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# Osteostimulative calcium phosphosilicate biomaterials partially restore the cytocompatibility of decontaminated titanium surfaces in a peri-implantitis model

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**Abstract:**
Bacterial peri-implant biofilms, and the chemotherapeutics for their removal alter titanium surface cytocompatibility. In this study we aimed to assess the adjunctive use of an osteostimulative biomaterial utilizing a peri-implantitis model under the hypothesis that it will increase cell migration towards treated titanium surfaces. Acid-etched titanium surfaces were inoculated with a multi-species biofilm model and treated with 1.5% NaOCl in a previously characterized *in vitro* peri-implantitis model. Cell migration of MG63 cells towards the treated titanium surface (CTRL) was significantly reduced following inoculation with biofilm and chemotherapeutic treatment as compared to sterile controls. Addition of a tricalcium phosphate biomaterial (TCP) as a control for $Ca^{+2}$ had a small non-significant effect, while BG significantly increased MG63 chemotaxis to titanium to levels comparable to sterile (STE). Similarly, cell viability at 5 days was increased in BG and TCP as compared to CTRL. SEM imaging confirmed the improved cytocompatibility of BG and TCP surfaces as compared to CTRL. Osteostimulative BG exhibited a strong chemotactic effect to osteoblasts, which was stronger than what was expected due to the chemotactic effect of $Ca^{+2}$ alone (TCP). In addition, substantially increased cell attachment and viability was found on treated implant surfaces as compared to CTRL. © 2018 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2018.

**Key Words:** calcium phosphosilicate, bioactive glass, titanium, osteoblast, peri-implantitis, dental

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## INTRODUCTION

Titanium dental implants are the premier treatment option for the rehabilitation of edentulism enjoying excellent initial success rates.[1] However, recent epidemiologic data have raised awareness for the alarming prevalence of peri-implantitis, which ranges from 13 to 26% on a person level.[2,3] Peri-implantitis is an inflammatory process around implants, which is associated to peri-implant bone loss and patient-perceived discomfort.[2,3] Its primary etiology is considered to be bacterial, thus treatment strategies are based on the removal of implant-bound biofilms in an effort to allow for bone regeneration in direct contact with the treated titanium implant surfaces, that is, reosseointegration. Nonetheless, existing treatment approaches mostly yield only short-term therapeutic benefits with some interventions showing up to 100% recurrence of disease after 12 months.[4] The majority of these treatment approaches have been based upon antibacterial assays to determine decontamination strategies that can maximize biofilm removal from titanium implant surfaces.[5] Importantly, recent data have revised our nascent understanding of the peri-implantitis healing cascade by demonstrating that antibacterial treatment strategies can alter titanium surface cytocompatibility.[6]

Preclinical studies utilizing multispecies biofilm models of peri-implantitis have consistently highlighted the uncoupling between: (1) biofilm removal, and (2) favorable healing response around titanium surfaces.[6,7] For instance, the use of chlorhexidine, an established antibacterial agent for gingivitis, is contraindicated for use in the treatment of peri-implantitis due to its adsorption on titanium surfaces and its deleterious effect on osteoblastic cell attachment on

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titanium.[6] In fact, physicochemical alterations of the titanium surface due to bacterial contamination[8] and/or treatment interventions[6] may be more influential than residual bacteria are for the cytocompatibility of titanium following surface treatment. Osteoconductive biomaterials are often used in conjunction with surgical peri-implantitis treatment to enhance bone regeneration in direct contact with the affected titanium implant surfaces. However, the clinical benefit from their use is small. Schou et al.[9] found that the addition of an osteoconductive anorganic bovine bone biomaterial in peri-implantitis defects in cynomolgus monkeys had a positive effect on reosseointegration, however, subpar to the effect of autogenous bone grafting. The peri-implant defect is a challenging environment for regeneration to occur given the reduced cytocompatibility of the treated titanium surface and the limited blood supply due to the presence of avascular metal component in one wall of the defect. Biomaterials that can enhance osteoblast chemotaxis and differentiation have been considered as treatment adjuncts to make the local microenvironment favorable for successful bone regeneration.[10]

Bioactive glass (BG) biomaterials have been extensively investigated for enhancing osseointegration of titanium implants owing to their osteostimulative properties, which are unique among alloplastic bone substitutes.[11–13] BG is primarily composed of silica ($SiO_2$), calcium, sodium, and phosphorus and forms a silica-rich hydroxyl carbonated apatite that resembles the bone's hydroxyapatite when in contact with biological fluids.[14] This apatite layer has a strong affinity for growth factors that interact with local macrophages which exhibit a strong chemical gradient for osteoblast progenitor cells.[14] In a series of investigations Xynos et al.[15–17] demonstrated that bioglass enhanced osteoblast proliferation by 150% and promoted osteoblast commitment and differentiation. Therefore, the potential addition of BG in the peri-implantitis treatment repertoire to increase the influx of osteoblasts and stimulate their activity warrants further investigation. In fact, Foppiano et al.[12] reported that BG significantly enhanced the cytocompatibility of sterile titanium alloys through promoting Runx2-mediated osteoblastic differentiation. Yet, the osteostimulatory effect of BG biomaterials on titanium surfaces that have undergone disease-related contamination remains unexplored. To determine whether the osteostimulatory effect of BG biomaterials can enhance the cytocompatibility of previously contaminated titanium implant surfaces we utilized an Food and Drug Administration (FDA) approved inorganic calcium phosphosilicate, thermally formed and bound together in a sodium silicate network (Novabone Morsels, Novabone, Alachua, FL).

This study tests the hypothesis that BG ions enhance cell migration toward the treated titanium surface and restore its cytocompatibility. To assess this hypothesis we utilized a previously validated *in vitro* peri-implantitis model[6] under the following aims: (1) to demonstrate that BG ions increase osteoblastic cell migration toward the treated titanium surface in addition to what is expected from a $Ca^{+2}$ gradient alone; (2) assess whether BG ions affect osteoblastic cell attachment and viability on the treated titanium surfaces.

## METHODS

### Peri-implant biofilm model

For all experiments we utilized a previously characterized *in vitro* peri-implantitis model. In brief, clinically relevant microrough titanium surfaces (Bioner Implants, Barcelona, Spain) were inoculated with a multispecies biofilm from peri-implantitis plaque samples. The original inoculum was obtained from a submucosal site with clinically confirmed peri-implant disease and expanded in basal mucin medium (BMM)[18] in anaerobic conditions as previously described.[6] For inoculation of disks we resuspended frozen stocks of the expanded samples in BMM and cultured anaerobically overnight at 37°C. After overnight incubation we standardized the approximate number of bacteria in the liquid culture by measuring the optical density at 600 nm (OD600) and diluted to OD600 = 0.2 for standardizing the inoculum per sample. The titanium disks were then placed in 48-well plates with 1 mL of inoculum per disk and cultured anaerobically for 48 h at 37°C (refreshed media once at 24 h with sterile BMM). This approach led to reliable disk coverage with a multilayered biofilm consisting of >30 oral taxa as described elsewhere.[6]

### Decontamination

The ideal outcome of peri-implantitis treatment is "reosseointegration."[8] Reosseointegration refers to the regeneration of the alveolar bone that was lost as part of the disease process in direct relation to a previously contaminated titanium surface. One of the most critical steps of peri-implantitis therapy is the decontamination of the biofilm-covered titanium surface to establish a surface compatible with health that can be repopulated with osteoblasts, that is, can be reosseointegrated. Based on our preliminary work we utilized a clinically relevant chemotherapeutic agent for titanium surface decontamination across all groups to avoid confounding by varying chemotherapeutic agent use; following biofilm inoculation, all experimental samples were immediately burnished with 1.5% NaOCl, as previously described.[6] Prior to seeding cells for all assays the titanium disks were rinsed twice with sterile water, dried and autoclaved in 120°C for 30 min. Identical, sterile titanium disks served as positive controls.

### Experimental groups

We utilized a calcium phosphosilicate particulate biomaterial (BG) owing to its osteostimulative properties. Briefly, osteostimulation is biomaterial property unique to BG that is defined by FDA as the active stimulation of osteoblast proliferation and differentiation as evidenced by increased levels of deoxyribonucleic acid (DNA) synthesis and the expression of osteoblast-related proteins, such as osteocalcin and alkaline phosphatase.[15,17,19] To assess whether any additional advantage of this biomaterial was due to osteostimulation, or due to simply providing a source of $Ca^{+2}$, we used a commercially available tricalcium phosphate (ß-TCP) biomaterial (TRT granules Streptodont, France) as a clinically relevant control.[17]

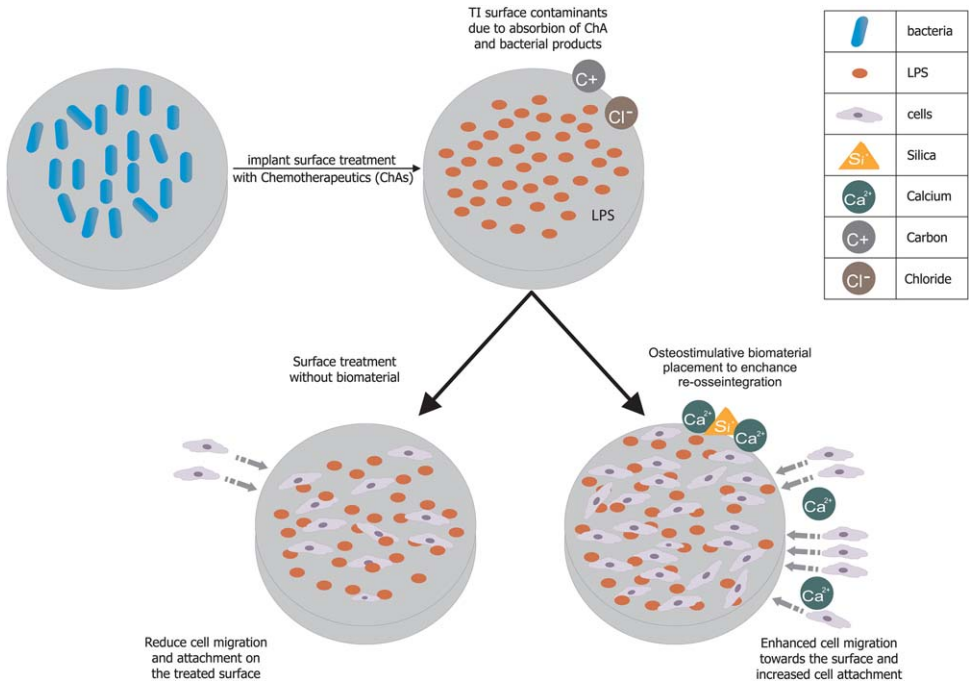


**FIGURE 1**. Experimental model.

Experimental workflow is described in Figure 1. For all experiments each titanium disk was seeded with 50 mg of each biomaterial. The following experimental and control groups were utilized:

- Sterile disks
- Disks with biofilm,treated with 1.5% NaOCl (CTRL)
- CTRL + ß-TCP (TCP)
- CTRL + BG (BG)
- Tissue culture plate (CP)

### Cell cultures

MG-63osteoblast-like cells were cultured using Dulbecco's modified eagle medium low glucose and phenol free supplemented with 10% fetal bovine serum (Biochrom, Germany) and 1% penicillin–streptomycin according to the instructions provided by the manufacturer. All experiments were performed with cells of the fourth passage in the Tissue Culture Laboratory, Department of Periodontics, National and Kapodistrian University of Athens. Unless otherwise noted all supplies were from Invitrogen. Experimental groups included decontaminated disks (10 mm diameter) with or without a biomaterial as described above. Sterile titanium disks served as controls. The experiments were performed in 48-well tissue CPs.

### Cell migration

For cell migration, 6.5 mm transwells with 8.0 μm pore polycarbonate filter (Transwell®, Corning) were used and cells were seeded at densities of 10,000 cells/cm$^2$ in the top chamber. One hundred microliter of cell solution was placed on top of the filter membrane in the transwell insert and incubated for 2 h at 37°C and 5% $CO_2$. The inserts were then positioned in the wells, each containing an experimental disk randomly allocated to the groups reported above. After 6 h of incubation in standard conditions the transwells were removed and the wells were incubated with 5 μg/mL of methylthiazolyldiphenyl-tetrazolium bromide (MTT) reagent. The number of cells that migrated through the inserts was reported as values of absorbance of the formazan product measured through MTT assay at 570 nm of a standard curve.

### Cell attachment

Following cell migration, we then assessed the attachment of MG63 on the titanium surfaces at 3 and 5 days. Cells were fixed with 2% glutaraldehyde in a sodium cacodylate 1*M* solution in preparation for scanning electron microscopic imaging (SEM). Fixed cells were incubated in a solution of osmium tetroxide in 1% sodium cacodylate for 60 min at 4°C and were then dehydrated in ascending concentrations of ethanol up to 100%. The samples were then mounted on SEM stubs, coated with a gold-palladium mixture and observed under SEM microscope at 20 kV (TM3030Plus, Hitachi, Krefeld, Germany).

### Cell viability

In order to detect cell viability, cells were seeded at densities of 5000 cells/cm$^2$ on the titanium disks. After five days of culture all the experimental disks were double stained with Fluorescein diacetate and propidium iodide stains (FDA/PI) (Sigma Aldrich). FDA/PI solution was added to stain live and dead cells, respectively, and solution was allowed to stand at room temperature for 5 min in the dark. An inverted fluorescence microscope (OLYMPUS IX71-12FL/PH-DP70, Olympus, Tokyo, Japan) was utilized to observe the stained cells.

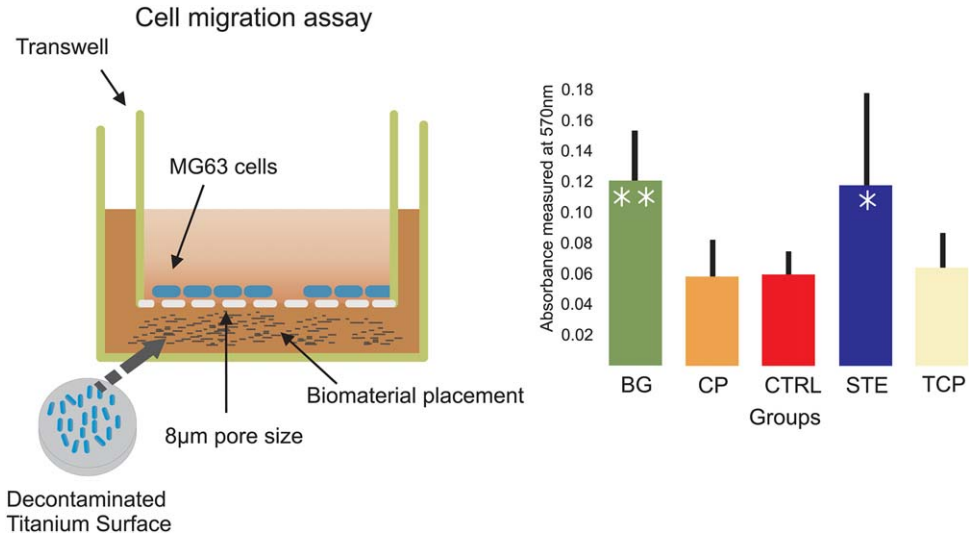


**FIGURE 2**. BG dissolution products increase osteoblastic cell migration toward the treated titanium surface in addition to what is expected from a $Ca^{+2}$ gradient alone.

### Gene expression analysis

MG-63 cells were seeded in 48 well-plates at a density of 10,000 cells/$mm^2$ on titanium disks treated according to the experimental groups described above (BG, TCP, STE, CTRL). Ribose nucleic acid (RNA) was extracted using Ultra-Clean Tissue and Cells RNA Isolation Kit (MoBio) according to the manufacturer protocol at 7, 14 and 21 days. RNA was then quantified with a bio-nanospectrophotometer (Shimatzu, Japan) and samples were kept at −80°C until further processing. Complementary DNA (cDNA) was performed using the Protoscript II first strand cDNA synthesis kit and was stored at −20°C until gene expression analysis.

Finally, quantitative reverse transcription polymerase chain reaction (PCR) reaction was performed using the universal SYBR Green Supermix (Biorad) and primers specific for osteogenic differentiation. Reactions were run in duplicates of 20 μL with the use of Biorad IQ5 real time PCR cycler (Biorad). For DNA amplification we performed 1 cycle at 95°C for 5 min, followed by 40 cycles of heating at 95°C for 30 s and at 60°C for 30 s for annealing/extension and 51 cycles of 0.5°C increase/cycle for melt curve analysis. The expression was normalized to glyceraldehyde 3-phosphate dehydrogenase and analysis was performed using the Delta-Delta CT (DDCt) method for relative quantification in relation to control group. Amplification curves were obtained from each sample in order to calculate the cycle threshold (Ct) and melt curves to verify specific product formation (Supporting Information, Figure S1)

### Statistical analysis

Summary statistics were expressed as mean ± standard error. One way analysis of variance (alpha = 0.05) with *post hoc* testing was utilized to determine between-group differences. Statistical analysis was performed using JMP Pro 12 (SAS). All experiments were performed in at least duplicates.

## RESULTS

### Cell migration

To determine if the calcium phosphosilicate osteostimulative particles stimulated the migration of osteoblastic cells toward the decontaminated titanium surfaces we performed transwell migration assays using a 8.0 μm transwell filter as illustrated in Figure 2(A). Cell migration assays revealed that inoculation of the surface with biofilm and subsequent treatment for biofilm removal (i.e., CTRL group) decreased the number of osteoblastic cells migrating toward the titanium surface by twofold as compared to pristine titanium surfaces.[3] In fact, the migratory pattern of cells toward CTRL surfaces was similar to CP controls without titanium [Figure 2(B)]. The presence of calcium and phosphate ions in the bottom chamber following the provision of ß-TCP particles (TCP group) seeded on the treated titanium surfaces provided a nonsignificant increase in the migratory trajectories of the MG63 cells toward the titanium surface as compared to controls. However, the presence of BG dissolution products in the lower chamber of the transwell model provided strong chemotactic stimuli to the MG63 cells as evidence to significant increase of cell migration toward the treated titanium surface in the BG group, which was in addition to what was noted due to a $Ca^{+2}$ gradient alone in the TCP group. Results in Figure 2(A) show that seeding of BG particles on the previously treated Ti surfaces restored their chemotactic abilities toward MG63 cells to levels comparable to that of sterile titanium.[3]

### Cell attachment

Cell attachment was assessed through SEM. The morphology of representative cells attached onto each surface at 3 and 5 days is displayed in Figure 3(A). After 3 days in culture the morphology of cells and extent of surface coverage varied across groups; cells on STE surfaces had good coverage demonstrating spherical shape at 3 days, while CTRL surfaces without addition of any biomaterial following decontamination

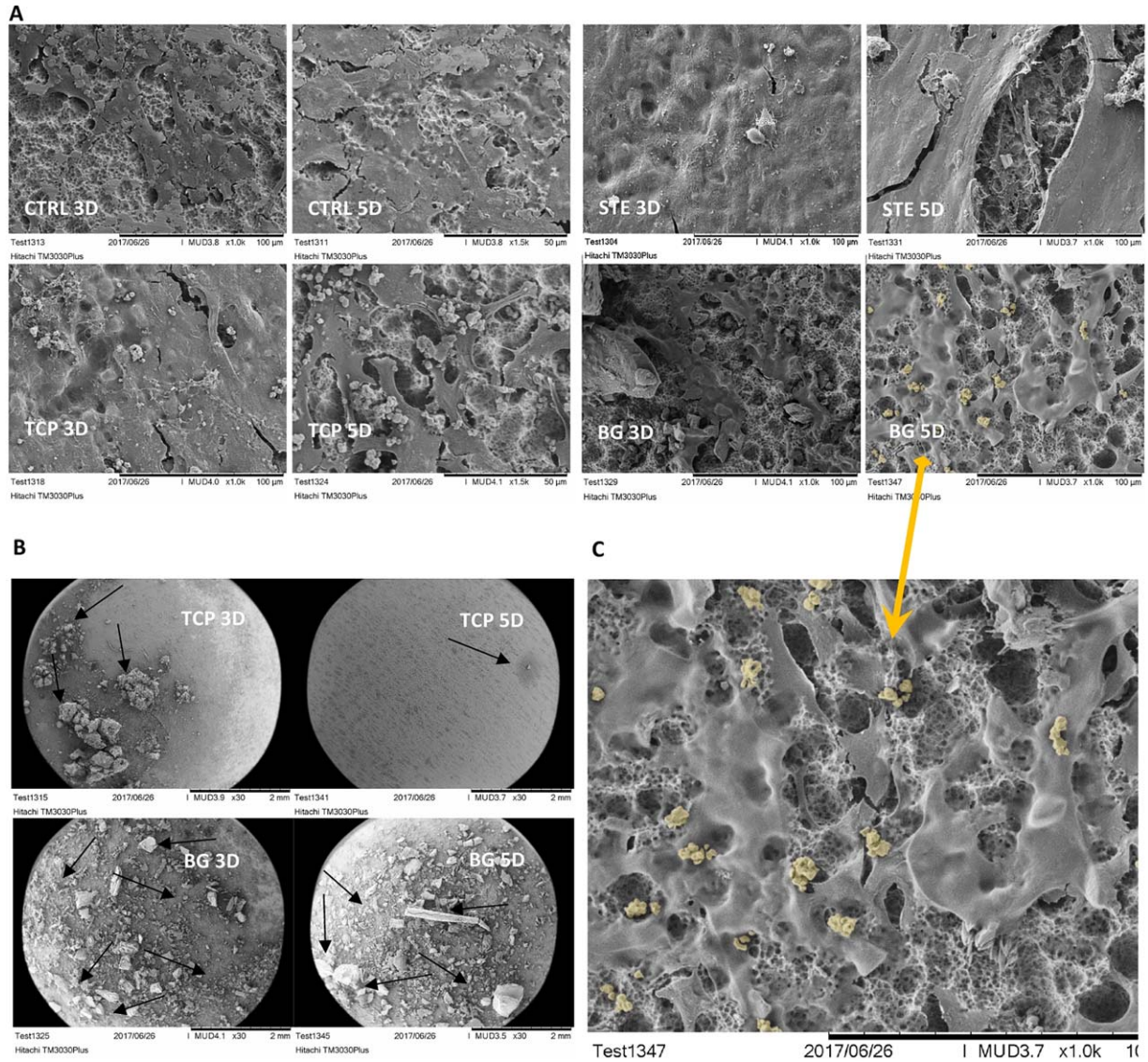


**FIGURE 3**. A time-dependent effect of biomaterial dissolution was noted with substantial differences between TCP and BG. The former showed a rapid rate of dissolution with almost the entirety of the particles (noted with black arrows) being dissolved within 5 days in culture, while the latter exhibited substantivity with a lower rate of dissolution of particles. Remaining BG particles were in direct contact with elongated MG63 cells attaching onto the titanium surfaces [BG particles have been highlighted in yellow to show their location in relation to osteoblastic cells at 5 days; see (C)].

presented with fewer adherent cells with mostly round-shape and scarce coverage of the surface. Among the two test surfaces, specimens demonstrated initial spreading of cells across the surfaces with residual biomaterial particles evident in both BG and TCP groups.

At 5 days, STE surfaces were covered with dense cell multilayers with cells exhibiting characteristic cuboidal shape with lamellipodia and filopodia extensions with extensive coverage of the material surface after 5 days. BG surfaces had good cell coverage at 5 days with cells demonstrating lamellopodia and fillopodia actin extensions and cell bodies being in intimate contact with residual particles. BG particles are highlighted with yellow color to demonstrate the presence of multiple cells immediately adjacent to BG particles in Figure 3(A). In TCP, less particles were noted at 5 days with fewer splindle-shaped cells with extensive fillopodia noted on the surfaces in proximity to TCP particles. Lower magnification SEM images (30×) were obtained to investigate the particle dissolution dynamics from the surface. The representative low power microphotographs from the two test groups in Figure 3(B) reveal the rapid dissolution of TCP particles within 5 days in culture, while BG particles demonstrate a substantivity with gradual dissolution noted. energy dispersive spectroscopy confirmed the presence of calcium ions in both groups (data not shown).

### Cell viability

Cell viability assays were performed using fluorescent labeling of live (FDA; green) and dead (PI; red) eukaryotic cells to study their differential proliferation and variability on the

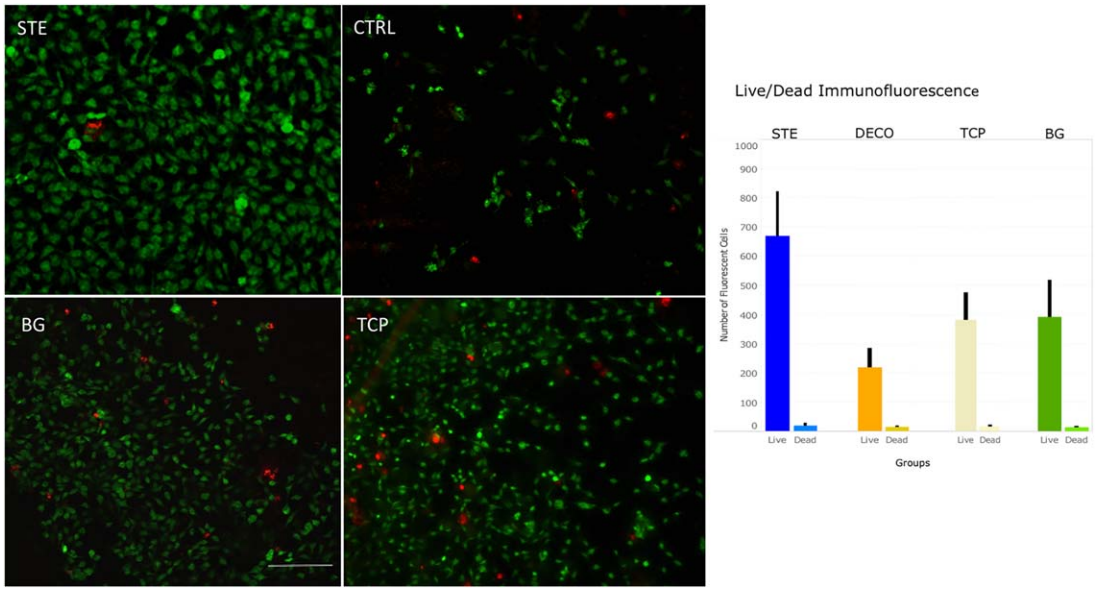


**FIGURE 4**. Cell viability results after 5 days of culture (5000 cells/cm$^2$, 20× magnification). Live cells are stained green; dead cells are stained red. STE surfaces demonstrated near confluent presence of live cells across the titanium surface. In contrast, CTRL surfaces had scarce presence of cells with large titanium areas free of adherent cells. BG and TCP cells demonstrated good cell coverage of the Ti surface with slightly fewer cells as compared to STE. The live/dead cell ratio was superior for BG as compared to TCP (scale bar: 100 μm). The barplots depict average cell counts at four regions per interest per disk.

Ti surfaces. Figure 4 depicts characteristics regions of interest from each group under fluorescent microscopy following channel merging. Major differences were noted across groups with STE surfaces showing complete coverage by live MG63 cells at 5 days as compared to CTRL that had scarce presence of live and fewer dead cells. The large areas of CTRL surfaces that were denuded of cells reveal the cytotoxicity of the bacteria-inoculated and subsequently treated Ti surface. More cells had grown on BG and TCP surfaces compared to the control group at 5 days. The quantity of live cells on BG surfaces was almost equal to that of STE, while the TCP group had good coverage but a larger number of dead cells as compared to BG.

### Cell differentiation

We determined the mRNA expression of known osteogenic genes of MG63 cells seeded on Ti surfaces at 7, 14 and 21 days to determine which gene expression pathways are involved in the differentiation of osteoblastic cells in this model of peri-implant bone. Sterile Ti surfaces served as controls. Gene expression was assessed through quantitative PCR using preselected primers; melt and amplification curves can be found in Supporting Information Figure S1.

Alkaline Phosphatase (ALP) expression was increased on STE at 7 days with a progressive reduction at 14 and 21 days, as expected based on ALP's known role in early osteoblastic differentiation. All remaining groups showed a delayed differentiation pattern with ALP expression levels peaking at 14 days with no significant intergroup differences. The uniformly delayed response across all groups with biofilm inoculation and subsequent treatment point to a regulatory effect of either the residual bacterial cell components or the chemotherapeutic agent used for surface treatment on ALP expression (Supporting Information Figure 1). The most striking difference among experimental groups was noted in the gene expression levels of Epidermal Growth Factor (EGF) with BG and CTRL exhibiting substantially greater EGF expression at 14 and 7 days respectively, which was indicative of continued proliferation of osteoblastic cells.[20] The peak in EGF gene expression at 14 days for cells grown on BG surfaces is consistent with the osteostimulation of BG that is dependent on EGF-mediated proliferation of osteoblastic cells[21] and provides cues for the investigation of mitogen-activated protein kinase/Extracellular signal-regulated kinases (ERK) activation by BG.

## DISCUSSION

Results from the present experiments indicate that a BG osteostimulative biomaterial (BG) exhibited a strong chemotactic effect toward MG63 osteoblastic cells, which was stronger than what was expected due to the chemotactic effect of $Ca^{+2}$ alone (ß-TCP). In addition to increased chemotaxis, substantially increased cell attachment and viability on treated implant surfaces were observed as compared to CTRL. These results using a clinically relevant model of implant surface contamination are very relevant to healing following treatment of peri-implantitis. As previously shown[6] the colonization of the implant surface with oral bacteria as well as with chemical contaminants during the cleaning process lead to loss of its cytocompatibility. In particular, the most detrimental effect seems to be related to the lack of support of previously contaminated titanium surfaces toward osteoblastic cell attachment and proliferation.[6] Notably, osteoblastic cells that manage to attach on these surfaces seem to differentiate and function properly.[6]

Findings of a strong chemotactic effect of BG toward osteoblastic cells in the present study are consistent with existing information on osteostimulation; a property unique to BG alloplastic biomaterials.[15,17] In a series of publications, Xynos et al. have demonstrated that BG biomaterials induce cell mitosis and DNA synthesis in preosteoblasts[15] and provided evidence that these stimulatory effects of BG are due to the releasing of its ions, that is, $Na^{+}$, $Ca^{+2}$ and $Si+^{2}$ into solution.[15] Hench who first engineered BG biomaterials has extensively demonstrated that the ions released from the BG surface during early immersion precipitate on the biomaterial surface forming an amorphous calcium phosphosilicate layer with chemotactic properties toward osteoblastic cells[22] supporting the cell migration results observed in the present study.

The interpretation of the increased cell migration toward a previously contaminated implant surface provides proof of principle data for the validation of osteostimulation in the context of peri-implantitis. Such data is crucial for the development of clinical therapeutic strategies that can predictably yield reosseointegration. Presently, the restoration of the titanium surface's cell migration properties presented herein can be clinically valuable in ascertaining that an adequate number of osteoblastic cells will be present in proximity to the titanium surfaces at the early stages of healing. Such abundance of cell populations that are well suited for reosseointegration can preferentially skew the cellular attachment on the titanium surface toward osteoblasts. In fact, the cellular attachment noted in our study for MG63 osteoblastic cells in the presence of BG particles was very favorable with mature adherent osteoblasts with actin projections on the treated titanium surfaces being present as early as 3 days postseeding. This becomes increasingly important since previous *in vivo* preclinical studies have shown that when the contamination of the implant surface either with plaque or unsuitable chemotherapeutic agents (e.g., Chlorhexidine) is not supportive toward osteoblastic attachment on the surfaces, which are in turn covered by long junctional epithelium.[23,24]

Direct comparisons with existing studies assessing the cytompatibility of inoculated titanium surfaces are limited by the use of single species biofilms in earlier studies. It is now well established that single species titanium-bound biofilms have distinctly different responses to decontamination strategies and are not simulating clinical conditions in a relevant fashion.[25] Importantly, cell proliferation results from the present study are in agreement with our previously published results showing substantially reduced cytocompatibility of previously contaminated titanium surfaces.[6] Indirect comparisons can be made to *ex vivo* studies utilizing plaque biofilms forming on titanium disks retained for at least 24 h in the oral cavity through retentive splints[26] such models lead to the formation of robust biofilms that resemble the supragingival microbiota. Specifically, Schwarz et al. assessed the effect of chlorhexidine in conjunction plastic curettes using a relevant *ex vivo* model and concluded that the metabolic activity of osteoblastic cells seeded on titanium surfaces treated with this approach was 10-fold less than that of cells on sterile disks.[26] These results, are concordant with findings of the present study. The reduced cytocompatibility of titanium surfaces can be attributed to residual bacterial cell components, the adsorption of chemotherapeutic agents on the surface as well as morphological alterations of the titanium surface particularly in the case of acidic agents.[6,27]

The present study has notable strengths. First, the utilized *in vitro* model for peri-implantitis is validated and clinically relevant.[6] The titanium surfaces utilized are modified to exhibit microroughness similar to that of commercially available implants, thus providing a realistic simulation for dental implant surfaces. The microcosm biofilm used to inoculate these surfaces stems from a clinical peri-implantitis sample and is based on nonselective growing media that provide retention of multiple oral taxa frequently encountered in human peri-implantitis.[6] In fact, in a validation study we have found that the aforementioned inoculation approach consistently yielded complete coverage of the titanium specimens with layered multispecies biofilms. Further, the surface treatment method used in the present investigation is based on a concentration of NaOCl, which has selective cytotoxicity as shown through robust cell-based testing, therefore has potential for clinical use.[28] The importance of cell-based cytocompatibility assays in peri-implantitis models cannot be overstated. For example, although the cytotoxic effect of chlorhexidine on osteoblastic cells has been extensively documented in the context of dental procedures and peri-implantitis in particular,[29,30] even to this date preclinical studies are recommending the use of chlorhexidine against peri-implant biofilms solely based on antimicrobial assays.[31,32] In the present study, osteoblastic cell response to the surfaces following the adjunctive use of the aforementioned biomaterials is extensively characterized providing a relevant model for reosseointegration. Nevertheless, the present study shares the limitation of *in vitro* investigations for drawing clinical conclusions. Results occurring in an *in vitro* model can provide clues for clinical performance but cannot be directly translated into clinical care guidelines without further clinical testing due to the complex interactions occurring in a living organism that cannot be replicated in their entirety in the lab. Second, standard tissue culture practices necessitate the sterilization of inoculated titanium surfaces prior to cell seeding. Autoclaving may to a certain extent alter the physical properties of these surfaces thus modifying cell response.[33] The latter, however, does not pose a major threat to the internal validity of the present investigation since the experimental focus is on the comparative performance of the treated surfaces as compared to controls.

## CONCLUSION

Results indicate that osteostimulative BG exhibited a strong chemotactic effect to osteoblasts, which was stronger than what was expected due to the chemotactic effect of $Ca^{+2}$ alone (ß-TCP). The increased chemotaxis was associated to substantially increased cell attachment and viability on treated

implant surfaces as compared to CTRL. The function of osteoblasts was also enhanced with upregulation of mineralization genes occurring at 14 days. Osteostimulative biomaterials should be assessed in clinical studies as peri-implantitis treatment adjuncts to enhance reosseointegration.


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