The Role Of Titanium Surface Properties In Directing Macrophage Polarization And Promoting Osseointegration

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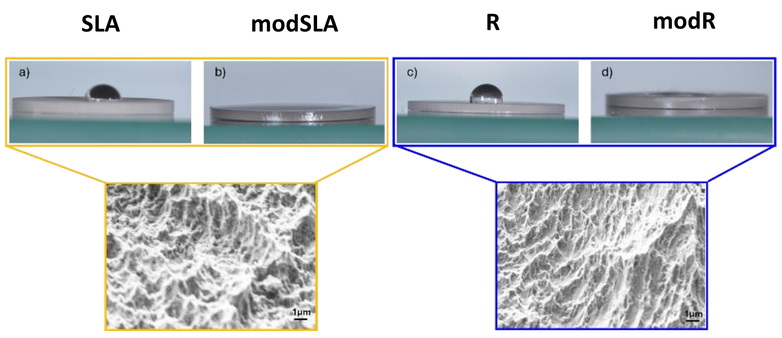
BACKGROUND AND RATIONALE

Dental implant success depends on osseointegration, a coordinated biological process shaped by implant surface properties. Historically, bone-forming cells were viewed as primary drivers; however, macrophages are now recognized as the first cells to engage the implant surface after placement.
Macrophages exhibit high phenotypic plasticity, polarizing toward a pro-inflammatory **M1** or an anti-inflammatory **M2** phenotype in response to local cues — a balance that critically regulates the immune-bone forming cell crosstalk.

Key question: How do surface chemistry and hydrophilicity of titanium vs. titanium/zirconia surfaces modulate macrophage polarization and downstream osteoblast mineralization?

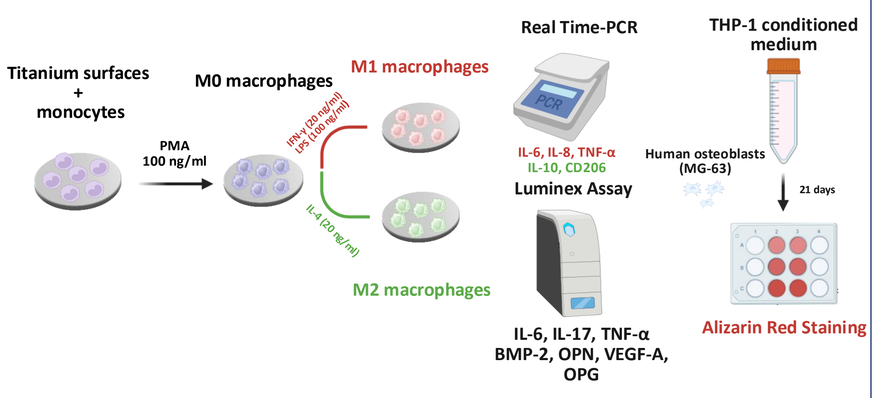
MATERIALS AND METHODS

Ti-SURFACES TESTED



Surface composition	Groups
Polished titanium surface (not reported in the figure)	P
Sand-blasted and acid etched titanium surface	SLA
Sand-blasted and acid etched hydrophilic titanium surface	modSLA
Sand-blasted and acid etched 85% titanium and 15% zirconia surface	R
Sand-blasted and acid etched 85% titanium and 15% zirconia hydrophilic surface	modR

EXPERIMENTAL WORKFLOW



The polarization of THP-1 monocytes through **M1/M2 phenotype** has been induced on the **SLA, SLActive (modSLA), Roxolid (R), Roxolid Active (modR)** surfaces and **gene expression** (e.g. IL-6, IL-8, TNF-α, IL-10, etc.), as well as **cytokines release** (e.g. BMP-2, IL-4, IL-6, IL-10, OPG, etc.) have been observed through **Real Time-PCR** and **Luminex assay**. Simultaneously, the **culturing medium** resulting by THP-1 has been used to **stimulate the mineralization** of MG-63 to corroborate the effectiveness of **the cross-talk between the lineages**

RESULTS

ADDITION OF ZIRCONIA TO TITANIUM IMPLANT SURFACE PROMOTES THE PRO-INFLAMMATORY M1 POLARIZATION

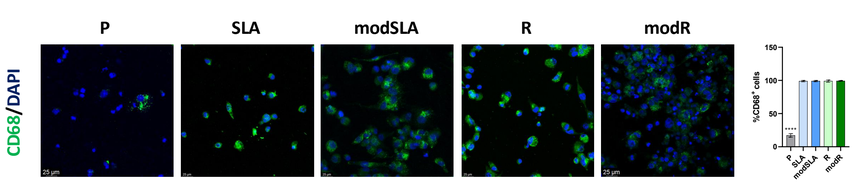


***Figure 1**: Monocyte-to-macrophage transition on dental implant-like surfaces. Immunofluorescence images of THP-1 cells polarized into steady-state M0 macrophages on different implantable surfaces.
The histogram reports the quantification of CD68-positive cells found on each surface. **** p<0.0001.*

Immediately after implantation, **monocytes interact with the implant surface** and differentiate into M0 macrophages. To mimic this in vitro, THP-1 cells were cultured on P, SLA, modSLA, R, and modR surfaces and differentiated with PMA. **CD68 staining confirmed M0 polarization** (Fig. 1).
Microrough surfaces (SLA, modSLA, R, modR) **showed higher CD68 expression and macrophage activation compared to smooth surfaces (P)**, consistent with previous findings and validating the model.

MACROPHAGE PHENOTYPIC CHARACTERIZATION ON DIFFERENT TITANIUM SURFACES

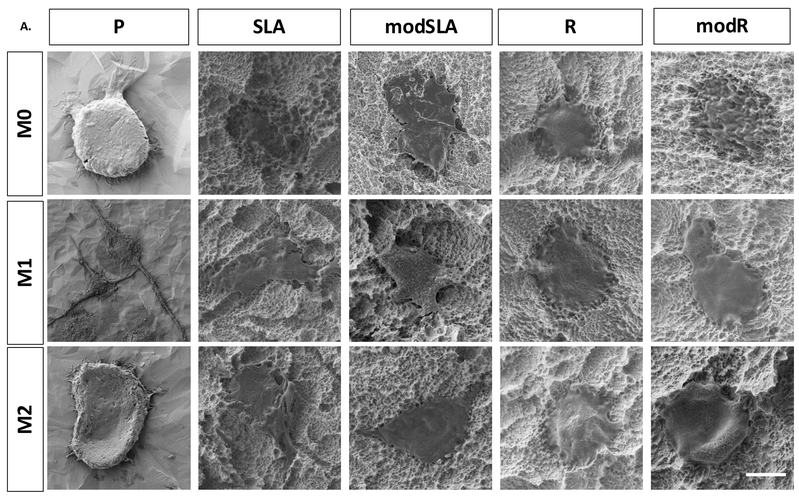


***Figure 2A**. SEM images of THP-1 cells induced into steady-state macrophages (M0) or polarized into M1 and M2 macrophages on different implantable surfaces. Scale bar:2 µm*

Cell morphology was similar across all considered surfaces (Fig. 2A). However, the **smooth P surface did not support the monocyte-to-macrophage transition**, and consequently **neither M1 nor M2 differentiation was observed**, highlighting the key role of surface roughness in macrophage activation.

IMMUNOFLUORESCENCE STAINING OF M1/M2 MACROPHAGE MARKERS

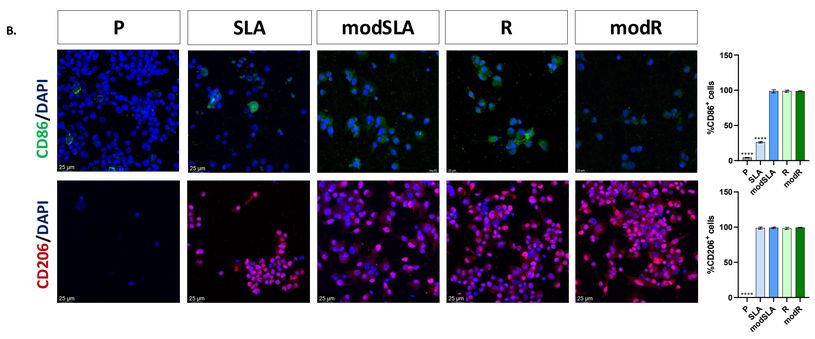


***Figure 2B**. Immunofluorescence images of CD86 (green) and CD206 (red) positive cells on different implantable surfaces. Histograms to the right report the quantification of CD86- and CD206-positive cells found on each surface. ****p<0.0001*

While the morphology of the cells was similar on all the considered surfaces (Figure 2A), the CD86 expression (Figure 2B) revealed that the **M1 phenotype was promoted by the presence of zirconia** (R and modR) and **hydrophilic titanium** (modSLA).

ZIRCONIA-DRIVEN MODULATION OF MACROPHAGE INFLAMMATORY RESPONSE

mRNA expression of immuno-related markers – Real Time PCR

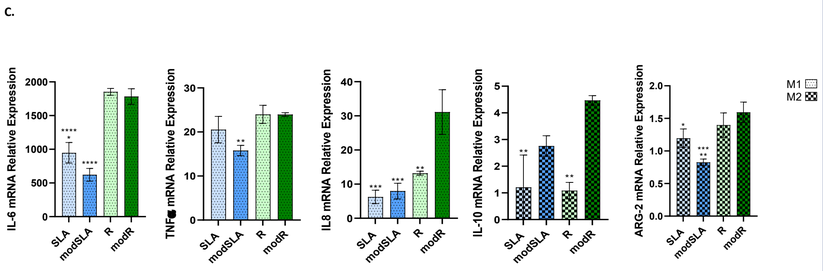


***Figure 2C**. mRNA levels of IL-6, TNF-α, and IL-8, and of IL-10, and ARG-2 on different implantable surfaces, after M1 or M2 polarization, respectively. **** p<0.0001, *** p=0.0008, ** p=0.0051, p=0.0365 .*

Cytokines quantification – Luminex Assay

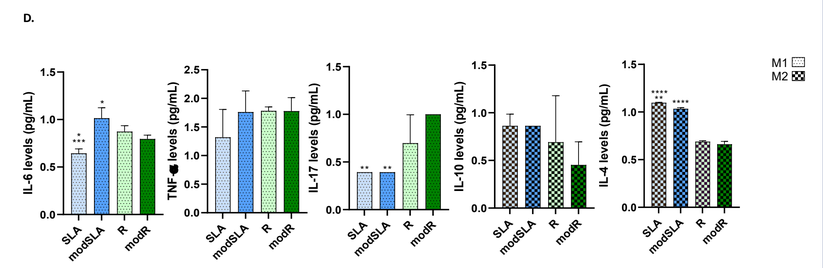


***Figure 2D**. Quantification of secreted pro-inflammatory (IL-6, TNF-α, and IL-17) and anti-inflammatory (IL-4 and IL-10) cytokines on different implantable surfaces, after M1 and M2 polarization, respectively. **** p<0.0001, *** p=0.0009, ** p=0.0080, * p=0.0209.*

Real Time-PCR (Fig. 2C) and Luminex analysis (Fig. 2D) confirmed that **zirconia-containing surfaces** (R, modR) **promoted higher expression and secretion of pro-inflammatory cytokines** (IL-6, TNF-α, IL-8, IL-17), **while reducing anti-inflammatory markers** (IL-10, IL-4).
Although a comparable number of CD206-positive cells was observed across most surfaces (except P), cytokine profiling revealed a clear **shift toward a pro-inflammatory microenvironment in the presence of zirconia**.
Overall, these findings indicate that surface chemical composition plays a key role in early macrophage polarization, whereas hydrophilicity appears to have a limited impact.

M1 POLARIZATION PROMOTES A PRO-OSTEOGENIC ENVIRONMENT, ENHANCED BY ZIRCONIA-DRIVEN CYTOKINE RELEASE

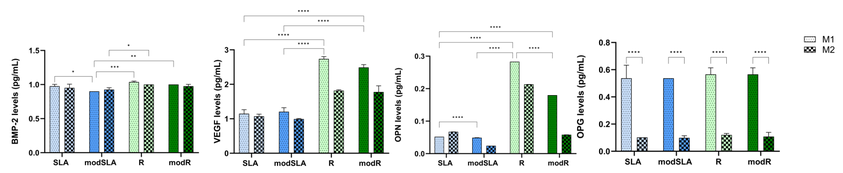


***Figure 3**: THP-1 secretion of bone-stimulating factors. Quantification of secreted BMP2, OPG, OPN and VEGF-A on different implantable surfaces, after M1 and M2 polarization, respectively. **** p<0.0001, *** p=0.0002, ** p=0.0050, * p=0.0487.*

Cytokines associated with bone formation (BMP2, VEGF-A, OPN, OPG) were quantified in THP-1 macrophage supernatants polarized on SLA, modSLA, R, and modR surfaces.

Zirconia-enriched surfaces further amplified VEGF-A and OPN release from M1 macrophages vs. SLA — both critical mediators of angiogenesis and osteoblast differentiation

These results suggest that early M1 activation contributes to a pro-osteogenic cytokine milieu, and that implant surface chemistry modulates this response

THE PRESENCE OF ZIRCONIA IN THE IMPLANTABLE SURFACE CONTRIBUTES TO THE MINERALIZATION OF HUMAN OSTEOBLASTS, SIMILARLY TO AN ENHANCED HYDROPHILICITY OF A TITANIUM SURFACE.

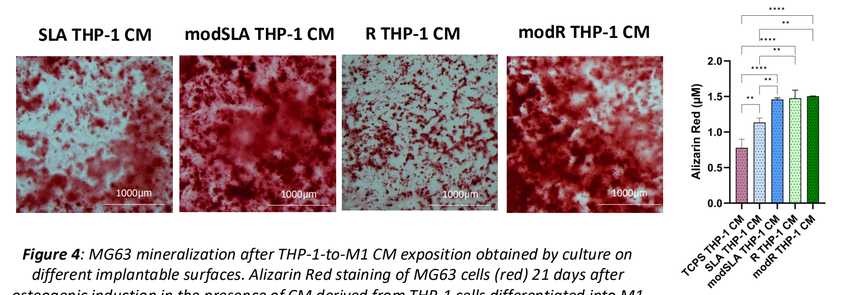


***Figure 4**: MG63 mineralization after THP-1-to-M1 CM exposition obtained by culture on different implantable surfaces. Alizarin Red staining of MG63 cells (red) 21 days after osteogenic induction in the presence of CM derived from THP-1 cells differentiated into M1 macrophages on SLA, modSLA, R and modR discs.
The histogram to the right reports the Alizarin Red quantification. Scale bar 1000µm;
**** P<0.0001, * p=0.0025, *** p=0.0055, ** p=0.0020.*

MG-63 osteoblast-like cells were cultured with conditioned media (CM) from M1-polarized THP-1 macrophages generated on SLA, modSLA, R, and modR surfaces, and mineralization was assessed after 21 days (Fig. 4).

R and modR conditioned media → highest Alizarin Red signal
Mineralization levels were comparable to modSLA, suggesting zirconia recapitulates the effect of enhanced hydrophilicity on bone-forming cell activity

Overall, these findings suggest that both zirconia incorporation and surface hydrophilicity contribute to generating a pro-osteogenic microenvironment that promotes osteoblast mineralization.

CONCLUSIONS

Osteoimmune framework:
Zirconia-Ti alloys support the interpretation of osseointegration as an osteoimmune process, not merely classic bone healing.

M1/M2 balance Is the key:
Early M1 dominance drives osteogenic cytokine release; imbalance may underlie peri-implant bone loss

Clinical relevance:
Ti/Zr implant formulations may offer superior osteoimmunomodulatory properties and improved osseointegration outcomes.

TAKE HOME MESSAGES

1. Surface **chemistry** (zirconia content) plays a greater role than hydrophilicity in early macrophage phenotype modulation
2. **M1 polarization is a necessary step for creating an osteogenic immune microenvironment**, promoting OPG, BMP2, VEGF and OPN release on all microrough surfaces.
3. Zirconia's capacity to amplify M1 activation while maintaining sufficient M2 markers supports a **balanced macrophagic response** favorable to bone regeneration.

TERMIS EU-Chapter 2026 PALMA DE MALLORCA 21-24 APRIL / SPAIN

Funding: This work was supported by the ITI Foundation (Large Grant n. 1535_2020, PI Prof. Simone Lumetti)

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