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ORIGINAL ARTICLE

Evaluation of peri-implant crevicular fluid prostaglandin E_2 levels in augmented extraction sockets by different biomaterials

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ABSTRACT

Objectives: This study compares peri-implant crevicular fluid (PICF) prostaglandin E_2 (PGE_2) levels, clinical parameters and implant stability quotient (ISQ) values around implants placed in augmented extraction sockets.

Materials and methods: The sockets (24 in total) were randomly augmented using either EMD or Bio-Oss Collagen. Implant placements were performed after three months of healing. ISQ readings were evaluated at three points: at the time of surgery, at the first month and at the third month. PICF was collected for PGE_2 evaluation after the first and the third months of implant surgery.

Results: After the first month, a higher level of PICF PGE_2 was observed in the EMD group than in the Bio-Oss Collagen group, and this increase was of statistical significance; however, at the third month there was no statistically significant difference in PICF PGE_2 levels between the two groups. For implants placed in EMD sites, ISQ values were statistically higher at the third month than at the first month, while no significant differences in ISQ value were detected between the first and third months in Bio-Oss Collagen sites.

Conclusions: The results of this research suggest that both EMD and Bio-Oss Collagen are effective treatment modalities for stimulating the formation of new bone at extraction sites prior to implant surgery.

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Introduction

Alveolar ridge resorption following tooth removal is an undesirable but unavoidable physiological phenomenon.[1] An average of 40 to 60% of the original bone height and width is expected to be lost after tooth extraction.[2] This healing process results in various complications, including the lack of available alveolar bone for implant placement, an unfavorable crown–implant ratio and aesthetic problems.[3,4]

Socket preservation is a procedure in which autogenous bone, graft materials or biological agents are placed in the socket of the extracted tooth at the time of extraction.[5–8]

Various materials have been tested, and many have been demonstrated to be effective in comparison with the natural healing of the socket.[4,9,10] Due to its excellent biocompatibility, deproteinized bovine bone graft has been widely used in bone grafting, and there is a wide range of research [4,7,9,11] showing effective clinical outcomes in many forms of bone loss using this technique.

Enamel matrix derivatives (EMD) have been used to promote periodontal regeneration. They are derived from embryonal enamel of porcine origin, and rely on the high degree of homology between porcine and human enamel proteins.[12] The mechanism is as yet unknown, although it is believed that EMD imitates the function of enamel matrix proteins in cementogenesis in the early stages of root growth.[12–14] EMD has attracted interest from researchers due to its effect on osteogenic gene expression and cell adhesion.[15]

Lekic et al. [16] have suggested that mesenchymal cells from the periodontal ligament (PDL) may participate in the healing of the socket wound. It is therefore possible that EMD may facilitate PDL cell attachment and growth. Moreover, the attachment of PDL cells to the EMD appears to generate an intracellular signal which increases their proliferation and the secretion of various autocrine growth factors. In particular, transforming growth factor-beta 1 (TGF-ß1) has been shown to affect osteoblast proliferation, chemotaxis and extracellular matrix deposition.[17] It has been reported that the production of TGF-ß1 is stimulated by EMD.[18] The use of EMD in extraction sites emerged from these results suggesting that it may play a role in encouraging bone formation.

Prostaglandin E_2 (PGE_2), a major arachidonic acid metabolite, is released locally, and has many pro-inflammatory effects on periodontal tissues including vasodilatation, enhancement of vascular permeability at sites of inflammation release of collagenase by inflammatory cells, activation of osteoclasts and mediation of bone resorption. Macrophages are believed

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to be a major source of PGE_2 in periodontal tissues.[19] Prostaglandins are associated with tissue destruction, changes in fibroblast metabolism and bone resorption.[20] PGE_2 can induce bone resorption, and increases the number of osteoclasts.[21]

To our knowledge, no research exists which examines inflammatory markers within peri-implant crevicular fluid (PICF) from implants into extraction sockets which have been treated with different biomaterials. It may be possible to detect the onset of inflammation around such implants by identifying these markers within PICF. The objective of this research is therefore to analyse implants inserted into extraction sites which have previously been filled with either EMD or Bio-Oss Collagen, to draw a comparison of PICF PGE_2 levels, clinical data and implant stability quotient (ISQ) values at the first and third month after surgery.

Materials and methods

Patient selection

Twelve patients (five males, seven females; all aged 40–60 years) having symmetrical single-rooted teeth condemned for extraction in bilateral quadrants of the upper jaw were included in this randomized clinical study. The patients participating in the study were drawn from individuals referred to the Department of Periodontology within the Faculty of Dentistry at Gazi University in Ankara, Turkey. The study protocol was approved by the Ethical Committee of the Faculty of Dentistry at Gazi University, in accordance with the Helsinki Declaration of 1975 (revised 2000). Informed written consent was obtained from all patients following a full explanation of the details of the clinical procedures involved, including periodontal measurements, PICF sampling and surgical procedure. All of the subjects were in good general health and none were smokers, and none of the women were pregnant, postmenopausal or lactating. None of the the patients were taking any medication which could inhibit normal bone healing. At least two teeth in each patient were to be extracted and replaced with endosseous implants. Criteria for excluding patients from the study were the presence of acute infection around the alveolar bone in the surgical site and coagulation disorders, sockets with a complete loss of a bone wall and the presence of severe untreated periodontal disease.

The initial patient examination comprised clinical and radiographic examination. Following this, periodontal therapy was carried out in patients where it was warranted, and surgical intervention was arranged.

Surgical procedure

I. Operation (tooth extraction and socket augmentation)

All surgery was performed by a single surgeon (EAA). Treatments with EMD and Bio-Oss were determined using a simple coin toss to ensure a random distribution. Following gentle tooth extractions without flap elevation, the fresh sockets were debrided to remove all of the soft tissue. Every effort was made to perform atraumatic extraction and preservation of all bone crest. In order to minimize surgical trauma to the surrounding tissue, a periotome and the appropriate dental forceps were used, since for implantation all four walls of the extraction sockets had to be intact. The gingival walls at the socket orifice were gently de-epithelialized. One fresh extraction socket site received EMD (EMD, Straumann, Malmö, Sweden) while the other symmetric socket was filled with Bio-Oss Collagen (Geistlich Pharma AG, Wolhusen, Switzerland). Primary coverage of the socket was achieved using a mucosal punch graft harvested from the palate. A suitable site for graft harvesting was chosen distal of the rugae of the palate at a distance of 4 to 5 mm from the gingival margin. With the selected punch, a free gingival graft of 2 to 3 mm thickness was cut and removed using all-around ball-attached scalpel blade. The harvested graft was placed on top of the socket orifice and sutured to the marginal gingiva of the extracted teeth with six interrupted sutures (Figure 1).

Postoperative systemic antibiotic of 500 mg amoxicillin for one week and 0.12% chlorhexidine mouthwash (twice daily) were prescribed. Sutures were removed ten days after the surgery.

II. Operation (re-entry surgery and implant placement)

After three months of healing, a surgical re-entry procedure was performed. Local anesthesia was given, and following this, crestal and intrasulcular incisions were made and mucoperiosteal flaps were reflected to allow access to the alveolar ridge.

Each patient received two dental implants (Standard ϕ4.1–10 mm, SLA coating, Institut Straumann AG, Waldenburg, Switzerland). The study followed a one-stage surgical protocol. All implants were inserted by the same surgeon, following the surgical procedure described by the manufacturer (Figure 2).

Resonance frequency analysis (RFA) measurements

The numeric stability values of implants were assessed by a single examiner using RFA. The captured data are presented as a quantitative value (ISQ) on a scale from 1 to 100. Higher ISQ values indicate higher stability, and lower values represent lower implant stability.[22] The ISQ readings were obtained for each implant at the time of surgery, and before flap closure using Osstell Mentor (Figure 2). All readings were taken in the buccal direction for each implant. To ensure that the stability value was correctly identified, measurements were determined in duplicate, with a third reading being taken if there was a difference of greater than two ISQ units between readings. These measurements were repeated after one and three months of healing, with a single investigator carrying out all of the measurements.

Patients were rehabilitated with both fixed and removable implant-supported prostheses. Prosthetic rehabilitation was started three months after implant placement.

Clinical measurements

All measurements were recorded using a calibrated Williams periodontal probe. The full-mouth plaque index (PI),[23] and

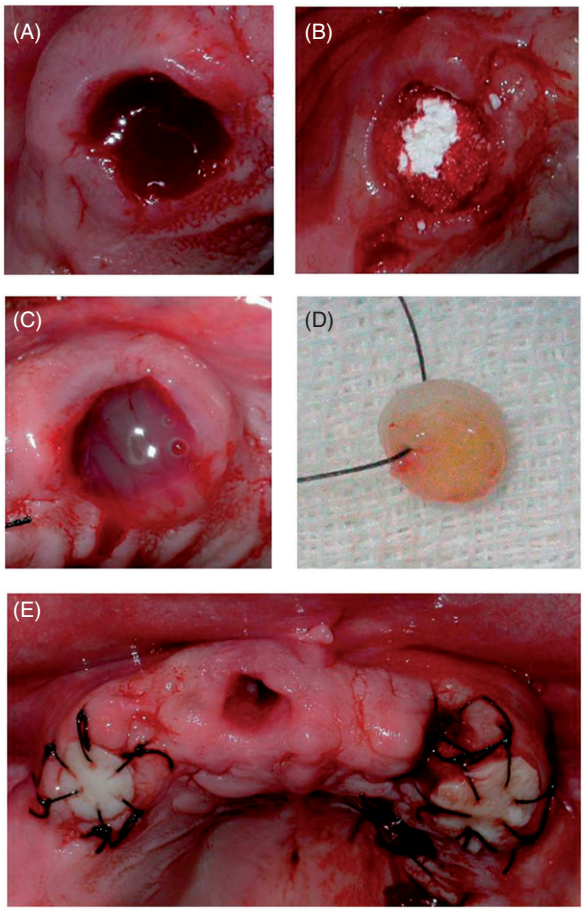

Figure 1. Clinical aspect of the first operation (A) Clinical view of the extraction site (B) Bio-Oss Collagen inserted into the extraction socket (C) EMD application into the extraction site (D) Punch graft (E) Punch grafts sutured to marginal gingiva.

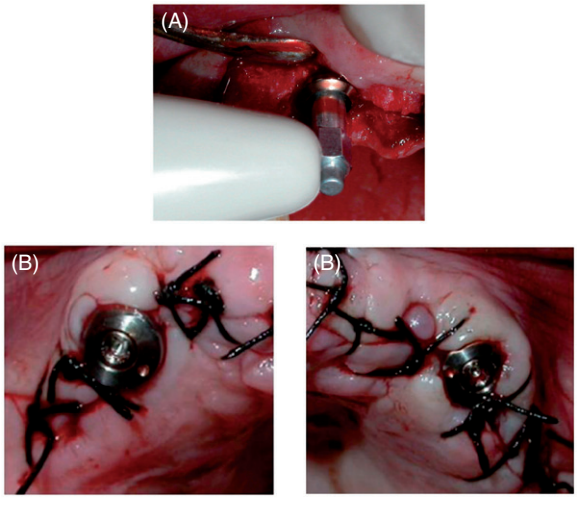

Figure 2. Clinical view at the re-entry operation (A) Obtaining the ISQ readings for each implant at the time of surgery before flap closure using Osstell Mentor (B) Suturing after implant placement, right and left maxillary canine sites.

full-mouth gingival index (GI) [24] scores were recorded in order to assess gingival health throughout the study.

An examination was performed around implants at the first and third months after surgery, including recordings of PI and GI scores.

PICF sampling and processing

All PICF samples were collected both mesially and distally of each implant after the first and the third months of implant surgery. The sample site was gently air dried and the area was isolated with cotton rolls in order to prevent contamination of the samples. Standardized sterile paper strips (Periopaper, Proflow Inc., Amitylville, NY) were inserted into the sulcus until slight resistance was felt and left in place for 4 min.[25] Strips contaminated by bleeding or exudate were discarded. Samples were then placed separately into coded, sealed plastic micro-centrifuge tubes, covered with paraffin and stored at −70 °C until processing.

PICF enzyme-linked immunoabsorbent assay (ELISA) analysis for PGE_2

Levels of PGE2 were determined using a sandwich ELISA kit (Biosource, Invitrogen Corporation, Carlsbad, CA), following the process recommended by the manufacturer. Micro-centrifuge tubes containing periopaper strips with the absorbed PICF sample were left at room temperature for 30 min and then eluted using a centrifugal method.[26] The strips were then removed and the fluid assayed using the ELISA kit for PGE_2.

Statistical evaluation

Data analysis was performed by using Statistical Package for Social Sciences (SPSS) software version 11.5 (SPSS Inc., Chicago, IL). Data were expressed as mean ± SD. While, the differences among more than two repeated measurements were evaluated by Friedman test, otherwise, Wilcoxon Sign Rank test was used for determining the differences between EMD and Bio-Oss Collagen groups. When the p value from the Friedman test is statistically significant to know which time differ from which others, non-parametric multiple comparison test was used. Pearson's correlation coefficient were also calculated for determining the degree of associations between continuous. A p value less than 0.05 was considered statistically significant. But, all possible multiple comparisons the Bonferroni correction was applied for controlling type I error. The power of the study was 77.1%.

Results

Clinical findings

All experimental sites healed uneventfully after the first operation. One patient showed necrosis in two punch grafts, and this patient was excluded from the study. Full-mouth GI and PI scores were maintained below 20%.

Peri-implant PI and GI scores at the first and third months for the EMD and Bio-Oss Collagen groups are shown in Tables 1 and 2. In both groups, there was a statistically significant decrease in peri-implant GI scores at the third month ($p = 0.023$). Although there was no statistically significant difference between the scores in the Bio-Oss Collagen groups

Table 1. Comparison of peri-implant PI and GI scores at first and third months in both of the groups.

Clinical parameters	Groups	One month mean ± SD	Three months mean ± SD	*p*
PI	EMD ($n = 12$)	0.32 ± 0.21	0.06 ± 0.12	0.023
	Bio-Oss Collagen ($n = 12$)	0.36 ± 0.19	0.12 ± 0.15	0.033[a]
GI	EMD ($n = 12$)	0.26 ± 0.19	0.0 ± 0.0	0.023
	Bio-Oss Collagen ($n = 12$)	0.25 ± 0.23	0.0 ± 0.0	0.023

[a]Statistically not significant according to the Bonferroni correction ($p > 0.025$).

Table 2. Comparison of peri-implant PI and GI scores at first and third months between the groups.

Clinical parameters	EMD	Bio-Oss Collagen	*p*
PI difference (1–3 mo)	−0.26 ± 0.19	−0.25 ± 0.23	1.000
GI difference (1–3 mo)	−0.26 ± 0,19	−0.25 ± 0.23	1.000

in the first and third months peri-implant PI ($p = 0.033$), there was a significant decrease in PI scores in the EMD group at the third month ($p = 0.023$). There was no statistically significant difference between EMD and Bio-Oss Collagen groups PI scores at first and third months ($p = 0.317$, $p = 0.317$). There was no statistically significant difference in GI scores between the EMD and Bio-Oss Collagen groups at either the first month or third month ($p = 1.000$, $p = 1.000$).

The statistical analyses for the PICF volume for the EMD and Bio-Oss Collagen groups at the first and third months are shown in Table 3. The Bio-Oss Collagen group showed significantly higher PICF volume than the EMD group at first month ($p = 0.023$). Although the PICF volume increased in both the EMD and Bio-Oss Collagen groups at the third month, the difference was not statistically significant ($p = 0.374$).

PICF PGE_2 levels

The statistical analyses for the PGE_2 levels at first and third months for the EMD and Bio-Oss Collagen groups are shown in Table 4. The PGE_2 levels in the EMD group were statistically higher than the Bio-Oss Collagen group at the first month ($p = 0.008$). There was no statistically significant difference between the groups at the third month ($p = 0.523$).

A comparison of PGE_2 levels within the groups at the first and third months shows a statistically significant decrease in PICF PGE_2 levels in the EMD group ($p = 0.011$). The decrease in Bio-Oss Collagen group was not statistically significant ($p = 0.717$).

RFA results

The average ISQ measurements taken at each sampling point, with a comparison of each group at each sampling point, are shown in Tables 5 and 6. Eleven of the 12 patients completed the study with three RFA measurements, and all ISQ values were between 55 and 80. There were no statistically significant differences in ISQ values for either group at baseline level and at the third month. The ISQ readings can be seen to increase for implants placed in EMD sites at the

Table 3. Comparison of PICF volume amounts at first and third months in both groups.

PICF (µl)	One month	Three months	p^a	Differences (1–3 months)	p^b
EMD	1.5 ± 1.3	2.6 ± 2.5	0.348	1.1 ± 3.2	0.798
Bio-oss Collagen	3.1 ± 1.3	3.9 ± 1.5	0.374	0.8 ± 2.2	
p^c	0.023	0.292			

[a]Comparison of PICF volume at first and third months in both groups.
[b]Difference between-group comparison at first and third months.
[c]Comparison of PICF volume at first and third months between groups.

Table 4. Comparison of PICF PGE_2 levels at first and third months.

$PGE_{2\ pg/site}$	One month mean ± SD	Three months mean ± SD	p^a	Differences (1–3 months)	p^b
EMD	394.8 ± 77.8	293.4 ± 81.0	0.011	−101.4 ± 84.3	0.042
Bio-oss Collagen	331.2 ± 80.3	315.8 ± 111.1	0.717	−15.4 ± 115.4	
p^c	0.008	0.523			

[a]Comparison of PICF PGE_2 levels at first and third months in both groups.
[b]Difference between-group comparisonat first and third months.
[c]Comparison of PICF PGE_2 levels at first and third months between groups.

first and third months ($p = 0.012$), while no significant differences were seen in the ISQ readings for implants placed in Bio-Oss Collagen sites ($p = 0.082$). For implants placed in EMD sites, ISQ values were statistically higher at the third month than the first month, while for implants placed in Bio-Oss Collagen sites no significant differences were seen in ISQ values at the first and third months (Tables 5 and 6).

There was no statistically significant correlation between clinical parameters and PICF PGE_2 levels in either group ($p > 0.05$). There was also no correlation between clinical parameters and ISQ values in either group ($p > 0.05$) (Table 7), and no statistically significant correlation between ISQ values and PICF PGE_2 levels was observed ($p > 0.05$).

Discussion

The aim of this investigation is to compare PICF PGE_2 levels in implant patients with extraction sites enhanced with either EMD or Bio-Oss Collagen. Existing research suggests that EMD directly promotes the formation of new bone, while Bio-Oss Collagen provides a more indirect support for bone deposition. At present it is not known whether implants in grafted bone will be as stable in the longer-term as implants in non grafted bone. Mixed results have been reported on the quality of regenerated deproteinized bovine bone grafted sites and dental implant contact. Although some studies have reported a proper osseointegration,[27,28] others have found no contribution, and did not recommend the use of this bone derivative for enhancing vital bone-implant contact.

It has been suggested that the quality of the bone in grafted sockets may not be adequate for implant placement. When extraction sockets are filled with grafting material, graft remnants usually remain at the time of implant placement.[29] Becker et al. [30] reported that the Bio-Oss Collagen particles remained in the extraction sockets for up to seven months in their study.

Dental implant stability has been put forward as an important factor in the identification of the ideal timing for

Table 5. Comparisons of ISQ readings (mean ± SD) of implants placed in EMD and Bio-Oss Collagen sites at baseline, first and third months postoperatively.

	Baseline	One month	Three months	*p*
ISQ readings of implants placed in EMD sites ($n = 11$)	66.57 ± 6.53	69.97 ± 7.35[a]	73.07 ± 5.13[b]	0.012
ISQ readings of implants placed in Bio-Oss collagen sites ($n = 11$)	65.77 ± 7.99	69.82 ± 5.70	71.80 ± 5.19	0.082

[a]Comparisons of ISQ readings at baseline and first month.
[b]Comparisons of ISQ readings at first and third months.

Table 6. Comparison of ISQ readings (mean ± SD) of implants placed in EMD and Bio-Oss Collagen sites.

	EMD p	Bio-Oss Collagen P	EMD/Bio-oss Collagen P
Baseline- One month	3.0 ± 3.02	4.75 ± 4.46	0.751
Baseline- Three months	6.0 ± 4.53	6.62 ± 7.76	0.135
1–3 Months	4.0 ± 4.28	2.87 ± 4.61	0.672

Table 7. The correlation between clinical parameters and PICF PGE_2 levels and also between clinical parameters and ISQ values in both groups.

	EMD PGE_2 levels				Bio-oss Collagen PGE_2 levels			
	One month		Three months		One month		Three months	
Clinical parametes	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
PI	−0.091	0.830	−0.147	0.728	0.017	0.969	−0.436	0.280
GI	−0.005	0.991	−0.177	0.676	−0.019	0.964	−0.008	0.985
ISQ readinds	0.335	0.417	−0.167	0.692	−0.258	0.537	−0.331	0.423

the loading of surgically inserted dental implants.[31] Implant stability is evaluated on the basis of RFA. This technique has proved to be sensitive in monitoring changes in implant stability.[32] Several factors influence the ISQ values of dental implants, including implant length, width, surface and density of bone.[33, 34] In this study, all experimental sites were within the maxillary arch, and identical dental implants were used, giving homogeneity of the experimental sites.

The results presented here show no statistically significant difference for baseline ISQ readings between two groups. Cochran et al. [35] have reported that the primary stability of a dental implant obtained during surgical placement is purely mechanical. During the first few weeks of healing, bone remodelling takes place around the dental implant surface. This phase, with the formation of lamellar bone from woven bone, may cause a decrease in primary bone contact. Woven bone formation around the implant surface has been reported to occur in 4–6 weeks.[36] Compared to the baseline values, implants in EMD sites showed statistically increased ISQ values at one and three months after surgery, although implants in Bio-Oss Collagen sites showed no significant difference.These results may be explained by residual graft chips occuring at the implant-bone interface during the early healing phase. The stability of the implants can be affected by healing time, as has been shown in previous studies.[34,37]

Arachidonic acid metabolites such as PGE_2 are known to play a central role in the progression of periodontal tissue destruction. Periodontitis patients exhibit elevated gingival crevicular fluid levels of pro-inflammatory cytokines, such as interleukin-1-beta (IL-1β), and PGE_2. PGE_2, a metabolite of the cyclooxygenase pathway, is the most potent mediator of alveolar bone loss in periodontitis.[38] PGE_2 is known to have an activity on fibroblasts and osteoclasts to induce the synthesis of MMPs, IL-1β and other cytokines. Preshav et al. reported that levels of PGE_2 were elevated in the gingival tissue and gingival fluid of patients with periodontitis, compared with periodontally healthy subjects.[39] In addition, it has been demonstrated that PGE_2 concentrations in gingival crevicular fluid offer a reliable predictive marker for the progression of periodontitis.[19]

Inflammatory changes in the peri-implant tissues may cause bone loss. PGE_2 has been shown to have pro-inflammatory effects on peri-implant tissues, including mediation of bone resorption.[40] The most common reason for the failure of a dental implant is the loosening of the implant without any clinically observable special event.[25] Analysis of PICF offers a non-invasive means of studying the host response in periodontal and peri-implant disease and may provide an early indication of the patient at risk for active disease. Peri-implant sulcus fluid analysis may help in detecting early metabolic and biochemical changes.[41]

For the evaluation of dental implants, this study used several clinical and laboratory measurements; each of these measurements is likely to provide an important aspect of information regarding the complex series of events at the dental implant sites.

According to the results presented here, although the EMD group showed statistically significant decreased PICF PGE_2 levels ($p = 0.011$), the decrease in the Bio-Oss Collagen group was not statistically significant ($p = 0.717$) at the third month. The improvement in clinical parameters was accompanied by a parallel decrease in PICF PGE_2 levels.

The correlation between PGE_2 levels and clinical parameters was not found to be significant; in addition, there was no correlation between clinical parameters and ISQ values in either group ($p > 0.05$). To our knowledge, there are no previous research studies concerning the interaction between clinical parameters and PICF PGE_2 levels around the implants placed in augmented extraction sockets, which would enable a comparison of the results presented here.

The primary limitation of our study was the small number of subjects. Further split-mouth studies using larger patient groups are needed in order to confirm these results.

However, these initial results appear to show that the enhancement of extraction sockets with either EMD or Bio-Oss Collagen can promote wound healing in the osseointegration process, and that these biomaterials are suitable for use in socket preservation, although larger studies will be required in order to determine the influence of each material within the osseointegration process.

Disclosure statement

The authors declare that they have no conflict of interest.

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