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Effects of titanium surface roughness and wettability on immune and stromal cell responses: a systematic review protocol and meta-analysis of in vitro studies

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ADMINISTRATIVE INFORMATION

Support - No specific financial support was received for this review.

Review Stage at time of this submission - Formal screening of search results against eligibility criteria.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 26 September 2026 and was last updated on 26 September 2026.

INTRODUCTION

Review question / Objective The aim of this systematic review is to determine how quantitatively characterized titanium surface properties, particularly surface roughness and wettability/contact angle, influence in vitro immune and stromal/osteogenic cellular responses, and to evaluate whether these physicochemical parameters explain variation in biological effect sizes across studies.

Primary review question: Among mammalian immune and stromal/osteogenic cells cultured directly on titanium or titanium-alloy substrates, how are quantitative surface roughness and wettability/contact angle associated with immunoinflammatory and stromal/osteogenic cellular responses?

The review will address two prespecified biological outcome domains rather than assigning one cell lineage as intrinsically primary and the other as secondary:

• Outcome domain 1 – Immunoinflammatory and immunomodulatory responses of immune cells.
• Outcome domain 2 – Stromal and osteogenic responses of stromal/osteogenic cells.

Individual biomarkers and assay-specific outcomes will be synthesized separately whenever sufficient comparable data are available.

Population (P): Mammalian immune cells (e.g., macrophages and monocytes) and stromal/osteogenic cells (e.g., mesenchymal stromal cells, osteoblasts or osteoblast-like cells, periodontal ligament stromal cells, and fibroblasts) cultured directly on titanium or titanium-alloy substrates in vitro.

Intervention/Exposure (I/E): Titanium or titanium-alloy surfaces with quantitatively characterized physicochemical properties, focusing on surface roughness (e.g., Ra, Rq, Sa, Sq) and wettability/contact angle. Surface properties may result from machining, sandblasting, acid etching, SLA treatment, laser texturing, anodization, hydrothermal treatment, alkali/chemical

modification, or other physical/chemical surface treatments that do not introduce an independent organic bioactive agent.
Comparator (C): Another titanium or titanium-alloy surface condition with quantitatively characterized surface roughness and/or wettability. A machined, polished, minimally modified, or commercially established titanium surface may serve as a reference, but an unmodified/machined titanium control is not mandatory when two or more eligible titanium surfaces can be directly compared.
Outcomes (O): Quantitative biological outcomes within the two prespecified outcome domains. Surface roughness and contact-angle measurements are exposure variables or potential moderators and are not biological outcomes.
Study design (S): Controlled in vitro experimental studies comparing two or more eligible titanium surface conditions.

Rationale Titanium and titanium-alloy implants are widely used in dental and orthopaedic applications. Surface modification techniques, particularly sandblasting, acid etching, and sandblasted large-grit acid-etched (SLA) treatment, are used to alter early host-cell interactions and subsequent tissue integration. However, nominally similar treatment categories may produce substantially different surface characteristics across laboratories and manufacturers.

Cellular behaviour at the material-cell interface may be more directly related to the physicochemical characteristics produced by surface treatment than to the treatment name alone. In particular, the quantitative relationships between surface roughness, including line-based parameters such as Ra and areal parameters such as Sa, wettability/contact angle, and in vitro immune or stromal/osteogenic cellular responses remain incompletely characterized. Existing studies also vary in cell models, exposure periods, assays, surface comparisons, and reported outcomes, making their findings difficult to interpret collectively.

This systematic review will therefore synthesize controlled in vitro evidence using quantitative surface properties as exposures and potential effect modifiers. For sufficiently comparable outcomes, Hedges' g will be synthesized using multilevel random-effects meta-analysis to account for multiple effect sizes derived from the same study. Study-level CR2 robust variance estimation with Satterthwaite-approximated degrees of freedom will be used for statistical inference. If sufficient independent studies and adequate variation in surface parameters are available, meta-regression will explore whether Ra, Sa, or contact angle explains variation in biological effect sizes. Outcomes unsuitable for quantitative pooling will be summarized using structured tables and narrative synthesis.

Condition being studied This review examines in vitro cellular responses relevant to the biological integration of titanium and titanium-alloy implants. The condition of interest is the inflammatory, immunomodulatory, stromal, and osteogenic response occurring at the titanium-cell interface during early peri-implant healing and osseointegration.

The review includes mammalian immune cells, such as macrophages and monocytes, and stromal/osteogenic cells, such as mesenchymal stromal cells, osteoblasts, osteoblast-like cells, periodontal ligament stromal cells, and fibroblasts. Surface roughness and wettability/contact angle are considered material exposures or potential effect modifiers rather than health outcomes. The included evidence is derived from controlled in vitro studies of titanium or titanium-alloy surfaces, particularly SLA, sandblasted, and acid-etched surfaces. This review does not directly assess clinical peri-implantitis, implant failure, or patient-level outcomes.

METHODS

Search strategy PubMed, Web of Science, Google Scholar were searched from database inception using the following core search strategy:

(titanium OR Ti OR Ti-6Al-4V) AND (SLA OR sandblasted OR sandblasting OR "acid etching" OR acid-etched) AND (inflammation OR inflammatory OR cytokine OR immune OR macrophage OR M1 OR M2 OR immunomodulation OR integrin)

The syntax was adapted as necessary for each database without changing the main search concepts. Google Scholar was used as a supplementary source for citation searching and identifying additional eligible studies. The reference lists of included studies and relevant reviews were also screened manually. Only English-language, peer-reviewed full-text journal articles were considered. Literature searching began in August 2026, and an updated search will be conducted before completion of the review, anticipated in December 2026.

Participant or population Primary cells or immortalized cell lines of human or other

mammalian origin relevant to implantology and peri-implant healing.
Immune cells, including macrophages and monocytes (e.g., THP-1-derived macrophages, RAW 264.7 cells, primary bone-marrow-derived macrophages, primary human monocytes/macrophages).
Stromal and musculoskeletal/bone-related cells, including osteoblasts/osteoblast-like cells (e.g., MG-63, MC3T3-E1), mesenchymal stem/stromal cells, periodontal ligament stromal/stem cells, and fibroblasts.
Cells must be cultured directly on titanium or titanium-alloy substrates for the exposure of interest.

Intervention Titanium or titanium-alloy surfaces with quantitatively characterized physicochemical properties, particularly surface roughness (e.g., Ra, Rq, Sa, or Sq) and wettability/contact angle. These surface properties may result from machining, sandblasting, acid etching, sandblasted large-grit acid-etched (SLA) treatment, laser texturing, anodization, hydrothermal treatment, alkali/chemical modification, or other physical or chemical surface treatments that do not introduce an independent organic bioactive agent. Eligible cells must be cultured directly on the investigated titanium or titanium-alloy surfaces.

Comparator Another titanium or titanium-alloy surface condition with quantitatively characterized surface roughness and/or wettability. A machined, polished, minimally modified, or commercially established titanium surface may serve as a reference, but an unmodified/machined titanium control is not mandatory when two or more eligible titanium surfaces can be directly compared.

Study designs to be included Included studies must be controlled in vitro experiments with at least two titanium or titanium-alloy surface conditions and must report at least one quantifiable eligible surface parameter (surface roughness and/or wettability/contact angle) together with at least one eligible quantitative cellular outcome.

Eligibility criteria Cell models – included
• Primary cells or immortalized cell lines of human or other mammalian origin relevant to implantology and peri-implant healing.
• Immune cells, including macrophages and monocytes (e.g., THP-1-derived macrophages, RAW 264.7 cells, primary bone-marrow-derived macrophages, primary human monocytes/macrophages).
• Stromal and musculoskeletal/bone-related cells, including osteoblasts/osteoblast-like cells (e.g., MG-63, MC3T3-E1), mesenchymal stem/stromal cells, periodontal ligament stromal/stem cells, and fibroblasts.
• Cells must be cultured directly on titanium or titanium-alloy substrates for the exposure of interest.

Cell models – excluded
• Live-animal in vivo studies and human clinical studies.
• Non-mammalian cell lines.
• Cell-free models or studies limited to acellular protein adsorption without cell culture.
• Studies in which the relevant cell response is measured only on non-titanium substrates and titanium-specific data cannot be isolated.

Surface interventions/exposures – included
• Commercially pure titanium or titanium alloys (e.g., Ti-6Al-4V), including discs, plates, or comparable substrates suitable for direct cell culture.
• Any physical or inorganic/chemical surface modification technique, provided the resulting surface roughness and/or wettability is quantitatively characterized and the treatment does not introduce an independent organic bioactive agent.
• At least one quantifiable roughness parameter (e.g., Ra, Rq, Sa, Sq) and/or contact-angle/wettability measurement must be reported.
• Ra/Rq (line-based roughness) and Sa/Sq (areal roughness) will be treated as distinct measurement families and will not be pooled as interchangeable measures without a prespecified and defensible harmonization method.
• Contact-angle measurements will be interpreted in relation to measurement method, testing liquid, and timing; substantially different wettability assessment methods will be analyzed separately when appropriate.
Surface interventions/exposures – excluded
• Non-titanium metallic substrates or non-metallic substrates when titanium-specific eligible groups cannot be isolated.
• Titanium surfaces carrying organic bioactive coatings or added agents such as growth factors, peptides, drugs, extracellular matrix components, or living-cell coatings that introduce an independent organic bioactive agent.
• Studies that provide no quantitative measurement of surface roughness or wettability/contact angle.

Comparator rules
Any eligible titanium surface may serve as the comparator if the comparison involves two or more

titanium surface conditions with quantitatively characterized surface properties. Machined/polished titanium is preferred as a conventional reference when available, but it is not required for study eligibility. TCPS or glass may be reported as background/reference conditions but will not substitute for an eligible titanium-to-titanium comparison in the principal synthesis.

Other selection criteria
Reports will be excluded if they are reviews, systematic reviews, meta-analyses, book chapters, conference abstracts, editorials, letters, commentaries, or non-peer-reviewed preprints. Duplicate reports from the same experiment will be linked and the most complete source used for each outcome; complementary data from multiple reports may be combined when clearly attributable to the same experiment.
Insufficient reporting of mean, dispersion, or sample size will not by itself exclude an otherwise eligible study from the systematic review. Such studies may remain eligible for narrative synthesis but may be excluded from a particular quantitative meta-analysis if an effect size and sampling variance cannot be reconstructed after reasonable attempts to obtain or derive the missing data.

Information sources The primary bibliographic databases will include PubMed and Web of Science. Google Scholar may be used as a supplementary source for citation searching and identification of additional eligible reports. Reference lists of included studies and relevant reviews will also be screened.
Search period: From database inception to the final search date. Literature searching began on August 2026, and review completion is anticipated in December 2026. An update search should be performed before final manuscript submission if a substantial interval has elapsed.
Language: English-language publications.
Publication status: Peer-reviewed full-text journal articles. Conference abstracts, editorials, commentaries, non-peer-reviewed preprints, and dissertations/theses without peer review will not be included in quantitative synthesis.

Main outcome(s) Outcome domain 1 – Immunoinflammatory and immunomodulatory responses
• Individual pro-inflammatory and anti-inflammatory cytokines or chemokines (e.g., TNF-α, IL-1β, IL-6, IL-10, TGF-β), measured quantitatively by ELISA, multiplex protein assays, other validated protein assays, or quantitative gene-expression methods.
• Macrophage polarization/phenotype markers, including M1-associated markers (e.g., CD86, iNOS/NOS2, TNF, IL1B) and M2-associated markers (e.g., CD206/MRC1, ARG1, IL10), measured by quantitative gene expression, protein assays, flow cytometry, or other quantitative phenotyping methods.
Different biomarkers will not be pooled simply because they belong to the same biological pathway or inflammatory phenotype. Meta-analysis will be conducted outcome-by-outcome when data are sufficiently comparable.
Outcome domain 2 – Stromal and osteogenic responses
• Osteogenic differentiation markers such as RUNX2, OCN/BGLAP, OPN/SPP1, and related prespecified markers, analyzed separately by biomarker and assay type where appropriate.
• ALP activity and ALP gene/protein expression, treated as distinct outcome types when necessary.
• Extracellular matrix mineralization, including quantitative Alizarin Red or comparable mineral deposition measurements.
• Cell-adhesion-related outcomes, including integrin expression and adhesion-signaling markers such as integrin β1, integrin β3, and FAK, where quantitatively reported.

Data management Study selection
Two reviewers will independently conduct title/abstract screening and full-text eligibility assessment using prespecified criteria. Records will be deduplicated in reference-management and/or systematic-review software. Reasons for exclusion at full-text stage will be documented, and the study-selection process will be reported in a PRISMA flow diagram. Disagreements will be resolved by discussion and re-examination of the source; if consensus cannot be reached, a third reviewer should adjudicate.
Prioritized exclusion criteria
Title/abstract screening:
• Ineligible publication/research type.
• Clearly unrelated topic.
• Ineligible substrate/material with no eligible titanium group.
• No indication of a controlled in vitro titanium-surface comparison.
Full-text screening:
• No eligible titanium-to-titanium surface comparison.
• No quantitative eligible surface roughness or wettability/contact-angle measurement.
• Surface treatment introduces an independent organic bioactive agent, including an organic, drug, or biologic coating.
• No eligible quantitative cellular outcome.

• Titanium-specific data cannot be isolated from mixed materials/conditions.

Data extraction

Two reviewers will independently extract data using a standardized, piloted form. Numerical values presented only in graphs may be digitized using WebPlotDigitizer or equivalent software. When essential quantitative information is unclear or missing, corresponding authors may be contacted up to two times over approximately two weeks.

The following information will be extracted where available:

• Bibliographic information, country/institution, funding, and conflicts of interest.

• Titanium material/alloy, specimen geometry, and surface modification method.

• Surface roughness metrics (Ra, Rq, Sa, Sq), units, and measurement technique.

• Contact angle/wettability, testing liquid, measurement method, timing, and surface energy if reported.

• Cell species, source, cell line, primary vs immortalized status, culture conditions, and exposure/incubation duration.

• Number of experimental groups, statistical unit, independent biological/experimental replicates, technical replicates, and reported sample size.

• For each eligible outcome: mean, SD (or convertible measure of dispersion), n, assay/method, time point, and any normalization performed by the original investigators.

Quality assessment / Risk of bias analysis Risk of bias and methodological quality will be assessed independently by two reviewers using the RoBDEMAT tool (Risk of Bias tool for pre-clinical dental materials research) according to the original tool instructions and signaling questions. The assessment will not be replaced by an ad hoc Cochrane-style domain structure. Any adaptations required for this specific in vitro cell-culture context will be prespecified and transparently reported rather than presented as the original RoBDEMAT instrument.

Reference: Delgado AHS, Sauro S, Lima AF, et al. RoBDEMAT: a risk of bias tool and guideline to support reporting of pre-clinical dental materials research and assessment of systematic reviews. Journal of Dentistry. 2022;127:104350.

Strategy of data synthesis General approach

Both quantitative synthesis and structured narrative synthesis are planned. Quantitative pooling will be considered when at least three independent studies provide sufficiently comparable biological outcomes, exposure definitions, and extractable effect-size data. When quantitative pooling is not appropriate because of clinical/methodological heterogeneity or insufficient data, findings will be summarized narratively using structured tables and effect-direction displays.

Biological outcomes will be stratified by cell lineage and individual biomarker/outcome. Immune-cell and stromal/osteogenic-cell results will not be pooled together. Different biomarkers will not be combined merely by standardizing their measurement scales.

Effect measures

Primary effect measure: Hedges' g (standardized mean difference with small-sample correction) will be calculated from group means, standard deviations, and sample sizes for comparable continuous outcomes. Pooled effects will be reported with 95% confidence intervals using the CR2 and Satterthwaite approach described below. Hedges' g will not be used to justify pooling biologically distinct biomarkers or outcomes.

If an original study reports only normalized fold-change values, those data will be used only when the corresponding dispersion and sample size are reported or can be validly reconstructed. A fold-change mean alone without information needed to estimate sampling variance will not be treated as sufficient for inverse-variance meta-analysis.

Effect model

Multilevel random-effects meta-analysis will be the primary synthesis model because true effects are expected to vary across cell models, surface-processing methods, incubation periods, and assay protocols. Variance components will be estimated using restricted maximum likelihood (REML). Cluster-robust variance estimation with the CR2 small-sample correction will be applied at the study level. Pooled estimates will be presented with 95% confidence intervals, and confidence intervals and two-sided tests will use a t distribution with Satterthwaite-approximated degrees of freedom. The same approach will be used for meta-regression coefficients when estimable. The exact software and packages will be reported in the final review.

Heterogeneity

Statistical heterogeneity will be evaluated using Cochran's Q and I^2, together with the estimated between-study variance (τ^2) when available. A Q-test p value <0.10 will be interpreted as evidence of statistically detectable heterogeneity, not as proof that heterogeneity is necessarily "substantial." I^2 will be used descriptively to quantify inconsistency; interpretation will consider the number and size of studies, the magnitude/direction of effects, and methodological differences. Prediction intervals may be reported

when sufficiently supported by the number of studies.
Meta-regression
Meta-regression will generally be undertaken only when there are at least 10 independent studies/ effect clusters for a given outcome–moderator analysis and adequate variation in the moderator.
Multiple surfaces, shared controls, and dependent effect sizes
When one study includes multiple eligible surface groups sharing a common comparator, effect-size dependency will be addressed using multilevel meta-analysis with study-level CR2 cluster-robust variance estimation and Satterthwaite-approximated degrees of freedom. The selected within-study covariance assumptions will be reported for each synthesis, and multiple comparisons from the same experiment will not be treated as fully independent observations.

Subgroup analysis Cell lineage/type: immune cells vs stromal/osteogenic cells; analyses will usually be performed separately rather than directly pooled.
Cell origin: human primary cells vs other mammalian primary cells vs immortalized cell lines, where meaningful.
Roughness measurement family: Ra/Rq vs Sa/Sq; values from different measurement families will not be treated as interchangeable.
Contact-angle method and testing liquid.
Culture/exposure duration or time point.
Surface modification class (e.g., SLA, anodization, sandblasting, acid etching, laser/nanostructuring) as a categorical descriptor, while recognizing that the primary exposure concept is the resulting quantitative surface property.

Sensitivity analysis Leave-one-study-out analysis when the number of studies permits meaningful interpretation.
Exclusion of studies judged at high risk of bias or with important methodological limitations.
Exclusion of studies requiring extensive data reconstruction, figure digitization, or statistical imputation.
Sensitivity analyses will retain Hedges' g as the effect measure and use the multilevel model with study-level CR2 correction and Satterthwaite-approximated degrees of freedom where estimable. Model non-convergence or non-estimable robust inference will be reported.

Language restriction Only studies published in English will be included.

Country(ies) involved Taiwan.

Keywords Titanium implants; titanium alloy; surface modification; surface roughness; Ra; Sa; contact angle; wettability; immunomodulation; inflammatory response; macrophage polarization; osteogenesis; stromal.

Dissemination plans The findings of this systematic review and meta-analysis will be submitted for publication in a peer-reviewed scientific journal.

Contributions of each author
Author 1 - Ting-Yi Su - Author 1 contributed to the study conception and design, protocol development, literature searching, study selection, data extraction, statistical analysis, and drafting of the manuscript.
Author 2 - Ting-Han Chang - Author 2 contributed to protocol development, independent study selection, data extraction, risk-of-bias assessment, data verification, and critical revision of the manuscript.
Author 3 - Da-Yo Yuh - Author 3 supervised the review, provided methodological guidance, adjudicated disagreements when necessary, and critically revised the manuscript. All authors reviewed and approved the final protocol and registration information.