The Effects of the Coupling of Titanium Implants and Dissimilar Metal Abutments on Osteoblast Differentiation In Vitro

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This study evaluated the effect of titanium endosseous dental implants coupled to dissimilar materials on the capacity of preosteoblasts in bone marrow culture to differentiate, to form alkaline phosphatase–positive colonies, and to mineralize. Ten UCLA abutments were cast in each of 4 alloys: Type III gold, ceramometal gold, commercially pure grade I titanium, and titanium-aluminum-vanadium (Ti-6Al-4V); 10 ceramic abutments and 30 sterile Brånemark System implants were also used. Five abutments of each material and 5 implants were incubated individually in rat bone marrow culture, as were 5 of each abutment attached to an implant; bone marrow cultures not containing test samples were used as controls. Following 17 days of culture, the solution potentials of individual abutments (except ceramic), the implant, and the implant-abutment couples were measured in the test medium. One dish of each group of 5 was then stained for bone nodule mineralization; the remainder were quantified by area for alkaline phosphatase staining. Statistical analysis of measured in vitro potentials showed that the uncoupled samples formed 2 groups, and coupled samples formed 3 groups. Analysis of variance for alkaline phosphatase–positive area values showed no significant differences between coupled or uncoupled groups and the control. Normal cell differentiation and morphology, as well as a lack of zones of inhibition, were observed. Bone nodule mineralization was evident in all groups. It was concluded that the presence of these commonly used implant abutment biomaterials coupled to titanium endosseous dental implants had no adverse effects on the in vitro capacity of preosteoblasts in marrow to differentiate and to form mineralized bone nodules, despite measured differences in solution potentials.
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Galvanic coupling occurs when dissimilar metals are placed in physical contact in an electrically conductive medium[1]; the attachment of a dissimilar metal abutment to an implant forms a galvanic couple in the mouth. The more electropositive metal becomes the cathode, and the more electronegative metal becomes the anode. Galvanic corrosion takes place at the anode and is

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accelerated by the polarizing effect of the reduction reaction at the cathode. The magnitude of the resulting galvanic current is dependent partly on the potential difference of the couple components, ΔE_{couple}, which is the difference between the anodic (E_a) and cathodic (E_c) potentials. E_a and E_c are measured in the corrosive medium before electrically coupling the dissimilar metals; the potential of the couple (E_{couple}) is measured once the dissimilar metals are in physical contact. E_{couple} is the mixed potential of the electrically coupled metals and must lie between E_a and E_c.

The pioneering research of Bothe and coworkers[2] revealed titanium to be a promising implant biomaterial. In that era, orthopedic clinical observations of the corrosion of surgical implants[3] had led to the development of the adage "thou shalt not mix the metals."[4] Since that time, while it has been recognized that the in vivo degradation of functionally loaded biomaterials is indeed an interaction between both electrochemical and mechanical processes,[5] the galvanic corrosion produced by metallic prosthetic components directly contacting titanium implants in vivo and in vitro has been studied and reported in the orthopedic, prosthodontic, and biomaterials literature; the dental technology literature is also following this issue.[6]

In their studies on 139 retrieved modular femoral components of human hip prostheses, Collier and coworkers[7–9] found that galvanically accelerated crevice corrosion had occurred in vivo at the interface between the cobalt-alloy head and titanium-alloy stem components, a phenomenon that was not observed when both components were composed of either titanium alloy or cobalt alloy. They suggested the use of single-alloy modular systems or the combination of ceramic modular components with those of a single alloy.

Gilbert et al[10] examined 148 retrieved modular human hip prostheses of both mixed (titanium-aluminum-vanadium [Ti-6Al-4V]/CoCr) and similar (CoCr/CoCr) metal combinations, finding significant corrosion in both mixed and similar metal combinations in the region between the head and stem. However, in mixed-metal cases, the CoCr heads demonstrated moderate to severe corrosive attack more frequently and earlier. The authors noted that this corrosion could result in the loss of mechanical integrity of the implants in vivo and the release of particles into the surrounding tissue.

Nilner and Lekholm[11] measured in vivo corrosion potentials and polarization behavior of uncoupled titanium implants/abutments and their gold alloy suprastructures. They noted that, despite the assumed galvanic activity in the functional environment, the patients were asymptomatic, and there was no evidence of damage to components.

Geis-Gerstorfer et al[12] studied the effects of the in vitro galvanic coupling of pure titanium implant alloy with 2 prosthodontic nickel-chromium (Ni-Cr) alloys, finding an enhanced substance loss (as compared to their uncoupled corrosion rates) from the Ni-Cr alloys. They suggested adopting a threshold value for maximum allowable ion release for individual alloys as measured in in vitro immersion studies, as discussed in DIN.[13]

Engelman and Avera[14] evaluated the in vitro corrosion resistance of Brånemark titanium implants; samples comprised either cast-to-gold cylinders (with interposed titanium abutments) or cast UCLA abutments, both in a variety of alloys, connected to titanium implants. No corrosion products were shown on the surfaces of the gold cylinder samples, but corrosion was identified in the UCLA abutment castings fabricated from gold-palladium (Au-Pd) and Type III gold alloys.

Ravnholt,[15] in an investigation of the in vitro coupling of commercially pure (cp) grade 1 titanium (Ti) with a variety of restorative alloys, found that the amalgam samples provoked a significant corrosion current and a significant rise in cathodic pH values around the titanium electrode. It was proposed that the possibility of resulting tissue damage, unfavorable alteration of microbial flora, and an influence on the rate of bone healing and remodeling should be considered when combining titanium with other metals in vivo. Ravnholt and Jensen[16] also studied the in vitro coupling of carbon fiber/polymethyl methacrylate composite and brazed and unbrazed Au-Pd alloy supraconstructions with cp 1 Ti implants and found that significant corrosion occurred only in the brazed joints of the Au-Pd. Improved casting accuracy to "avoid cutting and brazing" were suggested, and higher corrosion rates in the in vivo milieu were predicted.

Lemons, Lucas, and others have also studied this phenomenon.[17–20] In an ongoing retrospective retrieval analysis of 1,712 failed dental implants of various designs,[17] examination of prostheses involving Ti and Ti-6Al-4V coupled with other alloys suggested that this factor had played a role in their failure in some cases. It was proposed that this situation be avoided by the careful selection of combinations of prosthetic alloys of noble or at least electrochemically similar potentials, especially when dealing with subgingival interfaces.[18,19] In an in vitro study,[20] the corrosion potentials and corrosion currents were measured for passivated implant alloys (Ti, Ti-6Al-4V, 316L stainless steel, and cobalt-chromium-molybdenum) as well as

cast/polished prosthodontic alloys. It was found that the nickel and copper alloys all showed higher corrosion rates and more active corrosion potentials than the Au-Pd or implant alloys. The authors advised the careful evaluation of prosthodontic alloy combinations "to avoid adverse electrochemical couples which may cause corrosion and degradation, leading to implant loss and bone resorption."

Marshak et al[21] examined the in vitro attachment of a prosthetic abutment (comprising Au-Pd alloy cast to a Ti-alloy abutment) to a Ti-alloy implant with a Ti-alloy abutment screw. Surface deposition of corrosion products was revealed at the Ti-alloy/Au-Pd junction on the abutment as well as on the abutment screw. The authors discouraged the use of low gold and base metal alloys for the fabrication of implant suprastructures and encouraged the use of high noble and titanium alloys.

Iimuro and coworkers[22] studied the in vitro corrosion of cp 1 Ti coupled to ferromagnetic stainless steel (447J1, used in magnetic attachment "keepers") or prosthodontic gold alloys, and found the corrosion of titanium to have been accelerated by the coupling.

Reclaru and Meyer[23] performed a comprehensive in vitro study of the corrosion behavior of 15 prosthodontic and prosthetic alloys coupled to cp 1 Ti. They found that the Au-based, Pd-based, and silver-palladium (Ag-Pd) alloys caused negligible galvanism with no risk of triggering the crevice corrosion phenomenon; while the Ni-Cr and stainless steel alloys also showed negligible galvanism, crevice corrosion was clearly a concern. The authors suggested electrochemical requisites for the selection of alloys for the fabrication of implant-supported prostheses.

Oda and Okabe[24] examined the in vitro potentiodynamic polarization behavior of soldered joints of cp 1 Ti and Ti-6Al-4V rods. Specimens joined with Ti-based solders showed no transpassive regions or breakdown in the natural electrode range, while those joined with Au-based and Ag-based solders showed transpassive regions or breakdown potentials at < 0 mV. The authors recommended that Ti-based solder be used for the joining of titanium and its alloys.

Hildebrand and coworkers[25] studied the electrochemical behavior of titanium alloys (cast/annealed titanium-tantalum-30 and titanium-niobium-30) coupled with cp 1 Ti and gold-platinum (Au-Pt) alloy in vitro. The Au-Pt alloy couples demonstrated significantly greater corrosion potentials than the cp 1 Ti couples.

Cortada and coworkers[26] evaluated the in vitro coupling of wrought cp 1 Ti oral implants with various cast prosthodontic alloys, namely cp 2 Ti, palladium-copper-gallium alloy, Ni-Cr alloy, and Au-Cu-Ag alloy. The Ni-based alloy showed significantly greater corrosion potential, corrosion current density, and ion release, with the Ti samples showing significantly lower values than all other groups. The authors suggested that the galvanic effects of the coupling of dissimilar metals to Ti implants in vivo could well diminish their prognosis.

There is no standard metal for dental implant restorations. Most clinicians seem to base their choice on past experience with conventional dental prostheses and on cost. The work of the above investigators on biomaterial systems relevant to implant prosthodontics suggests that material selection is indeed a concern, as it can provoke galvanic corrosion and related phenomena throughout the prosthesis as well as on the implant and its contiguous tissues. The biologic sequelae of many of these phenomena are poorly understood.

The osseointegration of a titanium implant is a complex process, which requires that a stringent set of biologic, biochemical, and biomechanical criteria be met.[27,28] The possibility therefore exists that some of the electrochemical properties that allow titanium to establish or maintain osseointegration (including low current density and therefore diminished perturbance of ion fluxes in the surrounding tissues,[29] high dielectric constant[30] of surface oxides, favorable corrosion characteristics,[31] and the in vivo growth of its biopassivating oxide layer[32]) may be detrimentally altered by the galvanic coupling of the endosseous and extraosseous components.[19,29] The present study investigated the in vitro effects in bone marrow culture of the coupling of several commonly used types of prosthodontic biomaterials (employed as the restorative component) to a titanium endosseous dental implant.

Materials and Methods

Preparation of Samples. Ten abutment samples were cast using Delrin non-hexed UCLA abutment burnout patterns (component UCAB2CY, Implant Innovations, West Palm Beach, FL) (Fig 1) in each of the following materials: Type III gold, ceramometal gold, cp grade 1 titanium, and Ti-6Al-4V alloy. The Type III gold samples (Ney-Oro B2, JM Ney, Bloomfield, CT) were prepared by investing patterns in Beauty Cast Investment (Whip Mix, Louisville, KY), casting, and devesting according to manufacturer's instructions. The ceramometal

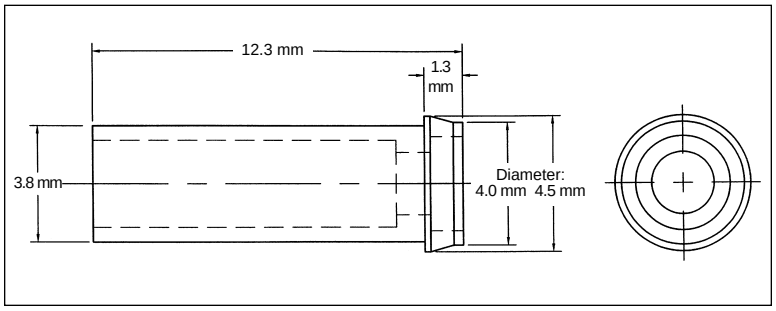

Fig 1 Dimensions of abutment casting pattern.

gold samples (Olympia, Jelenko Dental Health Products, Armonk, NY) were prepared by investing patterns in High-Span II Investment (Jelenko Dental Health Products), casting, and devesting according to manufacturer's instructions. The titanium samples of cp grade 1 titanium (Tytanium R1, Jeneric/Pentron, Wallingford, CT) and Ti-6Al-4V alloy (Tytanium R2, Jeneric/Pentron) were prepared by surface-coating and backup-investing patterns (Ty-Coat and Ty-Investment, Jeneric/Pentron), casting, and devesting according to manufacturer's instructions. The devesting protocol for all samples included the grit-blasting of all surfaces with 50 μm alumina, followed by ultrasonic cleaning for 5 minutes in 95% ethanol.[33]

Cast samples then underwent a standard lapping/reaming protocol, per the manufacturer's instructions (components RH100, LT100/LM100 & PPIA3, Implant Innovations) using a fresh set of implements for each material group so as to prevent the possibility of cross-contamination. All cast samples were then reduced to a standardized length of 8 mm. The para-implant cuff of each abutment sample was prepared to have a flattened side (to prevent sample movement on a flat surface) and then polished with a series of abrasive rubber wheels (Shofu Dental, Menlo Park, CA), again using a new set for each material group. Final polishing of the cuff was performed as described by Craig and Hanks[34] using Tripoli and rouge (with separate wheels for each material group), followed by ultrasonic cleaning for 10 minutes in Jelenko Polishing Compound Remover (Jelenko Dental Health Products) diluted 1:3 with distilled water. Ten prefabricated alumina ceramic abutments (CerAdapt prototype SPC 1150, Nobel Biocare AB, Göteborg, Sweden) were also procured. It should be noted that while the cast metal abutments are supplied with titanium abutment screws, the ceramic abutments are supplied with proprietary gold alloy abutment screws.

The abutments in their material groups were scrubbed under tap water with a soft nylon brush and then ultrasonically cleaned, first for 10 minutes in 95% ethanol and then for 10 minutes in double-distilled water.[34] Twenty cp 1 Ti abutment screws (component UCABH, Implant Innovations) were similarly cleaned. All were packaged in their material groups and steam-autoclaved for 15 minutes at 121˚C/1.4 kg/cm^2. Ninety presterilized and packaged 3.75 × 7-mm titanium implants (SDCA 002, Nobel Biocare AB) were also procured. The above cleaning and sterilization protocol was applied to all abutment samples for each of the 3 repeats; new implants were used for each repeat.[35]

Assembly of Samples. Assembly and plating of samples took place under sterile conditions with talc-free gloves in a laminar flow hood. Seventy sterile 35 × 10-mm culture dishes (Falcon 3001, Becton Dickinson Labware, Lincoln Park, NJ) were numbered and assigned to material groups as outlined in Table 1; ie, along with controls, there were 5 abutment samples of each material and 5 implants, all individually, and then one each of 5 abutment sample materials was attached to an implant. Coupled samples were assembled using a sterile titanium instrument tray with titanium forceps and screwdrivers (DIA 310, DIA 293, DIB 034, DIA 313, and DIB 038, Nobel Biocare AB). A clotting medium of bovine-citrated plasma (BCP) (Sigma Diagnostics, St. Louis, MO) with 100 μg/mL penicillin, 50 μg/mL gentamycin, and 0.3 μg/mL fungizone was prepared and 60 μL added to the center of each dish. The samples were then placed in the BCP using titanium forceps. In the medium control group (#1-5), no cells or samples were added; in the marrow control group (#61-65), no BCP or samples were added; in the BCP control group (#66-70), no samples were added to the BCP. Dishes were covered and placed in an incubator at 37˚C/5% CO_2 for 5 hours to allow clotting. Then, 4 mL of medium comprising α-

Table 1 Test and Control Sample Combinations

Sample	BCP + marrow suspension		No BCP	BCP
	No implant	Implant		
No abutment	#1 to 5 (medium control, no cells)	#6 to 10		
Type III gold alloy abutment	#11 to 15	#16 to 20		
Ceramometal gold alloy abutment	#21 to 25	#26 to 30		
Cp grade 1 titanium abutment	#31 to 35	#36 to 40		
Ti-6Al-4V alloy abutment	#41 to 45	#46 to 50		
Ceramic abutment	#51 to 55	#56 to 60		
Marrow suspension			#61 to 65 (marrow control)	#66 to 70 (BCP control)

minimal essential medium (α-MEM) containing 10% fetal bovine serum (FBS) (Life Technologies, Grand Island, NY), 100 µg/mL penicillin, 50 µg/mL gentamycin, 0.3 µg/mL fungizone and 10^{-8} mol/L dexamethasone (Dx, ICN Pharmaceuticals, Montréal, Canada) were added to each dish. The samples were incubated for 20 hours, after which time the medium, which had congealed slightly because of initial leaching of the clotting medium, was pipetted off. The same protocol was employed for each of the 3 repeats.

Harvesting and Plating of Marrow Cells. Bone marrow harvest and culture was performed ad modum Maniatopoulos et al[36] to allow osteoblast precursor cells to differentiate in contact with or proximity to the various biomaterials and combinations of biomaterials being investigated. A mixed marrow cell suspension was chosen as the source of osteoblast precursors for several reasons. This model mimics the complex biologic milieu into which the endosseous implant is placed, in that it is not immediately apposed to a pure population of bone cells but rather an interacting mixture of cell types derived from the surgical coagulum and marrow cells. The choice of marrow precursors also allows the possibility of differentiation of these contacting cells along alternate lineages (eg, fibroblastic), if they are not in an environment conducive to osteoblast differentiation. In favorable conditions, the preosteoblasts will differentiate into osteoblasts that express alkaline phosphatase activity and are capable of forming mineralized nodules. The use of primary bone cells in this application is preferable to the use of immortalized cell lines, as the primary cells are capable of the full range of expression of osteoblast markers of differentiation and are normal cells that have not undergone neoplastic transformation or transfection to immortalize them.[36–38]

The femora and tibiae of 2 young adult male Wistar rats, each weighing 125 to 150 g, were excised aseptically, cleaned of soft tissues, and stored temporarily in phosphate-buffered saline (PBS, Sigma Diagnostics) supplemented with 100 mg/mL penicillin, 50 mg/mL gentamycin, and 0.3 mg/mL fungizone. The ends of the bones were removed and the marrow was flushed out using fully supplemented medium expelled from a sterile syringe. This study's animal-subject usage protocol was approved by the Dalhousie Biomedical Research Review Board and all experimentation was done in accordance with ethical and humane principles of research and under the direct supervision of an ACVS-diplomate veterinary surgeon. A cell suspension was created in the medium and 4 mL added to each dish, except for the medium control group (#1-5) (see Table 1), and the dishes were returned to the incubator. The cell density of the suspension was estimated using a hemocytometer (American Optical Company, Buffalo, NY). The initial numbers of cells per dish for repeats A, B, and C were found to be 2.0, 9.3, and 5.3×10^6, respectively. The plating density of repeat A was initially selected on the basis of previous marrow culture studies.[36,39] Plating density was then increased for repeats B and C to achieve a greater confluence of osteoblast colonies.

The dishes were removed from the incubator and the medium was changed every other day for 16 days. The medium was enhanced beginning on day 7 with 50 µg/mL ascorbic acid and beginning on day 11 with 5 mmol/L ß-glycerophosphate (ß-GP, Sigma Diagnostics). The former is required as a cofactor for the activation of lysyl oxidase, used in collagen cross-linking, while the latter serves as a source of phosphate ions to allow mineralization of the matrix synthesized by the differentiating osteoblasts. At day 17, the dishes were removed

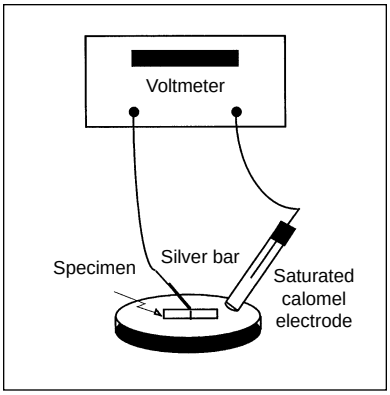

Fig 2a Diagram of solution potential measurement technique showing positions of silver bar and saturated calomel electrode relative to specimen.

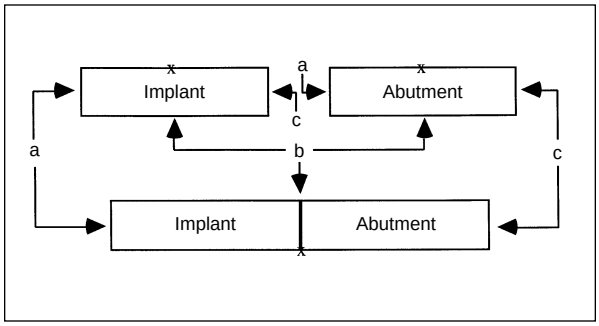

Fig 2b Locations (a, b, c) of saturated calomel electrode measuring tip and silver bar contact (x) for making potential measurements of coupled (*bottom*) and uncoupled (*top*) implant and abutment samples.

from the incubator and viewed at 100× magnification under phase-contrast light microscopy using a phase-contrast microscope (model CK2, Olympus Optical, Japan) equipped for photography. Selected regions in each dish were photographed.

Measurement of Solution Potentials. Immediately following light microscopic viewing, the potentials of individual abutments, the implant and implant/abutment couples were measured[40] in the test medium using a high-impedance voltmeter (Millivolt Meter Model HI 8418, Hanna Instruments, Woonsocket, RI) (Fig 2a) relative to a saturated calomel electrode (SCE). Sufficient medium was removed from each dish to expose a small portion of the specimen, thereby permitting a dry electrical contact to one input of the voltmeter via a pointed silver bar (SB). Specimen potentials were measured in 3 different locations (a, b, c) along the length of the implants, abutments, and couples, as shown in Fig 2b. The SCE was positioned approximately 2 mm from the specimen at each measurement location.

The solution potential data for groups for the 3 repeats (A, B, C) were combined (n = 15) and analyzed by analysis of variance (ANOVA) followed by Tukey Multiple Range Test (at a confidence level of $P = .05$) to determine whether differences existed among the driving forces of the 5 abutment and implant combinations, ΔE_{couple}, and among the 5 implant/abutment couples, E_{couple}. The potential of the implant/ceramic abutment couple was considered in statistical analysis of the couples, although its potential did not result from dissimilar material contact between the implant and the abutment itself.

Alkaline Phosphatase and von Kossa Staining. Immediately following the measurement of solution potentials, dishes were fixed using 10% formal saline for 1 hour at 4˚C; staining was then performed. One dish from each group of 5 was stained for the presence of mineralized bone nodules by the von Kossa technique.[37] The dishes were rinsed twice in cold phosphate-buffered saline (PBS); then 2 mL of 2.5% silver nitrate in deionized distilled water was added to each dish. After 30 minutes in a dark chamber, the dishes were washed with distilled water. Five percent sodium carbonate in formaldehyde was added to each dish and removed after 1 minute, followed by washing with distilled water. The remaining dishes were stained for alkaline phosphatase (APase) activity using a modification of a method previously described by Burstone.[41] The dishes were stained for APase activity by incubating for 45 minutes at room temperature in filtered Tris-hydrochloric acid buffer (0.2 mol/L at pH 8.3) with Naphthol AS MX-Phosphate (Sigma Diagnostics) dissolved in N,N-dimethylformamide as a substrate and Fast Blue B salt (Sigma Diagnostics) as a stain. All dishes were then macroscopically photographed at 0.63× magnification using a 35-mm camera body on a Zeiss Tessovar microscope (Carl Zeiss Jena GmbH, Jena, Germany).

Analysis of Osteoblast-Like Cell Development. The area of the dish covered by cells expressing APase staining was quantified using a dissecting microscope at 7× magnification by screening intercepts of an overlay grid ruled in 2-mm squares for the presence or absence of such cells, as previously described by Grigoriadis et al[42] (Fig 3). The

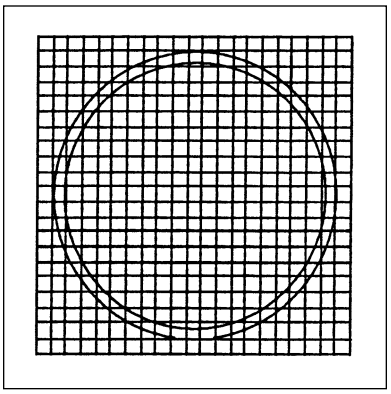

Fig 3 Overlay grid used for quantification of cell culture dishes (actual size).

Table 2 Mean Solution Potentials for Uncoupled Samples

Sample	Potential (mV)
Implant	–48 ± 29 (–15 to –95)
Ceramometal gold alloy	–56 ± 26 (–10 to –110)
Type III gold alloy	–75 ± 16 (–38 to –107)
Cp grade 1 titanium	–120 ± 52 (–21 to –179)
Ti-6Al-4V alloy	–140 ± 31 (–81 to –175)

Vertical lines join groups that are not significantly different at the 95% confidence level (Tukey test).

ratio of those intercepts with APase-positive cells to the total number of intercepts (245) was used to estimate the percentage coverage of the dish. Percent area of mineralization was similarly determined by quantifying grid intercepts with von Kossa-stained nodules. The staining data for groups for the 3 repeats (A, B, C) were combined (n = 12 for APase staining, n = 3 for von Kossa staining), and statistical analysis of the data was performed using ANOVA to compare sample groups to the cell suspension plus BCP control group.

Table 3 Mean Solution Potentials for Coupled Samples

Sample	Potential (mV)
Ceramometal gold alloy	–49 ± 19 (–23 to –79)
Type III gold alloy	–57 ± 22 (–16 to –86)
Ceramic	–95 ± 30 (–19 to –135)
Ti-6Al-4V alloy	–125 ± 22 (–87 to –162)
Cp grade 1 titanium	–128 ± 24 (–90 to –160)

Vertical lines join groups that are not significantly different at the 95% confidence level (Tukey test).

Results

Solution Potentials. At a confidence level of $P = .05$ (Tukey), no difference in the measured values existed between the 3 measurement sites per specimen (a, b, c). Reported potentials for each specimen are the average of the values measured at these 3 locations. Values are shown in Table 2 for uncoupled samples and in Table 3 for coupled samples. Uncoupled samples formed 2 groups (Ti-6Al-4V and cp 1 Ti/Type III gold, ceramometal gold, implant), and coupled samples formed 3 groups (cp 1 Ti and Ti-6Al-4V/ceramic/Type III gold and ceramometal gold) at a confidence level of $P = .05$ (Tukey).

Phase Contrast Microscopy. Representative areas are shown (×100) at 17 days for an uncoupled sample in Fig 4 and for a coupled sample in Fig 5. The cells showed no zone of inhibition around any of the materials or combinations of materials examined. The majority of the implant and abutment surfaces showed areas of excellent cell contact in all of the specimens, although the entire surfaces of the specimens were not uniformly covered with cells. The morphology of the day-17 cell colonies near the specimens showed well-differentiated cuboidal osteoblast-like cells forming a cobblestone appearance. In all specimens there was evidence of piling up of these cells, which is typical of cells forming mineralizing nodules (see Fig 4). There were no differences seen in the uncoupled versus coupled configurations with respect to cell morphology. The osteoblast colonies developing near all samples had an appearance typical of those seen in the marrow controls. Nodule formation occurred in all groups where cells were present. No zones of inhibition were noted in proximity to any of the biomaterials studied, and intimate cell contact with all of the biomaterials was observed.

Alkaline Phosphatase Staining. A representative sample of a coupled group is shown (×0.63) in Fig 6. Quantification of APase activity is shown graphically as percent area normalized to the control group for the combined values of the 3 experiments for uncoupled and coupled samples (Fig 7). An

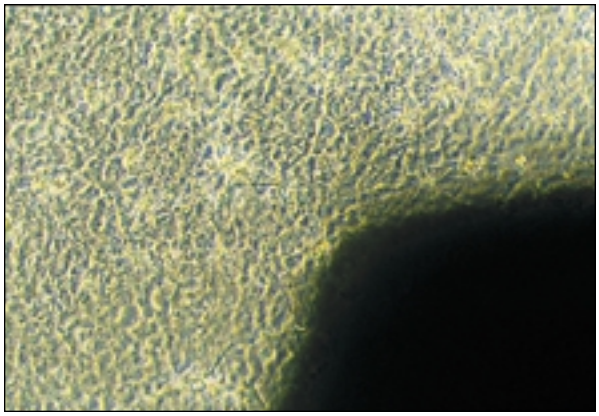

Fig 4 Uncoupled cp 1 Ti abutment (×100).

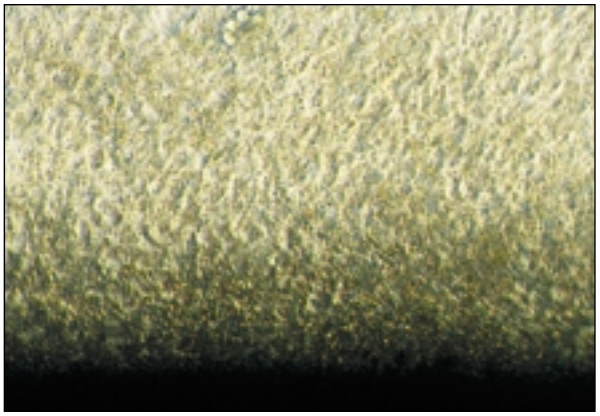

Fig 5 Coupled cp 1 Ti abutment and implant (×100).

Fig 6 Coupled ceramic abutment and implant (Fast Blue B stain, magnification ×0.63).

ANOVA for APase staining area values showed no significant differences (at $P = .05$) between coupled or uncoupled groups and the control (marrow suspension + BCP). There were no observed differences in the distribution pattern of the APase-positive colonies relative to the centrally placed samples.

Von Kossa Staining. All dishes revealed areas of mineralization consistent with bone nodule formation. A representative sample is shown in Fig 8. Quantification of von Kossa staining is shown graphically as percent area normalized to the control group for the combined values of the 3 experiments for uncoupled and coupled samples (Fig 9). An ANOVA for von Kossa staining area values showed no significant differences (at $P = .05$) between the coupled or uncoupled groups. Mineralization was observed immediately adjacent to all specimens.

Discussion

Table 2 shows that the uncoupled samples formed 2 statistically different groups based on their solution potentials ($P = .05$). The more electrically positive group contained the 2 noble alloys (Type III gold alloy and ceramometal gold alloy) and the base metal titanium implant. The more electrically negative group contained the 2 base metal abutments (cp grade 1 titanium and Ti-6Al-4V alloy). The fact that the values for cp grade 1 titanium abutment and the implant (which in the Brånemark System is also cp 1 titanium) are different could well be explained by their very dissimilar processing protocols. The latter is wrought, milled, and subjected to a variety of standardized surface treatments in a biologically and metallurgically controlled environment, then stored until use in a sterile evacuated ampule. The former is cast (albeit in very controlled atmospheric and physical conditions) and then processed by standard dental laboratory techniques. Cast titanium, by virtue of its susceptibility to heat effects and incorporation of contaminants,[43] differs in properties and composition from wrought and/or milled titanium, a fact that could further support the use of precision-milled versus cast fitting surfaces on prosthetic components. Similar findings were recently reported by Cortada et al.[26]

The grouping in Table 2 indicates that the coupling of this titanium implant material and the

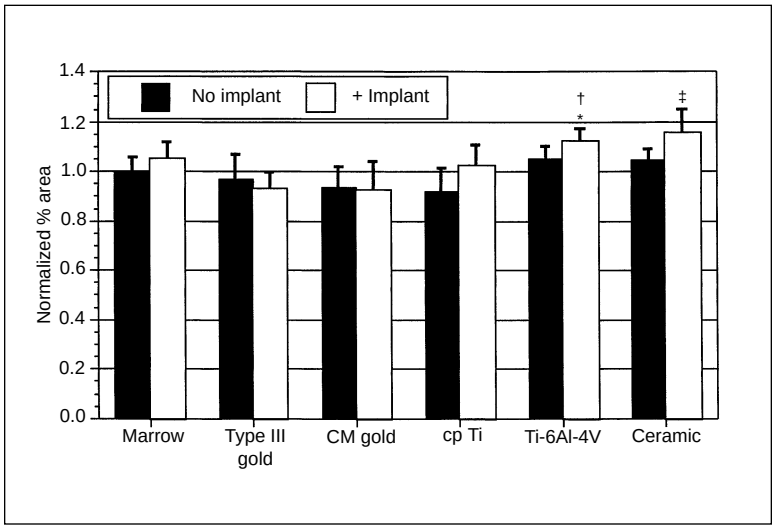

Fig 7 Normalized percent area covered by alkaline phosphatase stained cells; error bars represent standard error. *P = .0568 relative to marrow; †P = .036 relative to Type III gold; ‡P = .0622 relative to Type III gold.

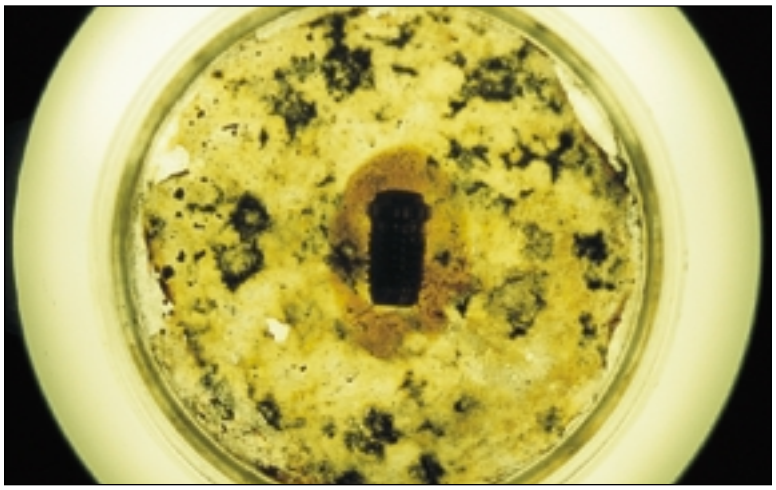

Fig 8 Uncoupled implant (von Kossa stain, magnification ×0.63).

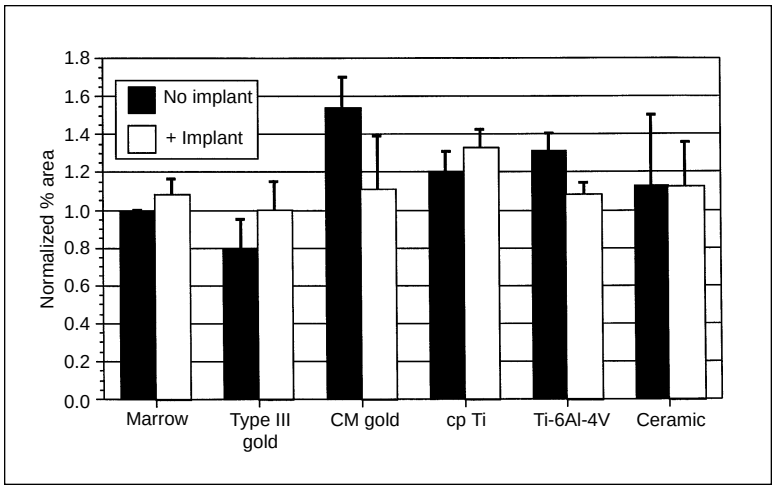

Fig 9 Normalized percent area covered by von Kossa positive colonies; error bars represent standard error.

noble alloy abutments would tend to produce less galvanic polarizing effect (ΔE_{couple}) as compared to coupling with the base metal alloys. Whether the larger values of ΔE_{couple} would produce significant corrosion currents for these highly corrosion-resistant metals and alloys is not known. Previous investigations suggest that resulting corrosion currents would be of low magnitude. Arvidson and Johansson[44] measured the current produced by the galvanic coupling of high noble content gold and cobalt-chromium alloys for 3 weeks in vitro and reported no measurable current densities (< 0.001 $\mu A/cm^2$). The cp grade 1 titanium and the Ti-6Al-4V alloy tested in the present investigation are much more corrosion-resistant than stainless steels or nickel-based casting alloys.[19] Additionally, Ravnholt[15] reported no measurable currents for cp titanium when galvanically coupled for 20 days in vitro to a high noble content gold alloy, Co-Cr alloy, or Type 304 stainless steel (< 1.0 $\mu A/cm^2$). Reclaru and Meyer[23] found minimal currents (< 1.0 $\mu A/cm^2$) for the galvanic coupling of titanium with gold-based alloys, stainless steels (excepting Type 316L), or nickel-based alloys in vitro.

The potential range data in Table 2 show that the titanium implant would not necessarily form the cathode in every galvanic couple tested. For example, the implant at the most negative value of its potential range (–95 mV) could be coupled to a Ti-6Al-4V abutment at the most positive value of its potential range (–80 mV). Under these conditions, the implant would form the anodic component in the couple, making it liable to increased corrosion rates, even though the implant is part of the more electrically positive group. The potential for the resultant surface degradation that becomes possible in this situation and its concomitant disruption of the surface characteristics of the implant[45] may be cause for concern, given the limited parameters of the dynamic exchange that must continue to take place at the Ti-Ti_xO_x-tissue interface of the osseointegrated implant for its continued success.[46] Even in couples where the implant forms the cathode, a high ΔE_{couple} could just as well affect this interface as characteristic current densities, electromagnetic fluxes, and ion exchange are affected, with possible deleterious effects on osseointegration and the remodeling characteristics of the surrounding bone.[15,19,20,29,45,47,48]

Table 3 shows the solution potentials resulting from the coupling of the abutment samples discussed in Table 2 to implants. Statistical analysis revealed 3 different groups (P = .05). The most electrically positive group contained the Type III gold alloy and the ceramometal gold alloy abutments, while the most electrically negative group contained the cp grade 1 titanium and the Ti-6Al-4V alloy abutments. The coupled ceramic abutment was intermediate. While measured potential data are useful to help characterize the electrochemical behavior of metals,[49,50] it must be understood that they are not by themselves capable of predicting corrosion rates for galvanically coupled metals,[1] since the sign and magnitude of the potentials do not indicate the level of corrosion activity. Therefore, it cannot be concluded from these data alone that any one of the couples had higher corrosion currents resulting from galvanic coupling than the others.

An interesting comparison may be made between the E_{couple} data in Table 3 and measurements made in previous in vivo investigations of solution potentials of conventional (ie, non-implant) gold alloy and ceramometal restorations in the mouth. Measured values fall within –200 to +100 mV (versus a SCE) in studies reported by Nomoto et al,[51] Johansson et al,[52] Yontchev et al,[53] and Muller et al.[50] Yontchev et al[53] reported no evidence of a correlation between patient orofacial complaints and the in vivo measured potentials. The potentials measured in the current study involving the components of implant restorations (Table 3) fall within the reported range of clinical acceptability for conventional restorations.

It is not known whether concentration cell corrosion in the form of crevice corrosion (in this case potentially due to a differential in oxygen concentration with respect to the surrounding medium) was affecting the measured potential of coupled samples. When abutments are coupled to an implant, crevices are formed at the surface contact areas; the abutment screw also forms crevices where it contacts the abutment and the implant. It is unlikely, however, that crevice corrosion occurred during this experiment. Since the tested abutments and implant biomaterials are highly resistant to crevice corrosion, the test period of 17 days would probably be insufficient to incubate crevice corrosion in static components.[54] Nevertheless, when the ceramic abutment was attached to the implant, the mean potential was –95 ± 30 mV (Table 3), compared to a mean potential for the implant alone of –48 ± 29 mV (Table 2). A Student t test showed these means are significantly different at P = .05. This difference indicates that the solution potential may be affected by the crevice formed by attachment of the ceramic abutment or by the dissimilar metal screw (gold alloy) used to attach the abutment. The remainder of the abutment screws, while indeed creating crevices (the effect of which on this

system having been deemed negligible, as discussed above) by their very attachment to their abutment and the implant, were assumed not to have any greater "dissimilar metal effect" than the implant itself, both being composed of milled cp 1 Ti.

Uncoupled dental casting alloys have been investigated extensively to evaluate their biocompatibility by studying their cytotoxicity in cell culture, generally in transformed fibroblast lines. Kawahara et al[55] found that the cytotoxicity of pure metals was related to their position on the periodic table of the elements: high for Group I, II, and III elements of low atomic number (eg, Cu) and negligible for those of high atomic number (eg, Au). Wright et al[56] determined the corrosion of Au-Cu-Ag alloys and found a correlation between corrosion and cytotoxicity and the concentration of copper in the culture medium. Pourbaix[57] studied the electrochemical corrosion of gold and silver-based biomaterials and found that it occurred from Ag-rich and Cu-rich segregations.

Craig and Hanks[27] studied the effects of dental casting alloys in both their as-cast and polished forms on cell morphology, succinic dehydrogenase (SDHase) activity, and optical density in fibroblast culture following a 2- to 3- day incubation period. Included in their study were the ceramometal gold alloy used in the present study and a Type III gold alloy very similar to the one used in the present study. They found the ceramometal gold alloy to exhibit very low cytotoxicity, especially in its polished form, attributing this to the biopassivating effect of the adherent base metal oxide layer produced by the indium in the alloy. The Type III gold alloy was found to exhibit moderate cytotoxicity, higher in its as-cast form; this was attributed to the release of copper, which was exacerbated in the unpolished form of these alloy samples. These investigators completed a similarly designed subsequent study,[34] this time studying a wide variety of experimental alloys (including Ti-6Al-4V) as well as some pure metals (including Ti, Au, Pd, Ag, and Cu). Titanium, Ti-6Al-4V, Au, and Pd were found to exhibit very low cytotoxicity, with Ag exhibiting low cytotoxicity; Cu showed very high cytotoxicity. The high biocompatibility of gold and palladium was attributed to their high nobility, while that of titanium and its alloy was attributed to their highly adherent biopassivating oxide layers. Copper cytotoxicity was imputed to its ready dissolution in the culture medium. A further study was undertaken by Wataha et al[58] investigating elemental release from high-noble, noble, and Ag-based alloys in fibroblast culture. It was determined that silver and copper release appeared to be the primary cause of cytotoxicity with these alloys, as determined by SDHase activity. Cortada et al[26] have also expressed concern with observed Ag and Cu ion release from Au-Cu-Ag and Pd-Cu-Ga alloys coupled to cp grade 1 Ti implants in artificial saliva in vitro, postulating that the levels observed would result in cytotoxic effects, in accordance with the aforementioned work of Wataha et al.[58]

Preosteoblasts from bone marrow were observed to differentiate and form mature, mineralized bone matrix nodules in the presence of all biomaterials tested in this study during the 17-day incubation period. The normal cell morphology, APase-positive staining, and nodule formation all reflect the lack of significant negative effects on osteoblast differentiation with these biomaterials as compared to the marrow controls, whether in their coupled or uncoupled configurations. The lack of zones of inhibition associated with any of the specimens also suggests that the cells were not experiencing significant negative effects from the electrochemical potentials that were shown to occur with the coupled and uncoupled biomaterials observed in this investigation. The trend toward greater APase-positive area observed in the Ti-6Al-4V (P = .0568) and ceramic combinations reflects the tendency of these materials to facilitate calcium and phosphate ion exchange at their surfaces (relative to the gold alloys), which may be conducive to osteoblast differentiation. The tendency of Type III gold alloy combinations to show lesser APase-positive area than observed in the Ti-6Al-4V (P = .036) and ceramic (P = .062) combinations may be attributable to the cytotoxic effect of the release of copper by these alloys in cell culture, especially in their as-cast form, as demonstrated by Craig and Hanks[33,34] and Wataha et al.[58] It is important to note, however, that the Type III gold alloy combinations did not perform statistically more poorly than the marrow controls with respect to the development of APase-positive colony area or percent area mineralized. Thus, although statistically significant differences were seen between Type III gold alloy and Ti-6Al-4V alloy with respect to APase-positive colony area, these differences represent the extremes within the biomaterials tested, and neither was significantly different to the marrow controls, although the Ti-6Al-4V approached significant improvement at P = .0568.

The body of implant literature shows the continuing conflict in the philosophy toward electrical potential and current as they pertain to the osseointegration of titanium; some workers propose deleterious effects, some propose beneficial

effects, while some propose no effect at all. The present study, while its statistical data and cytologic observations place it in the last of these 3 groups, shows data trends that suggest that the phenomena discussed by the other groups may bear further consideration. Further research is indicated, starting with long-term controlled prospective studies on endosseous titanium implants in animal models involving the application of electric potentials, currents, and fields, as well as the measurement of galvanic potentials and currents resulting from the coupling of oral prostheses. The effects of these electrical phenomena on both the host tissues and the prosthetic biomaterials must be definitively described if evidence-based choices are to be made when selecting implant prosthesis biomaterials and optimizing the prosthetic outcome for patients.

Conclusions

1. Differences were observed in solution potentials measured in bone marrow culture among the test groups of prosthodontic biomaterials both uncoupled and coupled to titanium endosseous dental implants.
2. Measured in vitro potentials of abutments and couples fell within the range of previously reported in vivo potentials of clinically successful non-implant restorations.
3. No differences were observed in alkaline phosphatase staining area or von Kossa staining area and controls measured in bone marrow culture among the tested groups of prosthodontic biomaterials, both uncoupled and coupled to titanium endosseous dental implants.
4. The presence of the tested commonly used implant abutment biomaterials, both uncoupled and coupled to titanium endosseous dental implants, had no adverse effects on the in vitro capacity of preosteoblasts in bone marrow culture to differentiate and form mineralized bone nodules, despite measured differences in solution potentials.

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