Osseointegration of Magnesium-Incorporated Sand-Blasted Acid-Etched Implant in the Dog Mandible: Resonance Frequency Measurements and Histomorphometric Analysis

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The aim of this pilot study was to investigate the bone responses of novel magnesium (Mg)-incorporated sand-blasted and acid-etched (SLA) titanium (Ti) implant in an experimental animal model. Novel Mg-incorporated SLA Ti implant was obtained via vacuum arc source ion implantation method and Mg-ions were implanted into the SLA implant surface. Control group consisted of two commercial implants; resorbable blasting media (RBM) and SLA. Twelve implants from each group were placed into the mandibles of 6 mongrel dogs. Experimental animals were divided into 2 groups of 3 animals, with 4 weeks and 8 weeks healing time points. Resonance frequency analysis was performed at the time of fixture installation, 1, 2, 4, and 8 weeks after installation. Bone to implant contact (BIC) measurements were assessed at the 4 and 8 weeks healing time points. The overall implant survival rate was 97.2%. The Mg-incorporated SLA Ti implants showed more rapid osseointegration than control group implants at follow-up periods of 4 weeks. Histomorphometric analysis showed a tendency for BIC% values of Mg-incorporated SLA Ti implant to be higher than that of other the implant groups. The results of this study suggest that Mg-incorporated SLA Ti implant may be effective in enhancing the bone responses by rapid osseointegration in early healing periods.

Key Words: Dental implant; Animal study; Surface treatment; Bone to implant contact; Bone metabolism

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INTRODUCTION

The introduction of the osseointegrated dental implants has revolutionized the rehabilitation of oral function in clinical dentistry. In an effort to enhance osseointegration of dental implants, various surface treatments have been developed, such as grit-blasting (i.e., sand-blasting), acid-etching, plasma-spray, and others [1-5]. The basic premise was to increase implant surface roughness, thereby increasing bone-to-implant contact (BIC) during the initial bone healing phase. One blasting technique uses resorbable blasting materials (RBM). Experimental studies have demonstrated a higher BIC with RBM-treated surface when compared with machine-turned surfaces [6,7]. In addition, using both sand-blast and acid etching (SLA) techniques combine the advantages of both to impart macro-roughness and micro pits to the implant surface [8]. These modifications provide for biomechanical interlocking between the implant surface and the surrounding bone. In addition, biochemical bonding also has been proposed to promote successful osseointegration [8,9].

Recently, chemical modification of dental titanium implants has been proposed to enhance osseointegration. Several studies reported surface treatment with magnesium (Mg), sulfur (S), calcium (Ca), phosphorous, or fluoride [10-12]. From these techniques, the surface chemistry of the titanium implants has been modified to incorporated specific ions in the titanium oxide layer, and these specific ions seem to reinforce the biochemical bonding of the titanium implant to bone [3,4,13,14].
Bioactive, Mg-incorporated titanium implant have also been widely investigated. Sul et al. [4] reported that Mg surface chemistry stimulates faster and stronger osseointegration. In addition, several studies demonstrated positive effects of Mg-incorporated titanium implants in osteoblastic cells [8,15,16] and in animals [4,17,18]. Although these studies have shown that Mg ions enhance the cellular response of osteoblastic cells and bone regeneration in vivo, the effects of combined surface treatment such as SLA with Mg-incorporation have not yet been evaluated in the clinical setting. While there is evidence of possible enhanced osseointegration of surface treated implants at the cellular level [8], further animal and clinical studies are needed to establish the clinical use of this implant.

There are various biomechanical methods for evaluating osseointegration [19,20]. However, almost of them, including removal torque quotient, push/pull out test and tensile pull-off tests, are invasive methods and needed to sacrifice objects for evaluation, therefore, they are not suitable for evaluation of osseointegration during early healing period. The resonance frequency analysis (RFA) is a noninvasive measurement of implant stability, and it can be used to evaluate the degree of osseointegration of implant indirectly [21,22]. Furthermore, RFA is useful tool to examine the effect of different surface treatments on implant stability [23]. To our knowledge, little is known about the implant stability changes and bone responses of SLA with Mg-incorporated implants in vivo.

Therefore, the aims of this study were to 1) compare the early stability measurements using RFA between three different surface-treated implants: SLA, RBM, and SLA with Mg-incorporated implants and 2) to compare the bone response by histomorphometric analysis, especially BIC measurement in mandible of dogs. The hypothesis was that the Mg-incorporated SLA Ti-implant enhances clinical and histological outcomes of osseointegration more than other implants.

MATERIALS AND METHODS

Implant design and preparation
A total of 36 titanium implants (Dio Inc., Busan, Korea) sized 3.8 mm diameter and 8 mm in length were used. Twelve experimental implants had Mg-incorporated SLA surfaces (group SLA-Mg). Whereas control groups consisted of SLA implants (n=12, group SLA) and RBM implants (n=12, group RBM). The RBM surface fixtures were blasted with 180 to 425 μm sized Ca phosphate. The SLA surface was prepared with large-grit blasting followed by acid etching. For Mg-ion implantation on SLA surface, PSII processing was performed [8,24]. Ionized Mg plasma was generated when Mg ion plasma was released from an arc spot in a negatively charged surface and then injected into 90 electromagnetic filters. The ionized Mg plasma accelerated within the electric field between the substrate and sheath and then reached the surface of the substrate. The electric energy of the implantation field was 15 keV.

Surface characterization
The surface morphology of the specimens was observed by scanning electron microscopy (SEM; JSM-5800, JEOL, Tokyo, Japan). To analyze the chemistry of the implanted layer, Auger electron spectroscopy (AES; PHI650; Physical Electronics, Chanhassen, MN, USA) was used. For electron excitation in the AES analysis, a primary electron beam (3 keV and 2.6 μA) with a diameter of 40 μm was used. The samples were sputtered by two symmetrically inclined 1 keV argon ion beams at an ion incidence angle of 47° with respect to the surface normal during depth profiling. Using the relative sensitivity factor provided by the instrument producer, the atomic concentrations were calculated. For Rutherford backscattering spectroscopy (RBS) analysis, helium ions were used as an ion beam source and accelerated using a Pelltro apparatus (6SDH2, NEC) at a voltage of 2 MeV. The charge of the helium ions was 20 μC, the incident angle was 0°, and the scattered angle was 10°. The measured spectrum was fitted to a theoretical spectrum and a 16-layer sample was measured and quantified (Figs. 1 and 2).

Animals
Six mature 24 month old mongrel dogs with a mean weight of 25 kg were used in this study, which was approved by the animal ethics committee at the Pusan National University, Korea. All subjects were healthy enough to receive implantation on mandible and had no periodontal problems.

Surgical technique
Teeth extraction
Two premolars were extracted from each dog bilaterally under general anesthesia. Every subject has treated identically with the same anesthesia protocol. All, dogs were premedicated with atropine (IV, 0.01 mg/kg, Huons Co., Seoul, Korea), and received presurgical antibiotics (IV, cephazoline 1 g+gentamicin 5 mg, Shinpoong Co. Ltd., Seoul, Korea) and analgesics (IM, tramadol, 100 mg/25 kg, Shinpoong Co. Ltd., Seoul, Korea). Anesthesia was induced with IV propofol (6.6 mg/kg, Jeil Pharm Co. Ltd., Seoul, Korea). The dogs were intubated and mechanically ventilated using muscle relaxants (vecuronium, 0.1 mg/kg, Korea United Pharm. Inc., Seoul, Korea). Additional propofol was added as necessary to maintain anesthesia.

Local anesthesia with 2% lidocaine (Lidocaine HCL; Huons Co., Seoul, Korea) was injected prior to extraction. Full thick-