Buccal and Lingual Differences of Peri-Implant Bone Quality

THESIS

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Abstract

Objective: The objective of the current study was to examine whether peri-implant bone tissue properties at the buccal region are different from those at the lingual region as a result of growth factor treatments at post-implantation healing periods. Methods: Four dental implant groups were used: titanium (Ti) implants, alumina-blasted zirconia implants (ATZ-N), alumina-blasted zirconia implants with demineralized bone matrix (DBM) (ATZ-D), and alumina-blasted zirconia implants with rhBMP-2 (ATZ-B). These implants were placed in mandibles of six male dogs. Nanoindentation elastic modulus (E) and plastic hardness (H) were measured for the buccal and lingual bone tissues adjacent and away from the implants at 3 and 6 weeks post-implantation. A total of 2281 indentations were conducted for 48 placed implants. Results: The peri-implant buccal region had less bone quantity resulting from lower height and narrower width of bone tissue than the lingual region. Buccal bone tissues had significant greater mean values of E and H than lingual bone tissues at each distance and healing period ($p<0.007$). Nearly all implant treatment groups displayed lower mean values of the E at the lingual bone tissues than at the buccal bone tissues ($p<0.046$) although the difference was not significant for the Ti implant group ($p=0.758$). Conclusions: The DBM and rhBMP-2 treatments stimulated more peri-implant bone remodeling at the lingual region, producing more immature new bone tissues with lower E than at the buccal region. Clinical Significance: This finding suggests that the growth factor treatments to the zirconia

implant system may help balance the quantity and quality differences between the peri-implant bone tissues.

Dedication

This document is dedicated to my family.

Acknowledgments

I would like to thank all those who have supported and encouraged me throughout the years. I would like to extend a special thank you to Drs. Lee, Brantley, and Kim whom are all amazing scientists, mentors, and individuals. I will surely take the lessons that I have learned from each of you and apply them to my life as I strive to do something meaningful.

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Chapter 1: Introduction

Conventional implant systems have achieved high success with respect to osseointegration and implant survival. However, it has been reported that while single implants within the anterior maxilla have a 96% success rate in terms of function and survival only 9% of these implants were considered successful in esthetic terms [1, 2]. The esthetic outcome of a restoration is largely dependent on the alveolar bone quantity. Sufficient bone allows for implants to be placed at the appropriate buccolingual orientation and this allows for the implant to be restored with an emergence profile that mimics a natural tooth. When insufficient bone exists, this poses a challenge to restoration of the site and a multitude of approaches have been considered for bone augmentation [3-5]. Another important esthetic consideration is the crestal bone height at interproximal sites and over facial surfaces of teeth. The lack of bone at interproximal sites will result in an atrophic inter-dental papilla [6] while localized dehiscence, defined as a condition where portion of root is without bony covering, may be associated with gingival recession [7] that disproportionately effects the buccal. Therefore, it is clear that while esthetic qualities of soft and hard tissues may be evaluated independently according to published white (tooth and bone) and pink (gingival) scores [7, 8] that they are interrelated.

Alveolar Development & Dimensional Changes Following Tooth Loss:

The alveolar bone is continuous with basal bone and develops in a vertical and buccal direction as teeth erupt. When there is tooth agenesis or ankylosis the normal alveolar development is disturbed [9]. The alveolar process is composed of an outer cortical wall, inner cancellous bone, and the alveolar bone proper or bundle bone that in conjunction with the periodontal ligament (PDL) supports the dentition. The bundle bone is reported to be approximately 0.4 mm in width, is continuous with the outer cortical bone and derives its vascular supply from the outer cortical plate periosteum, PDL, or marrow space. The latter is a predominate feature of the lingual alveolar bone [10].

The total bone volume of the lingual alveolar bone is greater than buccal bone [11] and buccal alveolar bone is thinner and more fragile than the lingual alveolar bone for the all sites in the maxilla [12]. The mandible is similar except that the excluding the lower incisors where both the buccal and lingual bone is thin [12]. Physical measurements from dentulous regions within a human cadaver demonstrate buccal alveolar bone that is thickest in the molar region and thinnest in the incisor region with a range from 1.6 to 2.2 mm [13]. Similarly, Han et al. showed that the buccal bone at the maxillary central incisors and mandibular incisors were the thinnest alveolar structures ($P<0.05$) [14]. A non-invasive method to evaluate alveolar bone dimensions utilizing Cone Beam CT suggests that only 15% of crestal, buccal alveolar bone in the anterior maxilla is thicker than 1 mm [12]. Similarly canine models also show increased lingual alveolar thickness relative to the buccal aspect [11].

When a tooth is extracted the alveolar proper and PDL is destroyed and the alveolar ridge undergoes disuse atrophy [15]. The healing process following tooth extraction has been extensively studied in human studies [16] as well as in animal models [11, 17]. Histologically it was shown that alveolar ridge resorption occurred in two phases. In the first phase, the woven bone (that replace bundle bone) is resorbed and then in an overlapping second phase the outer cortical plate demonstrated external resorption[11, 17]. The reason for this external resorption is not clear; however, the three-dimensional changes that result are well documented. In both the maxilla and mandible edentulous sites resorb more on the facial than the lingual/palatal and the ridge shifts lingual/palatal and effectively reduces arch length [18]. The bone loss is greatest in the transverse; however, there is also a loss in vertical bone height that is greater at the buccal alveolar bone [11, 19, 20]. In a recent meta-analysis the average transverse alveolar loss was 3.87 mm while the vertical bone loss was 1.61-2.03 and 1. 5mm when accessed, respectively, by clinical and radiographic methodology [21]. The rate of ridge atrophy can vary four-fold and is effected by gender, site (mandible, maxilla), age at time of tooth loss, as well as from local and systemic factors [22]. Resorption occurs throughout life but occurs most rapidly over the 6 months following extractions [21]. At 3, 6, and 12 months post-extraction bone loss was estimated as 21, 36, and 44% [22]. This period of rapid alveolar changes coincide with data showing that that majority of recession occurs within the first 3 months [23] and on the buccal aspect in 80% all cases [24].

In order to minimize bone loss following dental extractions, it was proposed that placement of immediate implants would maintain bone volume and ultimately improve the esthetic treatment outcomes. Conflicting findings are in the literature and although it has been shown that immediate implant placement maintains the transverse alveolar dimension better than delayed implant placement [25] other studies have shown less promising results and have shown vertical and transverse loss most significantly effecting the buccal alveolar bone [25, 26]. The clinical consensus, according to a 2010 Cochrane review, there is no clear advantage or disadvantage to immediate, immediate-delayed, or delayed implants [26]. Some attribute the disproportionate buccal bone loss to bone quantity [11] and it has been proposed that an initial buccal bone thickness of 1-2 mm at time of implant placement will maintain alveolar bone levels [27].

Mechanical Characterization of Alveolar Bone:

In addition to peri-implant bone quantity, bone quality also contributes to mechanical stability and regional properties (buccal, lingual) may contribute to the susceptibility for vertical and transverse dimensional changes within buccal bone. However, few studies have evaluated the mechanical properties of alveolar bone [28-32] and only one has distinguished between the buccal and lingual alveolar bone [33]. It was found significant variation in the elastic modulus of a dentate arch depending on jaw (maxillary<mandibular), location within jaw (anterior<posterior), and region within

alveolus (alveolar bone vs. bundle bone) [33]. No comparisons have been made between buccal and lingual peri-implant bone.

Objective:

The purpose of this study is to evaluate the mechanical properties of buccal and lingual peri-implant alveolar bone. The experimental matrix will allow for comparison of quality of new and established bone and may help to elucidate properties that predispose buccal bone to dehiscence formation. Nanoindentation may be used to fully characterize the elastic, plastic, and viscoelastic properties according to well-established methodology.

Chapter 2: Materials & Methods

Animal Model & Implant Treatment Groups [34]:

The experimental model and treatment groups have been thoroughly described elsewhere. For the purposes of describing the origins of samples evaluated in this work the samples will be briefly described.

Male beagle dogs 20 months of age were used in this study. Mandibular premolars and molars were atraumatically extracted and allowed to heal for 12 weeks. For implant placement a mid-crestal incision was made, mucoperiosteal flaps were reflected, and a 3 mm diameter osteotomy site was prepared. Following implant installation flaps were sutured and dogs were fed a soft food diet for 3 days containing analgesics and antibiotics. The implants were allowed to heal for 3 or 6 weeks prior to anesthetization and sacrifice of the animals. Implants were isolated en-block and processed into 50 μm sections for nanoindentation. All animal care protocols were reviewed and approved by the Institution of Animal Care and Use Committee at the Seoul National University School of Dentistry.

Titanium implants (Dentium, Korea) and alumina-toughened polycrystalline zirconia polycrystalline (Acucera, Korea) were custom designed to a 3 mm diameter and 8 mm length. Grade IV titanium (Ti) implants were treated with resorbable blast media while alumina toughened zirconia (ATZ) implants were sandblasted to achieve similar

surface roughness to the Ti implant. In some instances, ATZ implant sites were filled with a demineralized bone matrix gel (DBM, 30% human DBM, 70% porcine collagen gel; Rafugen) or recombinant BMP-2 gel (25% DBM, 22.5% carboxymethyl cellulose, 52.5% porcine collagen gel, 50 µg/ml rhBMP-2; Rafugen). Osteotomy sites were filled with DBM or BMP-2 gel and excess material was removed following implant placement. Implants were assigned randomly utilizing a two by two Latin square.

Nanoindentation:

The mechanical properties of peri-implant bone were measured using a Nano-XP nanoindenter (MTS; Oakridge, TN) equipped with a camera, light source, microscope, and mechanical stage allowing for site selection. Regional variation in properties will be evaluated for buccal and lingual alveolar bone as well as for old (away) and new (adjacent) bone. The new bone is defined as bone internal to the crest of the implant threads and has been shown to have the histological appearance of woven bone in other work. Indentations were made with a diamond, pyramidal Berkovich tip and a grid of nanoindentations is shown in Figure 3. The indentations had a standard 30 µm distance between sites and pore, cracks, osteons, and the bone/embedding matrix interfaces were avoided. Two areas were selected within the buccal and lingual bone (adjacent and away) consisting of approximately 9 nanoindentations each.

Indentation parameters included advanced the tip at a rate of 10 nm/sec to a depth of 500 nm followed by a 30 second hold at peak load and a 10 nm/sec unloading phase. Elastic, plastic, and viscoelastic properties may be derived from the acquired data. The

plastic hardness (H) and reduced elastic modulus (E_R) were evaluated in this study.

Contact hardness (H) is calculated at peak displacement according to Eq. (1) where P_{max} is the peak load (mN) and Ac is the projected contact area (mm) that can be estimated according to indentation depth, Eq. (2).

$$H = \frac{P_{max}}{A_c} \qquad (1)$$

The reduced modulus (Er) was calculated from the unloading slope according to Eq. (2) where *s* and *i* that, respectively, refer to sample and indenter properties and V is Poisson's ratio. Hardness and Poisson's ratio values for the diamond indenter tip are Ei =1141 GPa and Vi = 0.07. Poisson's ratio of 0.3 for bone was obtained from the literature. [35]

$$\frac{1}{E_R} = \frac{(1-V_S^2)}{E_s} + \frac{(1-V_i^2)}{E_i} \qquad (2)$$

Statistics:

The mechanical properties measured by nanoindentation will be strongly influenced by regional variation in mineral content and crystal orientation. The inherent heterogeneity and is anticipated to have a greater influence that inter-animal variability.

For this reason, each indentation is considered an independent measurement for statistical purposes. Initial sample size was determined from 198 indentations at the bone-implant interface (61 buccal, 137 lingual). It was found that 125 indentations per region were required to obtain significance ($p \leq 0.01$) with 95 % power. All groups investigated exceeded the minimum number of anticipated indentations and ANOVA analysis was used to access statistical significance at $p \leq 0.05$.

Chapter 3: Manuscript

INTRODUCTION

Dental implantation has been developed to restore masticatory function at the site of tooth extraction [35, 36]. Many clinical cases have observed alveolar bone resorption following tooth extraction, which reduces the amount of bone needed to achieve primary stability of an implant system [37]. In particular, it is documented that buccal bone resorbs more than lingual bone at the extracted site and the bone resorption could continue after implantation [11, 18, 19, 38]. These morphological changes of bone involve active bone modeling and remodeling that produce a heterogeneous distribution of bone tissue minerals [39]. Additional bone remodeling activated by the peri-implant bone tissue damage occurring during implantation surgery provides more alterations of bone tissue mineral distribution [40]. As mechanical properties of bone tissue are closely associated with its degree of mineralization [41, 42], the changes of peri-implant bone tissue mineral distribution are directly responsible for determining the primary and long-term stability of the implant system. However, differences of mechanical properties of bone tissues between buccal and lingual peri-implant regions have not been fully examined.

While bone grafting is most commonly recommended to treat oral bone deficiency [43-45], its use is restricted due to significant limitations, which include donor site morbidity, risk of infection, inappropriate synthetic architecture, and post-implantation failures [43, 46-55]. Alternatively, many studies have observed that growth factors, including demineralized bone matrix (DBM) and bone morphogenetic proteins (BMP), successfully enhance oral bone augmentation [56-59]. While those results observed substantial increase in bone quantity, there is lack of knowledge about their bone quality, including mechanical properties of bone at the tissue level. These mechanical properties play an important role in triggering bone remodeling by controlling micro-level deformation of bone tissue, which may result in micro-crack initiation and propagation.

The objective of the current study was to examine whether peri-implant bone tissue properties at the buccal region are different from those at the lingual region when growth factor treatments are employed at post-implantation healing periods. The current study used nanoindentation to measure the peri-implant bone properties because this test has been previously utilized to assess the mechanical properties of bone at the tissue level [60, 61]. With high measurement resolution extending to the nanoscale level, the nanoindentation test has the capability of characterizing detailed interfacial bone properties at micrometer distances from the implant [62-64]. The current study was able to examine the variation in peri-implant bone quantity and quality adjacent to traditional titanium and zirconia implant interfaces where different bone remodeling activities have been previously observed [34, 65, 66], as well as examine the effects of different implant surface treatments.

MATERIALS AND METHODS

Specimen preparation:

The current animal experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC Approval Number: SNU-090502-2) of the School of Dentistry, Seoul National University, Korea. Detailed information about the implantation surgery and specimen preparation has been presented in a previous study [34]. All mandibular premolars and first molars of six male beagle dogs (10 to 15 kg) were extracted. After a healing period of 12 weeks, a total of 48 implants (8 implants/dog) were placed. There were four groups of implants: CP Ti (Titanium), ATZ-N [alumina-toughened yttria and niobia co-doped tetragonal polycrystalline zirconia (ATZ), ATZ-D [ATZ with demineralized bone matrix (DBM) gel], and ATZ-B [ATZ with recombinant human bone morphogenetic protein-2 (rhBMP-2) in DBM gel (50 μg/ml)]. Oxytetracycline hydrochloride (Merck, Amsterdam, The Netherlands; 20 mg/kg SQ), xylenol orange (Sigma, Zwijdrecht, The Netherlands; 90 mg/kg SQ), and calcein blue (Sigma; 90 mg/kg SQ) were injected to label newly forming bone tissues at weeks 2, 4, and 5 after implantation. Three dogs were sacrificed after 3 and 6 weeks of post-implantation healing. Each implant system, consisting of an implant and peri-implant bone tissues, was dissected and embalmed in 4% neutral formaldehyde. Then, the specimens were embedded in light-cured resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany) were sectioned in the buccolingual direction to expose the bone-implant

interface using a cutting–grinding technique (EXAKT Apparatebau, Norderstedt, Germany) (Fig. 1). The final thickness of the specimens after this step was approximately 50 μm, and specimens were further polished with 1 μm diamond paste for nanoindentation. For histological examination, specimens were stained with hematoxylin and eosin.

Nanoindentation:

A nanoindenter (Nano-XP, MTS, Oak Ridge, TN) was used to measure the elastic modulus (E) and plastic hardness (H) of the peri-implant bone tissues, which represent the capacity of these tissues to resist elastic and plastic deformations, respectively. Bone tissues adjacent the implant within the borderline between threads (termed "Adjacent") and those outside the borderline far away from the implant surface (termed "Away") were identified by comparing the fluorescent-labeled bone in histologic images and nanoindenter microscopic images (Fig. 2). A 3×3 array of indentations was performed at each region of interest, as shown in Fig. 2 (c).

Indentations were made using the load-control mode, at a displacement rate of 10 nm/sec, until attaining a depth equivalent to 500 nm. The plastic hardness was obtained by dividing the peak indenting load (P_{max}) by the indenter contact area (A) [67].

After the 30-second hold period, the elastic modulus was measured during unloading of the indenter at the same displacement rate of 10 nm/sec. The 30-second hold period was used to minimize indentation creep-related experimental errors for the measurement

of elastic modulus during unloading [68]. The conventional equation of contact mechanics was employed to compute the nanoindentation elastic modulus (Eq. 1) [67].

$$\frac{1}{E_r} = \frac{(1-\nu_s^2)}{E_s} + \frac{(1-\nu_i^2)}{E_i} \qquad \text{(Eq. 1)}$$

The E_r (reduced elastic modulus) is obtained from the slope of the unloading force-displacement curve. Values of E_i = 1141 GPa and Poisson's ratio□□□$_i$) = 0.07 for the diamond Berkovich indenter and 0.3 for □$_s$ of bone were utilized in a previous study [69]. The elastic modulus (E_s) of the bone tissue specimen can then be computed, using Eq. 1.

Statistical analysis:

A total of 2281 nanoindentation was analyzed for the 74 implant sites (Table 1). Analysis of variance (SPSS 22, IBM), followed by the least significant difference (LSD) *post hoc* test, was conducted to compare differences of the nanoindentation parameters (E and H) between the buccal and lingual peri-implant bone tissues with respect to healing times (3 and 6 weeks), distance (Adjacent and Away), and treatments (Ti, ATZ-N, ATZ-D, and ATZ-B). Statistical significance was set at $p<0.05$.

RESULTS

Microscopic images of the four implant systems indicated that the peri-implant buccal region had less overall bone quantity resulting from lower height and narrower width of bone tissue than the lingual region (Fig. 1).

Overall values of the elastic modulus (E) and plastic hardness (H) were 12.15±6.53 GPa and 0.52±0.33 GPa, respectively. Bone tissues adjacent the implant (Adjacent) had significantly lower mean values of E and H than those away from the implant (Away) ($p<0.001$) (Table 2). The mean values of E and H at week 3 were significantly greater than those at week 6 ($p<0.001$). The adjacent region had significantly lower mean values of E and H than the away region at week 3 ($p<0.001$) and of H at week 6 ($p=0.002$). However, the difference of E values between the two regions was not significant at week 6 ($p=0.989$). The Ti implant group had significantly greater mean values of E than the ATZ-D and ATZ-B implant groups ($p<0.001$) but not significantly different from ATZ-N implant group ($p=0.067$). The mean values of H were not significantly different among the implant treatment groups ($p>0.073$).

Buccal bone tissues had significant greater mean values of E and H than lingual bone tissues ($p<0.001$) [Table 2 and Figs. 3 (a) and (b)]. The higher mean values of E and H for the buccal bone tissue compared to those for the lingual bone tissue were also found for the Adjacent and Away region groups ($p<0.002$) [Figs. 3 (c) and (d)], and at week 3 and week 6 ($p<0.007$) [Figs. 3 (e) and (f)]).

For the Ti implant group, the mean values of E were not significantly different between the buccal and lingual bone tissues ($p=0.758$) [Fig. 4 (a)]. However, the mean

values of H for the Ti implant group, and both E and H for all other treated implant interface groups, were significantly higher at the buccal bone tissue than at the lingual bone tissue ($p<0.046$) [Figs. 4 (a) and (b)]. Further analyses for the elastic modulus among treatment groups showed that the mean values of E at the buccal bone tissues were not significantly different between the treatment groups ($p>0.071$) (Fig. 4a). On the other hand, for elastic modulus at the lingual bone tissue, the Ti implant group had the highest mean value ($p<0.008$), and the ATZ-N group had a significantly higher mean value than the ATZ-D and ATZ-B groups ($p<0.013$), and there were no significant differences in mean values for the ATZ-D and ATZ-B groups ($p=0.076$).

For the Ti implant group, the buccal bone tissue had a significantly higher mean value of elastic modulus at week 3 ($p=0.016$), but a significantly lower mean value at week 6 ($p=0.003$), than the lingual bone tissue [Fig. 4 (c)]. There was no significant difference for the mean value of E between buccal and lingual bone tissues for the adjacent region ($p=0.519$) and the away region ($p=0.782$) [Fig. 4 (d)].

DISCUSSION

The peri-implant buccal region had less bone quantity but better bone quality, based upon values of elastic modulus (E) and plastic hardness (H) of bone tissue, than the lingual region. This trend was maintained at different post-implantation healing periods, distances from the implant, and implant treatments except for the conventional Ti implant system without treatment, which showed no significant difference in E between buccal and lingual regions. These findings suggest that the treatments likely stimulated more

active peri-implant bone remodeling in the lingual region than the buccal region, which could progressively produce more newly formed immature bone tissues that have lower elastic modulus than pre-existing mature bone tissues.

Nanoindentation has been utilized to characterize bone at the tissue level [60, 61, 70]. Although the high indentation resolution with this technology allows assessment of material properties over micrometer distance range, a single indentation cannot represent the heterogeneous bone tissue properties in the peri-implant regions of interest. However, the 3×3 array of indentations used in the current study is considered adequate to enable the assessment of the local heterogeneous distribution of bone tissue properties.

It was previously observed that the buccal bone is more resorbed than the lingual bone after tooth extraction [11, 71]. It was also previously found that density of the buccal bone tissue is higher than that of the lingual bone tissue [10]. On the other hand, the values of E and H obtained by nanoindentation have been found to correlate significantly with the degree of bone mineral density [41, 42]. Consistent with these previous results, the current study found that the buccal region had less peri-implant bone height and width but higher mechanical properties (E and H) of bone tissues than the lingual region. This higher peri-implant buccal bone quality could compensate for its relatively poor quantity to support the implant when masticatory loading is applied.

The inevitably vigorous implantation surgery triggers active bone remodeling, resulting in newly formed, less mineralized, bone tissues at the peri-implant region. Increasing these less mineralized immature bone tissues reduces the mechanical properties of the local region. As such, differences of the buccal and lingual peri-implant

bone tissue properties would be altered, dependent on the degree of bone remodeling activities. We anticipated that more bone remodeling might occur in the region adjacent the implant, which may have direct surgical damages compared to the region away from the implant. Also, it was expected that interfacial bone tissue mineralization progressively increased at longer healing periods. However, the difference in buccal and lingual bone properties was maintained, independent of both the distance from the implant and the healing periods. Furthermore, the values of mechanical properties (E and H) decreased as the healing period increased. These findings indicate that similar patterns of active bone remodeling continuously developed at the wide peri-implant region during the entire post-implantation healing periods examined in the current study.

Differences between buccal and lingual bone tissue properties were found to be substantially higher for the DBM (ATZ-D) and BMP (ATZ-B) treatment implant groups than for the Ti and ATZ-N implant groups. As no significant elastic modulus differences of the buccal bone tissues were observed among the implant groups, it is likely that the bone growth-factor treatments stimulated more bone remodeling at the lingual region, which increased the amount of more newly formed immature bone tissues with lower elastic moduli. This distribution of elastic moduli at the peri-implant region of the treatment groups could provide improved mechanical stability of the implant systems when masticatory force is applied. Under uniform loading, more deformation of bone tissue occurs when the elastic modulus is lower than when it is higher. As a result, the lingual bone tissue could be deformed more than the buccal bone tissue surrounding an implant system under masticatory loading. However, the greater amount of bone tissue at

the lingual region would tend to counteract its reduced mechanical properties, while providing some balance with the smaller amount of bone that possesses higher mechanical properties at the buccal region when the implant systems are loaded.

On the other hand, the untreated Ti implant group had the lower mean values for bone properties (E and H) at the buccal region than at the lingual region, but the differences between these two regions were not statistically significant with respect to distances from the implant. If a masticatory loading were applied on the Ti implant system under these peri-implant bone conditions, more deformation was likely to be developed at the buccal region having the smaller amounts of bone with weaker properties than at the lingual region. This unbalanced deformation may accelerate progressive loss of the buccal bone tissue around the implant system under long-term masticatory loading.

The current research has shown that the elastic modulus was more influenced by the different implant treatments, while the plastic hardness maintained a similar trend between the implant groups. This suggests that the treatment may provide more control of the elastic deformation of the peri-implant bone tissues and enable activation of bone remodeling, rather than undesirable plastic damage such as microcracks when loading is applied.

One limitation of the current study was that the specimens were dried and fixed using formaldehyde prior to nanoindentation, which may alter the measured values of elastic modulus, hardness and viscoelastic properties of bone tissue [72, 73]. This is a necessary laboratory procedure to prevent decay of bone tissues during the time-consuming process of embedding the bone-implant construct to enable holding it for longitudinal dissection.

The mean values of E and H obtained in the present investigation are in good agreement with those reported in previous studies (10.70 to 16.54 GPa) [69]. A second limitation is that the current results were obtained from the peri-implant bone tissues during post-implantation healing periods without applying any loading. A clinical retrieval study observed that active bone remodeling continues adjacent to the implant in function up to 5 years, resulting in lower values of nanoindentation modulus for the peri-implant bone tissues than would occur in the absence of functional forces [64]. This finding suggests that the differences in buccal and lingual peri-implant bone properties observed in the current study, which result from active bone remodeling during post-implantation healing periods, are likely maintained by the continuous bone remodeling that occurs under functional masticatory loading. Further retrieval studies combined with nanoindentation measurements of bone properties are needed to clarify this speculation.

In conclusion, the peri-implant buccal bone tissue has less bone quantity but stronger bone properties (elastic modulus and plastic hardness) than the lingual bone tissue during the entire healing periods after implantation except for bone adjacent to the conventional Ti implant group. The bone growth factor treatments induce more bone remodeling at the lingual region of peri-implant bone tissue than at the buccal region, balancing the regional differences for amount and properties of bone tissues.

ACKNOWLEDGMENTS

This investigation was partially supported (Kim, D-G) by an American Association of Orthodontists Foundation Award.

	B	L	Weeks				Distance				Treatments							
			3		6		Adjacent		Away		Ti		ATZ-N		ATZ-D		ATZ-B	
			B	L	B	L	B	L	B	L	B	L	B	L	B	L	B	L
Implant sites	34	41	16	17	18	24	34	40	33	39	7	7	6	7	10	14	11	13
Nano-indentation	1323	958	610	312	713	646	651	421	672	537	234	125	225	151	421	360	443	322

Table 1. Summary of implant sites and nanoindentations for buccal (B) and lingual (L) bone tissue.

Groups	Weeks	Distance	E (GPa)	H (GPa)
Ti	3	Adjacent	12.467±3.91	0.468±0.224
		Away	16.748±3.693	0.669±0.155
	6	Adjacent	11.01±3.769	0.427±0.167
		Away	14.042±5.313	0.509±0.234
ATZ-N	3	Adjacent	11.507±5.743	0.459±0.378
		Away	19.914±3.305	0.792±0.18
	6	Adjacent	12.699±3.305	0.476±0.157
		Away	8.866±7.109	0.418±0.141
ATZ-D	3	Adjacent	10.885±5.237	0.733±0.914
		Away	15.405±7.008	0.682±0.208
	6	Adjacent	9.872±5.248	0.406±0.203
		Away	9.627±7.637	0.431±0.24
ATZ- B	3	Adjacent	11.935±6.014	0.488±0.36
		Away	15.959±6.504	0.656±0.211
	6	Adjacent	10.717±4.18	0.407±0.173
		Away	11.513±8.087	0.524±0.234

Table 2. Nanoindentation values (E, elastic modulus; H, hardness) of 4 bone-implant system.

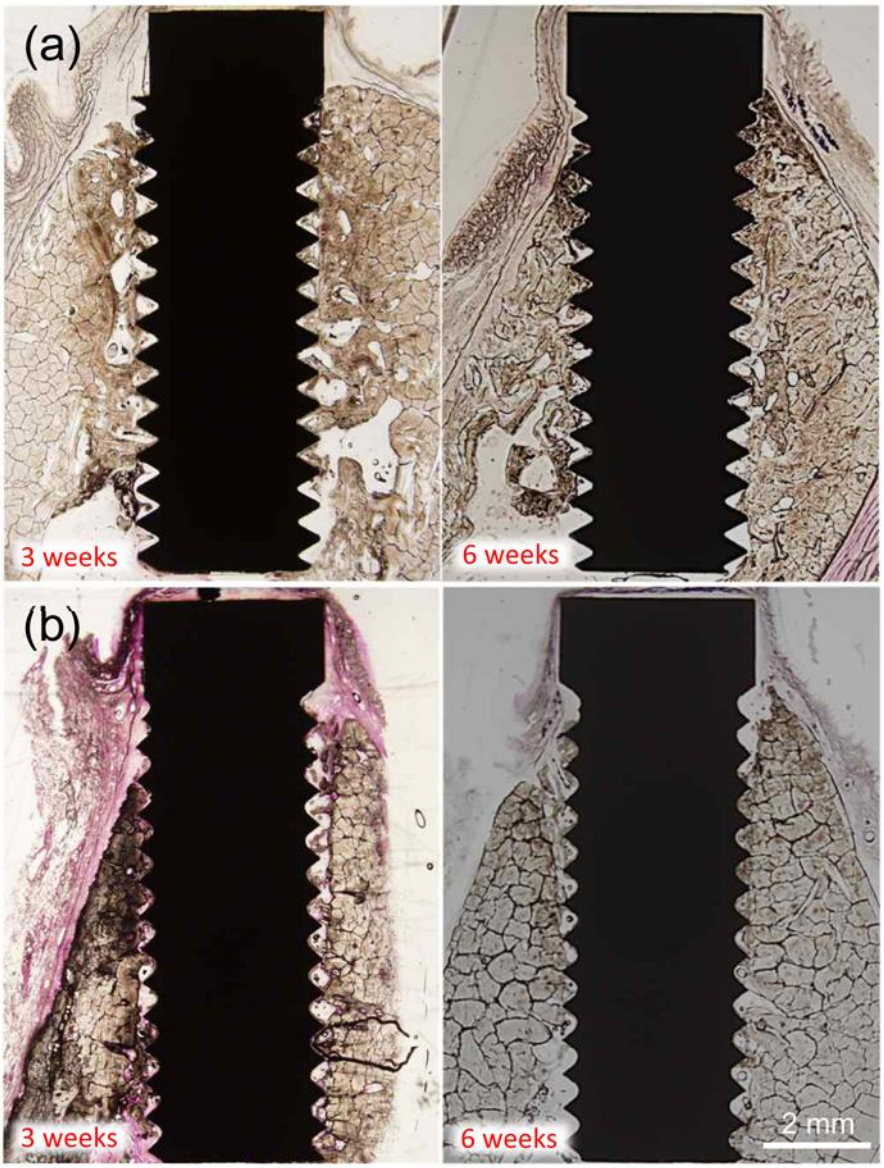

Figure 1**.** Microscopic images of buccal and lingual bone. Bone surrounding (a) Ti implant system and (b) ATZ-B system at 3 and 6 weeks post-implantation.

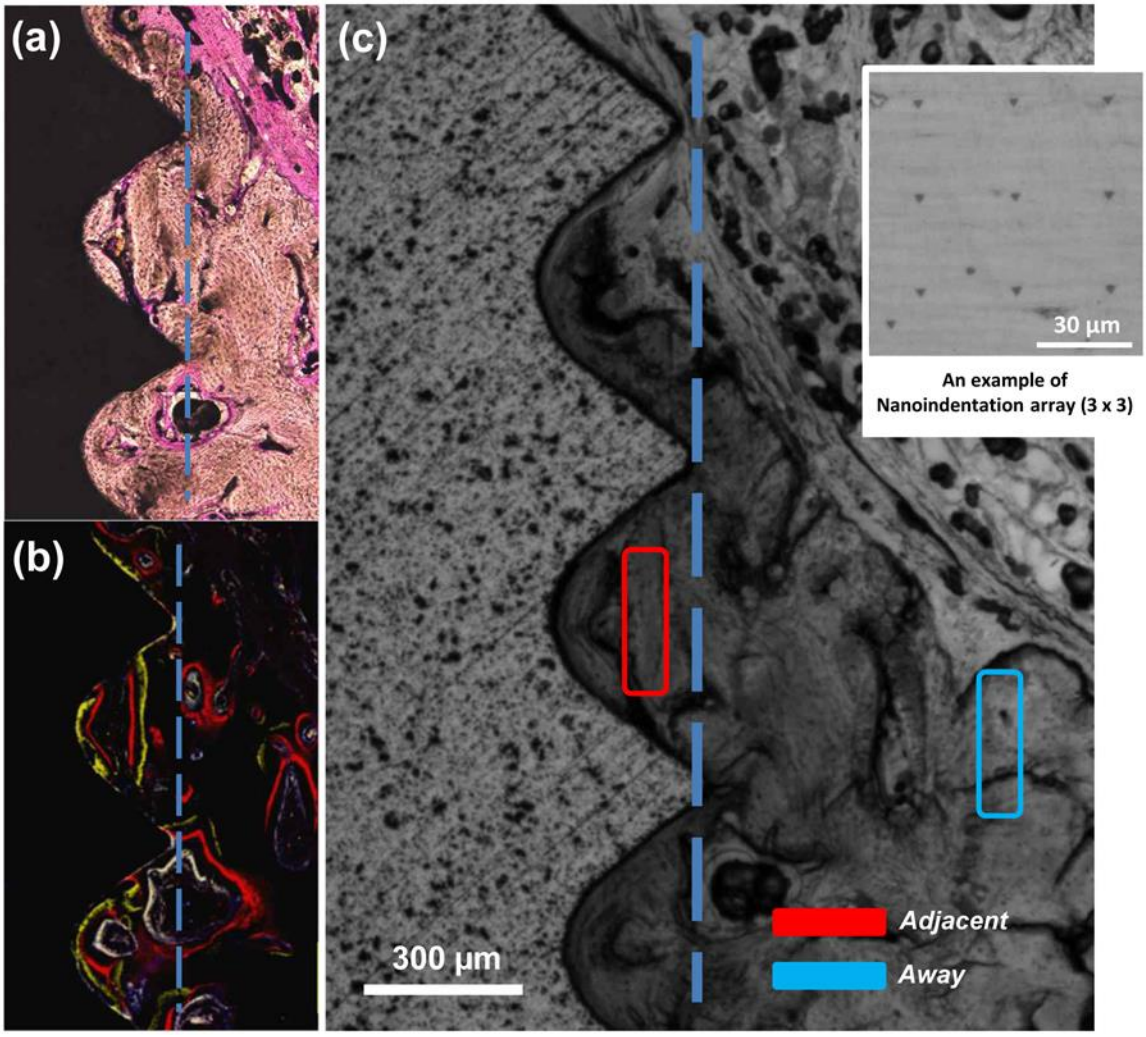

Figure 2. Nanoindentation locations at the bone-implant interface. Regions for indentation were identified by comparing (a) histologic (hematoxylin and eosin stain) and (b) fluorescence images. The blue dotted line indicates the border between adjacent and away bone tissue. The dimension of indentation sites is shown in (c).

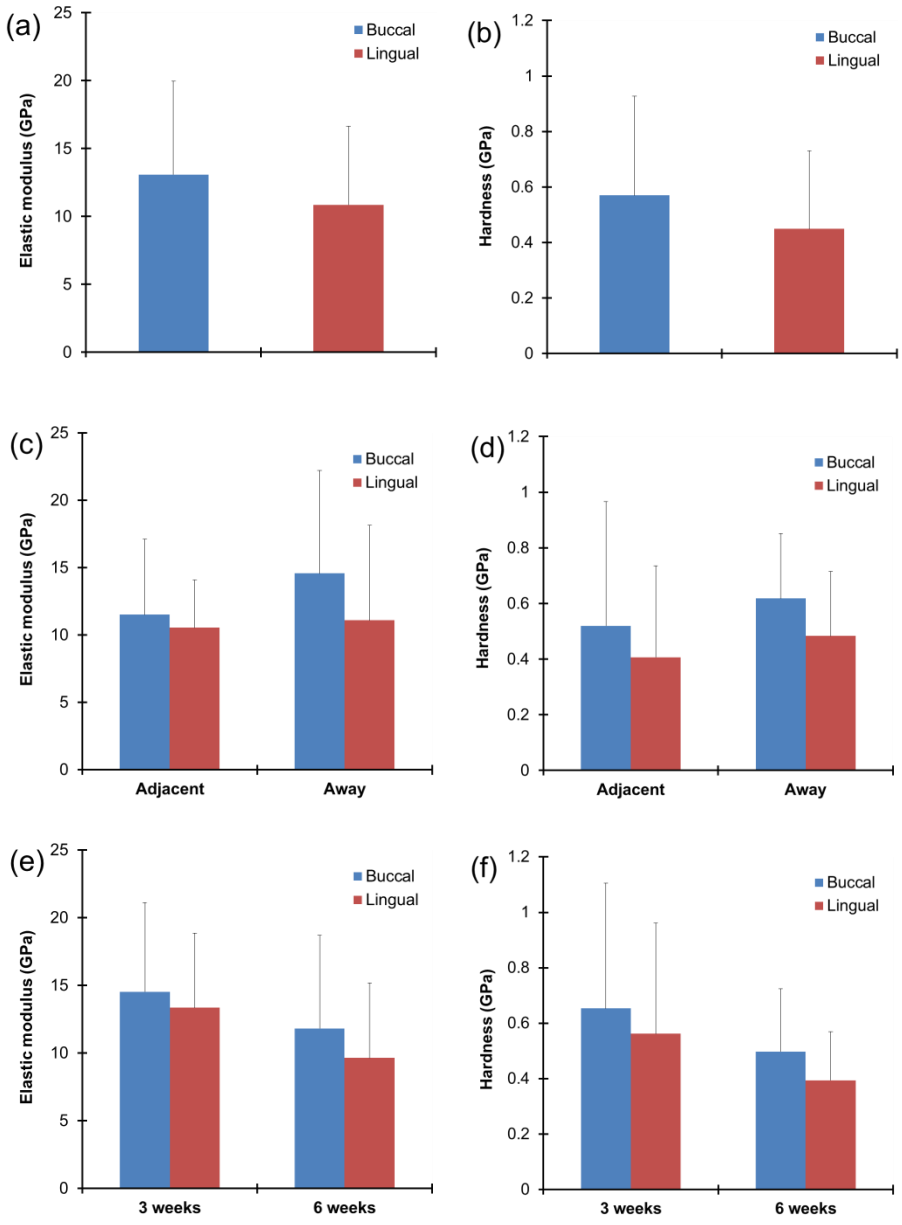

Figure 3. Differences in buccal and lingual peri-implant bone quality. Elastic modulus (E) and hardness (H) for all specimens (a, b), distances from the implant (c, d), and for post-implantation healing times. Significant differences between buccal and lingual were found for all comparisons ($p<0.01$).

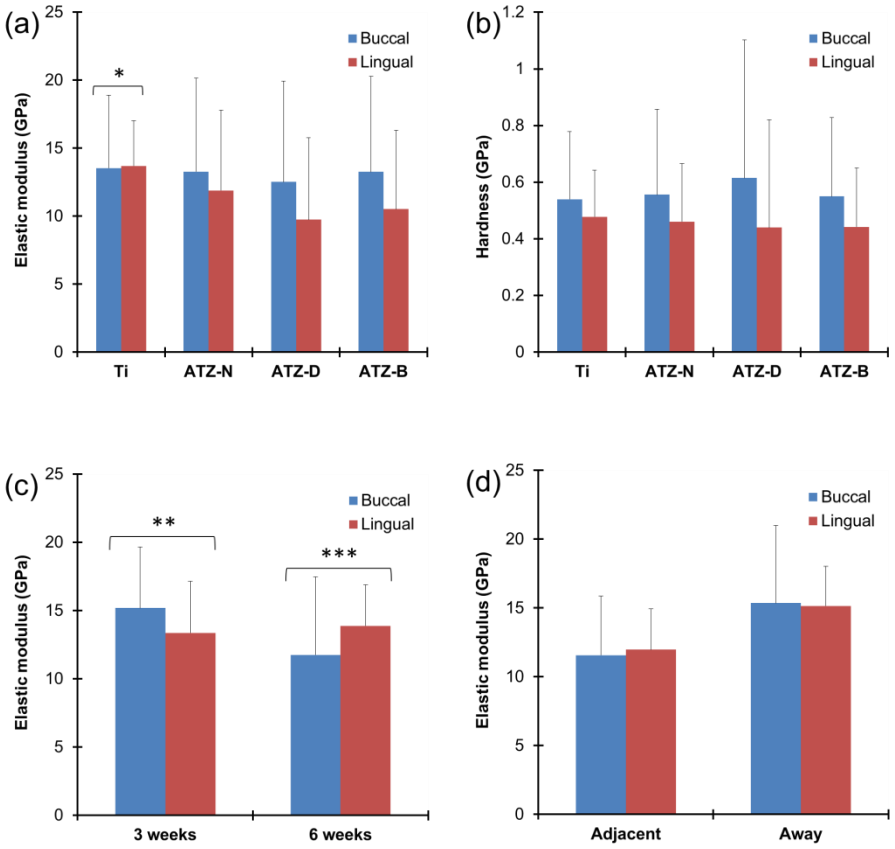

Figure 4. Buccal and lingual implant system comparisons. Significant difference in elastic modulus (a) and hardness (b) measurements between buccal and lingual bone tissue were found in 4 bone-implant systems (Ti, ATZ-N, ATZ-D, and ATZ-B) with the exception of modulus of the implant group (*; p=0.758) as well as for the buccal and lingual elastic moduli of the Ti implant group (c) at 3 and 6 weeks after implantation (**, p=0.016; ***, p=0.003), and (d) for adjacent and away distance from the implant (p>0.519).

Chapter 4: Discussion

The three-dimensional changes following tooth loss are well documented in human and animal models. Due to volumetric changes, alveolar bone augmentation is often required for implant placement and stability. The buccal bone is more sensitive to vertical and transverse change and bone augmentation is often required for proper implant placement and stability. The changes that have been observed post-extraction continue following implant placement and pose an esthetic challenge. It has been reported that a large proportion of implants placed in the anterior maxilla have unaesthetic outcomes. The esthetic outcome is directly related to buccal alveolar bone changes and has largely been attributed to the thin, fragile nature of the buccal bone. The regional variation of alveolar bone is unclear and was the motivation for this work. Although a number of variable were considered (treatment healing period, tissue maturity, and treatment group) the most significant finding was that the buccal bone had higher bone quality (increased reduced modulus and hardness).

The mechanical properties surrounding a healthy tooth structure were not evaluated in this work and it is reasonable to assume that the "away" bone in the buccal and lingual alveolus approximate a naïve alveolar structure. In this instance, it may be extrapolated that the buccal bone has increased mechanical properties to compensate for its thin nature. Perhaps the increased hardness and modulus of the buccal bone pre-

disposes this tissue area to increased microfractures, tissue necrosis, and subsequent dehiscence. In contrast, the lingual bone is less stiff, more compliant. These features in combination with the increased bony volume may make lingual alveolar bone better equipped to withstand microfracture. Microfracture may be detected with multiple histological techniques and it would be interesting to compare the presence of microfractures both buccal and lingual alveolar bone following osteotomy preparation and implantation.

The "adjacent" buccal bone also demonstrates increased mechanical properties (hardness and modulus) than the lingual bone. This may be indicative of variation in buccal and lingual bone remodeling process. It is possible that the enhanced blood supply to the lingual alveolar bone (from periosteum and bone marrow) in comparison to the buccal alveolar bone (periosteum only) allows for more rapid progression of the bone remodeling process. Perhaps supplementation of extraction sites with angiogenic growth factors may promote the formation of a more uniform vascular supply to buccal and lingual alveolar bone and enhance bone remodeling.

The influence of healing time was also evaluated in this work. When comparing 3 to 6-weeks post-implantation groups, the modulus and hardness properties were higher at 3 weeks. This suggests that at 3-weeks, there is more mature tissue present and that as healing progresses and the 6-week period is reached that the mechanical properties of the "adjacent" bone is reduced and represents more immature bone. Time dependent variation was anticipated as the tissue level properties are heavily influenced by collagen structure (such as the degree of cross-linking) as well as mineral properties (crystal size,

orientation, and degree of mineralization). It was, however, surprising that the properties were higher at 3-weeks and contraindicates the histological observation that these samples had characteristic woven bone at 3 weeks and more mature, lamellar bone was at 6-weeks.

When considering buccal and lingual properties in relationship to implant group comparisons the buccal properties were greater than lingual for all except the titanium group. Titanium implants are traditionally considered the gold-standard. However, ATZ implants may be esthetically advantageous when there is bony dehiscence with soft tissue recession or a thin gingival biotype with implant show through. The observation that the buccal and lingual peri-implant bone properties are similar suggests that the chemistry of the ceramic implant may influence the bone remodeling processes. This may be related to surface charge or factors that differentially adhere to the ceramic surface and serve to promote the remodeling process.

In a related study of the same sample set, BMP2 (promotes osteoblast proliferation, differentiation and function) was shown to significantly increase bone mineral deposition internal and external to the dental implant thread. This indicates that in a model free of critical sized defects, sufficient amount of growth factor was present to increase bone mineral deposition at a distance away from an the implant surfaces. The pattern of increased bone properties in the buccal (in comparison to the lingual) and in "away" bone (in comparison to "adjacent" bone) is maintained for the ATZ-BMP2 group; however, it may be useful to analyze the bone properties of "away" bone between ATZ treatment groups as well. Increased observed mineral deposition (identified by florescent

calcium chelating agent binding to mineralizing surfaces) may not only stimulate increased bone deposition and volume but increase bone quality. This would be a clinically relevant observation and support BMP2 supplementation in the absence of critical size defects.

The clinical application of this work is very interesting. Growth factors and other treatments have been widely utilized to augment bony defects and much is known regarding the biological response to BMP and DMP. The effect of surface chemistry on healing response is less well understood. However, it is fascinating to consider not only the effects of these treatments on bone fill and quantity but also on the resulting bone quality. Such information may allow for optimization of the healing process and generate bone that have the properties to withstand earlier mechanical loading or support the healing process so less bony changes are observed.

There is a large degree of variability in the data set and this is largely attributed to the high level of bone heterogeneity and bone remodeling. It is for this reason that approximately 18 indentations were made per section and each indentation was evaluated as an independent sample. This is common in the literature and justified as the tissue level variability is great due to material heterogeneity and that this is anticipated to supersede any inter-animal variability.

In summary, the regional variation found in this study is very clinically relevant and a good first step to elucidate mechanisms that contribute to buccal bone fragility. This work may be expanded to compare bone along the implant long axis to provide further insight into regional bone variation that influences differential bone response to

mechanical loading. Finally, advancing our understanding of alveolar bone quality may provide opportunities to tailor implant treatment protocols that optimize mechanical properties and buccal bone maintenance.

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