

學術專題 壁報論文比賽作品欣賞

診所組 第一名

Molecular and structural assessment of alveolar bone during tooth eruption and function in the miniature pig, *Sus scrofa*

臨床聯想：立即植牙後立即承載咬合力量是否可加速齒槽骨成熟

葉光大, DDS, MDS, PhD
梵谷美學牙醫診所

Introduction

Alveolar bone is well known to develop during a tooth's eruption into function, however, the transitions in bone structure adjacent to the developing tooth root and mechanisms driving these changes are not well understood. Woven bone forms a crypt that surrounds tooth buds during development and remodels as tooth bud grows.^{1, 2} With crown formation complete intraosseous tooth eruption begins, corresponding with the resorption of the coronal portion of the alveolar crypt and apposition occurring adjacent to the developing root.^{3, 4, 5} The underlying space left by the erupting tooth has been observed to be filled with premature woven bone.⁶ On the other hand, a fully erupted and functional tooth relies on the anchorage of the periodontal ligament (PDL) within the alveolar bone proper (ABP) for immediate occlusal support. Distal to the tooth root, cancellous bone fills the interior spaces of the alveolar crest and cortical plates support the outer surface of the dental arches. Although the ABP and cortical bone differentiate themselves from cancellous bone by higher bone density, it is still not clear that they are constituted by mature lamellar or premature woven bone structures as well as the potentially associated molecular mechanisms

This study aims to define the structural transition in pig alveolar bone during tooth eruption through molecular and structural assessment of alveolar bone of miniature pigs at the mucosal penetration stage of tooth eruption and the onset of functional occlusion. In particular, this study tested the hypotheses that predominantly woven bone forms adjacent to the tooth root during the mucosal penetration stage of tooth eruption, but that this immature structure transitions to lamellar bone during early tooth function. In addition to the increased lamellar bone formation corresponding with the onset of tooth function, osteoclasts are predicted to play a key role in resorbing the woven bone accrued during tooth eruption.

Materials and Methods

Hanford miniature pigs were acquired at 13 and 23 weeks of age, corresponding with the mucosal penetration stage of M1 tooth eruption and early occlusion of M1/M1 (Fig. 1). Tissues for histological processing were collected postmortem, decalcified in 8% EDTA or solution. Decalcified tissues were paraffin embedded and sectioned sagittally at 7 μ m. Sections including the entire length of the tooth root were stained with Hemotoxylin and Eosin (H&E) and TRAP, examined using light microscopy. In TRAP

stained tissues, the bone posterior to the tooth root was divided into cervical (adjacent to the CEJ), middle and apical thirds. A 1 x 1 mm region was identified within the alveolar bone proper (adjacent to the periodontal ligament) and further distal within the cancellous bone (midway between the ABP and the distal M2 crypt). Osteoclasts were identified as darkly staining multi-nucleated cells that were sometimes adjacent to Howship lacunae and counted.

Alveolar bone samples from the mandibular diastema and distal M1 regions were collected from 3 pigs of each age group (Fig. 2). The diastema region which lacks teeth was used to represent gene expression patterns in non-tooth supporting regions. Gene expression associated with the regulation of osteoclast formation, such as the receptor activator of NF- κ B ligand (Rankl), osteoprotegerin (Opg), and colony stimulating factor (Csf-1) was measured in order to assess the role of osteoclast activity in bone adjacent to tooth roots during eruption. Additionally, genes associated with bone formation, such as Runx2 and Bsp were compared between eruptive stages to determine whether the osteoblast activity differed between eruptive stages. Cellular RNA was isolated from tissues using Trizol Reagent For PCR analysis. cDNA was synthesized using 1.0 μ g total RNA with a cDNA synthesis kit (Roche). Quantitative Real-Time PCR (qRT-PCR) reactions were carried out with the LC 480 DNA Master SYBR Green I kit (Roche). Primer sequences were designed from pig partial coding sequences (Table 1) to amplify specific cDNA. Glyceraldehyde-3-phosphate dehydrogenase (Gapdh) served as a reference gene for normalization. Cultured porcine bone marrow cells were induced to differentiate into osteoblast cell lineages to provide stable ratio of target to reference genes and to normalize all samples within each run for qRT-PCR calibration. Template DNA was replaced with PCR-grade water as a negative control. The amplification profile used on the Lightcycler was: 95oC /0s; 58oC /7s; 72oC /20s and 50-55 cycles.

Each sample was amplified in triplicate for each gene. The mean value for triplicate experiments was used to compare samples from different age groups and locations using non-parametric statistical analyses (Mann-Whitney U tests). A p value of less than 0.05 was considered significant.

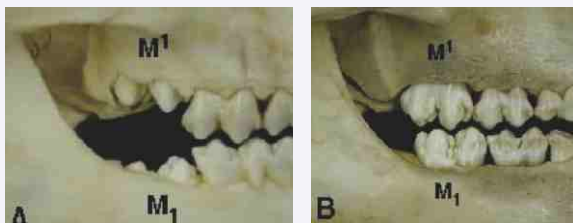


Figure 1. First mandibular molar, M1 at the mucosal penetration stage of tooth eruption (A) and after it has fully erupted into occlusion (B).

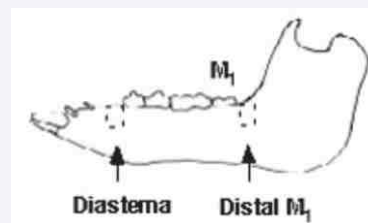


Figure 2. Bone samples were collected from the diastema and distal to the erupting and occluding M1.

	GenBank accession number	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>Rankl</i>	AY60682	TGTGAGACTACTAAGCGG	GCAGGTTCCAGCATGA
<i>Opg</i>	EF543195	GGGGCTCCTTCTAACT	CTCCGCAAGAAGTCCA
<i>Csf-1</i>	AJ583506	TCGTGCCAAATTGCCT	CGTCTCATAGAAAGTTCGGA
<i>Bsp</i>	L10363	ACGAAGGCATGAATTGT	ACGGGTAGGTATCGTGA
<i>Runx2</i>	EU668154	CAAAGCCAGAGCGGAC	AATTTGGATTTAATAGCGTGC
<i>Gapdh</i>	AF017079	GATCGTCAGCAATGCC	CCGGTAGAAGCAGGGA

Table 1. Primer sequences for *Sus scrofa* specific genes.

Bone Structure



Results

Figure 3.

Reticular fibrolamellar bone from the midroot region of a 13 week erupting M1 (A) and 23 week, occluding M1 (B). The younger bone appears more porous with thinner woven bone trabeculae, whereas the older bone appears denser with filling in of primary osteons (arrows).

- ◆ In both groups, the bone distal to the M1 showed mainly of reticular fibrolamellar structure.
- ◆ However the woven bone matrix appeared thicker in the older group than in the younger group.
- ◆ No secondary osteon (bone remodeling) was found in both groups.

Osteoclast Cell Numbers

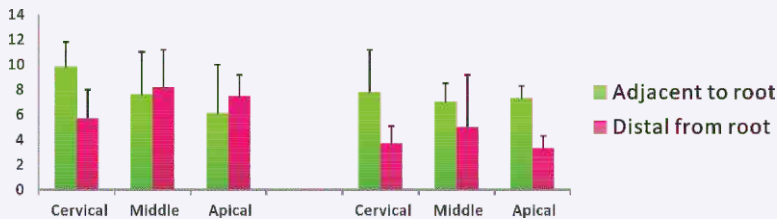


Figure 4.

Osteoclast cell counts from bone distal to the 13 week erupting M1 and 23 week, occluding M1.

- ◆ TRAP-stained osteoclasts were observed in all alveolar bone proper and cancellous bone regions.
- ◆ There was no marked differences between any region or age group.

Real Time PCR

Table 2. Target gene expression relative to Gapdh from bone samples.

Pig number	age	location	Rankl	Opg	Csf1	BSP	Runx2
4425	13 wk	diastema	0.13±0.00	24.68±40.5	0.63±0.04	27,808±156	4.19±0.13
5060	13 wk	diastema	0.25±0.02	188.32±308	0.70±0.03	20,378±3640	6.32±0.31
5061	13 wk	diastema	0.22±0.03	7.17±8.7	0.49±0.07	20,538±1612	3.61±0.58
Mean	13 wk	diastema	0.20±0.06		0.61±0.11	22,908±4244	4.71±1.42
4501	23 wk	diastema	0.07±0.01	18.99±20	0.23±0.10	11,411±1384	2.14±0.07
5069	23 wk	diastema	0.77±0.04	164.07±245.7	1.11±0.12	211,673±16,868	10.05±0.37
5071	23 wk	diastema	0.34±0.02	178.49±222	0.66±0.02	66,639±851	7.43±0.33
Mean	23 wk	diastema	0.39±0.36		0.67±0.44	96,574±103,432	6.54±4.03
5060	13 wk	M ₁ distal	0.61±0.03	6800±11,611	1.29±0.16	44,838±14,325	12.61±2.04
5061	13 wk	M ₁ distal	0.43±0.03	14,841±20,981	0.54±0.06	44,796±8683	6.18±0.84
Mean	13 wk	M ₁ distal	0.52±0.13		0.92±0.53	44,817±29	9.39±4.55
4501	23 wk	M ₁ distal	0.22±0.03	177.25±167.4	0.38±0.04	27,925±3724	2.15±0.17
5069	23 wk	M ₁ distal	0.19±0.03	4.49±5.7	0.66±0.14	3801±334	4.20±1.48
5071	23 wk	M ₁ distal	1.10±0.11	66.20±61.9	1.50±0.25	52,224±4721	12.38±1.33
Mean	23 wk	M ₁ distal	0.50±0.52		0.84±0.58	27,983±24,211	6.24±5.41

- ◆ Gene expression values were similar between locations and age groups.



Conclusions

1. The alveolar bone showed no mature lamellar structure but dense fibrolamellar structure that included primary osteons in both groups.
2. Younger pigs had less dense and more finely reticulated alveolar bone structure than the older pigs.
3. Osteoclast cell counts indicate a lesser role for resorption in the osseous changes surrounding M1 tooth roots.
4. The consistent levels of expression of Rankl and Csf-1 in alveolar bone associated with erupting and functioning molars further indicates the presence of constitutive levels of resorption.
5. Consistency in expression of genes associated with bone modeling, Runx2 and Bsp, suggest that incremental bone apposition may generate the structural differences in alveolar bone between 13 and 23 week pigs.
6. Occlusal loading, in contrast to expectation, did not lead to early remodeling of supporting alveolar bone structure which can fortify the strength of bone.
7. These data support the importance of primary stability in the success of immediate implant, especially when immediate occlusal loading is applied.

References

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學術專題 壁報論文比賽作品欣賞

診所組 第二名



植牙合併引導骨再生手術於上顎後牙懸臂式局部固定義齒：病例報告

Implantation with Simultaneous Guided Bone Regeneration for Cantilever Fixed Partial Denture over Maxillary Posterior Edentulous Area: A Case Report

葉立維 曾莉婷 劉謙美 王敏瑩
當代牙醫診所 國立台灣大學醫學院附設醫院牙科部

背景

植體周圍骨寬度至少要有1-2毫米,才不致義齒負載後有垂直骨喪失或是造成植牙維護困難。於是植牙合併引導骨再生手術應用於齒槽骨寬度高度不足、植體表面露出於理想植體位置或是植牙於拔牙窩等臨床狀況。但在骨條件嚴重不足、牙根走向、鼻竇過近等原因下,人工植牙手術困難度提升,且患者術後併發症比率,治療時間及費用提高。故在義齒採用懸臂式設計成為治療選項。

病例

54歲女性患者,主訴為14搖晃無法進食。診斷為全口慢性牙周炎及局部缺牙。經牙周基本治療及拔牙、補綴科醫師會診、CBCT檢查。實行15、16植牙合併引導骨再生手術。待半年後實行植牙二階段手術合併游離牙齦移植手術。臨時義齒於2個月後負載,正式義齒已追蹤18個月,功能正常。

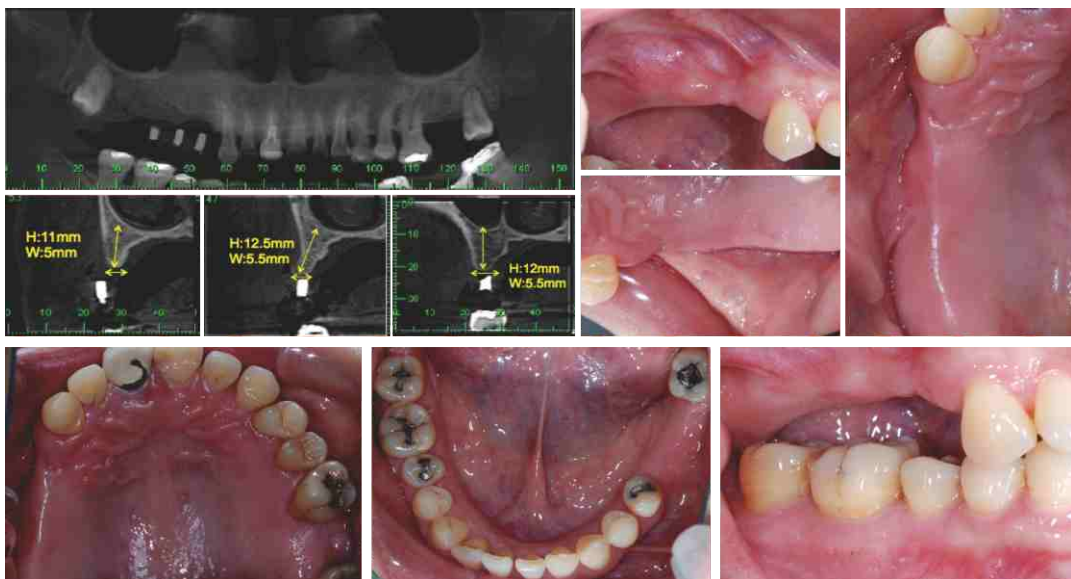
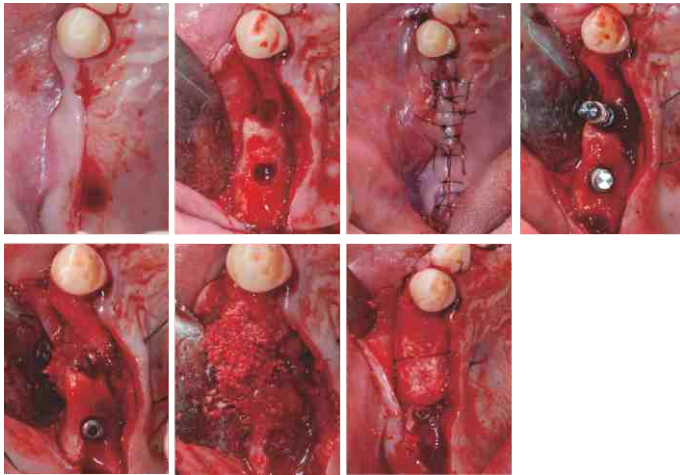


Figure 1. Pre-operative clinical photos and radiographs



Tooth	#16	#15
Implant	StraumannBL F 4.8mm 10mm	StraumannBL F 4.1mm 10mm
Crestal Bone Width	7 mm	5 mm
Residual Buccal Bone Plate	1.5mm	0 mm (2mm dehiscence)
Residual Lingual Bone Plate	2 mm	1 mm
Graft	Layered Graft Placement 1st layer: autogenous bone graft 2nd layer: Cerasorb(500-1000µm)	
Membrane	Collagen membrane (BioMendExtend 20X30mm)	

Figure 2. Clinical photos of implant surgery

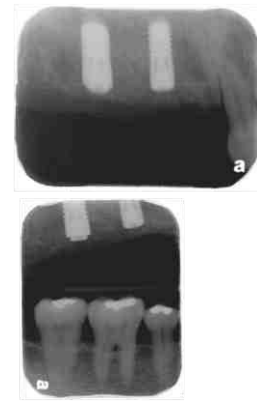


Figure 3. Radiographic examination after implant surgery

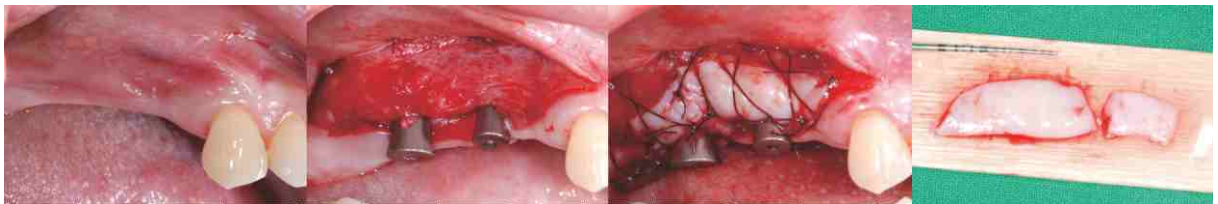


Figure 4. Clinical photos of 2nd stage surgery combined with free gingival graft



Figure 5. Prosthetic loading

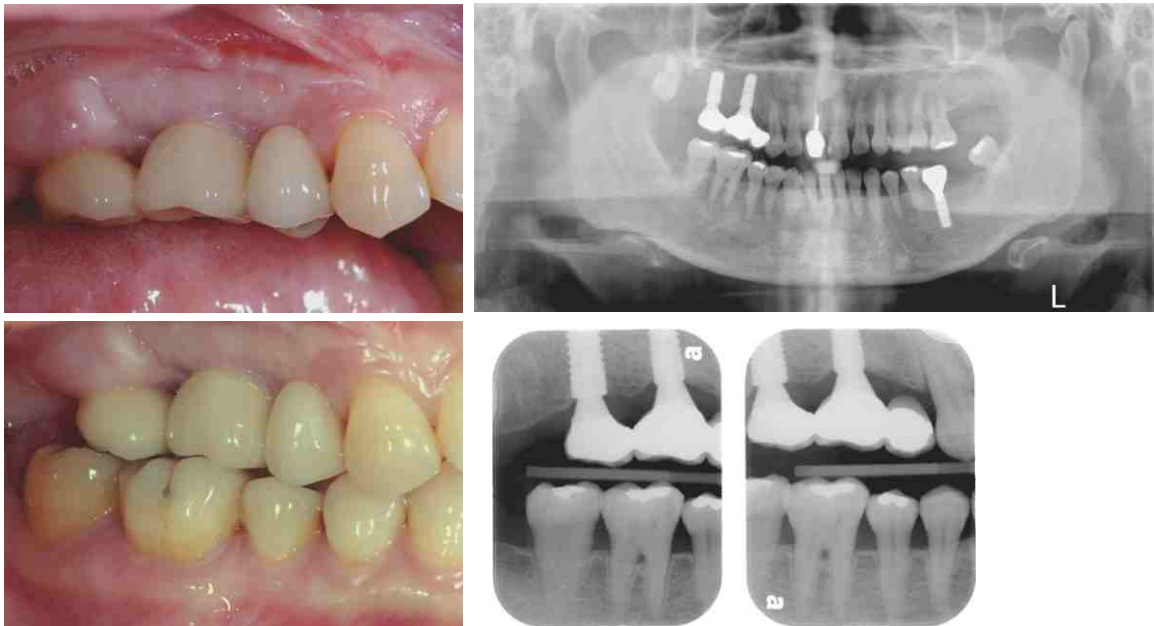


Figure 6. Prosthetic loading for 18 months

討論與結論

人工植牙條件為齒槽骨脊的骨質及骨量影響。本案例依硬組織狀況有兩個治療方案，其一是以階段式重建14硬組織再進行植牙手術。其二是在15及16置放植體，義齒14橋體部分採近心懸臂式設計。因患者具有高血壓病史且因血壓過高放棄兩次手術約診。故選擇後者方案，控制高血壓且減少手術次數。本案例目前功能正常，植體周圍骨喪失量尚在合理範圍。相關文獻亦顯示，人工植牙支撐義齒採用懸臂式設計對於植體存活率以及植體周圍骨喪失量與非懸臂式設計並無差異。



學術專題 壁報論文比賽作品欣賞

診所組 第三名



Medical Device Development of Functional Bone Graft in Bone Regeneration

吳啓明 (Chi-Ming, Wu)

Background:

Previously studies showed that platelets would be release growth factors to promote damaged tissue repair in injury site. The aim of this study was to develop an functional bone grafts for improving bone injury by combining with autologous Platelet Rich Plasma (PRP) and calcium sulfate bone grafts. Additionally, we also found that the degradation rate and growth rate are consistent with the periphery bone tissue in bone defects.

Materials and Methods:

We have already made up functional bone grafts for bone healing, which included autologous derived factors. The below design demonstrated that the functional bone grafts have higher potential to efficiently promote bone healing for chick limb organ culture system.



1 Incubation
18 days of egg



2 Harvest chicken
embryo



3 Sacrifice



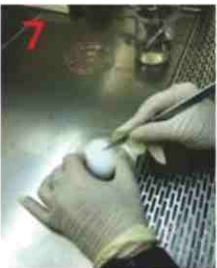
4 Take the legs



5 Harvest chickens
femur



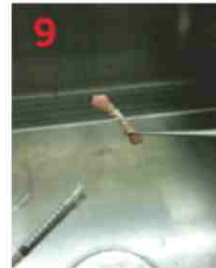
6 Record the femur
length



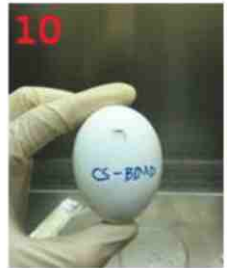
7 Cut the shell of
Development
7 days egg



8 Strip the Inner
Membrane



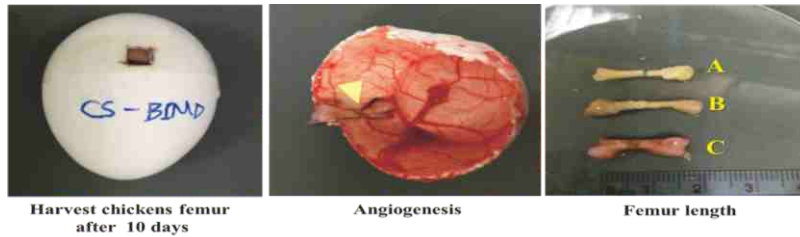
9 Implant the femur
into the egg



10 Recover
the wound

Results:

This study could be utilized as a functional bone grafts to prevent bone injury and, due to promoted bone healing. Therefore, we predict the functional bone grafts is a biomedical device that has good potential to induce bone regeneration and supplement treatment in clinical application.



Group		Bone Length
A	Control	18 mm → 18 mm (+ 0mm)
B	CS-Con	23 mm → 24 mm (+ 1mm)
C	Functional bone grafts	19 mm → 21 mm (+ 2mm)



Conclusion:

All results have showed that Functional bone grafts are possibly considered as an effective clinical treatment through autologous PRP and promoting bone healing healing.

References:

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學術專題 壁報論文比賽作品欣賞

診所組 佳作

Total Dentition Distalization in Patient with Bimaxillary Dentoalveolar Protrusion

Ya-Ying Teng (鄧雅音)

Department of Craniofacial Orthodontics
Chang Gung Memorial Hospital, Linkou, Taiwan

Introduction

Extraction of teeth and anterior retraction either by orthodontic approach or by surgery can effectively retract anterior teeth in a proper position and reduce the lip procumbency for patients with bimaxillary protrusion. The case report illustrated the non-extraction orthodontic treatment in patients with bimaxillary protrusion with the aid of temporary anchorage devices.

Case

16 years 2 months old Chinese female presented with her chief complaint of protrusive teeth and lips. The frontal photo showed slight facial asymmetry with transverse discrepancy, mentalis muscle strain and no gingival display upon smiling. The profile photo revealed convex profile, shallow labiomental fold, protrusive upper and lower lip relative to the E-line (Fig 1). She had a 1.5mm overbite, 2.0mm overjet, bilateral Class III molar relation, #12 lingual cross bite and 0.5mm shift of lower dental midline to her right. Clinical examination revealed four first premolars were extracted before. X-ray presented proclined incisors and had four third molars (Fig 2). According to cephalometric analysis (Table 1), she was diagnosed Skeletal Class I and Angle Class III malocclusion with bimaxillary dentoalveolar protrusion, #14 #24 #34 #44 missing.

Fig 1. Initial extraoral photo



Fig 2. Initial intraoral photo





Treatment Strategy and Result

Due to no remarkable skeletal discrepancy & lack motivation of orthognathic surgery, the non-extraction orthodontic tx plan was offered: Extract #18 #28 #38 #48 and reduce protrusion with en masse distal movement by aid of temporary anchorage device (TAD) placed at infrazygomatic area and buccal shelf of mandible on both sides (Fig 3).

After one and half year of orthodontic treatment, the upper and lower dentitions were distalized by 3mm. Class I molar relationship was established with proper OB and OJ. The facial profile and lip posture were improved with remarkable retraction of upper and lower lips. (Fig 4, Table 1).

Fig 3. (A) Retraction direction: from TAD to the hook distal to lateral incisor; (B) Profile change

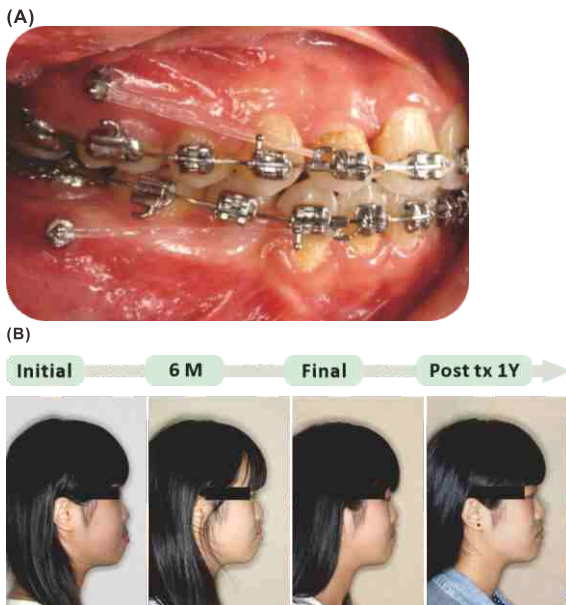
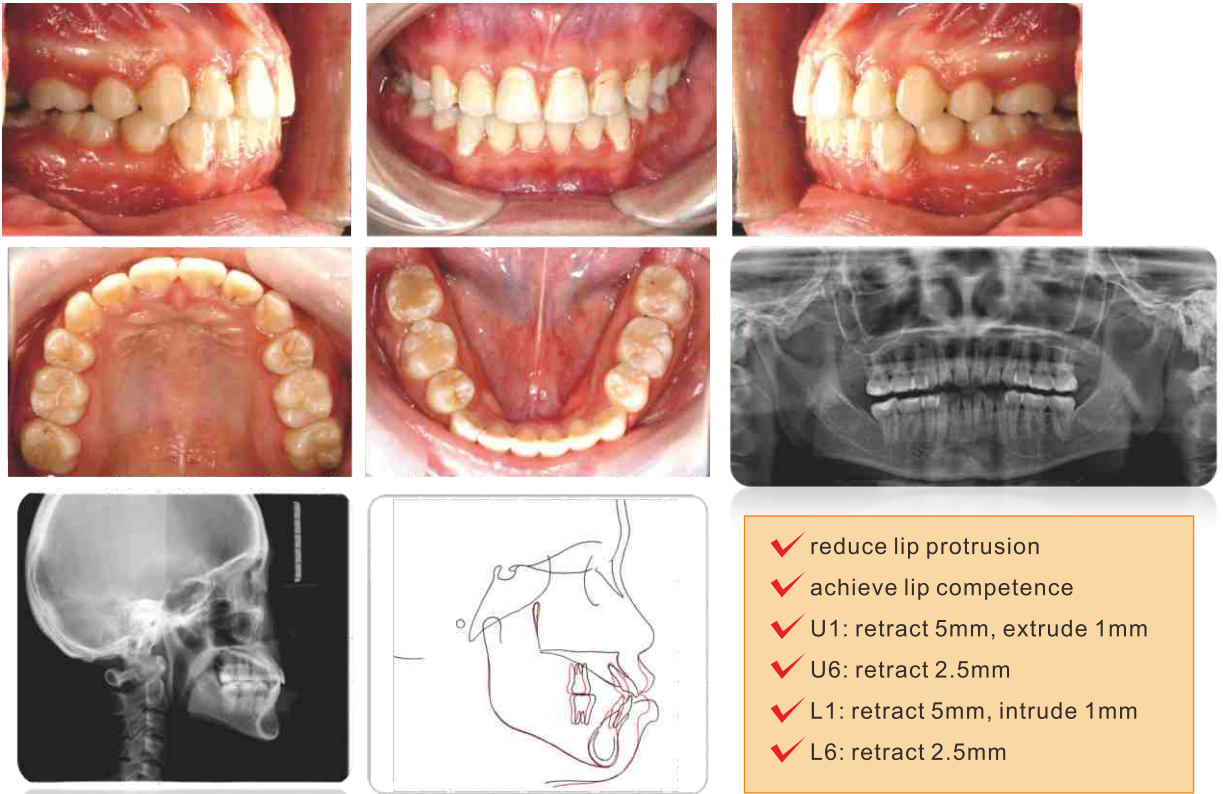


Table 1. Cephalometric analysis before and after orthodontic treatment.

Measurement	Skeletal		Dental		
	Initial	Final	Measurement	Initial	Final
SNA (°)	83.0	82.5	UI-SN (°)	124.0	108.0
SNB (°)	82.0	81.0	UI-NA (mm)	10.0	5.0
ANB (°)	1.0	1.5	UI-NA (°)	38.0	23.5
Wits (mm)	-1.5	-2.0	L1-NB (mm)	7.0	3.0
SN-FH (°)	5.0	5.0	L1-NB (°)	32.0	23.0
SN-MP (°)	26.0	26.0	UI-L1 (°)	109.0	131.0
A-Nv (mm)	-0.5	-0.5	L1-MP (°)	96.0	90.0
Pog-Nv (mm)	-2.0	-2.5	Soft Tissue		
UFH /LFH (%)	46 /54	45.6 /54.4	NLA (°)	88.0	103.0
			UL-E line	3.0	0.5
			LL-E line	9.0	3.0

Fig 4. Final photo





- ✓ reduce lip protrusion
- ✓ achieve lip competence
- ✓ U1: retract 5mm, extrude 1mm
- ✓ U6: retract 2.5mm
- ✓ L1: retract 5mm, intrude 1mm
- ✓ L6: retract 2.5mm

Conclusion and Discussion

- Extraction modality either in orthodontic or orthognathic approach is always the primary decision and is effective in treating patient with bimaxillary dentoalveolar protrusion.
- Due to missing four first premolars in this case, we reduced protrusion with distalization of upper and lower dentitions by miniscrews.
- Miniscrew-assisted distalization of dentition could be an effective and relatively easy modality in treating bimaxillary dentoalveolar protrusion with less skeletal discrepancy, mild crowding, mild dentoalveolar protrusion.
- Biological boundary should not be overwhelmed when distal movement of the whole dentition.
- The long-term stability about distalization with implant anchorage has not yet been reported, more follow-up is necessary to determine the long-term stability.