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Differences between buccal and lingual bone quality and quantity of peri-implant regions

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Abstract

The objective of the current study was to examine whether peri-implant bone tissue properties are different between the buccal and lingual regions treated by growth factors. 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. 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 ($p = 0.758$). 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. 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.

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Conflict of interest Statement
None declared.

Keywords

Peri-implant bone; nanoindentation; growth factors; bone morphogenetic protein-2; bone remodeling

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INTRODUCTION

Dental implantation has been developed to restore masticatory function at the site of tooth extraction (Branemark, et al. 1983, Brunski 1992). 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 (Al-Juboori, et al. 2013). 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 (Araujo & Lindhe 2005, Araujo, et al. 2005, Lekovic, et al. 1997, Pietrokovski & Massler 1967). These morphological changes of bone involve active bone modeling and remodeling that produce a heterogeneous distribution of bone tissue minerals (Roschger, et al. 2008). Additional bone remodeling activated by the peri-implant bone tissue damage occurring during implantation surgery provides more alterations of bone tissue mineral distribution (Wang, et al. 2014). As mechanical properties of bone tissue are closely associated with its degree of mineralization (Mulder, et al. 2008, Mulder, et al. 2007), 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 (Chen & Jin 2010, Mao, et al. 2006, Pellegrini, et al. 2009), its use is restricted due to significant limitations, which include donor site morbidity, risk of infection, inappropriate synthetic architecture, and post-implantation failures (Alpdogan & van den Brink 2012, Becktor, et al. 2002, Bishop, et al. 2011, Blanco, et al. 2005, Brunel, et al. 2001, Chen & Jin 2010, Delloye, et al. 2007, Rios, et al. 2011, Spin-Neto, et al. 2013, Spin-Neto, et al. 2014, Waasdorp & Reynolds 2010). Alternatively, many studies have observed that growth factors, including demineralized bone matrix (DBM) and bone morphogenetic proteins (BMP), successfully enhance oral bone augmentation (Gruskin, et al. 2012, Higuchi, et al. 1999, Kim, et al. 2014, Wallace, et al. 2014). 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 are different between the buccal and lingual regions treated by growth factors. The current study used nanoindentation to measure mechanical properties of bone tissue. 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 (Anchieta, et al. 2013, Baldassarri, et al. 2012, Jimbo, et al.

2012). Thus, this technology allows us to examine the variation in peri-implant bone quantity and quality adjacent to traditional titanium and zirconia implant interfaces in the current study.

MATERIAL 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 (Lee, et al. 2013). 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 grade 4), 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, Zwijndrecht, 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, respectively. Thus, the dogs were injected once at week 2 for the week 3 group and three times at weeks 2, 4, and 5 for the week 6 group. 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) and 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. Bone remodeling activities on the surface of bone specimens were examined using fluorescent images taken by a confocal microscope (Fluoview 300; Confocal Laser Scanning Microscope, Olympus, Tokyo, Japan). Then, 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 to 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) (Oliver & Pharr 2004).

After a 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 (Roy, et al. 1999). The conventional equation of contact mechanics was employed to compute the nanoindentation elastic modulus (Eq. 1) (Oliver & Pharr 2004).

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

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 (ν_i) = 0.07 for the diamond Berkovich indenter and 0.3 for ν_s of bone were utilized in a previous study (Huja, et al. 2007). The elastic modulus (E_s) of the bone tissue specimen can then be computed, using Eq. 1.

Statistical analysis

Data at 1 buccal Adjacent, 1 buccal Away, and 2 lingual Away regions were missed by machine errors that have also been found in the previous study (Hoffler, et al. 2005, Rho, et al. 1997). This error may arise from difficulty of indentation contact when dirt or a defect exists on the surface of bone specimen. As a result, a total of 2281 nanoindentations was successfully 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 12.147 ± 6.530 GPa and 0.519 ± 0.333 GPa, respectively. Bone tissues adjacent to 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 ATZN 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 significantly 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 ($p<0.013$); 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 (Hoffler, Guo, Zysset & Goldstein 2005, Kim & Elias 2014, Rho, Tsui & Pharr 1997). 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 $\sim 30\text{ }\mu\text{m}$ -

spaced 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 (Araujo & Lindhe 2005, Hatakeyama, et al. 2014). It was also previously found that density of the buccal bone tissue is higher than that of the lingual bone tissue (Novaes, et al. 2011). 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 (Mulder, Koolstra, den Toonder & van Eijden 2008, Mulder, Koolstra, den Toonder & van Eijden 2007). 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. It might be anticipated that more bone remodeling might occur in the region adjacent to 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 would progressively increase 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 four 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 masticatory loading were applied on the Ti implant system under these peri-implant bone conditions, more deformation would likely 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 these implant 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 (Pathak, et al. 2011, Rho & Pharr 1999). 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) (Huja, Fernandez, Hill & Gulati 2007). 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 (Baldassarri, Bonfante, Suzuki, Marin, Granato, Tovar & Coelho 2012). 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.

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

Fig. 2. Nanoindentation locations at the bone-implant interface (ATZ-D at week 6), which is identified by comparing (a) histologic (hematoxylin and eosin stain) and (b) fluorescent images. The blue dotted line indicates the border between bone tissues adjacent and away from the implant. The dimensions of the indentation sites are also shown in the inset for (c).

Fig. 3.

Differences of buccal and lingual peri-implant bone quality. (a) elastic modulus (E) and (b) hardness (H) for all specimens; (c) and (d) distances from the implant (Adjacent and Away); and (e) and (f) 3 and 6 weeks after implantation. Significant differences between buccal and lingual were found for all cases ($p < 0.01$).

Fig. 4. Significant differences in (a) elastic modulus and (b) hardness between buccal and lingual bone tissues for 4 bone-implant systems (Ti, ATZ-N, ATZ-D, and ATZ-B) ($p < 0.01$), except for elastic modulus of Ti implant group (*: $p = 0.758$), and buccal and lingual bone elastic moduli of the Ti implant group (c) at 3 and 6 weeks after implantation (**: $p = 0.016$ and ***: $p = 0.003$), and (d) for adjacent and away distances from the implant ($p > 0.519$).

The number of implant sites and nanoindentations for the buccal (B) and lingual (L) bone tissues of each group.

Table 1

		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
				16	16	18	24	33	40	33	38	7	6	6	7	11	14	10	13
Implant sites		34	40																
Nanoindentations		1323	958	610	312	713	646	651	421	672	537	234	125	225	151	421	360	443	322

Table 2

Nanoindentation values (E: elastic modulus and H: hardness) of 4 bone-implant systems (Ti, ATZ-N, ATZ-D, and ATZ-B) for distances from the implant (Adjacent and Away) at weeks 3 and 6 after implantation (mean $\pm$ standard deviation).

Groups	Weeks	Distance	E (GPa)	H (GPa)
Ti	3	Adjacent	12.467 $\pm$ 3.910	0.468 $\pm$ 0.224
		Away	16.748 $\pm$ 3.693	0.669 $\pm$ 0.155
	6	Adjacent	11.01 $\pm$ 3.769	0.427 $\pm$ 0.167
		Away	14.042 $\pm$ 5.313	0.509 $\pm$ 0.234
ATZ-N	3	Adjacent	11.507 $\pm$ 5.743	0.459 $\pm$ 0.378
		Away	19.914 $\pm$ 3.305	0.792 $\pm$ 0.180
	6	Adjacent	12.699 $\pm$ 3.305	0.476 $\pm$ 0.157
		Away	8.866 $\pm$ 7.109	0.418 $\pm$ 0.141
ATZ-D	3	Adjacent	10.885 $\pm$ 5.237	0.733 $\pm$ 0.914
		Away	15.405 $\pm$ 7.008	0.682 $\pm$ 0.208
	6	Adjacent	9.872 $\pm$ 5.248	0.406 $\pm$ 0.203
		Away	9.627 $\pm$ 7.637	0.431 $\pm$ 0.240
ATZ- B	3	Adjacent	11.935 $\pm$ 6.014	0.488 $\pm$ 0.360
		Away	15.959 $\pm$ 6.504	0.656 $\pm$ 0.211
	6	Adjacent	10.717 $\pm$ 4.180	0.407 $\pm$ 0.173
		Away	11.513 $\pm$ 8.087	0.524 $\pm$ 0.234