



Cite this: *J. Mater. Chem. B*,
2024, 12, 8778

Combining sandblasting and pink anodisation of Ti implants as a promising method for improving fibroblast adhesion and immune response†

Carlos Arias-Mainer,  ^a Francisco Romero-Gavilán,  ^a Andreia Cerqueira,  ^a
David Peñarocha-Oltra, ^b Juan Carlos Bernabeu-Mira, ^b Félix Elortza, ^c
Mikel Azkargorta, ^c Mariló Gurruchaga,  ^d Isabel Goñi^d and Julio Suay^a

This study examined the effect of combining the sandblasting and anodising of titanium alloys used in implants on the cell response and protein adsorption patterns. The titanium samples were divided into four groups depending on the surface treatment: machining (MC), pink anodisation (PA), sandblasting (MC04) and a combination of the last two (MC04 + PA). Their physicochemical properties were analysed by SEM/EDX, Raman, contact angle measurements and profilometry. *In vitro* responses were examined using human gingival fibroblastic (HGF) cells and THP-1 macrophages. Cytokine secretion, macrophage adhesion and gene expression were measured by ELISA, confocal microscopy and RT-PCR. Cell adhesion and collagen secretion were evaluated in HGF cultures. The adsorption of immune and regenerative proteins onto the surfaces was assessed employing *n*LC-MS/MS. MC04 + PA surfaces exhibited a change in the roughness, chemical composition and hydrophilicity of the material, showing more elongated HGF cells and a considerable increase in the area of cells exposed to the MC04 + PA surfaces. Moreover, cells cultured on MC04 + PA generally showed a reduction in the expression of proinflammatory genes (TNF- α , MCP-1, C5, NF- κ B and ICAM-1) and an increase in the secretion of anti-inflammatory cytokines, such as IL-4. These results correlated with the proteomic data; we found preferential adsorption of proteins favouring cell adhesion, such as DSC1 and PCOC1. A considerable reduction in the adsorption of immunoglobulins and proteins associated with acute inflammatory response (including SAA4) was also observed. The study highlights the potential advantages of MC04 + PA surface treatment to modify dental implant abutments; it enhances their compatibility with soft tissues and reduces the inflammatory response.

Received 8th May 2024,
Accepted 2nd August 2024

DOI: 10.1039/d4tb00992d

rsc.li/materials-b

1. Introduction

Dental implants are the commonly used option to compensate for tooth loss. The method has a high success rate; however, the treatment has to face one of the biggest challenges in the field of oral health, *i.e.*, prevention of complications after implant placement.¹ The success of the procedure relies not only on

osseointegration but also on the integration of implant abutment into the soft tissue.² It is necessary to improve both hard- and soft-tissue interactions with implants to avoid complications, such as periimplantitis, that can lead to tissue damage and implant rejection.³

Titanium (Ti) is commonly used in medicine and orthodontics due to its mechanical and anticorrosive properties and its excellent biocompatibility.⁴ Some of the main reasons for dental implant failures are *peri-implant* issues,⁵ caused by poor integration between the soft tissues and the insert.⁶ Lack of appropriate barrier formation means that pathogens can enter easily, leading to diseases such as periimplantitis.⁷ This disease is caused by bacterial colonisation on dental implants, primarily by the bacterium *Porphyromonas gingivalis*. The infection is characterized by the inflammation of the connective tissue surrounding the implant and a progressive loss of the supporting bone.⁸

The gingiva directional attachment to the tooth root results in the formation of epithelial and connective tissues that serve

^a Department of Industrial Systems Engineering and Design, Universitat Jaume I, Castellón de la Plana, Spain. E-mail: carias@uji.es

^b Department of Stomatology, Valencia University Medical and Dental School, Valencia, Spain

^c 3Proteomics Platform, CIC bioGUNE, Basque Research and Technology Alliance (BRTA), CIBERehd, ProteoRed-ISCIII, Bizkaia Science and Technology Park, Derio, Spain

^d Department of Polymers and Advanced Materials: Physics, Chemistry and Technology, Universidad del País Vasco, San Sebastián, Spain

† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d4tb00992d>

as a barrier against the penetration of oral bacteria.⁹ The soft tissue is mainly composed of human gingival fibroblasts (HGF),¹⁰ the cells most critical in implant–soft tissue integration and *peri* implant mucosa healing.¹¹ However, after implantation, the newly regenerated tissue forms disordered collagen fibres parallel to the implant surface. This structural aberration weakens the tissue seal, leaving the area vulnerable to bacterial infiltration and potential *peri*-implant issues.¹² To improve the seal between the implant and connective tissues, it is imperative to produce enhanced Ti surfaces, promoting the secretion and appropriate alignment of collagen fibres. Thus, the newly developed Ti surface treatments attempt to overcome these limitations, *i.e.*, to increase the effectiveness of the integration of the abutment and the surrounding *peri*-implant tissue. These treatments range from acid-etching and alkali-heat treatment to anodising, roughening and sandblasting.¹³ Some studies have reported that surfaces with high roughness increase fibroblast proliferation and adhesion.¹⁴ Moreover, the anodisation of Ti alloys improves the proliferation of human gingival epithelial cells (HGEPP) while preserving the physicochemical properties supporting fibroblast proliferation and collagen-I production.¹⁵ Anodised samples¹⁶ show improved integration and aesthetics. Such materials also effectively reduce the expression of pro-inflammatory cytokines such as monocyte chemoattractant protein 1 (MCP-1) and tumour necrosis factor α (TNF- α).¹⁷

When an implant comes in contact with tissues, one of the first events is protein adsorption onto its surface. The biological response to the implant can be determined by the characteristics of the protein layer formed on it.¹⁸ Changing the properties of the biomaterial surface can lead to changes in protein adsorption and, consequently, in cell responses, such as cell adhesion, proliferation, differentiation and immune reaction.¹⁹ Thus, studying the protein layer deposited on implant surfaces could help in understanding the body response.

This article presents a study of the combined effects of sandblasting and pink anodising surface treatments of titanium surfaces on soft tissue regeneration. The changes in the abutment surface roughness, wettability, morphology and chemical composition and their impact on HGF cytoskeleton arrangement, collagen secretion and macrophage immune responses *in vitro* will be examined. Moreover, the correlation between these results and the protein adsorption onto the developed surfaces will be analysed.

2. Materials and methods

2.1. Sample preparation

Grade V Titanium discs (6-mm diameter; Nueva Galimplant S.L., Lugo, Spain) were used in the study. All samples (MC, PA, MC04 and MC04 + PA) were initially machined. Once the disks were machined on a CNC lathe (CNC Machines; Winter Springs, Florida, USA), the fabrication process for the MC group was completed. The MC04 samples were then subjected to sandblasting with aluminium oxide particles (4- μ m). The discs were loaded into the sample holder, which was based on a

rotating carousel with six sample holder satellites. Inside the sandblasting cabin, there were three blasting stations: two stations with a single nozzle each, and a central station with two fixed nozzles. All nozzles were positioned 8 cm from the sample, each oriented to cover different sample sections to ensure uniform vertical blasting. At each sandblasting station, the sample was blasted for 3.6 seconds, with pressure gradually decreasing from 7.4 bar to 4 bar. During this process, the sample holder rotated to ensure uniform blasting around the entire perimeter of the sample, resulting in rough surfaces. Additionally, after the sandblasting process a soft acid pickling of the surface is carried out to remove any traces of Al₂O₃ that may have been embedded in the surface. The PA discs were anodised following the established procedures. Initially, surface cleaning was carried out, eliminating the natural layer of oxide and subsequently immersing the disc in an electrolytic cell containing a solution based on phosphoric acid, generating a pink anodised surface. Finally, to obtain the MC04 + PA samples, MC discs were sandblasted and anodised as described previously, giving the combination of the two treatments. The MC samples were used as references, and the individual treatments (PA and MC04) were employed as controls. Discs were sterilised using UV radiation before use.

2.2. Physicochemical characterisation

The effects of machining, anodising, sandblasting and the combined effect of anodising and sandblasting on surface topography were examined using a scanning electron microscope (SEM; Leica-Zeiss LEO, Leica, Wetzlar, Germany) under vacuum conditions coupled to an Oxford INCA 250 energy dispersive X-ray spectrometer. Before analysis, the materials were pretreated by platinum sputtering to improve conductivity. The surface roughness was determined using an optical profilometer (interferometric and confocal) PLm2300 (Sensofar, Barcelona, Spain). The experiments were performed in triplicate for each condition, and the results were reported as the arithmetic average roughness parameter (R_a ; μ m). The wettability was analysed after contact angle measurements using an Ossila Contact Angle Goniometer (Ossila Ltd, Sheffield, UK). A drop of 5 μ L of ultrapure water was deposited on each of the samples, and pictures were taken after 20 s. Five discs of each surface type were studied, MC04 + PA samples. Finally, Raman spectra were acquired using a Raman Confocal Microscope Apyron (WITec, Ulm, Germany) equipped with a 532 nm incident laser at 10 mW. The measurements were conducted with an integration time of 2 s per point. The area scanning method was employed, consisting of 20 points per line and 20 lines per image. Each point underwent 30 accumulations.

2.3. *In vitro* characterisation

2.3.1. Cell culture. Human gingival fibroblasts (HGF; LGC Standards, Barcelona, Spain) were cultured on the discs placed in 96 well NUNC plates (Thermo Fisher Scientific, Waltham, MA, US). The cells were cultured in Fibroblast Basal Medium (ATCC, American Type Culture Collection; Manassas, VA, USA) and supplemented with Fibroblast Growth Kit-Low Serum (ATCC) and 1% penicillin/streptomycin (ATCC). The HGF cell

medium was replaced every two days. The human monocytic cell line (THP-1; ECACC, Public Health England, Porton Down, Salisbury, UK) was employed for the analysis of the inflammatory response. THP-1 cells were cultured on the samples in 96-well NUNC plates using Roswell Park Memorial Institute medium (RPMI; Gibco, Life Technologies, Grand Island, NY, USA) supplemented with 1% penicillin/streptomycin and 10% FBS (Merck, Waltham, MA, USA). THP-1 cells were differentiated into macrophages by treating them with phorbol 12 myristate-13-acetate, 50 ng mL⁻¹ (PMA; Merck). Cultures were performed at 37 °C in a 90% humidified atmosphere and 5% CO₂ flow rate. The wells without biomaterials served as blanks.

2.3.2. Cytokine quantification. For the evaluation of the inflammatory response of macrophages, the secretion of TNF- α , transforming growth factor β (TGF- β) and interleukin 4 (IL-4) cytokines was measured. THP-1 cells were seeded on the samples in 96-well NUNC plates at a density of 3.0×10^5 cells cm⁻² and 1.5×10^5 cells cm⁻² and cultured for 1 and 3 days, respectively. After culture, the medium was collected and stored at -80 °C until analysis. Finally, TNF- α , TGF- β , and IL-4 concentrations were determined using ELISA (Invitrogen, Thermo Fisher Scientific), according to the manufacturer's instructions. Absorbance was measured at 450 nm in a Multiskan FC microplate reader (Thermo Fisher Scientific). Experiments were carried out in triplicate.

2.3.3. RNA extraction, cDNA synthesis and quantitative real-time PCR measurements. After 1 and 3 days, total RNA of THP-1 cells cultured on the tested surfaces was extracted by using TRIzol (1 M guanidine thiocyanate, 1 M ammonium thiocyanate, 3 M sodium acetate, 5% glycerol, 38% AquaPhenol). Aliquots of 300 μ L of TRIzol were added to each sample, RNA was released by scraping briefly, and samples were incubated at room temperature for 5 min. Then, 250 μ L of chloroform (CHCl₃) was added, and the samples were incubated at room temperature for 5 min and centrifugated (5 min, 10 000 rpm, 4 °C). The top layer (aqueous phase) was mixed with 550 μ L of isopropanol and incubated at room temperature for 10 min. Samples were centrifugated (20 min, 14 000 rpm, 4 °C), washed twice with 0.5 mL of 70% EtOH and air-dried. Finally, the resulting pellet was dissolved in 20 μ L of RNase-free water and frozen at -80 °C. RNA concentration and quality were measured with a NanoPhotometer N50 (Implen, Westlake Village, CA, USA). Approximately 1 μ g of total RNA was converted into cDNA using the PrimeScript RT Reagent Kit (Perfect Real Time; TAKARA Bio Inc., Shiga, Japan) in a reaction volume of 20 μ L. The reaction was conducted under the following conditions: 37 °C for 15 min, 85 °C for 5 s and a final hold at 4 °C. The resulting cDNA quality and concentration were measured using a NanoPhotometer N50; the sample was diluted in DNase-free water to a concentration suitable for reliable qRT-PCR analysis and stored at -80 °C until further analysis.

Quantitative real-time PCRs (qRT-PCR) were carried out in 96-well plates (Applied Biosystems[®], Thermo Fisher Scientific); the genes of interest were analysed for each sample using housekeeping gene (glyceraldehyde phosphate dehydrogenase (GAPDH)) as a reference. Primers for each gene were designed using the PRIMER3plus software tool (<https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>); specific DNA

sequences were obtained from NCBI (<https://www.ncbi.nlm.nih.gov/nucleotide/>) and purchased from Thermo Fischer Scientific. Specific primers for each gene are shown in Table S1 (ESI[†]). Individual reactions contained 1 μ L of cDNA, 0.2 μ L of specific primers (forward and reverse at a concentration of 10 μ M L⁻¹) and 5 μ L of SYBR Premix Ex Taq (Tli RNase H Plus; TAKARA) at a final volume of 10 μ L, in autoclaved distilled water. Reactions were carried out in a StepOne Plus[™]Real-Time PCR System (AppliedBiosystems[®], Thermo Fisher Scientific) at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, 60 °C for 34 s, 95 °C for 15 s and 60 °C for 60 s. The data were obtained using the StepOne Plus[™]Software 2.3 (AppliedBiosystems[®], Thermo Fisher Scientific). Fold changes were calculated using the 2^{- $\Delta\Delta C_t$} method, and the data were normalised to the blank wells (without any biomaterials). Six technical replicates for each sample were analysed. The expression levels of selected genes, TNF- α , MCP-1, interleukin 10 (IL-10), intercellular adhesion molecule 1 (ICAM-1), nuclear factor kappa B (NF- κ B) and complement component 5 (C5) were measured.

2.3.4. Macrophage adhesion. To observe THP-1 macrophage adhesion, the number of macrophages adhering to samples was measured. Cells were seeded at densities of 3.0×10^5 and 1.5×10^5 cells per cm² and cultured for 1 and 3 days, respectively. After the culture period, samples were washed with PBS and fixed with 4% PFA (Merck) for 10 min at room temperature. Macrophages were then permeabilized with 0.1% Triton X-100 (Merck) and incubated with (5% BSA) PBS for 2 hours. Following two PBS washes, nuclei were stained by incubating the samples in DAPI (Merck) for 5 min and washed twice. Images were captured using LAS X software (Leica), and nuclei counts were obtained using ImageJ software (National Institutes of Health, Maryland, USA).

2.3.5. Cytoskeleton arrangement. To examine the cytoskeleton arrangement, HGF cells were seeded on the biomaterial samples at a density of 10×10^3 cells cm⁻² and cultured for 1 and 3 days. Then, the samples were washed with PBS and fixed with 4% PFA (Merck) for 20 min at room temperature. The HGFs were permeabilised with 0.1% Triton X-100 and incubated for 1 h with phalloidin (1:100; Abcam, Cambridge, UK) diluted in 0.1% w/v bovine serum albumin (BSA)-PBS. After two washes with PBS, the nuclei were stained by incubating the samples in DAPI with a mounting medium. A Leica TCS SP8 Confocal Laser Scanning Microscope was employed for fluorescence detection. The images were acquired using LAS X software (Leica) and cell areas were obtained at day 1 employing Image J software.

2.3.6. Collagen secretion. Collagen secretion by HGFs was analysed using Sirius Red (SR) in a saturated picric acid solution (Merck). The cells were seeded on the evaluated surfaces in 96-well plates at densities of 20×10^3 , 10×10^3 and 5×10^3 cells cm⁻², to be cultured for 1, 3 and 7 days, respectively. Then, they were fixed with 4% PFA (Merck) for 20 min, washed with PBS and incubated in SR solution for 24 h. To remove the excess SR dye, the samples were washed with water and, finally, treated with 1 M NaOH (Merck). Absorbance

was measured at 570 nm in a Multiskan FC microplate reader (Thermo Fisher Scientific). The experiments were carried out in triplicate.

2.3.7. Protein layer elution. To analyse the adsorbed protein layer, the material samples were incubated in 200 μ L of human serum (Male AB plasma; Merck) for 3 hours in a cell incubator (37 $^{\circ}$ C, 5% CO₂, 90% relative humidity). To remove non-adsorbed proteins, the samples were washed five times with ddH₂O and once with washing buffer (100 mM NaCl, 50 mM Tris-HCl, pH 7). The adsorbed proteins were eluted with 100 μ L of lysis buffer (2 M thiourea, 7 M urea, 4% CHAPS and 200 mM dithiothreitol). Four independent replicates, with each replicate containing a pool of four discs were used for elution. All reagents were purchased from Merck.

2.3.8. nLC-MS/MS measurements and bioinformatics analysis. An Evosep ONE chromatograph (Evosep, Odense C, Denmark) coupled to a hybrid trapped ion mobility-quadrupole time of flight mass spectrometer (timsTOF Pro with PASEF; Bruker, Billerica, MA, USA) was employed for the analysis of eluted protein layer, as described in Romero-Gavilán *et al.*²⁰ Each profile was analysed in quadruplicate. Proteins were identified employing the MaxQuant software (<https://maxquant.org/>). Differential analysis (comparing MC04 + PA with PA and MC04) was performed using the Perseus platform (<https://maxquant.org/perseus/>). Functional analyses of differential proteins were carried out using PANTHER (<https://www.pantherdb.org/>) and the UniProt database (<https://www.uniprot.org/>). The UniProt ID nomenclature of proteins was employed.

2.3.9. Statistical analysis. The effects of MC04 + PA treatment *versus* the individual treatments (PA and MC04) used as

controls were analysed with GraphPad Prism[®] software version 5.04 (Graphpad Software Inc., La Jolla, CA, USA). The method employed was one-way variance analysis (ANOVA) with Tukey's *post hoc* test. Differences were considered statistically significant at $p < 0.05$. In the figures, the asterisks (****, $p < 0.0001$. ***, $p < 0.001$. **, $p < 0.01$. *, $p < 0.1$.) indicate statistical differences between MC and PA, MC04 and MC04 + PA. The black rhombuses (◆◆◆, $p < 0.0001$. ◆◆◆, $p < 0.001$. ◆◆, $p < 0.01$. ◆, $p < 0.1$.) indicate statistical differences between PA and MC04 + PA, and the white rhombuses (◇◇◇◇, $p < 0.0001$. ◇◇◇, $p < 0.001$. ◇◇, $p < 0.01$. ◇, $p < 0.1$.), between MC04 and MC04 + PA. Statistical differences in protein adsorption were examined using the Student's *t*-test (the Perseus platform). Proteins were considered differentially adsorbed when the difference was statistically significant ($p \leq 0.05$), and the abundance ratio between conditions was larger than 1.5 in either direction. Results are shown as means $\pm$ SD.

3. Results

3.1. Physicochemical characterisation

Fig. 1 shows the representative SEM micrographs of MC, PA, MC04 and MC04 + PA surfaces. The formation of surface grooves was induced by the machined treatment, as seen in MC samples (Fig. 1(a) and (a')). The anodising process (Fig. 1(b) and (b')) led to the formation of a TiO₂ layer, which effectively smoothed out the grooves formed during the machining. The roughness treatment of MC04 and MC04 + PA (Fig. 1(c), (c') and (d), (d')) resulted in a complete loss of the grooves observed on

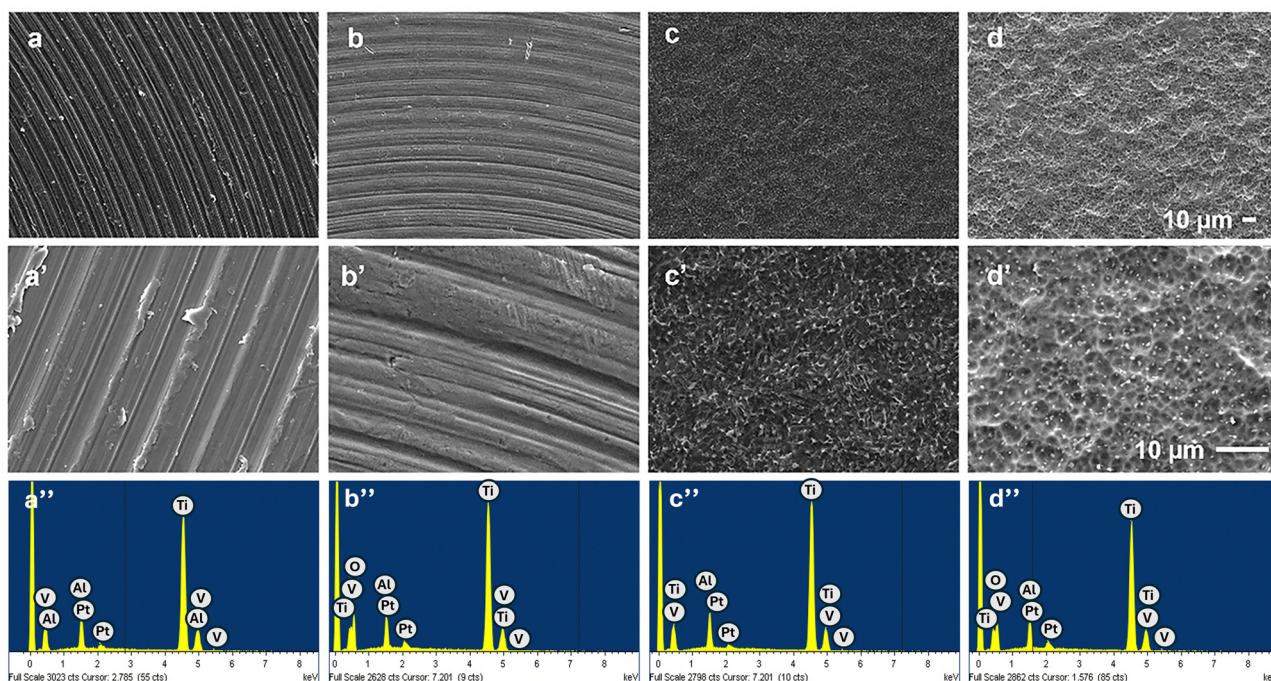


Fig. 1 SEM micrograph of MC (a) and (a'), PA (b), (b') and (b''), MC04 (c) and (c') and MC04 + PA (d) and (d') surfaces. Scale bars: 10 μ m. EDX measurements of MC (a''), PA (b''), MC04 (c'') and MC04 + PA (d'') surfaces.

MC and PA. Fig. 1(a'')–(d'') presents the EDX results, showing the characteristic peaks of Ti grade V (Ti, V, Al) for all compositions. The anodised samples (PA, MC04 + PA) exhibited the presence of oxygen, indicating the formation of a TiO_2 layer. Conversely, oxygen was not detected in the MC and MC04 samples. Sandblasting significantly increased Ra in MC04 ($0.503\ \mu\text{m}$) and MC04 + PA ($0.330\ \mu\text{m}$) in comparison to MC. However, anodisation reduced the Ra value of MC04 + PA surfaces in comparison with MC04. The Ra values for PA and MC materials did not differ significantly (Fig. 2(a)).

The wettability tests showed that MC04 was the most hydrophobic material (Fig. 2(b)), with contact angle values reaching 110° . The other surfaces displayed similar hydrophilicity, with contact angles above 80° .

The Raman spectra shown in Fig. 2(c) indicated the presence of TiO_2 in the MC and MC04 samples, showing an anatase (A) peak at $137\ \text{cm}^{-1}$. In the anodized samples (PA and MC04 + PA), higher signal intensities were observed, suggesting a thicker TiO_2 layer had formed compared to MC and MC04. For PA and MC04 + PA, Raman spectra identified four characteristic vibrational modes of A TiO_2 with the following symmetries and corresponding Raman shifts: E_g at $137\ \text{cm}^{-1}$, E_g at $197\ \text{cm}^{-1}$, B_{1g} at $399\ \text{cm}^{-1}$, and another E_g mode at $639\ \text{cm}^{-1}$.²¹ Also, peaks at $447\ \text{cm}^{-1}$, $612\ \text{cm}^{-1}$ with E_g and A_{1g} symmetries respectively were observed, indicating the presence of a Rutile (R) phase.²²

3.2. In vitro characterisation

3.2.1. Cytoskeleton arrangement and collagen secretion.

As can be seen in Fig. 3(a)–(h) and (a')–(h'), the HGF cells adapted to the morphological shapes of the material. On the MC and PA surfaces, the cells followed the circular groove patterns formed during machining (Fig. 3(a), (a'), (e) (e'), (b), (b') and (f), (f')). Cell area measurements (Fig. 3(i)) showed a decreased HGF area on MC04 surfaces compared with MC after 1 day of culture. In contrast, on MC04 + PA, a significant increase in the cell area was observed after 1 day compared to the individual PA and MC04 treatments. Fig. 3(j) displays the HGF collagen secretion levels after 1, 3 and 7 days of culture.

Cells cultured on MC04 + PA for 1 day showed a higher secretion of collagen than those cultured on MC.

3.2.2. Immune response evaluation. To evaluate the inflammatory potential of the MC04 + PA in comparison with individual treatments, the secretion of proinflammatory and anti-inflammatory cytokines was measured after 1 and 3 days using ELISA (Fig. 4). Anodising decreased the secretion of $\text{TNF-}\alpha$ in cultures on PA (compared to MC) after 1 day, while no differences were found between the MC04 + PA and MC (Fig. 4(a)). Anodising did not affect the $\text{TGF-}\beta$ (Fig. 4(b)) and IL-4 levels (Fig. 4(c)) for any of the compositions. In contrast, an increase in IL-4 levels was observed for MC04 + PA compared to MC after 1 and 3 days of incubation (Fig. 4(c)). Sandblasting reduced $\text{TNF-}\alpha$ secretion in MC04 cultures compared to MC (after 1 day). A significant increase in the IL-4 levels was observed after 1 and 3 days for MC04 and MC04 + PA surfaces in comparison with MC (Fig. 4(c)). The $\text{TGF-}\beta$ secretion was not affected by culturing the cells with sandblasted materials (Fig. 4(b)). To test the influence of material properties on macrophage adhesion, a study was conducted at 1 and 3 days. All samples had similar levels of macrophage adhesion except for MC04 (Fig. 4(e)), which exhibited a significant reduction in the number of adherent THP-1 macrophages with respect to the other surfaces.

To reach a better understanding of the effects of MC04 + PA on the immune response, the expression of selected genes ($\text{TNF-}\alpha$, MCP-1, ICAM-1, C5, IL-10 and $\text{NF-}\kappa\text{B}$) in THP-1 cells cultured on the studied surfaces was measured after 1 and 3 days (Fig. 5). The expression of $\text{TNF-}\alpha$, MCP-1, ICAM-1, C5 (proinflammatory markers; Fig. 5(a), (c), (d) and (f)), IL-10 (related to an anti-inflammatory behaviour; Fig. 5(b)) and $\text{NF-}\kappa\text{B}$ (with both pro- and anti-inflammatory roles (Fig. 5(e))) showed similar patterns for all the examined conditions. The expression of $\text{TNF-}\alpha$, MCP-1, ICAM-1, C5, IL-10 and $\text{NF-}\kappa\text{B}$ decreased for PA, MC04 and MC04 + PA compared to MC after 1 day of culture. However, after 1 day, the expression of C5 (Fig. 5(f)) decreased for MC04 than for PA. After 3 days, $\text{TNF-}\alpha$, MCP-1, ICAM-1 and IL-10 genes increased their expression in MC04 cultures with respect to MC. In contrast, the expression

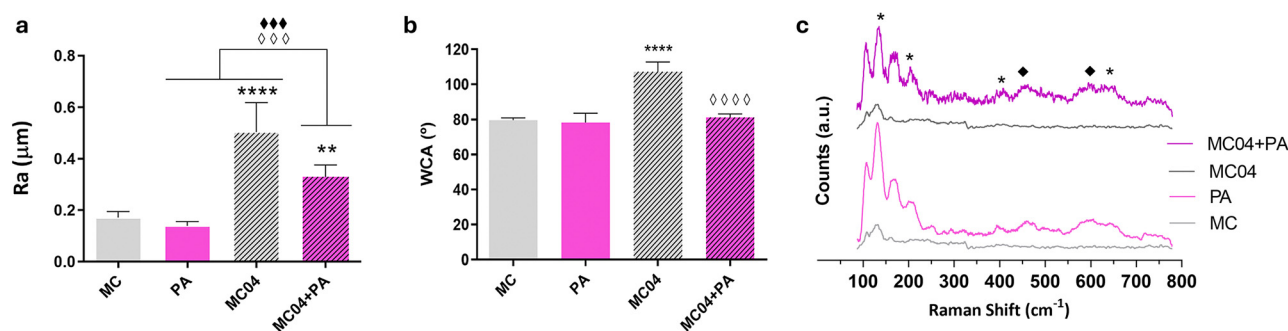


Fig. 2 Roughness (Ra; (a)), contact angle (WCA°; (b)) and Raman spectra (c) are displayed. Results are shown as means $\pm$ SD. Asterisks (*) indicate significant statistical differences between MC and PA, MC04 and MC04 + PA, black rhombuses (◼), significant statistical differences between PA and MC04 + PA, and white rhombuses (◻), between MC04 and MC04 + PA. For the Raman spectrum, asterisks (*) indicate peaks corresponding to anatase formation, while black rhombuses (◼) indicate rutile formation.

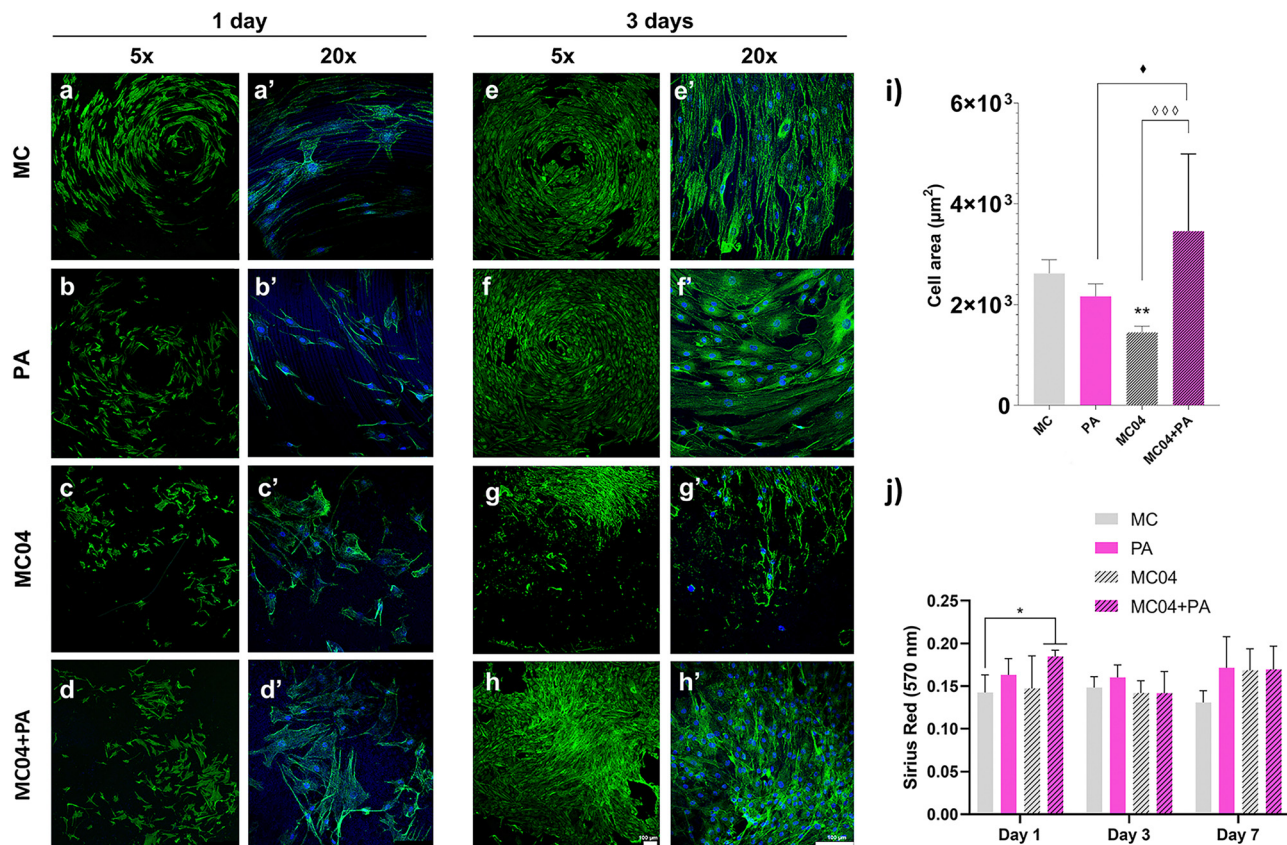


Fig. 3 Fluorescent confocal images of cytoskeleton arrangement of HGF on MC (a), (e), PA; (b), (f), MC04 (c), (g) and MC04 + PA (d), (h) surfaces. Images at 5 $\times$ (a)–(h) and 20 $\times$ (a')–(h') magnifications were obtained. Actin is stained with Phalloidin-iFluor 488 (green) and the nuclei with DAPI (blue). HGF cell areas are shown (i) for cultures on MC, PA, MC04 and MC04 + PA. Collagen secretion from HGF cells after 1, 3 and 7 days of culture on different surfaces are displayed in (j). Results are shown as means $\pm$ SD. Asterisks (*) indicate significant statistical differences between MC and PA, MC04 and MC04 + PA; the black rhombus (•) between PA and MC04 + PA, and the white rhombuses (◊) between MC04 and MC04 + PA.

of TNF- α , MCP-1, ICAM-1 and NF- κ B genes was reduced in cells grown on PA in comparison with MC. In the case of NF- κ B, it was also lower for MC04 + PA than for MC cultures. The analysis of the effect of sandblasting (MC04 + PA vs. PA) showed that the expression of TNF- α , MCP-1, ICAM-1, NF- κ B and IL-10 genes was higher in cells exposed to MC04 + PA than to PA (after 3 days). However, anodising reduced the TNF- α , MCP-1, ICAM-1, NF- κ B and IL-10 expression (in cultures on MC04 + PA compared to MC04, after 3 days). After the same period, the expression of the C5 gene was lower for PA, MC04 and MC04 + PA than for MC, and for MC04 + PA in comparison with PA.

3.3. Proteomic analysis

Table S2 (ESI[†]) shows the proteins differentially adsorbed onto PA and MC04 surfaces compared to MC04 + PA. Forty-one proteins were identified using nLC-MS/MS. Of those, 37 proteins were differentially adsorbed on MC04 + PA (relative to MC04). Seventeen proteins were differentially adsorbed when comparing MC04 + PA and PA.

3.3.1. The effect of sandblasting on PA: MC04 + PA vs. PA.

The differential proteins identified in the comparison of MC04 + PA with PA were classified according to their protein class and pathways using PANTHER and UniProt. The proteins

that were more abundant on the MC04 + PA surface than on PA (PANTHER results, Fig. 6) were assigned to a class of defence/immune proteins, transfer/carrier proteins or protein-binding activity modulators. The less abundant proteins were classified as protein-modifying enzymes, scaffold/adaptor proteins, intracellular signal molecules, or defence/immunity proteins. However, PANTHER did not find significant alterations in the pathways, so only the results obtained as a function of protein class are shown.

Based on key functions of soft tissue regeneration, the differentially adsorbed proteins could be classified by their roles in the immune response and coagulation/fibrinolysis (Table 1). MC04 + PA had increased affinity to two proteins associated with defence/immunity functions compared to PA: FHR5, associated with immune response regulation, and SPB12, related to trypsin and plasmin inhibition. Conversely, MC04 + PA showed reduced affinity to six proteins related to the immune system: three complement system proteins (C1QA, CO2 and C1QC), properdin (PROP) and two immunoglobulins (HV372 and HV102). Among the coagulation/fibrinolysis-associated proteins, coagulation factor IX (FA9), platelet factor 4 (PLF4) and its variant PF4V, and carboxypeptidase B2 (CBPB2, involved in fibrinolysis), were also less adsorbed onto MC04 + PA than PA surfaces.

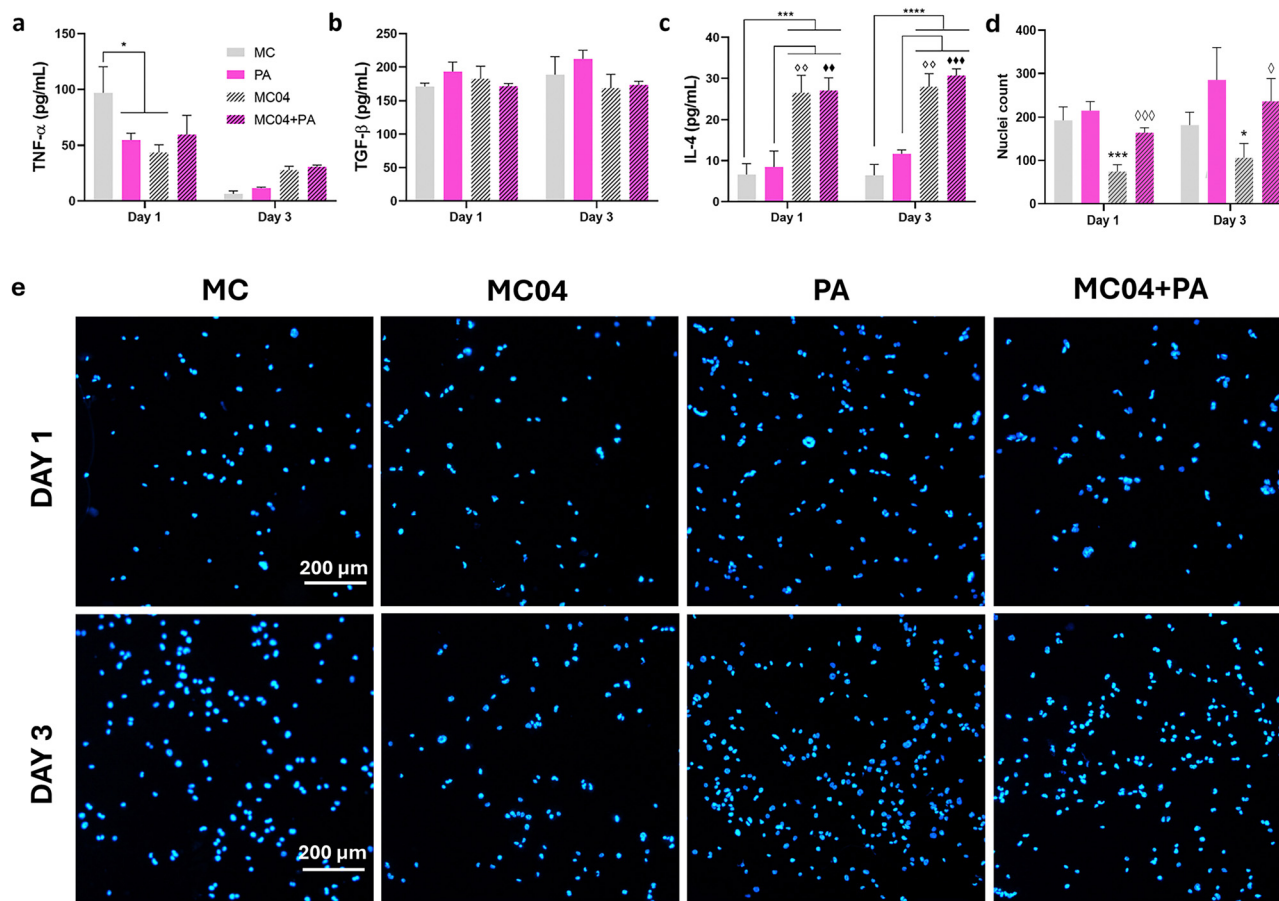


Fig. 4 Cytokine quantification by ELISA for TNF- α (a), TGF- β (b) and IL-4 (c) for THP-1 cultures at 1 and 3 days. THP-1 macrophage nuclei count (d). Fluorescent confocal images (e) for THP-1 cultures at 1 and 3 days. Nuclei w/DAPI (blue). Results are shown as means $\pm$ SD. Asterisks (*, $p < 0.05$) indicates significant statistical differences between MC and PA, MC04, MC04 + PA. (***, $p < 0.001$) represent significant statistical differences between PA and MC04 + PA. (♦♦♦, $p < 0.001$) represent significant statistical differences between MC04 and MC04 + PA. (♦♦, $p < 0.01$) represent significant statistical differences between MC04 and MC04 + PA.

3.3.2. The effect of anodising MC04: MC04 + PA vs. MC04.

The PANTHER system was employed to classify differential proteins based on the protein class and their functions in pathways (Fig. 7). The results revealed that the proteins with increased abundance on MC04 + PA belonged to the protein-modifying enzyme, intercellular signal molecule, protein-binding activity modulator, cell adhesion and defence/immunity protein classes. The proteins with decreased affinity to altered surfaces were classified as protein-modifying enzymes, transfer/carrier proteins, intercellular signal molecules, protein-binding activity modulators and defence/immunity proteins. The biological pathway analysis showed that proteins with increased adsorption on the MC04 + PA material were related to blood coagulation, while those with reduced adsorption were associated with blood coagulation, plasminogen activation and inflammation mediated by chemokine and cytokine cascades.

Based on the key functions in soft tissue regeneration, the differentially adsorbed proteins were classified by their roles in immune response, coagulation/fibrinolysis and cell adhesion (Table 2). Some immune response proteins increased their adsorption on MC04 + PA surfaces, including the complement

protein C8 beta chain (CO8B) and serum amyloid P-component (SAMP, involved in the activation of complement system pathway), complement factor H-related protein 5 (FHR5) and C4b-binding protein beta chain (C4BPB), related to their inhibition. Sixteen proteins associated with immune response showed a reduced affinity for this surface. This cluster included CO8A, VTNC, PROP, SAA4, CRP and 11 immunoglobulins (HV102, HV118, HV307, KV315, HV372, KV401, HV459, LV211, HV43D, HV146 and KV108). Furthermore, there was an increase in the adsorption of coagulation factor X (FA10), SPB12 and HRG, well known for their functions in coagulation. In contrast, six coagulation/fibrinolysis proteins (ANT3, KLKB1, PLF4, KNG1, FA11 and CBPB2) showed a decreased affinity for MC04 + PA. Moreover, preferential adsorption of cell adhesion-associated proteins (PCOC1 and DSC1) was also observed on this material.

4. Discussion

The long-term success of implantation is highly dependent on the degree of integration between the implant and the surrounding soft tissue.²³ It is of the utmost importance that the

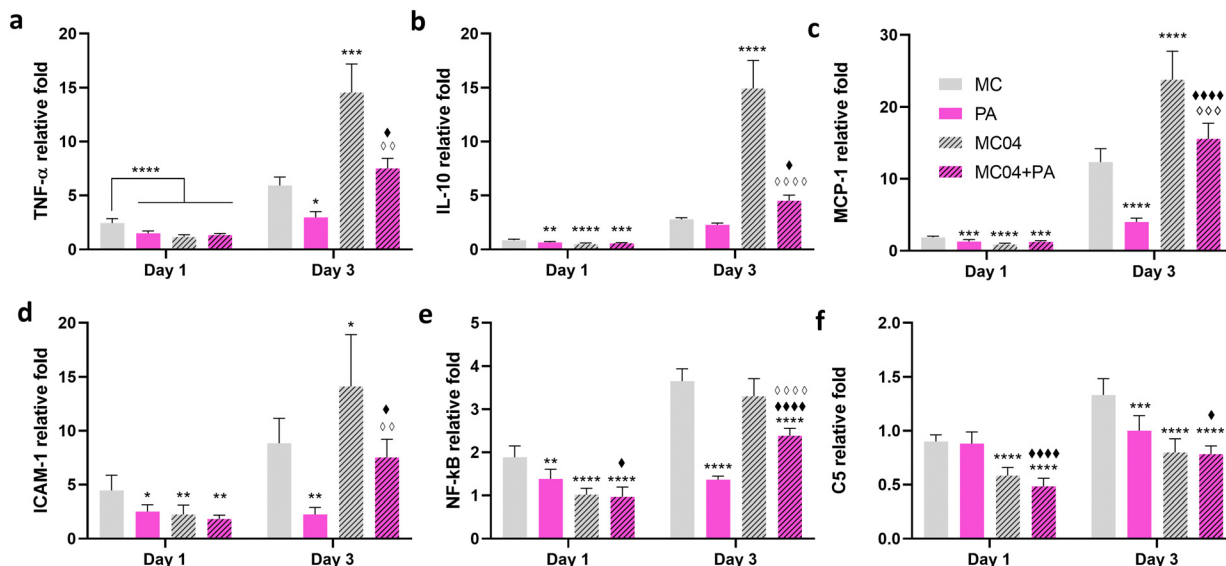


Fig. 5 RT-PCR measurement of TNF- α (a), IL-10 (b), MCP-1 (c), ICAM-1 (d), NF- κ B (e) and C5 (f) gene expression in THP-1 cells after 1 and 3 days of culture. Results are shown as means $\pm$ SD. Asterisks (*) indicate significant statistical differences between MC and PA, MC04 and MC04 + PA. Black rhombuses (♦) indicate significant statistical differences between PA and MC04 + PA, and white rhombuses (◊) between MC04 and MC04 + PA.

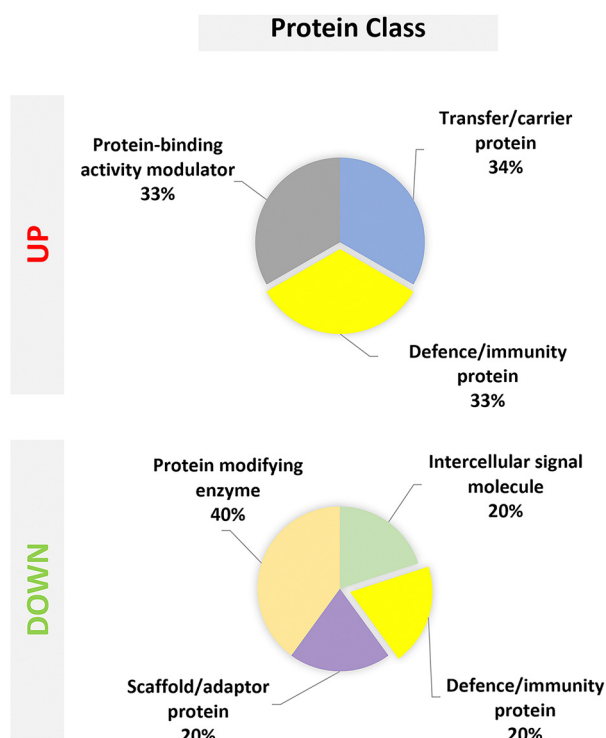


Fig. 6 Pie charts with protein class and pathway PANTHER classifications of the UP and DOWN differential proteins (MC04 + PA vs. MC04).

soft tissue barrier is formed to shield the *peri*-implant tissue from harmful bacterial plaque buildup effectively. Thus, many surface treatments are being explored, looking for morphological and chemical changes in the biomaterials that might improve this seal in the transmucosal region.²⁴

In this study, we evaluated the potential of combined sandblasting and pink anodising treatments for improving the biological interaction between dental abutments and *peri*-implant soft tissues. SEM analysis revealed that sandblasting caused a significant alteration in the morphology and topography of machined surfaces, resulting in increased roughness and eliminating the grooves formed during machining.²⁵ The anodisation process changes the chemical composition of the material due to the formation of TiO₂,²⁶ thus smoothing the surface. Raman spectra demonstrated the presence of the TiO₂ layer on the samples. The increased intensities of TiO₂ characteristic signals for the anodized samples (PA, MC04 + PA) can be associated with a thicker TiO₂ layer compared to MC and MC04.²⁷ These observations were verified through EDX measurements, as oxygen was detected in the anodized samples. Riivari *et al.*²⁸ found that TiO₂ enhanced the HGF adhesion to implant surfaces.

The combination of the two surface treatments (MC04 + PA) led to changes in the morphology, roughness and chemical composition of the material. The adhesion of HGFs to Ti surfaces is affected by several factors, including roughness, morphology, wettability, and chemical composition.²⁹ Nevertheless, few studies have explored the behaviour of HGFs on surfaces with different characteristics. One *in vitro* study has reported that fibroblast proliferation and adhesion increase on blasted Ti surfaces with high roughness in comparison with machined materials.¹⁴ However, Beheshti Maal *et al.*³⁰ have found that smooth surfaces boost HGF proliferation and adhesion, suggesting that cell adhesion to Ti does not depend only on the material roughness. Our results showed that MC04, with higher Ra, showed a lower cell adhesion than MC and MC04 + PA. Conversely, MC04 + PA displayed an improved HGF adhesion compared to PA, which had a smoother surface.

Table 1 Differentially (up/down) proteins adsorbed on MC04 + PA surfaces compared to PA materials. Proteins with a $p \leq 0.05$ and a ratio greater than 1.5 in either direction were considered. The proteins were classified into two functional groups corresponding to immune response and coagulation/fibrinolysis

Functional group		Protein
Immune response	Up	FHR5
	Down	C1QA, CO2, PROP, HV372, C1QC, HV102
Coagulation/fibrinolysis	Up	SPB12
	Down	FA9, CBPB2, PF4V, PLF4

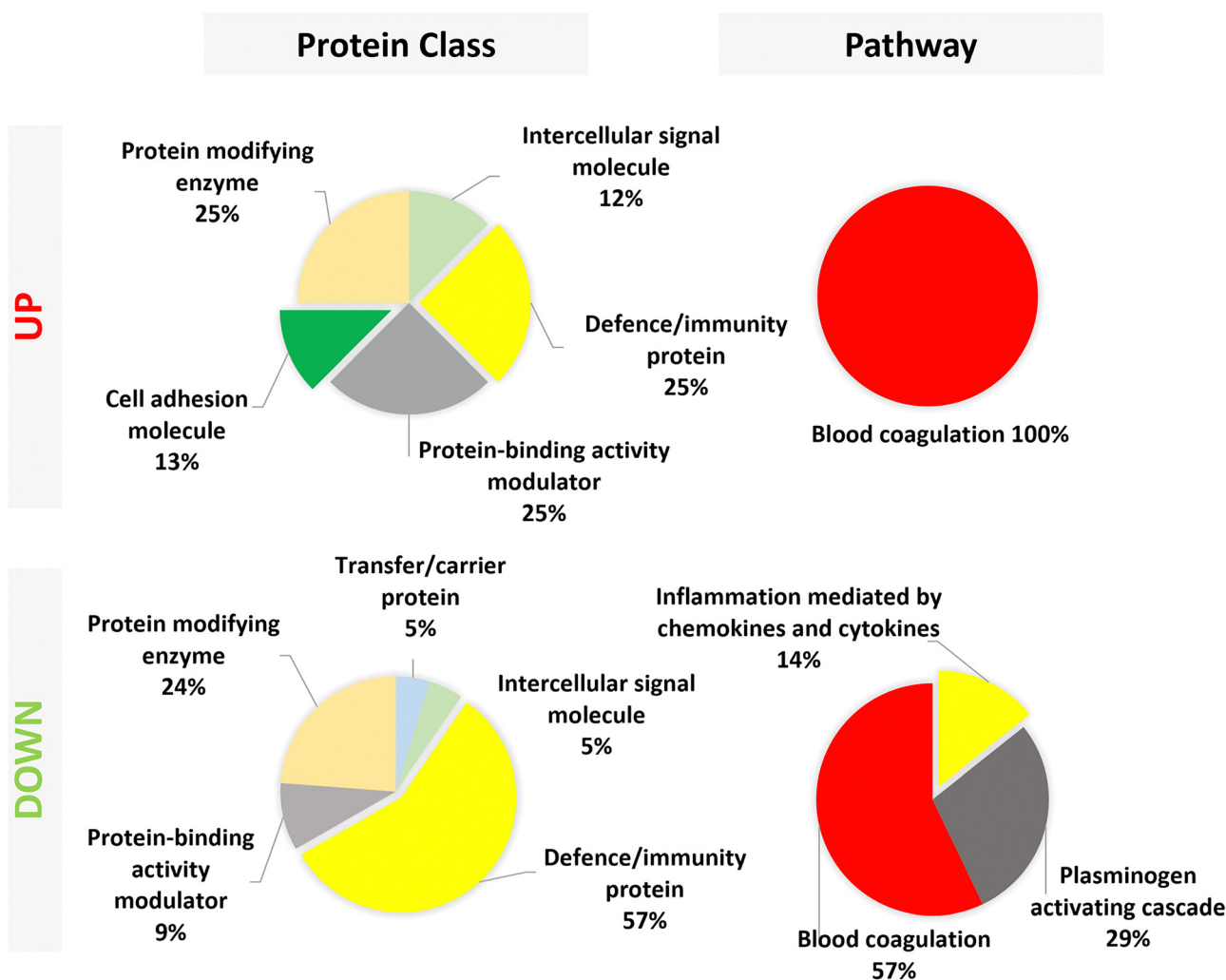


Fig. 7 PANTHER protein classification of the proteins differentially adsorbed on MC04 + PA vs. PA. Proteins were classified according to the protein class and the signalling pathway to which it is associated.

These results show that the behaviour of HGFs is affected by several factors, such as morphology or chemical composition, indicating that the roughness alone does not determine the final response. Proteomic analyses were conducted to better understand the biological regenerative potential of MC04 + PA surfaces. Forty-one proteins were adsorbed onto the studied materials. In addition, comparisons between MC04 + PA and surfaces obtained using the individual treatments (PA and MC04) were made to examine the specific effect of each

modification. Two proteins (DSCL1, PCOC1) associated with cell adhesion showed stronger affinity for MC04 + PA surfaces than for MC04. Garrod *et al.*³¹ have reported that DSCL1, a type of desmocollin, plays a crucial role in mediating cell adhesion at desmosomal junctions. These proteins belong to the cadherin superfamily and are essential for maintaining cell adhesion.³² Moreover, PCOC1 has been reported as a positive regulator of collagen deposition;³³ an increase in PCOC1 levels may lead to a rise in collagen production. Somaiah *et al.*³⁴ have

Table 2 Differentially (up/down) adsorbed proteins on the MC04 + PA surface relative to the MC04 material. Proteins with a $p \leq 0.05$ and a ratio greater than 1.5 in either direction were considered. The proteins were classified into three functional groups corresponding to immune response, coagulation/fibrinolysis and cell adhesion

Functional group		Protein
Immune response	Up	CO8B, FHR5, SAMP, C4BPB
	Down	HV102, HV118, HV307, CO8A, KV315, VTNC, HV372, KV401, PROP, HV459, LV211, HV43D, HV146, SAA4, CRP, KV108
Coagulation/fibrinolysis	Up	SPB12, FA10, HRG
	Down	ANT3, KLKB1, PLF4, CBPB2, KNG1, FA11
Cell adhesion	Up	DSC1, PCOC1
	Down	—

shown that such an increase in collagen levels results in a boost in MSC proliferation and cell adhesion. Overall, these findings suggest that DSCL1 and PCOC1 are important factors contributing to cell adhesion and proliferation. These data are in agreement with the results of the cytoskeleton arrangement assay, where the HGFs showed an increased adhesion to MC04 + PA surfaces. Moreover, a rise in 1-day collagen secretion by HGFs was observed in MC04 + PA cultures in comparison to MC. Banlue *et al.*³⁵ have demonstrated that augmented collagen synthesis favours wound healing. This improvement was not observed for surfaces obtained using individual treatments (PA and MC04). Therefore, MC04 + PA samples should result in a surface with a stronger soft tissue regenerative potential. Integration of an implant with soft tissue is not solely dependent on cell adhesion and tissue regeneration. The body's immune response to the implant material also plays a crucial role, and it can be either innate or adaptive.³⁶ The innate response is triggered by a variety of factors and involves cells such as macrophages. The macrophage polarisation to the M1 or M2 phenotype can lead to the secretion of proinflammatory or anti-inflammatory cytokines, respectively.³⁷ Goransson *et al.*³⁸ have examined monocytes exposed to Ti surfaces modified by different treatments (including anodisation and sandblasting). They have not found significant changes in the secretion of either inflammatory (TNF- α) or anti-inflammatory (IL-10) cytokines. Here, we found a reduction in the release of TNF- α by cells exposed to PA and MC04 materials for 1 day (compared to MC). This was supported by RT-PCR results, where a reduction in TNF- α expression was also observed for PA, MC04 and MC04 + PA compared to MC (after 1 day). Similar effects were noted for other proinflammatory markers, such as MCP-1 and ICAM-1 (an adhesion molecule also associated with a proinflammatory effect).³⁹ In addition, a significant reduction in the number of THP-1 macrophages adhering to MC04 surfaces was observed compared to the other surfaces. Conversely, despite this fact, cell adhered to MC04 exhibited higher secretion of IL-4; and this surface treatment increased TNF- α , IL-10, MCP-1 and ICAM-1 gene expression, which can indicate an augmented macrophage activity for MC04.

Taken together, these results show that the proinflammatory potential can be reduced by sandblasting or anodising machined surfaces. The macrophage exposure to sandblasted

surfaces also increased the secretion of IL-4, an anti-inflammatory cytokine (at all time points). Lü *et al.*¹⁷ have found that contact with anodised materials reduces the levels of TNF- α and MCP-1 produced in the culture on THP-1 cells. This is consistent with our results. In our experiments, after 3 days, THP-1 macrophages exposed to anodised surfaces reduced the expression of TNF- α , MCP-1 and ICAM-1 (as shown by MC vs. PA and MC04 vs. MC04 + PA comparisons), confirming the potential of this technique for reducing the immune response. The proteomic analyses corroborated these observations, showing increased adsorption on MC04 + PA (compared to MC04) of four proteins associated with the immune response: CO8B, FHR5, C4BPB and SAMP. FHR5 was also differentially more adsorbed to MC04 + PA than to PA. The CO8B protein is linked to the activation of the complement system and the formation of the membrane attack complex (MAC),⁴⁰ while FHR5 and C4BPB are involved in the regulation/inhibition of this cascade.^{41,42} SAMP, also associated with the complement system, is involved in inflammation, coagulation, tissue remodelling and defence against bacterial infections.⁴³ Although the exact function of SAMP is not yet clear, a study carried out by Wang *et al.*⁴⁴ have reported that low levels of SAMP are linked to aggravated periodontitis mediated through the C5a-C5aR pathway. In contrast, a remarkable decrease in the adsorption of immune-related proteins was observed on MC04 + PA in comparison with PA and MC04 surfaces. This result indicates that the combination of sandblasting and anodising treatments can reduce the inflammatory potential of the materials. In fact, there was a notable reduction in the immunoglobulin adsorption on MC04 + PA compared to PA and MC04. The amounts of two of the immunoglobulins (HV372 and HV102) adsorbed on the MC04 + PA material were lower than that on PA surfaces. In addition, other 11 immunoglobulins (HV102, HV118, HV307, KV315, HV372, KV401, HV459, LV211, HV43D, HV146 and KV108) showed reduced affinity to the MC04 + PA surface compared to MC04. Immunoglobulins are glycoproteins produced by plasma cells in charge of immune response.⁴⁵ High levels of immunoglobulins are associated with inflammatory responses to pathogens, as it has been verified for periimplantitis.⁴⁶ Moreover, proteins linked to the complement system (PROP, VTNC, CRP and CO8A) were less adsorbed on MC04 + PA compared to MC04.

Similarly, the amounts of C1QC1 adhering to MC04 + PA were smaller than those on PA. The decrease in complement system response to MC04 + PA was consistently observed in both the proteomic and RT-PCR results. Specifically, a reduction in the expression of C5, a protein related to the activation of the complement system, was noted after 1 and 3 days when comparing PA with MC04 + PA. Moreover, anodising (MC04 + PA compared to MC04) reduced the adsorption of C1QC1, CO8A, and CRP. These proteins are involved in activating the complement system cascade, initiating the immune response, while PROP and VTNC can regulate this reaction.^{47,48} SAA4 and CRP, both adsorbed less to MC04 + PA than to MC04, have been associated with acute inflammatory reaction.⁴⁹ Markedly increased levels of these proteins have been reported in patients with periodontitis and periimplantitis in comparison with healthy individuals.^{50,51} Furthermore, the adsorption of CRP on biomaterials has been linked to biocompatibility problems and the formation of fibrous capsules *in vivo*,⁵² which could lead to implant failure.⁵³ The NF- κ B serves as a pivotal mediator of inflammatory responses, inducing the expression of various proinflammatory genes.⁵⁴ Our study showed a reduction in the expression of this gene in cells exposed to MC04 + PA for 1 day (compared to PA) and for 3 days (compared with MC04). This decrease could indicate a down-regulation of this pathway and, therefore, reduced inflammation, which is consistent with the rest of the *in vitro* results.

Blood coagulation also plays a crucial role in tissue healing, not only by preventing excessive blood loss but also by providing a natural scaffold for tissue repair and regeneration.⁵⁵ Moreover, the coagulation process is closely associated with inflammation as both processes play a role in homeostasis during wound healing.⁵⁶ The proteomic analysis showed that a cluster of proteins that play roles in coagulation has an increased affinity for MC04 + PA surfaces. Sandblasting reduced the adsorption of pro-coagulation proteins such as FA9, CBPB2, PF4V and PLF4, which might diminish the procoagulant response to MC04 + PA. Regarding anodising, some variations in the adsorption of coagulation-related proteins were identified on PA surfaces compared to MC04 + PA. However, their impact remains uncertain and further experimentation will be necessary to observe potential differences in the coagulation process. The protein adsorption patterns, as well as their correlation with *in vitro* results, indicate that the MC04 + PA modified surfaces might become a promising material in the field of implantology. This combination of surface treatments boosts tissue regeneration by favouring HGF adhesion, increasing collagen production and reducing immune response. These properties might help in forming an optimal seal between the abutment and the soft tissue, increasing the implantation success rate.

5. Conclusions

The aim of this study was the creation of new Ti surface modifications by combining sandblasting and pink anodising to improve the response of cells (THP-1 macrophages and HGF

fibroblasts) exposed to such materials. Moreover, we examined the correlation between the results of *in vitro* experiments and the patterns of protein adsorption on the material surfaces through proteomics. The combined MC04 + PA treatment altered the surface morphology, roughness, and chemical composition of Ti, producing changes in both cellular response and protein adsorption patterns. Fibroblast adhesion on MC04 + PA was significantly increased compared to PA and MC04; the cells exposed to this material showed higher collagen secretion than those grown on the MC surfaces. Moreover, MC04 + PA displayed an improved anti-inflammatory *potential in vitro*, with an increased release of IL-4. This result was corroborated by the changes in the expression of the studied genes (such as IL-1 β , MCP-1 and C5). A remarkable reduction in the adsorption of immune-related proteins was observed, especially for immunoglobulins on MC04 + PA compared to MC04 and PA. This study highlights the potential advantages of MC04 + PA surface treatment in the production of dental implant abutments, enhancing their compatibility with soft tissues and reducing the inflammatory response.

Author contributions

Carlos Arias-Mainer: writing – original draft, data curation, investigation, and formal analysis. Francisco Romero-Gavilán: data curation, investigation, formal analysis, and writing – review & editing. Andreia Cerqueira: data curation, investigation, formal analysis, and writing – review & editing. Mikel Azkargorta: data curation and investigation. Felix Elortza: resources and investigation. Marilo Gurruchaga: writing – review & editing, funding acquisition, and project administration. Isabel Goñi: writing – review & editing, funding acquisition, and project administration. Julio Suay: writing – review & editing, funding acquisition, and project administration.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

The authors declare that there are no conflicts to declare.

Acknowledgements

This work was supported by the Science and Innovation Ministry of Spain [PDC2021-120924-I00/AEI/10.13039/501100011033/ Unión Europea NextGenerationEU/PRTR], Universitat Jaume I [UJI-B2021-25] and the Basque Government [MARSA21/07]. Andreia Cerqueira was supported by the Margarita Salas post-doctoral contract MGS/2022/10 (UP2022-024) financed by the European Union-NextGenerationEU. The authors would like to thank Raquel Oliver, José Ortega and Iraide Escobés for their valuable technical assistance.

Notes and references

- 1 S. Raikar, P. Talukdar, S. Kumari, S. K. Panda, V. M. Oommen and A. Prasad, *J. Int. Soc. Prev. Community Dent.*, 2017, **7**, 351–355.
- 2 D. French, R. Ofec and L. Levin, *Clin. Implant Dent. Relat. Res.*, 2021, **23**, 289–297.
- 3 B. Schminke, F. V. Orde, R. Gruber, H. Schliephake, R. Bürgers and N. Miosge, *J. Dent. Res.*, 2015, **94**, 354–361.
- 4 J. Barthes, M. Cazzola, C. Muller, C. Dollinger, C. Debry, S. Ferraris, S. Spriano and N. E. Vrana, *Mater. Sci. Eng., C*, 2020, **112**, 110845.
- 5 S. R. Dutta, D. Passi, P. Singh, M. Atri, S. Mohan and A. Sharma, *J. Maxillofac. Surg.*, 2020, **11**, 14–19.
- 6 D. Lops, E. Romeo, S. Calza, A. Palazzolo, L. Viviani, S. Salgarello, B. Buffoli and M. Mensi, *J. Clin. Med.*, 2022, **11**, 6243.
- 7 V. John, D. Shin, A. Marlow and Y. Hamada, *Case Rep. Dent.*, 2016, **2016**, 2491714.
- 8 Y. Jao, S. J. Ding and C. C. Chen, *J. Dent. Sci.*, 2023, **18**, 1453–1466.
- 9 N. Takahashi, B. Sulijaya, M. Yamada-Hara, T. Tsuzuno, K. Tabeta and K. Yamazaki, *Tissue Barriers*, 2019, **7**(3), e1651158.
- 10 P. Ahangar, S. J. Mills, L. E. Smith, S. Gronthos and A. J. Cowin, *npj Regen. Med.*, 2020, **5**(1), 24.
- 11 S. Gibbs, S. Roffe, M. Meyer and A. Gasser, *Eur. Cells Mater.*, 2019, **38**, 63–78.
- 12 V. R. Kearns, R. L. Williams, F. Mirvakily, P. J. Doherty and N. Martin, *J. Clin. Periodontol.*, 2013, **40**, 99–108.
- 13 I. S. Luke Yeo, *Materials*, 2020, **13**, 89.
- 14 H. Yildiz, E. Sen, H. Dalcik and S. E. Meseli, *Folia Morphol.*, 2023, **82**, 63–71.
- 15 M. Nossowitz, M. Teale, S. Mathes, A. Venturato and A. Gasser, *J. Oral Rehabil.*, 2019, 1–7.
- 16 T. Wang, L. Wang, Q. Lu and Z. Fan, *J. Prosthet. Dent.*, 2021, **125**, 445–452.
- 17 W. L. Lü, N. Wang, P. Gao, C. Y. Li, H. S. Zhao and Z. T. Zhang, *Cell Proliferation*, 2015, **48**, 95–104.
- 18 Z. Othman, B. Cillero Pastor, S. van Rijt and P. Habibovic, *Biomaterials*, 2018, **167**, 191–204.
- 19 H. P. Felgueiras, J. C. Antunes, M. C. L. Martins and M. A. Barbosa, *Peptides and Proteins as Biomaterials for Tissue Regeneration and Repair*, Elsevier Inc., Cambridge, 1st edn, 2018, pp. 1–27.
- 20 F. Romero-Gavilán, A. Cerqueira, I. García-Arnáez, M. Azkargorta, F. Elortza, M. Gurruchaga, I. Goñi and J. Suay, *J. Biomed. Mater. Res., Part A*, 2023, **111**, 45–59.
- 21 Z. Starowicz, M. Lipiński, R. P. Socha, K. Berent, G. Kulesza and P. Ozga, *J. Sol-Gel Sci. Technol.*, 2015, **73**, 563–571.
- 22 O. Frank, M. Zukalova, B. Laskova, J. Kürti, J. Koltai and L. Kavan, *Phys. Chem. Chem. Phys.*, 2012, **14**, 14567–14572.
- 23 M. Gulati, V. Govila, V. Anand and B. Anand, *Int. Scholarly Res. Not.*, 2014, **2014**, 1–8.
- 24 T. Guo, K. Gulati, H. Arora, P. Han, B. Fournier and S. Ivanovski, *Acta Biomater.*, 2021, **124**, 33–49.
- 25 P. Osak, J. Maszybrocka, M. Zubko, J. Rak, S. Bogunia and B. Łosiewicz, *Materials*, 2021, **14**, 7536.
- 26 E. J. Ekoi, A. Gowen, R. Dorrepaal and D. P. Dowling, *Results Phys.*, 2019, **12**, 1574–1585.
- 27 S. Riivari, E. Närvä, I. Kangasniemi, J. Willberg and T. Närhi, *Clin. Implant Dent. Relat. Res.*, 2023, **25**, 583–591.
- 28 Z. Su, L. Zhang, F. Jiang and M. Hong, *Prog. Nat. Sci.: Mater. Int.*, 2013, **23**, 294–301.
- 29 M. A. F. Martinez, Í. de Fátima Balderrama, P. S. B. H. Karam, R. C. de Oliveira, F. A. de Oliveira, C. R. Grandini, F. B. Vicente, A. Stavropoulos, M. S. R. Zangrando and A. C. P. Sant'Ana, *Int. J. Implant Dent.*, 2020, **6**, 46.
- 30 M. B. Maal, S. A. Ellingsen, J. E. Reseland and A. Verket, *BMC Oral Health*, 2020, **20**(1), 25.
- 31 D. Garrod and M. Chidgey, *Biochim. Biophys. Acta, Bio-membr.*, 2008, **1778**, 572–587.
- 32 C. F. Kline and P. J. Mohler, *Current Topics in Membranes*, Academic Press Inc., 2013, **72**, 121–158.
- 33 T. Weiss, S. Ricard-Blum, L. Moschovich, E. Wineman, S. Mesilaty and E. Kessler, *J. Biol. Chem.*, 2010, **285**, 33867–33874.
- 34 C. Somaiah, A. Kumar, D. Mawrie, A. Sharma, S. D. Patil, J. Bhattacharyya, R. Swaminathan and B. G. Jaganathan, *PLoS One*, 2015, **10**, 12.
- 35 A. Banlue, J. Kaewmuangmoon, K. Janebodin and K. Tansriratanawong, *Eur. J. Dent.*, 2024, **18**, 219–227.
- 36 L. Amengual-Peñafiel, L. A. Córdova, M. Constanza Jara-Sepúlveda, M. Brañes-Aroca, F. Marchesani-Carrasco and R. Cartes-Velásquez, *Jpn. Dent. Sci. Rev.*, 2021, **57**, 12–19.
- 37 Y. Yao, X. H. Xu and L. Jin, *Front. Immunol.*, 2019, **10**, 792.
- 38 A. Göransson, C. Gretzer, A. Johansson, Y. T. Sul and A. Wennerberg, *Clin. Implant Dent. Relat. Res.*, 2006, **8**, 210–217.
- 39 T. Brzicova, E. Javorkova, K. Vrbova, A. Zajicova, V. Holan, D. Pinkas, V. Philimonenko, J. Sikorova, J. Klema, J. Topinka and P. Rossner, *Nanomaterials*, 2019, **9**, 687.
- 40 D. Bubeck, P. Roversi, R. Donev, B. P. Morgan, O. Llorca and S. M. Lea, *J. Mol. Biol.*, 2011, **405**, 325–330.
- 41 C. Bechtler, S. Koutsogiannaki, E. Umnyakova, A. Hamid, A. Gautam, Y. Sarigiannis, R. B. Pouw, C. Lamers, S. Rabbani, C. Q. Schmidt, J. D. Lambris and D. Ricklin, *Acta Biomater.*, 2023, **155**, 123–138.
- 42 A. M. Blom, B. O. Villoutreix and B. Dahlbäck, *Mol. Immunol.*, 2004, **40**, 1333–1346.
- 43 A. Doni, R. Parente, I. Laface, E. Magrini, C. Cunha, F. S. Colombo, J. F. Lacerda, A. Campos, S. N. Mapelli, F. Petroni, R. Porte, T. Schorn, A. Inforzato, T. Mercier, K. Lagrou, J. Maertens, J. D. Lambris, B. Bottazzi, C. Garlanda, M. Botto, A. Carvalho and A. Mantovani, *Nat. Commun.*, 2021, **12**, 3739.
- 44 L. Wang, D. Liang, Y. Huang, Y. Chen, X. Yang, Z. Huang, Y. Jiang, H. Su, L. Wang, J. L. Pathak and L. Ge, *J. Adv. Res.*, 2023, **50**, 55–68.
- 45 K. B. Megha and P. V. Mohanan, *Int. J. Biol. Macromol.*, 2021, **169**, 28–38.

- 46 J. R. Pires, M. R. S. Nogueira, A. J. F. Nunes, D. R. F. Degand, L. C. Pessoa, C. A. Damante, M. S. R. Zangrando, S. L. A. Gregghi, M. L. R. de Rezende and A. C. P. Sant'Ana, *Front. Immunol.*, 2021, **12**, 591236.
- 47 B. Singh, Y. C. Su and K. Riesbeck, *Mol. Microbiol.*, 2010, **78**, 545–560.
- 48 J. Y. Chen, C. Cortes and V. P. Ferreira, *Mol. Immunol.*, 2018, **102**, 58–72.
- 49 Y. Zhang, J. Zhang, H. Sheng, H. Li and R. Wang, *Adv. Clin. Chem.*, 2019, **vol. 90**, 25–80.
- 50 C. M. Ardila and I. C. Guzmán, *J. Periodontal Implant Sci.*, 2015, **45**, 14–22.
- 51 S. Kulkarni, P. Oswal, M. Kulkarni, S. Sawant, A. Vas and K. Rajguru, *J. Adv. Med. Dent. Sci. Res.*, 2020, **8**, 116–118.
- 52 F. Romero-Gavilan, A. M. Sánchez-Pérez, N. Araújo-Gomes, M. Azkargorta, I. Iloro, F. Elortza, M. Gurruchaga, I. Goñi and J. Suay, *Biofouling*, 2017, **33**, 676–689.
- 53 N. Noskovicova, B. Hinz and P. Pakshir, *Cells*, 2021, **10**, 7.
- 54 T. Liu, L. Zhang, D. Joo and S. C. Sun, *Signal Transduction Targeted Ther.*, 2017, **2**, 17023.
- 55 Y. Yang and Y. Xiao, *Adv. Healthcare Mater.*, 2020, **9**, 23.
- 56 K. Oikonomopoulou, D. Ricklin, P. A. Ward and J. D. Lambris, *Semin. Immunopathol.*, 2012, **34**, 151–165.