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All outputs in CLoK are protected by Intellectual Property Rights law, including Copyright law. Copyright, IPR and Moral Rights for the works on this site are retained by the individual authors and/or other copyright owners. Terms and conditions for use of this material are defined in the <u>http://clok.uclan.ac.uk/policies/</u> Possible anti-inflammatory property of bioactive glass – an exciting material in implant

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Introduction

Bioactive glass (BG) is a widely used bone graft material for the regeneration of bone defects caused by trauma or various diseases [1]. Variations of BG such as 45S5 and S53P4 are FDA approved biomaterials that have been investigated as coatings over orthopaedic, and more recently, dental implants [2-4]. In Implant Dentistry, a common issue that has deleterious impact on implant failure is periimplantitis, which is characterised by aseptic inflammation in the peri-implant mucosa due to the release of metallic particles and ions from the implants [5, 6].

Titanium dioxide particles of Grade 5 titanium alloy (Ti-6AI-4V) implants in particular have been reported both *in vitro* and in animal models to induce cellular toxicity as well marked upregulation of pro-inflammatory markers such as interleukin 1 beta (IL-1 β), IL-6 and tumour necrosis factor alpha (TNF- α), leading to inflammation-induced osteoclastogenesis and periprosthetic osteolysis [7-9]. The increase in pH, as the result of BG dissolution, contributes to their anti-microbial properties [1, 10]. However, the effects of BG on peri-implant cell populations and inflammatory cells remain unclear. Previous studies reported that in the presence particulate 45S5 BG, human macrophages secreted lower pro-inflammatory cytokine such as IL-6 and TNF- α when compared to zinc phosphate glasses, suggesting BG might be of clinical use in conditions associated with inflammation [11, 12].

Authors hypothesise that S53P4 BG also possess anti-inflammatory effects on not only macrophages, including the cytokine secretion and polarisation, but also periimplant cell populations such as gingival fibroblasts, osteoblasts and bone marrow derived stromal cells.

Materials and methods

All chemicals and reagents were purchased from Merck Life Science (Dorset, UK) or ThermoFisher Scientific (Paisley, UK)

BG synthesis and dissolution product preparation

Prior to melting, powder mixture of the following chemical composition were prepared: high purity silica (SiO₂, 79.45 mol %), calcium carbonate (CaCO₃, 53,62 mol %), sodium carbonate (NaCO₃, 59.13 mol %) and phosphorous pentoxide (P₂O₅, 5.94 mol %). The mixture were melted at 1400 °C for 1.5 h in a Pt-5%Au crucible. The melt was quenched into deionised water, the frit was collected and dried at 100 °C. Frit was ball milled and sieved to yield particles with diameters less than 32 μ m. Dissolution products were prepared by submerging S54P5 BG at a ratio of 75 mg to 50 ml in relevant cell culture medium for 72 h on an orbital roller at 37 °C. Dissolution products were filter sterilised priot to use in cell culture.

Cell culture

Human gingival fibroblasts (HGFs, PCS-201-018[™], ATCC, UK), SaOS₂ human osteoblastic cell line (HTB-85[™], ATCC, UK) were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10 % (v/v) foetal bovine serum (FBS), 1 % (v/v) P-S (100 unit.mL⁻¹ penicillin and 100 µg.mL⁻¹ streptomycin). Human bone marrow derived stromal cells (HBMSCs, 2M-302, Lonza, UK) were cultured in Minimum Essential Medium (MEM) with 10 % (v/v) FBS and 1 % (v/v) P-S.

THP-1 human monocytic cell line was cultured in Roswell Park Memorial Institute medium (RPMI-1640) with 10 % (v/v) non-heat inactivated FBS and 50 pM β -mercaptoethanol (BME). THP-1 monocytic cells were differentiated into macrophages by 24 h incubation with 150 nM phorbol 12-myristate 13-acetate (PMA) and 24 h incubation with RPMI-1640.

HGFs, SaOS₂, HBMSCs and THP-1 derived macrophages were cultured in the presence of Ti-CP4 (commercially pure titanium), Ti-6AI-4V (grade 5 titanium alloy), Ti-15Zr (titanium-zirconium alloy) or Zr (zirconia) particles (~ 32μ m) at a ratio of 1.5 mg to 1 mL medium with or without S53P4 dissolution products for 24 hours.

Immunohistochemical staining

2.5 × 10⁴ THP-1 cells were seeded and differentiated into macrophages in ibidi[®] microscopy μ -Dish (Thistle Scientific, Glasgow, UK) as described above.

Undifferentiated THP-1 cells were fixated on microscopy μ -Dish by drying 100 μ L PBS containing 2.5 × 10⁴ cells. Samples were fixed in 4% paraformaldehyde in PBS (phosphate buffered saline). Following wash and blocking with 1 % (w/v) BSA (bovine serum albumin) in PBS, samples were incubated at 4 °C overnight with following primary antisera from ThermoFisher Scientific (Paisley, UK): CD14 (MA5-35200, 1:100 dilution), CD36 (PA1-16813, 1:100 dilution) and CD68 (PA5-32330, 1:100 dilution). This was followed by hour-long incubation with Alexa Fluor[®] 488-conjugated secondary antibody (1:1000 dilution). All samples were counter-stained with 0.1 μ g.mL⁻¹ DAPI and imaged under Leica SP5 confocal microscope.

Enzyme-linked immunosorbent assay (ELISA)

The release of cytokines of interest in culture medium was quantified using ELISA kits following manufacturers' instructions. The following ELISA kits were used: TNF- α (ADI-902-099, Enzo Life Sciences, UK), IL-1 β (ADI-900-130, Enzo Life Sciences, UK), IL-6 (ENZ-KIT178-0001, Enzo Life Sciences, UK) and mannose receptor (MMR/CD206, ThermoFisher Scientific, Paisley, UK).

Statistical analysis

Results were presented as mean \pm S.D. Non-parametric test with Kruskal-Wallis test (for multiple groups) were performed using GraphPad Prism. Results were deemed significant if the probability of occurrence by random chance alone was less than 5 %.

Results

Grade 5 titanium alloy Ti-6AI-4V implant particles significantly induced the secretion of TNF-a, IL-1 and IL-6 cytokines in HGFs, SaOS2 and HBMSCs (Figure 1). Ti-CP4, Ti-15Zr and Zr implant particles appeared to certain pro-inflammatory markers in certain cell type, albeit not statistically significant. The presence of S53P4 dissolution product reduced the elevated level of all 3 pro-inflammatory markers induced by Ti-6AI-4V implant particles to a level comparable to basal control. Human THP-1 monocytic cells were differentiated into macrophages using 150 nM PMA. Suspension THP-1 cells became adherent and, the expression of CD14 decreased while CD36 and CD68 increased, confirming the macrophage differentiation (Figure 2). In the presence of Ti-6AI-4V implant particles, typical pro-inflammatory/M1 marker TNF-a was significantly increased (Figure 3a) while anti-inflammatory/M2 marker CD206 was significantly decreased (Figure 3b). The addition of S53P4 dissolution product suppressed the elevation of TNF-a and reduction of CD206. All other implant particles did not appear to affect macrophage polarisation.

The mechanism through which BG stimulates and interacts with these cells has not been fully understood and further investigations are needed.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contribution

FB: conceptualisation, investigation, data curation, writing - original draft preparation, writing - reviewing and editing; SL: conceptualisation, methodology, investigation, data curation, validation, visualization, writing - original draft preparation, writing - reviewing and editing; JRJ: funding acquisition, supervision, writing - reviewing and editing.

For polarisation controls, M1 macrophages were generated by incubation with 20 ng.mL⁻¹ interferon gamma (IFN-γ, R&D Systems, Abingdon, UK) and 10 pg.mL⁻¹ lipopolysaccharides (LPS) while M2 macrophages were generated incubation with 20 ng.mL⁻¹ of interleukin 4 (IL4, R&D Systems, Abingdon, UK) and interleukin 13 (IL13, R&D Systems, Abingdon, UK).

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