Zeta potential titration and Kelvin probe force microscopy as tools for the design of biomaterials

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Keywords: Surface charge; functionalization; coatings; peptoids; tocopheryl phosphat

Hem. Ind. **78(1S)** 56 (2024)

Available on-line at the Journal web address: http://www.ache.org.rs/HI/

INTRODUCTION: The biomaterials' surface is the place where biomaterials, physiological fluids, proteins, and cells meet determining the host response. Surface features such as chemical composition, exposed functional groups, zeta and electric potential, wettability, topography, and roughness strongly affect this response. Surface functionalization and coating with biomolecules are strategic tools to modulate surface properties and control the biomaterial outcome. The design of a functionalization or coating process goes through the following main steps: selection of the biomolecule and process parameters, identification of the effective presence of the biomolecule on the surface and type of bond, and evaluation of the biological response. The role of advanced characterization techniques in these steps is here discussed.

EXPERIMENTAL: Ti6Al4V discs (10 mm in diameter-2 mm thick) were polished (MP), washed, chemically treated (CT), and irradiated with UV light. Alpha-tocopheryl phosphate (α-TP) was purchased (Sigma-Aldrich) while the GN2-Npm9 peptoid was synthesized at Roskilde University. For functionalization, a 100-µl drop of a solution of PBS and 1 mg/mL GN2.Npm9 was dropped onto the CT samples. The samples were left at 37 °C for 2 h, rinsed, and dried under a hood (CT_GN2-Npm9). Samples for coating were pre-soaked in a solution of $CaCl_2$, dried, soaked in a solution of α-TP in TRIS-HCL (5 mg/mL) for 3 h at 37 °C, dried, rinsed 3 times in ultrapure water, and dried again. An atomic force microscope (KPFM-Innova atomic force microscope, Bruker) equipped with a conductive tip (Sb-doped Si, frequency 75 kHz, SCM-PIT-V2, Bruker) was used. Surface zeta potential as a function of pH was measured by electrokinetic measurements (SurPASS, Anton Paar) equipped with an adjustable gap cell in an electrolyte solution of 0.001 M KCl.

RESULTS AND DISCUSSION: Two examples will be described: coating with tocopheryl phosphate [1] and functionalization with a peptoid for antibacterial purposes [2]. The role of Ca^{2+} in the chemisorption of the biomolecules and their surface distribution was evidenced by KPFM. The orientation and exposed functional groups of the biomolecules in solution and after adsorption on the CT surface were deduced from zeta potential titration curves. Biological tests evidenced different biological responses of the coated/functionalized surfaces according to different process parameters in terms of cytocompatibility (human mesenchymal stem cells) and antibacterial action (Staphylococcus Epidermidis).

CONCLUSIONS: Zeta potential titration curves as the function of pH and KPFM give significant information on the surface features of the substrate and functionalization solution, allowing the selection of process parameters and speculations on the mechanism of physical or chemical adsorption. Moreover, they allow detection and imaging of the adsorbed biomolecule on the functionalized surface. Peptoids and tocopheryl phosphate have a good potential as antibiofilm agents.

REFERENCES

[1] F. Gamna, A.M. Wiecek, A. Cochis, J. Barberi, A.C. Scalia, L. Rimondini, S. Spriano. Grafting of alpha-tocopheryl phosphate on chemically treated Ti-6Al-4V for antibacterial bone implants. Applied Surface Science 2023; 619: 156681, https://doi.org/10.1016/j.apsusc.2023.156681.

[2] F. Gamna, A. Cochis, B. Mojsoska, A. Kumar, L. Rimondini, S. Spriano. functionalization with the synthetic peptoid GN2-Npm9 as a strategy for antibacterial and biocompatible titanium implants. Helyon 2024; 10: e24246, https://doi.org/10.1016/j.heliyon.2024.e24246

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