Titanium dental abutments functionalization with flavonoids to increase gingival sealing

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Introduction

Titanium (Ti) and its alloys are frequently employed in the fabrication of dental implants due to their biocompatibility. However, these materials are biologically inert, and its necessity of modification and functionalization are growing to enhance tissue regeneration[1]. Abutment is the piece of implants in direct contact of gingival tissue, frequently undergoing anodization treatments. However, the weak sealing of the gingiva around titanium abutments contributes to an increased incidence of peri-implant infections, including peri-implantitis, which finally lead to an implant lose[2]. **The objective of this study is to enhance the properties of anodised Ti surfaces by functionalization with a variety of flavonoids (FLV) to promote gingival sealing and thus reduce the risk of peri-implantitis.**

Surface Characterization

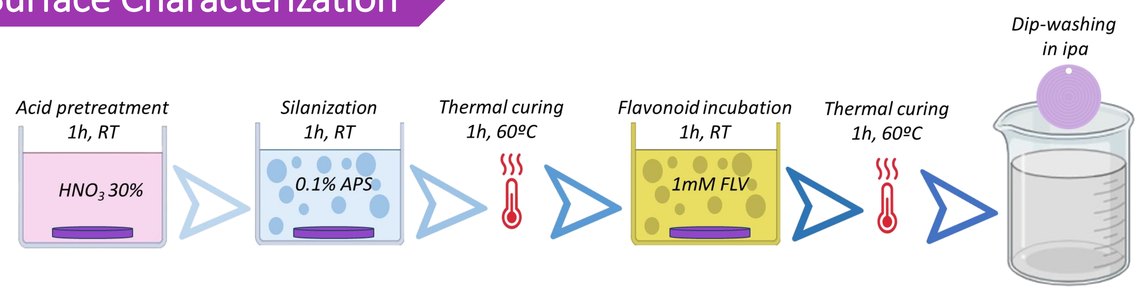


Fig. 1 *Preparation methodology of functionalized titanium grade V mechanized anodized discs with flavonoids*

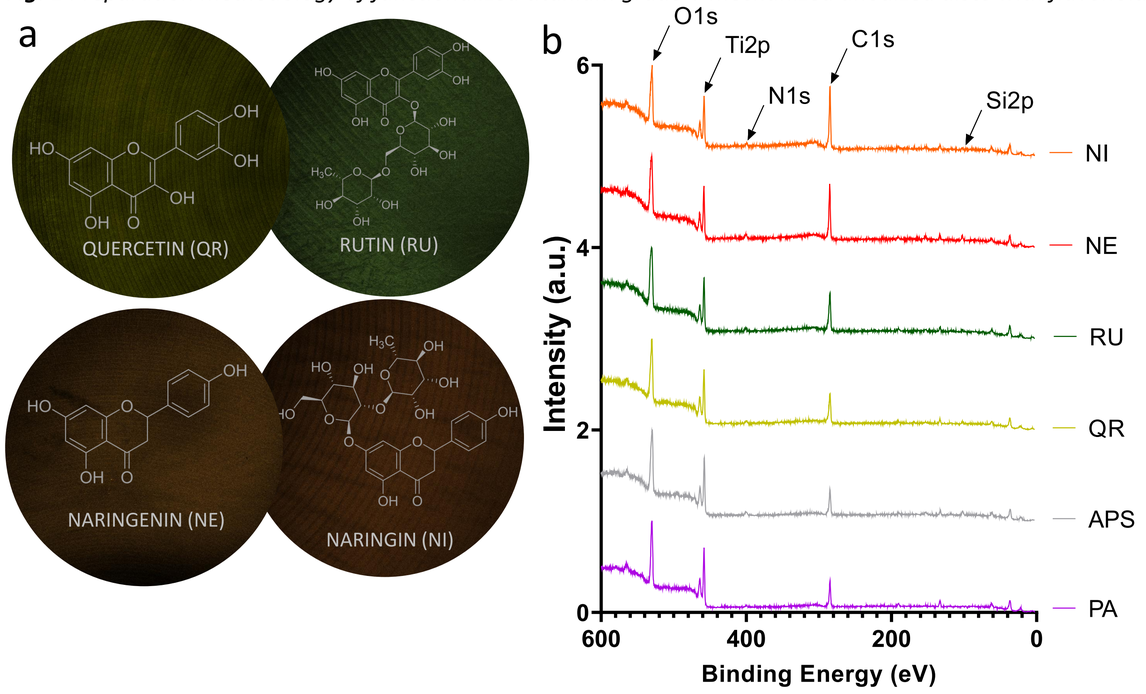


Fig. 2 *Fluorescence images of the flavonoids grafted and symbols used for each (a) and X-ray photoelectron spectroscopy (XPS) survey scan spectra of functionalized discs (b).*

HGF analysis with modified surfaces

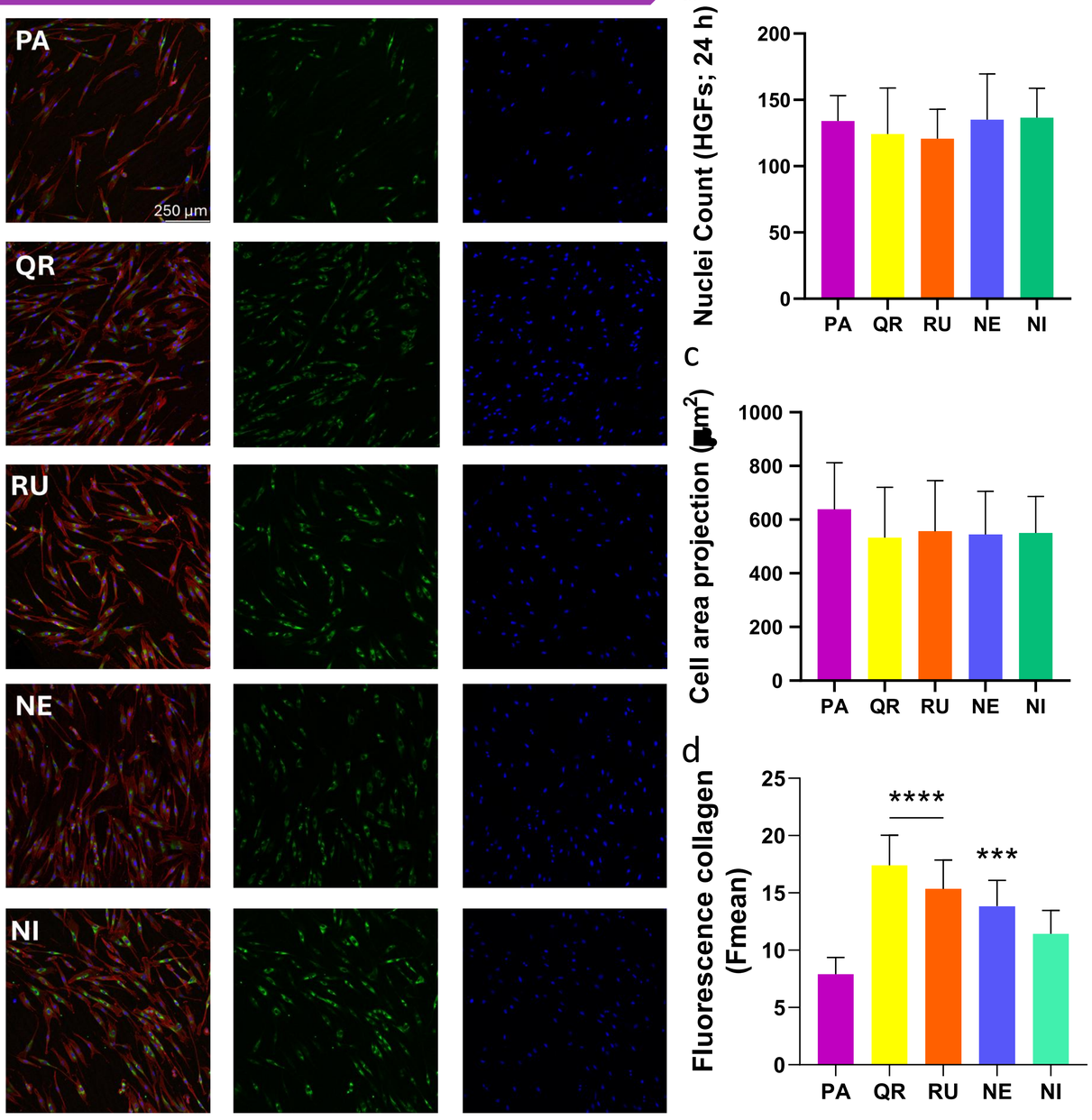


Fig. 3 *Cytoskeletal arrangement of HGF visualized by fluorescent confocal microscopy functionalized surfaces (a). Actin filaments were labeled with Phalloidin-iFluor 488 (red), collagen I deposits with anti-col1A1 (green) and nuclei were counterstained with DAPI (blue). HGF nuclei count (b), cell area projection (c) and collagen deposits (d) are showed. (ANOVA; p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***)) and p ≤ 0.0001 (****)).*

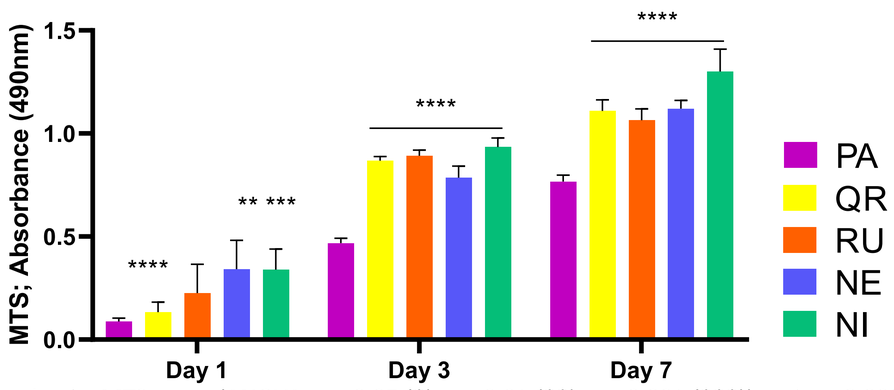


Fig. 4 *Cell proliferation by MTS assay (ANOVA; p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***)) and p ≤ 0.0001 (****)).*

Proteomic

a

BIOLOGICAL FUNCTION		QR/PA	RU/PA	NE/PA	NI/PA
Immune	↑UP	HV64D, MASP2, CLUS, C4BPB	C4BPA, CFHR2, C4BPB, IGHV3-64D	CFHR5	CFHR5, IGHV3-64D
	↓DOWN	KVD21, KV240, KV106, HV169, HV103, LV147	KVD21, KV240, KV106, HV169, HV103, LV147, C7, MASP1, C8B, C6	C1S, C6, C2, C8B, IKV6D-21, IGKV3-20, CFB, IGHV1-3, IGHV1-2, IGHV1-69, MASP1	C8B, IKV6D-21, IGKV3-20, IGKV2-40, CFB, IGHV1-3, IGHV1-2, IGHV1-69, MASP1
Coagulation	↑UP	PROC, AGT, F10	PROC, AGT, F10, F2	PROC, PROS1 AGT, F5, F10, F2, SERPINA5	PROC, AGT, F5, F10
	↓DOWN	KNG1, F11, F12, PLF4	KLKB1, SERPIND1, F11, F12, PLF4	KNG1, KLKB1, F12, F13B, PF4, SERPINA4, F11	KLKB1, F12, F13B, PF4, SERPINA4, F11

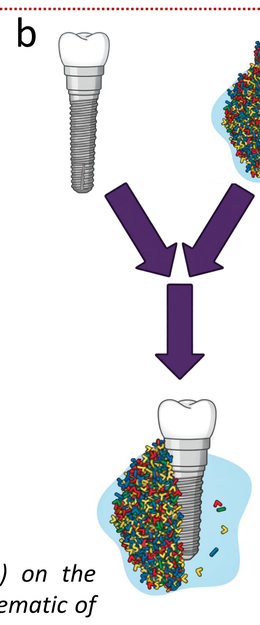


Fig. 6 *Differentially adsorbed proteins (upregulated or downregulated) on the surfaces of treated samples relative to the untreated material (a) and schematic of the protein adsorption process on the implant (b).*

Immune interactions

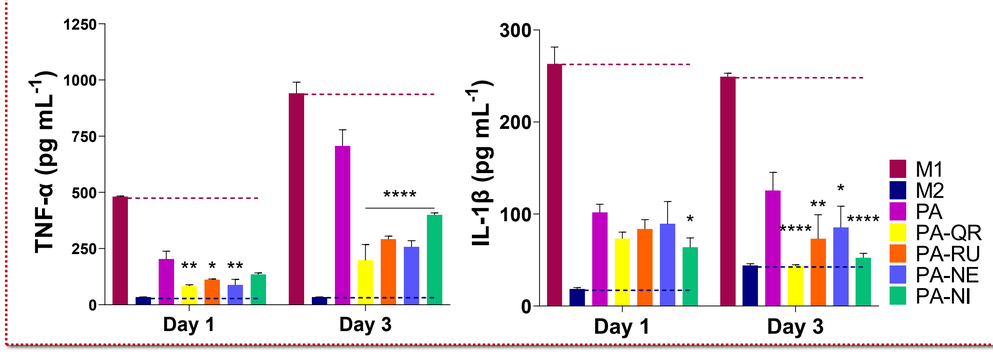


Fig. 5 *Cytokine levels measured by ELISA for TNF-α (a), IL-1β (b) in THP-1 cells at two time points (1 and 3 days). (ANOVA; p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***)) and p ≤ 0.0001 (****)).*

Discussion and conclusion

X-ray spectroscopy (XPS) and fluorescence (**Fig. 2**) analysis **confirmed the complete immobilization of flavonoids on the surface of the discs.** Contact between the modified surfaces and HGFs led to enhanced collagen secretion, with the exception of NI (**Fig. 3**). Fibroblast proliferation was increased at days 3 and 7, suggesting improved adhesion of fibroblasts to the modified surfaces (**Fig. 4**). Additionally, a significant capacity to reduce the secretion of pro-inflammatory cytokines was observed. Notably, **IL-1β levels were markedly decreased** in the QR and NI conditions (**Fig. 5).**

Most notably, on **Fig. 6** a **significant decrease in immunoglobulin adsorption** is observed across all modified surfaces. Concurrently, all surfaces exhibit an increase in proteins that regulate the complement cascade (C4BPA, C4BPB, CFHR2, CFHR5 and CLUS). In the case of QR, an increased adsorption of MASP2 is detected, indicative of activation of the lectin pathway, whereas the remaining surfaces show reduced levels of MASP1, suggesting downregulation of this pathway. Furthermore, RU, NE, and NI also display a decrease in complement cascade proteins (C1S, C2, C6, C7 and C8B). **A reduction in the coagulation-related protein KNG1** is observed in QR and NE. As KNG1 plays a role in modulating the activation of the coagulation cascade, its decrease may influence downstream inflammatory processes. The proteomic profile is consistent with the in vitro findings.

The incorporation of flavonoids appears to modulate inflammatory responses to varying extents, while simultaneously promoting fibroblast proliferation. This combined effect suggests an enhanced regenerative potential and, ultimately, an improvement in gingival sealing.

References

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Acknowledgements

Universitat Jaume I [UJI-B2021-25]; Ministerio de Ciencia, Innovación y Universidades [CPP2021-008492/AEI/10.13039/501100011033/ Unión Europea NextGenerationEU/PRTR] [PID2023-150429OB-I00/MCIU/AEI/10.13039/501100011033/FEDER,UE]; Generalitat Valenciana [CIPROM/2023/62]