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Enhancing osseointegration: A proteomic evaluation and in vitro study of sol-gel surfaces doped with quercetin

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Abstract

One of the main challenges in dental implantology is the success of osseointegration, the process by which a solid union is produced between a dental implant and the bone. In this context, sol-gel technology is beginning to be used to obtain biocompatible surfaces that interact favorably with the environment. By incorporating quercetin (QCT), a flavonoid with anti-inflammatory properties to this sol-gel technology, this study aims to make a comparison between these sol-gel surfaces doped with quercetin (QCT sol-gel) and Ti surfaces, through a proteomic evaluation and in vitro HOb adhesion studies through confocal microscopy, observing a decrease of proteins related to the activation of the immune response and coagulation for the QCT sol-gel surfaces. In addition, an increase in the cellular area of HOb adhered to the QCT sol-gel surfaces was also observed.

Keywords: *osseointegration, sol-gel, quercetin, proteomics, osteoblast, cell adhesion*

Resumen

Uno de los principales retos en implantología dental, es el éxito de la osteointegración, proceso por el cual se produce una unión sólida entre un implante dental y el hueso. En este contexto, la tecnología sol-gel se está comenzando a emplear con la finalidad de obtener superficies biocompatibles que interactúen favorablemente con el entorno. Mediante la incorporación de quercetina (QCT), un flavonoide con propiedades antinflamatorias a esta tecnología sol-gel, este estudio pretende realizar una comparativa entre estas superficies de sol-gel dopadas con quercetina (QCT sol-gel) y superficies de Ti, mediante evaluación proteómica y estudios in vitro de adhesión de HOb a través de microscopía confocal, observándose una disminución de proteínas relacionadas con la activación de la respuesta inmune y la coagulación para las superficies QCT sol-gel. Además de un aumento en el área celular de los HOb adheridos a las superficies QCT sol-gel.

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Introducción

In the field of oral implantology, the quest for innovative materials and techniques continues progress toward enhanced biocompatibility, longevity, and overall performance of implant surfaces[1]. A prominent contender in this pursuit is the utilization of sol-gel technology, a versatile and dynamic process with significant potential to revolutionize the field[2].

Sol-gel, a method originating from the chemical synthesis, has garnered increasing attention for its applicability in creating thin, uniform coatings on diverse substrates. In the context of implantology, these coatings offer a promising way to modulate surface properties, influence cellular responses, and ultimately improve the osseointegration process[3].

The utilization of the sol-gel technique offers a versatile means to obtain surface coatings, providing a multitude of advantages. Notably, this method enables enhanced control over both the chemical composition of the coating and the microstructure of the resulting film[4]. Moreover, sol-gel coatings can be employed as a release vehicle for both organic[5] and inorganic[6] compounds, thus improving the properties of these coatings. In our case, we have incorporated Quercetin (QCT) into the sol-gel network to improve the properties of the sol-gel coating (QCT sol-gel).

QCT belongs to the flavonoid's family; a class of polyphenolic compounds present in numerous fruits and vegetables. These kinds of compounds have gained significant interest in the field of biomaterials due to their diverse beneficial properties. Among these flavonoids, QCT has stood out for its remarkable anti-inflammatory characteristics[7]. These properties have sparked a growing interest in their integration into biomaterials with the aim of improving their functional properties in various biomedical applications.

Moreover, some studies, have reported that sol-gel exhibits a notable capacity to enhance cell adhesion, underscoring its effectiveness in promoting favorable cellular interactions[8]. In this sense, the incorporation of QCT to the sol gel network may lead to an improved cell adhesion. This may be due to the presence of the catechol group in QCT characterized by their ability to form strong adhesive bonds, also associated with enhanced cell-material interactions[9].

This paper aims to delve into the pivotal role of sol-gel coatings in implantology, exploring their influence on surface morphology, biocompatibility, and the overall success of implant integration. Moreover, the incorporation of QCT into the sol-gel network has the capacity to improve osteoblast cell adhesion while fostering a more controlled immune response.

Materials and methods

Sol-gel synthesis

For the development of the selected surface (QCT sol-gel), QCT hydrate and organically modified alkoxysilanes were employed for the sol-gel synthesis. Methyltrimethoxysilane (MTMOS; M) and tetraethyl orthosilicate (TEOS; T) (Sigma–Aldrich, Merck KGaA, Darmstadt, Germany) were used as precursors in a ratio (7:3) MTMOS and TEOS respectively. 2-Propanol, was employed as solvent for the sol-gel coatings in a volume ratio alcohol:siloxane 1:1. Hydrolysis was performed by adding H_2O acidified with nitric acid (HNO_3 0.1 M) at a rate of 1 drop s^{-1}. QCT Hydrate were added in a 2% and sol gel mixtures were stirred for 1 hour and were keep under resting for another hour. Once sol-gel was synthesized, it was applied as a coating on grade-4 Ti discs (10- mm diameter, 1-mm thick) with a dip-coater (KSV DC; KSV NIMA, Espoo, Finland). Ti discs were immersed for 1 minute in sol gel mixture at a velocity of 60 cm min^{-1} and removed at 100 cm min^{-1}. Finally, samples were incubated for 2 h at 80 °C.

Physico-Chemical characterization

The impact of QCT sol-gel on surface topography was assessed using scanning electron microscopy (SEM) conducted with Leica–ZeissLEO equipment under vacuum conditions (Leica, Wetzlar, Germany). To enhance conductivity, materials underwent pretreatment via platinum sputtering. Surface roughness was further characterized using an optical profilometer PLm2300 (Sensofar, Barcelona, Spain), with assays performed in triplicate for each sample. Contact angles were determined using an automatic contact angle meter OCA 20 (DataPhysics Instruments, Filderstadt, Germany). Analysis involved the study of six discs for each material.

In vitro characterization

Cell culture

The Human Osteoblast cell line (HOb) was cultured on biomaterial surfaces within 48-well NUNC plates (Thermo Fisher Scientific, Waltham, MA, US). HOb cells were incubated at

37 °C in a humidified atmosphere with 95% humidity and 5% CO_2. The culture medium for Hob comprised Dulbecco's Modified Eagle Medium (DMEM; Ref), supplemented with 1% penicillin/streptomycin (Biowest Inc., USA), and 10% Fetal Bovine Serum (FBS; Gibco, Life Technologies). To maintain optimal growth conditions, the cell culture medium was replenished every two days.

HOb adhesion

To assess cytoskeleton arrangement, HOb cells were seeded on the samples at a density of 1 x 10^4 cells cm^{-2} and cultured for 1 day. Subsequently, the samples underwent PBS washing and were fixed with 4% paraformaldehyde (PFA) for 20 minutes at room temperature. Following fixation, HOb cells were permeabilized with 0.1% Triton X-100 and incubated with phalloidin (1:100; Abcam, Cambridge, UK) diluted in 0.1% w/v bovine serum albumin (BSA)-PBS for 1 hour. After two PBS washes, nuclei were stained by incubating samples in DAPI with mounting medium for 5 minutes. For this purpose, a Leica TCS SP8 Confocal microscopy was employed. Image acquisition was obtained through LAS X software (Leica) and cell areas were subsequently analyzed using Image J software (National Institutes of Health, Maryland, USA).

Statistical analysis

After obtaining results of normal distribution analysis of the data and equal variance, the statistical analysis was carried out by one-way analysis of variance (one-way ANOVA) with Tukey post-hoc test. Statistical analysis was performed using GraphPad Prism 5.04 software (GraphPad Software Inc., La Jolla, CA, USA). Differences between Ti materials (Ti) and QCT sol-gel were considered significant at $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***), and $p \leq 0.0001$ (****).

Proteomic analysis

Protein elution

To obtain the protein layer, samples were incubated in 200 µL of human serum (Male AB plasma; Merck) for 3 hours in a cell incubator set at 37 °C, 5% CO_2, and 95% relative humidity. To eliminate non-adsorbed proteins, samples underwent five washes with ddH2O followed by an additional wash with a buffer solution (100 mM NaCl, 50 mM Tris-HCl, pH 7). Adsorbed proteins were eluted by adding 100 µL of lysis buffer (2 M thiourea, 7 M urea, 4% CHAPS, and 200 mM dithiothreitol). A protein pool was created by combining eluted proteins from four samples. All reagents were purchased from Merck.

nLC-MS/MS measurements and bioinformatic 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 the eluted protein layer, following the procedure outlined in Romero-Gavilán et al.[10] Each profile was analyzed in quadruplicate, and protein identification was conducted using MaxQuant software (http://maxquant.org/). The differential analysis was carried out through the Perseus platform (http://maxquant.org/perseus/). Functional analyses of differential proteins were performed using PANTHER (http://www.pantherdb.org/) and the UniProt database (https://www.uniprot.org/). The UniProt ID nomenclature was employed for protein identification.

Results

Physico-Chemical Analysis

Following the development of the QCT sol-gel surface, a comprehensive characterization was undertaken to scrutinize the resulting physicochemical changes on this surface. A comparative analysis was conducted, contrasting the titanium (Ti) commonly employed in implantology with the surface specifically formulated for this study.

The morphological attributes of both surfaces (Ti and QCT sol-gel) underwent meticulous evaluation through scanning electron microscopy (SEM), as depicted in the micrographs of Fig.1. Notably, in the case of titanium variants (Fig.1 a, b and c) surfaces showed a high roughness, a consequence of Ti undergoing shot-blasting treatment. This treatment gave rise to characteristic shapes evident in the Ti micrographs.

Conversely, the sol-gel coating was observed to cover the entire surface area, affirming the success of the coating process. Furthermore, specific sol-gel accumulations were noted in the biomaterial valleys, primarily attributed to irregularities stemming from Ti's shot-blasting treatment. However, no pores or QCT accumulations were detected in the sol-gel network.

Post-application assessments revealed that the sol-gel coating yielded surface roughness values lower than those exhibited by Ti (Table.1.) This

reduction can be attributed to the sol-gel's effective smoothing of imperfections introduced during Ti's shot-blasting treatment.

^{29}Si solid NMR spectrum is presented in Fig.2. This spectrum reveals two distinct types of bands: T^n bands, indicative of the presence of MTMOS, and Q^n bands, corresponding to the condensation bands of the TEOS alkoxysilane (both within the sol-gel network). The higher relative intensity of the T^3 peak with respect to the T^2 peak indicates a high degree of cross-linking of the network for the case of MTMOS, showing a correct condensation. For the case of TEOS, the results are very similar, being able to observe a high cross-linking given the intensity of the Q^3 and Q^4 signals. These findings collectively affirm the success on cross-linking of the sol-gel network.

In addition, the incorporation of QCT into the network was confirmed through UV release studies, as depicted in Fig.3. The controlled release profile illustrates a gradual and regulated liberation of QCT over time.

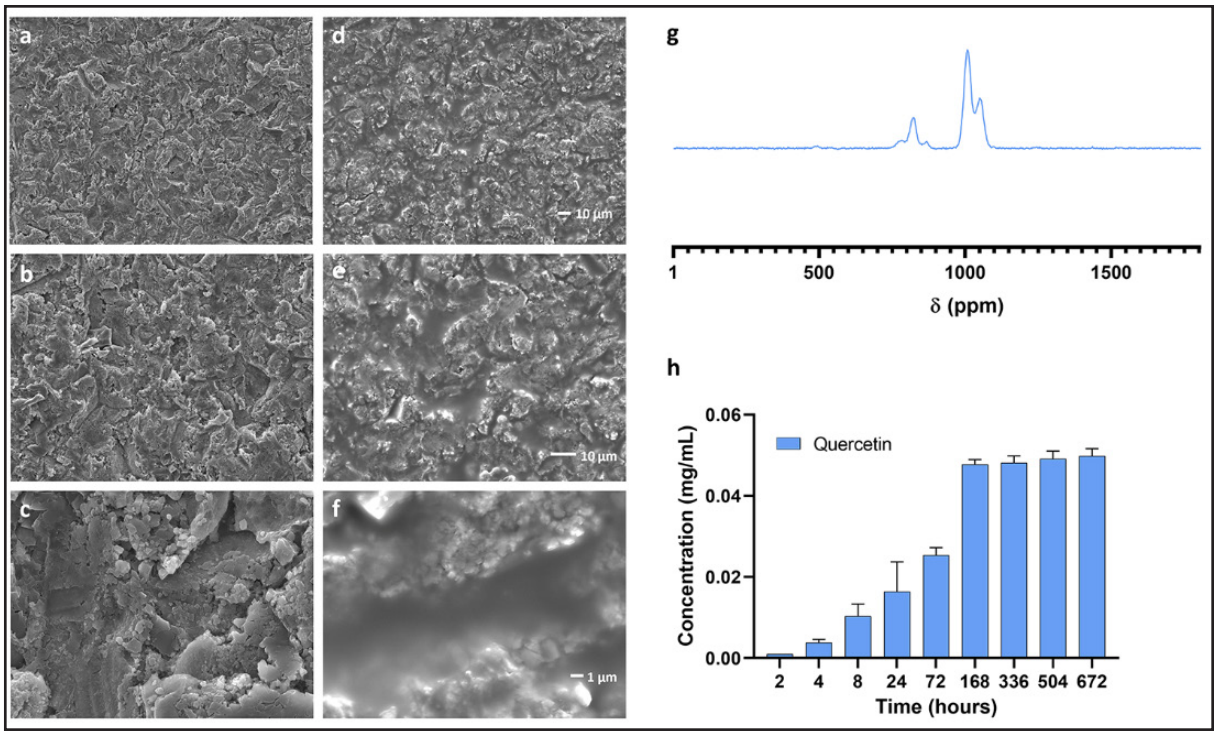


Fig.1. (a-f) SEM micrographs of Ti (a-c) and QCT sol-gel materials (d-f), images were purchased at x500 (a, d), x1000 (b, e) and x5000 (c, f). Scale bars: 10 µm for x500 and x1000, 1 µm for x5000. (g) ^{29}Si NMR spectra of QCT sol-gel materials. (h) UV release of QCT from the QCT sol-gel materials. Bars indicate standard errors.

	Ti	QCT sol-gel
WCA (°)	**Mean**	**Mean**
	79,55 ± 7,51	80,75 ± 1,657
Ra (µm)	**Mean**	**Mean**
	2,231 ± 0,338	0,933 ± 0,071

Table 1. Contact angle (WCA) results are shown as mean ± SD. Roughness measures (Ra) are also displayed as mean ± SD.

In vitro characterization

To assess the organization of the cytoskeleton, HOb cells were subjected to staining after a one-day culture period (Fig.2.). Phalloidin was utilized to highlight the cytoskeleton, depicted in green, while DAPI, incorporated into the mounting medium, was employed to stain the nucleus in blue. Remarkably, cells cultured on QCT sol-gel exhibited a significantly increased and more elongated cell area compared to those on Ti surfaces (Fig.2.b).

Proteomic evaluation

Table.2. summarizes the proteins identified as differentially adsorbed onto QCT sol-gel surfaces with respect to Ti surfaces. Based on key functions, these proteins could be classified by their roles on immune response, coagulation/fibrinolysis and cell adhesion.

Regarding the immune response, 22 proteins showed reduced affinity for QCT sol-gel surfaces. This cluster included (HPTR, SAMP, PRG4,

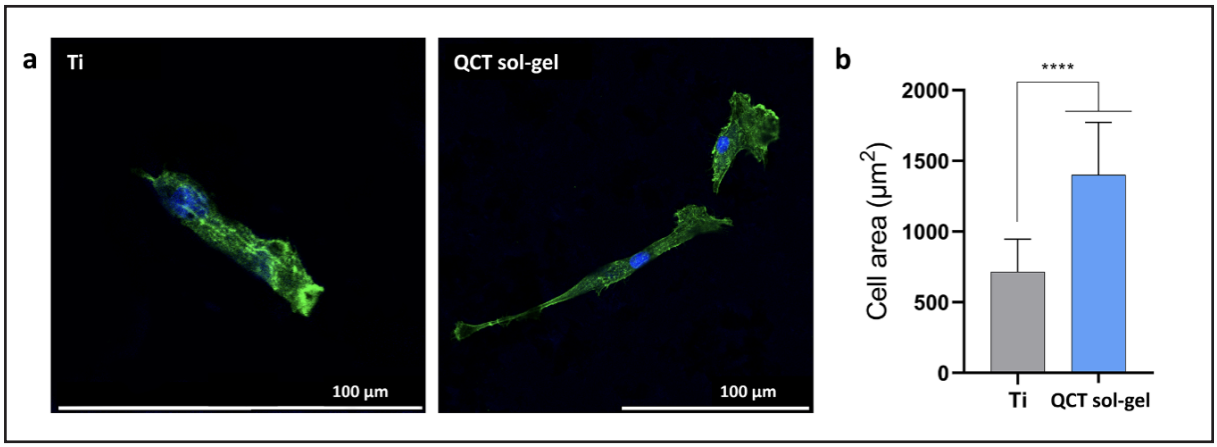


Fig.2. (a) Representative fluorescent confocal images of cytoskeleton arrangement of HOb on (a) Ti and QCT sol-gel. Actin filaments were stained with phalloidin (green), and nuclei were stained with DAPI (blue). Scale bars: 100 µm. (b) Area of the cells adhered to the materials. Results are shown as means ± SE. The asterisks $p \leq 0.0001$ (****) indicate statistically significant differences between Ti and QCT sol-gel.

Functional Group	**Protein Class**	
Immune response	UP	IGLL1, RARR2, CLUS
	DOWN	HPTR, LV140, HV118, KVD15, KV116, KVD16, KV105, KV106, SAMP, HV320, PRG4, MASP1, PIGR, LBP, KV117, HV373, SAA4, FCN2, FCN3, LECT2, C4BPA, C4BPB
Coagulation/fibrinolysis	UP	FIBA, HRG
	DOWN	PON1, HEP2, KLKB1, A2AP, KNG1, ANGI, IPSP, FIBG, ANT3, FA5, THRB, FA7, TFPI1, FA11, FA9, PLF4, FA10, PROC
Cell adhesion	UP	IBP2, COLA1, FBLN1, GELS
	DOWN	PCOC1, CADH1, ACTB, FBLN7, VTNC

Table 2. Differentially (UP/DOWN) adsorbed proteins on QCT sol-gel compared to Ti. 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: immune response, coagulation/fibrinolysis and cell adhesion.

MASP1, PIGR, LBP, SAA4, FCN2, FCN3, LECT2, SAMP, SAA4) and 10 immunoglobulins (LV140, HV118, KVD15, KV116, KVD16, KV105, KV106, HV320, KV117, HV373) were also found less adsorbed onto QCT sol-gel surfaces with respect to Ti. On the other hand, there was an increased adsorption of 3 proteins related to the immune system (IGLL1, RARR2, CLUS). Furthermore, there was a decrease in the adsorption of some proteins related to coagulation/fibrinolysis (PON1, HEP2, KLKB1, A2AP, KNG1, ANGI, IPSP, FIBG, ANT3, FA5, THRB, FA7, TFPI1, FA11, FA9, PLF4, FA10, PROC). Many of them associated with procoagulant effects belonging to the coagulation cascade, such as the coagulation factors (FA5, FA7, FA9, FA10 and FA11). On the other hand, QCT sol-gel surface showed an increased affinity for two proteins related to coagulation/fibrinolysis (FIBA, HRG). In terms of cell adhesion, QCT sol-gel surfaces exhibited a preferential adsorption for cell adhesion-associated proteins (IBP2, COLA1, FBLN1, GELS), while there was a decrease in the adsorption of (C4BPA, PCOC1, C4BPB, CADH1, ACTB, FBLN7, VTNC).

The differential proteins identified in the comparison QCT sol-gel vs Ti were classified according to their pathways using PANTHER (Fig.3.). PANTHER results show that downregulated pathways were classified as blood coagulation, plasminogen activating cascade, integrin signaling pathway, CCKR signaling map, Wnt signaling pathway, inflammation mediated by chemokine and cytokine, insulin IGF pathway, cadherin signaling pathway angiogenesis and cytoskeletal regulation by Rho GTPase. Conversely the upregulated pathways on the QCT sol-gel surfaces were classified as blood coagulation, plasminogen activating cascade, integrin signaling pathway, CCKR signaling map, FAS signaling pathway.

Discussion

Osseointegration, a complex process characterized by the direct structural and functional connection between living bone and the implant surface, has long been recognized to ensure implant success, ensuring implant stability and functionality[11]. While the challenges of obtaining optimal surface characteristics and promoting a microenvironment for bone integration persist[12], the sol-gel technique is gaining attention as a promising technology[13]. For this purpose, we have employed the sol-gel approach as release vehicle to enhance QCT anti-inflammatory properties[7] through the development of QCT sol-gel surfaces.

Although no significant differences in contact angle was observed in the comparison between Ti and QCT sol-gel surfaces, a significant reduction in roughness (Ra) was observed for the QCT sol-gel surfaces compared to Ti surfaces. Furthermore, ^{29}Si solid NMR spectrum revealed a good cross-linking of the network. Finally, UV release studies showed the presence and release of QCT in the sol-gel network, showing a controlled release in time, thus creating a prolonged effect. These

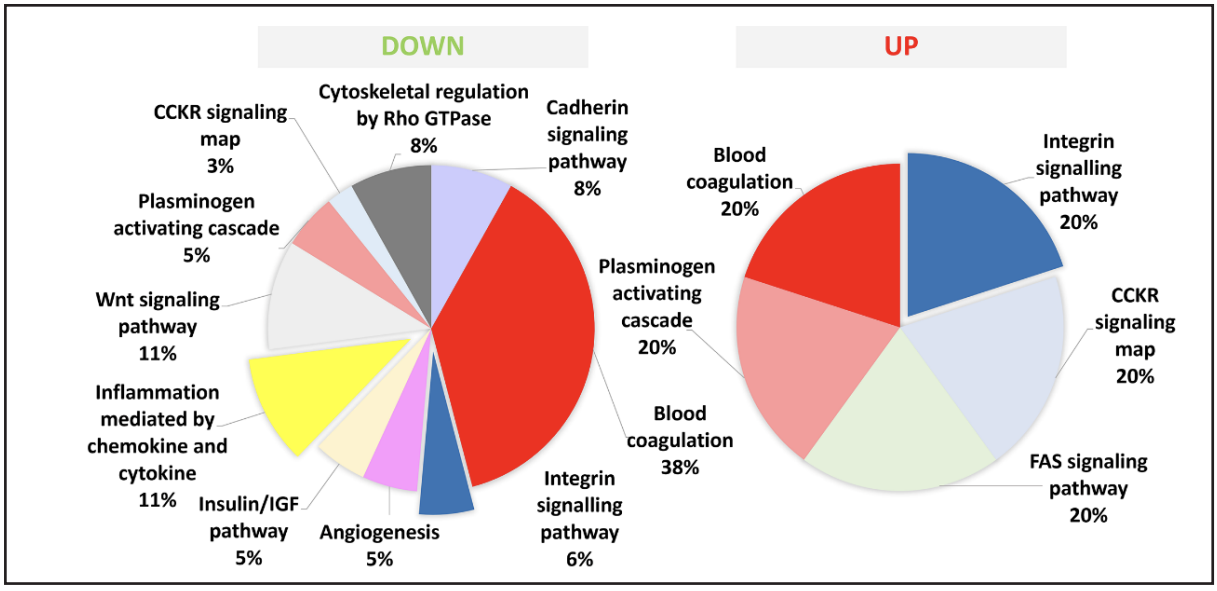


Fig.3. PANTHER pathway of the proteins identified as differentially adsorbed on Ti vs QCT sol-gel.

physicochemical changes can alter both protein adsorption and in vitro effects on HOb and therefore altering the microenvironment.

The osseointegration process is highly related to the immune response. A controlled immune response could be the key to a successful osseointegration[14]. The relationship between osseointegration and the immune response in dental implants is a complex and dynamic interplay that determines the success or failure of the implant integration process[15]. The immune response involves the body's defense mechanisms responding to the presence of foreign materials[16]. In this sense, proteomics is a powerful analytical approach that can be applied to study the biological effects of dental implants. Proteomic analysis revealed a cluster of proteins related to the immune response that were less adsorbed on QCT sol-gel surfaces compared to Ti surfaces. Among these, SAMP is intricately connected with the complement system, playing a role in inflammation, coagulation, tissue remodeling, and protection against bacterial infections[17]. While the precise function of SAMP remains elusive, Wang et al. investigated the levels of SAMP protein expression in both patients with periodontitis and healthy patients and found that the expression levels of SAMP was elevated in the periodontitis group in comparison to the healthy control group[18]. Furthermore, a reduced adsorption of proteins such as SAA4 was found for QCT sol-gel surfaces. SAA proteins, including SAA4, are a group of acute-phase proteins that are typically associated with the systemic inflammatory response. High levels of these proteins are related to an acute immune response, which could lead to the creation of an environment of chronic inflammation[19], thus worsening the osseointegration process.

In addition, 10 immunoglobulins (LV140, HV118, KVD15, KV116, KVD16, KV105, KV106, HV320, KV117, HV373) exhibited lower adsorption in QCT sol-gel surfaces compared to Ti surfaces. Immunoglobulins are glycoproteins produced by plasma cells that play a pivotal role in orchestrating the immune response. Elevated levels of immunoglobulins are linked to inflammatory responses triggered by infections from pathogens[20]. In addition to the effect of these proteins associated with a marked increase in the immune response, we also found a reduction in the adsorption of FCN2, FCN3 and MASP1 for QCT sol-gel surfaces compared to Ti surfaces, three proteins associated with the activation of the complex cascade via the lectin pathway[21]. The complement system is made up of many distinct plasma proteins that react with one another to opsonize pathogens and induce a series of inflammatory responses that help to fight infection. Therefore, the reduced response of all these proteins could represent an important reduction in the immune response for QCT sol-gel surfaces. On the other hand, a preferential adsorption by 2 proteins involved in the regulation of the immune response and the complement cascade[22] (RARR2, CLUS) was found for QCT sol-gel surfaces compared to Ti surfaces. In summary, through proteomics analysis, it was observed that for QCT sol-gel, there was a clear reduction in the levels of proteins associated with an acute immune response (SAMP, SAA4), 10 immunoglobulins (LV140, HV118, KVD15, KV116, KVD16, KV105, KV106, HV320, KV117, HV373), also responsible for an increase in the inflammatory load, as well as 3 proteins responsible for the activation of the complement cascade (FCN2, FCN3 and MASP1), while there was an increase in the adsorption of proteins related to the regulation of the immune response and the complement system (RARR2, CLUS). These findings collectively indicate a distinct decrease in the immune response observed in QCT sol-gel surfaces in comparison to Ti surfaces. This reduction has the potential to mitigate inflammation levels and foster the creation of an optimal environment conducive to the osseointegration of dental implants[23]. In addition, the PANTHER analysis showed a clear downregulation on the immune response functions related to differentially adsorbed proteins on QCT sol-gel surfaces.

Another key process in bone regeneration is coagulation. 18 proteins (PON1, HEP2, KLKB1, A2AP, KNG1, ANGI, IPSP, FIBG, ANT3, FA5, THRB, FA7, TFPI1, FA11, FA9, PLF4, FA10, PROC) related to coagulation processes were found less adsorbed on QCT sol-gel surfaces. On the other hand, two proteins also associated with coagulation were preferentially adsorbed on QCT sol-gel surfaces, FIBA, and HRG, a protein in charge to prevent the creation of heparin-antithrombin III complexes, thus impeding the inhibition of activated coagulation factors[24]. It is not uncommon to observe a remarkable reduction of coagulation-associated proteins as well as a downregulation in blood coagulation associated pathway showed on PANTHER results, given the clear reduction of immune-related proteins for QCT sol-gel surfaces since, as is well known, the processes of coagulation and immune response are intimately related[25], therefore should be normal to observe this reduction of coagulation-related proteins.

The success of an implant hinges on various factors. Among these is the controlled immune response, and equally crucial is the cellular adhesion of osteoblasts to the surfaces of the engineered materials, playing a pivotal role in determining osseointegration success[26]. Although proteomic analysis revealed a decrease in the adsorption of PCOC1, a protein in charge to enhance procollagen C-proteinase activity, VTCN and CADH1, both related to cell adhesion and two proteins related to cytoskeleton arrangement (ACTB, FBLN7). There was an increase in the adsorption of COLA1,

a crucial component of the extracellular matrix within bone tissue, recognized for play an essential role in the maturation of osteoblasts[27]. Also, there was an increase adsorption of two proteins related to cytoskeleton arrangement (GELS, FBLN1) and a protein related to either inhibit or stimulate the growth promoting effects of the insulin growth factors (IGFs) on cell culture and could potentially interact with integrins, facilitating and encouraging cell migration[28]. On the other hand, in vitro results, obtained through confocal microscopy, showed more elongated HOb cells on QCT sol-gel surfaces, resulting in an increased cell area. QCT sol-gel surfaces, showed significantly enhanced cell spreading compared to Ti surfaces. Also, panther analysis showed the integrin signaling pathway associated to the proteins adsorbed in our materials in both up and down regulated proteins.

However, although the developed material gives promising results regarding immune response and HOb adhesion to QCT sol-gel surfaces, in vivo studies are needed to create a surface applicable to the field of dental implantology.

Conclusion

To conclude, QCT sol-gel coatings were synthesized correctly, obtaining stable surfaces with a controlled release of QCT. By proteomics assays, a considerable reduction in the number of proteins related to immune response and complement system activation was observed for the QCT-sol-gel surfaces. This reduction was also accompanied by a reduction in coagulation-related proteins, with promising results. In addition, the QCT sol-gel surfaces also led to an improvement in HOb cell adhesion to these surfaces, thus creating a favorable environment for osseointegration due to this reduction in immune response and improvement in cell adhesion.

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Bibliography

1. **Inchingolo AM, Malcangi G, Ferrante L, et al.** Surface Coatings of Dental Implants: A Review. J Funct Biomater. 2023;14(5). doi:10.3390/jfb14050287
2. **Romero-Gavilan F, Araújo-Gomes N, Sánchez-Pérez AM, et al.** Bioactive potential of silica coatings and its effect on the adhesion of proteins to titanium implants. Colloids Surf B Biointerfaces. 2018;162:316-325. doi:10.1016/j.colsurfb.2017.11.072
3. **Fernández-Hernán JP, Torres B, López AJ, Rams J.** The Role of the Sol-Gel Synthesis Process in the Biomedical Field and Its Use to Enhance the Performance of Bioabsorbable Magnesium Implants. Gels. 2022;8(7). doi:10.3390/gels8070426
4. **Oshiro JA, Abuçafy MP, Manaia EB, Da Silva BL, Chiari-Andréo BG, Chiavacci LA.** Drug delivery systems obtained from silica based organic-inorganic hybrids. Polymers (Basel). 2016;8(4). doi:10.3390/polym8040091
5. **Cerqueira A, Romero-Gavilán F, Araújo-Gomes N, et al.** A possible use of melatonin in the dental field: Protein adsorption and in vitro cell response on coated titanium. Materials Science and Engineering C. 2020;116. doi:10.1016/j.msec.2020.111262
6. **Romero-Gavilán F, Araújo-Gomes N, Cerqueira A, et al.** Proteomic analysis of calcium-enriched sol–gel biomaterials. Journal of Biological Inorganic Chemistry. 2019;24(4):563-574. doi:10.1007/s00775-019-01662-5
7. **Wong SK, Chin KY, Ima-Nirwana S.** Quercetin as an agent for protecting the bone: A review of the current evidence. Int J Mol Sci. 2020;21(17):1-37. doi:10.3390/ijms21176448
8. **Meretoja V V., Rossi S, Peltola T, Pelliniemi LJ, Närhi TO.** Adhesion and proliferation of human fibroblasts on sol-gel coated titania. J Biomed Mater Res A. 2010;95(1):269-275. doi:10.1002/jbm.a.32829

9. **Spaans S, Fransen PPKH, Ippel BD, et al.** Supramolecular surface functionalization: Via catechols for the improvement of cell-material interactions. Biomater Sci. 2017;5(8):1541-1548. doi:10.1039/c7bm00407a
10. **Romero-Gavilan F, Sánchez-Pérez A, Araújo-Gomes N, et al.** Title Proteomic Analysis of Silica Hybrid Sol-Gel Coatings: A Potential Tool for Predicting Biocompatibility of Implants in Vivo.
11. **Parithimarkalaignan S, Padmanabhan T V.** Osseointegration: An update. Journal of Indian Prosthodontist Society. 2013;13(1):2-6. doi:10.1007/s13191-013-0252-z
12. **Accioni F, Vázquez J, Merinero M, Begines B, Alcudia A.** Latest Trends in Surface Modification for Dental Implantology: Innovative Developments and Analytical Applications. Pharmaceutics. 2022;14(2). doi:10.3390/pharmaceutics14020455
13. **Fernández-Hernán JP, Torres B, López AJ, Rams J.** The Role of the Sol-Gel Synthesis Process in the Biomedical Field and Its Use to Enhance the Performance of Bioabsorbable Magnesium Implants. Gels. 2022;8(7). doi:10.3390/gels8070426
14. **Albrektsson T, Tengvall P, Amengual L, Coli P, Kotsakis GA, Cochran D.** Osteoimmune regulation underlies oral implant osseointegration and its perturbation. Front Immunol. 2023;13. doi:10.3389/fimmu.2022.1056914
15. **Supra R, K Agrawal D.** Innate Immune Response in Orthopedic Implant Failure. Journal of Orthopaedics and Sports Medicine. 2023;05(01). doi:10.26502/josm.511500073
16. **Amengual-Peñafiel L, Córdova LA, Constanza Jara-Sepúlveda M, Brañes-Aroca M, Marchesani-Carrasco F, Cartes-Velásquez R.** Osteoimmunology drives dental implant osseointegration: A new paradigm for implant dentistry. Japanese Dental Science Review. 2021;57:12-19. doi:10.1016/j.jdsr.2021.01.001
17. **Doni A, Parente R, Laface I, et al.** Serum amyloid P component is an essential element of resistance against Aspergillus fumigatus. Nat Commun. 2021;12(1). doi:10.1038/s41467-021-24021-y
18. **Wang L, Liang D, Huang Y, et al.** SAP deficiency aggravates periodontitis possibly via C5a-C5aR signaling-mediated defective macrophage phagocytosis of Porphyromonas gingivalis. J Adv Res. Published online October 2022. doi:10.1016/j.jare.2022.10.003
19. **P. Azevedo E, Foguel D.** The Role of Inflammation in Amyloid Diseases. In: Amyloid Diseases. IntechOpen; 2019. doi:10.5772/intechopen.81888
20. **Schroeder HW, Cavacini L.** Structure and function of immunoglobulins. Journal of Allergy and Clinical Immunology. 2010;125(2 SUPPL. 2). doi:10.1016/j.jaci.2009.09.046
21. **Endo Y, Matsushita M, Fujita T.** The role of ficolins in the lectin pathway of innate immunity. International Journal of Biochemistry and Cell Biology. 2011;43(5):705-712. doi:10.1016/j.biocel.2011.02.003
22. **Chauhan AK, Moore TL.** Presence of plasma complement regulatory proteins clusterin (Apo J) and vitronectin (S40) on circulating immune complexes (CIC). Clin Exp Immunol. 2006;145(3):398-406. doi:10.1111/j.1365-2249.2006.03135.x
23. **Amengual-Peñafiel L, Brañes-Aroca M, Marchesani-Carrasco F, Jara-Sepúlveda MC, Parada-Pozas L, Cartes-Velásquez R.** Coupling between osseointegration and mechanotransduction to maintain foreign body equilibrium in the long-term: A comprehensive overview. J Clin Med. 2019;8(2). doi:10.3390/jcm8020139
24. **Cerqueira A, García-Arnáez I, Muriach M, et al.** The effect of calcium-magnesium mixtures in sol-gel coatings on bone tissue regeneration. Biomater Sci. 2022;10(19):5634-5647. doi:10.1039/d2bm00742h
25. **Burzynski LC, Humphry M, Pyrillou K, et al.** The Coagulation and Immune Systems Are Directly Linked through the Activation of Interleukin-1α by Thrombin. Immunity. 2019;50(4):1033-1042.e6. doi:10.1016/j.immuni.2019.03.003
26. **Chen S, Guo Y, Liu R, et al.** Tuning surface properties of bone biomaterials to manipulate osteoblastic cell adhesion and the signaling pathways for the enhancement of early osseointegration. Colloids Surf B Biointerfaces. 2018;164:58-69. doi:10.1016/j.colsurfb.2018.01.022
27. **Zhang Y, Wang L, Kang H, Lin CY, Fan Y.** Dose-dependent tandem responses of osteoblasts during osteogenesis. Med Nov Technol Devices. 2023;18. doi:10.1016/j.medntd.2023.100227
28. **Feng N, Zhang Z, Wang Z, et al.** Insulin-Like Growth Factor Binding Protein-2 Promotes Adhesion of Endothelial Progenitor Cells to Endothelial Cells via Integrin α5β1. Journal of Molecular Neuroscience. 2015;57(3):426-434. doi:10.1007/s12031-015-0589-3