

Study on the Morphology of Micro-implant-bone Interface Cells and Bone Histomorphology Under Different Implantation

Estudio sobre la Morfología de las Células de la Interfaz Microscópica
Implante-Hueso y la Histomorfología Ósea Bajo Diferentes Implantaciones

Li Bing¹; Hee-Moon Kyung²; Fan-Yu Xu¹; Tae-Geon Kwon² & Xiu-Ping Wu¹

LI, B.; HEE-MOON, K. ; FAN-YU, X. ; TAE-GEON, K. & XIU-PING, W. Study on the morphology of micro-implant-bone interface cells and bone histomorphology under different implantation. *Int. J. Morphol.*, 36(1):279-283, 2018.

SUMMARY: Micro-implant stability has always been the focus of orthodontic clinical research. In the experiment, the morphological changes of bone tissue around the micro-implants in self-tapping and assisting implantation were investigated to explore the effect of different implantation on the osseointegration of micro-implants in order to provide some theoretical basis for clinical practice. Six adult male Beagle dogs were selected, three implants were implanted into the left and right maxillary bone of Beagle dogs at the 0th, 4th and 6th week, respectively. One side to self-tapping implantation, the opposite side to assisting implantation. At the 8th week of the experiment, the animals were sacrificed and the micro-implant-bone tissue specimens with the healing time of 8w, 4w and 2w were obtained. The specimens were stained with Toluidine Blue (TB) and photographed under 100X, 200X microscope. Morphology of micro-implant-bone interface cells was observed under light microscope. In self-tapping group, there were some fibrous tissues surrounding the micro-implants at the 2th week, the formation of osteoblasts and osteoid was observed at the 4th week, the wavy and lamellar bone tissues were seen at the 8th week. In assisting group, more collagen fibers were deposited around the micro-implant at the 2th week, there were a large number of osteoid-like cells, and the collagen was gradually replaced by the bone tissue at the 4th week, the osteoblasts were active and the osteoblasts were linearly arranged and formed a laminate bone at the 8th week. Whether implanted self-tapping or assisted implantation, micro-implant-bone interface reconstruction can occur. If the clinical need for early loading force, micro-implant try to choose self-tapping implantation. By appropriately prolonging the healing time, the initial stability of the micro-implant under assistive implantation can be improved.

KEY WORDS: Beagle dog; Micro-implant anchors; Morphology ; Bone histomorphology.

INTRODUCTION

Stable and effective anchorage control is the key to good effect of orthodontic treatment. Micro-implants, as the absolute anchorage of orthodontic treatment, are gradually being applied by more clinicians (Khan *et al.*, 2016; Bozkaya *et al.*, 2017; Melsen & Dalstra, 2017). The initial stability of micro-implants has been restricted due to the influence of load intensity and rotation force. The stability of micro-implants anchorage has been a hot research topic in orthodontics (Davoody *et al.*, 2013; Chang & Tseng, 2014; Maino *et al.*, 2016). The biocompatibility and mechanical compatibility of the implant with the bone tissue are reflected in the interface between the implant and the bone tissue and the response of

the surrounding bone tissue to the implant. When the micro-implants are implanted, the micro-implant-bone interface undergoes a series of inflammatory response?absorption?bone reconstruction and bone plastic construction and other changes, which may eventually lead to the formation of fibrous bone retention and osseointegration (Shah *et al.*, 2016; Buser *et al.*, 2017; Liu *et al.*, 2017). In the experiment, we investigated the changes of implant-bone interface cells and bone histomorphometry around micro-implant by different implantation methods, to explore the effect of implantation on micro-implant osseointegration in order to provide some theoretical basis for clinical practice.

¹ Stomatology Hospital, Shanxi Medical University, Taiyuan, China.

² School of Dentistry, Kyungpook National University, Daegu, Korea.

Funding: Shanxi Province International Science and Technology Cooperation Project (China-Korea), No. 2015081030; Shanxi Province returned overseas students research funding projects, No. 2015-053.

MATERIAL AND METHOD

Experimental animals. Six healthy male beagles aged 18–20 months and weighing 12–14 kg were provided by the Animal Experimental Center, Shanxi Medical University, China. All dogs had intact dentition and normal occlusion, and they were free from dental caries and periodontal disease.

Experimental materials and equipment. Micro-implant 36 (diameter: 1.5 mm, length: 7 mm, gingival length: 2.5 mm) and supporting implantation equipment (Dentos, Korea); Leica2035 Tissue Slicers (Germany); NikonEM1200 photographic microscope; S-450 Scanning Electron Microscope (SEM, Hitachi, Japan); BI-2000 medical image acquisition and analysis system (Chengdu Thai League Technology Co, Ltd).

Experimental reagents. Anesthetic: 3 % Pentobarbital sodium, 2 % Lidocaine; Anti-inflammatory drugs: Penicillin, 1 % Iodine, 3 % H₂O₂; Fixed liquid: Formaldehyde, Paraformaldehyde, Glutaric acid aldehyde, Ketamine, Phosphate buffer; Toluidine blue staining reagent.

Experimental grouping. The root of the second, third and fourth premolar teeth of bilateral maxilla was selected as the implantation site of micro-implant. At the 0th, 4th and 6th week, three micro-implants were implanted on the right and left sides of the maxillary bone in Beagle dogs. One side was implanted by self-tapping and the other was implanted by assisting. At the 8th week of the experiment, the animals were sacrificed and the micro-implant-bone tissue specimens with the healing time of 8w, 4w and 2w were obtained. There were 18 micro-implants in the self-tapping implant group and the assistive implant group.

Preparation of hard tissue specimens. Three days after the operation of implanting micro implants with self-tapping

implantation and assisting implantation, the planting area was rinsed alternately with 3 % hydrogen peroxide solution and 0.9 % saline solution. The penicillin 800,000 units were intramuscularly injected. Check the micro-implant daily for damage or loss. After 8 weeks, all experimental dogs were over-anesthetized and the maxillary bone with implant was removed. Tissue pieces of about 2.0 cm × 1.0 cm × 1.0 cm were prepared and routinely fixed, dehydrated, soaked and embedded. The long axis was gently cut from the buccal tongue to the tissue block with a slice thickness of 60-80 mm.

Histological observation. The tissue sections of Toluidine Blue staining (TB) was performed on 100 x and 200X microscopy using BI-2000 medical image acquisition and analysis system. The morphology of implant-bone interface was observed under light microscope.

RESULTS

Morphological observation of implant-bone interface cells and bone histomorphology at different healing time using self-tapping implantation method. There were some fibrous tissues surrounding the micro-implants at the 2th week (Fig. 1), the formation of osteoblasts and osteoid was observed at the 4th week (Fig. 2), the wavy and lamellar bone tissues were seen at the 8th week (Fig. 3).

Morphological observation of implant-bone interface cells and bone histomorphology at different healing time using assisting implantation method. More collagen fibers were deposited around the micro-implant at the 2th week (Fig. 4), there were a large number of osteoid-like cells, and the collagen was gradually replaced by the bone tissue at the 4th week (Fig. 5), the osteoblasts were active and the osteoblasts were linear arrange and form a laminate bone at the 8th week. (Fig. 6).

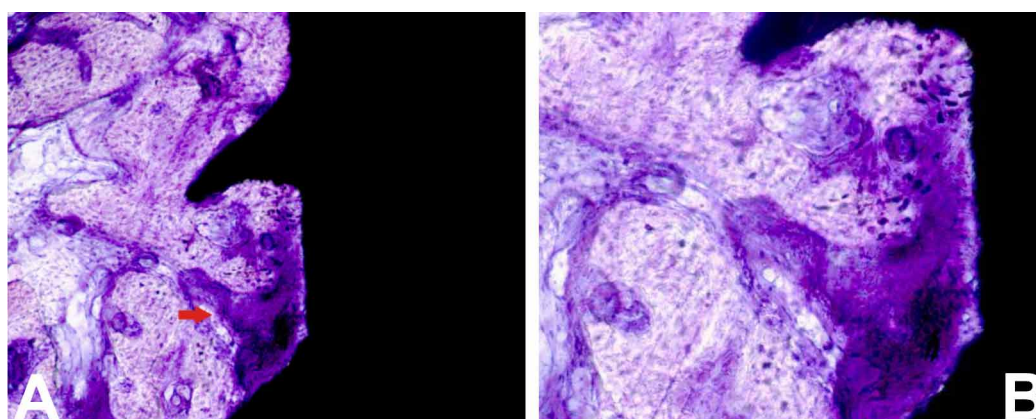


Fig. 1. Morphological observation of self-tapping implantation group at 2W (A: ×100, B: ×200)

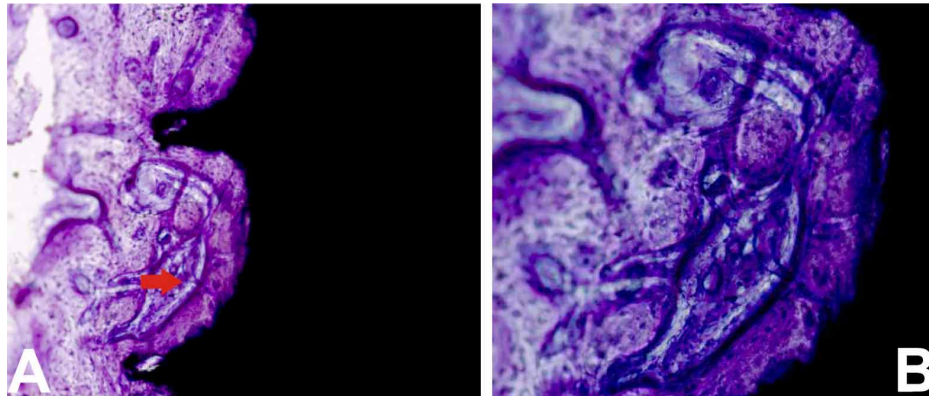


Fig. 2. Morphological observation of self-tapping implantation group at 4W(A: $\times 100$, B: $\times 200$)

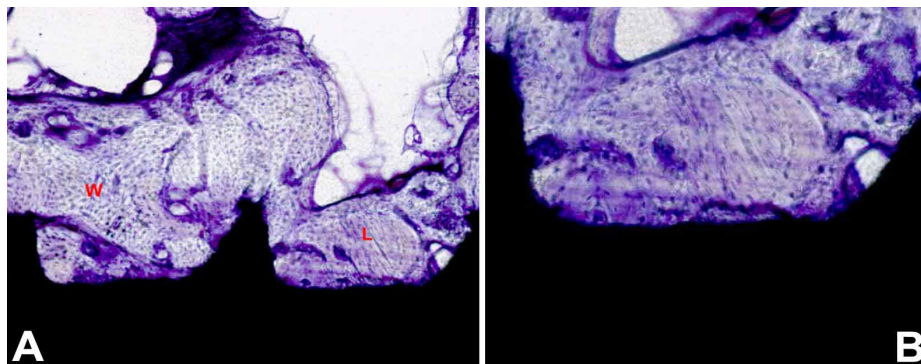


Fig. 3. Morphological observation of self-tapping implantation group at 8W(A: $\times 100$, B: $\times 200$)

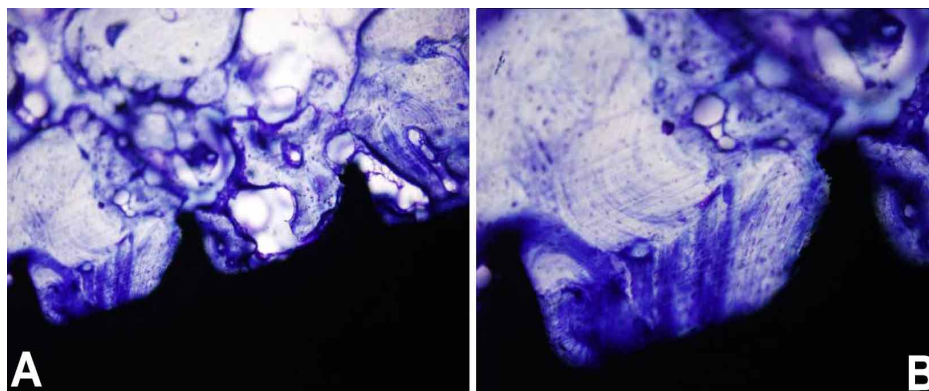


Fig. 4. Morphological observation of assisting implantation group at 2W(A: $\times 100$, B: $\times 200$)

DISCUSSION

Micro-implant anchorage has been widely used in orthodontic clinical practice, and the research on the stability of micro-implant has been a hotspot in this field because of its practical effect? convenient and flexible implantation comfortable wearing and no dependence on the characteristics of patient cooperation (Favero *et al.*, 2010; Wahabuddin *et al.*, 2015; Turkkahraman & Sarioglu, 2016). The biocompatibility and mechanical compatibility of the implant with the bone tissue are reflected in the interface between the implant and the bone tissue and the response of the surrounding bone tissue to the implant (Seifi & Matini, 2016). Branemark Osseointegration concept was proposed in the 1970 s, and a good combination of state phalanx of tissue implant material. In this study, 60-80 mm superhard tissue sections were successfully fabricated by Leical600 sawing machine. The sections were stained with toluidine blue to clearly observe the morphology of the cells around the implant and the surrounding bone histomorpholog.

The main purpose of this study is to investigate the effect of micro-implants implanted with different surgical techniques on osseointegration. Self-tapping implantation is the micro-implant directly into the cortical bone, micro-implants in the implantation process may cause extrusion of the

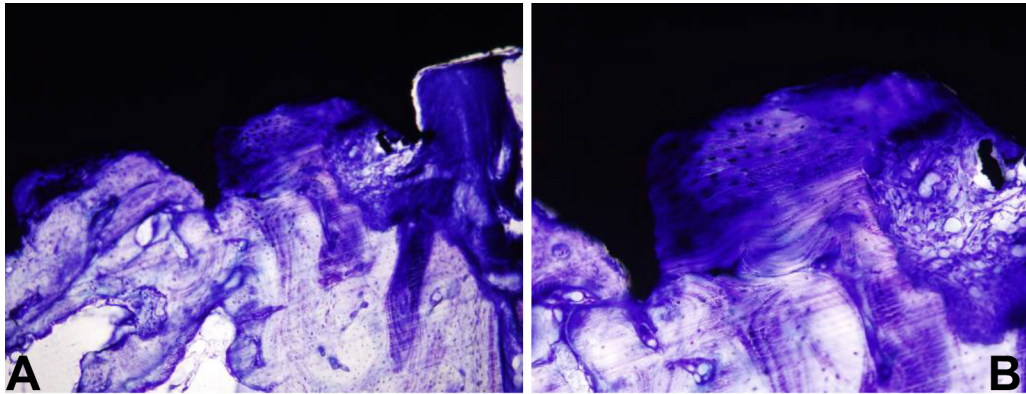


Fig. 5. Morphological observation of assisting implantation group at 4W(A: $\times 100$,B: $\times 200$)

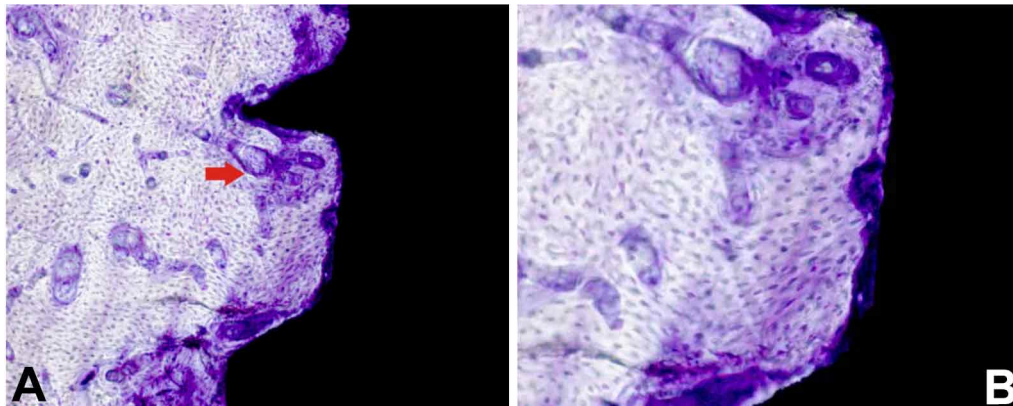


Fig. 6. Morphological observation of assisting implantation group at 8W(A: $\times 100$,B: $\times 200$)

surrounding bone tissue, the pressure may promote osteoblast response, stimulate bone formation. But at the same time, excessive pressure may also cause local bone ischemia and necrosis, while self-tapping bone debris is not easy to discharge, resistance is too large in the local bone easily lead to stress concentration, implant stability is difficult to guarantee (Gupta *et al.*, 2012; Jasoria *et al.*, 2013). Assisted implantation of the channel intact, smooth, screwed into the screw basically no resistance, fixed after the formation of axial tension, so you can make micro-implant to obtain greater retention. If the hole diameter is too large, it will easily lead to micro-implant loose after implantation. And assistive implant surgery greater trauma, too much heat can also affect the stability (Wu *et al.*, 2008; Sivakumar & Sivakumar, 2014).

The combination of micro-implant with the maxilla first heals the fiber and then gradually produces a small amount of bone healing (Basha *et al.*, 2010; Antoszewska-Smith *et al.*, 2017). Whether the micro-screw anchorage

implant and jaw healing form is the healing of fibrous tissue or bone tissue, as the supporting force can not exceed a certain range. At the initial stage of implant implantation, implants and bone tissue interface between the micro-mobility of more than 50 mm ~ 150 mm will be formed on the surface of the implant fibers wrapped. In this study, we observed that regardless of the implanting method, fiber healing and bone healing were observed in each time period. The difference between groups was only in the degree of bone healing different. Complete bone contact was not formed at 8 weeks, and more fibroids formed at 2 weeks to meet the clinical needs of orthodontics.

Whether implanted self-tapping or assisted implantation, micro-implant-bone interface reconstruction can occur. If the clinical need for early loading force, micro-implant try to choose self-tapping implantation. By appropriately prolonging the healing time, the initial stability of the micro-implant under assistive implantation can be improved.

LI, B.; HEE-MOON, K. ; FAN-YU, X. ; TAE-GEON, K. & XIU-PING, W. Estudio sobre la morfología de las células de la interfaz microscópica implante-hueso y la histomorfología ósea bajo diferentes implantaciones. *Int. J. Morphol.*, 36(1):279-283, 2018.

RESUMEN: La estabilidad del microimplante siempre ha sido el foco de la investigación clínica en ortodoncia. En este trabajo se investigaron los cambios morfológicos del tejido óseo alrededor de los microimplantes autorroscantes y se ayudó a la implantación para explorar el efecto de diferentes implantes en la osteointegración de microimplantes con el fin de proporcionar alguna base teórica para la práctica clínica. Se seleccionaron seis perros Beagle machos adultos, y se colocaron tres implantes en los huesos maxilares izquierdo y derecho en la 0ª, 4ª y 6ª semana, respectivamente. De un lado se colocó el implante autorroscante, y del otro lado el implante asistido. En la octava semana, se sacrificaron los animales y se obtuvieron las muestras de microimplante-hueso con el tiempo de cicatrización de 8, 4 y 2 semanas. Las muestras fueron teñidas con azul de toluidina (TB) y fotografiadas bajo aumento de 100X, y microscopio de 200X. La morfología de las células de la interfaz microimplante-hueso se observó bajo microscopio óptico. En el grupo autorroscante, había tejido fibroso que rodeaba los microimplantes a la 2ª semana, se observó la formación de osteoblastos y osteoide a la 4ª semana y de tejido óseo ondulado y lamelar a la 8ª semana. En el grupo asistido, se depositaron más fibras de colágeno alrededor del microimplante en la 2ª semana, hubo un gran número de células similares a osteoide, y el colágeno fue reemplazado gradualmente por el tejido óseo en la 4ª semana; los osteoblastos estaban activos y se ubicaron linealmente formando un hueso laminado en la 8ª semana. Ya sea que el implante sea con autoasistencia o con implantación asistida, puede ocurrir la reconstrucción de la interfaz microimplante-hueso. Si existe la necesidad clínica de una fuerza de carga temprana, el microimplante de elección sería la implantación autorroscante. Al prolongar apropiadamente el tiempo de curación, se puede mejorar la estabilidad inicial del microimplante bajo implantación asistida.

PALABRAS CLAVE: Perro Beagle; Anclajes de microimplantes; Morfología; Histomorfología

REFERENCES

- Antoszewska-Smith, J.; Sarul, M.; ?yczek, J.; Konopka, T. & Kawala, B. Effectiveness of orthodontic miniscrew implants in anchorage reinforcement during en-masse retraction: A systematic review and meta-analysis. *Am. J. Orthod. Dentofacial Orthop.*, 151(3):440-55, 2017.
- Basha, A. G.; Shantaraj, R. & Mogegowda, S. B. Comparative study between conventional en-masse retraction (sliding mechanics) and en-masse retraction using orthodontic micro implant. *Implant Dent.*, 19(2):128-36, 2010.
- Bozkaya, E.; Yüksel, A. S. & Bozkaya, S. Zygomatic miniplates for skeletal anchorage in orthopedic correction of Class III malocclusion: A controlled clinical trial. *Korean J. Orthod.*, 47(2):118-29, 2017.
- Buser, D.; Sennerby, L. & De Bruyn, H. Modern implant dentistry based on osseointegration: 50 years of progress, current trends and open questions. *Periodontol.* 2000, 73(1):7-21, 2017.
- Chang, H. P. & Tseng, Y. C. Miniscrew implant applications in contemporary orthodontics. *Kaohsiung J. Med. Sci.*, 30(3):111-5, 2014.
- Davoody, A. R.; Posada, L.; Utreja, A.; Janakiraman, N.; Neace, W. P.; Uribe, F. & Nanda, R. A prospective comparative study between differential moments and miniscrews in anchorage control. *Eur. J. Orthod.*, 35(5):568-76, 2013.
- Favero, L.; Giagnorio, C. & Cocilovo, F. Comparative analysis of anchorage systems for micro implant orthodontics. *Prog. Orthod.*, 11(2):105-17, 2010.

- Gupta, N.; Kotrashetti, S. M. & Naik, V. A comparative clinical study between self tapping and drill free screws as a source of rigid orthodontic anchorage. *J. Maxillofac. Oral Surg.*, 11(1):29-33, 2012.
- Jasoria, G.; Naik, V. R.; Manchanda, M.; Kalra, A. & Pai, V. Comparison between drill and drill free screws as a source of rigid orthodontic anchorage: a prospective clinical study. *Int. J. Orthod. Milwaukee*, 24(3):51-7, 2013.
- Khan, B. I.; Singaraju, G. S.; Mandava, P.; Reddy, G. V.; Nettam, V. & Bhavikati, V. N. Comparison of anchorage pattern under two types of orthodontic mini- implant loading during retraction in type A anchorage cases. *J. Clin. Diagn. Res.*, 10(10):ZC98-ZC102, 2016.
- Liu, Y.; Hu, J.; Liu, B.; Jiang, X. & Li, Y. The effect of osteoprotegerin on implant osseointegration in ovariectomized rats. *Arch. Med. Sci.*, 13(2):489-95, 2017.
- Maino, G. B.; Maino, G.; Dalessandri, D. & Paganelli, C. Orthodontic correction of malpositioned teeth before restorative treatment: efficiency improvement using Temporary Anchorage Devices (TADs). *Orthod. Fr.*, 87(4):367-73, 2016.
- Melsen, B. & Dalstra, M. Skeletal anchorage in the past, today and tomorrow. *Orthod. Fr.*, 88(1):35-44, 2017.
- Seifi, M. & Matini, N. S. Evaluation of primary stability of innovated orthodontic miniscrew system (STS): An ex-vivo study. *J. Clin. Exp. Dent.*, 8(3):e255-9, 2016.
- Shah, F. A.; Johansson, M. L.; Omar, O.; Simonsson, H.; Palmquist, A. & Thomsen, P. Laser-modified surface enhances osseointegration and biomechanical anchorage of commercially pure titanium implants for bone-anchored hearing systems. *PLoS One*, 11(6):e0157504, 2016.
- Sivakumar, I. & Sivakumar, A. Intrusion of an overerupted molar using orthodontic miniscrew implant: A preprosthodontic therapy. *Contemp. Clin. Dent.*, 5(3):422-4, 2014.
- Turkkahraman, H. & Sarioglu, M. Are temporary anchorage devices truly effective in the treatment of skeletal open bites? *Eur. J. Dent.*, 10(4):447-53, 2016.
- Wahabuddin, S.; Mascarenhas, R.; Iqbal, M. & Husain, A. Clinical application of micro-implant anchorage in initial orthodontic retraction. *J. Oral Implantol.*, 41(1):77-84, 2015.
- Wu, X.; Deng, F.; Wang, Z.; Zhao, Z. & Wang, J. Biomechanical and histomorphometric analyses of the osseointegration of microscrews with different surgical techniques in beagle dogs. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.*, 106(5):644-50, 2008.

Corresponding author:

Dr. Xiu-Ping Wu
Stomatology Hospital
Shanxi Medical University
63 Xinjian Road, Taiyuan 030001
CHINA

Email: libing-1975@163.com

Received : 20-05-2017

Accepted : 05-07-2017