CASE REPORT

Bony spicules trapped in peri-implant soft tissue: a common unrecognized finding

Teeratida Sampatanukul1, Pravej Serichetaphongse2, Pichet Sampatanukul3 & Atiphan Pimkhaokham4

1Esthetic Restorative and Implant Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
2Department of Prosthodontics, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
3Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
4Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

Correspondence
Atiphan Pimkhaokham, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chulalongkorn University, 34 Henri-Dunant Rd., Wangmai, Pathumwan, Bangkok, 10330 Thailand. Tel: +66-2218-8662; Fax: +66-2218-8664; E-mail: atiphan.p@chula.ac.th

Funding Information
This article was partially supported by THE 90th ANNIVERSARY OF CHULALONGKORN UNIVERSITY FUND (Ratchadaphiseksomphot Endowment Fund).

Received: 31 August 2016; Revised: 30 August 2017; Accepted: 1 September 2017

Clinical Case Reports 2017; 5(11): 1856–1861
doi: 10.1002/ccr3.1207

Key Clinical Message
According to the study, there were unexpected tiny bone spicules being inspected in peri-implant soft tissue. These displaced autogenous bone chips were probably presented when preparing implant sites. The displaced bone spicules seemed not induced significant inflammatory reactions; on contrary, defects of specimens and dissolving bone spicules pictures were demonstrated.

Keywords
Autogeneous bone, bone spicules, Bone–Implant Interface, dental implant, dental implant abutment, peri-implant tissue, soft tissue response.

Introduction
Dental implant is currently considered a treatment of preference for patients whose tooth have been missing and want to have replacements that imitate natural teeth. The structure of peri-implant tissue is similar to that of teeth, but the major difference is the attachment of connective tissue fibers, which is much weaker and prone to be infected than natural teeth [1, 2]. Moreover, to restore teeth in cosmetic area, several factors are to be considered. One factor that affects both function and esthetic demand of implant restoration is the abutment materials, which will affect different tissue reaction and color perception through gingival tissue [3–6]. By investigating the effect of the types of abutment materials on the attachment formation and inflammatory response of the peri-implant soft tissue in human subjects, the authors have observed an unrecognized finding of tiny bony spicules scattered in the peri-implant soft tissue. The numbers, characters as well as incidences of these bony particles were determined to speculate the nature and impacts of their presence.

Material and Methods
This article was a by study of the clinical research entitled Histological evaluation and inflammatory response of different abutment materials: An experimental study in human, which had been approved by the Ethics Committee of Faculty of Dentistry of Chulalongkorn University (HREC-DCU 2014-051). Patients who have posterior edentulous site with adequate bone for placing implant without bone grafting procedures and agreed to participate in the study were included in this study. The exclusion criteria included patients who were smokers, had
systemic diseases requiring routine use of antibiotics, or those who were pregnant. All patients included were agreed to participate in this study, with their signatures on the consent forms. All implants fixtures used in the research were Aster tech OsseoSpeed™ implant (Dentsply, Mölndal, Sweden) with diameters of 4.5 and 5.0, length 9 mm and 11 mm, were placed by post graduate students under a supervision of one experience surgeon. All implants were placed at posterior edentulous sites with a standard implant surgical protocol according to the manufacturer’s instruction. Briefly, patients were local anesthetized and, then, crestal incision line was performed. Flap operation followed by drilling protocol was performed at the implant sites. Then, the implant fixtures were installed. Participants were randomly assigned to the abutment groups. Three different abutment types, titanium, zirconia, and gold alloy were used and randomly allocated to patients after implant fixtures were installed to blind operators.

The biopsy procedure was taken at 8 weeks after implant installation. One operator performed the biopsy procedure using surgical blade no. 12D and 15C. The blade angle was parallel to the abutment surface, and the cutting blade was placed 1 mm away from abutment surface, which resulted in a circular shape of biopsy tissue. Then, the abutment and peri-implant tissue attached to the abutment were carefully removed. Regular healing abutments were inserted on the implant fixtures. After tissue biopsy obtained at 8 weeks, the sample had been processed for histological observations. Two tissue preparations techniques were operated as described below.

**Resin embedding technique**

The technique was previously introduced [7] and had been used to observe peri-implant tissue in many studies [8–10], which aimed to preserve the tissue–implant interface. Briefly, biopsy specimen underwent serial dehydration procedures with various concentrations of ethyl alcohol and then immersed with resin (Technovit 7200 VLC; Heraeus Kulzer, Wehrheim, Germany). The harvested specimen was positioned in a plastic block, filled up with resin (Technovit 7200 VLC; Heraeus Kulzer), and light cured (Exakt 520 Light Polymerization Unit, Norderstedt, Germany) for 12 h. The resin block was mounted on plastic slides. Then, the block was cut and grinded with Exakt cutting and grinding machines (Exakt Apparatebau, Norderstedt, Germany).

**Paraffin embedding technique**

After being fixed in 10% formalin overnight, the outer surface of tissue samples was marked with blue ink (CDI’s Tissue Marking Dyes®; Cancer Diagnostics, Durham, NC, USA). Then, specimens were cut into four pieces parallel to long axis of abutment, and the tissue parts were removed from abutment for processing. A routine histological processing technique was employed. Tissue sections were dehydrated in graded ethanol series (70–100%) and xylene and embedded in paraffin (Tissue Processing Center TPC 15 Duo/Trio; Medite GmbH, Wollweberstr, Germany). The histological sections were cut with microtome (Leica RM2235; Leica Biosystems, Richmond, IL, USA) and adhered to a glass slide.

All sections were stained with hematoxylin and eosin (Leica Biosystems) and observed under light microscope (Olympus BX53, Tokyo, Japan).

**Results**

A total of 12 healthy patients, six males and six females, age 37–60, enrolled in this study. Patients who had edentulous areas on both sides were treated with two implants, and a total of 18 implants were placed. Three 4.5-diameter implants were placed in the second premolars, and 15 5.0-diameter implants were placed in the first and second molar areas.

Of the 18 studied samples, 15 were processed by resin embedded technique, and three cases were paraffin embedded. The slides processed by resin embedded technique presented tissue with metal abutments, while the slides from paraffin embedded revealed only peri-implant tissue. The occurrences of bony spicules were found in 13 of 18 cases (72.2%). Three cases elicited more than 15 pieces of bone were seen in histological slides (Fig. 1). Most cases (10 of 13) disclosed 1–2 bony chips per section (Figs. 2 and 3).

From both embedded techniques, the histological slides revealed fibrous connective tissue aligned around pieces of bones with mild degree of inflammatory cell presented. In some specimens, giant cells presented near the small pieces of bones, showing some irregularity of osseous surface (Figs. 1 and 2). In larger pieces of bone spicule, osteocytes could be seen. The location of displaced bony spicules was found at inner connective tissue part. Distances varied from 200 to 1000 micron away from subepithelium.

Mild-to-moderate inflammation was mostly observed at subepithelial area, with different degrees among abutment materials. The infiltrated areas were separated from bony chips by the alignment of fibrous connective tissue around bony tissue. Therefore, no relationships of bone spicules to inflammatory responses resulted from abutment materials were found (Figs. 1–3).

Follow-up of all patients, after tissue biopsy and 3 months after delivery of final crowns, was uneventful.
Figure 1. Representative histological section of peri-implant tissue attached to zirconia abutment processed by resin embedding technique (A) many bone spicules presented in the connective tissue part, magnification of 40× (B) fibrous connective tissue orientated around bony spicules with very mild inflammatory cells presented, magnification of 100× (C) giant cells presented along lower boarder of a small osseous surface, magnification of 400×.

Figure 2. Representative histological section of peri-implant tissue from gold alloy abutment processed by paraffin embedding technique (A) groups of small pieces of bone spicules presented in the connective tissue part near inflammatory infiltrated area at subepithelium, magnification of 40× (B) fibrous connective tissue orientated around bony spicules with inflammatory cells and giant cells presented, magnification of 400× (C) large piece of bony chip presented at lower boarder of biopsy tissue, magnification of 100×.
No gingival inflammation or others complications were reported.

**Discussion**

Bony particles deposited in dental peri-implant soft tissue had not been previously described. The reason might be that no histological evaluation was routinely required. The authors had an opportunity to look at the tissue formed around implant abutments after 8 weeks of installment for the clinical research. This by study finding of bony particles was likely to be from displacement of the bony debris when drilling the bone in order to prepare implant sites. In three cases, which many pieces of bone spicules were found, the operators might use the autogenous bone debris collected with drilling burs to place at buccal site of implant fixtures to correct very minor bone dehiscence occurred at buccal site. However, no guided bone regeneration procedures were recorded in all cases.

The supporting evidences were common observations in 72.2% of the cases presented bony spicules in the connective tissue part. It seemed that during healing process, epithelial migration toward implant abutment materials and the spicules of bone was entrapped by connective tissue fibers. In three cases, many spicules presented and tissue reaction occurred toward the bones with some degrees of inflammatory cells and giant cells. However, some cases presented only fiber orientation toward this bone without inflammatory cells. Therefore, differential diagnosis of bony metaplasia was excluded.

Not many dental implant researchers studied on the peri-implant tissue were performed in human models. And to the author knowledge, these findings have not been mentioned before in the previous studies.

Previous studies related to autogenous particulate bone reported that the size of autogenous particulate bone affected the amount of new bone formation around the defect walls of periodontal tissue. Small sizes from 300 to 500 microns were found to be effective in restoring periodontal defect [11]. Various instruments were reported for the uses of harvesting particulate bones. High or slow speed handpieces, chisels, bone mills, piezosurgical instruments, rongeurs, or bone scrappers may be used to harvest bone from
donor sites [12, 13]. The previous studies suggested that harvesting autogeneic bone using bone mill and bone scraper techniques contains more viable cells and resulted in better osteoblastic cells adhesion and function [14]. In contrast, clinical evidence in animal models did not review significances of bone healing when using difference bone harvesting methods, between using bone mill, bone scraper, piezo drill, and bone slurry collected from drilling [15].

Moreover, to treat bone defect size, researcher suggested using resorbable or nonresorbable membrane to separate the healing of bone from soft tissue [16, 17]. This study could possibly explain the situation of using particulate bone graft without membrane and that some bone chips displaced to the soft tissue area.

As the presented bone spicules in soft tissue have not been studied, their clinical impact was not well established. Although severe inflammatory response was not found along with the bone chips, histological slides reviewed that bone chips were not likely to survive, due to limited blood supply. These bones were displaced from bone beds, and few vascular tissues were presented in connective tissue part 1. It seemed that pieces of bone in connective tissue would be dissolved over time. However, their impact on the harvesting technique was possible. Blade may hit the bony substance. Therefore, tissue preparation was affected resulting in tissue distortion.

**Conclusion**

Bony spicules could be displaced from bone bed to peri-implant tissue when preparing implant sites. At 8 weeks healing period, these bone spicules could be detected with some degree of tissue responses. As this finding has not been recognized, further studies should be conducted to investigate the impact of these bony spicules.

**Acknowledgments**

The authors gratefully acknowledge the cooperation and support of Professor Prasit Pavasant for his valuable suggestions in laboratory part. This research was financially supported by THE 90th ANNIVERSARY OF CHULA-LONGKORN UNIVERSITY FUND (Ratchadaphiseksomphon Endowment Fund). All the authors have no conflict of interest to declare.

**Authorship**

TS: collected and analyzed data, drafted the article, made critical review of the article. PS: designed the concept, made critical review of the article, approved the article. PS: interpreted and analyzed data, drafted the article, made critical review of the article. AP: interpreted and analyzed data, drafted the article, made critical review of the article, approved the article.

**Conflict of interest**

All authors declare no conflict of interest.

**References**

1. Cochran, D. L. 2000. The scientific basis for and clinical experiences with Straumann implants including the ITI Dental Implant System: a consensus report. Clin. Oral Implants Res. 11(Suppl 1):33–58.
2. Listgarten, M. A. 1996. Soft and hard tissue response to endosseous dental implants. Anat. Rec. 245:410–425.
3. Abrahamsson, I., T. Berglundh, P. O. Glantz, and J. Lindhe. 1998. The mucosal attachment at different abutments. An experimental study in dogs. J. Clin. Periodontol. 25:721–727.
4. Linkevicius, T., and P. Apse. 2008. Influence of abutment material on stability of peri-implant tissues: a systematic review. Int. J. Oral Maxillofac. Implants 23:449–456.
5. Welander, M., I. Abrahamsson, and T. Berglundh. 2008. The mucosal barrier at implant abutments of different materials. Clin. Oral Implants Res. 19:635–641.
6. van Brakel, R., G. J. Meijer, J. W. Verhoeven, J. Jansen, C. de Putter, and M. S. Cune. 2012. Soft tissue response to zirconia and titanium implant abutments: an in vivo within-subject comparison. J. Clin. Periodontol. 39:995–1001.
7. Donath, K., and G. Breuner. 1982. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. J. Oral Pathol. 11:318–326.
8. Schwarz, F., I. Mihatovic, J. Becker, K. H. Bormann, P. L. Keeve, and A. Friedmann. 2013. Histological evaluation of different abutments in the posterior maxilla and mandible: an experimental study in humans. J. Clin. Periodontol. 40:807–815.
9. Canullo, L., G. Wiel Marin, M. Tallarico, E. Canciani, F. Musto, and C. Dellavia. 2015. Histological and histomorphometrical evaluation of postextractive sites grafted with Mg-enriched nano-hydroxyapatite: a randomized controlled trial comparing 4 versus 12 months of healing. Clin. Implant Dent. Relat. Res. 18:973–983.
10. Chai, W. L., K. Moharamzadeh, I. M. Brook, and R. Van Noort. 2011. A review of histomorphometric analysis techniques for assessing implant-soft tissue interface. Biotech. Histochem. 86:242–254.
11. Zaner, D. J., and R. A. Yukna. 1984. Particle size of periodontal bone grafting materials. J. Periodontol. 55:406–409.
12. Zaffe, D., and F. D’Avenia. 2007. A novel bone scraper for intraoral harvesting: a device for filling small bone defects. Clin. Oral Implants Res. 18:525–533.
13. Miron, R. J., E. Hedbom, N. Saulacic, Y. Zhang, A. Sculean, D. D. Bosshardt, et al. 2011. Osteogenic potential of autogenous bone grafts harvested with four different surgical techniques. J. Dent. Res. 90:1428–1433.
14. Miron, R. J., R. Gruber, E. Hedbom, N. Saulacic, Y. Zhang, A. Sculean, et al. 2013. Impact of bone harvesting techniques on cell viability and the release of growth factors of autografts. Clin. Implant Dent. Relat. Res. 15:481–489.
15. Saulacic, N., D. D. Bosshardt, S. S. Jensen, R. J. Miron, R. Gruber, and D. Buser. 2015. Impact of bone graft harvesting techniques on bone formation and graft resorption: a histomorphometric study in the mandibles of minipigs. Clin. Oral Implants Res. 26:383–391.
16. Gielkens, P. F., R. R. Bos, G. M. Raghoebar, and B. Stegenga. 2007. Is there evidence that barrier membranes prevent bone resorption in autologous bone grafts during the healing period? A systematic review. Int. J. Oral Maxillofac. Implants 22:390–398.
17. Becker, W., M. Urist, B. E. Becker, W. Jackson, D. A. Parry, M. Bartold, et al. 1996. Clinical and histologic observations of sites implanted with intraoral autologous bone grafts or allografts. 15 human case reports. J. Periodontol. 67:1025–1033.