Simplified stress analysis on the temporomandibular joint in Class III patients with and without mandibular asymmetry using a rigid body spring model

著者	Ueki Koichiro, Takeuchi N., Nakagawa Kiyomasa,
	Yamamoto Etsuhide
journal or	Orthodontics and Craniofacial Research
publication title	
volume	12
number	4
page range	312-318
year	2009-11-01
URL	http://hdl.handle.net/2297/19831

doi: 10.1111/j.1601-6343.2009.01467.x

Simplified stress analysis on the temporomandibular joint in Class III patients with and without mandibular asymmetry using a rigid bodies spring model

K Ueki¹
N Takeuchi²
K Nakagawa¹
E Yamamoto¹

¹Department of Oral and Maxillofacial Surgery, Graduate School of Medicine, Kanazawa University

13-1 Takaramachi, Kanazawa 920-8641, Japan

²Department of Civil Engineering, Housei University

3-7-1, Kajinocho, Koganei, Tokyo, 184-8584, Japan

Running title: Stress analysis on temporomandibular joint using RBSM

Address correspondence to: Koichiro Ueki, DDS, PhD

Department of Oral and Maxillofacial Surgery

Graduate School of Medicine, Kanazawa University

13-1 Takaramachi, Kanazawa 920-8641, Japan

Tel: +81-76-265-2444; Fax: +81-76-234-4268

E-mail: kueki@med.kanazawa-u.ac.jp

Date: 1st May 2009

Structured abstract

Authors- K. Ueki, N. Takeuchi, K. Nakagawa, E. Yamamoto

Objective- Aim of this study is to investigate the differences in stress on the temporomandibular joint (TMJ) between Class III mandibular asymmetry and symmetry patients using a rigid bodies spring model (RBSM).

Design- Menton (Me), the center point of occlusal force on the line that connected the bilateral buccal cusps of the second molars and the most lateral, superior, and medial points of the condyle were plotted on frontal cephalograms and stress on the condyles was calculated with the 2-dimensional RBSM program of FORTRAN.

Setting and Sample Population- Eighty Japanese patients with diagnosed mandibular prognathism were divided into 2 groups, a symmetry group and asymmetry group on the basis of the Mx-Md midline position.

Outcome measure- The degree (force partition) of the resultant force, the direction (angulation) and displacement (X, Y) of each condyle were calculated. The horizontal displacement vector (u), the vertical displacement vector (v) and rotation angle (θ) of the mandibular body at Menton were also calculated.

Results- There were significant differences between the deviated and non-deviated sides of both groups regarding resultant force (symmetry group: P=0.0372, asymmetry group: P=0.0054), X (symmetry group: P<0.0001, asymmetry group: P=0.0001) and Y-component (symmetry group: P=0.0354, asymmetry group: P=0.0043). For angulation, there was a significant difference between the deviated and non-deviated sides in the asymmetry group (P=0.0095)

Conclusion- The results of this study suggest that difference in stress angulation on the condyles could be associated with asymmetry in mandibular prognathism.

Key words: Class III; Stress; Temporomandibular joint; Asymmetry; Rigid body spring model

Clinical relevance

For patients with a mandibular asymmetry a dynamical simulation including a stress analysis of both condyles might be helpful in diagnosis and treatment planning. The development of a rigid body spring model analysis (RBSM) for frontal cephalograms made it possible to simulate the stress on the TMJ. The RBSM suggested that a difference in stress direction on the condyles might be associated with mandibular prognathism with asymmetry.

Introduction

An association between temporomandibular joint (TMJ) dysfunction and mandibular asymmetry has been suggested. Nickerson and Moystad (1), Talents et al (2), and Katzberg et al (3) have all shown that degenerative joint disease might be associated with unilateral mandibular asymmetry. A study of 100 patients with mandibular asymmetry by Schellhas et al suggested that disc displacement, internal derangement, or degenerative joint disease could be major causes of mild and moderate mandibular asymmetry (4). Various studies have investigated occlusal disharmony as a predisposing factor of TMJ internal derangement. Occlusal instability, midline discrepancy, right-left differences in molar relationship, and inclination of the frontal occlusal plane has been considered to be important occlusal characteristics in patients with TMJ disorders (5, 6). Differences in heights of the right and left rami, have also been suggested as important skeletal problems associated with TMJ pathology (7, 8). A similar tendency has been recognized in mandibular prognathism with asymmetry (9), although the incidence of TMJ dysfunction in mandibular prognathism is lower than mandibular retrognathism (10).

Most studies agree that the external and internal morphologies of a given bone or joint in an adult are determined by the biomechanical loads placed upon them during growth. Stresses on the TMJ are considered to be important for maintaining normal structure and function of the TMJ (11-13). Stress analysis could elucidate the relation between mandibular asymmetry and the difference in bilateral TMJ structure.

Several theoretical approaches have been used in an attempt to understand various aspects of TMJ biomechanics (14-20). Finite element models (FEM) of the TMJ have been developed to simulate condylar motion or stress change. However, a reliable FEM model requires input of the material properties, which is currently not available. Therefore a stress distribution analysis method using a rigid body spring model (RBSM) is to be preferred. We have investigated stress on the TMJ using lateral cephalograms and sagittal tomography with RBSM (21-23). However, frontal cephalograms are used frequently in asymmetry patients and therefore a RBSM program using frontal cephalograms is to be preferred. The

purpose of this study was to investigate the differences in stress on the TMJ between Class III mandibular asymmetry patients and symmetry patients using RBSM for frontal cephalogram.

Patients and Methods

Patients

The 80 Japanese adults (22 males and 59 females) in this study presented with jaw deformities diagnosed as mandibular prognathism with or without asymmetry, and mandibular prognathism with bimaxillary asymmetry. The age ranged from 15 to 39 years, with a mean age of 23.7 years (standard deviation 5.5 years).

Frontal cephalometric analysis

All patients had lateral and frontal cephalograms. The cephalograms were analyzed using appropriate computer software (Cephalometric A to Z, Yasunaga Labo Com, Fukui, Japan). All patients were diagnosed as skeletal Class III from the cephalometric measurements. On the frontal cephalogram, the angle between the ANS-Menton line and the line perpendicular to the bilateral zygomatic frontal suture line was defined as the Mx-Md midline angle. A positive value of this Mx-Md midline angle represents mandibular deviation to the left and a negative value represents mandibular deviation to the right. The Mx-Md midline angles of all cases were then given a positive value so that all consecutive measurements could be attributed to either the deviated or the non-deviated side (9). The subjects were divided into a symmetry and an asymmetry group according to the Mx-Md midline. Asymmetry was diagnosed when the Mx-Md midline angle was >3 degrees. Occlusal cant was defined as the angle between bilateral zygomatic frontal suture line and the line between the most lateral mid-points of the bilateral upper first molar crown.

Determination of occlusal force center

A pressure-sensitive system was used in this study (Fig.1). This system consists of a

pressure-sensitive sheet (Dental Prescale; Fuji Photo Film Co., Tokyo, Japan) and its analyzing apparatus (Dental Occlusion Pressuregraph FPD-705; Fuji Photo Film Co.) that was connected with a personal computer. Data on the reproducibility and the method of calibration has been reported earlier (24-27). Each patient was seated with the head in natural head position, looking forward. The pressure-sensitive sheet was placed between the maxillary and mandibular teeth and the patient was instructed to bite as forcefully as possible for about 3 seconds. The sheet was read and analyzed by the Dental Occlusion Pressuregraph and the results were inputted into the computer and visualized on the display screen. The occlusal force center was determined on the basis of the occlusal balance in this system. Since the assumption that the direction of the maximum occlusal force was perpendicular to the occlusal plane on the frontal cephalogram was required for the RBSM, realistic vertical direction of the resultant occlusal force from the pressure sensitive system was not necessary.

Input data for calculations

The most lateral point of the buccal cusp of the lower second molar (Mn7), Menton, and the most medial, superior, and lateral points on both condyles were plotted on the frontal cephalogram. Only the value for the X-coordinate of the occlusal force center was inputted on the occlusal plane (the line between right Mn7 and left Mn7). The mandibular two-dimensional RBSM using the frontal cephalometric data was analyzed with the FORTRAN program according to the method previously reported (21-23). The calculation was performed according to our previous report as follows.

RBSM using frontal cephalogram

The RBSM model was based on a frontal cephalogram of each subject. The entire mandible can be considered as a single rigid element. A rigid displacement field is assumed in the mandible for the displacement (Fig.2).

In case of the numerical model for the temporomandibular joint, the integral points for calculating the contact stress are defined along the contours of the uppermost face of the

condyle (Fig.3). As this portion has a relatively smooth surface, only a vertical spring is fitted on each integral point, assuming that the surface bears the vertical surface force (or in other words, contact pressure only, and does not bear the shearing force. Fig. 4 shows the assumed spring along the contours of the uppermost face of the condyle. The glenoid fossa is assumed to be a rigid element, and the displacement of this rigid element is set to 0 so it may be treated as a supported element.

The occlusal force and its action position were determined using the pressure-sensitive system shown in Fig. 1. Only compressive force is transmitted in a contact surface. Redistribution of negative contact pressure was calculated, according to the following procedure: First, the contact pressure generated on integral points relative to the initially given muscular force was obtained; Second, if negative contact pressure was found on some integral points, it was temporarily removed and a constraint force was added to keep the balance; And third, as this constraint force does not actually exist, a force equal to this force was added in reverse direction.

The second and third steps were repeated until the negative contact pressure reduced to a neglectable value. Then, the contact pressure distribution without negative contact force could be obtained as shown in Fig.5.

Finally, as output data, the degree (force partition) of the resultant force, the direction (angulation) and the displacement (X,Y) of each condyle (Fig.6), the horizontal displacement vector (u), the vertical displacement vector (v) and rotation angle (θ) of the mandibular body at Me were calculated. The analysis was based on the definition that a stable condylar position is one in which stress is distributed equally over the condylar surface. When the final calculation is completed and contact pressure distributed equally over the condylar surface, any slight mandibular displacement may be disregarded. The displacement from vectors in the initial mandibular position to vectors in the final mandibular position after the calculations can be presented by conversion calculations from the displacement vector. This means that the higher the displacement vector, the less clinically stable the mandible and TMJ.

Statistical analysis

Data were compared between groups by non-paired t-test and between deviation and non-deviation side by paired t-test using the Stat ViewTM version 4.5 software program (Abacus Concepts, Inc., Berkeley, CA, USA). Differences were considered significant at p<0.05.

Results

There was a significant difference between the symmetry and asymmetry group regarding the Mx-Md midline (P<0.0001), however, there was no significant difference regarding the occlusal cant.

In vertical displacement, there were significant differences between the groups (P=0.0035). In the X-component on the deviation and non-deviation sides, the asymmetry group showed a higher value than the symmetry group (deviation side: P=0.0014, non-deviation side: P=0.0024). However, there were no significant differences between the groups regarding degree and angulation of resultant force and Y component on both sides. There were significant differences between the deviation and non-deviation side regarding resultant force (symmetry group: P=0.0372, asymmetry group: P=0.0054), X (symmetry group: P<0.0001, asymmetry group: P=0.0001) and Y-component (symmetry group: P=0.0354, asymmetry group: P=0.0043) in both groups. For angulation, there was no significant difference between both sides in the symmetry group, however, there was a significant difference between the deviation and non-deviation sides in the asymmetry group (P=0.0095) (Table 1-3).

Discussion

Some finite element models (FEM) of the TMJ have been developed to simulate condyle motion or stress change (12-20). FEM is suitable for calculating stress within elements, while the RBSM is used for calculating the surface force between elements. In

the RBSM, it is assumed that the treated material consists of rigid bodies. Therefore, there its limitation is that it is difficult to calculate stress within complicated elements, such as material with extreme elasticity. However, the RBSM has been used to analyze stress on the knee, hip, and wrist in the field of orthopaedic surgery (28, 29). This model was also employed in this study because many individual images had to be analyzed to provide a more comprehensive biomechanical description of the loading and the results had to be suitable for statistical analysis. Finally, the amount of data collected was rather large and a simple analysis was required.

From the results on FEM, Buranastidporn and colleagues concluded that the symptomatic sides were significantly related to the degree of inclination of the frontal occlusal plane and increasing its angulation resulted in a decrease in symptoms on the ipsilateral side and an increase on the contralateral side. In these asymmetrical-mandibular models, both TMJs were fixed at the same position and remained symmetrical in shape. Ten asymmetric models were created with the frontal occlusal and frontal mandibular planes inclined by 1-10 degrees in 1 degree increments ascending to the left side (30). However, in fact, it in other studies a significant difference in TMJ morphology between the deviated and non-deviated sides in asymmetry cases was found (21, 31). Thus, TMJ morphological adaptation may occur in asymmetry patients. Even if many material properties on the basis of previous data were considered in the calculation process using FEM, the lack of data on the realistic TMJ outline and occlusal force in individual patients could decrease the validity of the results.

On the other hand, our previous study using RBSM on sagittal tomography demonstrated that TMJ stress is associated with TMJ morphology in Class III patients regardless of their status of asymmetry. In the asymmetry group, stress angulation was significantly higher on the deviated than on the non-deviated side. There was also a significant correlation between disc position and stress angulation. In the asymmetry group, regression analysis indicated a significant correlation between the difference in stress angulation (between deviated and non-deviated side) and the degree of asymmetry (measured by the angle of asymmetry). These results proved that TMJ morphological

adaptation is strongly associated with occlusion and skeletal morphology (25).

When the frontal occlusal plane increased in FEM using frontal cephalograms, on the ipsilateral side the distribution showed a marked shift in direction, and the medial portions were loaded the least, with the stress to the lateral part increasing gradually. For the contralateral disc, the medial borders were additionally loaded. The mean stress values on the ipsilateral (shifted) disc were smaller than those in the standard model and those on the contralateral side (30). In contrast, in this study using RBSM, the resultant force on the deviated side was larger than that on the non-deviated side in both groups. However, since group division was determined by Mx-Md Midline, there was no significant difference in occlusal cant (frontal occlusal plane) between the symmetry and asymmetry groups. Furthermore the subjects in this study had mandibular prognathia with and without asymmetry. Apart from the difference between RBSM and FEM, these factors might also have affected the results making them different from those of the previous report. There was a significant difference in stress angulation between the deviated and non-deviated side in the asymmetry group. Although there was no significant difference in stress angulation between both sides in the symmetry group, that on the deviated side was significantly larger than that on the non-deviated side. Furthermore, although stress angulation on the bilateral condyles tended to incline to the opposite side in the symmetry group, the angulations tended to incline to the same (deviated) side in the asymmetry group. This tendency of stress angulation might promote mandibular asymmetry. Vertical displacement in the asymmetry group was larger than that in the symmetry group. This result suggested that a symmetrical mandible was more stable as an element in the vertical dimension of the asymmetry group. On the other hand, the X-component of the symmetry group was significantly smaller than that of the asymmetry group. This could imply that both condyles in the symmetry group were more dynamically stable than those in the asymmetry group in the horizontal dimension.

In conclusion, this study using RBSM on frontal cephalograms suggests that the difference in stress angulation on bilateral condyles could be associated with mandibular prognathism with asymmetry. Furthermore, the values of the direction (angulation) and the

degree (force partition) of the resultant force on each condyle, and the displacement (X, Y) of each condyle can be useful to determine the most suitable condylar position.

References

- 1. Nickerson JW Jr, Moystad A. Observations on individuals with radiographic bilateral condylar remodeling: clinical study. J Craniomandibular Pract 1983; 1: 20-37.
- 2. Tallents RH, Guay JA, Katzberg RW, Murphy W, Proskin H. Angular and linear comparisons with unilateral mandibular asymmetry. J Craniomandib Disord Facial Oral Pain 1991; 5: 135-41.
- 3. Katzberg R, Tallents RH, Hayakawa K, Miller T, Goske MJ, Wood BP. Internal derangements of the temporomandibular joint: findings in the pediatric group. Radiol 1985; 154: 125-7.
- 4. Schelhas KP, Piper MA, Omile MR. Facial skeleton remodeling due to temporomandibular joint degeneration: an imaging study of 100 patients. AJNR Am Neuroradiol 1990; 11: 541-51.
- 5. Solberg WK, Bibb CA, Nordstrom BB, Hansson TL. Malocclusion associated with temporomandibular joint changes in young adults at autopsy. Am J Orthod 1986; 89: 326-330.
- 6. Fushima K, Inui M, Sato S. Dental asymmetry in temporomandibular disorders. J Oral Rehabil 1999; 26: 752-6.
- 7. Inui M, Fushima K, Sato S. Facial asymmetry in temporomandibular disorders. J Oral Rehabil 1999; 26: 402-406.
- 8. Trpkova B, Major P, Nebbe B, Prasad N. Craniofacial asymmetry and temporomandibular joint internal derangement in female adolescents: a posteroanterior cephalometric study. Angle Orthod 2000; 70: 81-88.
- 9. Ueki K, Nakagawa K, Takatsuka S, Shimada M, Marukawa K, Takazakura D, Yamamoto E: Temporomandibular joint morphology and disc position skeletal Class III patients. J Cranio-Maxillofac Surg 2000; 28: 362-368.
- 10. Fernandez Sanroman J, Gomez Gonzalez JM, del Hoyo JA: Relationship between condylar position, dentofacial deformity and temporomandibular joint dysfunction: an MRI and CT prospective study. J Cranio-Maxillofac Surg 1998; 25:35-42.

- 11. Hylander WL: Mandibular function and temporomandibular joint loading. In: Developmental Aspects of Temporomandibular Joint Disorders, Carlson DS, McNamara JAJ, Ribben KA, editors. Ann Arbor: University of Michigan, 1985, pp. 19-35.
- 12. Korioth TWP, Romilly DP, Hannam AG: Three-dimensional finite element stress analysis of the dental human mandible. Am J Physic Anthropol 1992: 88: 69-96.
- 13. Hylander WL, Johnson KR: In vivo bone strain patterns in the craniofacial region of primates. In: Science and Practice of Occlusion, McNeill C, editor. Chicago, Quintessence, 1997, pp. 165-178.
- 14. Koolstra JH, van Eijden TM, Weijs WA, Naeije M. A three-dimensional mathematical model of the human masticatory system predicting maximum possible bite forces. J Biomech 1988;21:563-576
- 15. Korioth T W P, Hannam A G 1990 Effect of bilateral asymmetric tooth clenching on load distribution at the mandibular condyles, Journal of Prosthetic Dentistry 64: 62-73
- 16. Korioth T W P, Romilly D P, Hannam A G 1992 Three-dimensional finite element stress analysis of the dental human mandible. American Journal of Physical Anthropology 88: 69-96
- 17. Chen J, Xu L 1994 A finite element analysis of the human temporomandibular joint. Journal of Biomechanical Engineering 116:401-407
- 18. Tanaka E, Tanne K, Sakuda M 1994 A three-dimensional finite element model of the mandible including the TMJ and its application to stress analysis in the TMJ during clenching. Medical Engineering and Physics 16: 316-322
- 19. Tanne K, Tanaka E, Sakuda M. 1996 Stress distribution in the temporomandibular joint produced by orthopedic chincup forces applied in varying directions: a three-dimensional analytic approach with the finite element method. American Journal of Orthodontic and Dentofacial Orthopedics 110: 502-507
- 20. Devocht J W, Goel V K, Zeitler D L, Lew D 1996 A study of the control of disc movement within the temporomandibular joint using the finite element technique. Journal of Oral and Maxillifacial Surgery 54:1431-1437

- 21. Ueki K, Nakagawa K, Maruakwa K, Takatsuka S, Yamamoto E: The relationship between temporomandibular joint and stress angulation in skeletal Class III patients. Eur J Orthod 2005; 27:501-506.
- 22. Takeuchi N, Ueki K, Nakagawa K: Analysis of stress distribution on condyle including the effects of masticatory muscles. Fifth World Congress on computational Mechanics; Wien, Austria, July 7-12, 2002(Web Published, http://www.wccm.tuwien.ac.at/).
- 23. Ueki K, Nakagawa K, Takatsuka S, Yamamoto E, Laskin DM. Comparison of the stress direction on the TMJ in patients with class I, II, and III skeletal relationships. Orthod Craniofac Res 2008;11:43-50.
- 24. Harada K, Watanabe M, Okura K, Enomoto S. Measure of bite force and occlusal contact area before and after bilateral sagittal split ramus osteotomy of the mandible using a new pressure-sensitive device: A preliminary report. J Oral Maxillofac Surg 2000: 58: 370-373.
- 25. Hattori Y, Okugawa H, Watanabe M. Occlusal force measurement using dental prescale. J Jpn Prosthodont Soc 1994: 38: 835-41.
- 26. Maness WL, Benjamin M, Podoloff R, Boblick A, Golden RF. Computerized occlusal analysis: a new technology. Quintessence Int 1987: 18: 287-292.
- 27. Nagai I, Tanaka N, Noguchi M, Suda Y, Sonoda T, Kohama G. Change in occlusal state of patients with mandibular prognathism after orthognathic surgery: a pilot study. Br J Oral Maxillofac Surg 2001: 39: 429-433.
- 28. Schuind F, Cooney WP, Linscheid RL, An KN, Chao EY: Force and pressure transmission through the normal wrist. A theoretical two-dimensional study in the posteroanterior plan. J Biomech 1995; 28: 587-601.
- 29. Genda E, Konishi N, Hasegawa Y, Miura T: A computer simulation study of normal and abnormal hip joint contact pressure. Arch Orthop Trauma Surg 1995; 114:202-206.
- 30. Buranastidporn B, Hisano M, Soma K. Effect of biomechanical disturbance of the temporomandibular joint on the prevalence of internal derangement in mandibular asymmetry. 2006; 28: 199-205.
- 31. Kobayashi T, Honma K, Izumi K, Hayashi T, Shingaki S, Nakajima T.

Temporomandibular joint symptoms and disc displacement in patients with mandibular prognathism. Br J Oral Maxillofac Surg 1999; 37: 455-8.

Legends

- Figure 1. (a) Pressure sensitive sheet, (b) Results of occlusal force distribution
- Figure 2. RBSM numerical model.
- Figure 3. Integral points on the condylar surface.
- Figure 4. Spring model between the condylar surface and glenoid fossa. Kn shows spring coefficient in the calculation.
- Figure 5. Contact pressure distribution.
- Figure 6. Resultant forces. Arrows show the degree and angulation of resultant force on the condyles.

Table 1. Results of parameters and frontal cephalohram analysis. (u): horizontal displacement, (v): vertical displacement, (θ): rotational displacement. These show the displacement of the mandibular body at Menton. (u,v) have no unit, because these were coordinate values in the RBSM calculation. Mx-Md midline and occlusal cant are frontal cephalometric measurements that show the facial asymmetry.

					Mx-Md Midline	Occlusal cant
		(u)	(v)	(θ)		
				(degree)	(degree)	(degree)
Symmetry group	Mean	-0.0007	0.0107	0.0000	1.5473	-0.5765
	SD	0.0047	0.0013	0.0000	1.3753	2.1074
Asymmetry group	Mean	-0.0015	0.0117	0.0000	6.8570	-2.5080
	SD	0.0064	0.0016	0.0000	3.5046	2.5800
	P-value	0.5380	0.0035	0.0669	< 0.0001	0.6206

Table 2. Results of the RBSM analysis on the deviated and non-deviated side. The degree, angulation of the resultant force, horizontal displacement (X) and vertical displacement (Y) of each condyle were calculated. When the occlusal force is 1, the degree of resultant force shows relative value so that this could not have unit. (X,Y) have no unit, because these were coordinate values in the RBSM calculation. SD shows standard deviation.

Deviated side					
		Degree of resultant force	Angulation of resultant force	X-component	Y-component
			(degree)		
Symmetry group	Mean	0.5317	-1.3774	-0.0137	0.5309
	SD	0.0908	2.9148	0.0264	0.0908
Asymmetry group	Mean	0.5682	10.9476	0.0138	0.5661
	SD	0.1438	38.3450	0.0455	0.1442
	P-value	0.1795	0.5170	0.0014	0.1952

Non-deviated side						
		Degree of resultant force	Angulation of resultant force	X-component	Y-component	
			(degree)			
Symmetry group	Mean	0.4699	2.4372	0.0207	0.4684	
Symmetry group	SD	0.4099	4.1511	0.0207	0.0907	
	SD	0.0903	4.1311	0.0313	0.0907	
Asymmetry group	Mean	0.4344	6.0741	0.0506	0.4286	
	SD	0.1430	8.0671	0.0517	0.1423	
	P-value	0.1881	0.5546	0.0024	0.1400	

Table 3. Comparisons between the deviated side and non-deviated side (these were intra-group comparisons). * shows significant difference by paired t-test at P<0.05.

Deviated side	vs Non-deviated side				
		Degree of resultant force	Angulation of resultant force (degree)	X-component	Y-component
Symmetry group	P-value	0.0372*	0.2846	<0.0001*	0.0354*
Asymmetry group	P-value	0.0054*	0.0095*	0.0001*	0.0043*

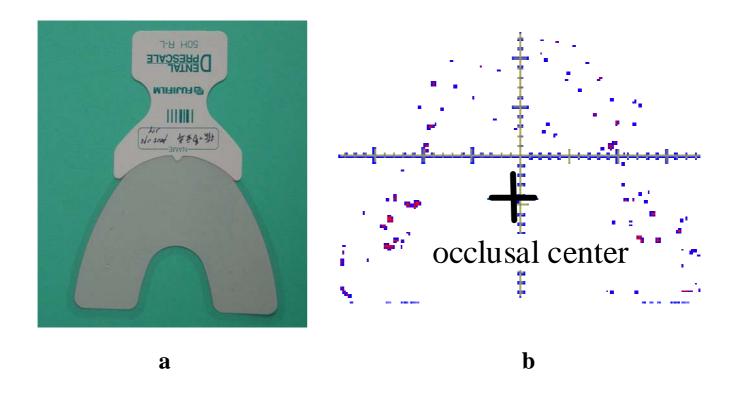


Fig. 1

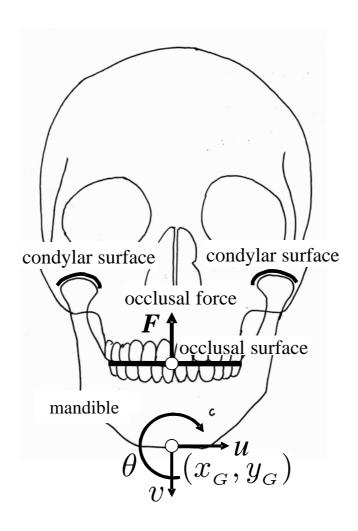


Fig. 2

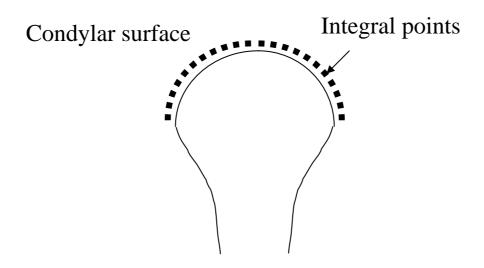


Fig. 3

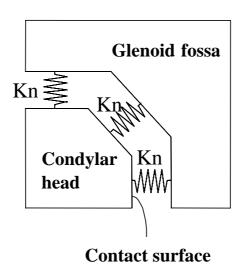


Fig. 4



Fig. 5

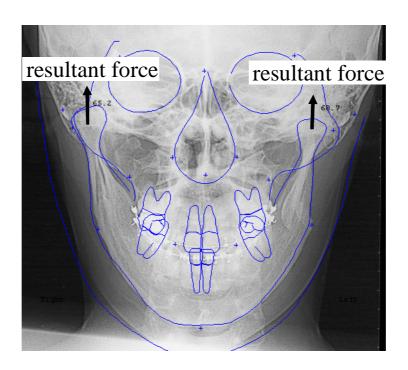


Fig. 6