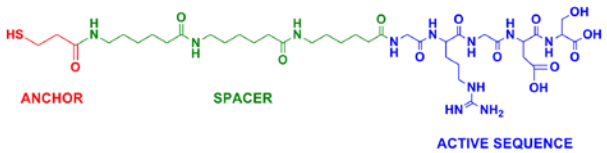

Fig 1. Chemical structure of the cell adhesive peptide containing the GRGDS active motif, three units of aminohexanoic acid as spacer and a mercaptopropionic acid as anchoring unit.

RESULTS AND DISCUSSION

Physicochemical characterization: Passivation with HNO_3 only reduced slightly the water contact angle of Ta samples. In contrast, activation with ozone drastically increased the wettability of the samples, resulting in highly hydrophilic surfaces. **1. Physical adsorption** of the RGD peptide on Ta/Ta HNO_3 samples was analyzed by the increase in the N1s signal by XPS. The peptide was physisorbed at a similar rate in both surfaces. However, peptide attachment was less efficient in ozone-activated samples. **2. Silanization with APTES** proved to be more efficient on ozone-treated Ta samples than on Ta/Ta HNO_3 samples, probably due to the difference in the presence of hydroxyl groups on the surfaces. The final amount of peptide bound to silanized-surfaces was nonetheless similar to all samples, regardless of the activation method used.

Biological characterization: 1. Physisorption of the RGD peptide on Ta and Ta-HNO_3 samples resulted in a significant increase in the surface's cell adhesive capacities, displaying higher number of cells attached and improved cellular spreading compared to non-functionalized samples. However, this effect was not observed on ozone-activated samples, due to the lower efficiency of peptide attachment observed for these samples. **2. The covalent immobilization** of the peptide (silanization) rendered also increased cell adhesion activities. Interestingly, the extent of cell adhesion was similar for all functionalized surfaces, consequent with an optimal silanization for all the activation condition tested.

CONCLUSION

We have characterized two methods of peptide immobilization to functionalize Ta. Both strategies have increased the number and spreading of Saos-2 cells onto the surfaces, thus representing viable approaches to increase the osteointegrative capacities of this material.

REFERENCES

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