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Research Article

Osseointegration Evaluation on Dental Implants Retrieved from a Cadaver Mandible

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Abstract

Osseointegration plays a crucial role to control implant stability and subsequent occlusal function. Histological investigation has been standardized and widely employed in animal studies to evaluate the stability and osseointegration of placed implants. In this paper, we have retrieved dental implants from a cadaver's mandible and the collected samples were subjected to optical and SEM observation and histological analysis. Excellent osseointegration was histologically noticed, and resembled the reported data from animal models. Accordingly, the applicability and validity of results obtained from animal models is confirmed.

Keywords: Osseointegration; Implant stability; Titanium implant; Histological study; *In vivo* cadaver study; Villanueva goldner staining; H-BIC

Introduction

Nowadays, dental implants represent a reliable treatment option for oral rehabilitation of partially or fully edentulous patients. Their longevity is strongly related to their stability, which is also related to osseointegration. There are a variety of methods to evaluate stability and the extent of osseointegration using *in vivo* animal tests as well as *in vitro* tests.

Osseointegration

Osseointegration is a time-dependent healing process and is defined as a direct structural and functional connection between vital bone and the surface of a load-carrying implant. To achieve a promising osseointegration, there are at least three compatibilities that the placed implant should exhibit, including biological compatibility, morphological compatibility and biomechanical compatibility [1-6]. Various surface modifications have been proposed and applied [2-5] for manipulating implant surfaces to exhibit biological, morphological and/or biomechanical compatibility.

Osseointegration is critical for implant stability and is considered as a prerequisite for implant loading and long-term clinical success of dental implants. The implant/tissue interface is an extremely dynamic region of interaction [7-9]. Histologic appearance of the osseointegration resembles a functional ankylosis with no intervention of fibrous or connective tissue between bone and implant surface [10]. Numerous pre-clinical studies have shown the superiority of particular surface modifications with respect to histomorphometric properties and biomechanical features. To guarantee long-term success in clinically challenging conditions, the development of multifunctional surface modifications and coatings is necessary [5,11]. Osseointegration is one important index to evaluate the success rates of dental implantation. Insufficiency of the osseointegration and increased micro-motion can increase the failure risk of the implant in the early stages of healing. Hence, osseointegration and implant stability are strongly related to each other [12].

Stability

Implant stability is a prerequisite characteristic of osseointegration. Continuous monitoring in a quantitative and objective manner is important to determine the status of implant stability [13,14]. Osseointegration is also a measure of implant stability that can occur in two stages: primary and secondary [15]. Primary stability mostly occurs from mechanical engagement with cortical bone. A key factor for implant primary stability is the Bone-to-Implant Contact (BIC) [16]. Therefore, the primary stability is affected by bone quality and quantity, surgical technique and implant geometry (length, diameter, surface characteristics). Secondary stability offers biological stability through bone regeneration and remodeling [17-20]. Secondary stability is affected by primary stability [19,21].

During the transition period from primary to secondary stability, the implant faces the risk of micromotion; possibly leading to implant failure. It is estimated that this period in humans occurs roughly 2-3 weeks after implant placement when osteoclastic activity decreases the initial mechanical stability of the implant, but not enough new bone has been produced to provide an equivalent or greater amount of compensatory biological stability [11,16,22]. This is related to the biologic reaction of the bone to surgical trauma during the initial bone remodeling phase; bone and necrotic materials resorbed by osteoclastic activity are reflected by a reduction in the Implant Stability Quotient (ISQ) value. The Implant Stability Quotient (ISQ) is the scale to indicate the level of stability and osseointegration in dental implants. The scale ranges from 1 to 100, with higher values indicating greater stability. The acceptable stability range lies between 55-85 ISQ [23]. This above-mentioned process is followed by new bone apposition initiated by osteoblastic activity, leading to adaptive bone remodeling around the implant [24,25].

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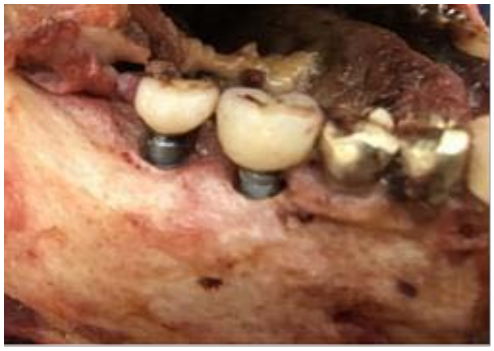

Figure 1: Two placed implants at #6 and #7 in the right mandible.

Summarizing, implant stability is one of the most important factors for the success of implant treatments. Although most studies show a correlation between bone densities and implant stability, some studies suggest the opposite, probably due to the differences in the methods used. Recent studies suggest that implant stability during the healing process only increases for implants with low initial stabilities; meanwhile, loss of stability during healing can be observed in implants with high initial stabilities [26-28].

In vitro evaluation of stability

In vitro cell culture models are routinely used to study the response of osteoblastic cells in contact with different substrates for implantation in bone tissue. Cell cultures focus on the morphological aspect, growth capacity and the state of differentiation of the cells on materials with various chemical, composition and topography [29,30]. It is well documented that the biochemistry and topography of biomaterial surfaces play a key role in the success or failure upon placement in a biological environment [31]. Wettability, texture, chemical composition and surface topography are properties of the biomaterials that directly influence their interaction with cells [32-34]. Historically, the gold standard method used to evaluate the degree of osseointegration was microscopic or histologic analysis. In addition, biological responses can be measured by cell morphology and cell activity (cell adhesion, differentiation and proliferation) [35-40].

Because cell interactions with extracellular matrix directly affect the cellular processes of adhesion, proliferation and differentiation [41], the surface properties of biomaterials are essential to the response of cells at the biomaterial interface, affecting the growth and quality of newly formed bone tissue [42]. Cell activity is strongly related to implant surface morphology and topology and is related to the process of osseointegration [30]. Actually, these surface characteristics collectively are one of the three major requirements for placed implants to exhibit subsequent retention in the bone (in other words, osseointegration)-known as morphological compatibility [2,3]. Two other requirements are biological compatibility and biomechanical compatibility [2,6]. To manipulate surface structure to maximize morphological compatibility to bone, various methods and techniques have been proposed including: as-machined, blasted surface, acid or alkaline etching, chemical treatment on blasted surface, hydroxyapatite coating, and more recently biomimetic calcium phosphate coatings [2].

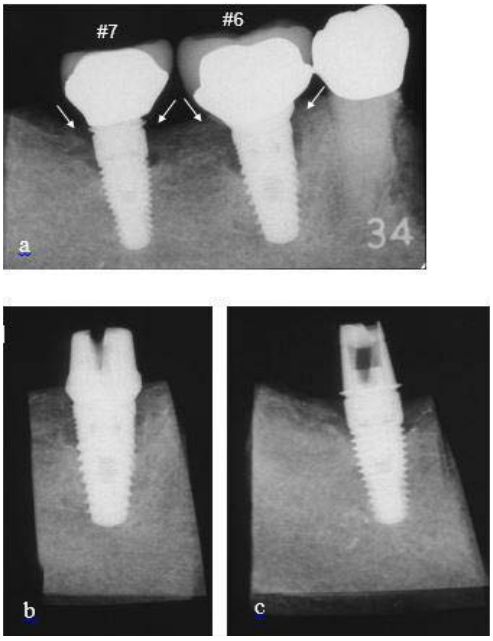

Figure 2: X-ray images; a: intraoral x-ray image in the condition in which the specimens were received; b: intraoral x-ray image after the superstructure was removed (#6); c: intraoral x-ray image after the superstructure was removed (#7).

In vivo animal evaluation of stability

It is generally believed that outcomes on the initial biological behavior of implantable materials obtained *in vitro* can't be fully correlated to *in vivo* performance. Cell cultures can't reproduce the dynamic environment that involves the *in vivo* bone/implant interaction, and their results can only be confirmed in animal models and subsequently in clinical trials [29,43,44]. Irrespective to the different animal models or surgical sites, valuable information can be retrieved from properly designed animal studies. Static and dynamic histomorphometric parameters plus biomechanical testing are recommended as measurable indicators of the host/implant response where different surface designs are compared. Bone-to-Implant Contact (BIC), which is the most often evaluated parameter in *in vivo* studies, together with bone density and amount and type of cellular content, are examples of static parameters [44].

Besides histological analyses, biomechanical tests (torque, push-out, pullout, etc.) can measure the amount of force that a torque needs to fail the bone-implant interface surrounding different implant surfaces [20, 45-48]. Considering the several factors that influence osseointegration, the evaluation of the largest possible number of host/implant response parameters is desirable to better understand bone healing adjacent to different implant surfaces. These tests can clarify indications of use and provide direction regarding immediate/early loading. These tests check clinically for mobility with the help of blunt ended instruments, cutting torque resistance, reverse torque and resonance frequency analysis [26,49,50].

In the majority of publications, canine [51,52], sheep/goat [53-55], pig [56], rabbit [40,57-60], rat and mice [61-64] are popular animal models, as are nonhuman primates [65,66]. Although animal models appear to be a well-established approach to provide

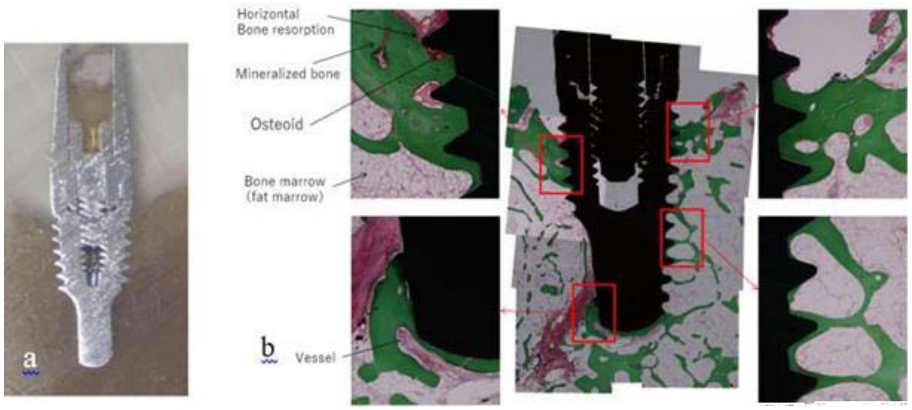

Figure 3: Photos for histology analysis.a: Resin-embedded #7 with methyl methacrylate; b: Villanueva Goldner stained sample #7.

valuable and applicable information to the human condition, there are still several concerns. Some results from *in vitro* studies can be difficult to extrapolate to the *in vivo* situation because there are differences in bone composition between the various species and humans. While no species fulfils all of the requirements of an ideal model, an understanding of the differences in bone architecture and remodeling between the species is likely to assist in the selection of a suitable species for a defined research question [66]. Another of the main problems associated with the *in vivo* tests using animal models is the test duration and its validity for application. Dziuba et al. [59], using rabbit's model, investigated the biological behavior of Mg implantable alloy over 12 months. Amerstorfer et al. [63] conducted *in vivo* tests using Sprague-Dawley rats for 12 months to investigate the biodegradability for Mg alloy as a potential implant material. Furthermore, Akens et al. [53], using sheep over for 18 months, studied the efficacy of photo-oxidized bovine osteochondral transplants. Although these tests showed promising results, a longer period of testing is required before applying the materials to human subjects.

The relevance of results obtained from animal models has been subject to great debate. The use of animal models in the study of dental implants has contributed greatly to understand many different devices in used and is often an essential step in the testing of orthopedic and dental implants prior to clinical use in humans [66]. Animal testing plays a major role in assessing the safety and efficacy of dental implants. To date, animal testing has shown the nature of soft tissue attachment to implants and the types of interfacial tissues within bone sites. There have been an increasing number of studies correlating animal tests with *in vitro* analysis and human studies.

Evaluation on human samples

Mangano et al. [67] placed a dental implant in the posterior maxilla (#14) of a 48-year-old female. The implant was an Anyridge type with a nanostructured calcium-incorporated surface as a biomimetic coating. The patient was subjected to immediate functional loading. A month later, due to a traumatic injury causing mobilization of the fixture, the implant was removed. The sample was investigated under SEM observation. It was reported that (i) the surface of the implant (for one month) showed a highly-structured texture, carved by irregular, multi-scale hollows reminiscent of a fractal structure, and (ii) the human specimen showed trabecular bone firmly anchored to the implant surface, bridging the screw threads and filling the spaces among them. They concluded that histological analysis indicated that the nanostructured calcium-incorporated surface was covered by new bone, one month after placement in the posterior maxilla, under an immediate functional loading protocol [67]. The research group conducted another human evaluation. Ten totally edentulous subjects (age ranging from 46 to 77 years old) received two transitional implants: one tapered implant with a nanostructured calcium-incorporated surface and one cylindrical implant with a sandblasted surface as a control. The implants were placed according to a split-mouth design and immediately loaded to support an interim complete denture. After 2 months, they were removed for histologic and histomorphometric analyses, and BIC and BD (bone density) calculations. It was concluded that in the posterior maxilla, under immediate loading conditions, implants with nanostructured calcium-incorporated surfaces seem to increase the peri-implant endosseous healing properties [68].

We have been reviewing implant-related research using animals, and human samples with implants placed for 1-2 months. The specific aims of this study were (i) to conduct SEM observation and histological analysis on implant samples retrieved from a cadaver who had implants placed for about 5 years, and (ii) to compare results to those obtained from animals (at longest 18 months) and human (1 and 2 months duration).

Subject and Methods

About the cadaver

The subject of this report died on February 2018 when he was 99 years old. The cadaver was immediately subjected to a topical disinfectant spray and wash for preservation purposes. Serology was performed and results indicated that Hepatitis B Surface Antigen was negative, Hepatitis C Virus Antibody was negative and HIV 1/0/2 Ab was also negative. According to the chart attached to the cadaver, the Genesis® implant was placed at #6 or #46 in his right mandible on November 2011 (when he was 92 years and 9 months old) and the PrimaConnex® implant was inserted at #7 (or #47) in his right mandible on May 2012 (when he was 93 years and 3 months old) (Figure 1).

Cutting the block for X-ray imaging

A block about 20mm apart from both mesial and distal sides was

removed from the cadaver mandible for X-ray imaging.

Histological analysis

For the histological evaluation of osseointegration of titanium implants, the cadaver samples were firstly treated with alcohol and acetone for washing and delipidation purpose. The thus treated samples were immersed in the following sequential solutions; a mixture of methyl methacrylate monomer and acetone with equal amount, single solution of methyl methacrylate monomer, and then a mixed solution (immersion-embedding solution) of methyl methacrylate monomer + methyl methacrylate polymer + benzoyl peroxide to embed the samples. The MMA resin blocks were fabricated by gradual heating up from 30°C for polymerization. Using the microtome cutter ISOMET1000 (BUEHLER) and micro cutting machine BS-300CL (EXACT), one samples was prepared with thickness of about 500μm along the cross sectional sagittal plane from the center portion of the implant main body. The sample was polished down to the thickness of about 40μm with the micro grinding machine MG-400CS (EXACT). The sample was finally subjected to the Villanueva Goldner staining [44,69,70] and histological investigation was conducted with the optical microscope ECLIPSE (E600) (Nikon Corp., Tokyo).

In general, it is believed that the Bone-to-Implant (BIC) is defined as the linear surface of a dental implant in direct contact with the mineralized bone which is expressed as percentage of the total surface of endosseous dental implant at the light microscopic level. Hence, the BIC value can be treated as an osseointegration indicator [71]. Histomorphometric examination on bone-to-implant relationship has been still considered the gold standard for analyzing bone formation and healing process, and assessing the osseointegration [16,44,68,72-75]. Although the original concept for BIC evaluation was developed for metallic dental implant, Han et al. [76] applied to nonmetallic implant (i.e., zirconina implant) and claimed its acceptable applicability. While keeping the same definition of BIC, there are several newly developed techniques [77,78]. Manresa et al. [77] used the back-scattered scanning electron microscopy and Balatsouka et al. [78] applied the stereological technique to show 3D BIC value. Accordingly, it is not good enough to mention the BIC value, rather it appears to be necessary to add a word by which the method for BIC evaluation to differentiate data obtained by other method. Hence, the authors would like to propose a term "H-BIC as a histomorphometric bone-to-implant contact".

SEM analysis

The samples were fixed with 2% Paraformaldehyde (PFA) and 2% Glutaraldehyde (GA) in 0.1 M cacodylate buffer pH 7.4 at 4°C overnight. The samples were additionally fixed with 1% tannic acid in 0.1 M cacodylate buffer pH 7.4 at 4°C for 2h. After the fixation the samples were washed 4 times with 0.1 M cacodylate buffer for 30 min each, and post-fixed with 2% Osmium Tetroxide (OsO_4) in 0.1 M cacodylate buffer at 4°C for 3 h. Then, the samples were dehydrated in graded ethanol solutions (50%, 70 %, 90 %, 100 %). The schedule was as follows: 50 % and 70 % for 30 min each at 4°C, 90 % for 30 min at room temperature, and 4 changes of 100 % for 30 min each at room temperature. After these dehydration processes, the samples were continuously dehydrated with 100 % ethanol at room temperature overnight. The samples were substituted into tert-butyl alcohol at room temperature. The schedule was as follows: 50:50 mixture of ethanol and tert-butyl alcohol for 1 h, 3 changes of 100 % tert-butyl alcohol for 1 h each followed by being frozen at 4 °C. The frozen samples were vacuum dried. After drying, the samples were coated with a thin layer (30 nm) of osmium. The samples were observed by a scanning electron microscope (JSM-7500F; JEOL Ltd., Tokyo, Japan) at an acceleration voltage of 3.0 kV.

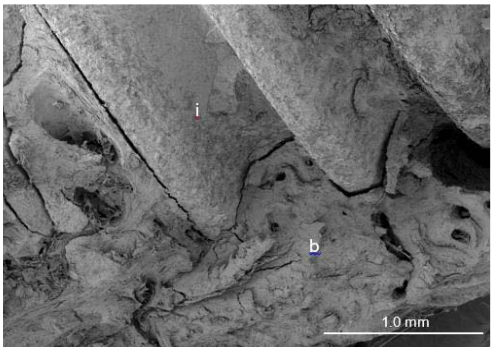

Figure 4: SEM image of #6 sample (50.6:1), where i indicates the implant and b is bone.

Results

General observation

A Keystone series Genesis® implant was placed at #6 and a Keystone series PrimaConnex® implant was inserted at #7, respectively. It appears that the final restoration was a single crown type and cement-bonded with porcelain-fused-to-metal crown. Moreover, a saucer-shaped bone defect (or saucerization) was observed about 5mm around the entire periphery of the placed implants, and the threaded portion was exposed around the supra-bony zone.

X-ray imaging and comments

Sub-marginal contour of the #6 final restoration was over-contour appearance and a space was found at the proximal sides between #6 and #7 see (Figure 2). Final restoration #7 was ill-fitted to the abutment and about 1mm gap there between was observed. Saucerization was found at both #6 and #7 and bone loss was indicated about 1/3 to 1/2 of the implant length and it was more emphasized at interdental area.

Due to improper implant morphology and insertion, and perhaps because of the man's age and intraoral hygiene, severe implant periodontal disease developed, causing exposure of the micro-thread portion of the implant in the supra-bony zone. This might occur for several reasons;

Fixture: The fixture was inserted slightly deeper than the surrounding bone ridge level and the micro gap appears to be set much deeper than the sub gingival margin. In particular, the reason for #7 revealing severe implant periodontal disease might be due to several factors, including smaller implant size compared to the #6 implant, possibly different implant material (accordingly different mechanical properties), and adverse influence of occlusal (jiggling) force than #6.

Final restoration: There was over-contour on #6, and #7 was poorly cement-bonded, causing a contact space between both implants.

Histological examination

Relatively thick calcified bone was noticed at the implant collar and apex sides with thick trabeculae. Proximally, scattered osteoid was evident (Figure 3a). The implant main body was covered with thin mineralized bone and vascular ingrowth was observed locally.

Mineralized surface (green area in Figure 3b) in direct contact with the implant was measured. The total implant surface length was 304.494 mm, and the mineralized portion was 169.528 mm. Accordingly, the H-BIC (histomorphometric bone-to-implant contact) at this site was 55.68%.

SEM observation

Analysis of the SEM showed that bone matrix was apposed to the implant surface and no connective tissue was found between the bone and implant, indicating excellent osseointegration (Figure 4).

Discussion

In general, osseointegration was recognized in a length span between 5 and 6 mm. Final prosthetic restorations for both implants were cement-bonded to the fixture and the restorations were single unit porcelain-fused-to-metal crown. Due to the relatively low height of the abutments, thick keratinized gingiva was not established. It appears that the platform switching method in which alveolar bone levels around the implants are preserved was not performed [79,80]. Implant placements were evaluated as satisfactory, except insertion depth was insufficient. Both exhibited peri-implantitis.

Judging from the x-ray image, the #6 implant was placed more deeply than necessary, resulting in more saucer-shaped bone resorption. The lingual side of the implant was completely immersed inside the bone structure, suggesting that the #6 implant was thicker in diameter and wider in bone width. Moreover, the fixture height was not sufficiently high, so that the connecting portion between the prosthesis and the abutment (cement line) is deeper. Figure 1 shows insufficient keratinized gingiva. The emergency profile from the platform shows over-contour, suggesting the prosthesis had high plaque retention [81].

There was a large gap in margin height between these two, resulting in difficulty for controlling the adjacent gingiva. Particularly on the mesial side of #7, bone resorption was noticeable. Moreover, the #6 prosthesis had a further problem in that there was excess cement extruded from the buccal side of #7 and plaque control was not easily performed.

Fixtures with collars having different height were used in this case, and the abutment of #6 was closer to the bone, causing pressure on the gingiva. The excess pressure might have caused the bone resorption.

It appears to be that the classic X-ray imaging technique is still effective in a wide range of treatments; including diagnosis [82], examining early stage of osseointegration and healing process [45] and even prognosis [83]. The H-BIC value of 55.68% is very acceptable in comparison with reported values of 29% [84] and about 55-60% [85,86].

Because this is a case report, it is impossible to conduct statistical analysis. Placing an implant in a patient who was over 90 years old should have contributed to enhancement of the patient's quality of life. At the same time, we should express our sincere respect to his dentist who diagnosed the patient's intraoral condition and pursued the implant surgery rather than the removable partial denture treatment. In the future, it is recommended that the implant be designed by considering the implant supported overdenture and high self-cleaning capability.

Conclusion

A man, who received dental implants on his right mandible when he was 92-93 years old, more than 5 years before his death, was the subject to this study. Surprisingly given his age, the placed implants exhibited satisfactory osseointegration. The H-BIC value was about 56%, which is fairly good for an aged patient. Results from the present study agree with results reported on animal models (eg, rat [87], rabbit [88] and dog [89]) and human samples (for a short period of time [67,68]). We conclude that ordinary animal test results are applicable to human beings for the evaluation of long-term longevity of oral implants.

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