International Conference on TMJ Replacement and Tissue Engineering in Hiroshima

平成17年度文部科学省科学研究費補助金一般研究C（企画調査）成果発表事業

顎関節部の再建と組織再生に関する国際カンファレンス-広島

International Conference on TMJ Replacement and Tissue Engineering in Hiroshima

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PREFACE

Temporomandibular joint disorder (TMD) is one of the major diseases affecting masticatory function. TMD has been defined as a result from intraarticular morphologic abnormalities, such as different forms of disc displacement, degenerative joint disease, inflammatory arthritis, synovitis and congenital and neoplastic anomalies. Epidemiologic surveys report that 20% to 25% of the population have symptoms of TMD, although only one fifth of those with TMD symptoms require treatment. It has been observed that up to 70% of patients with TMD suffer from displacement of the articular disc, which is called internal derangement of the temporomandibular joint (TMJ-ID).

TMJ-ID is defined as an abnormal positional relationship of the disc relative to the mandibular condyle and the glenoid fossa. Its major symptoms are TMJ pain, clicking and/or crepitus, muscle tenderness, and limitation of mouth opening. The recognition of the concept of TMJ-ID has contributed greatly to treatment of this type of TMJ dysfunction. The role of the disc in the progression of TMJ-ID is controversial. Initially, it was postulated that disc displacement preceded the onset of osteoarthritic changes in the TMJ. In contrast, the high association of articular degradation with disc malposition has led some investigators to suggest that the degenerative process predisposes to disc displacement.

From a review of etiological events of TMJ-ID and -osteoarthritis (-OA), trauma, functional overloading, joint laxity, and increased joint friction are considered to play a major role for the initiation of disc displacement and degradation of the condyle. It is documented in the literature indicates that excessive loading during jaw movement and the subsequent biomechanical imbalance in the TMJ may be assumed as an initial factor for a series of degenerative changes, resulting in condylar resorption and deformity. Therefore, an evaluation of the biomechanical environment in the TMJ would lead to a better understanding of the inducing mechanism of TMJ pain and disability, which result in proper diagnosis and appropriate treatment planning for TMD.

At the initial stage, signs and symptoms of TMDs can be treated by a nonsurgical approach such as splint therapy, physical therapy and pharmacotherapy. A surgical approach, such as total or partial alloplastic reconstruction of the TMJ, can be considered if the implemented treatment fails and the pathologic status becomes more severe. Surgical options include arthroplasty, disc repositioning and discectomy. Discectomy may become necessary when satisfactory repositioning or repair of the disc is not possible, for instance, when the morphology of the disc is so altered that it becomes an obstacle. However, it has been reported that surgical results with discectomy are not always satisfactory. Furthermore, although about 85% of discectomies are successful under ideal circumstances, these successes are not always accompanied with restoration of asymptomatic function. Apparently the loss of the articular disc has to be considered as a severe disadvantage for TMJ function. This suggests that a patient indicated for this surgical intervention would benefit from a suitable replacement. Tissue engineering offers this potential solution to replace the irreversibly damaged disc and cartilage, and alleviate the need for discectomy. Consequently, it is prominently expected to investigate tissue engineering of the TMJ components.

In the latest years much information has been obtained on the histological characteristics of the normal and osteoarthrotic TMJ. Obtaining information on the biomechanics of the TMJ has represented a much more difficult task. This information is not only important to provide insight into the normal and abnormal functioning of the joint but is also of a critical importance for the wear behavior and design of TMJ alloplastic implants. Thus far, several theoretical approaches have attempted to better understand the biomechanics of the TMJ. These studies have clearly shown that the disc and articular cartilage have an important function for stress distribution and shock absorption. However, for a quantitative analysis of stresses in the joint components the mechanical properties of the disc and cartilage have to be assessed adequately. Precise information on the biomechanical behavior of the disc contributes to develop suitable joint simulation models. These are a prerequisite to develop replacement materials for TMJ prosthesis and tissue engineering of TMJ components.

With this in mind, TMJ replacement and tissue engineering are considered absolutely necessary for fundamental treatment of TMDs in future. A proper understanding of the biomechanical behavior of the joint components and biomechanical environment within the TMJ presumably would provide us a better focus in the search and selection of mechanically compatible synthetic or regenerative biomaterials for TMJ reconstruction.

February 28, 2006.

Organizing committee
Eiji Tanaka
Kazuo Tanne
Theo MGJ van Eijden

Program

March 20 (Monday)

	Session and Speakers	Chairs
14:00-14:15	Opening Address Kazuo Tanne (Hiroshima University)	
14:15-17:00	Session 1: Biomechanical Responses of TMJ Jan Harm Koolstra (ACTA) Eiji Tanaka (Hiroshima University) Takanori Shibata (Health Science University of Hokkaido)	T.M.G.J. van Eijden

Welcome Dinner Party

19:00-21:00

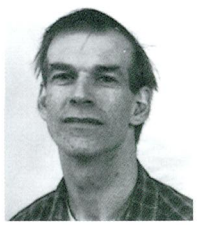
at Granvia Hotel Hiroshima

March 21 (Tuesday)

	Session and Speakers	Chairs
9:00-12:00	Session 2: Treatment of TMJ-ID and -OA Shumei Murakami (Osaka University) Takeshi Muramoto (Tokyo Medical and Dental University) Kazuo Tanne (Hiroshima University) Katsunori Ishibashi (Tsurumi University)	K. Tanne A. Nakajima
12:00-13:30	Lunch Time	
13:30-16:30	Session 3: TMJ Replacement and Tissue Engineering Toshio Sugahara (Okayama University) Kyriacos A. Athanasiou (Rice University) Michael S. Detamore (University of Kansas) Shigeru Ohno (Hiroshima University)	K.A. Athanasiou Y. Mori
16:30-16:45	Closing Address Eiji Tanaka (Hiroshima University)	

Contents

Contribution of Finite Element modeling to assessment of TMJ loading patterns.



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ABSTRACT

Simulations were performed with a dynamic biomechanical model of the human masticatory system that included the deformable cartilaginous structures of the temporomandibular joints as finite element models. This model predicted jaw movements as a result of force patterns of the masticatory muscles. Tension, compression and shear stresses as well as the pressure distribution were predicted during jaw open-close movements. It was found that the articular disc is able to reduce load concentrations between the articular eminence and mandibular condyle at the cost of relatively large shear stresses. Separately, predicted changes in pressure distribution inside the disc gave rise to the idea that its interstitial fluid could be subject to a continuous mixing flow pattern.

Introduction

The temporomandibular joint combines large movability with a large load bearing capacity. This is a challenging combination, and makes the joint susceptible to disorders that may lead to wear and unrecoverable damage. The mechanical load and its distribution over the various joint structures is considered to be the predominant factor that discriminates between function and dysfunction. Extrapolating to replacement of joint structures by artificial appliances, it most probably discriminates between success and failure.

Assessment of the distribution of mechanical loads in the temporomandibular joint in-vivo is hardly possible by direct measurement. Fortunately, biomechanical modeling provides a powerful tool to estimate joint load distributions. Provided that the mechanical behavior of the various tissues can be approximated adequately and the geometry of the various structures measured with sufficient accuracy, the Finite Element (FE) method can be applied successfully.

A FE model of the temporomandibular joint enables to predict local tensions and deformations in its deformable structures as a reaction on the displacements of the mandibular condyle with respect to the temporal bone. On the one hand, relatively small changes in imposed displacements may lead to large changes in the predicted tensions and deformations. On the other hand, the changes in condylar displacements due to altered muscle recruitment patterns leading to altered joint loading are generally beyond the measurement error. This makes FE analysis of the temporomandibular joint challenging.

Methods

Jaw movements are caused by the forces generated by the masticatory muscles. Furthermore, the joint reaction forces and external forces guide the jaw (Koolstra, 2002). Jaw movements can be simulated by biomechanical modeling, using a so-called rigid-body model (Koolstra and van Eijden, 1995). Taking into account all relevant forces jaw movements including realistic condylar displacements can be predicted. By incorporating FE models of the deformable joint structures in this dynamical rigid-body model of the human masticatory system (Fig. 1), the tensions and deformations in the temporomandibular joint can be approximated during such simulated jaw movements (Koolstra and van Eijden, 2005). As these tensions and deformations define the joint reaction force, they directly influence the jaw movement by guiding the condyle along the articular eminence.

The present model contains the skull and mandible as rigid bodies. They articulate with two six degree-of freedom temporomandibular joints. These joints consist of three FE models (temporal cartilage, articular disc and condylar cartilage) each. Their geometry had been obtained from a male human cadaver. The dentition is modeled by impermeable structures. Twelve pairs of muscle portions move the mandible with respect to the skull. They can be activated individually. They are of the Hill-type consisting of a contractile element, a parallel elastic element and a series elastic element. All relevant architectural parameters had been obtained from human cadavers. This model enables to asses the influence of joint-,

skull- and muscle geometry on jaw movements and concomitant deformations and tensions in the articular disc and cartilage layers of the temporomandibular joint. Furthermore, the influence of altered muscle activation patterns can be predicted. Finally, the influence of changes in material properties of the cartilaginous structures as occurring during ageing can be assessed.

Results

The model has been applied to estimate the stress and strain distribution in the articular disc and the articular cartilage layers in the temporomandibular joint during free and loaded jaw open-close movements. It was found that while the articular cartilage layers are primarily loaded with compressive stress, the articular disc is loaded with compressive, tensile and von Mises stress (Fig. 2). The latter is predominantly associated with shear. This points to large deformations of the disc without large changes in volume. During the jaw movements the predicted load on the condyle was concentrated at its superior aspect. On the temporal bone it was concentrated in the region of the articular eminence, while the mandibular fossa, in a more closed position, received a more moderate load, distributed over its entire surface. In the articular disc the largest stresses were observed at the lateral side of the intermediate zone as the jaw was open and more medially as the jaw was closed.

The model was also applied to predict the hydrostatic pressure distribution in the cartilaginous structures of the temporomandibular joint during jaw movements. Herewith the flow of fluid within, and between these structures and their environment can be assessed. These flows are considered to play a role in load bearing, friction and nutrition of these structures. During jaw closing pressurization of various regions of the articular cartilage was predicted. During jaw opening these regions relaxed. No dilatations were predicted, in contrast to the articular disc where both compression and dilatation was predicted. The pattern of hydrostatic stresses in the inferior layer of the articular disc had a predominant antero-posterior orientation, whereas in the superior layer the orientation was obliquely medio-lateral (Fig. 3).

Discussion

The human masticatory system is a complex musculoskeletal system where the masticatory muscles, tensions and deformations in the temporomandibular joint and jaw movements interact mutually. If one of these factors changes it will influence all the others. This especially concerns the activation patterns of the masticatory muscles which more or less easily adapt to a new environment. This may occur, for instance, to relieve pain in a deranged joint. Furthermore, the mechanical properties of the cartilaginous structures may change due to aging or wear. This most likely does not only affect the tensions and deformations in the joint, but also the movement patterns of the jaw. The present model enables to assess these interactions. The most important assumption to make for a proper analysis of this interaction concerns the activation pattern of the masticatory muscles.

Finite Element modeling relies on a proper description of the mechanical behavior of the included materials. For the majority of technical materials such a description can be formulated relatively easy. For biological materials like cartilage, however, such a formulation is more troublesome. One of the reasons is that their mechanical properties differ from subject to subject and change with age and degree of wear. In the present model the cartilaginous structures have been modeled with the Mooney-Rivlin material model as it behaves most reliably under the large deformations that occur in the temporomandibular joint. This model is not able to deal with viscoelastic properties as present in cartilage. Furthermore, it underestimates its hyperelastic nature. Therefore, as long as an unambiguously correct material model is unavailable predictions of tensions and deformations have to be regarded as qualitative.

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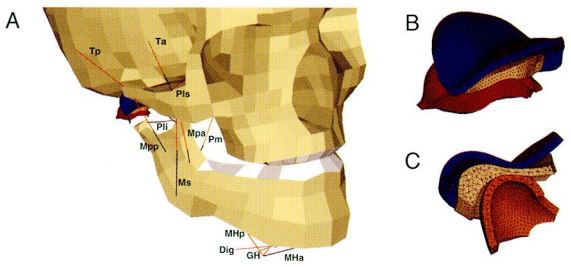
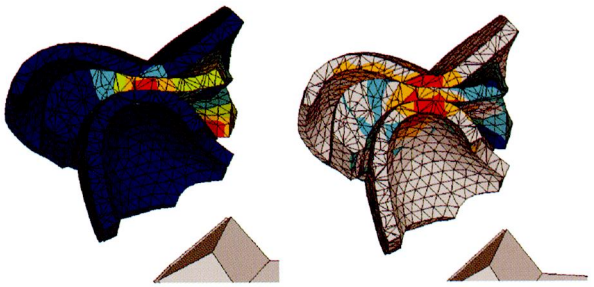
Figure 1.



Model of the human masticatory system.
A: Overview. Red lines: Muscle contractile element. Black lines: Series elastic element. Ta, Tp: anterior and posterior temporalis. Pls, Pli: superior and inferior lateral pterygoid. Mpa, Mpp: anterior and posterior deep masseter. Ms: superficial masseter. Pm: medial pterygoid. MHa, MHp: anterior and posterior mylohyoid. Dig: digastric. GH: geniohyoid.
B: Finite element model of the temporomandibular joint. View as in A. Blue: temporal cartilage. Orange: articular disc. Red: condylar cartilage.

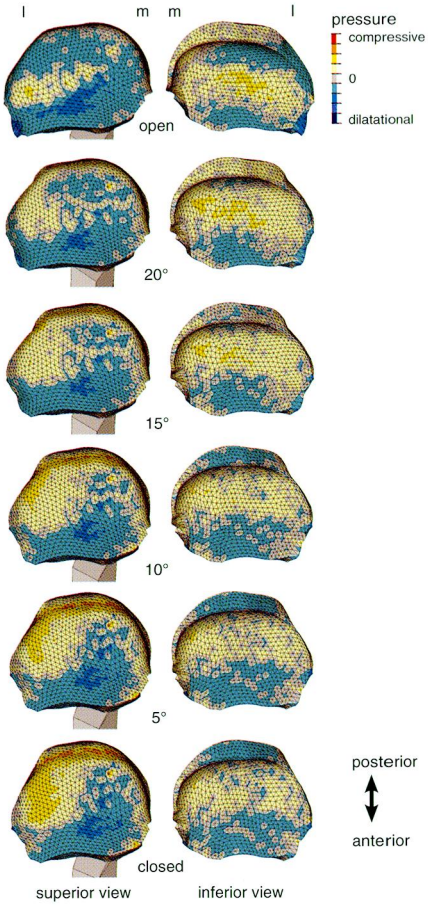
C: Finite element model of the temporomandibular joint in sagittal cross-section.

Figure 2.



Predicted stress in the temporomandibular joint.
Sagittal view, anterior side to the right. Left: von Mises stress, increasing from blue to red. Right: maximum principal stress, tensile stress increasing from green to blue, compressive stress increasing from orange to red.

Figure 3.



Hydrostatic pressure in the articular disc during jaw closing.
l: lateral, m: medial.

Biomechanical response of the temporomandibular joint disc complex to mechanical loads

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Key words. Stress and strain, viscoelasticity, elastic modulus, TMJ disc

ABSTRACT:

The temporomandibular joint (TMJ), one of the load-bearing organs in human body, is composed of bone components and soft tissues. Of the soft tissues, the TMJ disc consists mainly of collagen fibers and proteoglycans constrained in the interstices of the collagen network. This construction results in a viscoelastic response to loading and enables the disc to play an important role as a stress absorber during function. The viscoelastic properties depend on the direction (tension, compression, and shear) and the type of the applied loading (static and dynamic). For instance, upon dynamic loading the disc is likely to behave less stiff than under static loading because of the difference of fluid flow through and out of the tissue during loading. Furthermore, the retrodiscal tissue adjacent to the TMJ disc has an important joint-stabilizing function during mouth opening although this tissue exhibits less stiffness than the TMJ disc and therefore has no or less function as a stress absorber. Information about the viscoelastic behavior of the soft tissues in the TMJ is required to understand its function, which is a requirement, for instance, to develop a suitable replacement and tissue engineering of the TMJ. In the present paper, the biomechanical properties of the TMJ disc and retrodiscal tissue in response to various loading conditions are discussed.

INTRODUCTION

During mandibular movements, the temporomandibular joint (TMJ) undergoes various loadings as a load-bearing organ (Koolstra and van Eijden, 2005; Tanaka *et al.*, 2004). The modulation of the TMJ loading is dependent on the biomechanical response of the TMJ soft tissues located between the mandibular condyle and glenoid fossa. The articular surfaces of the TMJ are highly incongruent. Due to this incongruency, the contact areas of the opposing articular surfaces are very small. Upon joint loading this may lead to large peak loads, which may cause damage to the cartilage layers on the articular surfaces. The presence of fibrocartilaginous disc is believed to prevent these peak loads (Tanne *et al.*, 1991; Scapino *et al.*, 1996), as it is capable to deform and to adapt its shape to that of the articular surfaces.

The magnitude of the deformation and resulting stress in the disc is primarily determined by the nature of the applied loads and by its biomechanical properties. Therefore, a better understanding of these properties is of critical importance for several reasons. First, they determine the role of the disc as a stress distributing and load absorbing structure (Nickel and McLachlan, 1994; Beek *et al.*, 2001). Second, precise information on the biomechanical properties of the disc is required to develop suitable joint simulation models, with which the distribution of stress and strain in the structures of the joint can be estimated. Finally, information on the biomechanical properties of the disc is important to develop TMJ replacement and tissue engineering of the TMJ.

The retrodiscal tissue of the TMJ contains collagen and elastic fibers, and numerous blood vessels and nerves (Kino *et al.*, 1993). Of the components, collagen fibers are responsible for resistance to tensile forces and for maintaining the shape of the tissue, while elastin may function in the restoration of shape after load removal (Zhu *et al.*, 1994). The retrodiscal tissue consists of a temporal, condylar, and intermediate part (Scapino, 1997). The temporal and condylar part are directly attached to the posterior band of the disc and are supposed to play an important role in controlling the disc position during jaw opening and closing (Scapino, 1997). During jaw opening, the anteroposterior length of the temporal part increases, while the condylar part is folded beneath the posterior band (Scapino, 1997). In contrast, during jaw closing the length of the temporal part decreases, while that

of the condylar part increases. This suggests that the temporal and condylar part of the retrodiscal tissue contribute to maintain the position of the disc relative to the condyle during, respectively, jaw opening and closing. Therefore, insight into the viscoelastic properties of the retrodiscal tissue respond to various loading is also important to unravel the possible nature of secondary tissue changes.

In this paper, we provide a thorough review of biomechanical studies of the TMJ disc and retrodiscal tissue. The biomechanical properties of the disc and retrodiscal tissue, including elastic modulus and viscoelasticity, are summarized.

BIOMECHANICAL BEHAVIOR OF THE DISC AND THE RETRODISCAL TISSUE

Elastic constants

The relationship between stress and strain of an elastic material can be described by a stress-strain curve (Figure 1). The curve has both elastic and plastic deformation regions. In the elastic region a toe region and a transition zone can be distinguished (Fung, 1981), which are more or less arbitrarily divided by the so-called critical point (Tanne *et al.*, 1991; Tanaka *et al.*, 2000). In order to evaluate the basic biomechanical characteristic of a tissue, the elastic modulus E is commonly calculated. This modulus is defined as the slope of the elastic region of the stress-strain curve. The tensile and compressive moduli are a measure of the ability of the tissue to resist deformation in the direction of the applied load. The shear modulus G is a measure of the ability of the tissue to resist shear stress in a particular plane.

Tensile modulus

The tensile modulus is mainly dependent on the orientation of collagen fibers, because they can resist tension only in the direction parallel to their orientation. The intermediate zone of the disc consists mainly of anteroposteriorly oriented fibers (Detamore and Athanasiou, 2003). Therefore, the tensile modulus of the intermediate zone is larger in anteroposterior direction than in mediolateral direction (Teng *et al.*, 1991; Beatty *et al.*, 2001). For example, the tensile modulus of the porcine disc was 76.4 MPa in the anteroposterior direction, whereas it was 3.2 MPa in the mediolateral direction (strain rate: 500 mm/min; Beatty *et al.*, 2001). As in the anterior and posterior bands of the disc the collagen fibers run mainly mediolaterally they have a relatively large tensile modulus in mediolateral direction.

With respect to the retrodiscal tissue, the first attempt to investigate the elastic properties of the retrodiscal tissue under tension was reported in 2003 (Tanaka *et al.*, 2003). In the bovine retrodiscal tissue, the stress-strain relationships were also nonlinear in nature and were best represented as a power function ($\sigma = A\,\varepsilon^{B}$). Then, the non-linear stress-strain relationship could be represented by a bilinear relation of two line segments with a certain critical stress. The obtained critical stress was around 0.5 MPa, and the elastic moduli were 2.08 MPa for the low (0-0.5 MPa) and 4.30 MPa for the high (0.5-2.0 MPa) stress region, respectively. The both moduli are approximately 10-20% of the bovine disc elastic modulus (Tanaka *et al.*, 2001). This implies that the retrodiscal tissue has not sufficient strength to pull the disc back although it can support and control the disc position slightly.

Compressive modulus

The resistance to compression is mainly dependent on the density of proteoglycans, especially of the large chondroitin sulfated proteoglycans. As the distribution of the proteoglycans is different in various regions of the disc, regional differences in its compressive modulus can be expected. In the anterior and posterior bands, and in the central region of the intermediate zone the compressive modulus is higher than in the medial and lateral regions of the intermediate zone (del Pozo *et al.*, 2002). The possible explanation for this regional difference is that the large chondroitin-sulfate proteoglycans and the related chondroitin sulfate are preferentially localized in the central region of the intermediate zone and in the anterior and posterior bands (Mizoguchi *et al.*, 1998).

The retrodiscal tissue is subjected to tension during normal jaw opening (Scapino, 1997). However, this tissue seems also subjected to compression. A previous study suggested that when the condyle is nearing the fully closed position, the condylar part of the retrodiscal tissue may be under compressive load (Scapino, 1997). Furthermore, with an abnormal anterior disc displacement, the retodiscal tissue is subjected to continuous compression (Scapino and Mills, 1997). The compressive modulus of the retrodiscal tissue was 1.54 MPa (Tanaka *et al.*, 2002a), which is almost 1/20 of that in the disc. This indicates that the retrodiscal tissue has no or less function as a stress absorber.

Shear modulus

Investigation of shear properties in synovial joints is of particular interest, because shear stress can result in fatigue, damage and deformation of cartilage (Zhu *et al.*, 1993, 1994). Therefore, data on the shear modulus might contribute to a better understanding of secondary tissue damage. Shear stress is likely to occur during loading of the disc, because the articular surfaces that compress the disc do not run parallel. This causes that not all areas of the disc are deformed in the same direction leading to local shear. Another reason why shear stress occurs in the disc is its inhomogeneous structure. Its inner layer consists mainly of anteroposterior running collagen fibers and the 'leaflet-like' proteoglycans (Nakano and Scott, 1996), whereas the superior and inferior surface layers

mainly consist of anteroposteriorly and mediolaterally running collagen fibers and small proteoglycans (Nakano and Scott, 1996). Therefore, these layers are considered to have different biomechanical properties (Nakano and Scott, 1996; Mizoguchi *et al.*, 1998) which might lead to shear stress. This is supported by the results of a finite element study, in which a relative large shear stress was predicted in a disc consisting of three layers (Tanaka *et al.*, 1994).

With respect to the shear modulus of the disc, Lai *et al.* (1998) reported that the shear modulus of the intermediate zone of the human disc (strain rate: 0.02 mm/s) was 1.0-1.75 MPa. Furthermore, it appeared that in the central region the shear modulus (about 1.0 MPa) was lower than in the lateral and medial regions (about 1.75 MPa).

Viscoelastic properties

Quasi-static behavior-stress relaxation

How the viscoelastic properties of the disc change over time during constant loading can be characterized by stress-relaxation testes, creep tests, and restoration tests (Figure 2). The parameters obtained from these tests provide valuable information on the tissue behavior as a function of time and are of great importance for a better understanding of mechanical properties of the disc such as energy dissipation and stress absorption. In a stress-relaxation test, a stepwise deformation with a specific strain level is applied to a specimen and this strain level is kept constant until the stress reaches an almost steady level. From this test, the relaxation time and the relaxed modulus are obtained.

The relaxation time of the disc ranges from 3 to 45 s and the relaxed modulus ranges between approximately 5 and 50% of the initial modulus (Tanaka *et al.*, 1999; del Pozo *et al.*, 2002). This indicates that the movement of fluid out of the disc under loading is relatively slow and not proportional to the fluid pressure. Because of this relatively long relaxation time, the loaded disc becomes relatively stiff when it is cyclically loaded, during for example chewing and speaking (Beek *et al.*, 2001). After stress relaxation, a biomechanical equilibrium will occur eventually which implies a balance between the applied stress and the resistance to this stress in the disc. More than 50% of the initial stress is dissipated. This behavior implies that the disc functions as a stress absorber and a stress distribution material. Without the dissipation of strain energy, storage of excessive strain energy can lead to breakage of the disc and other components of the TMJ (Teng *et al.*, 1991; Nickel and McLachlan, 1994; Tanaka *et al.*, 1999; del Pozo *et al.*, 2002).

The retrodiscal tissue exhibited an almost similar stress relaxation response as the disc. In the case that the stress-strain curve was described by a two-segment piecewise linear function with a threshold stress of 0.5 MPa, the relaxation times τ_ε for the constant strain were 30.1 s in the low stress region and 39.1 s in the high stress region (Tanaka *et al.*, 2003). The stress relaxation ratio was approximately 55% after 5 min relaxation. This indicates that there exists an energy-dissipation mechanism in the retrodiscal tissue. The remaining 50-60% of the tensile stress after stress-relaxation works for tethering the disc during jaw closing. It can be concluded that the retrodiscal tissue has a great capacity for energy-dissipation and resistance to tensile forces, and thus might have a function to maintain the position of the disc relative to the condyle during jaw closing.

Dynamic behavior

The above-mentioned quasi-static experiments have provided valuable information on how the tissue behavior of the disc changes over time. With quasi-static experimental set-ups, however, only the linear viscoelastic behavior of the disc can be studied. The disc and retrodiscal tissue should essentially be approached as structures with a non-linear behavior and thus their dynamic viscoelastic properties need to be examined, although the mechanisms responsible for stress distribution, energy dissipation and stress absorption are the same as for quasi-static loading.

In dynamic tests (Figure 3), a complex dynamic modulus E* can be determined experimentally by applying a sinusoidal strain (Tanaka *et al.*, 2002b; Tanaka and van Eijden, 2003). It consists of a real part, the storage modulus E', and an imaginary part, the loss modulus E". E' describes the elastic deformation under stress and is directly proportional to the energy storage in a cycle of deformation. E" describes the viscous deformation and is proportional to the average dissipation or loss of energy as heat in a cycle of deformation. The values of dynamic moduli (|E*|, E' and E") increase as the frequency increased from 0.1 to 100 Hz (Tanaka *et al.*, 2002b). In a dynamic tensile test, the dynamic viscoelastic E-moduli were about 2 times larger at 100 Hz than those at 1 Hz (Tanaka *et al.*, 2002b). This non-linear dependency on the frequency is due to fluid flow and squeezing within the matrix of the disc. Below a certain frequency, the fluid flow may match up to the applied frequency. At higher frequencies, the proteoglycans occupying the interfibrilar spaces interfere with smooth fluid flow, which leads to strain energy dissipation, resulting in a higher stiffness.

CONCLUSIONS

With this in mind, a proper understanding of the viscoelastic properties of the disc and retrodiscal tissue under simulated loading conditions can presumably give us a better focus in the selection and development of mechanically compatible synthetic or autogenous substitutes for TMJ replacement and tissue engineering.

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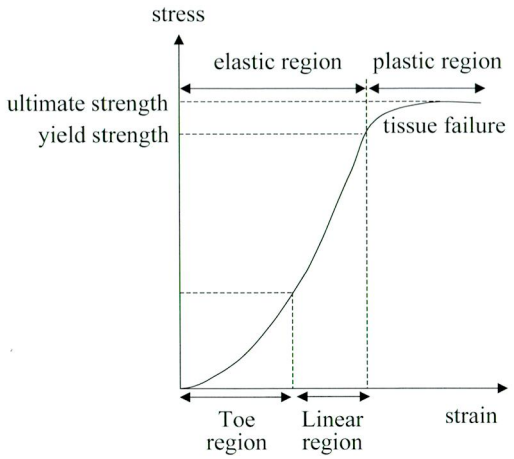

Figure 1 Stress-strain curve for soft tissue.
The curve has both elastic and plastic deformation regions. If the structure is not loaded beyond the elastic region, it will return to its original shape once the load is released. If the structure is loaded up to its plastic region it will not return to its original shape when the load is released. After plastic deformation, the stress will cause permanent damage of the tissue. The elastic region, a toe region and transition zone can be distinguished, which are more or less arbitrarily divided by the so-called critical point.

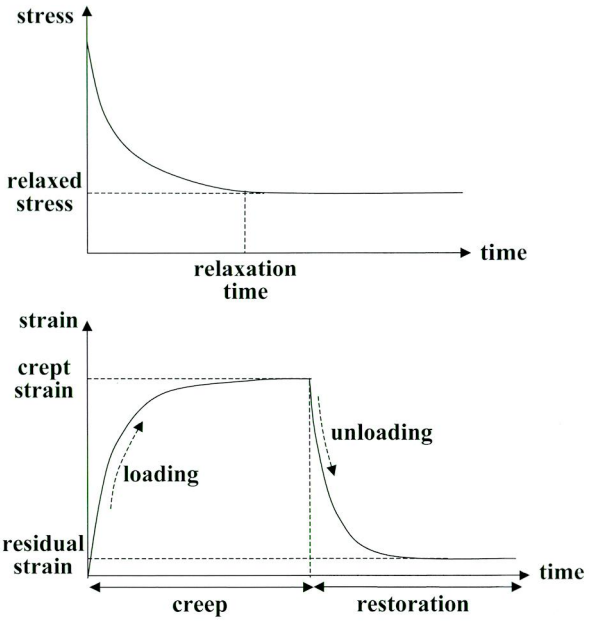

Figure 2 Stress relaxation curve (1) and creep and restoration curve (2).

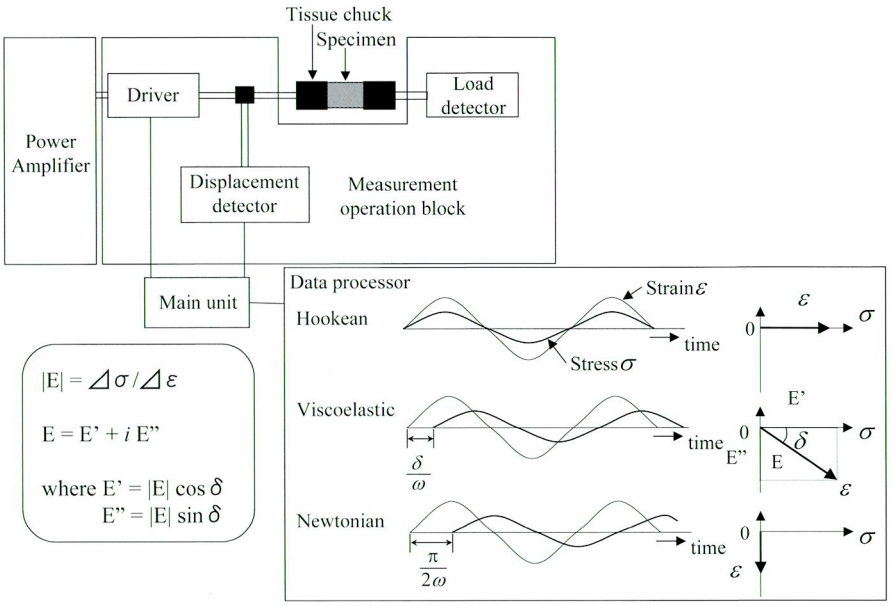

Figure 3 Block diagram of dynamic viscoelastometer and schematic representation of relationship between stress and strain of perfectly elastic solid (Hookean body), viscoelastic material and perfectly viscous liquid (Newtonian fluid) with sinusoidal varying stress.
The sinusoidal strain is produced by a tension control motor in the driver and the stress and strain are measured by means of load and displacement detectors and transmitted to a data processor. In a viscoelastic material, the phase difference between stress and strain is somewhere in between ($\pi/2 > \delta > 0$), and the complex modulus E* is resolved into two components, i.e., the storage modulus E' and the loss modulus E', shown vectorially. Furthermore, the tangent of the phase angle (δ) between stress and strain is a measure of the ratio of energy loss to energy stored during cyclic deformation.

Biochemical analysis of Temporomandibular Joint (TMJ) Synovial Fluid

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INTRODUCTION

Recent improvements in techniques for detecting trace amounts of biologic molecules in small volume of synovial fluid (SF) have lead to analysis on various inflammatory and cartilage degradation markers in the diseased temporomandibular joint(TMJ).

After the last of 1990's, in Japan, a lot of research groups have grown up in this field. They have been focusing and analyzing on various molecules in TMJ synovial fluid , e.g. interleukin 1-β (IL-1β),tumor necrosis factor-α (TNF-α), Interferon-γ , matrix metalloproteinase 1 and 3, tissue inhibitor matrix metalloproteinase 1 and 3 as inflammatory markers , and Chondroitin -4 and 6 sulfates(C4S, C6S), Hyaluronic acid(HA), Keratan sulfate (KS), procollagen Ⅱ C-peptide (pCol Ⅱ-C), pyridinoline and deoxypyrodinoline as cartilage degradation markers.

The purpose of this article is to report on our cross sectional study of the concentrations of the cartilage degradation molecules (HA, C4S, C6S, KS and pCol Ⅱ-C) and the inflammatory molecules(IL-1β , TNF-α) in the direct aspirated SF in patients with TMJ disc disorders, and to discuss their role in the joint pathology.

PATIENTS and METHODS

Patients

All of female patients were diagnosed as a normal disc position (NOR), an anterior displaced disc with (WR) or without reduction (WOR) by MR imagery. All of the joints showed evidence of joint effusion and joint pain.

Joint Fluid

The aspirates of TMJ SF were obtained and treated by the modified direct aspiration method as described previously. The previous study on the exact location of TM joint fluid using MR Imaging in patients with chronic internal derangements revealed that SF in a mouth closed condition was most frequently concentrated in the antero-lateral area of upper compartment of TMJ. This position is just under the articular tubercle and just inside of lateral wall of capsule. Based on this information, the point of needle entry was changed to be just under the articular tubercle. The samples were diluted with physiologic saline (approximately 1.5 g) in vitro. The diluted samples were collected in plastic tubes and then centrifuged at 8000 g for 20 minutes to remove cells and tissue debris. The supernatants were stored in aliquots at - 20° C until used.

Analysis

1) Cartilage degradation molecules

(1)HA, C4S, C6S

CHEMICALS

The standard unsaturated disaccharides, 2-acetamido-2-deoxy-3-0-(β -D-gluco-4- enepyranosyluronic acid)-D-glucose (Δ di-HA), 2-acetamido-2-deoxy-3-0-(β -D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose (Δ di-4S), 2-acetamido-2-deoxy-3-0-(β -D-gluco-4-enepyranosyluronic acid)-6-0-sulfo-D-galactose (Δ di-6S). chondroitinase ABC (EC 4.2.2.4) (CHase ABC), and hyaluronidase derived from Streptococcus dysgalactiae (EC 4.2.2.1) HAase SD) were obtained from Seikagaku Corporation (Tokyo. Japan): 2-cyanoacetamide was purchased from Atdrich(Milwaukee, WI). All other chemicals wcre of reagent gradc. YMC gel PA-120 (YMC Ltd. Kyoto, Japan), packed in a stainless steel column (4.6-mm inner diameter × 250 mm) was used to separate unsaturated disaccharides.

ENZYMATIC DIGESTION OF JOINT FLUIDS

Each joint fluid specimen was diluted 10-fold with distilled water and then digested with CHase ABC and HAase SD as follows. To 200 μL diluted joint fluid, 50 μL CHase ABC solution in distilled water (5 units/ml), 80 μL of 10 mmol/L sodium acetate buffer (pH 8.0), and 70 μL distilled water were added. The

mixture was incubated at 37℃ for 2 hours and then the mixture was ultrafiltered using an Ultrafree C3GC system (molecular size cut-off 10.000; Japan Millipore Ltd, Tokyo, Japan). Because a large mount of HA exists in the sample, and CHase ABC acts more slowly on HA than on CS, HA was not completely reduced to disaccharide by CHase ABC digestion only. Thus, toreduce HA completely to the disaccharide, 30 μL HAase SD solution in distilled water (0.5 units/-ml) and 30 μL of 100 mmol/L sodium acetate buffer (pH6.0) were added to 300 μL of the ultrafiltrate and the mixture was incubated at 37° C for 2 hours. HAase SD derived from Streptococcus reacts with the tetrasaccharides of HA and produces the unsaturated disaccharide of HA (Δ di-HA). After digestion with HAase SD, the mixture was ultrafiltered as previously described, and the filtrate obtained was analyzed by HPLC.

HPLC Analysis

HPLC analysis of the unsaturated disaccharides derived from HA and CS in SF was performed according to the method of Toyoda et al. The HPLC system used was constructed from two pumps (model 880-PU: Japan Spectroscopic Co, Ltd, Tokyo, Japan), an autosampling injector (Model 23 1 ; Gilson, Villiers le Bet, France), a stainless steel column packed with propylaminc-bound silica gel (YMC gel PA- 120). a dry reaction bath (DB-3: Shimamura Instrument Co. Tokyo,Japan), a spectrofluorometer (model 820-FP: Japan Spectroscopic). and an integrator (model 805-GI; Japan Spectroscopic).

The unsaturated disaccharides in each sample were eluted with a gradient of 0 to 100 mmol/L sodium sulfate for 60 minutes at a flow rate of 0.5 mL/min. To the eluent from the column, 100 mmol/L sodium tetraborate buffer (pH 9.0) containing 10 mg/mL 2-cyanoacetamide was added at a flow rate of 0.5 mL/min, and the mixture was passed through a polyetherketone reaction coil (0.8 mm inner dimension × 10 m) set in the dry reaction bath thermostated at 137℃. The effluent was monitored by the spectrofluorometer set at an excitation of 331 nm and emission of 383 nm. The area of each peak corresponding to unsaturated disaccharide was calculated by the integrator. After the concentrations of the Δ di-6S, Δ di-4S, and Δ di-HA were measured by HPLC the ratio of Δ di-6s or Δ di-4S to Δ di-HA, and Δ di-6S to Δ di-4S, were calculated.

(2) KS

KS concentrations was quantified by electrochemilumine-scence immunoassay (ECLIA) with a monoclonal antibody (1-20 5D4). The range of detection level of this assay is 1～500 ng/ml.

(3) pCol Ⅱ-C

pCol Ⅱ-C was quantified by enzyme immunoassay (EIA) kit with a polyclonal antiserum against the bovine propeptide (chondrocalcin test 'TeijinR', Teijin Ltd., Japan). The detection level of this kit is over 0.2 ng/ml.

2) Inflammatory molecules

(1) IL-1β

IL-1β was quantified by enzyme immunoassay (EIA) kit with a blend of monoclonal antibodies against distinct epitopes of IL-1β (MEDGENIX IL-β E ASIATM kit, BioSource Europe S.A. Ltd., Belgium). The detection level of this kit is over 2 pg/ml.

(2) TNF-α

TNF-α was quantified by immunoenzymometric assay kit with a blended of mono-clonal antibodies directd against distinct epitopes of TNF-α are used (TNF- EASIATM BioSource Europe S.A., Belgium). The detection level of this kit is over 3pg/ml.

Statistical analysing method

SAS Ver. 6.0 was used for statistical analysis. Statements in this study indicating a significant difference refer to a p value of 0.05 or less.

RESULTS

1) Cartilage degradation molecules

(1) HA, C4S, C6S

There were no significant differences in the concentration of Δ di-6S, Δ di-4S, and Δ di-HA. among the groups and in the ratio of Δ di-6S or Δ di-4S to Δ di-HA among the groups.

(2) KS

In 62 joints (100%) of 62 joints of 55 female patients (mean 34.1, mode 29 years old), KS in SF was detectable. The mode and ranges of KS concentrations in the WR (24 joints), WOR reduction (24 joints) and OA (14 joints) groups was 1779.4, 617.22 and 387.75, and 280.32～39495.5, 64～8740 and 126.48～3510.5 ng/ml, respectively.This difference among the groups was statistically significant ($p<0.05$).

(3) pCol Ⅱ-C

In 36 joints (73.5%) of 49 joints of 45 female patients (mean 31.8 years), pCol Ⅱ-C in SF was detectable. The frequency of TMJ with detectable pCol Ⅱ-C in the NOR (2 joints), WR (19 joints) and WOR reduction (28 joints) groups was 0%, 57.9% and 89.3%, respectively. This difference among the groups was statistically significant ($p< 0.01$).

2) Inflammatory molecules

(1) IL-1β

In 44 joints (80%) of 55 joints of 49 female patients (mean 34.9 years, mode 27 years), IL-1β in SF was detectable. The frequency of TMJ with detectable IL-β in the WR (19 joints), WOR (21 joints) and OA (15 joints) groups was 100%, 76.2% and 60%, respectively. This difference among the groups was statistically significant ($p< 0.05$).

(2) TNF-α

In 18 joints (32.7%) of 55 joints of 49 female patients (mean 34.9 years), TNF-α in SF was detectable. The frequency of TMJ with detectable TNF-α in the WR (19 joints), WOR (21 joints) and OA (15 joints) groups was 36.8%, 23.8% and 40%. There was no

statistically difference in the detection of TNF-α among the groups.

DISCUSSION and CONCLUSION

1) Cartilage degradation molecules

Although the concentration of C6S, C4S and HA. and the ratio of C6S or C4S to HA in SF dose not reflect the pathosis of patients with disc disorders, analysis of the GAG content in the joint fluid represents a unique way to study joint diseases. Because C6S is the chancteristic CS of cartilaginous tissues, and C4S may be the characteristic CS of synovial tissue, measurement of these molecules, in particular, should provide precise information about alterations in cartilage metabolism and synovial proliferation.

On the other hand, the increased levels of KS in SF may reflect an increased rate of turnover in the extraceller matrix of the TMJ cartilage and degenerative disc in patients with the early stage of the disc disorders, especially with an anterior displaced disc with reduction.The increased levels of pCol Ⅱ-C in SF may reflect an increased rate of synthesis of collagen Ⅱ in the TMJ cartilage of patients with disc disorders, especially with an anterior displaced disc without reduction.

2) Inflammatory molecules

The increased levels of IL-1β in SF may reflect inflammatory changes of the TMJ synovial tissue in the patients with disc disorders, especially with an anterior displaced disc with reduction. But the detection of TNF-α in SF dose not reflect the pathosis of patients with disc disorders.

ACKNOWLEDGMENT

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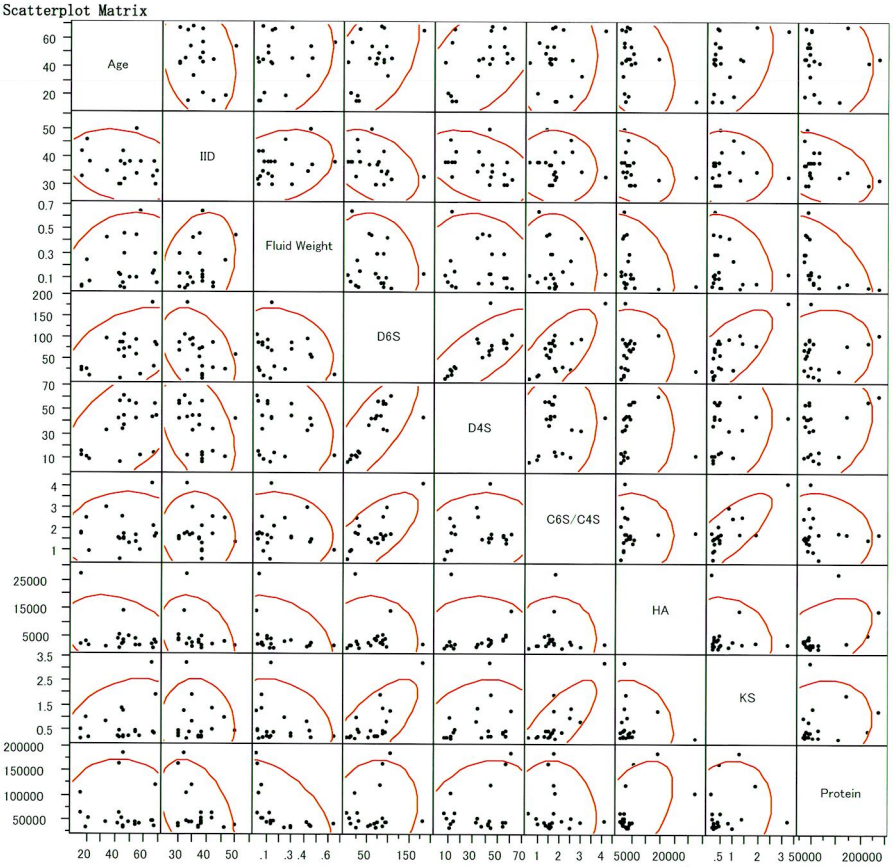
OA
Multivariate
Correlations

	Age	IID	Fluid Weight	D6S	D4S	C6S/C4S	HA	KS	Protein
Age	1.0000	-0.2336	0.2283	0.4758	0.5054	0.0952	-0.3596	0.3216	-0.0526
IID	-0.2336	1.0000	0.2807	-0.3952	-0.4170	-0.0618	-0.3318	-0.0662	-0.4145
Fluid Weight	0.2283	0.2807	1.0000	-0.1408	-0.1239	-0.0873	-0.3505	-0.2244	-0.5283
D6S	0.4758	-0.3952	-0.1408	1.0000	0.7617	0.6906	-0.0433	0.7138	0.1686
D4S	0.5054	-0.4170	-0.1239	0.7617	1.0000	0.1361	0.0263	0.2228	0.3007
C6S/C4S	0.0952	-0.0618	-0.0873	0.6906	0.1361	1.0000	-0.0187	0.7822	-0.0847
HA	-0.3596	-0.3318	-0.3505	-0.0433	0.0263	-0.0187	1.0000	-0.1076	0.5110
KS	0.3216	-0.0662	-0.2244	0.7138	0.2228	0.7822	-0.1076	1.0000	0.1850
Protein	-0.0526	-0.4145	-0.5283	0.1686	0.3007	-0.0847	0.5110	0.1850	1.0000

Scatterplot Matrix



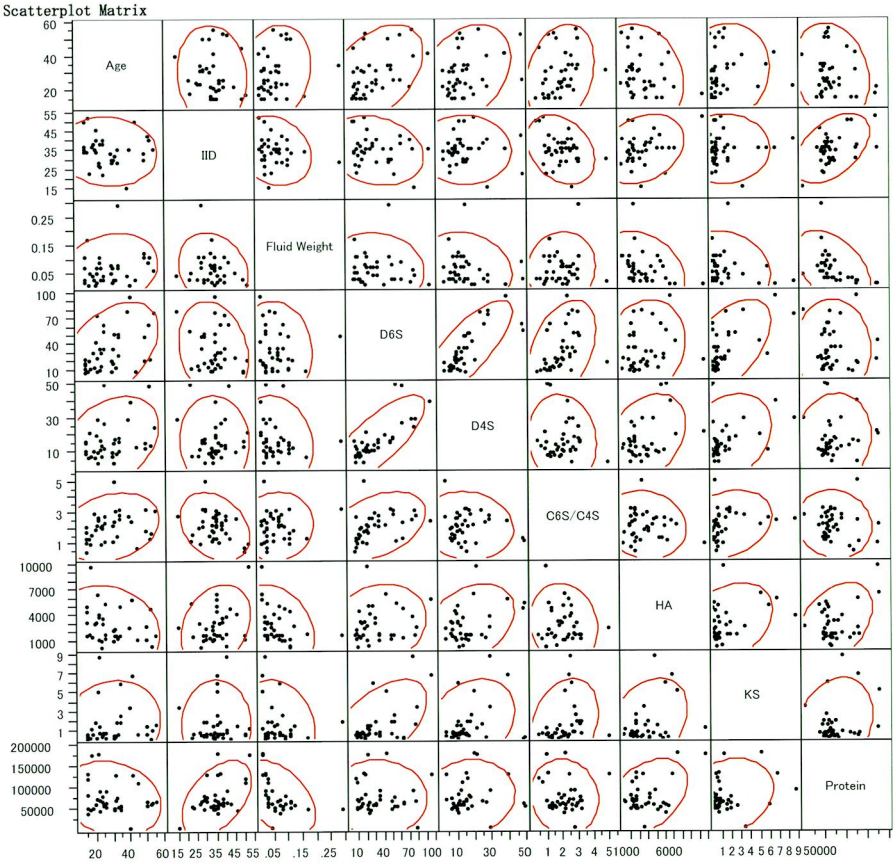
Nonparametric: Spearman's Rho

Variable	by Variable	Spearman Rho	Prob>|Rho|	
IID	Age	-0.1516	0.5234	------
Fluid Weight	Age	0.3273	0.1590	++++++++++++
Fluid Weight	IID	0.2082	0.3785	++++++++
D6S	Age	0.3762	0.1021	+++++++++++++
D6S	IID	-0.4970	0.0258	------------------
D6S	Fluid Weight	-0.1544	0.5158	------
D4S	Age	0.4516	0.0456	++++++++++++++++
D4S	IID	-0.5509	0.0118	--------------------
D4S	Fluid Weight	-0.2425	0.3030	---------
D4S	D6S	0.7759	<0.0001	++++++++++++++++++++++++++++++
C6S/C4S	Age	-0.0498	0.8349	
C6S/C4S	IID	-0.0992	0.6773	--
C6S/C4S	Fluid Weight	-0.1710	0.4710	------
C6S/C4S	D6S	0.4987	0.0252	++++++++++++++++++
C6S/C4S	D4S	0.0391	0.8700	
HA	Age	-0.1236	0.6035	-----
HA	IID	-0.5601	0.0102	--------------------
HA	Fluid Weight	-0.4834	0.0308	-----------------
HA	D6S	0.3308	0.1542	++++++++++++
HA	D4S	0.6451	0.0021	++++++++++++++++++++++++
HA	C6S/C4S	0.0466	0.8452	
KS	Age	0.2314	0.3262	++++++++
KS	IID	0.0357	0.8812	
KS	Fluid Weight	-0.2123	0.3687	---------
KS	D6S	0.6195	0.0036	++++++++++++++++++++++++
KS	D4S	0.2797	0.2323	++++++++++
KS	C6S/C4S	0.6709	0.0012	++++++++++++++++++++++++++
KS	HA	-0.0827	0.7289	--
Protein	Age	-0.1900	0.4224	-------
Protein	IID	-0.2341	0.3206	---------
Protein	Fluid Weight	-0.8358	0.0001	----------------------------------
Protein	D6S	0.1023	0.6679	++++
Protein	D4S	0.0827	0.7289	++
Protein	C6S/C4S	0.1181	0.6200	++++
Protein	HA	0.1353	0.5694	++++
Protein	KS	0.2150	0.3626	++++++++

WOR
Multivariate
Correlations

	Age	IID	Fluid Weight	D6S	D4S	C6S/C4S	HA	KS	Protein
Age	1.0000	-0.0761	0.2134	0.4584	0.3425	0.2473	-0.2229	0.1200	-0.1907
IID	-0.0761	1.0000	-0.2064	-0.2092	-0.0355	-0.3058	0.2800	0.0156	0.4899
Fluid Weight	0.2134	-0.2064	1.0000	-0.0645	-0.1569	0.0733	-0.3111	-0.1742	-0.4456
D6S	0.4584	-0.2092	-0.0645	1.0000	0.7808	0.4097	0.1865	0.5710	-0.0308
D4S	0.3425	-0.0355	-0.1569	0.7808	1.0000	-0.1001	0.3763	0.3242	0.0926
C6S/C4S	0.2473	-0.3058	0.0733	0.4097	-0.1001	1.0000	-0.1157	0.2742	-0.0894
HA	-0.2229	0.2800	-0.3111	0.1865	0.3763	-0.1157	1.0000	0.3589	0.4032
KS	0.1200	0.0156	-0.1742	0.5710	0.3242	0.2742	0.3589	1.0000	0.2184
Protein	-0.1907	0.4899	-0.4456	-0.0308	0.0926	-0.0894	0.4032	0.2184	1.0000

Scatterplot Matrix



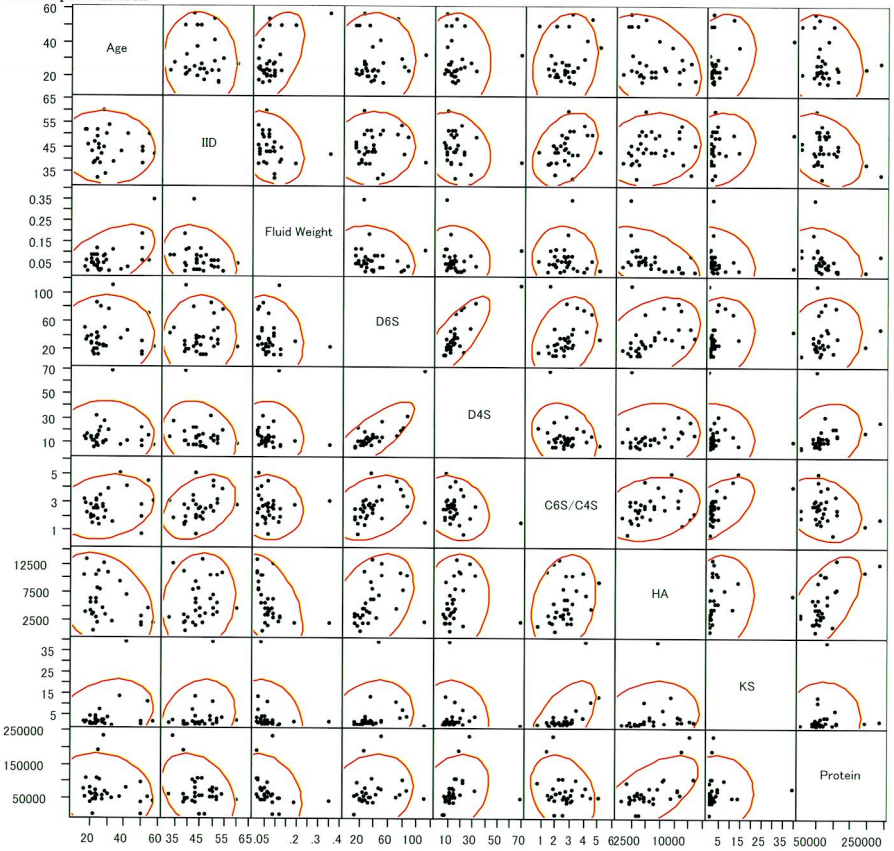
Nonparametric: Spearman's Rho

Variable	by Variable	Spearman Rho	Prob>|Rho|	
IID	Age	-0.1495	0.3705	----
Fluid Weight	Age	0.2674	0.1046	++++++++++
Fluid Weight	IID	-0.2302	0.1644	---------
D6S	Age	0.4845	0.0021	+++++++++++++++++++
D6S	IID	-0.0509	0.7616	--
D6S	Fluid Weight	-0.0677	0.6863	--
D4S	Age	0.3370	0.0386	++++++++++++
D4S	IID	0.1514	0.3643	++++++
D4S	Fluid Weight	-0.1929	0.2459	-------
D4S	D6S	0.7738	<0.0001	+++++++++++++++++++++++++++++++
C6S/C4S	Age	0.3699	0.0223	+++++++++++++++
C6S/C4S	IID	-0.1824	0.2731	-------
C6S/C4S	Fluid Weight	0.0695	0.6783	++
C6S/C4S	D6S	0.6084	<0.0001	++++++++++++++++++++++++
C6S/C4S	D4S	0.0998	0.5510	++
HA	Age	-0.2567	0.1198	----------
HA	IID	0.2487	0.1321	++++++++
HA	Fluid Weight	-0.3883	0.0160	----------------
HA	D6S	0.2369	0.1521	++++++++
HA	D4S	0.1662	0.3186	++++++
HA	C6S/C4S	-0.0194	0.9081	
KS	Age	0.2685	0.1031	++++++++++
KS	IID	0.1060	0.5266	++++
KS	Fluid Weight	-0.1394	0.4038	-----
KS	D6S	0.4337	0.0065	++++++++++++++++
KS	D4S	0.3008	0.0664	++++++++++++
KS	C6S/C4S	0.5049	0.0012	++++++++++++++++++++
KS	HA	0.1722	0.3011	++++++
Protein	Age	-0.1094	0.5131	----
Protein	IID	0.3326	0.0413	++++++++++++
Protein	Fluid Weight	-0.6063	<0.0001	------------------------
Protein	D6S	0.0159	0.9247	
Protein	D4S	0.2626	0.1112	++++++++++
Protein	C6S/C4S	-0.0498	0.7665	
Protein	HA	0.0297	0.8597	
Protein	KS	0.0791	0.6368	++

WR
Multivariate
Correlations

	Age	IID	Fluid Weight	D6S	D4S	C6S/C4S	HA	KS	Protein
Age	1.0000	-0.0605	0.5482	0.0278	0.0056	0.1998	-0.3100	0.2812	-0.1721
IID	-0.0605	1.0000	-0.2951	0.0520	-0.2180	0.3801	0.1474	0.2097	-0.3310
Fluid Weight	0.5482	-0.2951	1.0000	-0.1648	-0.0530	-0.0557	-0.4867	-0.2046	-0.2944
D6S	0.0278	0.0520	-0.1648	1.0000	0.7848	0.3253	0.3729	0.2009	0.1629
D4S	0.0056	-0.2180	-0.0530	0.7848	1.0000	-0.2468	0.1420	-0.0826	0.2745
C6S/C4S	0.1998	0.3801	-0.0557	0.3253	-0.2468	1.0000	0.2574	0.5694	-0.2598
HA	-0.3100	0.1474	-0.4867	0.3729	0.1420	0.2574	1.0000	0.1972	0.6295
KS	0.2812	0.2097	-0.2046	0.2009	-0.0826	0.5694	0.1972	1.0000	0.0505
Protein	-0.1721	-0.3310	-0.2944	0.1629	0.2745	-0.2598	0.6295	0.0505	1.0000

Scatterplot Matrix



Nonparametric: Spearman's Rho

Variable	by Variable	Spearman Rho	Prob>|Rho|	
IID	Age	-0.0789	0.6732	--
Fluid Weight	Age	0.2271	0.2191	+++++++
Fluid Weight	IID	-0.3453	0.0571	----------
D6S	Age	0.0245	0.8960	
D6S	IID	0.0909	0.6269	++
D6S	Fluid Weight	-0.2556	0.1651	----------
D4S	Age	-0.0378	0.8399	
D4S	IID	-0.1455	0.4348	-----
D4S	Fluid Weight	-0.2674	0.1459	----------
D4S	D6S	0.7214	<0.0001	+++++++++++++++++++++++++++
C6S/C4S	Age	0.1524	0.4132	++++++
C6S/C4S	IID	0.4379	0.0137	+++++++++++++++
C6S/C4S	Fluid Weight	-0.1641	0.3778	------
C6S/C4S	D6S	0.4881	0.0053	++++++++++++++++
C6S/C4S	D4S	-0.1117	0.5495	-----
HA	Age	-0.2178	0.2391	---------
HA	IID	0.2849	0.1203	++++++++++
HA	Fluid Weight	-0.6386	<0.0001	----------
HA	D6S	0.5851	0.0005	++++++++++++++++++++
HA	D4S	0.4379	0.0137	+++++++++++++++
HA	C6S/C4S	0.2828	0.1232	++++++++++
KS	Age	0.1873	0.3131	++++++
KS	IID	0.3497	0.0538	++++++++++++
KS	Fluid Weight	-0.4985	0.0043	----------
KS	D6S	0.4935	0.0048	++++++++++++++++
KS	D4S	0.1480	0.4269	++++
KS	C6S/C4S	0.5954	0.0004	+++++++++++++++++++++
KS	HA	0.5782	0.0007	+++++++++++++++++++++
Protein	Age	-0.1384	0.4579	-----
Protein	IID	-0.0907	0.6276	--
Protein	Fluid Weight	-0.5595	0.0011	----------
Protein	D6S	0.3583	0.0478	++++++++++++++
Protein	D4S	0.6411	<0.0001	++++++++++++++++++++++++
Protein	C6S/C4S	-0.2066	0.2649	---------
Protein	HA	0.5568	0.0011	+++++++++++++++++++++
Protein	KS	0.3099	0.0897	++++++++++++

Change of Temporomandibular Joint Disk Configuration and Clinical Findings following Conservative Treatment

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Short title; Change of TMJ disk and clinical findings after treatment
Key words; temporomandibular joint, magnetic resonance imaging, conservative treatment, follow-up study

Abstract

The objective of this study was to evaluate the relationship between changes in the disk status (position and reduction) and changes in the clinical findings following conservative temporomandibular joint (TMJ) treatment.

The subjects consisted of 164 patients (328 joints) having TMJ disorders. Clinical examination and MR imaging were performed on all patients before and after conservative treatment. Clinical assessments included joint noise, pain and inter- incisal distance (IID). Disk position and reduction was classified as "improved", "worsened", or "unchanged" following treatment. Joint noise and pain were similarly classified as "improved", "worsened", or "unchanged" foll owing treatment.

In the 55 joints where disk position was improved, noise was improved in 37 (67.2%) and worsened in 6 (10.9%). In the 48 joints where disk reduction was improved, noise was improved in 34 (70.8%) and worsened in 4 (8.3%). There was a significant relationship between improvement in the disk status and improvement in the noise. In 55 joints where the disk position was improved, pain symptoms were disappeared in 22 (40.0%) and newly occurred in 4 (7.3%). In the 56 joints where the pain was disappeared, disk position was improved in 22 (39.3%) but became worse in 22 (39.3%) joints. In 56 joints where the pain was disappeared, disk reduction improved in 17 (30.4%) joints but became worse in 32 (57.1%). There was no significant relationship between improvement in the disk status and improvement in the pain. Treatment has no significant influence on IID.

We conclude that there was no relationship between improvement in the disk status and improvement in the joint pain and IID after conservative treatment, but there was close relationship between the disk status and the joint noise in their changes.

Introduction

Of patients receiving treatment for disorders of the temporomandibular joint (TMJ), increasing numbers are diagnosed with internal derangement (Murakami et al., 1993). Internal derangement has been defined as the displacement of the disk in relation to the condyle. When a significant symptomatic internal derangement exists, a conservative course of treatment is frequently indicated because the success rates of conservative treatments are usually high (Anderson et al., 1991). Conservative treatment typically consists of attempted disk recapture and mandibular repositioning by occlusal splints (Summer and Westesson, 1997).

With the increasing desire to see symptomatic joint structures, arthrography, magnetic resonance (MR) imaging and arthroscopy have become useful diagnostic modalities (Muller et al., 1996). MR imaging is the most popular method to diagnose the disk position and reduction because neither ionizing irradiation nor anesthesia is used and any oblique tomographic section can be obtained.

The relationship between disk status and clinical findings has been examined by multiple authors (Merill et al., 1990, Kurita et al., 1998, Schellhas, 1989). In most studies, a significant relationship between disk position and patient symptoms has been recognized. However, there is little understanding of the relationship between change of disk status and change of the

clinical signs following treatment. Accordingly, the aim of this outcome study was to investigate the influence of conservative treatment on the relationship between disk status (position and reduction) and clinical findings.

Subjects and Methods

Patients

The subjects in this study consisted of 164 patients (328 joints) selected from 1829 patients who underwent MR imaging for TMJ diagnosis. There were 119 females and 45 males. The average age of the patients was 35.0 (18-78 years old). The inclusion criteria for selecting patients in this retrospective study were:

1. Patients were clinically diagnosed as having TMJ disorder.
2. MR imaging and clinical examinations were available at the beginning and end of treatment.
3. The period between two MR examinations was more than one month.
4. Patient received conservative treatment. This consisted of attempted disk recapture and mandibular repositioning by occlusal splints.

Imaging

MR images were obtained with a MR scanner using bilateral 8-cm diameter surface coils. Images were obtained in closed- and open-mouth positions in the oblique sagittal plane perpendicular to the long axis of the condylar heads. Eight 3-mm images were obtained in the open- and closed-mouth position. Scanning parameters included a TR of 50 ms and gradient echo sequence.

Clinical findings

Clinical findings which were recorded at the clinical examinations included pain, noise and inter-incisal distance (IID) at maximal opening. Pain was recorded as either present or absent. Noises at TMJ on opening were classified into 3 groups: crepitation, clicking or silent by palpation and stethoscope. IID was measured in millimeters by a caliper between the upper and lower central incisors.

Pain after treatment was classified into 3 groups. If the patient had no pain after treatment, they were scored as "improved." Patients were grouped as "worsened" if they claimed any pain after treatment. We did not consider any type, degree, or duration of pain. Patients were classified as "unchanged" if they reported no change in their pain experience.

Noise after treatment was classified into 3 groups. If clicking or crepitation disappeared or crepitation changed into clicking after treatment, they were scored as "improved." Patients were grouped as "worsened" if they reported any new noise or their clicking changed into crepitation after treatment. We did not consider any frequency nor duration of noise. If there were no change in noise, we classified into "unchanged."

MRI findings

Disk positions at closed mouth position were classified into 3 groups, such as superior position, anterior-superior position and anterior position, according to the criteria reported previously (Murakami et al., 1993). In the cases which were diagnosed as anterior or antero-superior position at closed mouth position, those were also diagnosed whether disks were reduced or not at open-mouth position.

As for change of the disk position after treatment, we classified changes into 3 groups. If the displaced disks changed into normal position or anteriorly displaced disk changed into antero-superior position after treatment, we grouped into "improved". And we grouped into "worsened", if any displacement occurred or antero-superior disk more displaced anteriorly after treatment. If there were no change in position, we classified into "unchanged"

As for change of the disk reduction after treatment, joints were classified into 3 groups. If the displaced disks changed into normal position or non-reduced disk changed into reducing one after treatment, we grouped into "improved". And we grouped into "worsened", if any displacement occurred or reducing disk changed into non-reduced one after treatment. If there were no change in reduction, we classified into "unchanged."

Statistical analysis

The relationship between improvement in the disk status and improvement in the noise and pain after treatment was evaluated statistically with using Chi-square test for independence. The difference of IID between before and after treatment according to the change of disk status was investigated statistically with using Wilcoxon signed-rank test and Paired two group t-test. All statistical analyses were performed on StatView (ver. 4.0) software.

Results

There was noise in 227 joints (clicking: n = 144, crepitation: n = 83) and pain in 112 joints. Average IID was 42.0 mm.

MR imaging revealed that 107 joints had superior position disks, 41 joints had antero-superior position disks and 180 joints had anterior position disk. In opened mouth position there were 22 (53.7%) reduced disks in 41 antero-superior position disks and 54 (30.0%) reduced disks in 180 anteriorly displaced disks.

As for noise, crepitation disappeared in 16 (19.3%) out of 83 joints and clicking disappeared in 54 (47.4%) out of 144 joints. Crepitation changed into clicking in 9

(10.8%) out of 83 joints. Crepitation or clicking occurred in 37 (36.6%) out of 101 joints which had not had any noise at the initial examination. Clicking changed into crepitation in 15 (10.4%) out of 144 joints. While the pain disappeared in 56 (50.0%) out of 112 joints, the pain occurred in 14 (6.5%) out of 216 joints which had not had any pain at the initial examination. Average of IID after treatment was 42.7 mm.

After treatment disk position was not changed in 221 (67.4%) disks. There was improvement in 55 joints (16.8%) and worsening in 52 joints (15.9%).

After treatment disk reduction was not changed in 202 (61.6%) disks. There was improvement in 48 joints (14.6%) and worsening in 78 joints (23.8%).

As for the relationship between change of disk position and change of noise, noise was improved in 37 (67.3%) and worsened in 6 (10.9%) out of 55 joints where disk position was improved. In 79 joints where the noise was improved, 37 (46.8%) disk position was improved and 12 (15.2%) disk got worse on disk position. There was significant relation between improvement in the disk position and improvement in the noise statistically ($0.001 < p < 0.01$, Chi-square value: 10.5).

As for the relationship between change of disk reduction and change of noise, noise was improved in 34 (70.8%) and worsened in 4 (8.3%) out of 48 joints where disk reduction was improved. In 79 joints where the noise was improved, 34 (43.0%) disk reduction was improved and 13 (16.4%) disk got worse on disk reduction. There was significant relation between improvement in the disk reduction and improvement in the noise statistically ($p < 0.0001$, Chi-square value: 25.7).

As for the relationship between change of disk position and change of pain, pain was disappeared in 22 (40.0%) and newly occurred 4 (7.3%) out of 55 joints where disk position was improved. In 56 joints where the pain was improved, although 22 (39.3%) joints were improved, the same number of joints got worse on disk position. There was no significant relation between improvement in the disk position and improvement in the pain statistically ($p = 0.48$, Chi-square value: 0.5).

As for the relationship between change of disk reduction and change of pain, pain was disappeared in 17 (35.4%) and newly occurred in 3 (6.3%) out of 48 joints where disk reduction was improved. In 56 joints where the pain was improved, although 17 (30.4%) joints were improved, 32 (57.1%) joints got worse on disk position. There was no significant relation between improvement in the disk reduction and improvement in the pain statistically ($p = 0.60$, Chi-square value: 0.3).

As for change of IID, although average of IID was increased after treatment, there was no significant difference in each groups statistically.

Discussion

In this study, we found the statistical significant correlation between improvement in the noise and improvement in the disk status after treatment. It makes sense that noise was improved because the positional relationship between the disk and the mandibular condyle was improved after treatment. The TMJ noise decreased when joint space was increased, allowing smoother condylar translation beyond disk surface irregularity and positional abnormality (Kirk et al., 1991). In some of our cases, joint space would widen after treatment. However, out of 79 joints where the noise improved, disk position and reduction were worsened in 12 and 13 disks (15.2% and 16.4%). In these cases it was considered that clicking was disappeared because the disk was more anteriorly displaced and posterior attachment would be elongated by the treatment.

As for relationship between improvement in the pain and improvement in the disk status, there was no significant relationship statistically, although pain hardly occur newly in cases where the disk status was improved. In 56 cases where the pain disappeared, however, the disk position was worsened in 22 joints (39.3%) and the disk reduction was also worsened in 32 joints (57.1%). As reported previously, improvement of the pain might due to the restoration of the disk mobility (Kirk et al., 1991). And it was said that by improving the disk mobility, without improving the disk position, the articulation in joint space might benefit and thereby alleviate the pain (Montgomery et al., 1991). From our results, we can say the pain could occur not only by the abnormal disk status. As for the joint pain there must be various kinds of factors, so clinicians do not have to pay attention only to the disk status during treatments. It might take longer time to make the pain decreased after treatment. There is possibility of difference in time between improvement in the pain and improvement in the disk status.

Although average IID was increased after treatment, there was no significant difference between before and after treatments. Increasing the IID did not always correspond to the improvement of the disk status. For example, if the disk was more anteriorly displaced, condyle had no longer obstruction. In these cases, posterior attachment of the TMJ would be elongated or perforated. It was said that the keys to the improvement of IID appeared to be loss of elasticity of the posterior attachment and release of the adhesive force in the joint space (Choi et al., 1994). On consideration of IID, not only disk status but also posterior attachment and joint space should be diagnosed in the MRI or other modalities.

In this study a few faults could be indicated concerning with the classifications. On the classification of the disk position, we did not consider any degree of the displacement, although we classified the

displacement disks into two groups, such as "anterio rly" or "supero-anterioly". As for pain, we classified into "yes" or "no", according to patients complaints without considering kinds, duration nor frequency of the joint noise. On the TMJ noise, we did not pay attention to any duration and frequency. We did not collect data in detail because there were lots of variations in the clinical findings.

It was reported that there was no relationship between improvement in clinical findings and improvement in the disk position after treatment (Perrott et al., 1990). The study was conducted using arthroscopy, and although their findings were interesting, the use of anesthesia in both arthroscopic and arthrographic methods may affect a patient's reported pain symptoms.

If these data collection is performed in detail, the result would be changed a little. And in the future study, we should diagnose the disk status more precisely with using higher resolution MR scanner. Moreover the condition of the posterior attachment and joint effusion which are considered to be linked clinical findings should be evaluated.

In conclusion, there was no relationship between change of the disk status and change of the pain and IID. Although diagnosing on disk position and reduction by MR imaging has been popular, we had better investigate not only the disk status. We should know to improve the disk position and the disk reduction is not main purpose of the treatment for TMJ disorder patients.

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Orthodontic treatment for patients with temporomandibular disorder

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1) Short title: Orthodontic treatment with TMD
2) Key words: orthodontic treatment, TMD, mandibular deviation, asymmetry

In an orthodontic clinic, we encounter diverse patients with mild crowding to severe malocclusion, starting from their early childhood to aged seniors. And it is one of our interests to actually estimate the number of TMD patients within our orthodontic patient pool. The investigation of pretreatment patients of the orthodontic clinic at the Tokyo medical and dental university showed that 37% of malocclusion patients with stable mandibular position indicate TMD symptoms. However, in case of patients with functional mandibular deviation, TMD symptoms were increased up to 59%. These results emphasize the importance of TMJ evaluation especially in patients with unstable mandibular position. In addition, high incidence of TMD was observed in mandibular asymmetry patients. Among other characteristics, asymmetry in the vertical dimension is significantly correlated to the TMD symptoms and therefore it is considered as an important contributing factor for TMD.

For a while, we aimed to establish systematic diagnostic steps for malocclusion patient with TMD by using diagnostic imaging such as MRI, cone beam CT along with functional analytic methods such as EMG and prescale (pressure-responsive film). Here, I would like to share some clinical steps in material taking, diagnosis and treatment flow and discuss measures for orthodontic patients associated with TMD.

Case 1

31-year-old male came to the clinic with the chief complaint of crossbite and pain on the masseter muscle. Tenderness pain was positive around the masseter and the posterior temporalis region. Movement restriction, TMJ sound was negative. From the EMG, spontaneous muscle activity of the masseter was detected even during the closing phase of jaw movement. Cognitive behavioral therapy for clenching along with splint to relieve pain by the raising the vertical dimension was used. Afterwards, orthodontic treatment was continued to establish ideal mandibular position followed by orthodontic treatment to establish appropriate mandibular position and occlusion.

Case 2

11-year-old girl came to the clinic with the chief complaint of upper anterior crowding and TMJ sounds. Reciprocal click on both TMJ was present, but tenderness pain or mandibular restriction was negative. Intraorally, premature contact of the malpositioned upper lateral incisor induced distal displacement of the mandible. From the MRI images, anterior and lateral displacement with reduction was confirmed. To correct the premature contact, upper arch was expanded and aligned. In addition, pumping manipulation was carried out to recapture the displaced disk.

Case 3

24-year-old female came to the clinic with the complaint of closed lock and facial asymmetry. Her TMD symptoms include bilateral click with intermittent lock on rising. Tenderness pain was present on the occipital region, sternocleidomastoid and temporalis muscle. Her upper right first premolar was extracted at the age of 16 to gain space for upper crowding, while one lower incisor was congenitally missing. From the MRI images, anterior displacement with reduction of the lower TMJ was noticed. For this case repositioning splint with open-closing exercise therapy was used to recapture the disk followed by orthodontic treatment to stabilize the occlusion.

Case 4

33-year-old female came to the clinic with anterior openbite. Mild pain on mandibular movement with click on the right TMJ was present. Mandibular restriction was negative. She had a history was bilateral TMJ pain with closed lock 5 years before coming in for consultation. Bilateral flattening of the condyle was observed from the CT images. She felt that the degree of her openbite worsened from around two years ago. The MRI indicated anterior displacement

without reduction on the right TMJ. Intraorally, no tooth contact was present anterior to the second premolar. After one year of close follow-up of the TMJ, we initiated the orthodontic treatment to establish ideal occlusion.

Mechanisms of Temporomandibular Joint-Osteoarthrosis (TMJ-OA) :Biomechanical, Histological and Biochemical Evidences

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ABSTRACT

Condylar resorption in the TMJ or TMJ-OA has been experienced occasionally in daily orthodontic practice and recognized to induce substantial influences on craniofacial morphology and the treatment outcomes. This study was designed to investigate the mechanisms of TMJ-OA by means of biomechanical, histological and biochemical approaches. Biomechanical study with finite element stress analysis revealed an existence of large compressive stresses in the anterior, middle and lateral areas on the condyle and prominent increases in the compressive stresses in association with vertical discrepancy of the craniofacial skeleton. Such skeletal discrepancy, simulated in growing rats by placing a metal plate on the upper molars, produced a decrease in the thickness of cartilage layers and an increase in the number of TRAP-positive cells, both of which lead to degenerative changes in the articular cartilage of the mandibular condyle. Furthermore, excessive tensile stresses, applied to articular chondrocytes with use of the Flexercell Strain Unit, induced an imbalance between matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs), which is assumed to induce lower resistance to external stimuli and degenerative changes leading to the resorption of bone and cartilage. It is thus shown that excessive or imbalanced mechanical loading on the TMJ components from occlusal and skeletal discrepancies induce various degenerative responses of cartilaginous tissues and articular chondrocytes, leading to the destruction of bone or cartilage in TMJ-OA.

KEY WORDS: temporomandibular joint disorder, osteoarthrosis, condylar resorption, mechanical stress, orthodontic treatment

INTRODUCTION

Temporomandibular joint disorder (TMD) has become an important disease in the field of dentistry and/or orthodontics (Egermark and Thilander, 1992; Greene, 1982; Tanne *et al.*, 1993). Under such clinical background, various studies have been conducted to elucidate the nature and causes of TMD in association with various etiologic factors (Arnett *et al.*, 1996; Artun *et al.*, 1992; Larsson and Ronnerman, 1981; Laskin, 1973; Ozawa *et al.*, 1999; Rinchuse, 1987; Sugiyama *et al.*, 1997). As a result, TMD is currently accepted as a multi-factorial disease, however, occlusal parameters have also been speculated to have a certain association with TMD (Riolo *et al.*, 1987; Roth, 1973).

Background of the present study

In order to elucidate the nature of TMD and the causative factors , we have been conducting a series of studies for the TMD-related problems or parameters. These subjects are 1) the nature and prevalence of TMD in orthodontic population, 2) association of malocclusion with TMD, 3) association of condylar position with disk displacement in TMD, 4) association of craniofacial morphology with TMD, and 5) influences of TMD, TMJ-OA in particular, on craniofacial morphology.

Firstly, prevalence of TMD was examined in the patients of our clinic. In this survey, the percentage of TMD patients to the total number of patients was approximately 14% (Ozawa, 1996). It is surprising to know very high prevalence of TMDs, which are mostly occupied by TMJ-internal derangement (ID) with various intra-articular pathologies, in adolescent patients with malocclusion, although most of the patients visited our clinic to undergo orthodontic treatment, without complaining of TMD symptoms. It is also of a clinical significance that adult population has higher prevalence (Nakamoto *et al.*, 1998), and jaw

deformity patients exhibit substantially higher prevalence (Nonoyama *et al.*, 1998) than adolescent patient group and asymptomatic adult volunteers, respectively. Furthermore, it is of a great interest that the prevalence of TMJ-OA is about 18 % in all the TMD patients and approximately 2.5 % in all the patients in our clinic (Ozawa, 1996).

The second topic is the association of malocclusion with TMD. The prevalence of TMD was considerably higher in open bite, deep bite, and posterior cross-bite (Tanne *et al.*, 1993). Thus, some specific types of malocclusion were significantly associated with the occurrence of TMD in the patient group. It is also suggested from this finding that condylar displacement in the TMJ space associated with these malocclusions may change disk position realtive to the displaced condyle and result in the onset of TMJ-ID.

As a next, we examined the association between condylar position in the TMJ space and pathologic status of TMJ-ID. Condylar position was more posterior in anterior disk displacement with reduction (AWWD), whereas concentric in anterior disk displacement without reduction (ADDWo) (Sugiyama *et al.*, 1997). It is indicated that condylar position is directly relevant to the disk displacement and the nature of TMJ-ID, or the progress in TMJ-ID from ADDW to ADDWo (Artun *et al.*, 1992; Ozawa *et al.*, 1999; Sugiyama *et al.*, 1997). These findings provide such clinical implication that repositioning of the condyle and disk should be performed as soon as possible in early-stage TMJ-ID, which is featured as a mild disk displacement with reduction associated with a clinical sign of early-type clicking during jaw movement.

Then, the association between craniofacial morphology and pathologic status of TMJ-ID was examined by means of a Spearman's rank correlation analysis. The size (Go-Me, r = -0.39) and position (SNB angle, r = -0.70) of the mandible presented significant negative correlations and the mandibular plane (SN/Mp, r = 0.39) and ANB (r = 0.57) angles exhibited significant positive correlations with the pathologic stages I through V defined by Wilkes (1989) (Ozawa, 1996; Ozawa *et al.*, 1994). It is shown from these results that the progress in pathologic status of TMJ-ID is highly related to more severe vertical discrepancy of the craniofacial skeleton, which is a morphologic feature of Skeletal 2 open bite with small and retropositioned mandible. It is thus speculated that vertical discrepancy of the craniofacial complex with less developed mandible and mandibular asymmetry are highly associated with the degree of TMJ-ID or the progress in pathologic status in the TMJ induced bilaterally and unilaterally, respectively (Nonoyama *et al.*, 1998; Ozawa, 1996; Schellhas, 1998).

The final topic is the influence of TMJ-OA or condylar resorption on craniofacial growth and morphology. The influence has already been demonstrated in the preceding section in terms of the association between craniofacial morphology and TMD. The evidence is examined here in an adolescent patient. This patient came to our clinic at the age of 16-year-old. She had a retrognathic lateral soft tissue profile due to Class II maxillary protrusion with small mandible. Since then, she came to our clinic twice, however, no treatments were performed. At the age of 28-year-old, 12 years after the initial visit, the retrognathic profile became more prominent in association with larger overjet. Changes in lateral soft tissue profile and craniofacial morphology are shown in Fig. 1. Please note backward and downward rotation of the mandible and the subsequent retrognathic profile, which are speculated due to progressive resorption of the condyle for the 12 years. These findings provide us with very interesting and useful clinical implications such that condylar resorption in TMJ-OA produces jaw deforminty with less developed and distally located mandible and affects the outcomes and stability of orthodontic treatment (Arnett *et al.*, 1996; Nonoyama *et al.*, 1998; Ozawa, 1996; Schellhas, 1998).

Another evidence is presented for a 21-year-old female (Tanaka *et al.*, 2000). She had TMJ pain in the left TMJ, muscle tenderness for the left masseter and difficulty in jaw opening. The amount of maximum mouth opening was 33.0 mm. Molar relation was Angle Class II and overjet and overbite were 6.0 mm and -3.0 mm. Open bite was found at the anterior to the premolar region (Fig. 2A). On the tomogram of left condyle, .severe flattening was observed on the anterior surface (Fig. 2B). On the MR images, anterior displacement of the disk without reduction and disk deformity were observed for both TMJs.

From these examinations, this case was diagnosed as ADDWo or stage IV TMJ-ID or TMJ-OA (Wilkes, 1989). Posterior bite splint was first used with manipulation to the TMJ to induce counter-clockwise rotation of the condyle and mandible followed by orthodontic occlusal reconstruction, aiming to reduce TMJ loading in the anterior region where condylar resorption was prominent. As a result of a series of treatment, stable occlusion was obtained (Fig. 3A), although disk repositioning was not achieved as was expected before treatment (Tanaka *et al.*, 2000). It is surprising that the left condyle was reformed or exhibited unexpected adaptive responses (Fig. 3B). It is demonstrated that the sable occlusion achieved by orthodontic occlusal reconstruction has produced biomechanical equilibrium in the TMJ and subsequently provided the condyle with a potential for adaptive or functional remodeling (Arnett *et al.*, 1996; Tanaka *et al.*, 2000).

Hypothesis

These findings are very useful for understanding the nature of TMD and can be used as a background for the following studies to elucidate the mechanisms of TMJ-OA, indicating that changes in occlusion and the relevant condylar position may produce disk displacement leading to TMJ-ID. It would also be hypothe-

sized from these findings that such occlusal or skeletal discrepancy produces an increase in TMJ loading, which further leads to degenerative changes in the articular cartilage of the mandibular condyle and resorption of bone and cartilage expressed as TMJ-OA.

The purpose of this study

This study was designed to elucidate the mechanisms of bone or cartilage resorption in the mandibular condyle in TMJ-OA by means of biomechanical, histological, and biochemical approaches.

MATERIALS & METHODS

Finite element analyses for TMJ loadings in response to vertical skeletal discrepancy

A three-dimensional model of the mandible including the TMJ was constructed for stress analysis with finite element method (FEM) from a young human dry skull (Tanaka *et al.*, 1994). The model is consisted of 2088 nodes and 1105 solid elements (Fig. 4). Hereafter this model is referred to as a standard model. For loading conditions, the magnitude of muscle forces was determined to exert a resultant force of 500 N, simulating the maximum clenching. During loading, the model was restrained at the superior region of the temporal bone to avoid sliding movement of the model. Modeling procedure in detail and the accuracy are described elsewhere (Tanaka *et al.*, 1994).

For stress analysis, the standard model was modified to represent vertical discrepancies of the craniofacial complex by changing the shape of mandible, maintaining the number of nodes and elements for the standard model (Tanne *et al.*, 1995). The gonial angle was changed from 110.1 to 134.1 degrees with a 6.0-degree increment (mean; 122.1 degrees). The mandibular plane angle to the Frankfort horizontal plane (FMA) was similarly varied from 18.5 to 42.5 degrees with a 6.0-degree increment (mean; 30.5 degrees). Stress analysis was executed on a personal computer with the FE software, ANSYS from ANSYS Inc. (Houston, USA). Three principal stresses were analyzed on the standard and modified models for the condyle, disk and glenoid fossa. The stresses were evaluated for five (anterior, middle, posterior, medial, and lateral) areas of the TMJ in association with various skeletal patterns.

Histomorphometric analyses for condylar responses to vertical skeletal discrepancy in growing rats

Thirty 4-week-old male Wistar strain rats were used. Under general anesthesia with sodium pentobarbital, a one mm-thick metal plate was bonded onto the occlusal surface of the maxillary molars to induce a backward and downward rotation of the mandible and to increase the TMJ loading on the concyle (Fig. 5) (Sugiyama *et al.*, 1999). Lateral cephalograms were taken of all the rats using a rat and mouse cephalometer (Asahi Roentgen, Kyoto, Japan) for morphometric analyses of the mandible.

For histological and histochemical examinations, the head of each animal was dissected and cut into serial frontal and sagittal sections of 6 μm thickness. The sections were stained with tartrate-resistant acid phosphatase (TRAP) and hematoxylin-eosin (H-E) for histomorphometric analyses of the thickness of cartilage layers, and the number of TRAP-positive osteoclasts (Sugiyama *et al.*, 1999).

Biochemical examination of condylar cartilage cells in response to a high magnitude cyclic tensile stress

Chondrocytes were isolated from the surface and middle zones of the knee joint cartilage of 4-week-old Japanese male rabbits. The cells were seeded at a cell density of 5 x 10^4 per 25 mm Flexercell plate dish. A high magnitude tensile stress of 17 kPa was applied, at a frequency of 30 cycles/minute, to the chondrocytes in the Flexercell type I flexible-bottomed dishes using the Flexercell strain unit (Flexcell Corp. Mckeesport, USA) for 12hr or 24hr (Fig. 6) (Honda *et al.*, 2000).

We examined the protein level of cartilage matrixes and the gene expression of matrix metalloproteinases (MMPs), tissue inhibitors of matrix metalloproteinases (TIMPs) and proinflammatory cytokines such as IL-1b and TNF-a in loading with the high magnitude cyclic tensile stress (Honda *et al.*, 2000). Control cultures were cultivated for the same period using Flexercell type II rigid-bottomed dishes.

RESULTS

TMJ loadings in response to vertical skeletal discrepancy

Mean stresses are shown for the condyle, disk and glenoid fossa (Table 1). Large compressive stresses were induced by the maximum clenching in the anterior, middle and lateral regions, whereas tensile stresses were found in the remaining areas (Tanaka *et al.*, 1994).

Meanwhile, these stresses were changed in association with varying mandibular plane angles (FMAs) and exhibited more substantial changes than those with varying gonial angles (Tanne *et al.*, 1995). Changes in the stresses were nonlinear and particularly drastic when the angle became more than 36.5 degrees. Mean stresses on the condyle and glenoid fossa were almost constant within the range from 18.5 to 36.5 degrees, and increased substantially in the anterior and posterior regions with FMAs greater than 36.5 degrees (Fig. 7).

It is shown that the maximum clenching produces large compressive stresses in the anterior and lateral areas of the condyle, where idiopathic condylar resorption is observed most frequently. It is also

demonstrated that vertical skeletal discrepancy induces an increase of TMJ loading and lack in biomechanical equilibrium for the TMJ components.

Histomorphometric changes in rat condylar cartilage layers from simulated vertical skeletal discrepancy

At the end of the experiment, decreased ramus height and large gonial angle were found in the experimental group, demonstrating an appearance of vertical skeletal discrepancy with less developed mandible (Sugiyama *et al.*, 1999).

During the initial phase of experiment, the thickness of proliferative and maturative/hypertrophic zones in the anterior and superior regions of the condyle was significantly smaller than in the controls (Table 2). The number of TRAP-positive cells was significantly greater in the experimental group than in the controls at the initial phase of experimental, and then adaptive responses were induced up to the end of experiment (Fig. 8) (Sugiyama *et al.*, 1999).

From these findings, it is shown that biomechanical changes in the intra-ariticular environment associated with vertical skeletal discrepancy influences or inhibits cartilaginous growth of the condyle or mandibular growth to a considerable extent, if induced during growing period.

Responses of condylar cartilage cells to a high magnitude cyclic tensile stress

A change in cell morphology from a polygonal to spindle-like shape was observed. Toludine blue staining, type II collagen immunostaining, and an assay of the incorporation of [^{35}S] sulfate into proteoglycans revealed a decrease in the level of cartilage specific matrices in chondrocyte cultures subjected to high magnitude cyclic tensile stress (Fig. 9). Furthermore, PCR-Southern blot analysis showed that the high magnitude cyclic tensile stress increased mRNA levels of MMP-1, MMP-3, MMP-9, IL-1b, TNF-a and TIMP-1 in the cultured chondrocytes, while the levels of MMP-2 and TIMP-2 were unchanged (Fig 10) (Honda *et al.*, 2000).

It is shown that excessive stresses induce changes in cartilage cell morphology, reducing a synthesis of cartilage matrices such as type II collagen and proteoglycan, leading to lower resistance of cartilage tissues to external stimuli. It is also demonstrated that induction of MMPs and proinflammatory cytokines and quantitative imbalance between MMPs and TIMPS directly produce the destruction of cartilage matrices leading to bone or cartilage resorption on the mandibular condyle.

DISCUSSION

TMD has been regarded as one of important diseases in dentistry. In the fields of orthodontics, TMD has become an important topic in terms of the substantial influences on stomatognathic functions, craniofacial morphology and orthodontic treatment outcomes (Roth, 1973; Janson and Hasund, 1981; Ozawa, 1996; Riolo *et al.*, 1987; Sadowsky and BeGole, 1980; Tanaka *et al.*, 2000).

Among TMDs, internal derangement of the TMJ is the most prevalent in orthodontic population during growing period (Ozawa, 1996), therefore, it is of a great significance to elucidate the causative factors, while TMD is a multi-factorial disease in nature. According to our previous studies, it is emphasized that a certain type of malocclusion with a lack in occlusal stability produces condylar displacement in the TMJ space, and then disk displacement is induced as the condyle occupies concentric position (Sugiyama *et al.*, 1997; Weinberg, 1979). Another explanation for TMJ-ID, derived from biomechanical studies on joint friction and synovial lubrication, is that pathologic changes in the TMJ space generate reduced viscosity of synovial fluid and greater friction, which finally induce disk displacement in TMJ-ID (Kawai *et al.*, 2004; Tanaka *et al.*, 2004 and 2005a).

As a result of the present study, it is shown that excessive TMJ stress, induced by vertical discrepancy of the occlusion and craniofacial skeleton, directly changes the metabolism of cartilage by reducing the matrix components and causing a quantitative imbalance between MMPs and TIMPs. It is also confirmed that excessive mechanical loading is essentially responsible for degenerative change and the subsequent resorption of bone and cartilage in the TMJ, whereas optimal mechanical stresses of 7 and 10 kPa significantly enhanced cell proliferation and syntheses of collagen and proteoglycan (Tanaka *et al.*, 2005b).

Based upon these findings, the mechanisms of TMJ-OA are summarized as a sequence depicted in the flow chart (Fig. 11). It is well understood that condylar resorption substantially affects the occlusion and maxillo-mandibular relation and produces jaw deformity in extreme cases, as was demonstrated in an adolescent case shown in Fig. 1 (Arnett *et al.*, 1996; Ozawa, 1996; Qzawa *et al.*, 1999; Schellhas, 1989). Condylar resorption, if induced by various factors at once, affects intra-articular mechanical environment which further induces degenerative changes and condylar resorption in a progressive manner. If such a sequence is interrupted by a certain treatment with an aid of sufficient host remodeling capacity, a functional and adaptive remodeling may be achieved as shown in Fig. 3 (Arnett *et al.*, 1996; Tanaka *et al.*, 2000).

For the treatment of TMJ-OA, we firstly have to perform appropriate and accurate examinations enough for differential diagnosis (Ozawa, 1996; Ozawa and Tanne, 1997). In addition to the conventional examinations, a highly advanced biochemical examination of urinary bone resorption markers (pyridinoline and deoxypyridinoline) has recently been used for the detection of bone or cartilage destruction in TMJ-OA

(Imada *et al.*, 2003; Tanimoto *et al.*, 2004). During a series of treatment after differential diagnosis, we have to achieve TMJ unloading or the biomechanical equilibrium by means of condylar repositioning and orthodontic occlusal reconstruction, although disk repositioning may not be succeeded in most cases. Thus, orthodontic approach is confirmed as an effective tool for the achievement of optimal intraarticular environment secured by stable occlusion, if performed appropriately without producing adverse influences on TMJ structures and functions (Artun *et al.*, 1992; Rendell *et al.*, 1992; Wyatt, 1987).

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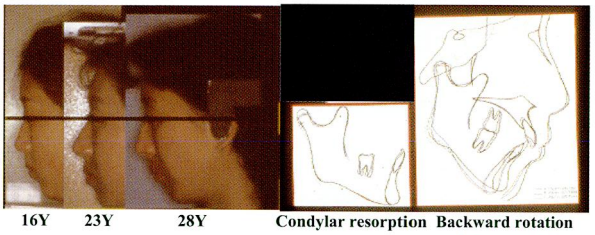

Fig. 1 A clinical evidence of TMJ-OA in adolescent female of 16-year-old and the changes in the lateral profile and craniofacial morphology for 12 years

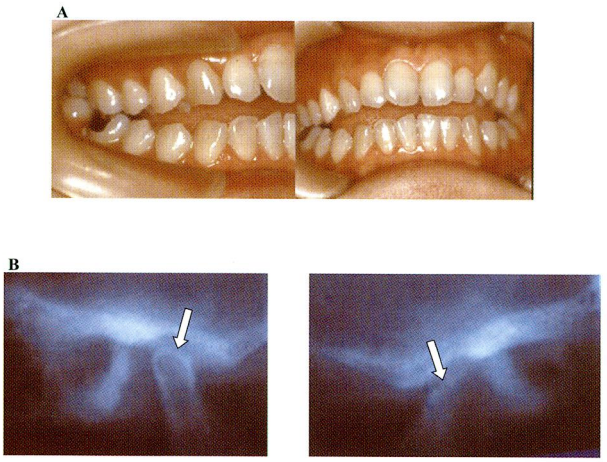

Fig. 2 An adult open bite case with TMJ-OA before treatment
A: Intraoral photos
B: Lateral tomograms, arrows indicate resorption of the condyle

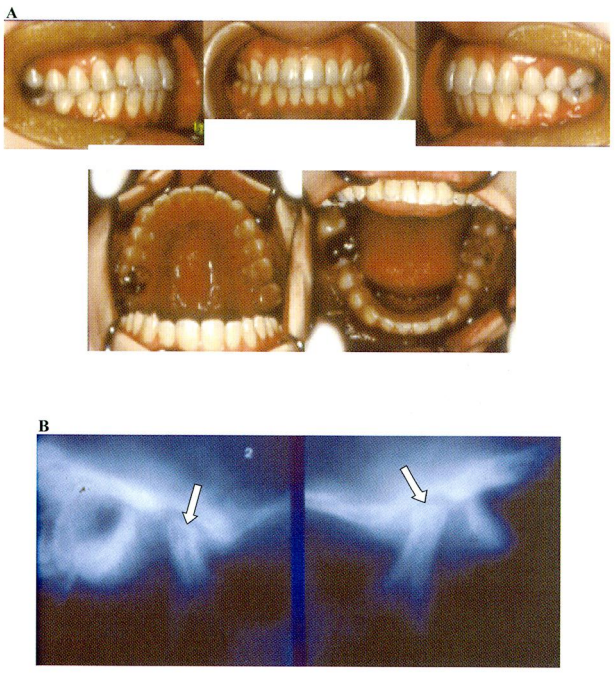

Fig. 3 An adult open bite case with TMJ-OA after treatment
A: Intraoral photos, stable occlusion is obtained.
B: Lateral tomograms, arrows indicate functional and adaptive remodeling of the condyle

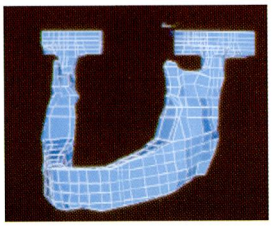

Fig. 4 A three-dimensional finite element model of the mandible including the TMJ

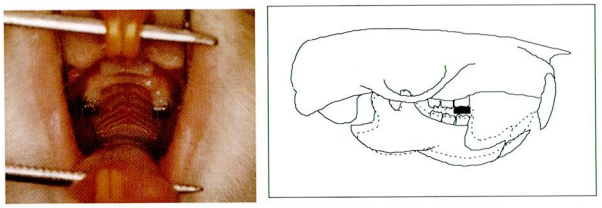

Fig. 5 Experimental appliance to induce backward and downward displacement of the mandible, simulating vertical skeletal discrepancy which is assumed to increase the TMJ loading

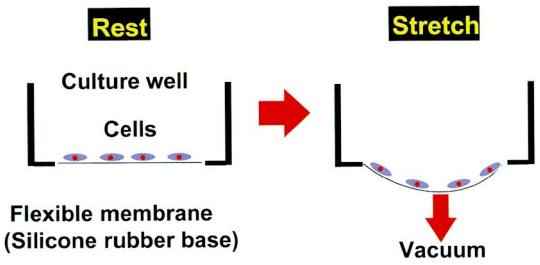

Fig. 6 Vacuum-induced flexion cycling with a Flex I culture plate membrane to apply a high magnitude cyclic tensile stress on cultured articular chondrocytes

Mean Stresses in the TMJ

	Anterior	Middle	Posterior	Lateral	Medial
Condyle	-1.642	-0.543	0.664	-1.017	0.521
Glenoid fossa	-0.440	-0.410	0.445	-0.351	0.103
Articular disk	-0.403	-0.200	0.258	-0.342	0.041

(Unit : MPa)

Table 1 Mean stresses on the TMJ structures

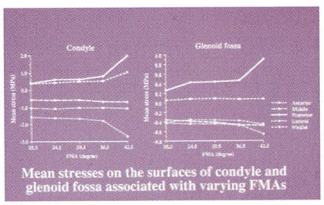

Fig. 7 Changes in the mean stresses on the condyle and glenoid fossa in association with vertical skeletal discrepancy simulated by varying mandibular plane angles

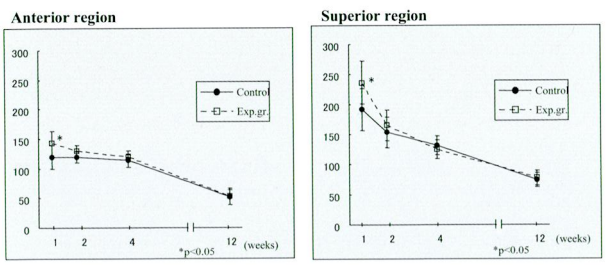

Fig. 8 Changes in the number of TRAP-positive cells in the cartilage layers on the mandibular condyle in response to vertical skeletal discrepancy, which is assumed to increase TMJ loading

Table 2 Changes in the thickness of articular cartilage layers in the anterior region of the mandibular condyle in response to vertical skeletal discrepancy

	Experimental period (weeks)							
	1		2		4		12	
	Control	Exp. gr.	Control	Exp. gr.	Control	Exp. gr.	Control	Exp. gr.
Fibrous layer	37.0 (11.6)	22.5 * (7.4)	27.8 (8.7)	20.2 (8.2)	26.9 (7.5)	27.0 (7.6)	32.1 (5.5)	31.9 (9.2)
Proliferative layer	44.4 (7.9)	29.2 ** (5.7)	35.9 (2.6)	29.2 ** (4.6)	35.4 (6.0)	29.7 (7.2)	38.8 (8.8)	31.3 (7.3)
Maturative/ hypertropic layer	173.6 (14.5)	147.5 * (21.9)	142.3 (21.8)	112.5 ** (12.0)	105.4 (18.5)	103.8 (9.3)	95.3 (15.7)	85.2 (15.2)
Total	255.0 (24.0)	199.3 ** (31.1)	205.9 (31.1)	161.9 ** (21.0)	168.2 (18.4)	159.8 (21.1)	167.6 (17.3)	148.4 (18.5)

( ); SD, *p<0.05, **p<0.01 (Unit : μm)

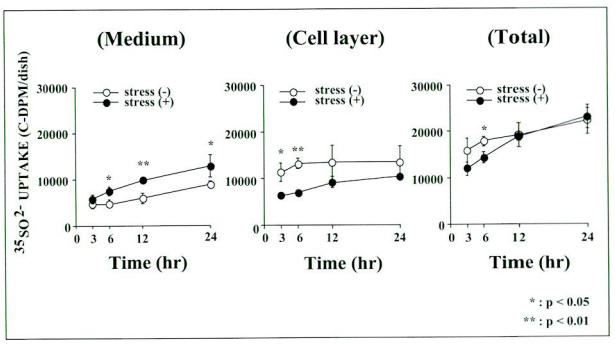

Fig. 9 Effect of a high magnitude cyclic tensile stress on proteoglycan synthesis in cultured rabbit articular chondrocytes

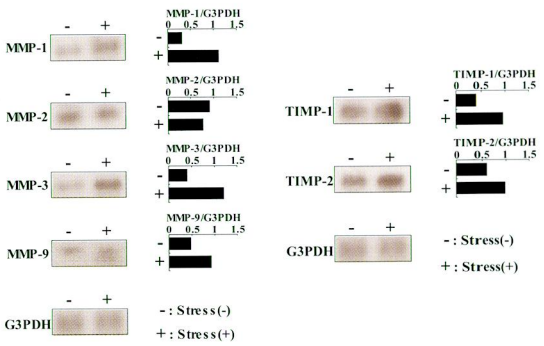

Fig. 10 Effects of a high magnitude cyclic tensile stress on the gene expression of MMPs and TIMPs in cultured rabbit articular chondrocytes

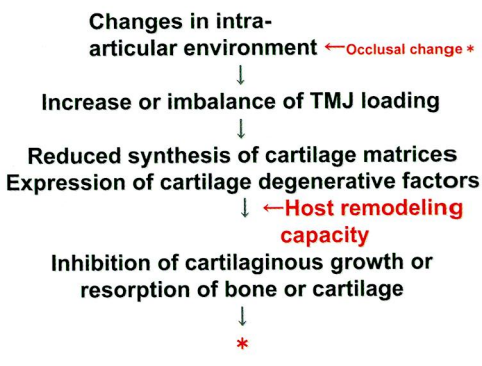

Fig. 11 Mechanisms of condylar resorption

Differential Diagnosis of TMJ Diseases and their Allied Conditions

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Various conditions require discrimination between diseases of the maxillo-facial and cervical regions. Above all, diseases related to the temporomandibular joint (TMJ) appear as disorders of function rather than tissue obstacles, and are thus represented by TMD. Difficulties in differentiation are not uncommon, partly because of the addition of psychogenic ornamentation.[1, 2]

In recent years, advances in diagnostic imaging have facilitated great progress in TMJ diseases, yielding useful information for differential diagnosis. However, making suitable use of the available information is important for correct diagnosis, requiring appropriate choices of examination based on detailed history and careful inspection of existing symptoms. Experience and knowledge are also known to exert a significant influence.

Differential diagnosis of TMJ diseases by signs and symptoms and related or associated disease of the TMJ are presented herein, mainly based on cases.

1. Essential points to distinguish diseases of the TMJ

Although observations of each image on radiographic views are important, attention must be paid to making a diagnosis that puts all findings together, such as the past history, present status and progress of symptoms. Furthermore, differential diagnosis tends to be difficult when patient condition involves psychogenic factors compounding physical findings.[3]
Essential conditions for differential diagnosis peculiar to the TMJ itself are as follows:

1) Does imaging diagnosis conform with morphological tissue changes in the TMJ lesion? Investigations with radiography (CT, arthrography) and magnetic resonance (MR) imaging are necessary. Occasionally, biochemical investigations and RI scans are helpful.
2) Is obstacle accompanied by functional disturbances of jaw movements such as deviation of the jaw on opening, chewing pain and trismus?
3) Do changes of the TMJ on imaging correspond to functional disturbances?
4) Are functional disturbances linked with pain?
5) Can psychogenic factors be ruled out?

2. Diseases of the TMJ not peculiar to the TMJ itself

1) Disturbance of mouth opening (trismus)

Various organs and tissues are relevant to opening and closing of the mouth, and transient and sustained disturbances of these can produce disturbances in opening movements. Opening disorders can involve various components, including masticatory muscles, the nervous system, and hard tissue of the maxilla and mandible. Obstacles can thus comprise soft tissues in addition to the TMJ itself.

Disturbance of mouth opening as the main sign

Square-shaped mandible[4, 5].

Manifestation of this disease, named by Isberg (1990), is characterized by severe disturbance of mouth opening and increased width of the mandibular ramus, which seems square in appearance. Cephalometric analysis reveals a small gonial angle and small angles between the SN and mandibular planes. No evidence of adhesions is seen in the TMJ joint cavity.

Elongation of the coronoid process (Trismus-pseudocamptodactyly syndrome)[6]

Elongation of the coronoid process collides with the maxillary or zygomatic bone and causes disturbance of mouth opening. As only partial symptoms of the syndrome may be present, attention is necessary to identify any sign of a hereditary background.

Tetanus

Clostridium tetani entering from a deep wound can produce neurotoxin that exerts actions on the central nervous system, causing spasm of the masticatory muscles. Strong opening obstacle (trismus) is characteristic.

Other disturbances of mouth opening by disease, not primarily originating from the TMJ itself:

Inflammatory: Acute (osteomyelitis, periosteitis and cellulitis of the maxillofacial region) and chronic (osteomyelitis, actinomycosis of the maxillofacial region).

Contracture of scar tissue: Following trauma, operation or radiation to the maxillofacial region

Tumor invasion to TMJ, masseter or adjacent tissues of the TMJ.

Masticatory muscle diseases: Difficulty of mouth opening caused by diseases of muscle itself, such as dermatomyositis and muscular dystrophy

Psychiatric diseases: Epilepsy, clonic and tonic convulsions

Drug induced: Oral dyskinesia, an adverse effect of psychiatric drugs

Trauma: Fractures of the mandible, contusion of the maxillofacial region

3. Pain in the cervical, TMJ and temporal regions not originating from the TMJ itself

Distinguishing whether a pain might originate from a disease of the TMJ itself or not is very important in differential diagnosis. Strict attention must be given to the course, properties, and presence of triggers of pain in connection with jaw movements. The following diseases that involve pain in the maxillofacial region must be distinguished:

Diseases of the teeth: pulpitis, periodontitis, pericoronitis of molars

Trigeminal neuralgia (including post-herpetic neuralgia): produces pain in the cervical region bilaterally, glossopharyngeal neuralgia

Diseases of the ears, nose and paranasal sinuses: otitis media and inflammation of the external auditory meatus

Salivary gland disease (sialoadenitis)

Lymphadenitis: anterior and posterior auricular lymph nodes, parotid lymphadenitis

Various headaches[7]:

Muscle-contraction headache (MCH)

Migraine headache, cluster headache

Tumor (tumor in the skull or central nervous system)

Blood vessel disorder (carotidynia, temporal arteritis)

Elongation of the styloid process (Eagle syndrome)

Pain around the angle of the mandible and noise on mouth opening due to pseudarthrosis of the elongated styloid process.

Carotidynia[8]

Carotidynia is characterized by intense pain in the carotid artery region and sometimes face and mandible, and was described by Fay in 1927. This condition is thought to be caused by stimulation of sympathetic nerves of the blood vessels, and can be categorized as type I,II or III.

4. Diseases of the TMJ diagnosed according to conditions peculiar to the TMJ itself

1) Inflammation-related disease

Suppurative arthritis of the TMJ[9]: Primary suppurative arthritis of the TMJ is very rare, and typically involves extension from suppurative inflammation of adjacent organs and tissues.

Rheumatoid arthritis of the TMJ[10]

Rheumatoid arthritis in the TMJ appears as local symptoms of connective tissue disease of the whole body. Initial manifestations in the TMJ are uncommon, but morbidity rate of the TMJ is high when symptoms appear in the other joint (the hands).

Crystal-induced inflammation - gout and pseudogout of the TMJ

Accompanied by joint sharp pain and formation of a gout node causing characteristic repeated acute joint pain as a first symptom. Symptoms are caused by hyperuricemia, due to abnormalities of uric acid metabolism. Family history may be present.

Gouty arthritis and gout nodes may be apparent in the first metatarsophalangeal joint, but are extremely rare in the TMJ.

Pseudogout of the TMJ - calcium pyrophosphate deposition disease (CPPD)[11]

Deposition of crystal of calcium pyrophosphate in joint tissue causes pseudogout, with deformative changes and spot-like and linear calcification images, and sometimes mass depositions, in the joint tissue. (Fig1-3) Few cases involving the TMJ have been reported. Findings of polarized light microscopy and electron microprobe analysis (EMPA) of joint fluid are useful for differential diagnosis from suppurative arthritis.

2) Tumor and tumorous conditions of the TMJ

Osteochondroma and chondroma: These are relatively frequent in the TMJ, involving enlargement of the condyle, and hyperplasia of condyle, growth abnormality and osteoma are also not particularly rare. Having both characteristics (hyperplasia and tumor) distinguishing enlargement from osteoma is difficult.

Fibrous dysplasia of bone: Usually the maxillary or mandibular body are affected, but invasion to the mandibular condyle is rare. In rare cases, this is accompanied by deformation of the condyle or temporal bone and an opening disorder can result.

Synovial (osteo)chondromatosis of the TMJ[12]: Metaplasia of cells in the synovial membrane produces a cartilaginous mass and lump in the joint cavity. (Fig.4)

Osteoid osteoma of the condyle[13]

This is rare in long bones, and development in the TMJ is extremely rare. The tumor forms rich osteoid tissue, and the main signs are nidus formation and a bone hardening image of the circumference on radiography, with a characteristic of sharp pain in the night. (Fig.5)

Eosinophilic granuloma of the condyle may rarely occur in the TMJ, appearing with sharp pain and the appearance of bone destruction on radiography.

3) Others

Avascular necrosis (AVN) of the condyle[14, 15]

Disturbance of a nutrient artery at the condyle, first reported in the TMJ by Schellhas (1989), shows as severe destruction of the condyle on radiography and MR imaging.

Summary

Numerous conditions and cases involving facial pain, including the TMJ, and masticatory disturbances, mainly as disturbance of mouth opening, have been identified using regional diagnostic approaches. The main signs and symptoms in TMJ diseases are pain and functional disturbance of the masticatory system. Careful evaluation of the history, characteristics of pain and trismus may reduce the number of etiological possibilities in differential diagnosis. Furthermore, image diagnosis and even biochemical investigations can assist in the differential diagnosis. Evaluation of mental factors that may contribute to the problem is also important, since the signs and symptoms of the maxillofacial region are readily affected by emotional influences. Most important are knowledge and awareness of the various conditions that may cause pain and functional disturbances, so that serious problems are not carelessly overlooked.

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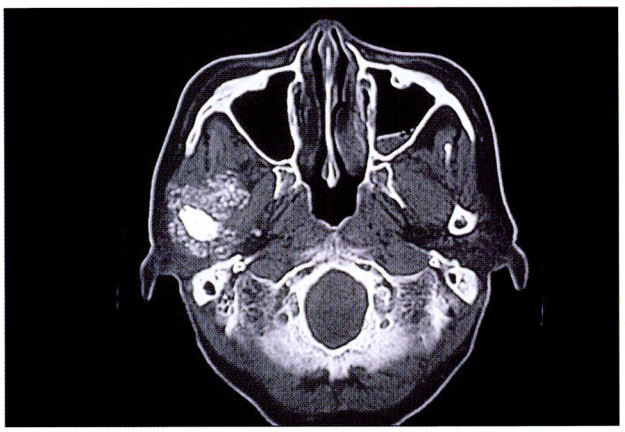

Fig. 1 CPPD of the TMJ: Axial CT image reveal a large nodular mass around the right TMJ.

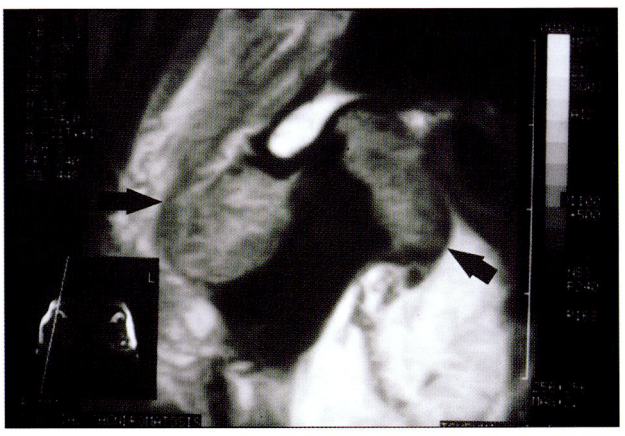

Fig. 2 CPPD:T1 MR image of the right TMJ shows amorphas mass surrounding the condyle.

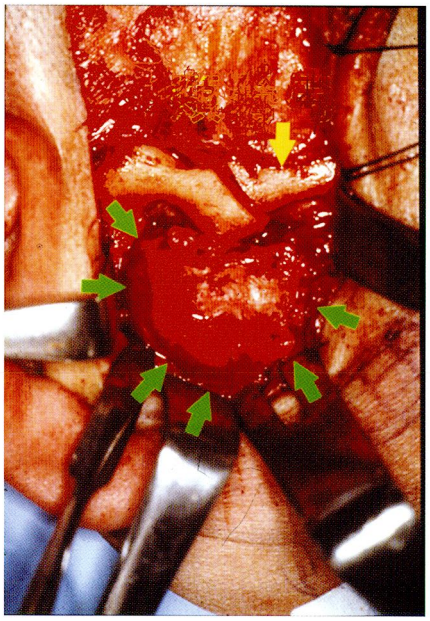

Fig. 3 CPPD:Exicion of the tumor (arrows) at surgery

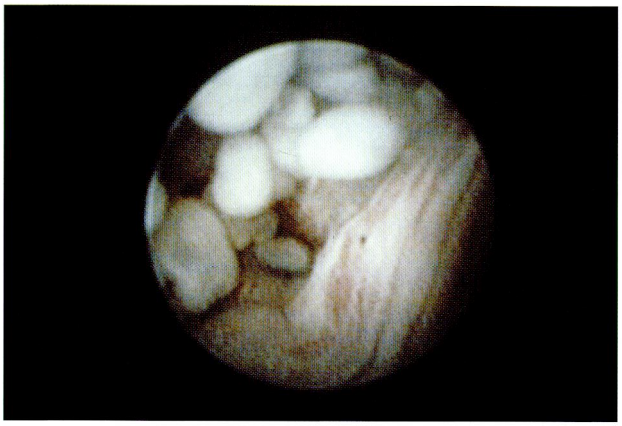

Fig. 4 Chondromatosis of the TMJ (arthroscopic finding in the joint cavity)

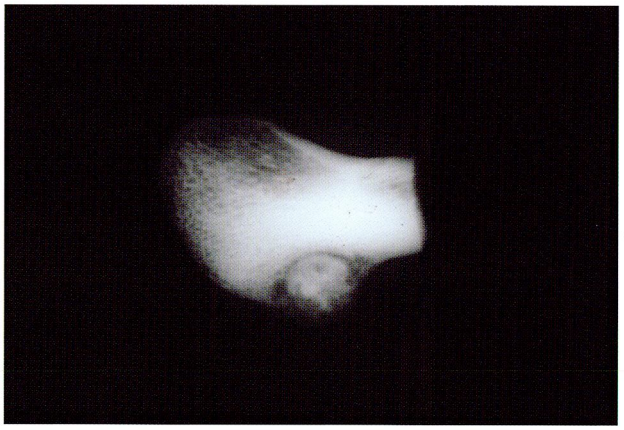

Fig. 5 Soft X-ray imaging of Osteoid osteoma (amptated spacimen)

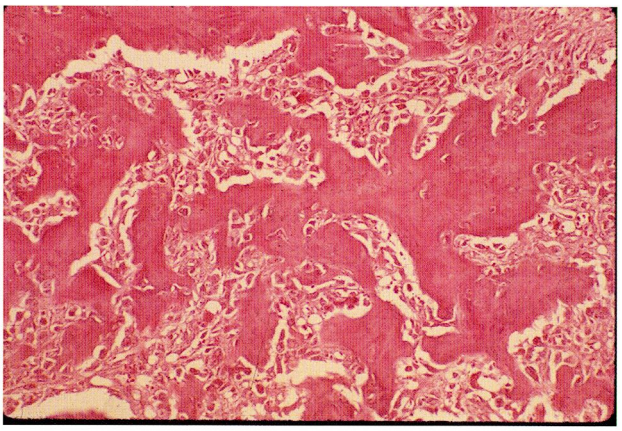

Fig. 6 Histopathogical finding of osteoid osteoma.

Total temporomandibular joint replacement in patients with rheumatoid arthritis

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Key words: rheumatoid arthritis, obstructive sleep apnea, total temporomandibular joint replacement, artificial temporomandibular joint

Introduction

Destruction of the temporomandibular joint (TMJ) is observed in many rheumatoid arthritis (RA) patients (Chalmers and Blair, 1973). Morphological changes may cause obstructive sleep apnea (OSAS), which can lead to sudden death during sleep (Redlund-Johnell, 1988). We performed total TMJ replacement in six patients with RA who had TMJ destruction to improve respiratory status and occlusion (Sugahara et al, 1994; Mishima et al, 2003).

Materials and Methods

All six patients had retrognathia and anterior open bite and could not masticate solid food.

The artificial TMJ consisted of an artificial glenoid fossa, custom-made of high-density polyethylene, and an artificial mandibular condyle, ready-made of Vitallium® alloy (Figure 1). The TMJ was approached through a preauricular incision. The posterior mandibular ramus was exposed up to the mandibular condyle through a submandibular incision. After intermaxillary fixation, the artificial TMJ was placed. The artificial fossa was adjusted and attached to the glenoid fossa with three titanium screws. The artificial condyle was then attached to the mandibular ramus. The posterior airway space (PAS), ramal height and respiratory status were measured preoperatively, 1 month postoperatively, and more than 1 year postoperatively. As for respiration, oxygen saturation and apnea and/or hypopnea index were measured.

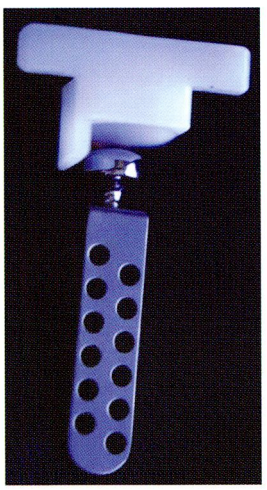

Figure 1. Artificial TMJ. The artificial TMJ consisted of an artificial glenoid fossa made of high-density polyethylene and an artificial mandibular condyle made of Vitallium® alloy.

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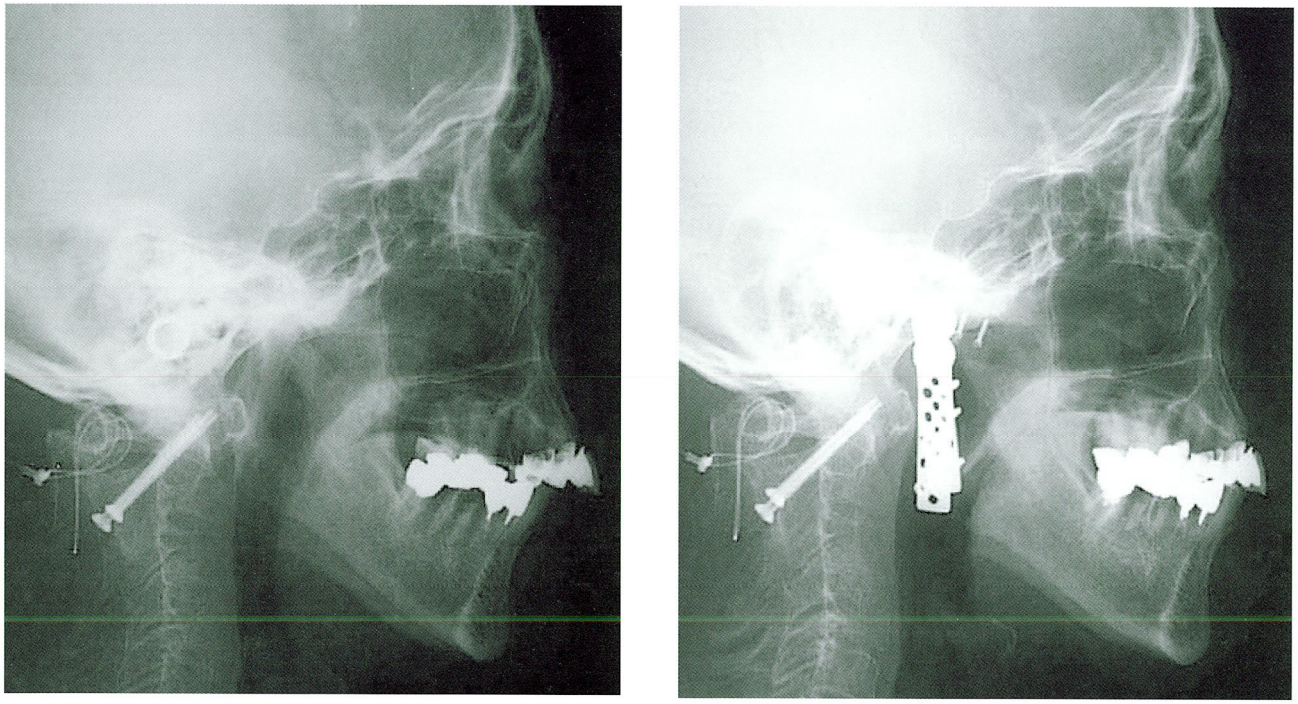

Figure 2. Lateral cephalograms obtained preoperatively and 1 month postoperatively.
Preoperative lateral cephalograms showing morphological changes, including retrognathia, an anterior open bite, low ramal height, and a narrow PAS. After total TMJ replacement, the occlusion and ramal height improved, and the posterior airway space increased.

Results

In all patients, symptoms such as snoring and daytime sleepiness improved after total TMJ replacement. In one patient, the tracheal tube was subsequently removed, and the tracheostomy stoma closed. After removal of intermaxillary fixation, all patients were able to masticate solid food.

Lateral cephalographic measurements revealed that both PAS and ramal height significantly improved postoperatively (Figure 2). The PAS of two patients decreased during the year after surgery; ramal height was unchanged. The PAS did not decrease after surgery in the other two patients.

Mean oxygen saturation 1 month postoperatively had significantly improved as compared with the value before surgery. However, oxygen saturation 1 year postoperatively did not differ significantly from the preoperative value.

Discussion

In patients with RA, upper airway obstruction can be caused by atolantoaxial subluxation and TMJ destruction (Redlund-Johnell, 1988). Postoperative cephalograms obtained more than 1 year after surgery demonstrated that the PAS had decreased in two patients. Apnea indices in the two patients who had not undergone posterior spinal fusion improved immediately after total TMJ replacement, but worsened subsequently. In the other two patients, who had undergone posterior spinal fusion before total TMJ replacement, indices improved. Future studies should investigate the optimal timing of treatment for TMJ destruction and atolantoaxial subluxation.

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Is tissue engineering of the TMJ disc a feasible process?

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Abstract

Temporomandibular joint (TMJ) disorders are common and difficult to remedy. Tissue engineering is one alternative that seeks to improve TMJ surgical treatment options. Tissue engineering aims to replace diseased or injured tissue with biologically engineered constructs. These constructs should reproduce native function and limit an immune response. To achieve tissue engineering success, it is important to first understand the tissue's cellular, biochemical, and mechanical properties in order to create validation and design criteria. Reviewed herein are the known properties of the TMJ disc and initial attempts toward TMJ disc tissue engineering. Important aspects of tissue engineering are scaffold selection, cell source, biochemical factors, and mechanical stimuli.

Motivation

The temporomandibular joint (TMJ), or jaw joint, is used throughout normal everyday functions such as eating or talking. Thus, disease or injury of this joint greatly decreases a patient's quality of life. Common activities become difficult and painful for patients with a TMJ disorder (TMD). The prevalence of TMJ dysfunction is surprisingly high; based on various epidemiological studies, 28-88% of the population exhibit some physical sign or symptom of a TMJ dysfunction (Solberg *et al.*, 1979).

Around one-fifth of patients exhibiting symptoms seek medical treatment for TMDs (Gray *et al.*, 1995). In the United States, there is an estimated 10 million TMD patients (TMJ Implants - A Consumer Information Update [FDA Report], 1999); around 70% of patients seeking treatment exhibit a displaced TMJ disc (Farrar and McCarty, 1979). Figure 1 illustrates the five stages of TMJ disc internal derangement as described by Wilkes (1989); the patient population from this study had an average age of 31 years and a female to male ratio of 7:1, common characteristics of the TMD patient population.

In addition to joint pain, TMD symptoms include headaches, earaches, jaw clicking, limited jaw opening, and jaw lock (Farrar and McCarty, 1979; LeResche, 1997; Solberg *et al.*, 1979). Unfortunately, TMD symptoms offer little aid in understanding the cause of TMDs. Numerous treatment options for TMD patients exist, but standard approaches and treatments are rarely agreed upon, even among experts. TMJ treatments and surgical approaches are presented in greater detail in reviews by Wong *et al.* (In Press) and Dimitroulis (2005). Briefly, non-surgical options are the first treatment modality and include pain medication and physical therapy. Minimally invasive surgery, like arthrocentesis or arthroplasty, may be attempted in dysfunctional joints with limited tissue degradation; these procedures aim to reduce inflammation or repair the disc/attachments. When the disc is beyond repair, it may be removed (discectomy). Post-discectomy the joint may be left empty or replaced with autologous tissue. Synthetic discs are no longer implanted due to extensive wear and immune response (Trumpy *et al.*, 1996). In the most extreme cases of degeneration, patients may opt for total joint replacement. Unfortunately, many TMDs are progressive, leading to extensive joint remodeling. Treatments primarily focus on the reduction of pain. This leaves the field of TMJ research primed for tissue engineering alternatives that have the potential to reduce pain and restore total function.

Disc characteristics

The TMJ disc is located between the mandibular condyle and fossa-eminence of the temporal bone (Figure 2). The joint is enclosed in a synovial capsule; the synovium serves to nourish and lubricate the joint (Piette, 1993). The TMJ is a ginglymo-diarthrodial

joint, meaning it exhibits both hinge-like and rotational motions. During normal movements, the disc translates anteriorly during jaw opening and posteriorly during closing. The presence of the disc's fibrous attachments is important to joint motions, but their exact mechanical function and location is heavily debated. The disc is believed to aid in joint lubrication as well as load distribution, jaw stabilization, and shock absorption.

The TMJ disc is divided into three regions: anterior band, posterior band, and intermediate zone (Figure 2). The posterior band is thicker than the anterior band; both bands are significantly thicker than the intermediate zone (Rees, 1954). The disc is generally divided into these three regions for characterization purposes. The bilaminar zone, a fourth element of the disc, exists between the posterior band and the posterior attachments, but generally, is not considered part of the disc. This region possesses some vasculature and is difficult to discern from the posterior attachment tissue.

While the disc is cartilaginous, it is very different from hyaline articular cartilage or even the knee meniscus (Almarza and Athanasiou, 2004a). A healthy TMJ disc is primarily avascular, although some vasculature can be found near the attachment regions. It is well hydrated, containing 70% water (Detamore *et al.*, 2006). Similar to the knee meniscus, the TMJ disc exhibits a mixed population of cell types. In the porcine disc, there are approximately 70% fibroblast-like cells and 30% chondrocyte-like cells (Detamore *et al.*, 2006). The percent of chondrocyte-like cells increases in the intermediate zone and decreases in the bands. This cell population is indicative of the disc's proper characterization as fibrocartilage.

The extracellular matrix (ECM) of the disc is essential to tissue function and important to thoroughly understand before attempting to engineer a construct. The TMJ disc is primarily collagen, and the collagen of the TMJ disc is nearly all collagen type I. Collagen type I makes up the majority of the disc's dry weight, approximately 85% (Nakano and Scott, 1989). Trace amounts of types II, III, VI, IX, and XII can be found in various animal models (Ali and Sharawy, 1996; Gage *et al.*, 1990; Gage *et al.*, 1995; Landesberg *et al.*, 1996; Milam *et al.*, 1991; Minarelli and Liberti, 1997). The fibers of the disc are primarily oriented circumferentially around the outer regions of the disc (Minarelli *et al.*, 1997). In the intermediate zone, fibers are more random but possess a primarily anteroposterior alignment. Collagen fibers in the porcine disc have an average diameter of 18 $\pm$ 9 μm with a range of 2.9 to 37.4 μm (Detamore *et al.*, 2005). Parallel to the collagen fibers are elastin fibers, which are found in all regions (Detamore *et al.*, 2005; Mills *et al.*, 1994; Minarelli and Liberti, 1997; O'Dell *et al.*, 1990).

Glycosaminoglycans (GAGs) and proteoglycans (PGs) are also important components of tissue ECM. The TMJ disc contains approximately 5% GAGs on a dry weight basis (Axelsson *et al.*, 1992; Detamore *et al.*, 2005; Nakano and Scott, 1989). Chondroitin sulfate is the most prevalent GAG in the disc, comprising 70-80% of the total GAG content (Detamore *et al.*, 2005; Kobayashi, 1992; Nakano and Scott, 1989). Aggrecan is an example of a chondroitin sulfate PG that is present in the disc and is important in hydration, lubrication, and compressive strength (Sindelar *et al.*, 2000). Dermatan sulfate is the next most abundant GAG in the disc, making up 15-25% of total GAG content (Kobayashi, 1992; Nakano and Scott, 1989; Nakano and Scott, 1996). Dermatan sulfate PGs include decorin and biglycan, which are important in controlling the collagen fiber lateral packing ability and diameter size (Sindelar *et al.*, 2000). Hyaluronic acid, which binds non-convanently to aggrecan, has been found in the range of 2.8-10% of the total GAG content (Axelsson *et al.*, 1992; Kobayashi, 1992; Nakano and Scott, 1989; Scott *et al.*, 1995). Heparan sulfate was found as 4.3% of total GAG content in the human disc (Axelsson *et al.*, 1992). Keratan sulfate GAGs are generally considered a trace component of the TMJ disc but have been measured up to 2% of the total GAGs (Detamore *et al.*, 2005; Kobayashi, 1992; Nakano and Scott, 1989; Nakano and Scott, 1996).

Mechanical properties of the TMJ disc are important to understand since engineered constructs must support the necessary load imparted on the native tissue. The tensile elastic modulus of the porcine TMJ disc is higher in the anteroposterior direction than the mediolateral direction at 76.4 MPa and 3.2 MPa, respectively (Beatty *et al.*, 2001). In the mediolateral direction, Detamore and Athanasiou (2003) found significant differences between the posterior band, anterior band, and intermediate zone with relaxation moduli of 23.4 MPa, 9.5 MPa, and 0.58 MPa, respectively. In the anteroposterior direction, the stiffest region was the central section followed by the medial section and then lateral section (Detamore and Athanasiou, 2003; Tanne *et al.*, 1991).

Several methods have proved useful in modeling the compressive properties of the TMJ disc. An elastic, compressive modulus for human discs was observed in the range of 211 kPa to 514 kPa, dependent on the strain rate (Chin *et al.*, 1996). The biphasic theory has been employed frequently since its conception to illustrate a tissue's viscoelastic characteristics (Mow *et al.*, 1980). Biphasic modeling of the porcine TMJ disc yielded properties of 20.1 kPa for the aggregate modulus, 0.45 for the Poisson's ratio, and 24.1×10^{-15} m^4/Ns for the permeability (Kim *et al.*, 2003). Most recently, unconfined compression, stress relaxation

tests were performed to give the surface-regional instantaneous and relaxation moduli of the porcine disc. These values were found to be strain dependent, ranging from 90-3870 kPa (instantaneous modulus) and from 16.9-74.6 kPa (relaxation modulus) for 10%-30% strain, respectively. The coefficient of viscosity was also strain dependent, ranging from 1.3-13.8 MPa*s (Allen and Athanasiou, 2005a).

Shear properties of the TMJ disc have recently received due attention. Tanaka *et al.* (2004a) found a storage modulus between 0.78-2.0 MPa depending on the compressive strain and percent shear. A loss modulus near 0.4 MPa and loss tangent ranging from 0.2-0.25 MPa was observed.

Tissue engineering

Tissue engineering is a potential option for the future treatment of diseased or injured discs. The general approach to tissue engineering involves selection of a cell source, seeding these cells on an appropriate scaffold, and applying external stimuli to encourage ECM production and organization. These external stimuli may be grouped into two general categories: biochemical and mechanical. Tissue engineering approaches may commence *ex vivo* or *in vivo* and may exclude one or more of the aforementioned factors (cells, scaffold, and stimuli). For example, skin therapies have been successful using acellular collagen scaffolds. However, all tissue engineering therapies aim to replace the native tissue characteristics through tissue remodeling or regeneration. TMJ tissue engineering has focused on the combination of scaffolds, cells, and stimuli *in vitro* as illustrated in figure 3.

Scaffolds

Scaffolds, an important part of a construct's initial mechanical integrity, provide surface area for cell attachment. The earliest tissue engineering study used a porous collagen scaffold; after two weeks the construct appeared similar to the disc in gross morphology and cell shape (Thomas *et al.*, 1991). Later, researchers attempting to create a replacement for the TMJ disc used fibers of polyglycolic acid (PGA) and polylactid acid (PLA) and concluded that both scaffold materials were able to support cell attachment, matrix production, and retain testable mechanical properties after 12 weeks (Puelacher *et al.*, 1994). Another study compared PGA, polyamide filaments, expanded polytetrafluoroethylene (ePTFE) filaments, and bone blocks (Springer *et al.*, 2001). While all these scaffolds supported cell attachment and a small amount collagen production, they were unable to form neotissue after 4 or 8 weeks. Tissue engineering studies in our lab have primarily used PGA non-woven meshes (Almarza and Athanasiou, 2005; Almarza and Athanasiou, 2006; Bean *et al.*, Accepted November 2005; Detamore and Athanasiou, 2004; Detamore and Athanasiou, 2005b). While PGA supports cell attachment and matrix production, it degrades very rapidly, leaving constructs with limited mechanical integrity after only a few weeks. PLA non-woven mesh, however, has shown promise in retaining tensile and compressive integrity over a similar time scale (Allen and Athanasiou, 2005b).

Some researchers have investigated novel materials for TMJ disc engineering that would allow custom-shaped scaffolds to be implanted through minimally invasive surgery (Poshusta and Anseth, 2001). Acrylated collagen type I scaffolds were successfully photopolymerized through a layer of rat skin; in this study, viability of osteoblasts in a photopolymerized poly(ethylene oxide) dimethacrylate was demonstrated, suggesting this process could be accomplished with other cell types. However, corresponding data for TMJ disc cells encapsulated in alginate showed a drastic decrease is cell numbers at 4 and 8 weeks of culture with no ECM production at any time point, suggesting TMJ disc cells may not survive an encapsulated environment (Almarza and Athanasiou, 2004b).

Cell source

The cell source for a tissue engineering study is tremendously important, but limited research has been conducted in TMJ disc engineering studies. The most commonly used cells for these experiments are derived from the TMJ disc (Almarza and Athanasiou, 2004b; Almarza and Athanasiou, 2005; Almarza and Athanasiou, 2006; Almarza and Athanasiou, Accepted August 2005; Bean *et al.*, Accepted November 2005; Detamore and Athanasiou, 2004; Detamore and Athanasiou, 2005a; Springer *et al.*, 2001; Thomas *et al.*, 1991) or articular cartilage (Girdler, 1998; Puelacher *et al.*, 1994; Springer *et al.*, 2001). A major hurdle to overcome in tissue engineering is that tissue engineering generally requires a large cell population to create a construct. While passaged cells may seem appealing, chondrocytes have been found to de-differentiate to a more fibroblastic phenotype after only a couple of passages (Darling and Athanasiou, 2005). Additionally, TMJ disc cells showed a decreased expression for ECM proteins with the exception of decorin and biglycan due to passage (Figure 4) (Allen and Athanasiou, Submitted 2006). Thus, for the future of TMJ disc engineering, a cell source that can yield a large population of TMJ disc cells, or a population of cells that rapidly fill a scaffold, must be identified.

As mentioned previously, after a discectomy, surgeons may replace the disc with some type of autologous tissue, such as skin, auricular cartilage, dura mater, temporalis muscle, or temporalis fascia (Puelacher *et al.*, 1994). Any of these tissues may serve as potential cell sources for the TMJ disc, but one of the most appealing in terms of clinical feasibility and patient comfort is dermis. Adult dermal fibroblasts have been

shown to produce matrix indicative of a chondrocytic phenotype when seeded on aggrecan-coated plates (Figure 4) (French *et al.*, 2004).

Biochemical factors

Growth factors are commonly used in tissue engineering studies. Four studies have demonstrated the potential of growth factors for TMJ disc tissue engineering. This potential was first observed using transforming growth factor-β_1 (TGF-β_1) and prostaglandin E_2 (PGE_2) on bovine TMJ disc cells in monolayer. TGF-β_1 increased cell proliferation by 250%, while PGE_2 had no significant effect (Landesberg *et al.*, 1996). Also in monolayer, the effects of platelet derived growth factor (PDGF), insulin like growth factor (IGF) and basic fibroblast growth factor (bFGF) on porcine TMJ disc cells demonstrated that lower concentrations of these growth factors favored biosynthesis, while higher concentrations favored proliferation (Detamore and Athanasiou, 2004). The most beneficial growth factors were IGF-I and bFGF, which both showed significant increases in collagen synthesis and cell proliferation. The effects of IGF-I, bFGF and TGF-β_1 on porcine TMJ disc cells in PGA scaffolds showed increased collagen production when exposed to low concentrations of IGF-I and TGF-β_1 (Detamore and Athanasiou, 2005b), but no other significant differences between the experimental groups existed. In the end, IGF-I was recommended for future tissue engineering studies due to low cost and beneficial collagen production. Of course, the native tissue is exposed to a variety of growth factors; so, it is possible growth factor combinations will be more beneficial than any single factor. IGF-I, bFGF, and TGF-β_1 have been investigated in combinations of two to determine if synergistic effects exist (Almarza and Athanasiou, 2006). All constructs exposed to growth factor combinations improved in structural integrity compared to a no growth factor control, but no combination was statistically significant in terms of biochemical or mechanical properties. While synergistic effects were not observed, improved overall cellularity of the constructs was noted when both growth factors were used at a high concentration.

Although growth factors have received the most attention, positive biochemical stimulation is also likely to come from culture conditions and cellular interactions as well. An ascorbic acid concentration of 25 μg/mL has been shown to produce constructs with higher total collagen content and higher aggregate modulus relative to concentrations of 0 μg/mL or 50 μg/mL (Bean *et al.*, Accepted November 2005). This was likely associated with improved seeding observed for the constructs cultured in 25 μg/mL of ascorbic acid. Initial cell seeding is another important consideration in any tissue engineering construct due to cell-to-cell interactions and signaling. Almarza and Athanasiou (2005) showed that PGA scaffolds seeded at saturation increased cellularity and ECM content relative to scaffolds seeded below saturation.

Mechanical stimulation

The native TMJ disc undergoes significant loading, which is often broken down into compression, tension, and shear components (Tanaka *et al.*, 2003). While cells proliferate and produce ECM in static culture, mechanical stimuli may be required to produce an optimal tissue engineered construct. A variety of mechanical stimuli may be beneficial including compression, tension, hydrostatic pressure, and fluid shear stress. Darling and Athanasiou (2003) have published an extensive review of the mechanical bioreactors that have been used in engineering cartilaginous tissues.

Three recent studies have investigated the effects of mechanical stimulation on TMJ disc constructs. A low-shear fluid environment by means of a rotating wall bioreactor created constructs with dense matrix and cell composition (Detamore and Athanasiou, 2005a); however, when the biochemical content of these constructs was compared to those grown in static culture, no clear benefit of the bioreactor was observed. When disc cells were exposed to hydrostatic pressure in monolayer or PGA scaffolds, constant hydrostatic pressure at 10 MPa increased collagen production compared to static culture (Almarza and Athanasiou, Accepted August 2005). In contrast, intermittent hydrostatic pressure from 0 to 10 MPa at 1 Hz frequency was detrimental to the constructs, producing less collagen and GAGs than unloaded controls. These results were consistent in both two and three-dimensional culture. In another recent study, dynamic tensile strain significantly reduced interleukin-1 β induced up regulation of matrix metalloproteinase (Deschner *et al.*, 2005). This may have implications on future tissue engineering studies since MMPs play an important role in ECM degradation and remodeling.

Future directions for TMJ disc tissue engineering

While TMJ disc tissue engineering is in its infancy, other musculoskeletal tissues have been studied to a greater extent. These tissues include articular cartilage, bone, and tendon. TMJ disc tissue engineering should build on not only past TMJ research but also successes in these other tissues, while keeping in mind the disc's structural and functional differences.

The issue of scaffold certainly requires further investigation. Scaffolds that degrade too quickly are unable to provide the necessary mechanical integrity; thus, future research may focus on polymers with longer degradation times or that encourage rapid ECM production. Alternatively, using natural polymers like

collagen may be effective since cells would simply remodel existing matrix instead of forming a new collagen network, thereby decreasing the time until the scaffold reaches a functional state. A third option is a scaffoldless or self-assembling process. Such approaches have been examined in both tendon and articular cartilage (Calve *et al.*, 2004; Hu and Athanasiou, March 2006). While these methods require refinement to increase mechanical strength, data suggest these approaches may offer a new direction in soft tissue engineering. Futhermore, by eliminating the scaffold material within an engineering construct, concerns over mechanical integrity and cell toxicity due to the scaffold degradation process are diminished.

An optimal cell source is necessary for tissue engineering to be realized. To date, no such source has been identified that is likely to be clinically sound. However, research in other musculoskeletal tissues like cartilage, tendon, and bone has explored the possibility of using mesenchymal stem cells for tissue engineering (Altman *et al.*, 2002; Awad *et al.*, 1999; Funakoshi *et al.*, 2005; Hankemeier *et al.*, 2005; Juncosa-Melvin *et al.*, 2005; Li *et al.*, 2005; Mao and Nah, 2004; Mauck *et al.*, 2005; Moreau *et al.*, 2005; Tanaka *et al.*, 2004b; Wang *et al.*, 2005; Wayne *et al.*, 2005). Using progenitor cells may also be desirable for the TMJ disc, since bone marrow or adipose tissue could potentially yield a large population of autologous, pluripotent cells. Alternatively, research on other potential cell sources, such as embryonic stem cells and dermis-derived fibroblasts, continues to demonstrate promise.

The inclusion of biochemical signaling will be an integral part of producing a TMJ disc tissue engineering construct. Significant work has been performed in both two- and three-dimensional cultures to determine optimal growth factor signaling for TMJ disc engineering. Recent work showed the growth factors IGF-I and TGF-β_1 used alone produced increases in collagen production (Detamore and Athanasiou, 2005b). This provides a basis for growth factor selection in future TMJ disc tissue engineering studies. Beyond growth factors, the media used for culturing should also be further investigated. Ascorbic acid concentration has influenced the outcome of engineered constructs (Bean *et al.*, Accepted November 2005); thus, other media supplements may need further optimization as well. Cell-to-cell interactions are important, and seeding the cells in scaffolds at saturation was shown to produce constructs with significant increases in ECM production (Almarza and Athanasiou, 2005). This is clearly vital for fabrication of an optimal TMJ disc construct.

Cartilage is a mechanical tissue; thus, mechanical stimulation should be expected for regeneration of any cartilaginous tissue. The most successful mechanical stimulation used to date for the TMJ disc has been constant hydrostatic pressure (Almarza and Athanasiou, Accepted August 2005). Hydrostatic pressure should certainly be pursued further, because there are likely to be other beneficial loading regiments. Tension has shown promise in monolayer culture and should be pursued for future three-dimensional tissue engineering studies (Deschner *et al.*, 2005). Success in engineering the knee meniscus has been seen using direct compression; these results may apply to the TMJ disc due to the fibrocartilaginous nature of both tissues (Aufderheide and Athanasiou, Submitted 2005). Additionally, perfusion increased cellularity and ECM production in articular chondrocytes and may hold the same potential for the TMJ disc (Davisson *et al.*, 2002). Perfusion may also create larger constructs due to increased nutrient circulation.

In conclusion, while the field of TMJ disc engineering remains young, significant progress has been achieved. With this progress have come new, challenging questions and a wealth of knowledge on the disc's characteristics. Related research may begin to merge with TMJ disc engineering due to the increased knowledge of TMJ disc design criteria. Tissue engineered TMJ constructs may now be validated with the increased fund of information on the tissue's native characteristics. With these tools at hand, TMJ research will continue to rapidly progress to, hopefully, a viable tissue engineering implant.

Acknowledgements

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Figures

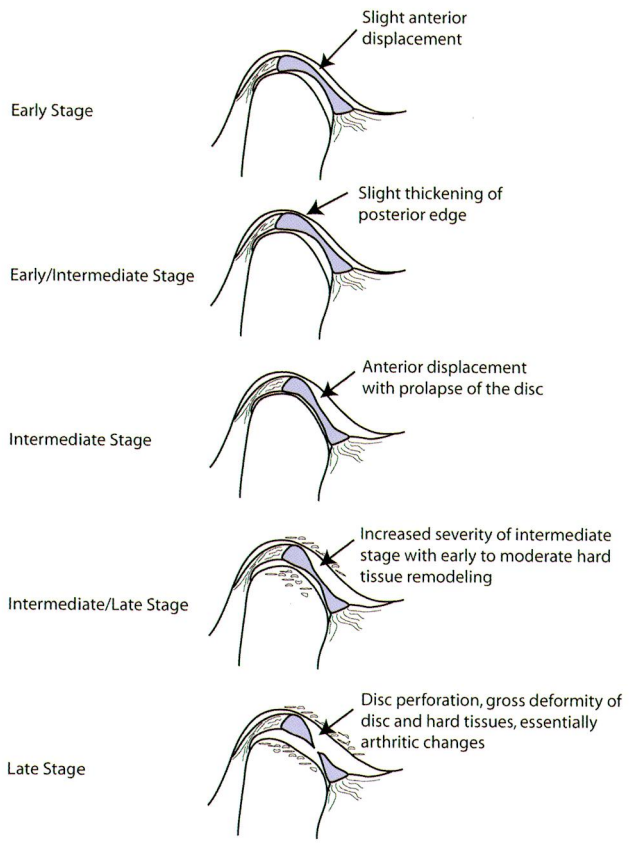

Figure 1: The stages of TMJ internal derangement as described by Wilkes (Wilkes, 1989).
Schematics describe the progression of TMJ internal derangement; these schematics were created based upon radiologic findings described by Wilkes (Wilkes, 1989). In early stages, clinical symptoms are limited (no significant pain or mechanical symptoms); however, a slight anterior displacement of the disc can be observed. As the derangement progresses towards the intermediate stage, a few episodes of pain along with occasional joint tenderness, headaches, and mechanical problems are reported. Here, the disc displacement is slightly more forwards and the posterior edge thickens. At the intermediate stage, pain intensifies along with other clinical symptoms; anterior displacement of the disc is significant and coupled with disc prolapse. As the disorder progresses toward late stages, chronic pain develops; disc displacements are severe and hard tissue remodeling ensues. In late stages, joint scraping and difficulty in function are evident. The disc may be out of position, degenerated, or perforated. Hard tissue remodeling is severe; the joint is essentially arthritic.

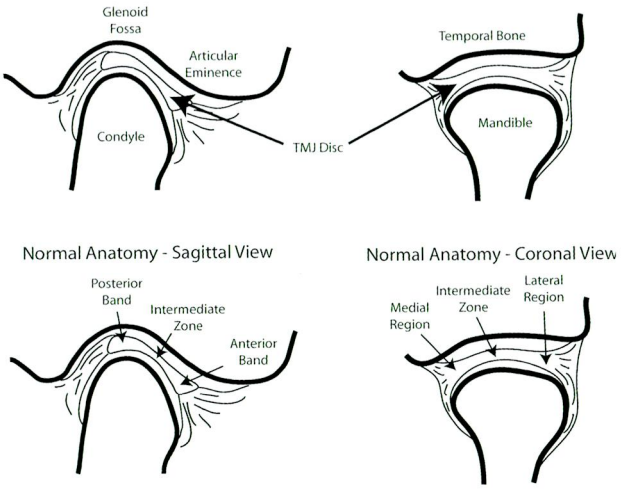

Figure 2: Joint anatomy and disc regions
The TMJ disc is located between the mandibular condyle and fossa-eminence of the temporal bone. The disc is fibrocartilaginous and has a biconcave shape in both sagittal and coronal views. Thickness variations are evident in the sagittal view, where the thick posterior and anterior bands differ significantly from the intermediate zone. In the coronal view, thickness variations are less pronounced; however, the medial and lateral extents of the disc are slightly thicker than the intermediate zone.

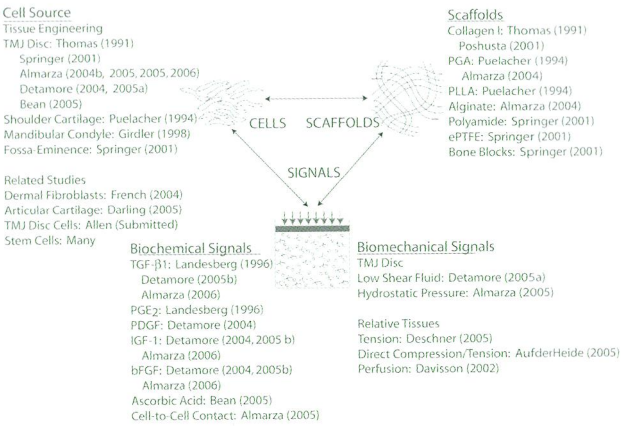

Figure 3: A tissue engineering paradigm: history of TMJ disc engineering
Tissue engineering, generally, is conducted by combining cells and signals on an appropriate scaffolding material. This approach has been the standard thus far in TMJ disc engineering. References to significant studies of scaffolding, signals, and cell source for the TMJ disc are placed within the classic paradigm figure. Clearly, TMJ disc engineering is very young; however, it is apparent that the field is rapidly expanding.

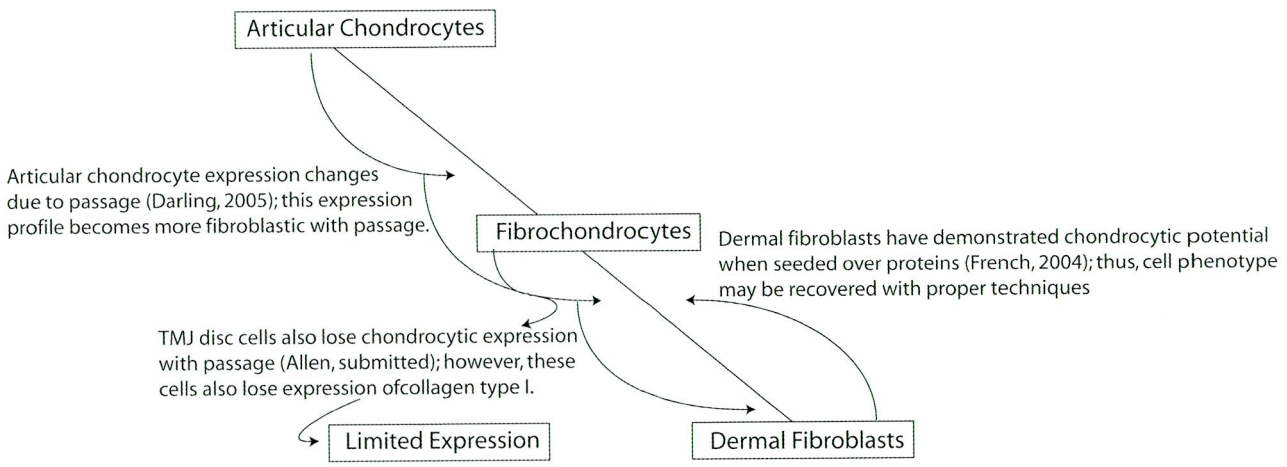

Figure 4: From chondrocyte to fibroblast
In our laboratory, we have investigated the relationship between chondrocytes, fibrochondrocytes, and dermal fibroblasts. First, chondrocytes progressively dedifferentiate as a function of monolayer culture (Darling and Athanasiou, 2005). As these cells are passaged, they become more fibroblastic in nature, characterized by a loss of chondrocytic ECM gene expression and a gain in fibroblastic expression. Fibrochondrocytes follow a similar loss in gene expression; however, fibroblastic gene expression is also lost as a function of passage (Allen and Athanasiou, Submitted 2006). However, it may be possible to regain these losses by seeding passaged cells over proteins; dermal fibroblasts have demonstrated a chondrocytic response when seeded over specific extracellular matrix proteins (French *et al.*, 2004).

TMJ tissue engineering: From the disc to the condyle

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ABSTRACT

The field of TMJ tissue engineering is blossoming, a field that was once far behind orthopaedic tissue engineering is rapidly gaining ground. Both TMJ disc and TMJ condyle tissue engineering efforts present unique challenges and will ultimately be necessary to regenerate TMJs for patients suffering from severe disorders. The TMJ disc is a fibrocartilaginous structure with complex attachments, where tensile mechanical integrity is a crucial design requirement. The TMJ condyle presents the challenge of engineering both bone and cartilage. Its cartilage is a fibrocartilage with four distinct zones, and the design requirement for mechanical integrity is the ability to resist compression and shear. We are focusing our efforts on the mandibular condyle, with efforts in all three areas of the tissue engineering triad: cell source, scaffold selection, and chemical signals. With regard to cell source, we are interested in comparing a traditional source of cartilage cells with an exciting new source in human umbilical cord matrix (HUCM) stem cells for engineering cartilage. The HUCM stem cells have shown promise in their ability to synthesize GAGs and collagen on poly(glycolic acid) scaffolds. Moreover, we have initial data to suggest that HUCM stem cells will be strong candidates for bone tissue engineering as well. Regarding scaffold selection, we are currently investigating new hydrogels and novel approaches to designing poly(lactic-co-glycolic acid) scaffolds. Our research in chemical signals has focused on the use of glucosamine, and on the use of proteoglycans along with growth factors. Our results have suggested that glucosamine may have a beneficial effect on TMJ condylar cartilage cells in culture, and effective concentration ranges are currently being investigated.

CELL SOURCE

HUCM stem cells develop from extraembryonic mesoderm, which forms the umbilical cord matrix (Mitchell *et al.*, 2003). They have been shown to be multipotential stem cells with properties between embryonic stem cells and adult stem cells. There is a lower incidence of graft vs. host disease than with bone marrow transplants, suggesting that umbilical cord cells have an innate mechanism to evade the immune system (Barker and Wagner, 2003). Currently, trials are underway using umbilical cord cells to treat a number of diseases. Only recently have HUCM stem cells been considered for tissue engineering, and for the most part have been focused on vascular tissue engineering. Recently, a German group reported that they were able to differentiate HUCM stem cells into cells with osteoblastic properties (Eblenkamp *et al.*, 2004). They suggested that HUCM stem cells are a promising source for cell-based therapies due to their ease of procurement and large supply. We intend to use this new cell source technology to engineer osteochondral constructs.

Cartilage Tissue Engineering With HUCM Stem Cells

Porcine TMJ condylar cartilage cells and HUCM cells were separately seeded onto PGA scaffolds for 6 days in a spinner flask. Spinner flasks for TMJ cells and HUCM cells contained control and chondrogenic medium, respectively. After seeding, constructs were then each cultured in either control medium or chondrogenic medium for an additional 4 wks. Although both groups were seeded at 5 million cells/mL, the HUCM cells were greater in number immediately after seeding and after 4 wks. After 4 wks, immunohistochemical staining demonstrated a strong presence of collagen I, and Saf-O/Fast green staining indicated a significant amount of GAG synthesis (Fig. 1). These results not only demonstrate the feasibility of using HUCM stem cells for chondrogenesis in 3D, but also suggest a superiority of these cells over a chondrogenic cell source.

Osteogenic Differentiation of HUCM Stem Cells

HUCM stem cells were cultured in monolayer for 12 days in control medium or osteogenic medium. Alizarin red staining demonstrated clear nodule formation and early osteogenic differentiation (Fig. 2). These encouraging results were the impetus for a

subsequent 3D investigation where HUCM stem cells were seeded directly onto PGA scaffolds and cultured in osteogenic medium for 1 wk. Again, alizarin red staining was positive, but more importantly, immunohistochemical analysis revealed an early presence of two osteogenic markers, alkaline phosphatase and osteopontin, in distinct isolated regions.

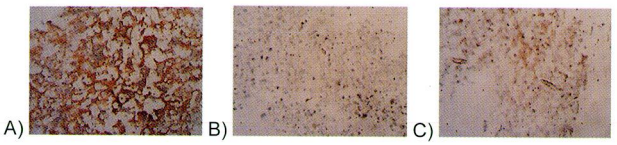

Figure 1: Saf-O/Fast green staining (200X), A) HUCM stem cells vs. B, C) condylar cartilage cells. Constructs were seeded for 6 days in a spinner flask (HUCM stem cells in chondrogenic medium, condylar cartilage cells in control medium), then cultured for 4 wks in static culture (A, B→control medium; C→chondrogenic medium).

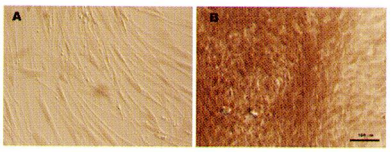

Figure 2: HUCM stem cells grown in A) control or B) osteogenic medium for 12 days, stained with alizarin red.

SCAFFOLD SELECTION

Microparticle-based scaffolds have recently incited enthusiasm in tissue engineering due to their ease of fabrication, the ability to discretely control particle physicochemical properties, and versatility for controlling the release kinetics of bioactive molecules. Microparticles fabricated from biodegradable polymers such as poly (glycolic acid) (PGA), poly(lactic acid) (PLA) or co-polymers of the two (PLGA) offer the added benefits of modulating degradation kinetics, and of FDA approval for use in humans. Chondrocytes and osteoblasts have been successfully cultured on scaffolds composed of PLGA microspheres containing active compounds such as BMP-2 and TGF-β (Elisseeff *et al.*, 2001; Lu *et al.*, 2001; Oldham *et al.*, 2000). With our collaborators, we have a unique ability to create uniform PLGA microspheres to facilitate production of tissue scaffolds with precisely reproducible physical features. Our goal with our present work is to control the spatial organization of encapsulated signal molecules, which we can then use to direct HUCM stem cell differentiation in a region-specific manner.

CHEMICAL SIGNALS

There is a plethora of evidence for the safety and efficacy of glucosamine and chondroitin sulfate for treating osteoarthritis, including several recent reports (Anderson *et al.*, 2005; Brief *et al.*, 2001; Bruyere *et al.*, 2004; Chalmers, 2005; Christgau *et al.*, 2004; Davenport, 2004; Einhorn, 2004; Nakamura *et al.*, 2004; Zerkak and Dougados, 2004). Successes with glucosamine were the impetus for the formation of the Glucosamine Arthritis Intervention Trial (GAIT), created in part with funding from the National Institute of Arthritis and Musculoskeletal and Skin Diseases. GAIT was a 24-wk placebo-controlled study of 1588 patients at 13 centers, with results being released in just the past few months, with the conclusion that glucosamine and chondroitin are effective in treating osteoarthritis. There is a strong possibility that glucosamine-chondroitin may provide for an effective new strategy in cartilage tissue engineering, which we are currently investigating.

Glucosamine in Monolayer Culture

In a 2-week monolayer study with confluent porcine ankle chondrocytes in 12-well plates, wells with only 0.1 mg/mL of glucosamine produced 20 ± 6 mg GAG/well compared to 11 ± 2 mg for the control (Fig 3).

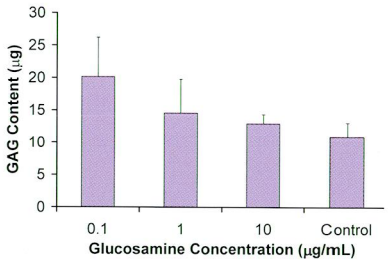

Figure 3: GAG content in monolayer cultures of chondrocytes in 2 weeks, based on varying concentration of glucosamine content. It is interesting to note that higher concentrations of glucosamine do not necessarily ensure a higher GAG content.

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Multi-differentiation Potential of Mesenchymal Stem Cells in Three-dimensional Collagen Gel Cultures

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Introduction

Transplantation from iliac or hip bone has been utilized for grafting bone into defects caused by congenital disease, trauma or tumor invasion, which generally results in a desirable outcome. However, a considerable tissue volume is required for the transplantation, which also necessitates substantial surgical invasion and results in increased morbidity. Additionally, while the restoration of degenerated cartilage in articular condyles has been attempted, this approach has not been successful because of a difficulty in expanding articular chondrocytes *in vitro*. The transplantation of cartilage constructed *in vitro* from expanded primary cells also has had limited success partially because of its inability to sustain function. A newer concept for cartilage regeneration proposes the construction of complex osteochondral tissues consisting of sequential cartilage and bone tissues which may enhance the success of these constructs due to the preferable affinity between bony tissues on transplantation. With these considerations, a new technique for regenerating combined osteochondral tissues *in vitro* has generated substantial research and clinical interest.

An anagenetic technique using mesenchymal stem cells (MSCs) has a good affinity between the organism and material, because MSCs can proliferate quickly and differentiate into various tissues[1]. In combination with a three-dimensional culture system, it seems to be possible to develop a tissue block or scaffold consisting of MSCs, which can alter itself to a tissue component equivalent to peripheral tissue. However, an appropriate scaffold has not yet been developed for a three-dimensional culture system of MSCs.

Of the various three-dimensional scaffold materials, collagen has many advantages over others. Collagen is the major component of extracellular matrix in bone and cartilage, participates in regulating cell growth and differentiation, and because of its mechanical properties and plasticity, provides the relevant physiological stiffness of tissues. Due to its biocompatibility, collagen has been used as a scaffold in constructing a three-dimensional cell culture system, and has demonstrated its ability to maintain a chondrocytic phenotype[2]. Prior to precartilage condensation, the initial stage of chondrogenesis, MSCs highly express type I collagen, and the expressions decrease in a series of chondrocytic differentiations[3,4]. Thus, collagens are essential for maintaining the homeostasis of MSCs, and would possibly be a good candidate as a scaffold for a three-dimensional culture system of MSCs. However, it has not been demonstrated whether or not MSCs can differentiate into osteoblasts or chondrocytes in three-dimensional collagen gels.

The high expression of hyaluronic acid (HA) during endchondral bone formation indicates an important role of HA in bone and cartilage development. In fetal tissues, HA is particularly rich, but the content decreases during development[5]. HA is also a highly and widely distributed component of extracellular matrices, like collagens, in various tissues. HA has been reported to promote the migration and proliferation of MSCs[6,7], and is associated with the acceleration of wound healing[8]. Furthermore, HA binds to specific cell-surface receptors, such as CD44 and the receptor for HA-mediated motility (RHAMM), and also binds to other matrix molecules, such as collagen and proteoglycans[5,9-11]. It would thus be assumed that HA exerts certain influences on the differentiation process from MSCs and becomes an appropriate scaffold for the three-dimensional culture system of MSCs.

The purpose of this study was to characterize the ability of MSCs to differentiate into osteoblastic or chondroctyic lineages in three-dimensional collagen gels under appropriate biological stimuli, and to

elucidate the availability of HA as an additional scaffold for the three-dimensional collagen gel culture system of MSCs.

Materials and Methods

Three-dimensional gel culture for MSCs: Human bone marrow MSCs and growth medium (MSCGM) were purchased from Bio-Whittaker Inc. For the construction of three-dimensional culture, MSCs were harvested from monolayer culture with trypsin treatment and embedded in 0.3% type I collagen gel obtained as a collagen gel kit according to the manufacture's instructions. Briefly, 1 ml collagen solution containing cells either in low density (5 x 10^5 cell/ml) or high density (5 x 10^6 cell/ml) was placed in 24-well cell culture dishes and incubated at 37°C for 30 minutes for gelatinization. For the investigation of HA effect, MSCs were cultured in HA-collagen hybrid gel composed of 0.15% collagen in combination with 0 or 0.5 mg/ml HA (Suvenyl®). The medium was changed every two days up to a maximum of 20 days of culture. All the cultures were maintained at 37°C in a humidified 5% CO_2 incubator.

Cell differentiation: For chondrogenic differentiation, human MSCs seeded at low or high density in collagen gels were placed in chondrogenic differentiation medium (CDM) consisting of serum-free MSCGM that contained 1 mM sodium pyruvate, 100 mg/ml L-ascorbic acid-2-phosphate (AsAP), 1 x 10^{-7} M dexamethasone (Dex), 1% ITS, 5.33 mg linolate, 1.25 mg/ml bovine serum albumin, 40 mg/ml proline, and 10 ng/ml recombinant human TGF-β3[12, 13]. For osteogenic differentiation, human MSCs seeded at low density were cultured osteoblastic differentiation medium (ODM) consisting of MSCGM supplemented with 100 nM Dex, 10 mM β-glycerophosphate, and 0.05 mM AsAP[14].

Toluidine blue staining in the chondrogenic gel: The gels were fixed with 100% ethanol and stained with 1% toluidine blue. To avoid the staining of exogenous HA, the staining procedure was performed at pH 2.5.

Measurement of sulfated glycosaminoglycan (GAG) contents in the chondrogenic gel: The sulfated GAG contents were quantified using a Blyscan Sulfated Glycosaminoglycan Assay kit. The gel cultures stimulated with CDM were digested with papain solution for 3 hours at 65°C. The total GAG contents were quantified by reading an optical density (OD) at 655 nm on a microplate reader. Standard plots were obtained from chondroitin 4-sulfate, and the absolute GAG contents in the gel cultures were determined according to the standard plots with a reference to the OD values.

Alizarin red and alkaline phosphatase (ALP) stainings: The gels cultured in ODM were fixed with 100% ethanol and stained with 1% alizarin red (pH6.3) as described previously. Alkaline phosphatase (ALP) staining was also performed for the gel cultures using fast 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium tablets.

Calcium incorporation assay: The gels were digested with 0.2% (v/v) triton-X-100, 0.02% collagenase and 6N HCl. The total amount of calcium was measured using a Calcium-C-kit and a microplate reader at an OD of 570 nm.

ALP activity analysis: The gels were digested with 0.2% (v/v) triton-X-100 and 0.02% collagenase, and incubated with 5 mM *p*-nitrophenyl phosphate in 50 mM glycine, 1 mM $MgCl_2$, pH10.5 at 37°C for 2 hours. The ALP activity was estimated by quantifying the absorbance of *p*-nitrophenol product formed at an OD of 405 nm on a microplate reader.

Quantitative real time reverse transcription polymerase chain-reaction (real time RT-PCR): Quantitative RT-PCR was performed for several bone and cartilage markers including type II collagen, type X collagen, type I collagen, bone sialoprotein (BSP) and ALP. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control gene. Total RNA was isolated from the gel cultures using a guanidine thiocyanate method. A single strand cDNA was synthesized from 1 mg of total RNA using Oligo $(dT)_{20}$ primer and a Rever Tra Ace-a first strand cDNA synthesis kit. The mRNA levels were determined by quantitative real time RT-PCR analysis, using a SYBR Green PCR master mix or a TaqMan Universal PCR master mix with an automated fluorometer. Quantification of the signals was performed by normalizing the signals of target genes relative to the GAPDH signals. Normalized Ct values were expressed relative to the controls.

Results

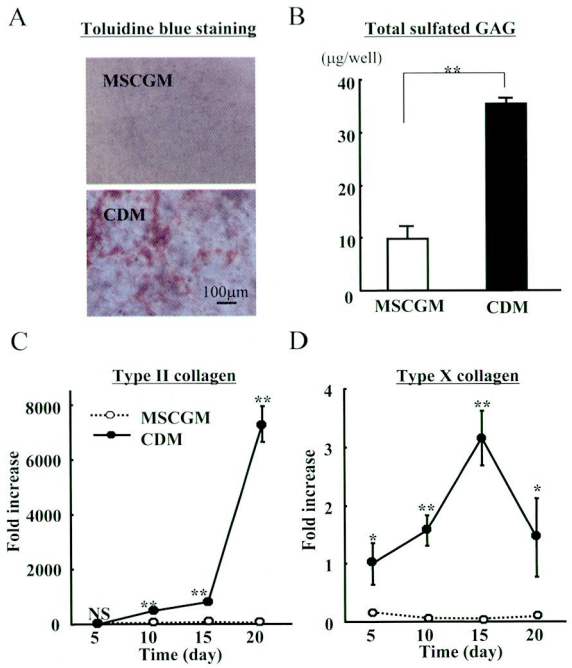

Figure 1 Chondrogenic differentiation in the three-dimensional collagen gel.
MSCs were cultured in three-dimensional collagen gel treated with MSCGM and CDM for 20 days. The gels were fixed and stained with toluidine blue (A). The gels were digested and the GAG contents were measured (B). The mRNA expressions of type II collagen (C) and type X collagen (D) were analyzed with quantitative real-time PCR. *: $p<0.05$, **: $p<0.01$

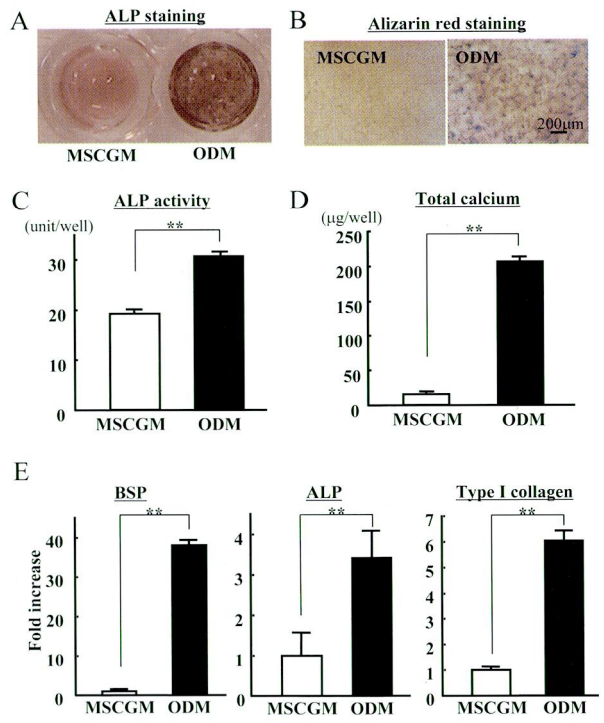

Figure 2 Osteogenic differentiation in the three-dimensional collagen gel.
MSCs were cultured in three-dimensional collagen gel treated with MSCGM and ODM for 14 days. The gels were stained with ALP(A) and Alizarin red (B). The gels were digested, and ALP activity (C) and calcium contents (D) were determined. The mRNA expressions of BSP, ALP and type I collagen in MSCs on day 5 were analyzed with quantitative real-time PCR (E). **: $p<0.01$

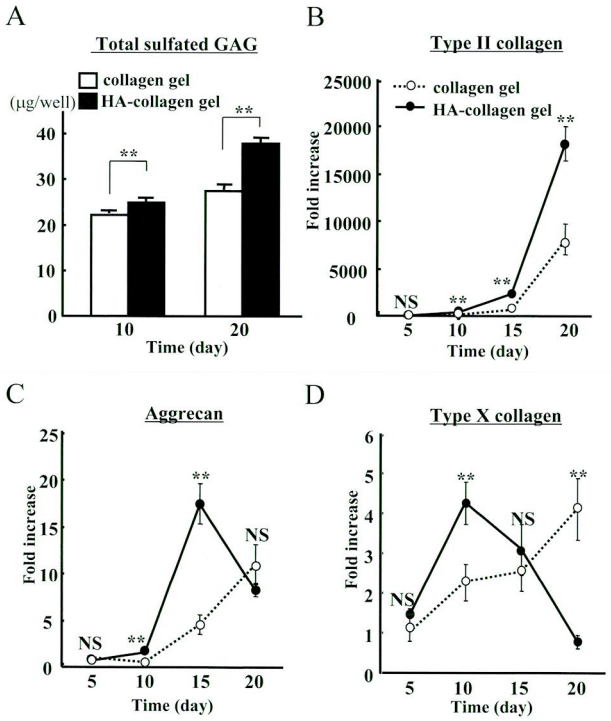

Figure 3 The effect of HA in the chondrogenic differentiation.
MSCs were cultured in HA-collagen hybrid gel treated with MSCGM and CDM for 20 days. The gels were digested and the GAG contents were measured (A). The mRNA expressions of type II collagen (B), Aggrecan (C) and type X collagen (D) were analyzed with quantitative real-time PCR. *: $p<0.05$, **: $p<0.01$

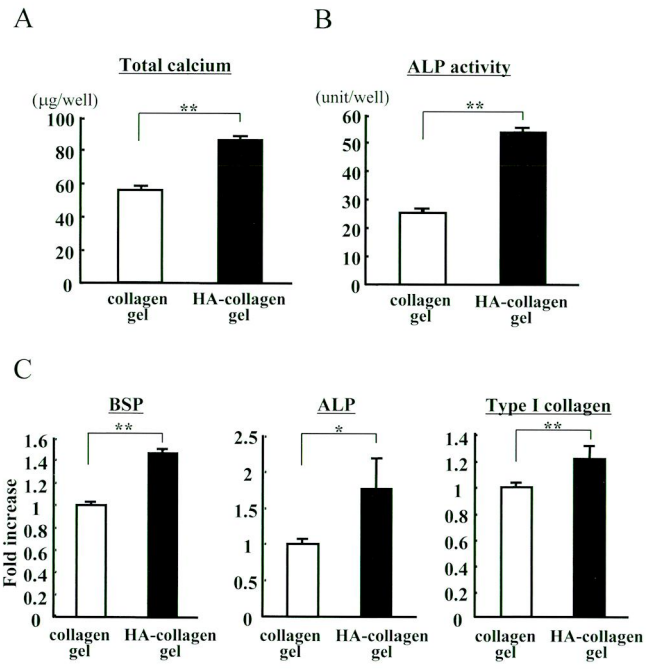

Figure 4 Figure 4 The effect of HA in the osteogenic differentiation
MSCs were cultured in HA-collagen hybrid gel treated with MSCGM and ODM. The gels were digested, and calcium contents (A) and ALP activity (B) were determined on day 14. The mRNA expressions of BSP, ALP and type I collagen in MSCs on day 5 were analyzed with quantitative real-time PCR(C). *: $p<0.05$, **: $p<0.01$

Chondrogenic differentiation in three-dimensional collagen gel culture: The GAG deposition on day 20 assessed by toluidine blue staining was remarkably higher in the high density gel cultures maintained in CDM than in MSCGM as the control (Fig. 1A). Similarly, the total amount of GAG per gel was also significantly higher in constructs maintained in CDM than in MSCGM (Fig. 1B). A time-dependent increase in the expression of type II collagen mRNA was observed in the high density gel cultures treated with CDM compared to MSCGM. This increase was significantly higher in CDM-stimulated gels over control gels at 10, 15 and 20 days of culture, but not at 5 days culture (Fig. 1C). A temporal increase in expression of type X collagen was also observed in the gels treated with CDM, which was significantly greater than that in the control gels at 5, 10, 15 and 20 days of culture (Fig. 1D).

In contrast to the increases in GAG and the expression of type II collagen and type X collagen mRNAs in high density cultures stimulated with CDM, low density gel-cell constructs did not show any significant increases in GAG after 20 days of culture (data not shown). Similarly, the expressions of type II collagen and type X collagen mRNAs were also not changed in the low-density cultures exposed to CDM as compared to those in MSCGM over 15 days (data not shown).

Osteogenic differentiation in three-dimensional collagen gel culture: Intensity of ALP staining was higher in the gel culture maintained in ODM for 14 days than in MSCGM as the control (Fig. 2A).

Alizarin red staining for the MSC gel culture showed enhanced calcium deposition in the extracellular regions of gels maintained in ODM over those in MSCGM (Fig. 2B). The ALP activity and incorporation of calcium into the extracellular matrix were also significantly higher in the gels maintained in ODM than in MSCGM (Fig. 2C, D). The expressions of bone markers BSP, ALP and type I collagen mRNAs were significantly higher in the MSC gel cultures maintained in ODM than those in MSCGM over a period of 5 days (Fig. 2E).

Chondrogenic differentiation in the HA-collagen hybrid gel: The total amount of sulfated GAG in the collagen gels was also significantly greater in gels containing HA than those without it (Fig. 3A). A time-dependent increase in the expression of type II collagen mRNA was observed in both collagen and HA-collagen gels; however, the expressions of type II collagen mRNA in the HA-collagen hybrid gels were substantially greater than those in the collagen mono gels on day 20 (Fig. 3B). A transient up-regulation of the aggrecan and type X collagen was observed in the MSCs cultured in the HA-collagen hybrid gels when treated with CDM, whereas the expression of these genes was increased in a time-dependent manner in those cultured in collagen mono gels (Fig. 3C, D).

Osteogenic differentiation in the HA-collagen hybrid gel: When the MSCs were stimulated by ODM, the incorporation of calcium into the extracellular matrix and ALP activity were significantly greater in the HA-collagen hybrid gel than in the collagen mono gel (Fig. 4A, B).

The gene expressions of all bone markers examined, BSP, ALP and type I collagen were significantly greater in the HA-collagen hybrid gel than in the collagen mono gel (Fig. 4C).

Conclusions

We have demonstrated that human MSCs have an ability to differentiate into both bone and cartilage tissues in three-dimensional collagen gel, and had a higher differentiation potential to hard tissues such as the bone and cartilage in the HA-collagen hybrid gel than in the collagen mono gel. This report indicates that HA treatment has become a useful tool for a more efficient and optimal hard tissue regeneration from MSCs.

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