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## Research Paper

# 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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## 1. 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 and Lindhe, 2005; Araujo et al. 2005; Lekovic et al., 1997; Pietrokovski and 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, 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 and 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 and van den Brink, 2012; Becktor et al., 2002; Bishop et al., 2011; Blanco et al., 2005; Brunel et al., 2001; Chen and Jin, 2010; Delloye et al., 2007; Rios et al., 2011; Spin-Neto et al., 2013, 2014; Waasdorp and 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., 2014; 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.

## 2. Material and methods

### 2.1. 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 (10–15 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 (ATZ)] 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.

### 2.2. 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

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