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Peri-implant Inflammation Defined by the Implant-Abutment Interface

ABSTRACT

An implant-abutment interface at the alveolar bone crest is associated with sustained peri-implant inflammation; however, whether magnitude of inflammation is proportionally dependent upon interface position remains unknown. This study compared the distribution and density of inflammatory cells surrounding implants with a supracrestal, crestal, or subcrestal implant-abutment interface. All implants developed a similar pattern of peri-implant inflammation: neutrophilic polymorphonuclear leukocytes (neutrophils) maximally accumulated at or immediately coronal to the interface. However, peri-implant neutrophil accrual increased progressively as the implant-abutment interface depth increased, *i.e.*, subcrestal interfaces promoted a significantly greater maximum density of neutrophils than did supracrestal interfaces (10,512 ± 691 *vs.* 2398 ± 1077 neutrophils/mm^2). Moreover, inflammatory cell accumulation below the original bone crest was significantly correlated with bone loss. Thus, the implant-abutment interface dictates the intensity and location of peri-implant inflammatory cell accumulation, a potential contributing component in the extent of implant-associated alveolar bone loss.

KEY WORDS: bone loss, implant-abutment interface, inflammation, microgap, neutrophil.

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INTRODUCTION

The placement of an implant-abutment interface at the level of alveolar bone is associated with a significant inflammatory cell infiltrate and bone loss, as compared with the complete absence of an interface (Broggini *et al.*, 2003). These observations suggest that (1) an inflammatory stimulus originates at the implant-abutment interface, and (2) there is a causal relationship between the extent of peri-implant inflammation and the magnitude of alveolar bone loss.

The pathophysiological consequences of the implant-abutment interface position have clinically important implications, since esthetic demands encourage the placement of implants in a more apical position (Buser and von Arx, 2000). Such placement could promote inflammation and bone loss, perhaps recession, and esthetic failure. Relative to the original alveolar crest, crestal and subcrestal implants have demonstrated greater bone loss than have implants placed supracrestally (Todescan *et al.*, 2002; Piattelli *et al.*, 2003), although the data observed under additional conditions of immediate/early load are difficult to interpret (Piattelli *et al.*, 2003; Siar *et al.*, 2003). Additionally, differences in response may exist for implants (Abrahamsson *et al.*, 1997) whose abutments have not been manipulated during healing (Todescan *et al.*, 2002). Furthermore, the magnitude and distribution of inflammatory cells along the implant surface remain to be established. The purpose of the current study was to determine whether an increasing apical position of the implant-abutment interface leads to a proportionally greater magnitude of inflammatory cells and associated bone loss under simulated clinical conditions.

MATERIALS & METHODS

Implant Design and Placement

Following approval by the Institutional Animal Care and Use Committee (UTHSCSA, San Antonio, TX, USA), two-piece, submerged implants varying in the apico-coronal location of the implant-abutment interface were placed either 1 mm coronal to, at, or 1 mm apical to the alveolar bone crest (supracrestal, crestal, or subcrestal, respectively) (Fig. 1A). Specimens were procured from a larger study involving six different implant designs, as previously reported (Hermann *et al.*, 1997). In brief, 30 implants fabricated of grade IV commercially pure titanium and with a screw-type sand-blasted, large-grit, acid-etched (SLA) surface (Institut Straumann AG, Waldenburg, Switzerland) were placed in duplicate (left and right) in partially edentulous mandibles of 5 male foxhound dogs. The SLA surface was 4.5 mm in length, with a rough-smooth border between the SLA surface and the machined coronal portion located 1.5 mm below the original alveolar bone crest, *i.e.*, the machined portion had apico-coronal lengths of 2.5 mm, 1.5 mm, and 0.5 mm for supracrestal, crestal, and subcrestal implants, respectively. Core implant diameter was 3.5 mm, while the thread diameter was 4.1 mm, the machined-

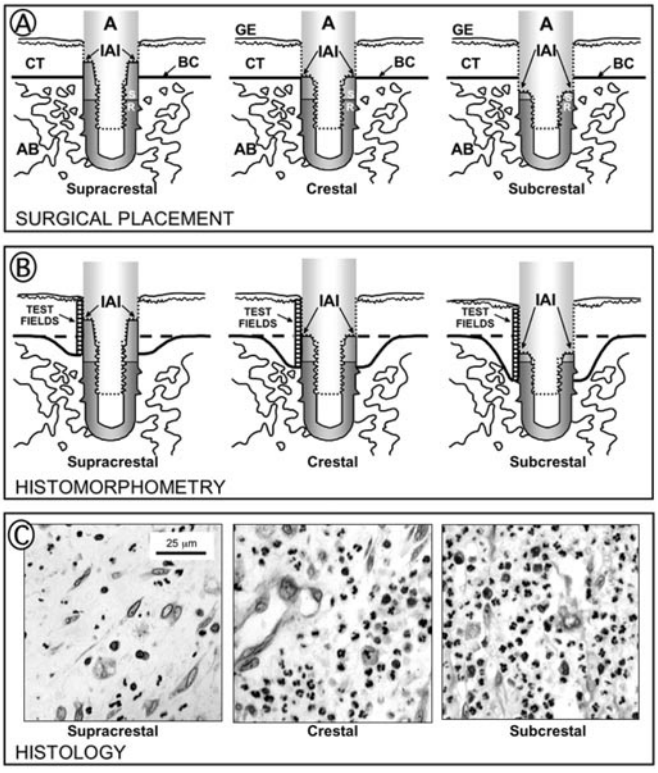

Figure 1. Implant design and placement, histomorphometric study area, and histology. **(A)** At the time of initial surgery, implants were positioned so that the implant-abutment interface was either supracrestal, crestal, or subcrestal to the alveolar bone, *i.e.*, 1 mm coronal to, at, or 1 mm apical to the alveolar bone crest, respectively. Three months later, abutments (outlined in a dashed line) were connected to implants. After 3 additional mos, specimens were derived for histomorphometric analyses. **(B)** Sequential histological (test) fields of apico-coronal peri-implant soft tissue were digitally captured; morphometric assessment of peri-implant tissue was confined to connective tissue immediately adjacent to the implant surface from gingival epithelium to alveolar bone. All non-vascular interstitial cells were assessed and designated as either neutrophils or mononuclear cells. For the latter, lymphocytes, plasma cells, monocytes, and macrophages were collectively considered as a single population of cells. **(C)** Photomicrographs of representative soft tissues immediately adjacent to the implant-abutment interface. Although neutrophils are abundant in the specimen with crestal or subcrestal placement, these cells were infrequent in the supracrestal specimen. Toluidine blue, basic fuchsin stain. A = abutment; AB = alveolar bone; BC = bone crest; CT = connective tissue; GE = gingival epithelium; IAI = implant-abutment interface; R = rough portion of implant (SLA surface); S = smooth-machined collar of implant.

surface abutment diameter was 3.5 mm, and the implant-abutment interface was 50 µm. Plaque control was performed three times a week with a soft toothbrush and sponge, in combination with a 0.2% chlorhexidine gel (PlakOut Gel, Hawe-Neos AG, Bioggio/TI, Switzerland). Abutments were connected 3 mos later. At 4, 8, and 10 wks following abutment connection, abutments were loosened and then immediately tightened, to imitate typical clinical procedures. Six months following initial implant placement, specimens were obtained and prepared for non-decalcified histology (Schenk *et al.*, 1984). Sections (~ 80 µm) were glued to Plexiglass with acrylic cement and stained superficially with toluidine blue, followed by basic fuchsin.

Histomorphometric Analyses

Light microscopic histomorphometry was performed as previously described (Broggini *et al.*, 2003). Histomorphometric software (Image-Pro Plus®, Media Cybernetics, Silver Spring, MD, USA) was used for digital image capture, enumeration of individual inflammatory cells, and measurement of peri-implant tissue areas.

Sequential peri-implant test fields (0.25 mm x 0.33 mm) along the entire implant surface (Fig. 1B) were evaluated after digital capture (at X340). The original bone crest was designated as the histological reference point for all specimens. This reference point coincided with the location of the implant-abutment interface for crestal implants, whereas, for supracrestal and subcrestal implants, the reference point was defined 1 mm apical or coronal to the interface, respectively. In captured images of each test field, neutrophils and mononuclear cells (lymphocytes, plasma cells, monocytes, and macrophages) were identified on the basis of cytoplasmic and nuclear morphology. Intravascular inflammatory cells were excluded, as were areas of alveolar bone, blood vessels, and gingival or junctional epithelium. Thus, extravascular (interstitial) soft tissues were assessed and used to calculate cell density, *i.e.*, cells/mm^2, for each field. Linear soft tissue distances were calculated by summation of the apico-coronal dimensions of test fields. This histomorphometric measurement of soft tissue distances below the original bone level confirmed results of a previous study that evaluated bone-to-implant distances in the same histological specimen (Hermann *et al.*, 2000). Histomorphometric data from crestal implants have been published previously (Broggini *et al.*, 2003).

Data Analysis

Test fields were evaluated individually and collectively as three different zones: the entire apico-coronal distance, coronal to the original bone crest, and apical to the original bone crest. The rationale for this subdivision was the hypothesis that a subset of cell accumulation could be correlated with bone loss. Results from corresponding implants in the right and left mandibles of each animal were averaged and used to calculate descriptive statistics (mean ± SEM; n = 5 animals). We used analysis of variance for repeated measures, with Tukey's multiple comparison test of least-squares means, to determine whether significant differences existed among implant types. These analyses included adjustments for variability in implant and animal. SAS software (SAS, Cary, NC, USA) was used for all statistical analyses; $p \leq 0.05$ was considered significant.

RESULTS

As the apical position of the implant-abutment interface was progressively increased, the total number of peri-implant inflammatory cells was increased in parallel, *i.e.*, the deeper the interface, the greater the magnitude of peri-implant inflammation (Fig. 2, Table). Further, regardless of the implant-abutment interface position relative to the original alveolar bone crest, the highest concentration of inflammatory cells was consistently at or immediately coronal to the interface, and progressively decreased thereafter toward bone or gingiva (Fig. 1c). For both crestal and implants, the neutrophil was the predominant peri-implant inflammatory cell. Comparatively, however, the apico-coronal accumulation of peri-implant neutrophils associated with subcrestal implants was significantly ($p < 0.005$) increased in

comparison with crestal implants (5934 ± 998 *vs.* 2968 ± 280 neutrophils). For supracrestal implants, cumulative peri-implant mononuclear cells were somewhat greater than neutrophils; nevertheless, among all implants, mononuclear cells were relatively uniformly distributed along the entire implant surface (Fig. 2, Table).

The implant-abutment interface position significantly influenced peri-implant inflammatory cell accumulation apical to the original bone crest (Table). In contrast, interface position had no significant effect on the cumulative collection of neutrophils or mononuclear cells coronal to the original bone crest (although there was a trend toward increased numbers of neutrophils with increased depth of the interface). Importantly, as the interface depth was progressively increased, the apical accumulation of neutrophils was sequentially and significantly increased ($p < 0.005$) (Table). Further, the maximum density of neutrophils adjacent to supracrestal implants (2398 ± 1077 cells/mm^2) was significantly less ($p < 0.005$) than for crestal and subcrestal implants, *i.e.*, 8276 ± 1031 cells/mm^2 and 10,512 ± 691 cells/mm^2, respectively. Moreover, the peri-implant location with maximum neutrophil density was also dependent upon the depth of the implant-abutment interface (Table). Thus, for supracrestal implants, this location was near the implant-abutment interface (1.00 ± 0.41 mm *above* the original bone crest), whereas for subcrestal implants, this location was immediately coronal to the implant-abutment interface (0.53 ± 0.35 mm *below* the original bone crest).

In parallel with differences in peri-implant inflammatory cell accumulation, the apico-coronal dimension of connective tissue was also progressively expanded as the depth of the implant-abutment interface was increased (Table). This primarily reflected increases in the connective tissue compartment apical to the original alveolar bone crest (*i.e.*, alveolar bone loss). Specifically, there was significantly greater ($p < 0.0005$) bone loss associated with subcrestal implants (2.45 ± 0.20 mm) as compared with implants placed in either a crestal (1.60 ± 0.17 mm) or supracrestal (1.40 ± 0.12 mm) position.

When the data derived from all implant types were considered, the relationship between bone loss and inflammatory cell accumulation *below* the original alveolar bone crest was highly significant ($p \leq 0.0001$) (Fig. 3). In contrast, there was no significant relationship between bone loss and the accumulation of inflammatory cells *above* the original alveolar bone crest.

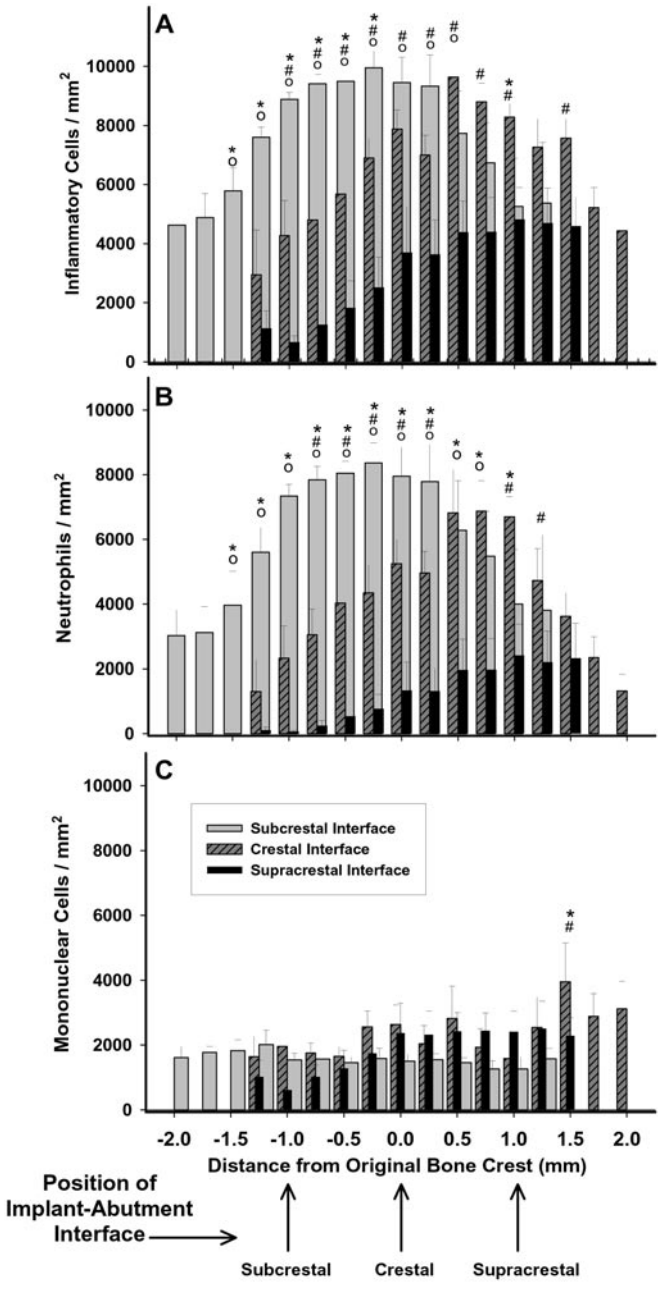

Figure 2. Effect of the implant-abutment interface position on the distribution of peri-implant inflammatory cells. Histomorphometric quantitation of neutrophils and mononuclear cells at specific locations relative to the original alveolar bone were averaged for a given implant type in each animal. These results were then used to calculate the group mean (± SEM; n = 5). **(A)** Total inflammatory cells. **(B)** Neutrophils. **(C)** Mononuclear cells. * = significant differences between crestal and subcrestal implants, # = significant differences between crestal and supracrestal implants, and o = significant differences between subcrestal and supracrestal implants ($p < 0.05$).

DISCUSSION

Current focus on the enhancement of peri-implant esthetic outcomes has led to recommendations regarding surgical incisions, implant placement relative to adjacent teeth and/or implants, and surgical handling of soft tissues (Buser and von Arx, 2000; Belser *et al.*, 2003). Ideal apico-coronal implant position for a harmonious emergence profile and adequate soft tissue support has also been postulated. The rationale behind most recommendations is that if implant placement is performed in a certain manner, soft tissue contours will mimic the natural dentition and render an esthetic result. Despite increasing progress, the actual determinants of soft tissue response remain to be clarified.

The current study investigated the influence of implant-abutment/crown interface position upon peri-implant soft tissue response. Specifically, the quantity and nature of peri-implant inflammation and the magnitude of associated alveolar bone loss were examined. Among all implants, the peak concentration of peri-implant inflammatory cells occurred consistently at or immediately coronal to the implant-abutment

Table. Effects of Implant-Abutment Interface Position on Peri-implant Inflammation and Alveolar Bone Loss

	Entire Apico-Coronal Distance[a]	Coronal to Original Bone Crest[a]	Apical to Original Bone Crest[a]	Maximum Cell Density[a] (cells/mm^2)	Location of Maximum Cell Density (mm)[a]
			Cumulative Cells		
Total cells					
Supracrestal	[c,d] 2333 ± 891[b]	1540 ± 542	[c,d]793 ± 377[c,d]	5,683 ± 1138	0.45 ± 0.05
Crestal	[c] 4677 ± 654	2676 ± 474	[c] 2001 ± 338	10,542 ± 847	0.48 ± 0.26
Subcrestal	7722 ± 921	2578 ± 828	5144 ± 368	11,777 ± 587	-0.53 ± 0.35
Neutrophils					
Supracrestal	[c,d] 937 ± 493 ([c,d]27 ± 11)	718 ± 389 ([c,d]31 ± 13)	[c,d]219 ± 132 ([c,d]14 ± 8)	[c,d]2,398 ± 1077	[c]1.00 ± 0.41
Crestal	[c] 2968 ± 280 (63 ± 4)	1795 ± 259 (64 ± 7)	[c]1173 ± 198 (58 ± 5)	8,276 ± 1030	0.35 ± 0.13
Subcrestal	5934 ± 998 (76 ± 4)	2032 ± 797 (75 ± 7)	3902 ± 326 (76 ± 3)	10,512 ± 691	-0.53 ± 0.35
Mononuclear Cells					
Supracrestal	1396 ± 536 ([c,d]73 ± 11)	822 ± 289 ([c,d]69 ± 13)	[c] 575 ± 252 ([c,d]86 ± 8)	3,869 ± 736	0.10 ± 0.21
Crestal	1709 ± 471 (37 ± 4)	881 ± 318 (36 ± 7)	828 ± 190 (43 ± 5)	5,451 ± 1009	0.25 ± 0.45
Subcrestal	1788 ± 232 (25 ± 4)	546 ± 167 (25 ± 7)	1242 ± 207 (24 ± 3)	3,606 ± 540	-1.03 ± 0.61
			Distance		
Connective Tissue (mm)					
Supracrestal	[c]3.08 ± 0.30	1.68 ± 0.31	[c]1.40 ± 0.12	n/a	n/a
Crestal	3.45 ± 0.26	1.85 ± 0.24	[c]1.60 ± 0.17	n/a	n/a
Subcrestal	4.00 ± 0.19	1.55 ± 0.33	2.45 ± 0.20	n/a	n/a

[a] Peri-implant soft tissue was examined in reference to the alveolar bone crest at the time of implant placement; the location of maximum cell density for a given implant was determined relative to the distance (mm) from the original alveolar bone crest.
[b] Results are presented as the mean ± SEM; numbers in parentheses are percentages of total cells. Data derived from duplicate implants in an individual animal were averaged and then used in calculation of the mean for each implant type; n = 5 animals/group.
[c] Significantly different ($p < 0.005$) from corresponding subcrestal implant.
[d] Significantly different ($p < 0.005$) from corresponding crestal implant.

interface, regardless of interface position. Despite this shared distribution pattern of inflammatory cells relative to the interface, increased apical implant-abutment depth was associated with significantly greater peri-implant inflammation and concomitant bone loss. Moreover, the extent of bone loss was significantly related to the magnitude of inflammatory cell accumulation below the original alveolar bone crest. In combination, these findings indicate that the implant-abutment interface position defines the degree of inflammatory cell accumulation, and suggest that inflammatory cells contribute, directly or indirectly, to the extent of alveolar bone destruction.

The present study confirmed earlier observations that neutrophils are the predominant peri-implant inflammatory cell (Broggini *et al.*, 2003). Increased accumulation of acute inflammatory cells near the implant-abutment interface suggests that a persistent chemotactic stimulus arises from this region to sustain continuous neutrophil recruitment. More importantly, this stimulus progressively increases as the interface is placed more apically. The source and nature of a persistent chemotactic stimulus remains unknown, but likely reflects the presence of microbes within the implant-abutment/crown interface, as demonstrated by previous clinical studies (Quirynen and van Steenberghe, 1993; Persson *et al.*, 1996). This presence of bacteria may result from either contamination during initial implant placement and/or abutment connection or transmission of micro-organisms from the oral environment after prosthetic installation (Persson *et al.*, 1996). Indeed, the phenomenon of microleakage has been described, regardless of implant system (Quirynen *et al.*, 1994; Jansen *et al.*, 1997; Guindy *et al.*, 1998; Gross *et al.*, 1999). Additionally, a reduction of microbial access through internal components has been demonstrated by utilization of the intermediate washers between components, cement *vs.* screw-retained restorations, or inter-component varnish application (Besimo *et al.*, 1999; Piattelli *et al.*, 2001; Rimondini *et al.*, 2001). Further evidence that a persistent chemotactic stimulus originates at the interface is supported by the fact that, in the complete absence of an interface, only scant peri-implant inflammatory cells accumulate (Broggini *et al.*, 2003). If such a microbial chemotactic stimulus could be contained, a transition from a neutrophilic inflammatory infiltrate into a mononuclear cell population—*e.g.*, monocyte/macrophages, lymphocytes, and/or plasma cells—would be observed. That this cellular transition did not occur in the current study, even after 6 mos, suggests that the stimulus associated with the interface persisted and could not be resolved. These findings are consistent with our working hypothesis, in which persistent microbes within the implant-abutment interface continually

produce a chemotactic signal to sustain the acute inflammatory cell infiltrate. Another possibility is that micromotion could result in fretting corrosion, which could produce fine particles and corrosion products that also contribute to the inflammatory response. However, scanning electron microscopy has not indicated such events (unpublished observations), and no other evidence indicates that this occurs.

This study has also identified a highly significant relationship between the amount of peri-implant inflammation and the magnitude of alveolar bone loss. A causal relationship is suggested, since this association was limited to the extent of inflammatory cell accumulation *below* the original alveolar bone crest, *i.e.*, no such relationship existed between bone loss and inflammatory cells above the original alveolar crest. Such detrimental tissue destruction as a consequence of inflammation is known to develop in diverse pathophysiological settings. Around teeth with periodontal disease, this has been described as an "extended arm" of gingival inflammation (Waerhaug, 1979), an "effective radius of action" (Garant, 1979), and an "inflammatory front" (Graves and Cochran, 2003). If the host inflammatory response is minimized, bone loss is greatly reduced, *e.g.*, as demonstrated with inhibitors of the pro-inflammatory molecules IL1 and TNFα (Assuma *et al.*, 1998). The current study demonstrated that moving the interface supracrestally, effectively changing the location of the inflammatory stimulus, also reduces peri-implant bone loss. Thus, minimal inflammation (and bone change) occurred when the interface was above the original bone crest, whereas the greatest inflammation (and bone loss) occurred when the interface was below the alveolar crest.

Our findings have several important clinical implications relative to limiting inflammation and bone loss around implants. First, implant design could be either one-part or transmucosal to eliminate the interface. Second, the interface could be positioned supracrestally. Or, third, the interface might be made in such a way that excludes microbes. In these scenarios, inflammation would not be expected to develop near the alveolar crest, consequently reducing the potential for bony changes. Support for this speculation comes from another animal study in which implants were placed with the interface approximately 3 mm above the original alveolar crest. Bone loss around these implants was minimal (Hermann *et al.*, 1997). Further, in patients with transmucosal implants placed so that the implant interface was approximately 3 mm above the original alveolar crest, minimal bone loss was observed over an eight-year period (Buser *et al.*, 1999).

Results from the current study are also consistent with those of a recent prospective clinical trial that identified a similar relationship between the location of the interface and the magnitude of bone loss (Hartman and Cochran, 2004). In this clinical investigation, when the implant interface was placed close to the original alveolar crest, greater bone loss occurred as compared with implants with the interface placed more supracrestally. Additionally, bone loss did not develop until the interface was created, and, when it occurred, bone loss progressed rapidly and then was relatively stable for up to 5 yrs. Thus, the location of the interface is an important determinant of alveolar bone loss in humans, as has been noted in initial observations (Bränemark *et al.*, 1969) and subsequently studied (Ericsson *et al.*, 1996; Hermann *et al.*, 1997). These clinical observations are highly relevant, since the maintenance of crestal bone height appears to be an important predictor of soft tissue margins in both natural dentition (Gargiulo *et al.*, 1961; Tarnow *et al.*, 1992) and implants (Hermann *et al.*, 2001).

In summary, this study has documented that an intense concentration of peri-implant inflammatory cells is associated with the implant-abutment/crown interface, regardless of whether the interface is placed at, above, or below the alveolar bone crest. Furthermore, implant-associated inflammation resulted in significant bone loss when the interface was located at or below the original bone crest level. Therefore, there is a direct relationship between implant configuration and peri-implant soft tissue outcome.

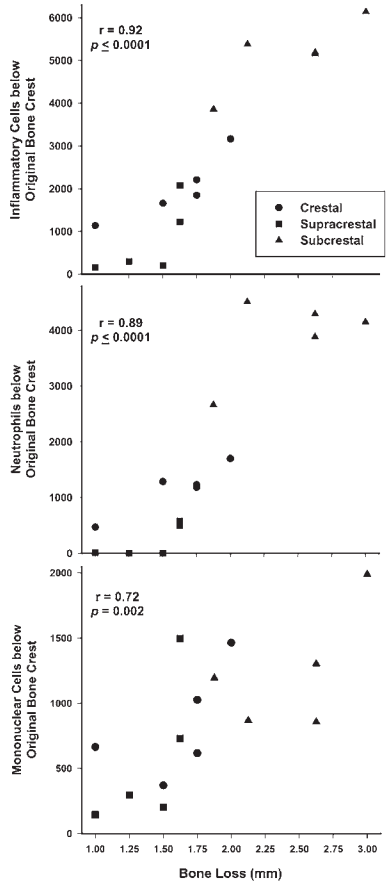

Figure 3. Relationship between inflammatory cells below the original bone crest and bone loss. For a given animal, results from duplicate samples were averaged.

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