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BIOACTIVE COATINGS FOR TITANIUM DENTAL IMPLANTS CONTAINING SR/ZN PHYTATES: OSTEOGENIC AND ANTIBACTERIAL PROPERTIES

Gerardo Asensio ¹, Ana M. Hernández-Arriaga ^{2,6}, Marcela Martín del campo ^{1,3}, Auxiliadora M. Prieto ^{2,6}, Agustín R. González-Elípe ^{4,6}, Blanca Vázquez-Lasa ^{1,5,6}, Luis Rojo ^{*1,5,6}

¹ Instituto de Ciencia y Tecnología de Polímeros (CSIC), Madrid, Spain; gerardo.asensio@ictp.csic.es; bvazquez@ictp.csic.es; rojodelolmo@ictp.csic.es; marcela@ictp.csic.es

² Centro de Investigaciones Biológicas – Margarita Salas (CSIC), Madrid, Spain; arriaga@cib.csic.es; auxi@cib.csic.es

³ Facultad de Estomatología, Universidad Autónoma San Luis Potosí (UASLP), San Luis Potosí, Mexico;

⁴ Instituto de Ciencia de Materiales de Sevilla (CSIC), Sevilla, Spain; arge@icmse.csic.es

⁵ Consorcio Centro de Investigación Biomédica en Red, Madrid, Spain.

⁶ Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy (CSIC), Madrid, Spain.

Abstract: Materials science in the field of oral implantology is focused on the development of bioactive surfaces able to promote both osteogenic and antibacterial mechanisms. In this work, we propose an affordable and reproducible methodology for the fabrication of bioactive SAMs containing Sr/Zn phytate complexes. Phytate SAMs were fabricated by condensation reaction (pH 4) promoted by casting evaporation, and physicochemical features were studied by XPS, SEM, AFM and profilometry. *In vitro* biological studies performed with hObs cultures demonstrated the ability of modified surfaces for sustaining the cellular colonization and stimulating the ALP activity and the mineralization degree. Moreover, antibacterial assays performed with *S. mutans* cultures seeded over modified substrates corroborated the potential application of phytate-SAMs as bioactive coatings for titanium dental implants.

Keywords: <antibacterial>, <osteogenic>, <phytic acid>, <strontium>, <zinc, titanium>



INTRODUCTION

The increasing tendency of dental implant insertions has encouraged the fabrication of bioactive surfaces designed to ensure a long-lasting performance even when treating complicated patients. In this sense, the development of simple and affordable functional coatings is urgently demanded to promote osteogenic regeneration around bone trauma and to avoid post-operational infections and peri-implantitis disease. Here, phytic acid (PA) and its derivatives with bioactive strontium and zinc (SrPhy and ZnPhy), are prepared as self-assembled monolayers (SAMs) of titanium surfaces to explore synergic biological effects provided from the combined action of both the ligand and the cations [1, 2], and their osteogenic and antibacterial properties are evaluated in vitro.

METHODS

Firstly, SrPhy and ZnPhy were synthesised by hydrothermal reaction following an adapted method described in a previous work [2]. Before use, titanium discs were activated (Ti-Act) by alkali treatment (NaOH 5 M). The fabrication of phytate-SAMs was carried out by five condensation cycles of the corresponding aqueous phytate solution (1 mM, pH 4) over Ti-Act discs promoted by casting evaporation at 80 °C. Four experimental groups were obtained comprising Ti-PA, Ti-SrPhy, Ti-ZnPhy and Ti-SrPhy/ZnPhy, and Ti-Act group was used as control. SAMs formation was assessed by XPS analysis, and the topography studied by SEM, AFM and profilometry. The adhesion and colonization of human osteoblast cells (hObs) over modified surfaces were evaluated by SEM imaging. Their osteogenic ability was assessed in vitro with hObs cultures in terms of ALP activity and matrix mineralization degree. The antibiofilm capacity was tested against *S. mutans* cultures by crystal violet staining, colony forming unit (CFU) viability, LIVE/DEAD microscopy and SEM observation.

RESULTS AND DISCUSSION

Bioactive phytate-based SAMs were successfully fabricated as confirmed by XPS analysis, and the topographic characterization revealed a significant roughness increase for Ti-SrPhy, Ti-ZnPhy and Ti-SrPhy/ZnPhy groups ($SA > 1.2 \mu\text{m}$) in comparison with Ti-PA and Ti-Act ($SA > 1.0 \mu\text{m}$). SEM imaging of hObs seeded onto experimental groups displayed correct adhesion, spreading and proliferation over time, compared to Ti-Act (Figure 1A). The quantification of calcium deposits showed significantly higher mineralization for all phytate-SAMs with respect to Ti-Act control group at 14 days, and for Ti-SrPhy samples at 7 days. Interestingly, the activity of ALP/DNA was only statistically overexpressed by Ti-SrPhy, Ti-ZnPhy and Ti-SrPhy/ZnPhy regarding the control group, and Ti-SrPhy even presented a significant increase when comparable to Ti-PA. On the other hand, the disruption of the biofilm produced by *S. mutans* cultures and the viability of adhered bacteria was significantly reduced by all phytate-SAMs surfaces without



differences between the experimental groups. Moreover, SEM and LIVE/DEAD assays suggested that the cell membrane integrity of bacteria was altered when cultured over phytate-modified samples (Figure 1B).

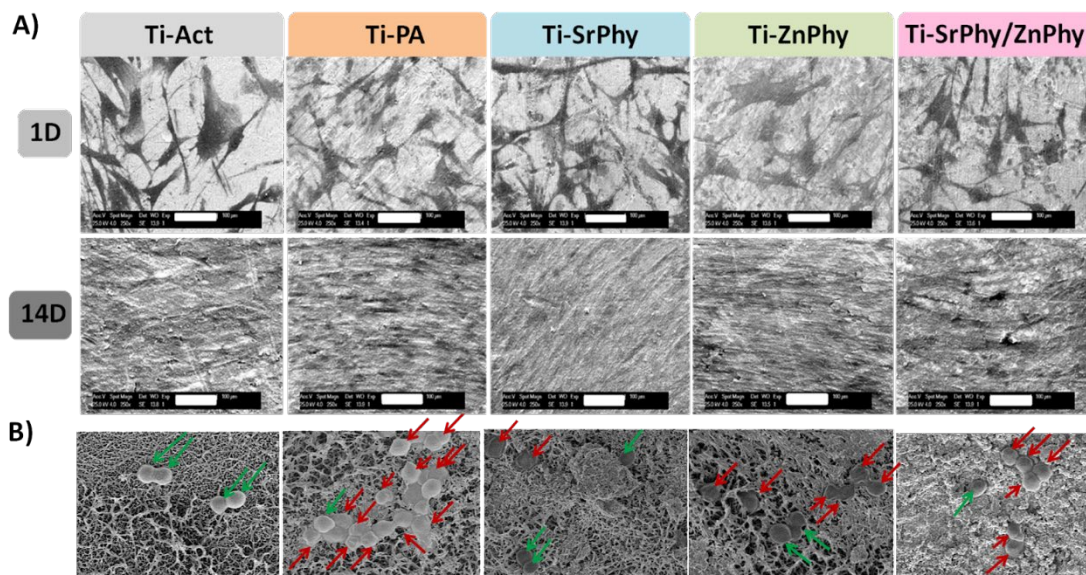


Figure 1. SEM imaging of A) hObs cells after 1 and 14 days of incubation, and B) *S. mutans* adhered to titanium surfaces indicating bacteria with the disrupted membrane (red arrows) and not affected (green arrows).

CONCLUSIONS

Bioactive phytate-based SAMs have been fabricated through an easy, reproducible and chemically-green process. The *in vitro* evaluation suggested adequate antibiofilm properties for all phytate-containing samples, and the quantification of ALP kinase activity results indicated evidence of the synergic effects produced by the bioactive cations and phytate moieties. Therefore, these promising findings envision an excellent potential of the developed SAMs as bioactive coatings for dental implants.

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